UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
DOCTORADO EN CIENCIAS BIOMÉDICAS
FACULTAD DE MEDICINA

ALTERACION DE LA ACTIVIDAD DE CELULAS DENDRITICAS POR ANTIGENOS SECRETADOS/EXCRETADOS DE *TAENIA CRASSICEPS*: SU IMPACTO EN LA MODULACION DE LA RESPUESTA INMUNE A ANTIGENOS HETEROLOGOS

TESIS
QUE PARA OPTAR POR EL GRADO DE:
DOCTOR EN CIENCIAS

PRESENTA:
CESAR AUGUSTO TERRAZAS ALDUCIN

DIRECTOR DE TESIS
DR. LUIS IGNACIO TERRAZAS VALDES
FACULTAD DE ESTUDIO SUPERIORES IZTACALA, UNAM

COMITÉ TUTOR
DRA. LAURA BONIFAZ
DEPARTAMENTO DE ENFERMEDADES AUTOINMUNES, IMSS

DRA. GLORIA SOLDEVILA
INSTITUTO DE INVESTIGACIONES BIOMÉDICAS, UNAM

MÉXICO, D. F. JULIO 2013

Este trabajo se realizó en su mayor parte en el laboratorio de Inmunoparasitología, Unidad de Biomedicina de la FES, Iztacala, UNAM y un 15 % en el laboratorio del Dr. Abhay Satoskar, en la Universidad Estatal de Ohio.

Durante el periodo de la investigación el autor fue becario de Doctorado del CONACYT bajo el numero 220758, además de haber recibido una beca para estancia de investigación deFulbright-COMEXUS.El autor también recibió apoyo del Posgrado en Ciencias Biomédicas, UNAM.

Agradecimientos:

Dr. Luis I. Terrazas
Dr. Abhay Satoskar
A todos los integrantes del laboratorio de Inmunoparasitología de la UBIMED.

Differential response of antigen presenting cells from susceptible and resistant strains of mice to *Taenia crassiceps* infection. Infection, Genetics and Evolution 2009, 9(6), 1115-1127.

Impaired pro-inflammatory cytokine production and increased Th2-biasing ability of dendritic cells exposed to *Taenia* excreted/secreted antigens: A critical role for carbohydrates but not for STAT6 signaling. International Journal for Parasitology 2010, 40(9), 1051-1062.

Cestode antigens induce a tolerogenic-like phenotype and inhibit LPS inflammatory responses in human dendritic cells. International Journal of Biological Sciences 2011, 7(9), 1391-1400.

Helminth-excreted/secreted products are recognized by multiple receptors on DCs to block the TLR response and bias Th2 polarization in a cRAF dependent pathway FASEB J, 2013 (En prensa).

Modulation of dendritic cell responses by parasites: A common strategy to survive.Journal of Biomedicine and Biotechnology, 2010.

Capítulos de libroCestoda: Tapeworm Infection. CA Terrazas, M Rodríguez-Sosa, LI Terrazas Immunity to Parasitic Infection, 307-322

Abstract

In cysticercosis, a parasitic disease caused by cestodes, the details of early interactions between parasite antigens and innate cells from the host are not well understood. In this study, the role of cestode-conditioneddendritic cells (DCs) in priming Th1 versus Th2 responses to bystander antigen was examined byusing CD11c+ DCs as antigen-presenting cells and naive CD4+ DO11.10 lymphocytes specific to ovalbumin(OVA) as responding cells. No conventional maturation was induced in DCs exposed to *Taenia crassiceps*excreted/secreted antigens (TcES). The ability of TcES to affect Toll-like receptor (TLR)-mediated maturationand the pro-inflammatory response was analyzed by co-pulsing DCs with TcES and several TLR ligands. DCs exposed to TcES blocked TLR4, TLR9 and *Toxoplasma* soluble antigen-induced phenotypic maturation. TcES-exposed DCs also blocked secretion of pro-inflammatory cytokines and alloreactive T cell proliferation, while preserving IL-10 production. DCs pulsed with TcES + OVA suppressed IFN-γ, whereas induced greater IL-4 production by CD4+ DO11.10 cells. Interestingly, TcES with chemically-altered glycans failed to modulate TLR-mediated activation of DCs and their Th1-inhibiting ability.

On the other hand, we also examined different responses of APCs in mice that are known to be susceptible(BALB/c) or resistant (C57BL/6) to *Taenia crassic*eps cysticercosis. Bone marrow-derived dendritic cells (BMDCs) from both strains of mice were exposed to TcES and, at the same time, to the TLR4 ligand LPS. BMDCs from BALB/c mice underwent a partial maturation when incubated with TcES and displayed decreased responses to TLR-dependent stimuli associated with low CD80, CD86, CD40 and CCR7 expression and impaired IL-15 production. These BMDCs-induced impaired allogenic responses. In contrast, BMDCs from C57BL/6 mice displayed normal maturation and induced strong allogenic responses. Moreover, the exposure to TcES resulted in a lower production of IL-12 and TNF-α by LPS-activated DCs from BALB/c mice compared to C57BL/6 DCs. Together, these findings suggest that APCactivation during *T. crass*iceps infection may be a possible mechanism that underlies the differential susceptibility to *T. crassiceps* infection displayed by these mouse strains.

We also investigated the receptors and intracellular signaling pathways involved in TcES modulation on DCs. We found that TcESnegatively affected p38 and NFkBp65 phosphorylation induced by LPS. Additionally we found that TcES products were recognized by DCs through MGL, MR andTLR2. MR and MGL triggered cRAF phosphorylation, whereas TLR2 contribution to enhance cRAF phosphorylation was minimal.cRAF phosphorylation induced by TcES was necessary to down regulate the TLR-mediated DC maturation and secretion of IL-12 and TNF-α. In line with this finding blocking MR, MGL and TLR2 together abolished the ability of TcES to downregulate pro-inflammatory cytokine production after TLR4 activation. Moreover, we show for the first timethat blocking cRAF in DCs abolishes their ability to induce Th2 polarization in vitro after TcES exposure. However, MR, MGL or TLR2 synergistically contributed to enhance Th2 responses primed by DCs pretreated with TcES.

Finally, we showed that human DCs weremodulated byTcES. TcES did not induce the maturation of human DCs judged by a lack of increase in the expression of CD83, HLA-DR, CD80 and CD86 molecules but enhanced the production of IL-10 and positively modulated the expression of the C-type lectin receptor *MGL* and negatively modulated the expression of DC-SIGN. Additionally, TcES were capable of down-modulating the inflammatory response induced by LPS in these cells by reducing the expression of the maturation markers and the production of the inflammatory cytokines IL-1β, TNF-α, IL-12 and IL-6. The effects of TcES upon the DCs responses to LPS were stronger if cells were exposed during their differentiation to the helminth antigens. All together, these findings suggest the ability of TcES to induce the differentiation of mouse and human DCs into a tolerogenic-like phenotype and to inhibit the effects of inflammatory stimuli.

Together this thesis shows that helminth excreted/secreted molecules can induce an alternative phenotype on DCs, capable to induce Th2 responses and modulate TLR signaling.

Resumen

Las células dendríticas (CDs) tienen un papelfundamental en el inicio de la respuesta inmune, reconociendo y descifrando señales intrínsecas en los patógenos, resultando en diferentes fenotipos de CDs con capacidad diferencial de activar linfocitos T en diferentes subclases. La respuesta inmune clásica en contra de infecciones con helmintos es una respuesta Th2, hasta ahora se carece de conocimiento de cómo se dicta el inicio de tal respuesta. Probablemente, por su capacidad de activar linfocitos T vírgenes, las CDs tienen un papel fundamental.

La investigación de esta tesis se enfoca en estudiar el fenotipo resultante de la interacción de las CDs con moléculas de excreción/secreciónde cestodo *Taenia crassiceps*(TcES). La estimulación de las CDs con los TcES, resultó en un fenotipo semi-maduro, con elevados niveles de expresión de MHCII en la membrana, moléculas co-estimuladoras en niveles basales, ausencia de citocinas pro-inflamatorias y poca producción de IL-10. Este fenotipo tuvo la capacidad de inducir una respuesta Th2 in vitro e in vivo. Por otro lado, el fenotipo de las CDs expuestas a los TcES mostraron una respuesta deficiente a diversos agonistas de los TLRs, como LPS, CpG y antígeno de *Toxoplasma gondii,* todos conocidos por inducir la maduración y secreción de citocinas pro-inflamatorias por las CDs. La capacidad de las CDs para inducir respuestas Th2 así como la tolerancia inducida por los TcES a agonistas para TLRs fue dependiente de los carbohidratos presentes en los TcES. Las moléculas presentes en los TcES fueron reconocidos por el receptor de manosa, el receptor MGL y el receptor tipo toll 2 (TLR2). Las vías de señalizaciónp38 y NFkBp65inducida por el LPS, fueron afectadas negativamente en las CDs expuestas a los TcES, mostrando menores niveles de fosforilación comparadas con las CDs estimuladas sólo con LPS.

Debido a que los TcES indujeron las fosforilación de cRAF, investigamos si esa vía de señalización se asocia a la inducción de respuestas Th2 y confiere tolerancia a estímulos TLR dependientes. Utilizando un inhibidor específico de cRAF (GW5074), o utilizando ARN de interferencia para cRAF demostramos que la vía cRAF es importante para bloquear la señalización e inducción de citocinas iniciada por TLR4.Debido a quelas CDs tratadas con el inhibidor de cRAF o ARN de interferencia y los TcES fueron capaces de responder adecuadamente al LPS recuperando la habilidad de maduración

y secreción de citocinas pro-inflamatorias como IL-12 y TNF-α.Adicionalmente demostramos que la vía RAF es clave para el inicio de la respuesta Th2 inducida por las CDs tratadas con los TcES. Finalmente, demostramos que el receptor de manosa (MR) y MGL contribuyen a la activación de la vía RAF y que bloqueando de manera conjunta MR, MGL y TLR2, las CDs expuestas a los TcES son capaces de responder de manera normal a la estimulación con LPS y pierden su capacidad de inducir respuestas Th2. Nuestros datos en conjunto, demuestran por primera vez que la vía de señalización a través RAF es importante para mediar una respuesta Th2 en respuesta a antígenos de helmintos, y que esta vía bloquea la señalización de TLR4.La manipulación de esta vía de señalización en las CDs puede ser útil para modular el balance Th1/Th2.

Introducción

Los parásitos helmintos son considerados un problema de salud pública principalmente en países en vías de desarrollo (Hotez et al., 2008). Las infecciones causadas por estos parásitos se caracterizan por ser crónicas y modular la inmunidad del hospedero contra infecciones no relacionadas, vacunas, y enfermedades autoinmunes (Maizels et al., 2004). Los parásitos helmintos se dividen filogenéticamente en trematodos, nematodos y cestodos. En general los helmintos tienen ciclos de vida complejos y utilizan vías de infección oral y cutánea. Algunos helmintos son transmitidos por vectores. En el hospedero, los helmintos pueden desarrollar diferentes estadios los cuales ocupan diferentes espacios anatómicos dentro del hospedero, como el tracto intestinal, músculo, el hígado, los pulmones, ojos, y el sistema nervioso central (Maizels et al., 2004). A pesar de la diversidad entre los helmintos, en general larespuesta inmune contra estos parásitos resulta en la polarización de la respuesta inmune a un tipo Th2 y/o Treg, con la generación de células T productoras de interleucina (IL-)-4, IL-5,IL-10 e IL-13, reclutamiento de células mieloides supresoras, macrófagos alternativamente activados, eosinofilia, mastocitosis y elevada producción de anticuerpos IgE (Maizels et al., 2004). Esta respuesta se ha asociado con protección contra infecciones causadas por helmintos intestinales. Sin embargo, existe evidencia contradictoria, donde una respuesta Th2 puede facilitar la sobrevivencia de estos parásitos cuando se alojan en tejidos distintos al intestino, como la cavidad peritoneal, hígado, músculo o el cerebro (Pearce et al., 2004; Terrazas et al., 1998). La respuesta Th2, es importante para la formación de granulomas, la contracción en el intestino y pulmón, la liberación de moco yla reparación de tejidos. Además la respuesta Th2 se asocia con respuestas alérgicas.Una de las interrogantes de la respuesta Th2, es como es iniciada y porque el sistema inmune desarrolla preferencialmente esta respuesta contra parásitos helmintos(MacDonald and Maizels, 2008; Palm et al., 2012).

Las interacciones tempranas entre el sistema inmune y los parásitos helmintos, pueden ser fundamentales para el inicio de una respuesta Th2. En la cisticercosis experimental, con el cestodo *Taenia crassiceps*, se ha establecido una inducción de una polarización a una respuesta Th2 en la fase crónica de la infección. Durante la etapa crónica, los macrófagos expresan arginasa, YM1, las moléculas de membrana PDL1 y PDL2 y son altamente supresores.En conjunto estas características se asocian a

macrófagos alternativamente activados o macrófagos tipo 2 (M2) (Rodriguez-Sosa et al., 2002b; Terrazas et al., 2005a). A pesar de la presencia de M2, y de evidencia sustentando que favorecen una respuesta Th2(Rodriguez-Sosa et al., 2002b), su participación en el inicio de la respuesta Th2 es poco probable, debido a que estos marcadores de activación alternativa son dependientes del receptor IL-4 y la molécula de señalización STAT6 (O'Connell et al., 2009; Rodriguez-Sosa et al., 2002a). Esto sugiere, que los M2 son producto y/o amplificadores de una respuesta Th2 y no iniciadores de dicha respuesta. En contraste, las células dendríticas, son las principales activadoras de linfocitos T vírgenes, y tienen un papel fundamental en el inicio de la respuesta Th1, Treg y Th17(Banchereau et al., 2000; Pulendran et al., 2008). Sin embargo, la participación de las CDs en el inicio de la respuesta Th2, es poco clara, y no existen reportes de su papel en la cisticercosis experimental(MacDonald and Maizels, 2008; Pulendran and Artis, 2012).

Antecedentes

Inicio de la respuesta Th2 en infecciones por helmintos.

El sistema inmune se encarga de reconocer señales de peligro presentes en el microambiente. Esta característica está presente preferencialmente en células del sistema inmune innato como CDs y macrófagos(Matzinger, 2002).Algunas de las señales de peligro son moléculas conservadas entre diferentes patógenos, por ejemplo el LPS en bacterias gram negativas, ácidos nucleicos ricos en regiones CpG en virus y bacterias. Estos patrones moleculares pueden ser reconocidos por receptores en células del sistema inmune, como los receptores tipo Toll. Después de este reconocimiento, las CDs son activadas y adquieren la capacidad de iniciar potentes respuestas adaptativas (Hemmi and Akira, 2005). En contraste con virus y bacterias, hasta ahora se desconoce si los helmintos comparten patrones moleculares que sean reconocidos por receptores del sistema inmune y de esta manera se pueda iniciar una respuesta Th2 (Kool et al., 2012; MacDonald and Maizels, 2008).

Algunos grupos de investigación proponen que estos patrones no se encuentran en los helmintos debido a que sus moléculas son filogenéticamente muy parecidas a los hospederos y proponen que el inicio de la respuesta Th2 puede ser iniciada por el daño tisular causado por la infección. Diversos factores pueden llevar a este daño, como el tamaño del parásito, la migración dentro del hospedero y a que algunos de ellos penetran la mucosa intestinal. El daño de tejido se asocia con la liberaciónsubstancias conocidas como alarminas, como TSLP, IL-33, Trefoil factor 3 derivado de células epiteliales, las cuales condicionan a las células presentadoras de antígeno a activar linfocitos que producirán IL-13, IL-4 y/o IL-5(Palm et al., 2012).Esta hipótesis es contrastante, debido a que se ha demostrado desde hace varios años que moléculas derivadas de los helmintos pueden inducir una respuesta Th2 in vitro e in vivo sin la presencia de la infección (Gomez-Garcia et al., 2006; Okano et al., 1999). Sin embargo, es posible, que proteasas presentes en estos extractos puedan causar daño en algunas células y de esta manera inducir la liberación de alarminas. Soportando esta idea, algunas moléculas con actividad proteolítica como la papaína generan respuestas Th2 (Liang et al., 2012). En contraste con esta hipótesis, carbohidratos derivados de helmintos parásitos pueden ser responsables de la polarización a una respuesta Th2

(Gomez-Garcia et al., 2006; Okano et al., 1999).Otros estudios han demostrado, que la desnaturalización de las proteínas no afecta la capacidad de las moléculas derivadas de los helmintos de polarizar a una respuesta Th2, argumentando contra la hipótesis del daño tisular (Massacand et al., 2009). Esto sugiere, que las moléculas glicosiladas presentes en los helmintos, podrían ser reconocidas por receptores específicos para carbohidratos y representar un patrón asociado a helmintos.

Por otro lado, aun no es claro cuales células del sistema inmune innato tienen un papel fundamental en el inicio de la respuesta Th2 (Kool et al., 2012). Por su eficiente capacidad de presentación de antígeno y capacidad de activar linfocitos T vírgenes, las células dendríticas son las candidatas naturales para asumir dicho papel(MacDonald and Maizels, 2008). Los basófilos también podrían tener un papel importante en el inicio de esta respuesta, debido a que pueden funcionar como fuente temprana de IL-4 en la infección con *Trichuris muris*, y presentar eficientemente antígeno asociado a MHCII (Perrigoue et al., 2009). Sin embargo, en la infección con *Schistosoma mansoni*, la presencia de las CDs es indispensable para el desarrollo de una respuesta Th2 (Phythian-Adams et al., 2010). En experimentos in vitro, las CDs expuestas a moléculas de helmintos son capaces de polarizar a linfocitos vírgenes para producir IL-13, IL-4 e IL-5 (Balic et al., 2004; Cervi et al., 2004; Jenkins and Mountford, 2005; Whelan et al., 2000). La transferencia adoptiva de CDs derivadas de médula ósea expuestas a moléculas derivadas de helmintos o expuestas al antígeno derivado del acaro, a ratones sanos, son suficientes para inducir eosinofilia y una respuesta Th2(Hammad et al., 2010). En contraste, la participación de las CDs *in vivo*, y la necesidad de otras células del sistema inmune en la eficiente inducción de una respuesta Th2 es poco clara.

La participación de la IL-4 en el inicio de la respuesta Th2 se ha propuesto como la principal inductora de la diferenciación a Th2. La IL-4 puede provenir de células innatas como eosinófilos, mastocitos o basófilos, también las células NK-T pueden ser una fuente temprana de IL-4 (Kool et al., 2012). Sin embargo, en la infección con *Nyppostrongylus brasiliensis*, los linfocitos T deficientes en el receptor alfa de IL-4, son capaces de producir IL-4 (Balic et al., 2006), sugiriendo que la IL-4 puede ser dispensable para el inicio de una respuesta Th2. Acorde con esto, STAT6, la principal molécula encargada de la transducción de señales del receptor de IL-4, es indispensable para la producción de IL-4 por eosinófilos y basófilos(Voehringer et al.,

2004). Otros factores solubles provenientes de células diferentes a las CDs, como las células epiteliales, pueden influir en el fenotipo de las CDs para desarrollar una respuesta Th2. El factor Trefoil, el cual es un péptido resistente a la actividad proteolítica liberado en el moco por células epiteliales intestinales,es necesario para la rápida producción de IL-33 en células dendríticas y macrófagos, lo que a su vez, induce una respuesta Th2, principalmente aumentando la producción de IL-13 en la infección con*Nippostrongylus brasiliensis*(Hung et al., 2013; Wills-Karp et al., 2012). Aunado a esto, la IL-4 e IL-13 son eficientes inductoras de la expresión del factor Trefoil en células epiteliales, probablemente de esta manera amplificando la respuesta Th2. Hasta el momento se desconoce, si moléculas derivadas de los helmintos pueden inducir directamente la producción de IL-33 en las CDs.Otra molécula relacionada con el inicio de la respuesta Th2, es la linfopoietina tímica estromal (TSLP), producida por las células epiteliales intestinales, y es crítica para la eliminación del parásito *Trichuris muris*. En el transcurso de la infección la TSLP regula de manera negativa la producción deIL-12 y en consecuenciala producción de IFN-γ. Sin embargo, el bloqueo de IL-12 o IFN-γ en los ratones deficientes en TSLP, restaura los niveles de citocinas tipo Th2 (Taylor et al., 2009). En contraste, ratones deficientes en el receptor de la TSLP tienen una respuesta Th2 normal en ratones infectados con *Heligmosomoides polygyrus* o *Nippostrogylus brasiliensis* (Massacand et al., 2009).Por otro lado, moléculas presentes en la membrana de células presentadoras de antígeno, tienen un papel importante en la polarización de los linfocitos T a un perfil Th2. Las moléculas OX40Le ICOS, presentes en CDs y macrófagos se han asociado positivamente en la respuesta Th2 (Blazquez and Berin, 2008; Shilling et al., 2009).

Inmunomodulación por *Taenia crassiceps*y sus antígenos.

El estadio de metacestodo *Taenia crassiceps*, ha sido usado como modelo de cisticercosis experimental. De manera natural el parásito adulto se encuentra alojado en el intestino de canidos, y la fase larvaria se encuentra en músculo y cavidad peritoneal de roedores. En el modelo murino, la infección se lleva a cabo de manera experimental inyectando larvas en la cavidad peritoneal. Este modelo ha sido útil para investigar los mecanismos de susceptibilidad y resistencia, desarrollo de vacunas, inmunomodulación, regulación del sistema endocrino, identificación de antígenos para diagnóstico(Ito et al., 1998; Morales-Montor and Larralde, 2005; Terrazas, 2008)(Sciutto et al., 2011).Durante

la fase aguda de la infección, las citocinas circulantes son de tipo Th1(IFN-γ). Sin embargo, en la segunda semana de infección, al progresar a una etapa crónica, las citocinas IL-4, IL-13, IL-5 se encuentran elevadas (Terrazas et al., 1998; Villa and Kuhn, 1996). De manera interesante, y en contraste a otros helmintos, la respuesta Th1 se asocia con la eliminación del parásito, ya que la administración de IFN-γ exógena genera inmunidad contra *T. crassiceps*(Terrazas et al., 1999), probablemente a través de la producción de óxido nítrico ya que este último se ha asociado a protección(Alonso-Trujillo et al., 2007).Además, los ratones deficientes en la molécula IL-12 o STAT4, ambas necesarias para la polarización a Th1, desarrollan altas cargas parasitarias(Rodriguez-Sosa et al., 2004; Rodriguez-Sosa et al., 2003). En contraparte, la respuesta Th2 se asocia con el establecimiento de la infección y con susceptibilidad a la misma, donde los macrófagos alternativamente activados pueden ser un factor importante para la sobrevivencia del parásito, debido a su función supresora sobre la proliferación de células T(Reyes et al., 2010; Rodriguez-Sosa et al., 2002b; Terrazas et al., 2005b).En soporte de esta hipótesis, la inmunización con péptidos provenientes de *Taenia crassiceps* son responsables de reducir las cargas parasitarias en la cisticercosis experimental murina y se asociada con una respuesta Th1 (Toledo et al., 2001; Toledo et al., 1999).

En la resistencia a la infección por *T. crassiceps* se puede observar un dimorfismo sexual, en el cual las hembras desarrollan cargas parasitarias altas, mientras que los machos restringen el crecimiento del parásito. La gonadectomía en machos incrementa la susceptibilidad, mientras que la gonadectomía en hembras reduce la carga parasitaria (Huerta et al., 1992), la cual puede ser revertida por la administración de 17 beta estradiol (Terrazas et al., 1994).La administración de testosterona después de la gonadectomía reduce las cargas parasitarias; lo cual se relaciona con la producción de IL-2 e IFN-γen células de bazo (Morales-Montor et al., 2002). Durante el curso de la infección, los niveles de estradiol incrementan en los machos, mientras que la testosterona disminuye, provocando un fenómeno de feminización que es regulado por la presencia de IL-6 (Morales-Montor et al., 2001). Estos datos demuestran un papel importante del sistema endocrino en la regulación del sistema inmune en el transcurso de la cisticercosis experimental.Por otro lado, se han demostrado efectos directos de las hormonas sexuales en el parásito, por ejemplo, 17 beta estradiol o progesterona, favorecen la reproducción del cisticerco *in vitro*, mientras

que la presencia de testosterona o dihidrotestosterona inhiben su reproducción(Escobedo et al., 2004), enfatizando la importancia de las hormonas sexuales en la sobrevivencia del cisticerco de*Taenia crassiceps*.

Las infecciones con helmintos son comunes en países en vías de desarrollo donde pueden generarse co-infecciones debido a las condiciones geográficas o socio culturales de dichos países. En este aspecto, se ha demostrado que la previa infección con *T. crassiceps* afecta la inmunidad a una posterior infección con parásitos intracelulares como*Trypanosoma cruzi y Leishmania mexicana*, donde los ratones son más susceptibles a la segunda infección (Rodriguez-Sosa et al., 2006; Rodriguez et al., 1999). Este fenómeno, se ha asociado con la presencia de macrófagos alternativamente activados, los cuales podrían ser más susceptibles a la infección con parásitos intracelulares debido a que son refractarios a la activación por IFNy (datos no publicados) o favorecer la infección secundaria debido a su actividad supresora de la proliferación de los linfocitos T (Terrazas et al., 2005a).

Por otro lado, se ha observado que en países desarrollados, donde la infección por helmintos es baja, el índice de enfermedades autoinmunes es mayor que en países en vías de desarrollo(Rook, 2007). En la hipótesis de la higiene, se propone a los helmintos como reguladores naturales de la respuesta inmune, y que la pérdida de contacto con estos, conlleva a una mayor incidencia de enfermedades autoinmunes (Rook, 2007). En línea con esta hipótesis, la previa infección de ratones con *T. crassiceps*, disminuye la patología causada por la inducción de diabetes tipo 1, y esclerosis múltiple experimental. En ambos casos, se observa una disminución en la producción de IFN-γy polarización a una respuesta Th2(Espinoza-Jimenez et al., 2010; Reyes et al., 2011). Dicha respuesta Th2, se asocia a su vez con la generación de macrófagos alternativamente activados, los cuales son supresores de la proliferación de células T (Terrazas et al., 2005a).

Lasmoléculas derivadas de *Taenia crassiceps*pueden recapitular la respuesta inmune generada por la infección y tienen un papel importante en la modulación de la respuesta inmune. Dentro de los antígenos solubles totales, se encuentran una amplia diversidad de moléculas.Sin embargo, pocas de ellas han sido estudiadas en el contexto de la regulacióninmunológica. Por ejemplo, se han descrito moléculas

presentes en los extractos solubles de *T. crassiceps*que poseen proteasas capaces de degradar lalL-2(Baig et al., 2005). Los antígenos totales de*T. crassiceps* son eficaces inductores de una respuesta Th2*in vivo*, funcionando también como adyuvantes a proteínas no relacionadas.Además de estos antígenos funcionan en el reclutamiento de células mieloides supresoras(Gomez-Garcia et al., 2005; Gomez-Garcia et al., 2006).Tales efectos inmunológicos son dependientes de la presencia de carbohidratos en los antígenos(Gomez-Garcia et al., 2005; Gomez-Garcia et al., 2006),en los cuales las glicosilaciones N-terminales son manosa, glucosa y fucosa (Lee et al., 2005). Esto sugiere que los receptores de carbohidratos podrían tener un papel importante en el reconocimiento de *T. crassiceps* y en la polarización a Th2. Por otro lado, sólo un estudio se ha reportado usando moléculas secretadas de *Taenia crassiceps*, en el cual se demuestra que la presencia de los TcES suprime la proliferación de células de bazo pre activadas con anti CD3/CD28 (Spolski et al., 2000), pero los mecanismos asociados a este fenómeno no fueron estudiados en ese trabajo.

CélulasDendríticas

El sistema inmune cuenta con una organización compleja de diferentes células especializadas en responder contra diferentes patógenos o señales de peligro. En 1970 Steinman et al descubrieron una célula encargada de reconocer patógenos y activar especialmente a linfocitos T vírgenes, las célulasdendríticas (CDs)(Steinman et al., 1979). Las CDs cuentan con un repertorio de moléculas capaces de reconocer patrones asociados a patógenos (PAMPs), estos receptores se localizan en la membrana celular y también en el citoplasma, entre ellos se encuentran receptores tipo Toll (TLRs), receptores tipo Lectina (CLRs), NOD, entre otros(Robinson et al., 2006; Takeda and Akira, 2004). Dichos receptores forman una amplia gama de arreglos para reconocer patógenos. Debido a que diversos patógenos contienen una compleja mezcla de moléculas que pueden ser agonistas de receptores presentes en las CDs, el mecanismo de decisión en la respuesta de las CDs es un proceso complejo y altamente regulado. Las CDs se encuentran en tejidos periféricos claves en el encuentro con patógenos, al ser activadas migran a los ganglios linfáticos.Duranteesta etapa presentan cambios en la expresión de moléculas de membrana como CD80, CD86, CD40, OX40L inducidas por el previo reconocimiento del patógeno. Además, la presencia de las moléculas MHCI y MHCII se incrementa en la membrana celular. Por otro lado, las CDs producen

citocinas como interferones tipo I, IL-1β, IL-6, IL-10, IL-12, IL-15 yTNF-α principalmente.Las CDs migran al ganglio linfático en repuesta a ligandos de CCR7, además se ha demostrado que la expresión de CD40 también es importante para la migración de la piel al ganglio linfático (Flores-Romo, 2001; Moodycliffe et al., 2000; Rivas-Caicedo et al., 2009). En conjunto, la presentación de antígeno en el contexto de MHCII, el arreglo de moléculas co-estimuladoras y las citocinas secretadas por las CDs, son las señales necesarias para dictarla polarizaciónde los linfocitos T cooperadores a las diferentes sub poblacionesTh1, Th2, Th9, Th17 o Treguladoras(Banchereau et al., 2000).Las CDs se han agrupado en diferentes subclases dependiendo de los marcadores de membrana, localización y función. Las principales activadoras de linfocitos T vírgenes son las CDs mieloides y linfoides. En bazo, ganglios linfáticos y dermis se encuentran las células dendríticas mieloides (CD11c+DCIR+CD8a-), las cuales se asocian al transporte de antígenos de la periferia a los ganglios linfáticos. Por otro lado las CDs linfoides se caracterizan por ser residentes del bazo (CD11c+DEC205+CD8a+)(Dudziak et al., 2007). Ambas poblaciones son potentes activadoras de linfocitos T vírgenes, la principal diferencia reportada hasta ahora se encuentra en que las CDs mieloides son mejores presentadoras de antígeno a linfocitos T CD4+, y son capaces de iniciar respuestas Th1 y Th2, mientras que las CDs linfoides son más eficientes activadoras de linfocitos CD8+ debido a su eficiencia de presentación cruzada. Además esta subpoblación se asocia a una polarización principalmente tipo Th1(Pulendran et al., 2008).

Receptores tipo Toll (TLRs)

El principal mecanismo de activación de las CDs es el reconocimiento de moléculas a través de receptores presentes en la membrana o citosol. Los receptores más estudiados son los TLRs, los cuales son proteínas transmembranales con ectodominios ricos en leucina y una parte intracelular semejante a la de la interterluecina 1 que funciona para la transducción de señales. Hasta ahora se conocen once TLRs. TLR1, TLR2, TLR4, TLR6 y TLR11 se encuentran en membrana y pueden formar heterodímeros para reconocer moléculas como lípidos, lipoproteínas y proteínas, presentes en patógenos. También existen TLRs en el retículo endoplasmático, endosomas, lisosomas y endolisosomas,como lo son TLR3, TLR7, TLR8 y TLR9 que reconocen ácidos nucleicos presentes en patógenos(Takeda and Akira, 2004).

A través del dominio TIR, los TLRs reclutan diferentes moléculas adaptadoras que funcionan en la transducción de señales. El reclutamiento de las diferentes moléculas adaptadoras es dependiente del TLR que inicia la cascada, así como también de la célula donde se lleva a cabo la señalización celular. TLR3 y TLR4 inducen la producción de interferones tipo I, además de citocinas pro-inflamatorias. En contraste, TLR2 induce principalmente citocinas inflamatorias. Esto es debido al reclutamiento diferencial de moléculas adaptadoras. La molécula adaptadora MyD88 funciona en la señalización de todos los TLRs excepto TLR3 y lleva a la activación del factor de transcripción NFkB y de las MAPK JNK, p38 y ERK1/2, que inducen la producción de citocinas inflamatorias. Por otro lado, TRIF, es una molécula adaptadora de TLR3 y TLR4 la cual induce una vía alternativa que lleva a la activación de los factores de transcripción IRF3 y NFkB los cuales inducen a su vez interferones tipo I y citocinas pro-inflamatorias respectivamente. También existen las moléculas adaptadoras TRAM y TIRAP las cuales funcionan como adaptadoras de TRIF para TLR4 y MyD88 para TLR2 respectivamente. De esta manera existen cascadas MyD88 dependientes que llevan a la activación del factor de transcripción NFkB el cual se transloca al núcleo e induce la transcripción de citocinas pro-inflamatorias (IL-1β, IL-6, IL-12, IL-15, TNF-α), y también de la citocina anti-inflamatoria IL-10. La cascada MyD88 independiente induce la producción de interferón alfa y beta además de la producción de citocinas inflamatorias. En el caso de TLR4, las cuatro moléculas adaptadoras tienen un papel importante para la transducción de señales. TLR4 recluta TIRAP en la membrana plasmática y facilita el reclutamiento de MyD88 que inicia la activación de las MAPK y de NFkB. Enseguida, TLR4 es endositado y en los endosomas forma un complejo con TRAM y TRIF lo cual inicia la cascada MyD88 independiente que lleva a la activación de IRF3 y a la activación de NFkB y MAPK. Ambas vías de señalización son necesarias para la inducción de citocinas e interferones tipo I(Akira and Takeda, 2004).

La molécula NFkB es un factor de transcripción que incluye a las moléculas p65/RelA, p50/NFkB1, p52/NFkB2, RelB and c-Rel. NFkB se encuentra en el citoplasma en su forma inactiva secuestrado por la molécula IkB. Después de la estimulación a través de los TLRs, IkB es fosforilado por IKKa y IKKb, lo cual induce la ubiquitación y degradación de IkB en el proteasoma lo que permite la liberación de NFkB y su subsecuente translocación al núcleo donde NFkB facilita la expresión de numerosos

genes entre ellos los de citocinas pro-inflamatorias como TNF-α, IL-6, IL-15, IL-12 entre otras (Takeda and Akira, 2004).

La transducción de señales iniciada por los TLRs también involucra a la vía de las MAP cinasas, donde la fosforilación de p38puede favorecer la translocación de NFkB y favorecer la producción de IL-12, mientras que la fosforilación de ERK1/2 favorece su propia translocación al núcleo y la activación del factor de transcripción AP1 (Dillon et al., 2004; Poncini et al., 2010).

Receptores tipo Lectina

Las CDs también expresan en su membrana receptores tipo lectina, estos pueden ser o no dependientes de calcio. Entre los receptores tipo lectina más importantes en el reconocimiento de patógenos se encuentran DCSIGN presente en CDs humanas, el receptor de manosa(MR), MGL, DECTINA-1. En general, los receptores tipo lectina (CLRs) se asocian a la internalización de parásitos intracelulares o a la fagocitosis de productos microbianos. Sin embargo, en la última década, se demostró que los CLRs pueden inducir señalización intracelular modulando la respuesta de las CDs. A diferencia de los receptores tipo Toll, poco es conocido acerca de la señalización intracelular de los receptores tipo lectina y su influencia sobre la maduración y activación de las CDs(Robinson et al., 2006).

Hasta el momento se conoce que Dectina 1, inicia una cascada intracelular a través de las moléculas SYK y CARD9, induciendo la producción de IL-12. También se ha demostrado que el reconocimiento de patógenos a través de Dectina 1 por las CDs les confiere la característica de inducir respuestas Th17, la cual es importante para generar inmunidad contra hongos y micobacterias(Gringhuis et al., 2009b). Por otro lado, DCSIGNes un receptor para una amplia variedad de patógenos como VIH, lipoarabinomamanos presentes en la pared celular de *Mycobacterium tuberculosis*, la bacteria *Helicobacter pylori*, el protozoario *Leishmania mexicana*, el helminto *Schistosoma mansoni*, y la molécula Salp15,una molécula presente en la saliva del vector de la enfermedad de Lyme, además también reconoce la molécula Lewis X(Geijtenbeek et al., 2000; Geijtenbeek et al., 2002; van Die et al., 2003). La respuesta generada a través de DCSIGN es contrastante, en algunos casos sinergiza con la

respuesta generada por los TLRs aumentando la producción de citocinas pro-inflamatorias, y en otros casos regula negativamente dicha respuesta. Este fenómenoparece depender del contenido de manosa o fucosa presentes en los ligandos. La cascada de señalización intracelular iniciada por DCSIGN se ha demostrado depender de las moléculasSYK y cRAF principalmente(Geijtenbeek and Gringhuis, 2009).

La molécula MGL reconoce estructuras glicosiladas con N-acetilgalactosamina y/o galactosa, hasta el momento sólo se ha demostrado que se une a antígenos derivados del helminto*Schistosoma mansoni*. En ensayos de señalización intracelular generadapor activación con anticuerpos que reconocen MGL, se reportó que la molécula ERK1/2 es importante en la señalización de este receptor(van Vliet et al., 2013). En contraste, el receptor de manosa (MR), reconoce principalmente moléculas glicosiladas con manosa y/o glucosa, y se han descrito un gran número de patógenos reconocidos por MR. Sin embargo, no se ha descrito una vía de señalización intracelular para este receptor(Geijtenbeek and Gringhuis, 2009; Robinson et al., 2006).

Regulación CLRs – TLRs

La respuesta inflamatoria de las CDs es altamente regulada. Los TLRs son iniciadores de la respuesta inflamatoria en las CDs.Algunos CLRs pueden influir en la respuesta iniciada por los TLRs. Dectina 1, por ejemplo, aumenta la producción de IL-12 inducida por TLR2 mediante laseñalización intracelular dependiente de las moléculas SYK-CARD9 que inducen la activación de NFkB. Sin embargo, la respuesta generada por Dectina 1 es independiente de la señalización de TLR2, y Dectina 1 por sí misma puede inducir la producción de IL-12(Gringhuis et al., 2009b). DCSIGN, actúa en sinergismo con los TLRs, se ha demostrado que después de la unión de ManLam o gp120 del VIH, induce la fosforilación de cRAF que conlleva a la acetilación de NFkB, lo que resulta en una prolongada transcripción de citocinas pro-inflamatorias (IL-12, IL-6, TNFα) y también IL-10(Gringhuis et al., 2009a; Gringhuis et al., 2007). En contraste, el reconocimiento de *H. pylori* o la molécula SALP15 a través de DCSIGN induce la fosforilación de cRAF, lo cual lleva a una disminución en la producción de IL-12 y TNF-α(Gringhuis et al., 2009a; Hovius et al., 2008). La molécula Lewis X, es reconocida a través de DCSIGN, pero en contraste con otros ligandos de DCSING no induce la

fosforilación de cRAF, pero resulta en la disgregación del complejo de señalización de DCSIGN, conformado por PKC,PKA, RAS y cRAF(Gringhuis et al., 2007).

Interacción Helmintos – Células Dendríticas

La importancia de las CDs como iniciadoras de la respuesta adaptativa, ha llevado a numerosos estudios enfocados en explicar el rol de las CDs en las infecciones por helmintos, el reconocimiento de moléculas presentes en este grupo de patógenos y la respuesta de las CDs. Además, debido a que los helmintos son los inductores naturales de potentes respuestas Th2, se usan como modelo para explicar el inicio de esta respuesta.

En general, diferentes estudios usando moléculas derivadas de helmintos, han reportado diferentes fenotipos de CDs, en los cuales se observa un patrón entre los diferentes experimentos: 1. Las CDs expuestas a antígenos de helmintos no sobre-expresan moléculas co-estimuladoras y no secretan citocinas pro-inflamatorias. En algunos reportes se demuestra una baja producción de IL-10 después de la estimulación con las moléculas derivadas de helmintos. 2. Las CDs condicionadas con moléculas derivadas de helmintos responden de manera inadecuada a estímulos TLR dependientes, con baja o nula producción de IL-12 y TNF-α.3. la exposición de las CDs a los derivados de helmintos las condiciona para inducir respuesta tipo Th2 y/o Treguladoras. Hasta el momento se desconocen los eventos intracelulares que conllevan a dichas respuestas, así como las moléculas co-estimuladoras o factores solubles presentes en las CDs condicionadas con moléculas de helmintos importantes para la polarización a Th2(MacDonald and Maizels, 2008; Pulendran and Artis, 2012; Terrazas et al., 2010). Para tratar de explicar la interacción helmintos-células dendríticas se han propuesto diferentes hipótesis. La hipótesis de la generación de Th2 por default, propone que las CDs expuestas a helmintos no producen IL-12, y esta es la principal razón por la que ocurre una polarización a un perfil Th2. Una segunda hipótesis, sugiere que las moléculas de los helmintos son reconocidas a través de receptores diferentes a los TLRs, y que de esta manera condiciona de manera diferencial a las CDs para inducir respuestas Th2. La tercera hipótesis, consiste en un modelo inhibitorio, donde las moléculas de helmintos son reconocidas por receptores diferentes a los TLRs, los cuales compiten o bloquean la señalización clásica de los TLRs, además estos mismos

receptores condicionan a las CDs a polarizar respuestas Th2(MacDonald and Maizels, 2008).

Hasta el momento, se carece de información sólida o irrefutable para aceptar alguna de estas hipótesis, la principal razón radica en la falta de conocimiento acerca de los ligandos presentes en los helmintos y sus receptores en las CDs. Pocos estudios se han publicado en esta área, uno de ellos muestra que los antígenos solubles de *Schistosoma mansoni*, son reconocidos a través de las lectinas MGL, MR, y DCSING por CDs humanas. Sin embargo, no se asoció directamente ninguno de estos receptores con el fenotipo inducido en las CDs.

Informaciónmás detallada sobre la interacción de helmintos y CDs se puede encontrar en el apéndice I: artículo de revisión escrito por el autor de esta tesis durante el doctorado:**Modulation of Dendritic Cell Responses by Parasites: A Common Strategy to Survive.**

Hipótesis

Las moléculas de excreción/secreción de *Taenia crassiceps* son reconocidas a través de receptores en células dendríticas capaces de unir estructuras glicosiladas, lo cual generaría una señalización intracelular regulando la activación de las CDs a responder a estímulos TLR dependientes y a favorecer una respuesta Th2.

Objetivos

1. Investigar la respuesta de las CDs a las moléculas de excreción/secreción de *Taenia crassiceps*,
2. Determinar como la previa exposición a los TcES influye en la respuesta de las CDs a agonistas de TLRs.
3. Explorar posibles receptores a los TcES.
4. Identificar alguna vía de señalización inducidapor TcES y relacionarla con la deriva a una respuesta Th2.

Estrategia experimental

Debido a que las células dendríticas son escasas en los tejidos, y su purificación es causante de maduración y/o activación, decidimos usar CDs derivadas de médula ósea, esta técnica tiene como ventaja que las CDs pueden obtenerse en estado inmaduro y en grandes cantidades. Por otro lado, decidimos usar moléculas de excreción/secreción de *T. crassiceps* debido a que ser liberados y entrar en contacto con las células del sistema inmune.

Materiales y métodos

Obtención de moléculas de excreción/secreción de *Taenia crassiceps*.

Los cisticercos de *Taenia crassiceps* fueron obtenidos de la cavidad peritoneal de ratones Balb/c infectados durante 8 semanas. Los cisticercos fueron lavados con solución salina y mantenidos en solución salina a 37C en la incubadora de CO2. Los sobrenadanes de los cultivos fueron obtenidos cada 24 horas durante tres días. Los sobrenantes fueron centrifugados a 5000 rpm durante 10 minutos. Enseguida, los sobreanadantes fueron concentrados por centrifugación en tubos AMICON (Millipore) con una membrana de 50kda. Las moléculas con peso molecular mayor a 50kda y las menores de 50kda fueron recuperadas y se realizó la cuantificación de proteínas presentes en ambas fracciones por el método de Bradford.

Diferenciación y estimulación de células dendríticas a partir derivadasde médula ósea.

Fémur y tibia fueron obtenidos de ratones Balb/c o C57BL6. Las células obtenidas fueron ajustadas a 1 $x10^6$ células/ml, y sembradas en cajas de Petri (Falcon), en medio RPMI suplementado con 10% de suero fetal bovino, 5% de penicilina/estreptomicina además de 10% de sobrenadante de células CHO productoras de GMCSF. En el día 3, diez ml de RPMI suplementado como anteriormente se menciona, se agregó a los cultivos. Las células dendríticas inmaduras, fueron recuperadas el día 6-7 sembradas en una concentración de 1 $x10^6$ células/ml. Un día después se realizaron los estímulos con LPS 1µg/ml, antígeno soluble de *Toxoplasma gondii* 5 µg/ml, CpG 1µg/ml y/o TcES en varias concentraciones.

Deglicosilación de los TcES.

La modificación de los glicanos del antígeno total, mediada por metaperyodato de sodio, fue realizada usando una modificación de Tawill *et a*l. 2004. 2 mg/ml de TcES fueron incubados durante pocos segundos (v/v) con 50 mM de acetato de sodio pH 4.5 (buffer de acetato de Na) a temperatura ambiente. La muestra fue dividida en dos para producir un tratamiento con peryodato del antígeno soluble de *T. crassiceps* (meta) y un control (mock) del tratamiento con peryodato de los TcES. 20 mM de metaperyodato de sodio (v/v) se añadió al antígeno (meta),

mientras que el mock se agregó buffer de acetato sin metaperyodato de sodio, ambos tubos se incubaron 30 minutos en la obscuridad a temperatura ambiente con agitación suave. La reacción se completó con la incubación de los tubos con 100 mM de boro hidróxido de sodio en PBS por 30 minutos a temperatura ambiente. El exceso de sales se removió usando Amicon Ultra Filter Units (Millipore, Billerica).

Selección de CD4+ y proliferación celular.

Mediante columnas magnéticas de separación, se purificaron células CD11c+ del cultivo de médula ósea de ratones BALB/c y células CD4+ de bazo de ratones DO11.10. Fueron separadas por medio de anticuerpos con esferas magnéticas dirigidos hacia estos marcadores específicos de células dendríticas y linfocitos T respectivamente.La evaluación de proliferación celular se llevó a cabo adicionando carboxifluoresceína diacetato succimidyl ester (CFSE) a las células CD4+ en una concentración de 0.5 µM de CFSE.

Transferencia de células dendríticas a ratones DO11.10

Para investigar si el fenotipo de las CDs estimuladas con los antígenos de *T. crassiceps*eran capaces de iniciar una respuesta adaptativa *in vivo*, las CDs se cargaroncon OVA y con antígeno total de *T. crassiceps* o LPS, después de 24 horas de tratamiento, las CDs fueron lavadas 3 veces en solución salina centrifugándolas a 2500 rpm por 5 minutos, al finalizar las células se ajustaron a 1x105 CDs/ml en solución salinaestéril, y posteriormente fueron inyectadas en el cojinete plantar de ratones hembras DO11.10.

Análisis de moléculas co-estimuladoras por citometría de flujo

Al tiempo indicado las CDs y/o CD4+, fueron obtenidas de los cultivos,concentrándolas por centrifugación a 2000 rpm por 5 minutos, se ajustaron en 1 ml debuffer para FACs , las células fueron contadas yajustadas a $1x10^6$/ml y se centrifugaron nuevamente, la pastilla se resuspendió en 200 µldel mismo buffer. Se agregaron los anticuerpos conjugados con distintos marcadores defluorescencia y un anticuerpo isotipo (IgG2a) como control para pegado inespecífico, auna concentración de 0.2 µg/ml, se mantuvo en incubación durante 30 minutos a 4° C, lascélulas fueron lavadas dos veces con

buffer para FACs. Finalmente la pastilla sesuspendió en 500 µl de Facs Flow, y se analizaron en el citómetro. (FACs Calibur).

Determinación de citocinas por ELISA

Los sobrenadantes de los cultivo celulares fueron analizados por medio de ELISA Sándwich para la detección de citocinas secretadas, se utilizaron kits Peprotech para la detección de IL-1b, IFN-_, IL-10, IL-4, IL-12, IL-15 y TNF-α.

Análisis de moléculas por citometría de flujo

Al tiempo indicado las CDs y/o CD4+, fueron obtenidas de los cultivos, concentrándolas por centrifugación a 2000 rpm por 5 minutos, se ajustaron en 1 ml de buffer para FACs las células fueron contadas en cámara de Neubauer yajustadas a $1x10^6$/ml y se centrifugaron nuevamente, la pastilla se re suspendió en 200 µldel mismo buffer. Se agregaron los anticuerpos conjugados con distintos marcadores de fluorescencia auna concentración de 0.2 µg/mla los diferentes tratamientos. Como control se usaron anticuerpos isotipos conjugados con los fluorocromos usados en el ensayo. Las células se mantuvieron en incubación durante 30 minutos a 4° C, lascélulas fueron lavadas dos veces con buffer para FACs. Finalmente la pastilla se resuspendió en 250µl de buffer, y se analizaron por citometría de flujo. (FACs Calibur).

Resultados

La interacción entre helmintos y células dendríticas es aún poco entendido, en el presente trabajo se caracterizó la respuesta de las células dendríticas a las moléculas de excreción/secreción del cestodo *Taenia crassiceps.* En esta tesis se presentan los resultados en forma de artículos publicados (Apéndice I).

En el artículo I. **Differential response of antigen presenting cells from susceptible and resistant strains of mice to *Taenia crassiceps* infection.Infection, Genetics and Evolution, 2009. 9(6), 1115-1127**.Se describe la interacción de los TcES con las CDs provenientes de ratones Balb/c o C57BL6. Los ratones BALB/c son susceptibles a la infección con *T. crassiceps*, mientras que los ratones C57BL/6 son resistentes (Sciutto et al., 1991). Al ser evaluada la expresión de moléculas co-stimuladoras, las CDs BALB/c tratadas con TcES y LPS presentaron niveles significativamente menores de expresión en membrana de las moléculas CD80,CD86 CD40 y CCR7 comparadas con la expresión de estas moléculas inducidas sólo con LPS. En contraste, las CDs C57BL/6 tratadas con TcES y LPS expresaron niveles CD80,CD86 CD40 y CCR7 que no fueron significativamente diferentes a la expresión de estas moléculas en CDs estimuladas con LPS. Por otro lado, la producción de IL-12 y TNFα inducida por LPS fue regulado negativamente en presencia de los TcES. Este efecto negativo solo se observó en las CDs derivadas de ratones BALB/c. En contraste, en las CDs derivadas de ratones C57BL/6 la inducción de IL-12 y TNFα por LPS no resultó afectada en presencia de los TcES. Finalmente, el efecto biológico de las diferencias encontradas en la expresión de moléculas co-estimuladoras y citocinas se evaluó en co-cultivos alogénicos de CDs y células T. Como resultado se observó que las CDs derivadas de ratones BALB/c tratadas con los TcES indujeron niveles bajos de proliferación celular mientras que las CDs derivadas de ratones C57BL/6 indujeron mayor proliferación celular. En conclusión, la maduración de las CDs derivadas de ratones BALB/c fue regulada negativamente por los TcES, mientras que las CDs derivadas de ratones C57BL/6 no fueron afectadas por los TcES.

En el artículo**Impaired pro-inflammatory cytokine production and increased Th2-biasing ability of dendritic cells exposed to Taenia excreted/secreted antigens: A critical role for carbohydrates but not for STAT6 signaling.International Journal for Parasitology, 2010. 40(9), 1051-1062,**demostramos que los TcES indujeron mayores niveles de MHCII localizados en la membrana celular. Por otro lado, los TcES no indujeron cambios significativos en las moléculas co-estimuladoras (CD80, CD86, OX40L), lo cual resultó en una baja inducción de proliferación celular en co-cultivos con células T. Debido a que la activación de los linfocitos T, se ha postulado que además de moléculas co-estimuladoras y reconocimiento del péptido unido al MHCII, requiere de la presencia de citocinas,por lo que evaluamos la producción de IL-12, IL-15, IL-6, TNFα, e IL-10, de las cuales sólo encontramos alterada la producción de IL-10 de manera no significativa. Este fenotipo de CDs fue eficiente inductor de la producción de IL-4 en células T vírgenes.

Debido a que el fenotipo inducido por los TcES es muy parecido al de una CD inmadura, investigamos si las CDs tratadas con los TcES responden de manera semejante que las CDs inmaduras a estímulos inflamatorios. Para ello usamos ligandos de TLRs como LPS, CpG y antígeno soluble de toxoplasma cuyo reconocimiento lleva a la maduración de las CDs y a la secreción de citocinas pro-inflamatorias. Las CDs expuestas a los TcES respondieron de manera deficiente a los ligandos de TLRs probados, reflejado en un estado semi maduro y con menor producción de IL-12, IL-15 y TNFα. De manera interesante la producción de IL-10 generada por los ligandos de los TLRs no fue afectada. La presencia de IL-10 en los cultivos de las CDs tratadas con TcES, y el hecho de que esta citocina no fue alterada cuando las CDs fueron co-estimuladas, nos llevó a investigar el papel de IL-10 en la regulación de la respuesta a los TLRs. Usando anticuerpos anti IL-10, o anti IL-10R, encontramos que la modulación de las CDs por TcES fue IL-10 independiente.

En investigaciones anteriores se demostró que los carbohidratos presentes en los antígenos totales de *T. crassiceps* fueron importantes para la inducción de una respuesta Th2*in vivo*. Para investigar el papel de los carbohidratos en la modulación de las CDs,los TcES fueron deglicosilados con metaperyodato. Los TcES tratados con

metaperyodato no modularon negativamente la secreción de IL-12 y TNFα inducidas por el LPS, además no condicionaron a las CDs a inducir respuestas Th2 in vitro.

Algunas investigaciones han reportado que los parásitos helmintos pueden contener moléculas semejantes a citocinas del hospedero, por ejemplo MIF. Debido a que la IL-4 es una molécula importante en la generación de macrófagos alternativamente activados con capacidad de inducir respuestas Th2, decidimos investigar si la vía de señalización de IL-4 tenía un papel en la modulación de las CDs. Los resultados muestran que las CDs deficientes en la molécula STAT6, fueron moduladas de la misma manera que CDs WT por los TcES, descartando la participación de STAT6 en la señalización de los TcES.

En el artículo,**Cestode antigens induce a tolerogenic-like phenotype and inhibit LPS inflammatory responses in human dendritic cells.International Journal of Biological Sciences 2011, 7(9), 1391-1400**, investigamos el efecto de los TcES sobre CDs derivadas de monocitos humanos. *T. crassiceps* es un helminto que generalmente infecta canidos y roedores. Sin embargo, se han reportado casos de infecciones en humanos inmunocomprometidos (Chermette et al., 1995; Francois et al., 1998). El interés en evaluar la modulación de las CDs humanas por los TcES se efectuó en base al potencial uso de los TcES como inmunoduladores en enfermedades autoinmunes. Los resultados muestran que los TcES no interfieren con la diferenciación de monocitos humanos a célulasdendríticas in vivo, ya que se encontraron niveles similares de expresión de la molécula CD11c en cultivos tratados con TcES durante el proceso de maduración y sus controles. Las CDs expuestas a los TcES no afectaron los niveles de expresión de CD86, HLA-DR, CD80 y CD86. Sin embargo mostraron una respuesta disminuida al estímulo con LPS, reflejada en menores niveles de expresión de CD83, CD80 y CD86 comparadas con CDs que recibieron solo LPS. De manera interesante la expresión de HLA-DR inducida por el LPS no fue afectada por los TcES. Los resultados de la producción de citocinas muestra que le exposición de las CDs a los TcES aumento la producción de IL-10, pero no de IL-1β IL-6, IL-12, IL-23, TNFα y TGFb. En contraste, la pre exposición de las CDs a los TcES redujo la capacidad de las CDs en la producción deIL-1β,IL-6, IL-12, y TNFα inducidas por el LPS. De manera interesante, la disminución en la capacidad de responder al LPS fue mayor en las CDs que fueron expuestas a los TcES mediante el proceso de diferenciación.Basados en

elhecho que en anteriores trabajos se demostró que la modulación de los TcES sobre las CDs fue dependiente de los carbohidratos, analizamos los mensajeros de posibles receptores para los TcES. El receptor MGL fue regulado positivamente por la presencia de los TcES, los mensajeros se conservaron significativamente altos en presencia de LPS y los TcES. En contraste, los TcES redujeron los mensajeros de DCSIGN, pero no alteraron la expresión inducida por el LPS. Los mensajeros delreceptor de manosa no fueron afectados por los TcES. Otras investigaciones han reportado que los receptores TLRs son capaces de reconocer moléculas presentes en *Schistosoma mansoni* y *A. vitae*(Goodridge et al., 2005). Los TcES no afectaron la expresión de ninguno de los TLRs evaluados. Sin embargo, sinergizaron con LPS en la inducción de la expresión de TLR2.

En el artículo**Helminth-excreted/secreted products are recognized by multiple receptors on DCs to block the TLRresponse and bias Th2 polarization in a cRAF dependent pathway (The FASEB Journal 2013, En prensa)**, evaluamos los receptores involucrados en el reconocimiento de los TcES, así como las vías de señalización intracelular inducidas y afectadas en las CDs. Mediante la tinción de los TcES con el fluorocromo FITC, encontramos que los TcES se unen a las CDs. Usando anticuerpos de bloqueo o ensayos de competencia, evaluamos diferentes receptores para los TcES. Los receptores MGL y MR fueron eficientes en el reconocimiento de TcES. La participación de MGL también fue evaluada usando CDs provenientes de ratones deficientes en dicha molécula, resultando en la disminución de fluorescencia de las CDs expuestas a los TcES-FITC. De la misma manera, usamos CDs de ratones deficientes en el receptor TLR2, las cuales mostraron menor unión de los TcES. Debido a que MR, TLR2 y MGL fueron importantes en el reconocimiento de los TcES, evaluamos si estos receptores son importantes para la actividad moduladora de los TcES. Previamente mostramos que la respuesta de las CDs a ligandos TLR dependientes como CpG o LPS, fue disminuida en presencia de los TcES.Para identificar el posible papel de de TLR2 o MGL usamos CDs provenientes de ratones deficientes en dichas moléculas. Los TcES fueron capaces de regular la respuesta al LPS en las CDs TLR2-/- o MGL-/- en manera similar que las CDs WT.

La unión de los TcES a TLR2 nos llevó a investigar si los TcES son capaces de activar la cascada intracelular característica de los TLRs. Para ello evaluamos la

actividad de los TcES en células HEK transfectadas con TLR2 ó TLR2/TLR6, las cuales inducen la activación del reportero IL-8, a través de dichos receptores. Los TcES no indujeron la secreción de IL-8 por las células HEK transfectadas con TLR2 o TLR2/6. Adicionalmente, usamos células HEK transfectadas con TLR4 y CD14, en las cuales los TcES no activaron la secreción de IL-8.

La siguiente estrategia fue investigar las señales intracelulares afectadas por los TcES. Después de 30 minutos de incubación con los TcES, encontramos que la fosforilación de cRAF fue aumentada por los TcES. En contraste, la fosforilación de p38, ERK1/2, MEK1/2 y NFkBp65 permanecieron en niveles basales. Por otro lado, investigamos si los TcES afectan la señalización inducida por el LPS. Las CDs tratadas con LPS incrementaron la fosforilación de ERK1/2, MEK1/2, p38 y NFkBp65. El tratamiento con los TcES, redujo la fosforilación de p38 y NFkBp65 inducida por el LPS. Sin embargo ERK1/2 y MEK1/2 permanecieron sin cambios. De manera interesante, las CDs que recibieron ambos estímulos mostraron menor fosforilación de RAF que las CDs tratadas solo con TcES. Esta modulación entre el LPS y los TcES sugiere que ambas cascadas de señalización se regulan mutuamente, o posiblemente compiten entre sí. Debido a la activación de la vía cRAF por los TcES, la siguiente pregunta fue si esta vía de señalizaciónestá involucrada en el bloqueo de la activación de las CDs por el TcES. Para ello, las CDs fueron pre-tratadas con el inhibidor específico de cRAF o transfectadas con siRNA específico para cRAF. El efecto de modulaciónde los TcES sobre la activación de la producción de TNFα e IL-12 inducida por el LPS fue dependiente de cRAF, ya que cuando las CDs fueron pre-tratadas con el inhibidor de cRAF y estimuladas con LPS y TcES, los niveles de IL-12, TNFα, CD80 y CD86 fueron semejantes a los niveles inducidos en las CDs que sólo se estimularon con el LPS. Acorde con estos resultados, encontramos que la activación de NFkB inducida por el LPS en células RAW, fue reducida en presencia de los TcES, dicha modulacióntambiénfue dependiente de cRAF. La siguiente pregunta fue si cRAF es necesaria para que las CDs inicien una respuesta Th2. Para responder esta pregunta, las CDs fueron cultivadas en presencia del péptido deovoalbúmina, tratadas con el inhibidor de cRAF y estimuladas con los TcES. Estas CDs fueron usadas como presentadoras de antígeno en co-cultivos con células CD4+ provenientes de ratones con TCR transgénico para reconocimiento de OVA (OTII). Las CDs expuestas a los TcES indujeron la producción de IL-4 e IL-13 por los linfocitos T. En contraste, en los co-

cultivos donde las CDs fueron pre tratadas con el inhibidor de cRAF y expuestas a los TcES, los niveles de IL-4 e IL-13 fueron reducidos significativamente.

Debido a que la vía de cRAF fue importante para modular negativamente la producción de IL-12, citocina clave para el desarrollo de una respuesta Th1, investigamos si los TcES reducen la producción de IFN-γ inducida por el LPS a través de un mecanismo cRAF dependiente. En acuerdo con experimentos anteriores, los TcES redujeron los niveles de IFNγ inducidos por el LPS en los co-cultivos. Sin embargo, cuando las CDs fueron pre-tratadas con el inhibidor de cRAF, los niveles de IFNγ permanecieron elevados aun en presencia de los TcES.

Finalmente, con el objetivo de asociar la señalización intracelular inducida por los TcES y sus receptores, realizamos el bloqueo conjunto de los receptores previamente identificados. Los resultados mostraron que el MR y MGL fueron necesarios para la fosforilación de cRAF inducida por los TcES. Mientras que el bloqueo de TLR2 resultó en una disminución no significativa de la fosforilación de cRAF. Sin embargo, el bloqueo simultaneo de MR, TLR2 y MGL, interfirió completamente con la activación de cRAF por los TcES. Debido a que previamente demostramos que las CDs deficientes en MGL o TLR2 respondieron de manera similar a los TcES, decidimos bloquear en combinación dichos receptores. El bloqueo individual de MGL o TLR2 resultó en el mismo fenotipo encontrado en las CDs deficientes en dichas moléculas. Adicionalmente, el bloqueo de MR resultó en la recuperación de la producción de TNFα, pero no de IL-12 en las CDs. Sin embargo, el bloqueo simultaneo de MR, MGL y TLR2 resultó en una recuperación de la respuesta de las CDs, reflejado en la producción normal de IL-12 y TNFα en CDs tratadas con los TcES y LPS. Finalmente evaluamos la participación individual de MR, MGL y TLR2 en la inducción de la respuesta Th2 por CDs estimuladas con los TcES. La producción de IL-4 fue disminuida parcialmente bloqueando cada uno de los receptores de manera individual. Sin embargo, el bloqueo simultaneo de MR, MGL y TLR2 resultó en la inhibición de la producción de IL-4.

Resultados no publicados

Los antígenos excretados/secretados de *Taenia crassiceps* disminuyen el curso de colitis inducida por DSS en ratones C57BL/6.

Uno de los objetivos de estudiar los TcES, es poder modular patologías asociadas a la exacerbación de la respuesta inmune a través de las CDs. Para estudiar si los TcES pueden modularla respuesta inmune *in vivo*, usamos un modelo de colitis aguda usando Dextran/sulfato de sodio (DSS). En este modelo se ha demostrado que la respuesta inmune innata juega un papel primordial en la patología produciendo altos niveles de óxido nítrico en el colon y citocinas proinflamatorias, como TNFα. También se ha demostrado que las CDs juegan un papel fundamental en el desarrollo de la colitis inducida por DSS, ya que las CDs responden al DSS produciendo citocinas pro-inflamatorias como TNFα. Adicionalmente se demostró que la eliminación de las CDs disminuye la progresión de la colitis (Berndt et al., 2007). Debido a la incapacidad de las CDs tratadas con los TcES de producir citocinas pro-inflamatorias a subsecuentes estímulos inflamatorios, nuestra hipótesis es que los TcES pueden afectar el desarrollo de la colitis inducida por DSS. Para comprobar nuestra hipótesis, desarrollamos un modelo de colitis usando DSS al 4% en ratones C57BL/6, inyectamos i.p. 50 µg de TcES i.p. diariamente durante 9 días.

La pérdida de peso característica del modelo de colitis fue menor en los ratones que recibieron DSS+TcES al igual que el score clínico (sangrado en las heces, diarrea) (Figura 1). En los cortes histológicos del colon de los ratones tratados con DSS se observó pérdida de la mucosa intestinal con evidente alteración de la morfología de las criptas y abundante infiltrado celular en la lámina propia. En contraste, los ratones que recibieron DSS y fueron inyectados con los TcES se observó la lámina propia y las criptas intestinales con poco daño aunque también se observó infiltrado celular (Figura 2). En suero, los niveles de IL-12 aumentaron a los 10 días en los ratones tratados con TcES, DSS o DSS+TcES. En contraste, la IL-10 aumentó significativamente solo en los ratones tratados con DSS+ TcES (Figura 3A). Una molécula importante en el desarrollo de la colitis es el óxido nítrico. En las secciones de colon evaluamos por PCR en tiempo real la expresión de iNOS. La expresión de iNOS aumentóen los ratones tratados con DSS comparados con los controles. En contraste, los ratones que recibieron DSS+TcES

mantuvieron bajos niveles de expresión de iNOS comparados con los ratones que recibieron DSS+PBS (Figura 3B). Finalmente, se ha demostrado que los macrófagos y las CDs pueden expresar moléculas relacionadas con anti-inflamación o con un estado de activación alternativa, por lo cual buscamos en el colon los marcadores de activación alternativa como Arginasa1, YM1 y RELMα. Encontramos que la expresión de arginasa fue menor en los ratones que recibieron DSS, lo cual muestra un balance entre arginasa e iNOS que compiten por el mismo substrato. En contraste la expresión de arginasa en los ratones que recibieron DSS+TcES no se afectó respecto al control. De manera interesante YM1 y RELMαse encontraron sobre expresados en los ratones tratados con DSS y también en los ratones tratados con DSS+TcES (Figura 3B).

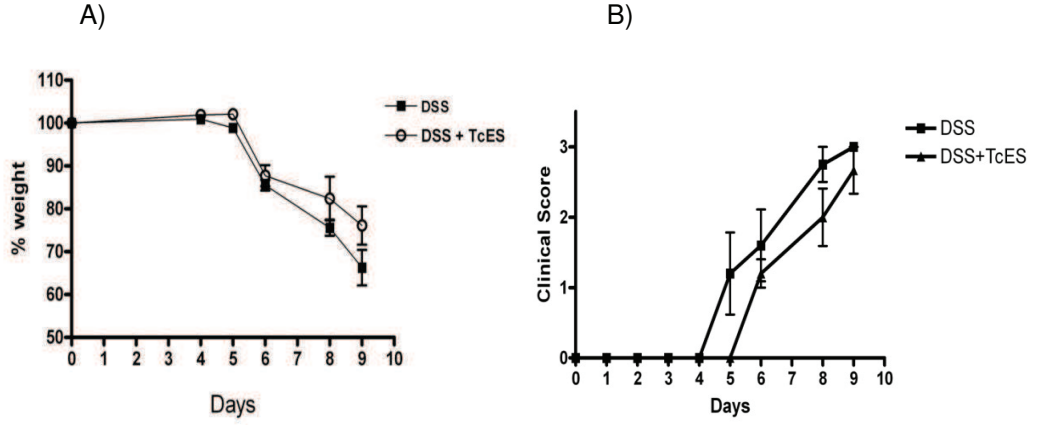


Figura 1. Los TcES reducen los signos clínicos de la colitis inducida por DSS. A) Porcentaje de pérdida de peso. B) Score clínico de patología por colitis. Figura representativa de dos experimentos independientes. N=4

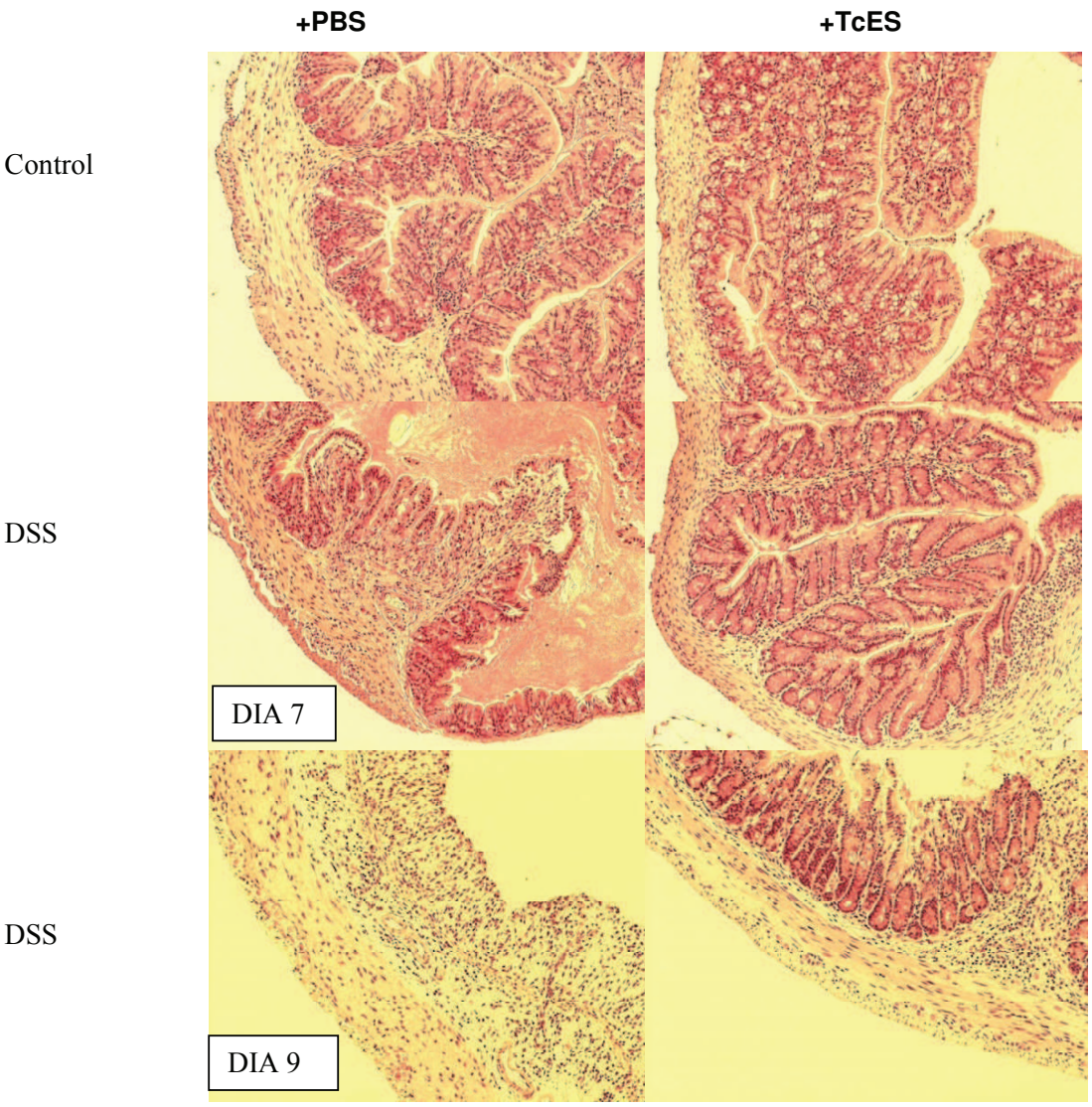
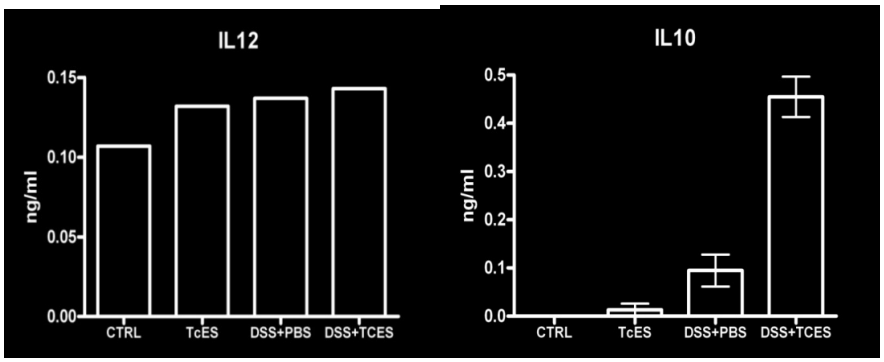


Figura 2. Cortes transversales de colon de ratones C57BL/6 teñidos con hematoxilina/eosina. Los ratones fueron mantenidos con agua o agua con DSS 4% e inyectados i.p. diariamente durante 9 días con TcES 50 µg/ml o PBS como control usando 4 ratones por grupo. Datos representativos de 2 experimentos.

A)



B)

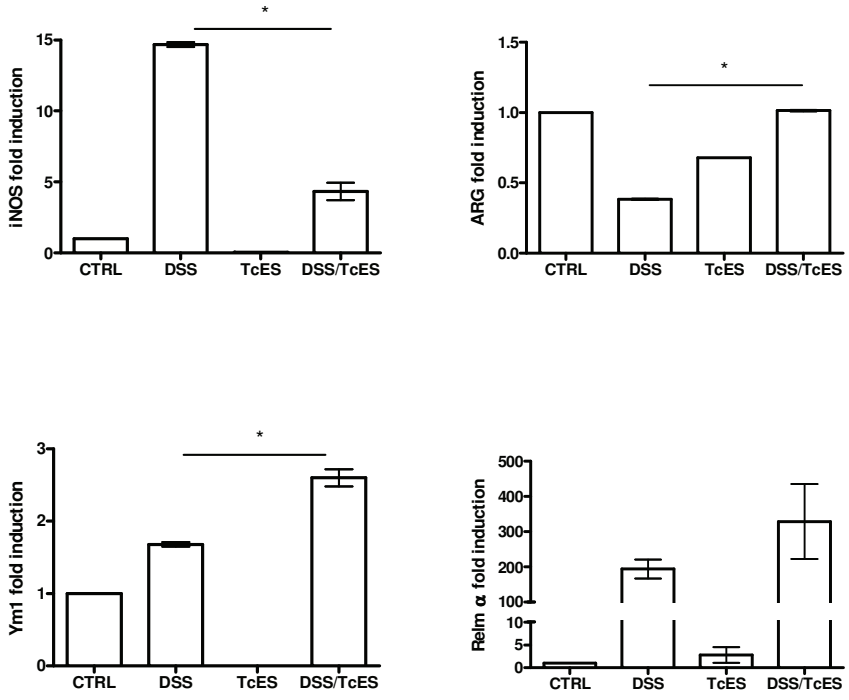


Figura 3. La inyección de TcES aumenta los niveles de IL-10 en suero en los ratones tratados con DSS y disminuyen la expresión de iNOS en el colon. A) IL-12 e IL-10 fueron cuantificados por ELISA en suero de ratones tratados durante 10 días con DSS e inyectados con PBS o TcES. B) PCR en tiempo real de secciones de colon después de 10 días de tratamiento. N=4 *$p<0.05$

Los TcES funcionan como adyuvantes para Th2 en un modelo de presentación de antígeno restringido a células dendríticas in vivo.

La función de los TcES como inductores de respuestas Th2 a través de las CDs fue clara en los estudios *in vitro*. Para demostrar la capacidad de los TcES para inducir respuestas Th2 *in vivo* a través de las CDs, usamos un modelo de presentación de antígeno el cual fue restringe la presentación de antígeno a células dendríticas in vivo.Este sistema fue desarrollado en el laboratorio del Dr. Ralph Steinman y consiste en anticuerpos dirigidos contra moléculas de membrana expresadas en las CDs o subclases de CDs los cuales están unidos a OVA (Bonifaz et al., 2004).

En este estudio usamos el anticuerpo anti 33D1 dirigido contra la lectina DCIR, la cual se encuentra expresada en CDs de bazo CD11c+CD8-DCIR+. Y un anticuerpo anti DEC205, cuyo antígeno se encuentra expresado en CDs de bazo CD11c+CD8+ (CDs linfoides). Ambos anticuerpos fueron conjugados con la proteína ovoalbúmina. De tal manera que las células a las que está dirigido el anticuerpo serán las únicas células capaces de presentar ovoalbúmina. Por otro lado, se realizó transferencia adoptivade células T CD4+ transgénicas para el reconocimiento del péptido de la ovoalbúmina provenientes de ratones DO11.10 a ratones WT, las cuales serán las únicas células que responderán a dicho péptido. En este sistema, desarrollamos dos tipos de inmunizaciones, intraperitoneal y subcutáneo usando el cojinete plantar del ratón. En el modelo intraperitoneal encontramos que los antígenos TcES no indujeron la producción de IFN-γ en los ratones recipientes. En contraste, activaronla producción deIL-4 cuando la ovoalbúmina se administró conjugada con el anticuerpo anti 33D1 en presencia de los TcES. Sin embargo, cuando se administró OVA conjugada con el anticuerpo DEC205, las células CD4+ no produjeron IL-4 en presencia de los TcES (Figura 4B).

En contraste, cuando los TcES y los anticuerpos se administraron de manera subcutánea, ambos anticuerpos fueron capaces de inducir la producción de citocinas tipo Th2. De manera interesante, el estímulo de las células del ganglio poplíteo y células de bazo con el péptido OVA indujo la producción de IL-4 (Figura 5B,D). Nuestros datos demuestran que las CDs pueden iniciar una respuesta Th2 in vivo y

sugieren que la vía de inmunización es importante en el diseño de vacunas que tienen como blanco las CDs. Además, sugieren que existen diferentes subclases de CDs que reconocen antiDEC205 o antiDCIR, en bazo y en el cojinete plantar del ratón. Estas subclases, dependiendo de su localización son capaces de inducir respuestas Th2. Experimentos adicionales son necesarios para caracterizar la subclase de CDs en el cojinete plantar del ratón que expresan DEC205 y son capaces de inducir una respuesta Th2.

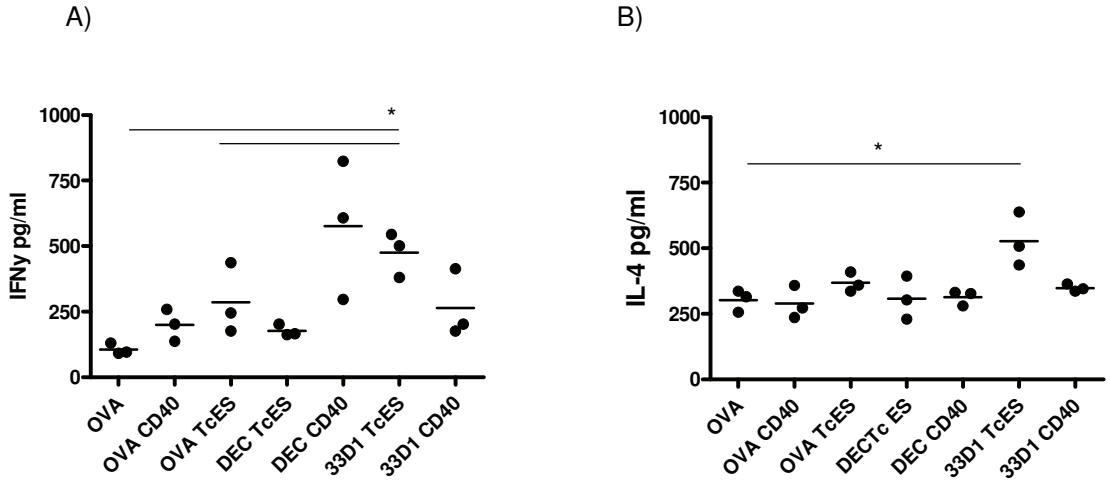


Figura 4. La administración de los TcES vía intraperitoneal induce la producción de IL-4 a través de células dendríticas 33D1 positivas. Ratones Balb/c recibieron vía intravenosa células CD4+ provenientes de ratones DO11.10 un día previo a la inmunización. Los ratones Balb/c recibieron 50 µg de TcES vía intraperitoneal en el cojinete plantar mezclado con OVA (25000ng), anti DEC205 conjugado con OVA (500ng) o anti 33D1 conjugado con OVA (500ng). Datos representativos de 3 experimentos. N=3 *$p<0.05$

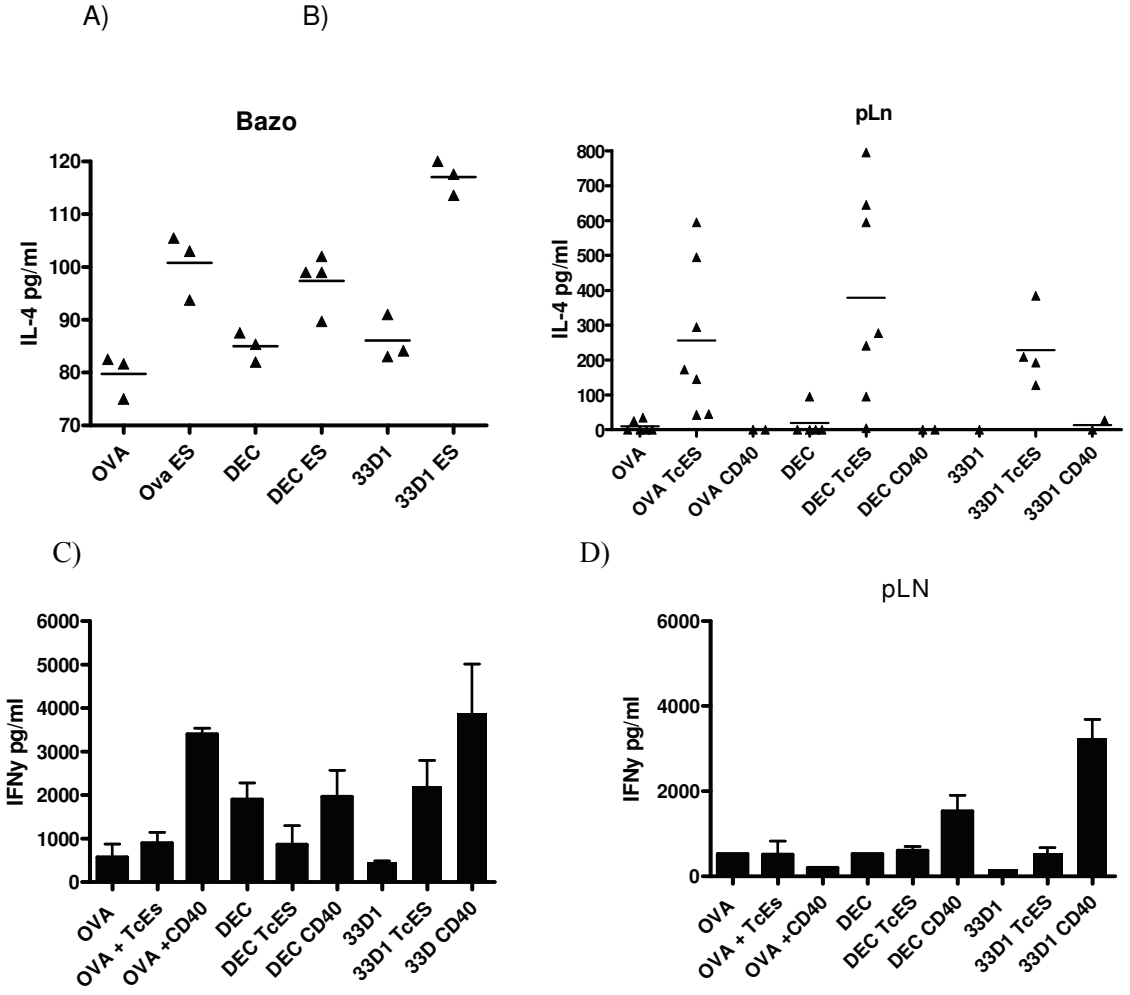


Figura 5. Los TcES funcionan como adyuvantes para inducir una respuesta Th2 a través de CDs DEC205+ y DCIR+ en la inmunización subcutánea. Ratones Balb/c recibieron vía intravenosa células CD4+ provenientes de ratones DO11.10 un día previo a la inmunización. Los ratones Balb/c recibieron 50 µg de TcES en el cojinete plantar mezclado con OVA (2500ng), anti DEC205 conjugado con OVA (500ng) o anti 33D1 conjugado con OVA (500ng). Siete días post inmunización las células del ganglio poplíteo (B,D) y el bazo (A,C) fueron re-estímuladas con OVA péptido durante 5 días. Las concentraciones de IL-4 o IFNy en los sobrenadantes fueron detectadas por ELISA. Datos representativos de 2 experimentos para los tratamientos usando CD40 y 3 experimentos usando TcES. N=3

Los TcES condicionan a las CDs a inducir respuestas Th2 a través de factores solubles.

El siguiente experimento se realizó con el objetivo de identificar si son factores solubles o de membrana los causantes de polarizar la respuesta a un tipo Th2 y que factores afecta cRAF para disminuir la respuesta Th2 a través de las DCs. Para ello se usaron las siguientes estrategias:

Las CDs fueron sembradas en placas de 24 pozos, 500.000/pozo, se incubaron en presencia de OVA péptido durante 2 horas, al finalizar se incubaron durante 2 horas con el inhibidor de cRAF, finalmente se agregaron los TcES (40µg/ml).Para asegurar que el TcES no internalizado por las DCs y el inhibidor remanente de cRaf no estuviera presente en los co-cultivos, la placa se lavó como sigue:24 h después la placa de 24 pozos fue centrifugada 2000 rpm durante 5 minutos, el sobrenadante fue retirado y se agregaron 500 µl de PBS por pozo y la placa fue centrifugada nuevamente, esto se repitió 3 veces.Al finalizar los lavados, se agregó paraformaldehído 4% para fijar las DCs en los tratamientos correspondientes, esto permitiría evaluar la participación de moléculas de membrana en ausencia de factores solubles en la polarización de las células T.Al finalizar la incubación durante 20 minutos, los pozos que recibieron paraformaldehído se lavaron exhaustivamente.

Para evitar el contacto de membrana entre las CDs que fueron expuestas a los TcES y los linfocitos T, se usó un transwell 0.4 micras, para permitir el paso de factores solubles y evitar el contacto de membrana.En la parte superior del transwell fueron sembradas CDs incubadas durante 24 horas sólo con el péptido OVA, usadas como presentadoras de antígeno y que no tuvieron contacto con los TcES. En algunos tratamientos las CDs en la parte superior fueron fijadas con paraformaldehído.

Finalmente se agregaron las células T aisladas de ratones OTII mediante selección negativa usando microbeads. Siete días después, los sobrenadantes fueron evaluados por ELISA.Los resultados muestran que la producción de IL-4 es dependiente de los factores solubles provenientes de las CDs tratadas con TcES, y que estos factores son dependientes de cRAF. Es probable que los factores solubles de las CDs tratadas con TcES induzcan la producción de los mismos factores solubles en las

CDs presentadoras (parte superior del transwell), ya que en el tratamiento CD+TcES TW la producción de IL-4 aumenta, y cuando las DCs presentadoras están fijadas la producción de IL-4 disminuye (Figura 6).

Las CDs tratadas con TcES aumentan la producción de IL-13 y fue dependiente de cRAF, la producción de IL-13 se conservó al usar transwells. Sin embargo, la producción de IL-13 fue disminuida al fijar las células presentadoras; esto sugiere que la inducción de IL-13 depende de factores solubles de las CDs expuestas a los TcES, y de moléculas de membrana inducidas en las CDs presentadoras, y que los factores solubles de las DCS+TcES actúan sobre las CDs presentadoras induciendo un fenotipo inductor de Th2.En conjunto, estos resultados demuestran que los factores solubles son importantes para iniciar la respuesta Th2 a través de las CDs. 2. Que la respuesta Th2 no es una respuesta por default. 3. Que las células CD4+ que producen IL-4 podrían ser diferentes a las que producen IL-13, o que las células CD4+ necesitan estímulos diferentes para producir IL-13 o IL-4. 4. Que células dendríticas expuestas a los TcES pueden modular a células dendríticas bystander, induciéndolas a un fenotipo DC2/o que células CD4+ expuestas a factores solubles de las CDsTcES, inducen un fenotipo en CDs que no tuvieron contacto con el antígeno capaz de inducir la secreción de IL-13. 5. Los factores solubles responsables de la inducción de IL-4 e IL-13 sonparcialmente cRAF-dependientes.

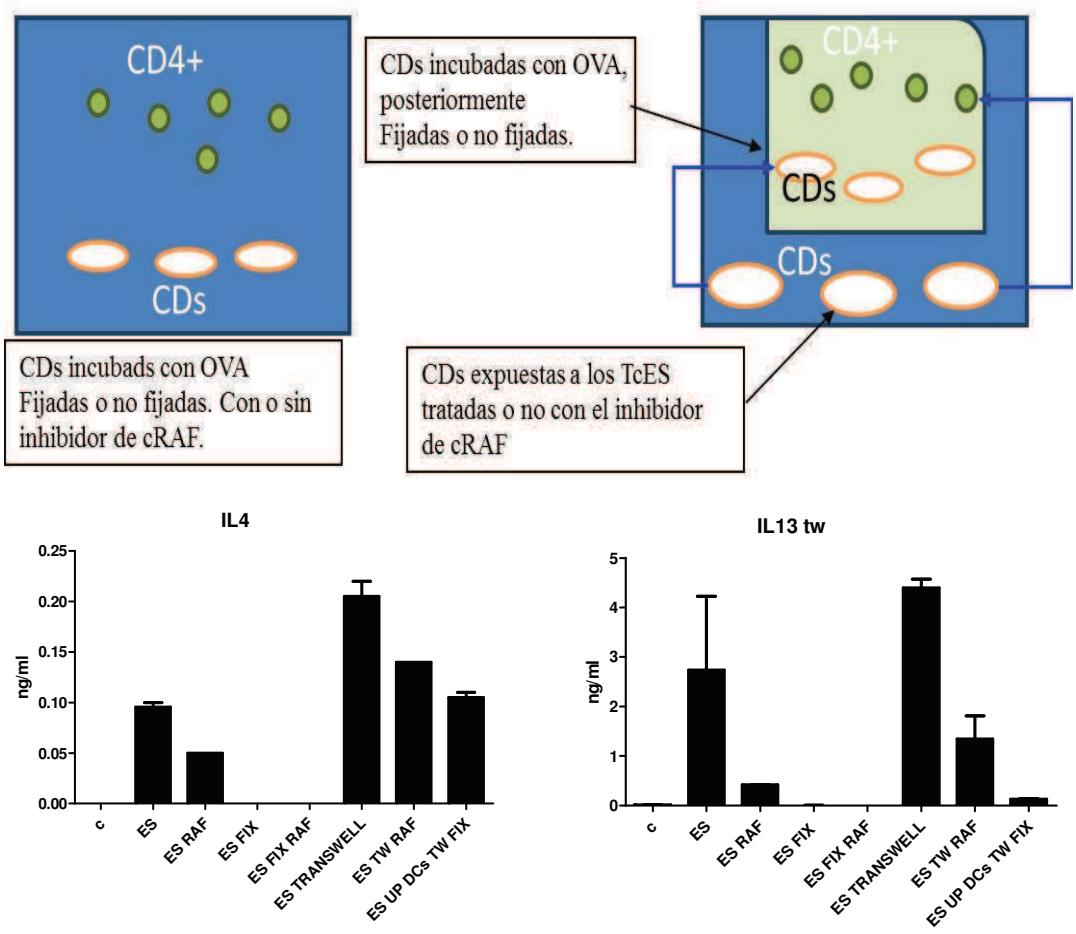


Figura 6. Las CDs tratadas con TcES inducen un fenotipo Th2 a través de la producción de factores solubles. Tratamientos: ES RAf: CDs pre-tratadas con el inhibidor de RAF. ES FIX: CDs fijadas con paraformaldehido. ES FIX transwell: Las CDs en la parte inferior fueron estimuladas con TcES y fijadas con paraformaldeído. ES TW RAF: Las CDs en la parte inferior fueron pre-tratadas con RAF y TcES. ES UP CDs TW FIX: Las CDs en la parte inferior fueron estimuladas con TcES, las CDs en la parte superior fueron estimuladas con OVA péptido y fijadas con paraformaldehído.

Identificación de moléculas presentes en los TcES con potencial inmunomodulador.

Los TcES fueron analizados por espectrofotometría de masas, resultando en el hallazgo de 11 moléculas asociadas al género *Taenia*. Para conocer la composición de los TcES, usamos la técnica de espectrometría de masas. Los datos obtenidos fueron analizados con el software Mascot, y se buscaron secuencias de moléculas del género *Taenia* (Tabla 1). Como resultado de la identificación de estas moléculas en los TcES, se muestra que la mayoría de ellas tienen un potencial rol en la inmunomodulación no sólo de las células dendríticas, sino de otras células como macrófagos.

Los resultados mostraron un alto contenido de albúmina en las secreciones de *Taenia crassiceps,* probablemente procedentes del hospedero. Debido a esto, es posible que algunas moléculas con poca abundancia puedan ser enmascaradas por las altas cantidades de albúmina y que los TcES contengan moléculas adicionales a los identificados en la tabla 1. Por esta razón, utilizamos cromatografía de intercambio iónico usando columnas aniónicas para colectar diferentes fracciones y poder identificar las fracciones con la propiedad de inhibir la producción de TNFα e IL-12 inducida por LPS y con actividad para inducir respuestas Th2 in vitro a través de las células dendríticas.

Los TcES fueron divididos en 10 fracciones (Figura 7), cada una de las fracciones fue añadida a los cultivos de CDs, e incubadas con o sin LPS. Por otro lado, las fracciones fueron usadas para estimular las CDs en presencia del péptido OVA y fueron co-cultivadas con células CD4+ aisladas de bazo de ratones C57BL/6 OTII.Los resultados muestran mayor actividad inhibitoria de la producción de citocinas IL-12 y TNFα, en las fracciones 8, 9 y 10 (Figura 8). De manera interesante la producción de IL-6 e IL-10 aumentó en los cultivos tratados con las fracciones 8, 9 y 10 en combinación con LPS (Figura 8). En los co-cultivos, el mayor porcentaje de células CD4+ IL-4+ se observó cuando las CDs se estimularon con la fracción 9 o 10. En contraste, IFNγ solo aumento en las CDs estimuladas con LPS (Figura 9). Con el objetivo de identificar alguna de las moléculas presente en las fracciones con mayor actividad moduladora de la respuesta al LPS e inductoras de la producción de IL-4 en los co-cultivos fueron combinadas, marcadas con fluorescencia (PE)y separadas en un gel de poliacrilamida. De la misma manera las fracciones inactivas fueron marcadas con fluorescencia (FITC)

y separadas en el mismo pozo que las fracciones activas. De esta manera se identificaron dos bandas en la fracción activa que fueron diferentes de las bandas en las fracciones inactivas (Figura 10). Estas bandas se extrajeron del gel y se sometieron a espectrofotometría de masas, los péptidos fueron analizados en el programa Mascot. Las secuencias presentes en *Taenia crassiceps* se muestran en la tabla 2.

prot_acc	prot_desc	prot_score	prot_mass
gi|45360118|gb|AAS59168.1|	Na+/K+-ATPase alpha subunit [Taenia solium]	538	113058
gi|124783335|gb|ABN14922.1|	actin 1 [Taenia asiatica]	306	25294
gi|124784704|gb|ABN14989.1|	polyubiquitin [Taenia asiatica]	195	22664
gi|283466488|emb|CBH36499.1|	phosphoenolpyruvate carboxykinase, partial [Taenia crassiceps]	177	54980
gi|161650|gb|AAA30092.1|	actin [Taenia solium]	109	42003
gi|261266611|gb|ACX56268.1|	enolase [Taenia asiatica]	97	46909
gi|223403630|gb|ACM89282.1|	2-cys peroxiredoxin [Taenia crassiceps]	94	21986
gi|295901404|dbj|BAJ07359.1|	cyclophilin [Taenia taeniaeformis]	91	17643
gi|155964455|gb|ABU40277.1|	Ts1 [Taenia solium]	64	25852
gi|21930119|gb|AAM82156.1|AF523312_1	oncosphere-specific antigen [Taenia solium]	63	9838
gi|283466484|emb|CBH36497.1|	phosphoenolpyruvate carboxykinase, partial [Taenia saginata]	61	55004

Tabla 1. Los TcES fueron sometidos a espectrofotometría de masas para la identificación de su las moléculas presentes. Los resultados fueron sometidos a análisis en el software Mascot. Los criterios de búsqueda fueron los siguientes: M/S M/S ion search. Enzima: Tripsina. Protein mass: unrestricted. Protein mass tolerance: +/- 1.5 Max mixed cleaveage: 2. Score mayor de 50 se consideraron positivos.

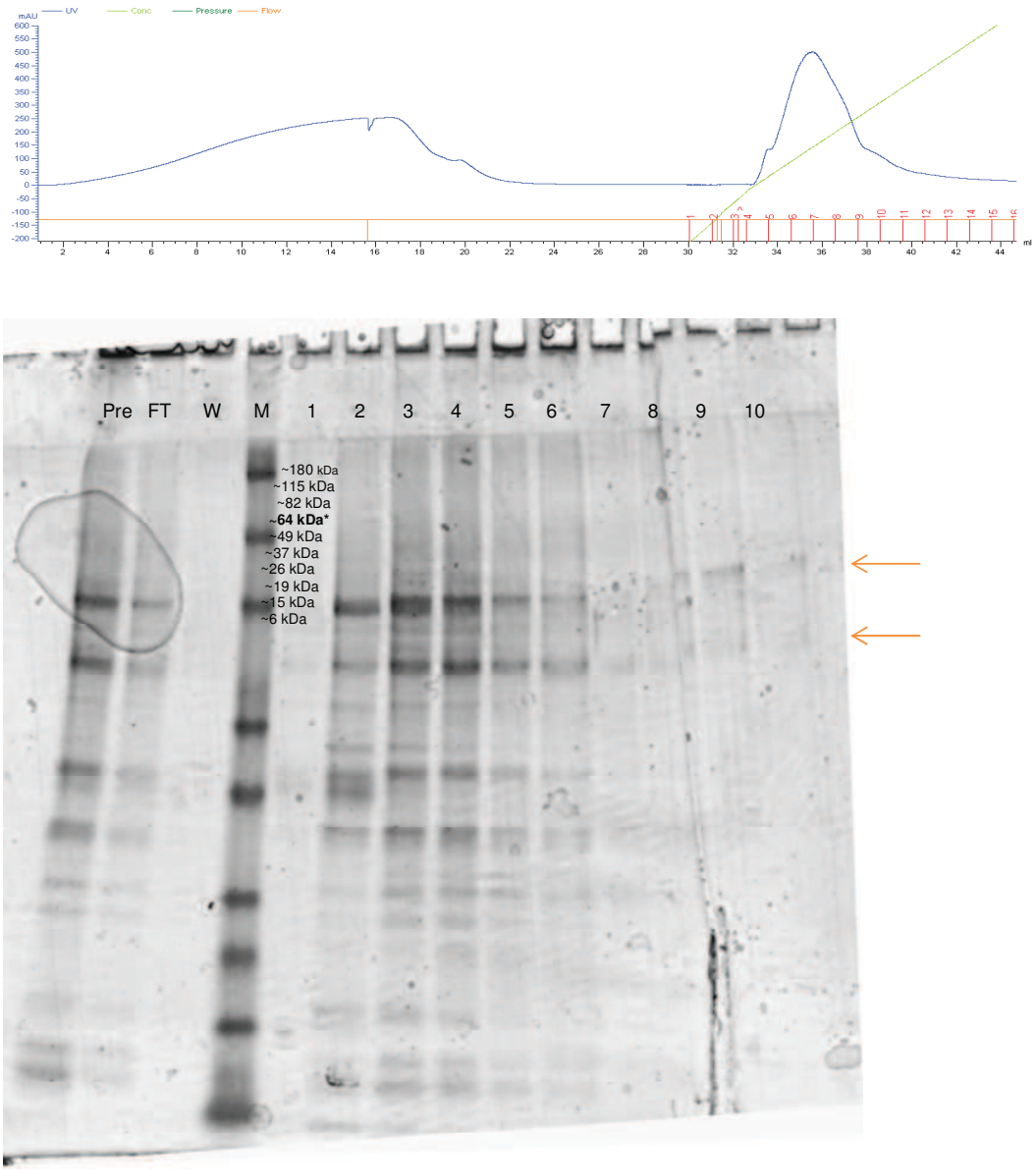


Figura 7. Los TcES fueron obtenidos de diferentes cultivos de *T. cassiceps* de ratones infectados durante 6-8 semanas. Los TcES fueron concentrados usando una membrana de 3kDa, 5 mg de TcES fueron vertidos en la columna de afinidad por compuestos anionicos, Q-XL column, y eluidos con el buffer 1M NaCl recuperando diez diferentes fracciones. Las fracciones 8, 9 y 10, comparten una banda de >82kDa.

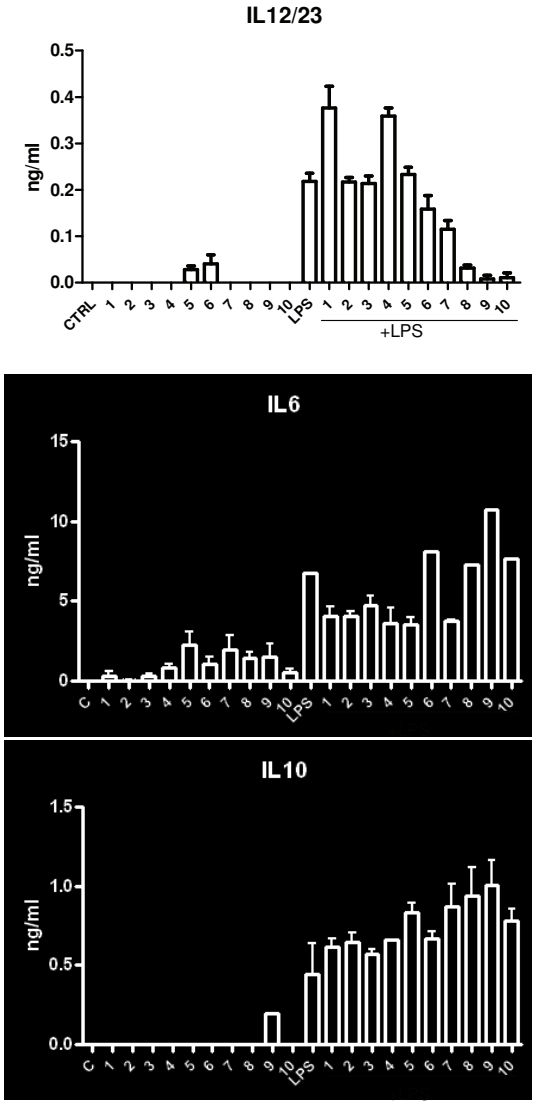


Figura 8. Diferentes fracciones de los TcES fueron obtenidas mediante cromatografía de intercambio iónico. Las fracciones 8, 9 y 10 mostraron mayor actividad inhibitoria de IL-12, en contraste aumentaron la producción de IL-10 en combinación con LPS. La fracción 9 aumentó la producción de IL-10 y en combinación con LPS aumento la producción de IL-10 e IL-6 por las células dendríticas.

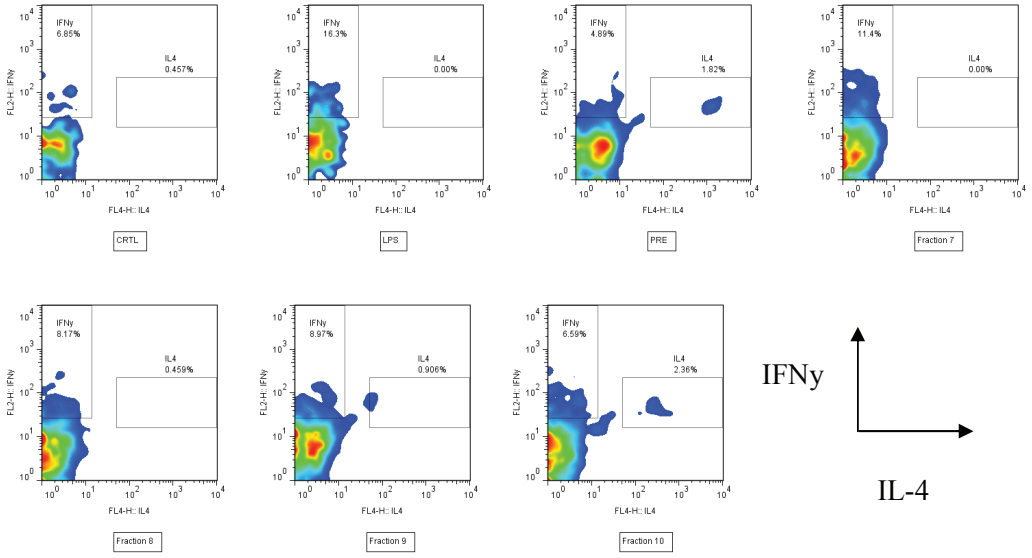


Figura 9. La fracción 9 y 10 de TcES obtenidas a través de cromatografía de intercambio iónico aumentan la producción de IL-4 en co-cultivos de células dendríticas y células CD4+. Las CDs fueron estimuladas con cada una de las fracciones durante 24 horas en presencia de OVA 2 µg/ml y co-cultivadas con células CD4+ aisladas de ratones OTII. Después de 5 días de co-cultivo las células fueron estimuladas con ionomicina/PMA durante 5 horas en presencia de brefeldina A. Las células marcadas con anti CD3, fueron fijadas, permeabilizadas tenidas con anti IFNγ e IL-4 y analizadas por citometría de flujo. Los plots muestran células seleccionadas de la región CD3 positivas. PRE= TcES antes de someterse a cromatografía de afinidad.

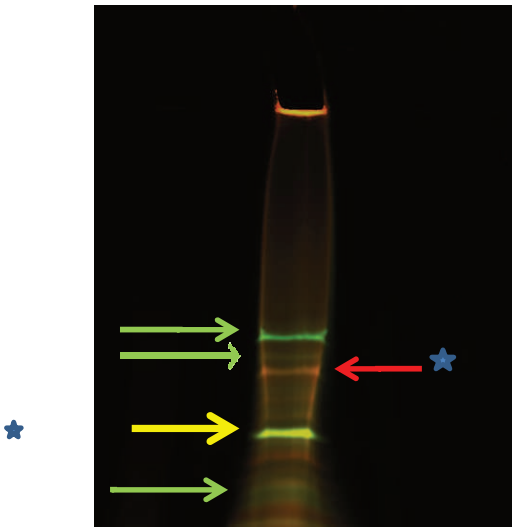


Figura 10. Las fracciones recuperadas por intercambio iónico. La fracción activa (F9 y F10) fue marcada con Cy3(rojo) y la fracción inactive fue marcada con Cy5 (verde). Ambas fracciones fueron mezcladas y separadas en un gel SDS-PAGE 4-15%. La imagen del gel fue adquirida en Typhoon Phosphorimager. La flecha roja corresponde a moléculas presentes sólo en la fracción activa. La flecha verde corresponde a moléculas presentes en la fracción inactiva. Líneas amarillas corresponden a moléculas conservadas en ambas fracciones.

	Score	Mass	Matches	Sequences	emPAI	
1	553	25294	13 (13)	7 (7)	2.45	actin 1 [Taenia asiatica]
2.1	430	42003	15 (15)	9 (9)	1.30	actin [Taenia solium]
2.2	64	21681	3 (3)	2 (2)	0.54	actin [Taenia asiatica]
3	310	36733	10 (10)	7 (7)	1.00	elongation factor 1 alpha [Taenia crassiceps]
4	148	46909	4 (4)	4 (4)	0.31	enolase [Taenia asiatica]

Tabla 2. Identificación de moléculas presentes en las fracciones activas de los TcES.
Las moléculas presentes en las bandas activas fueron obtenidas como se muestra en la figura 10. La banda teñida de color rojo fue sometida a espectrofotometría de masas y las secuencias obtenidas fueron analizadas con el software Mascot. La tabla muestra las secuencias con un Score mayor a 100.

Discusión general

Las infecciones por helmintos parásitos son aún un problema de salud pública a nivel global, presentando mayor incidencia en países en vías de desarrollo. Hasta el momento se carece de estrategias inmunológicas para la erradicación de estas infecciones. La carencia de una vacuna que eficazmente confiera inmunidad para algún helminto pone en evidencia los complejos mecanismos de evasión inmune de estos parásitos, y el escaso conocimiento sobre los mismos. Un ejemplo claro, es el desconocimiento de receptores en células inmunes innatas para moléculas parasitarias derivadas de los helmintos(MacDonald and Maizels, 2008; Maizels et al., 2004). Ya que estas células innatas como lo son las células dendríticas, pueden iniciar la compleja cascada de eventos que determinaran la eficacia de la respuesta inmune sobre la infección(Banchereau et al., 2000; Flores-Romo, 2001).

En general, la respuesta inmune generada contra los helmintos, se caracteriza por ser una respuesta tipo Th2, que involucra secreción de citocinas como IL-13, IL-4, IL-5, la activación de mastocitos, eosinófilos y basófilos, además de polarización de los macrófagos a un estado de activación alternativa(Palm et al., 2012; Pulendran and Artis, 2012). Se ha demostrado, para el caso de diversos nemátodos y tremátodos que en la fase intestinal, la respuesta Th2 confiere protección, a través de diferentes mecanismos como la activación alternativa de macrófagos, secreción de moco y de la inducción de contracciones en el intestino principalmente mediados por la IL-4 e IL-13 (Anthony et al., 2007; Reyes and Terrazas, 2007). Sin embargo, existen excepciones, por ejemplo en la infección intestinal por *Heligmosomoides polygyrus*, a pesar de que se desarrolla una respuesta Th2, el parásito no es expulsado. De manera interesante, cuando se administra medicamento antiparasitario y se elimina el parásito, el hospedero es inmune a una segunda infección con el mismo, de manera Th2 dependiente (Reynolds et al., 2012). En algunos casos, la respuesta Th2 no genera inmunidad cuando los helmintos invaden otros tejidos, como pulmón, hígado, músculo, cerebro, o la cavidad peritoneal(Chen et al., 2012; Faz-Lopez et al., 2013; Herbert et al., 2004). En otros tejidos excluyendo el intestino, la respuesta Th2 puede favorecer el establecimiento del parásito, y una respuesta Th1 restringe la infección(Pearce et al., 2004; Terrazas et al., 1998; Terrazas et al., 1999). Esto ha generado la pregunta, ¿Por qué el sistema inmune responde con una respuesta Th2 en infecciones extra intestinales contra estos parásitos

cuando pareciera ser inefectiva para la eliminación de los mismos? Recientemente, esta pregunta ha sido abordada por diferentes grupos de investigación, y se ha demostrado que la respuesta Th2 puede ser en beneficio del hospedero, manteniendo bajos niveles de infección, pero, sin eliminar al parásito completamente. Es decir, una respuesta Th1 exacerbada contra estos macro parásitos en tejidos como pulmón, cerebro o hígado, podría resultar en la destrucción del tejido y propiciar la muerte del hospedero, mientras que una respuesta Th2, principalmente a través de IL-4 e IL-10, contribuye a la formación de granulomas y la generación de células anti-inflamatorias como macrófagos alternativamente activados, que rodean a estos parásitos y favorecen la reparación de tejido (Chen et al., 2012; Faz-Lopez et al., 2013). Sin embargo, la respuesta Th2 puede resultar ineficiente para erradicar la infección, pero es importante para mantenerla controlada, evitando la migración del parásito y/o su reproducción (Herbert et al., 2004). Estas observaciones son congruentes con la hipótesis de Ruslan Medzhitov, en la cual propone que el sistema inmune es capaz de identificar el costo de eliminar a un patógeno sobre el beneficio de mantenerse vivo(Medzhitov et al., 2012). Por otro lado, Polly Matzinger ha propuesto que la clase inmune efectora es determinada por el tejido donde ocurra, y que ciertos tejidos tienen preferencia a inducir respuestas Th1, como la piel. Otros tejidos como las mucosas preferencialmente inducen respuestas Th2; se propone que esta preferencia podría mediarse por alarminas tejido específicas, liberadas en presencia de peligro (Matzinger and Kamala, 2011). Esta hipótesis, no es excluyente de la hipótesis, costo - beneficio expuesta por Medzhitov, debido a que el tipo de respuesta preferencial inducida en los tejidos, podría generarse para evitar una respuesta deletérea. Sin embargo, es aceptado por Polly Matzinger, que algunos patógenos podrían sobrepasar la regulación del tejido, y modificar la respuesta inmune para su propia sobrevivencia (Matzinger and Kamala, 2011). En el caso de parásitos protozoarios que generan infecciones crónicas, por ejemplo, *Toxoplasma gondii, Trypanosoma cruzi, o Leishmania donovani*, y que residen en tejidos en los cuales sería muy peligroso inducir una respuesta Th1 necesarias para su eliminación, como lo son cerebro, tejido cardiaco e hígado respectivamente, generan ambientes anti-inflamatorios, o modulan la activación de las CDs (Terrazas et al., 2010). De esta misma manera parásitos helmintos como el cisticerco de *Taenia solium* infecta músculos y cerebro en el humano, donde el último parece ser el de mayor tropismo para este parásito; o en el caso de *S. mansoni*, y *E. granulosus* los cuales infectan el hígado. La destrucción del tejido por el proceso invasivo de estos parásitos, puede producir las señales de peligro

para activar al sistema inmune. Es probable que las moléculas parasitarias puedan bloquear o modificar la respuesta de las células inmunes a estas señales de peligro. En el caso de los helmintos que residen en el intestino, algunas especies dañan las paredes intestinales, mientras que otros atraviesan la pared intestinal debido a su migración, este hecho involucra la translocación de bacterias del lumen extra-intestinal al tejido, hecho que derivaría en una inflamación que podría ser dañina para el helminto y para el hospedero. De tal manera, que esta inflamación o respuesta a productos bacterianos podría ser regulada por el helminto parásito.

Otra interrogante en la respuesta Th2 es ¿Cómo se inicia esta respuesta? Esta última pregunta es desafiante, y numerosos esfuerzos experimentales e hipótesis han tratado de responderla(MacDonald and Maizels, 2008; Palm et al., 2012; Pulendran and Artis, 2012). Se ha planteado que la carencia de IL-12 en estas infecciones da como resultado una respuesta por default, en la que la presentación de antígeno sucede en ausencia de IL-12, la principal señal para iniciar la producción de IFNγ (Jankovic et al., 2006). En esta situación, la respuesta del linfocito T, sería la producción de IL-4. Sin embargo, las CDs, por ejemplo en el intestino, o la piel, están expuestas a diferentes moléculas de manera natural. Dichas moléculas pueden ser procesadas por las CDs y ser presentadas en forma de péptidos a células T, si las CDs permanecen en estado inmaduro y sin producir IL-12, llevaría a que esos linfocitos T a producirIL-4 en respuesta al antígeno. Esto, resultaría en una respuesta Th2 a esencialmente cualquier molécula incapaz de inducir la producción de IL-12 por las CDs. Además, otros resultados han aportado evidencia contra esta hipótesis, como el hecho de que aún en presencia de IL-12, una respuesta Th2 puede ser inducida(Cervi et al., 2004). En modelos in vitro, incluyendo este trabajo, la presentación del péptido OVA por CDs inmaduras es incapaz de inducir la producción de citocinas Th2 en linfocitos CD4+ (Jenkins and Mountford, 2005; Massacand et al., 2009). Estos hallazgos sugieren que se necesita un fenotipo específico en las CDs para inducir respuestas Th2. Esto lleva a pensar que al igual que bacterias, virus y protozoarios, de los cuales se conoce que existen patrones asociados a estos patógenos, que son reconocidos por receptores en las CDs, y que dicho reconocimiento activa a las CDs para inducir la diferenciación de los linfocitos T CD4+ a producirIFNγ, podrían existir patrones moleculares en los helmintos que propicien un fenotipo en las CDs con la característica de inducir la producción de citocinas tipo Th2.

Uno de los patrones en las moléculas de helmintos es su alto contenido de glicanos, algunos de ellos con características únicas. En 1999, El Dr. Donald Harn, asoció la presencia de estructuras glicosiladas a la inducción de respuestas Th2, usando los antígenos solubles del huevo de *Schistosoma mansoni* (SEA), demostró que los carbohidratos presentes en SEA eran los responsables de inducir una respuesta Th2 *in vivo*(Okano et al., 1999; Okano et al., 2001). Este experimento, demuestraque las moléculas derivadas del parásito son responsables de la inducción de una respuesta Th2, sin necesidad de la presencia del parásito. Sugiriendo entonces, que el daño del tejido inducido por los parásitos helmintos podría jugar un papel secundario en el inicio de una respuesta tipo Th2. Acorde con estos experimentos, nuestro grupo demostró que los antígenos solubles del metacestodo de*Taenia crassiceps*, son capaces de inducir respuestas Th2 *in vivo*, de manera dependiente de los carbohidratos (Gomez-Garcia et al., 2006). Sin embargo, en ambos modelos, se desconocen las células blanco que propician la generación de células T productoras de IL-4, IL-5 e IL-13. En esta tesis, usando los TcES demostramos que la polarización a Th2 puede ser a través del condicionamiento de las CDs a un fenotipo capaz de activar la producción de IL-4 e IL-13 en linfocitos T, donde los carbohidratos tienen un papel fundamental en dicha polarización.

Nuestros datos sugieren que las CDs pueden reconocer a las moléculas derivadas de helmintos a través de receptores especializados en el reconocimiento de carbohidratos. Los receptores tipo lectina (CLRs) tienen la característica de reconocer diferentes carbohidratos, y se asocian principalmente con la fagocitosis de patógenos. En los últimos años, estos receptores han retomado importancia, debido al descubrimiento de que a través de algunos CLRs, las CDs pueden ser activadas, y algunos interactúan con la señalización intracelular de los TLRs (den Dunnen et al., 2010; Kawai and Akira, 2011). Las moléculas presentes en SEA, se unen a MR, MGL, Dectin-1 y DCSIGN, los cuales internalizan dichas moléculas en endosomas(Robinson et al., 2006). Hasta ahora, se desconoce el papel de esos receptores en el condicionamiento de las CDs para la inducción de una respuesta Th2. En nuestros experimentos, nosotros encontramos que los TcES se unieron a MR, MGL y TLR2, en conjunto los tres receptores indujeron la fosforilación de cRAF. Resulta interesante el hecho de que el receptor de manosa y MGL fueron necesarios de inducir la fosforilación

de cRAF. Sin embargo, las CDs deficientes en MGL fueron moduladas por los TcES de igual manera que las CDs WT. Lo que sugiere que en ausencia del receptor MGL, el MR, podría funcionar aumentando la fosforilación de cRAF. El rol de TLR2, es menos claro, debido a que la inducción de cRAF fue ligeramente afectada al bloquear dicho receptor. Sin embargo, para inhibir completamente los efectos de los TcES sobre las CDs, tanto la fosforilación de RAF, la modulación de la respuesta al LPS, y la inducción de TH2, fue necesario bloquear de manera conjunta MR, MGL y TLR2. Por ahora, no podemos asegurar que estos receptores actúan como co-receptores para una molécula, debido a que los TcES son una compleja mezcla de moléculas. Es probable, que diferentes moléculas se unan a diferentes receptores. Sin embargo, no descartamos que una molécula presente en los TcES pueda ser reconocida por la combinación de los tres receptores. Otros estudios usando antígenos solubles del *Taenia crassiceps* indujeron la producción de IL-6 en macrófagos a través del receptor TLR4. Sin embargo, los TcES fueron incapaces de inducir la activación de iniciada por TLR4/CD14. Estos resultados sugieren que los TcES no contienen las moléculas necesarias para activar TLR4, y que dichas moléculas pueden estar contenidas en otras estructuras del parásito. Apoyando esta idea, las moléculas de bajo peso molecular presentes en el fluido vesicular del cisticerco de *Taenia solium*, fueron las únicas capaces de inducir la producción de citocinas inflamatorias por leucocitos humanos (Amit et al., 2011).

Debido a que el inicio de la infección con *T. crassiceps* las citocinas relacionadas con una respuesta Th1 se encuentra elevadas (Terrazas et al., 1998), y a que las CDs no secretan IL-12 o TNFα en respuesta a la exposición de TcES o a los antígenos solubles del parásito completo, sugiere que existen otras células que pueden reconocer moléculas de *Taenia crassiceps* y producir citocinas pro-inflamatorias, por ejemplo los neutrófilos reclutados de manera temprana en la cavidad peritoneal en la infección con éste parásito. La respuesta diferencial entre CDs y neutrófilos, podría ser debido a la expresión diferencial de receptores que regulan la cascada de los TLRs, por ejemplo el MR y MGL, el cual reguló la fosforilaicón de RAF, y afectó señalización intracelular de los TLRs, se encuentra expresado preferencialmente en células dendríticas y macrófagos, mientras que los neutrófilos carecen de dichas moléculas. Es decir, algunas células, como macrófagos o células dendríticas, podrían ser más propensas a la regulación por moléculas provenientes del parásito. Esta idea, puede ser soportada debido a que en la infección con *Taenia crassiceps*, una gran cantidad de células como

linfocitos T, células B, eosinófilos, se encuentran en un estado de apoptosis (Zepeda et al., 2010). La inducción de cRAF en CDs, que posiblemente se lleve a cabo también en macrófagos, puede ser la clave de resistencia a la apoptosis de los macrófagos en la cavidad peritoneal, debido a que cRAF puede funcionar induciendo cascadas anti-apoptóticas, principalmente bloqueando la vía de las caspasas (Smalley et al., 2009). En consecuencia, la activación de cRAF, podría tener un papel importante en la modulación del inflamosoma, el cual requiere la activación de caspasas para la activación de IL-1β e IL-18 (Sutterwala et al., 2007).

La participación de cRAF en la modificación de la señal de los TLRs puede inducir o bloquear genes relacionados con la activación de NFkB. Por ejemplo, el virus de la inmunodeficiencia humana, o los lipoabiromananos presentes en *Mycobacterium tuberculosis* son reconocidos por DCSIGN e inducen la activación de cRAF, propiciando la acetilación de NFkBp65 y de esta manera aumentan la producción de, IL-10,IL-12 y TNFα inducida por TLR8 o TLR4 respectivamente (Gringhuis et al., 2007). Por otro lado, el reconocimiento de Salp15,una molécula presente en la saliva del vector de la enfermedad de Lyme, es mediado por DCSIGN el cual activa cRAF, pero en este caso, cRAF es necesario para regular negativamente la producción de citocinas pro-inflamatorias, mediando la remodelación de la cromatina que reduce la activación de IL-12, y desestabilizando los mensajeros del TNFα (Hovius et al., 2008). Existe evidencia que dependiendo del carbohidrato que se une a DCSIGN, la respuesta intracelular puede cambiar.Al reconocer manosa, la vía DCSIGN – cRAF aumenta la producción de citocinas pro-inflamatorias. En contraste, al reconocer fucosa, DCSIGN sufre la pérdida del señalosoma, lo cual resulta en menor producción de citocinas pro-inflamatorias(Gringhuis et al., 2009a). El hecho de que DCSING a través decRAF modifica de dos maneras opuestas la vía de los TLRs, sugiere que la naturaleza del ligando de DCSIGN puede inducir cascadas de señalización diferentes, o que DCSIGN puede reconocer diferentes ligandos en asociación a diferentes co-receptores que dictan el fenotipo de la CD. Recientemente se descubrió que la principal molécula presente en SEA responsable de bloquear la respuesta iniciada por TLR4 e inducir una respuesta Th2 a través de las CDs, es una ribonucleasa, la cual es reconocida y fagocitada a través del receptor de manosa. Al ser interiorizada, la ribonucleasa es capaz de degradar los RNA mensajeros de TNFα e IL-12 (Everts et al., 2012). En nuestro modelo el MR tuvo un papel importante en la regulación de las CDs. Aunado a

esto, los TcES se acumularon en mayor cantidad en CDs incubadas a 37°C comparadas con las incubadas a 4°C. Lo cual sugiere que los TcES pueden ser internalizados. Hasta el momento desconocemos, si la internalización de los TcES es necesaria para modificar el fenotipo de las CDs, o si el reconocimiento de los TcES a través de MR, MGL y TLR2 es suficiente para modificar las CDs. El bloqueo de la señalización a través de cRAF fue suficiente para bloquear los efectos de los TcES. La posibilidad de que cRAF influya en la interiorización de los TcES debe ser considerada en futuros experimentos enfocados a explicar cómo los TcES afectan la funcionalidad de las CDs.

Las CDs expuestas a moléculas derivadas de parásitos helmintos muestran poca respuesta a diversos agonistas de TLRs(Terrazas et al., 2010). Se ha propuesto que este fenómeno puede ser producto de una competencia o bloqueo entre vías de señalización inducidas por los TLRs y aquellas inducidas por los helmintos, hasta ahora no hay reportes que puedan sustentar esta hipótesis, y los mecanismos de regulación son totalmente desconocidos (Jankovic et al., 2006; MacDonald and Maizels, 2008). En nuestro estudio investigamos si las vías intracelulares de los TLRs eran afectadas por la presencia de los TcES. Encontramos que la fosforilación de NFkBp65 y p38 fueron menores en las CDs expuestas a los TcES e inmediatamente tratadas con LPS, en comparación a las CDs tratadas sólo con LPS, indicando una interrupción de la señalización río arriba de estas moléculas. La consecuencia de la reducción de la activación de esta vía puede ser la disminución de la translocación al núcleo del NFkB y la reducción en los niveles de producción de citocinas pro-inflamatorias.

Debido a que los TcES inducen la activación de cRAF en las CDs, y que cRAF es importante en la regulación de la respuesta pro-inflamatoria inducida por el LPS,sugiere la existencia de una interacción entre las vías intracelulares inducidas por los TLRs y las vías intracelulares de los receptores que reconocen los TcES. Donde cRAF es la vía de señalización que puede bloquear o competir con la vía inducida por el TLR4.Debido a la dicotomía de la función de cRAF en las CDs, existe la posibilidad que los receptores mediante los cuales son reconocidos los TcES confiera uncambio en cRAF capaz de disminuir la activación de NFkBp65 y p38 inducida por el LPS. La asociación directa de cRAF con NFkBp65 no ha sido reportada en la literatura. Sin embargo, cRAF se une e induce la fosforilación de IkB en células tumorales, evento

necesario para la activación de NFkB (Li and Sedivy, 1993). Este estudio sugiere, que la interacción de cRAF e IkB puede suceder en las CDs. En nuestro estudio, es probable que cRAF se una a IkB, inhibiendo su fosforilación, de esta manera bloqueando la liberación de NFkBp65 y manteniéndolo secuestrado en el citoplasma. Otra posibilidad es que cRAF pueda inducir la translocación al núcleo del heterodímero RelB/p52, evento conocido como la activación alternativa de NFkB. Acorde con esta hipótesis, el carbohidrato LNFPIII derivado de *S. mansoni*, activa la vía alternativa del NFkB en las CDs (Thomas et al., 2005). Se ha postulado que la vía clásica y la alternativa de NFkBdifieren en la activación de genes. Sin embargo, recientemente se ha demostrado que ambas combinaciones p65/p50 o Relb/p52 comparten sitios de unión al DNA. En el hallazgo de Thomas et al(Thomas et al., 2005), las CDs expuestas a LNFPIII activaron la vía alternativa de NFkB, sin embargo, no produjeron TNFα, o IL-12, citocinas controladas por la vía clásica del NFkB, sugiriendo que la vía clásica y alternativa difieren en la activación de genes en las CDs. La diferencia puede radicar en el remodelamiento de la cromatina causada por la vía alternativa, ya que se ha reportado que se asocia con el complejo de remodelación de la cromatina SWI/SNF(Sun, 2011). El reconocimiento de Salp15a través de DCSING y dependiente de la vía de señalización a través de cRAF lleva a la disminución en la remodelación de la cromatina en la región del promotor de la IL-12p35. En este caso, las CDs estimuladas con Salp15, no secretan IL-12 en respuesta al LPS(Hovius et al., 2008), el mecanismo por el cual se lleva a cabo la disminución en la remodelación de la cromatina inducido por Salp15-DCSIGN-cRAF no es conocido, pero es independiente de la activación de ERK1/2, molécula situada rio abajo de cRAF. Probablemente, la activación alternativa del NFkB pueda ser la causa de dicha remodelación. La evaluación de la activación de la vía alternativa de NFkB será necesaria para elucidar su participación en la vía de señalización de los TcES. Por otra parte, la fosforilación de p38 inducida por el LPS también resultó menor en las CDs expuestas a los TcES. La fosforilación de p38 es asociada con una eficiente producción de IL-12 por las CDs (Lu et al., 1999; Terrazas et al., 2011). Además, la activación de p38 en las células dendríticas es necesario para el desarrollo de la esclerosis múltiple experimental, asociada con la polarización de la respuesta inmune a un tipo Th1/Th17 (Huang et al., 2012). La regulación de p38 por los TcES, puede ser un mecanismo por el cual las CDs producen menores cantidades de IL-12 en respuesta al LPS. Esta regulación de los TcES sobre las CDs puede ser un mecanismo por el cual los ratones infectados con *T. crassiceps* desarrollan menor

patología en el transcurso de la encefalomielitis autoinmune experimental (EAE, un modelo de esclerosis múltiple) (Reyes et al., 2011). La transferencia de CDs expuestas a los TcES y pulsadas con mielina debe ser considerada en futuros experimentos destinados descifrar los mecanismos de regulación en el desarrollo de la EAE en ratones infectados con *Taenia crassiceps*. En conjunto, la supresión de la activación de NFkBp65 y p38, dos vías involucradas en procesos inflamatorios y autoinmunes, demuestran el potencial inmunomudulador de los TcES, y explican la modulación en la producción de citocinas pro-inflamatorias en respuesta al LPS y probablemente de otros ligandos de TLRs. El hecho de que la regulación sucede de manera temprana, sugiere que la disminución de la fosforilación de p38 y NFkBp65 es un efecto directo de la señalización de los TcES y no un efecto autócrino, aunado a esto, nuestros experimentos donde se bloqueó la IL-10 en los cultivos de CDs no afectó la disminución en la producción de citocinas pro-inflamatorias.

El papel de cRAF en la polarización a Th2 puede explicar la regulación mutua entre respuestas Th1 y Th2 iniciadas a través de las CDs. En nuestros experimentos, la fosforilación de cRAF aumentó cuando las CDs fueron estimuladas con los TcES. Dicha fosforilación fue atenuada cuando las CDs recibieron un estímulo a través de TLR4, resultando en un fenotipo de CDs con potencial de inducir una respuesta atenuada y mixta de IFN-γ e IL-4. Esta idea es soportada por el hecho de que la estimulación de las CDs a través de los TLRs puede reducir su capacidad de inducir una respuesta Th2, dependiente de MyD88 (Sun et al., 2005). Indicando que la regulación de las señales intracelulares iniciadas por receptores que inducen la activación de las CDs y los receptores que inducen un fenotipo tolerogénico o Th2, pueden regularse mutuamente probablemente río abajo de MyD88. La expresión diferencial de moléculas en los patógenos, y el arreglo de receptores en las células del hospedero, pueden dictar la respuesta de las CDs a diferentes patógenos, donde una compleja red de señales intracelulares determinarán la respuesta efectora. Al parecer, cuando las células dendríticas se encuentran ante diferentes estímulos, no es la suma de los estímulos lo que decide el fenotipo si no la manera en que sus vías de señalización interaccionan entre sí. El estudio de los procesos río abajo de cRAF serán de vital importancia para descifrar esta nueva vía de señalización y cómo impacta en el condicionamiento de las CDs a inducir respuestas Th2.

A pesar de que *in vitro*las CDs pueden inducir una respuesta Th2, el microambiente en el que se genera esta repuesta *in vivo*es más complejo. Además de la interacción directa de las CDs con el parásito, el microambiente generado por la infección por helmintos, puede sinergizar o modificar la respuesta iniciada por las CDs.Por ejemplo, se ha demostrado, que el daño tisular que generan estos macro parásitos, puede liberar señales de peligro, las cuales condicionan a las CDs a inducir respuestas Th2. El factor trefoil2 (TFF2), es liberado por células epiteliales del intestino al sufrir daño provocado por parásitos intestinales, las CDs y macrófagos que reconocen TFF2, liberan IL-33 la cual puede iniciar el desarrollo de una respuesta Th2, principalmente la producción de IL-13(Wills-Karp et al., 2012). Nuestros experimentos mostraron que los factores solubles liberados por las CDs, fueron importantes para la producción de IL-4 e IL-13 por las CDs. Además, los sobrenadantes de las CDs expuestas a los TcES indujeron la producción de IL-4 e IL-13 por células T activadas por CDs sin estimular. Uno de los factores solubles que puede estar implicado es la IL-33, hasta ahora se desconoce si las moléculas de algún helminto son capaces de inducir la producción de IL-33 por las CDs o macrófagos. Otro factor soluble liberado por las células epiteliales del intestino es el TSLP (Linfopoyetina tímica estromal), la cual es necesaria en la inmunidad contra el parásito *Trichuris* en el intestino. Los ratones deficientes en TSLP, desarrollan altas cargas parasitarias y los niveles de IL-12 e IFNy se encuentran elevados, mientras que la respuesta Th2 está disminuida (Taylor et al., 2009). En contraste, la respuesta Th2 no es afectada en ratones TSLP -/- infectados con *Nippostrongylus brasiliensis* o *H. polygyrus*. Adicionalmente, las CDs TSLP-/- expuestas a moléculas derivadas de ambos parásitos son eficientes inductoras de respuestas Th2 (Massacand et al., 2009). Estos datos sugieren que TSLP podría funcionar regulando de manera negativa la respuesta Th1, pero tiene un papel limitado en inducir una respuesta Th2.

Otros mecanismos del inicio de la respuesta Th2 han sido identificados, por los basófilos, los cuales pueden producir IL-4 de manera temprana, y funcionar como célula presentadora de antígeno con capacidad de activar linfocitos T vírgenes en respuesta a la papaína o al helminto *T. muris*(Perrigoue et al., 2009). En el mismo estudio Perrigoue et al, muestran que la eliminación de las CDs no afectó la respuesta Th2. Estos estudios han sido controversiales. Donde la estrategia para identificar el papel de las CDs puede llevar a conclusiones erróneas. Por ejemplo el uso de ratones que expresan el receptor de la toxina diftérica en el promotor CD11c+, necesitan la administración constante de la

toxina diftérica (DT), la administración frecuente de DT puede llevar a la muerte del ratón. Debido a esto, se usa la administración de DT en periodos cortos, en este escenario es muy probable que las CDs pueden repoblar los tejidos, y que la eliminación de las CDs no sea total después del tratamiento con DT. Con el objetivo de conocer si los TcES pueden inducir respuestas Th2 in vivo a través de las CDs, y si alguna subclase de CDs se especializa en promover una respuesta Th2, usamos la molécula OVA acoplada a anticuerpos dirigidos contra receptores que se expresan preferencialmente en CDs. Este modelo ha sido utilizado ampliamente en el estudio de la presentación de antígeno a través de las CDs. (Bonifaz et al., 2002; Bonifaz et al., 2004; Idoyaga et al., 2011). Los receptores fagocíticos 33D1 expresado en CDs mieloides (CD11c+CD8a-33D1+) y DEC205 expresado en CDs linfoides (CD11c+CD8a+DEC205+) en el bazo, son eficientes en la presentación de antígeno de la molécula fagocitada. La administración de los TcES en combinación con DCIR vía intraperitoneal indujo la secreción de IL-4 en los ratones recipientes en niveles similares a la administración de OVA sin conjugar y los TcEs, aun cuando la concentración de OVA unida al anticuerpo 33D1 fue cien veces menor a la de OVA sin conjugar. En contraste, las células DEC205+ no indujeron la secreción de IL-4. Esto concuerda con estudios previos, donde se asocia la producción de IFN-γ a la presentación de antígeno por CDs DEC205+, mientras que las CDs 33D1+ favorecen la producción de IL-4 (Soares et al., 2007). En contraste con la inyección intraperitoneal, la administración en el cojinete plantar de los TcES en combinación con los anticuerpos, llevó a la producción de cantidades similares de IL-4 cuando se administró la OVA acoplada a anti-33D1+ o anti-DEC205+. La diferencia de la respuesta encontrada entre la inyección en la cavidad peritoneal y el cojinete plantar, puede radicar en que las células dendríticas en la piel como las CDs dérmicas y las células de Langerhans también expresan el receptor DEC205, y ambas son capaces de migrar a los ganglios linfáticos y presentar antígeno en forma eficiente (Flacher et al., 2012). Debido a ello, es probable que alguna de estas dos subclases de CDs pueda tener relevancia en la inducción de la respuesta Th2 después de la inmunización subcutánea. En apoyo de esta hipótesis, se ha reportado que ratones depletados de CDs dérmicas langerina+ y células de Largerhans tienen una deficiencia en la producción de IgG1(Flacher et al., 2012), en otro estudio las células de Langerhans tienen mayor participación en inducir una respuesta Th2 (Nagao et al., 2009) sugiriendo que los TcES pueden inducir una respuesta Th2 a través de las células de Langerhans. Por otro lado, no descartamos

que los TcES puedan estimular o atraer otras células y que estas, mediante la producción de citocinas establezcan el microambiente adecuado para polarizar la respuesta a un tipo Th2. El hallazgo de que la respuesta Th2 fue dependiente del sitio y la subclase de célula dendrítica que presentó el antígeno puede impactar en el diseño de estrategias de inmunización, especialmente, cuando se pretende polarizar a una respuesta Th2, por ejemplo en contra de helmintos intestinales. Así mismo, la respuesta Th2 inducida a través de DEC205 vía cutánea, puede tener efectos desfavorables en el intento de inducir potentes respuestas Th1 y citotóxicas a través de este receptor.

La disminución de citocinas pro-inflamatorias como TNFα e IL-12 en las CDs tratadas con los TcES, nos llevó a investigar si los TcES podrían tener un efecto modulador en una patología inflamatoria como lo es la colitis (Perse and Cerar, 2012). En nuestro modelo de colitis aguda inducida con DSS, la respuesta inmune innata es la principal efectora del daño provocado al tejido, principalmente mediante la infiltración de monocitos y neutrófilos al colon (Hudcovic et al., 2001). En este modelo, la microflora intestinal es indispensable para el desarrollo de la colitis, ya que ratones libres de gérmenes no desarrollan signos de colitis (Hudcovic et al., 2001). Indicando que la activación de las vías de señalización dependientes de los TLRs pueden ser un factor temprano en la activación de las células innatas presentes en el colón para generar inflamación (Hoshi et al., 2012). El hecho de que el tratamiento con los TcES disminuyó los signos clínicos y la pérdida de peso de los ratones tratados con DSS, demuestra que los TcES tienen un impacto en la inflamación in vivo. La inyección de 50 microgramos de TcES diariamente tuvo resultados favorables en los signos y en el daño del tejido, probablemente una dosis mayor de TcES pudiera reducir la colitis en mayores niveles. Los mecanismos por los cuales los TcES redujeron la colitis necesitan mayor investigación, sin embargo, la reducción de iNOS, y la mayor expresión de arginasa sugieren que los TcES podrían condicionar la activación de las CDs o macrófagos en el colon en respuesta a la microflora intestinal, disminuyendo la producción de citocinas y/o quimiocinas inflamatorias. La producción de IL-10 en el colon es indispensable para mantener el tejido, ya que ratones deficientes en IL-10 desarrollan colitis de manera espontánea, o desarrollan signos más severos que cuando se induce colitis con fármacos (Hoshi et al., 2012). La administración de los TcES indujo mayor producción de IL-10, es probable que este sea uno de los mecanismos protectores contra la colitis, sin embargo, hasta el momento desconocemos que células afecta el TcES para inducir

la producción de la IL-10 in vivo. Adicionalmente, la inyección de los TcES en el peritoneo induce la acumulación de células mieloides supresoras. Es probable que estas células puedan tener un efecto en la modulación de la colitis, produciendo citocinas como IL-10 o migrando al colon y modulando la inflamación en el tejido. La administración del glicano LNFIII (derivado *de S. mansoni*) en la cavidad peritoneal genera la acumulación de macrófagos alternativamente activados (Atochina et al., 2008). Es probable que la constante inyección de los TcES induzca la acumulación de estos macrófagos alternativos con alto potencial inmunomodulador. En conjunto, los TcES podrían modular diferentes poblaciones celulares reduciendo la inflamación característica de enfermedades autoinmunes, como infiltración de neutrófilos y la activación de la respuesta Th1/Th17.

Finalmente, la elucidación de las moléculas en los TcES con actividad inmunomoduladora fueron evaluados. En un primer intento, los TcES fueron sometidos a espectrofotometría de masas, los resultados fueron analizados en el software Mascot, las proteínas identificadas se muestran en la Tabla 1. Los resultados muestran once moléculas, dentro de ellas la Na+ K+ ATPasa, la cual es una enzima que en el cisticerco de *T. solium* y *T. crassiceps*se localiza en las células de músculo y en el tegumento de la pared del parásito. De manera interesante también fue localizado en los ductos protronefridiales en el sistema excretor de *T. solium* y *T. crassiceps*(Willms et al., 2004). En *Schistosoma mansoni* cuyas moléculas también han sido asociadas a la modulación de las células dendríticas, la Na+ K+ ATPasa fue localizada en el musculo y también en los conductos excretores y se demostró que esta molécula podría tener un papel protegiendo al parásito del ataque del complemento (Skelly et al., 2001).

Fosfoenolpiruvato carboxi-cinasa está involucrada en glucogénesis y también tiene como substrato cortisona y se ha reportado que Fosfoenolpiruvato carboxi-cinasa de *Mycobacterium tuberculosis* aumenta la producción de citocinas como IL-12 e IFNy (Liu et al., 2006). Otra molécula identificada fue enolasa, la cual se ha encontrado en exosomas de parásitos protozoarios como *Leishmania* y *Trypanosom*a, en tegumento de *E. granulosus* y en excreciones/secreciones de *Fasciola hepatica y S. mansoni,* el cual sirve como receptor de plasminógeno, y podría funcionar en el proceso invasivo de algunos parásitos (Gan et al., 2010; Liu et al., 2009).Otra molécula identificada fue la 2-cys peroxiredoxin, la cual funciona eliminando radicales libres como H_2O_2, se ha

descrito en parásitos protozoarios y en las moléculas excretadas/secretadas de *Echinococcus granulosusFasciola hepatica* y *S. mansoni*(Virginio et al., 2012). Su papel en la evasión del sistema inmune se ha propuesto ser a través de la eliminación de radicales libres producidos en la explosión respiratoria. Sin embargo, recientemente se demostró que la peroxiredoxin activa de manera alternativa a los macrófagos, independiente de su función reductora, así los macrófagos expuestos a 2-cys peroxiredoxin de *Schistosoma mansoni* son capaces de polarizar la respuesta a un perfil Th2 (Donnelly et al., 2008). La ciclofilina es una molécula detectada en el citosol y también secretada, tiene como su principal característica unirse a la ciclosporina. Los virus como VIH, hongos y bacterias utilizan la ciclofilina para unirse a las células como macrófagos o células dendríticas. Se ha reportado que la ciclofilina recombinante humana es quimioatrayente para monocitos, eosinófilos y en altas concentraciones para neutrófilos. En monocitos estimula la secreción de IL-6. La ciclofilina también se ha reportado presente en las excreciones/secreciones de *Echinococcus granulosus*(Virginio et al., 2012)la cual tiene homología con el alérgeno Malf6 y con la ciclofilina humana. Sin embargo, los anticuerpos IgE que reconocen la ciclofilina de *E. granulosus* no reconocen la ciclofilina humana o el alérgeno Malf6, y se relacionó con síntomas alérgicos en pacientes infectados con *E. granulosus*(Ortona et al., 2002).

Algunas de estas moléculas no tienen secuencia de secreción, pero es poco probable que estas moléculas fueran liberadas por disrupción de los parásitos, debido a que otras proteínas citoplasmáticas abundantes, por ej. proteínas de choque térmico estuvieron ausentes de los TcES. Una explicación posible es que los helmintos tengan rutas alternativas de secreción, como la liberación del tegumento, característica de *E. granulosus*(Virginio et al., 2012). Otra posibilidad es la liberación de exosomas que contengan esta compleja mezcla de moléculas. Hasta el momento se ha demostrado que *Echinostoma caproni* y *Fasciola hepatica*liberan exosomas con contenido proteico del parásito donde se encuentran proteínas identificadas previamente en el contenido de excreción/secreción de estos parásitos; la existencia de estas microvesículas explica la secreción de proteínas atípicas (Marcilla et al., 2012). En conjunto, las proteínas identificadas en nuestros experimentos coinciden con las proteínas de otros helmintos como *S. mansoni, F. hepática,* y*E. granulosus*, las cuales son capaces de alterar la repuesta de CDs y macrófagos, y algunas de ellas inducir respuestas Th2. Debido a la conservación de estas moléculas de secreción entre diferentes helmintos, es posible

que alguna de las moléculas identificadas pueda ser un patrón molecular asociado a patógenos (PAMPs) de los helmintos.

En un análisis más detallado, usando las fracciones obtenidas mediante cromatografía de intercambio iónico, se encontró la molécula factor de elongación alfa y enolasa. El factor de elongación alfa es una molécula que se encarga de la exportación de moléculas del núcleo y el transporte de tRNA al ribosoma (Sasikumar et al., 2012). Esta molécula se ha reportado en productos de excreción/secreción de *E. granulosus*, y es inmunoreactiva con anticuerpos IgE, además de inducir una respuesta Th2 en PBMCs de pacientes infectados (Ortona et al., 2001), también se encuentra en exosomas de *E. caproni*(Marcilla et al., 2012), está expuesta en el tegumento de *Schistosoma japonicium*(Mulvenna et al., 2010), en secreciones de *Trypanosoma brucei*, y en exososomas de *Trypanosoma cruzi*(Bayer-Santos et al., 2013). De manera interesante, el factor de elongación alfa de *L. donovani*, es exportado del fagosoma en macrófagos, y es capaz de unirse y activar la fosfatasa SHP-1. El factor de elongación alfa de *L. donovani* pero no el factor de elongación del hospedero, es capaz de inhibir la producción de óxido nítrico inducida por IFN-γ en los macrófagos (Nandan et al., 2002; Silverman and Reiner, 2011). Es posible que la secreción del factor de elongación alfa, sea un mecanismo común entre parásitos para la modulación de la respuesta inmune del hospedero, a través de la modulación de las CDs y macrófagos (Sasikumar et al., 2012). Debido a que las moléculas identificadas en los TcES pueden estar contenidas en exosomas, es necesario investigar si los receptores involucrados en el reconocimiento de los TcES tienen un papel en el reconocimiento e interiorización de los exosomas, o si las moléculas solubles en los TcES son capaces de unirse a estos receptores. Por otro lado, es necesario purificar el factor de elongación alfa e investigar su función moduladora de las CDs.

La regulación de la respuesta innata en función de los estímulos provenientes de patógenos o del huésped, resulta en una red de eventos intracelulares que dictan la respuesta final de las CDs. Esta tesis, ejemplifica la compleja regulación de las CDs y la importancia de diferentes receptores para responder a un patógeno y cómo afecta su respuesta a un estímulo subsecuente. Mientras que la respuesta inmune adaptativa amplía su repertorio de reconocimiento de patógenos a través de la recombinación, la

respuesta innata lo hace en función de la combinación de sus receptores y la interconexión de su señalización intracelular.

Conclusiones

- Los TcES inducen un fenotiposemi-maduro en las CDs capaz de inducir respuestas Th2*in vivo*e *in vitro*.
- Las CDs expuestas a los TcES son tolerogénicas a estímulos TLR dependientes.
- Los carbohidratos presentes en los TcES, son responsables de la modulación de las CDs.
- Los TcES son reconocidos por las CDs al menos a través de MR, MGL y TLR2.
- Los TcES inducen la activación de cRAF a través de MR, MGL y TLR2.
- Los TcES regulan de manera negativa la fosforilación de p38 y NFkBp65.
- La vía de señalización dependiente de cRAF modula de manera negativa la vía de señalización de los TLRs, la maduración de las CDs y la producción de IL-12 y TNFα.
- Los TcES condicionan a las CDs a inducir respuestas Th2 de manera cRAF dependiente.

Bibliografía

Akira, S., and K. Takeda. 2004. Toll-like receptor signalling. *Nature reviews. Immunology* 4:499-511.

Alonso-Trujillo, J., I. Rivera-Montoya, M. Rodriguez-Sosa, and L.I. Terrazas. 2007. Nitric oxide contributes to host resistance against experimental Taenia crassiceps cysticercosis. *Parasitology research* 100:1341-1350.

Amit, P., K.N. Prasad, G.R. Kumar, T. Shweta, J. Sanjeev, P.V. Kumar, and T. Mukesh. 2011. Immune response to different fractions of Taenia solium cyst fluid antigens in patients with neurocysticercosis. *Experimental parasitology* 127:687-692.

Anthony, R.M., L.I. Rutitzky, J.F. Urban, Jr., M.J. Stadecker, and W.C. Gause. 2007. Protective immune mechanisms in helminth infection. *Nature reviews. Immunology* 7:975-987.

Atochina, O., A.A. Da'dara, M. Walker, and D.A. Harn. 2008. The immunomodulatory glycan LNFPIII initiates alternative activation of murine macrophages in vivo. *Immunology* 125:111-121.

Baig, S., R.T. Damian, J. Morales-Montor, P. Olecki, J. Talhouk, R. Hashmey, and A.C. White, Jr. 2005. Characterization of excretory/secretory endopeptidase and metallo-aminopeptidases from Taenia crassiceps metacestodes. *The Journal of parasitology* 91:983-987.

Balic, A., Y. Harcus, M.J. Holland, and R.M. Maizels. 2004. Selective maturation of dendritic cells by Nippostrongylus brasiliensis-secreted proteins drives Th2 immune responses. *European journal of immunology* 34:3047-3059.

Balic, A., Y.M. Harcus, M.D. Taylor, F. Brombacher, and R.M. Maizels. 2006. IL-4R signaling is required to induce IL-10 for the establishment of T(h)2 dominance. *International immunology* 18:1421-1431.

Banchereau, J., F. Briere, C. Caux, J. Davoust, S. Lebecque, Y.J. Liu, B. Pulendran, and K. Palucka. 2000. Immunobiology of dendritic cells. *Annual review of immunology* 18:767-811.

Bayer-Santos, E., C. Aguilar-Bonavides, S.P. Rodrigues, E.M. Cordero, A.F. Marques, A. Varela-Ramirez, H. Choi, N. Yoshida, J.F. da Silveira, and I.C. Almeida. 2013. Proteomic Analysis of Trypanosoma cruzi Secretome: Characterization of Two Populations of Extracellular Vesicles and Soluble Proteins. *J Proteome Res* 12:883-897.

Berndt, B.E., M. Zhang, G.H. Chen, G.B. Huffnagle, and J.Y. Kao. 2007. The role of dendritic cells in the development of acute dextran sulfate sodium colitis. *Journal of immunology* 179:6255-6262.

Blazquez, A.B., and M.C. Berin. 2008. Gastrointestinal dendritic cells promote Th2 skewing via OX40L. *Journal of immunology* 180:4441-4450.

Bonifaz, L., D. Bonnyay, K. Mahnke, M. Rivera, M.C. Nussenzweig, and R.M. Steinman. 2002. Efficient targeting of protein antigen to the dendritic cell receptor DEC-205 in the steady state leads to antigen presentation on major histocompatibility complex class I products and peripheral CD8+ T cell tolerance. *The Journal of experimental medicine* 196:1627-1638.

Bonifaz, L.C., D.P. Bonnyay, A. Charalambous, D.I. Darguste, S. Fujii, H. Soares, M.K. Brimnes, B. Moltedo, T.M. Moran, and R.M. Steinman. 2004. In vivo targeting of

antigens to maturing dendritic cells via the DEC-205 receptor improves T cell vaccination. *The Journal of experimental medicine* 199:815-824.

Cervi, L., A.S. MacDonald, C. Kane, F. Dzierszinski, and E.J. Pearce. 2004. Cutting edge: dendritic cells copulsed with microbial and helminth antigens undergo modified maturation, segregate the antigens to distinct intracellular compartments, and concurrently induce microbe-specific Th1 and helminth-specific Th2 responses. *Journal of immunology* 172:2016-2020.

Chen, F., Z. Liu, W. Wu, C. Rozo, S. Bowdridge, A. Millman, N. Van Rooijen, J.F. Urban, Jr., T.A. Wynn, and W.C. Gause. 2012. An essential role for TH2-type responses in limiting acute tissue damage during experimental helminth infection. *Nature medicine* 18:260-266.

Chermette, R., J. Bussieras, J. Marionneau, E. Boyer, C. Roubin, B. Prophette, H. Maillard, and B. Fabiani. 1995. [Invasive cysticercosis due to Taenia crassiceps in an AIDS patient]. *Bulletin de l'Academie nationale de medecine* 179:777-780; discussion 780-773.

den Dunnen, J., S.I. Gringhuis, and T.B. Geijtenbeek. 2010. Dusting the sugar fingerprint: C-type lectin signaling in adaptive immunity. *Immunology letters* 128:12-16.

Dillon, S., A. Agrawal, T. Van Dyke, G. Landreth, L. McCauley, A. Koh, C. Maliszewski, S. Akira, and B. Pulendran. 2004. A Toll-like receptor 2 ligand stimulates Th2 responses in vivo, via induction of extracellular signal-regulated kinase mitogen-activated protein kinase and c-Fos in dendritic cells. *Journal of immunology* 172:4733-4743.

Donnelly, S., C.M. Stack, S.M. O'Neill, A.A. Sayed, D.L. Williams, and J.P. Dalton. 2008. Helminth 2-Cys peroxiredoxin drives Th2 responses through a mechanism involving alternatively activated macrophages. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 22:4022-4032.

Dudziak, D., A.O. Kamphorst, G.F. Heidkamp, V.R. Buchholz, C. Trumpfheller, S. Yamazaki, C. Cheong, K. Liu, H.W. Lee, C.G. Park, R.M. Steinman, and M.C. Nussenzweig. 2007. Differential antigen processing by dendritic cell subsets in vivo. *Science* 315:107-111.

Escobedo, G., C. Larralde, A. Chavarria, M.A. Cerbon, and J. Morales-Montor. 2004. Molecular mechanisms involved in the differential effects of sex steroids on the reproduction and infectivity of Taenia crassiceps. *The Journal of parasitology* 90:1235-1244.

Espinoza-Jimenez, A., I. Rivera-Montoya, R. Cardenas-Arreola, L. Moran, and L.I. Terrazas. 2010. Taenia crassiceps infection attenuates multiple low-dose streptozotocin-induced diabetes. *Journal of biomedicine & biotechnology* 2010:850541.

Everts, B., L. Hussaarts, N.N. Driessen, M.H. Meevissen, G. Schramm, A.J. van der Ham, B. van der Hoeven, T. Scholzen, S. Burgdorf, M. Mohrs, E.J. Pearce, C.H. Hokke, H. Haas, H.H. Smits, and M. Yazdanbakhsh. 2012. Schistosome-derived omega-1 drives Th2 polarization by suppressing protein synthesis following internalization by the mannose receptor. *The Journal of experimental medicine* 209:1753-1767, S1751.

Faz-Lopez, B., Y. Ledesma-Soto, Y. Romero-Sanchez, E. Calleja, P. Martinez-Labat, and L.I. Terrazas. 2013. Signal transducer and activator of transcription factor 6 signaling contributes to control host lung pathology but favors susceptibility against Toxocara canis infection. *BioMed research international* 2013:696343.

Flacher, V., C.H. Tripp, B. Haid, A. Kissenpfennig, B. Malissen, P. Stoitzner, J. Idoyaga, and N. Romani. 2012. Skin langerin+ dendritic cells transport intradermally injected anti-DEC-205 antibodies but are not essential for subsequent cytotoxic CD8+ T cell responses. *Journal of immunology* 188:2146-2155.

Flores-Romo, L. 2001. In vivo maturation and migration of dendritic cells. *Immunology* 102:255-262.

Francois, A., L. Favennec, C. Cambon-Michot, I. Gueit, N. Biga, F. Tron, P. Brasseur, and J. Hemet. 1998. Taenia crassiceps invasive cysticercosis: a new human pathogen in acquired immunodeficiency syndrome? *The American journal of surgical pathology* 22:488-492.

Gan, W., G. Zhao, H. Xu, W. Wu, W. Du, J. Huang, X. Yu, and X. Hu. 2010. Reverse vaccinology approach identify an Echinococcus granulosus tegumental membrane protein enolase as vaccine candidate. *Parasitology research* 106:873-882.

Geijtenbeek, T.B., and S.I. Gringhuis. 2009. Signalling through C-type lectin receptors: shaping immune responses. *Nature reviews. Immunology* 9:465-479.

Geijtenbeek, T.B., D.S. Kwon, R. Torensma, S.J. van Vliet, G.C. van Duijnhoven, J. Middel, I.L. Cornelissen, H.S. Nottet, V.N. KewalRamani, D.R. Littman, C.G. Figdor, and Y. van Kooyk. 2000. DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells. *Cell* 100:587-597.

Geijtenbeek, T.B., G.C. van Duijnhoven, S.J. van Vliet, E. Krieger, G. Vriend, C.G. Figdor, and Y. van Kooyk. 2002. Identification of different binding sites in the dendritic cell-specific receptor DC-SIGN for intercellular adhesion molecule 3 and HIV-1. *The Journal of biological chemistry* 277:11314-11320.

Gomez-Garcia, L., L.M. Lopez-Marin, R. Saavedra, J.L. Reyes, M. Rodriguez-Sosa, and L.I. Terrazas. 2005. Intact glycans from cestode antigens are involved in innate activation of myeloid suppressor cells. *Parasite immunology* 27:395-405.

Gomez-Garcia, L., I. Rivera-Montoya, M. Rodriguez-Sosa, and L.I. Terrazas. 2006. Carbohydrate components of Taenia crassiceps metacestodes display Th2-adjuvant and anti-inflammatory properties when co-injected with bystander antigen. *Parasitology research* 99:440-448.

Goodridge, H.S., F.A. Marshall, K.J. Else, K.M. Houston, C. Egan, L. Al-Riyami, F.Y. Liew, W. Harnett, and M.M. Harnett. 2005. Immunomodulation via novel use of TLR4 by the filarial nematode phosphorylcholine-containing secreted product, ES-62. *Journal of immunology* 174:284-293.

Gringhuis, S.I., J. den Dunnen, M. Litjens, M. van der Vlist, and T.B. Geijtenbeek. 2009a. Carbohydrate-specific signaling through the DC-SIGN signalosome tailors immunity to Mycobacterium tuberculosis, HIV-1 and Helicobacter pylori. *Nature immunology* 10:1081-1088.

Gringhuis, S.I., J. den Dunnen, M. Litjens, M. van der Vlist, B. Wevers, S.C. Bruijns, and T.B. Geijtenbeek. 2009b. Dectin-1 directs T helper cell differentiation by controlling noncanonical NF-kappaB activation through Raf-1 and Syk. *Nature immunology* 10:203-213.

Gringhuis, S.I., J. den Dunnen, M. Litjens, B. van Het Hof, Y. van Kooyk, and T.B. Geijtenbeek. 2007. C-type lectin DC-SIGN modulates Toll-like receptor signaling via Raf-1 kinase-dependent acetylation of transcription factor NF-kappaB. *Immunity* 26:605-616.

Hammad, H., M. Plantinga, K. Deswarte, P. Pouliot, M.A. Willart, M. Kool, F. Muskens, and B.N. Lambrecht. 2010. Inflammatory dendritic cells--not basophils--are necessary and sufficient for induction of Th2 immunity to inhaled house dust mite allergen. *The Journal of experimental medicine* 207:2097-2111.

Hemmi, H., and S. Akira. 2005. TLR signalling and the function of dendritic cells. *Chemical immunology and allergy* 86:120-135.

Herbert, D.R., C. Holscher, M. Mohrs, B. Arendse, A. Schwegmann, M. Radwanska, M. Leeto, R. Kirsch, P. Hall, H. Mossmann, B. Claussen, I. Forster, and F. Brombacher. 2004. Alternative macrophage activation is essential for survival during schistosomiasis and downmodulates T helper 1 responses and immunopathology. *Immunity* 20:623-635.

Hoshi, N., D. Schenten, S.A. Nish, Z. Walther, N. Gagliani, R.A. Flavell, B. Reizis, Z. Shen, J.G. Fox, A. Iwasaki, and R. Medzhitov. 2012. MyD88 signalling in colonic mononuclear phagocytes drives colitis in IL-10-deficient mice. *Nature communications* 3:1120.

Hotez, P.J., P.J. Brindley, J.M. Bethony, C.H. King, E.J. Pearce, and J. Jacobson. 2008. Helminth infections: the great neglected tropical diseases. *J Clin Invest* 118:1311-1321.

Hovius, J.W., M.A. de Jong, J. den Dunnen, M. Litjens, E. Fikrig, T. van der Poll, S.I. Gringhuis, and T.B. Geijtenbeek. 2008. Salp15 binding to DC-SIGN inhibits cytokine expression by impairing both nucleosome remodeling and mRNA stabilization. *PLoS pathogens* 4:e31.

Huang, G., Y. Wang, P. Vogel, T.D. Kanneganti, K. Otsu, and H. Chi. 2012. Signaling via the kinase p38alpha programs dendritic cells to drive TH17 differentiation and autoimmune inflammation. *Nature immunology* 13:152-161.

Hudcovic, T., R. Stepankova, J. Cebra, and H. Tlaskalova-Hogenova. 2001. The role of microflora in the development of intestinal inflammation: acute and chronic colitis induced by dextran sulfate in germ-free and conventionally reared immunocompetent and immunodeficient mice. *Folia microbiologica* 46:565-572.

Huerta, L., L.I. Terrazas, E. Sciutto, and C. Larralde. 1992. Immunological mediation of gonadal effects on experimental murine cysticercosis caused by Taenia crassiceps metacestodes. *The Journal of parasitology* 78:471-476.

Hung, L.Y., I.P. Lewkowich, L.A. Dawson, J. Downey, Y. Yang, D.E. Smith, and D.R. Herbert. 2013. IL-33 drives biphasic IL-13 production for noncanonical Type 2 immunity against hookworms. *Proceedings of the National Academy of Sciences of the United States of America* 110:282-287.

Idoyaga, J., A. Lubkin, C. Fiorese, M.H. Lahoud, I. Caminschi, Y. Huang, A. Rodriguez, B.E. Clausen, C.G. Park, C. Trumpfheller, and R.M. Steinman. 2011. Comparable T helper 1 (Th1) and CD8 T-cell immunity by targeting HIV gag p24 to CD8 dendritic cells within antibodies to Langerin, DEC205, and Clec9A. *Proceedings of the National Academy of Sciences of the United States of America* 108:2384-2389.

Ito, A., A. Plancarte, L. Ma, Y. Kong, A. Flisser, S.Y. Cho, Y.H. Liu, S. Kamhawi, M.W. Lightowlers, and P.M. Schantz. 1998. Novel antigens for neurocysticercosis: simple method for preparation and evaluation for serodiagnosis. *The American journal of tropical medicine and hygiene* 59:291-294.

Jankovic, D., S. Steinfelder, M.C. Kullberg, and A. Sher. 2006. Mechanisms underlying helminth- induced Th2 polarization: default, negative or positive pathways? *Chemical immunology and allergy* 90:65-81.

Jenkins, S.J., and A.P. Mountford. 2005. Dendritic cells activated with products released by schistosome larvae drive Th2-type immune responses, which can be inhibited by manipulation of CD40 costimulation. *Infection and immunity* 73:395-402.

Kawai, T., and S. Akira. 2011. Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. *Immunity* 34:637-650.

Kool, M., H. Hammad, and B.N. Lambrecht. 2012. Cellular networks controlling Th2 polarization in allergy and immunity. *F1000 biology reports* 4:6.

Lee, J.J., S. Dissanayake, M. Panico, H.R. Morris, A. Dell, and S.M. Haslam. 2005. Mass spectrometric characterisation of Taenia crassiceps metacestode N-glycans. *Molecular and biochemical parasitology* 143:245-249.

Li, S., and J.M. Sedivy. 1993. Raf-1 protein kinase activates the NF-kappa B transcription factor by dissociating the cytoplasmic NF-kappa B-I kappa B complex. *Proceedings of the National Academy of Sciences of the United States of America* 90:9247-9251.

Liang, G., T. Barker, Z. Xie, N. Charles, J. Rivera, and K.M. Druey. 2012. Naive T cells sense the cysteine protease allergen papain through protease-activated receptor 2 and propel TH2 immunity. *The Journal of allergy and clinical immunology* 129:1377-1386 e1313.

Liu, F., S.J. Cui, W. Hu, Z. Feng, Z.Q. Wang, and Z.G. Han. 2009. Excretory/secretory proteome of the adult developmental stage of human blood fluke, Schistosoma japonicum. *Molecular & cellular proteomics : MCP* 8:1236-1251.

Liu, K., X. Ba, J. Yu, J. Li, Q. Wei, G. Han, G. Li, and Y. Cui. 2006. The phosphoenolpyruvate carboxykinase of Mycobacterium tuberculosis induces strong cell-mediated immune responses in mice. *Molecular and cellular biochemistry* 288:65-71.

Lu, H.T., D.D. Yang, M. Wysk, E. Gatti, I. Mellman, R.J. Davis, and R.A. Flavell. 1999. Defective IL-12 production in mitogen-activated protein (MAP) kinase kinase 3 (Mkk3)-deficient mice. *The EMBO journal* 18:1845-1857.

MacDonald, A.S., and R.M. Maizels. 2008. Alarming dendritic cells for Th2 induction. *The Journal of experimental medicine* 205:13-17.

Maizels, R.M., A. Balic, N. Gomez-Escobar, M. Nair, M.D. Taylor, and J.E. Allen. 2004. Helminth parasites--masters of regulation. *Immunological reviews* 201:89-116.

Marcilla, A., M. Trelis, A. Cortes, J. Sotillo, F. Cantalapiedra, M.T. Minguez, M.L. Valero, M.M. Sanchez del Pino, C. Munoz-Antoli, R. Toledo, and D. Bernal. 2012. Extracellular vesicles from parasitic helminths contain specific excretory/secretory proteins and are internalized in intestinal host cells. *PloS one* 7:e45974.

Massacand, J.C., R.C. Stettler, R. Meier, N.E. Humphreys, R.K. Grencis, B.J. Marsland, and N.L. Harris. 2009. Helminth products bypass the need for TSLP in Th2

immune responses by directly modulating dendritic cell function. *Proceedings of the National Academy of Sciences of the United States of America* 106:13968-13973.

Matzinger, P. 2002. The danger model: a renewed sense of self. *Science* 296:301-305.

Matzinger, P., and T. Kamala. 2011. Tissue-based class control: the other side of tolerance. *Nature reviews. Immunology* 11:221-230.

Medzhitov, R., D.S. Schneider, and M.P. Soares. 2012. Disease tolerance as a defense strategy. *Science* 335:936-941.

Moodycliffe, A.M., V. Shreedhar, S.E. Ullrich, J. Walterscheid, C. Bucana, M.L. Kripke, and L. Flores-Romo. 2000. CD40-CD40 ligand interactions in vivo regulate migration of antigen-bearing dendritic cells from the skin to draining lymph nodes. *The Journal of experimental medicine* 191:2011-2020.

Morales-Montor, J., S. Baig, C. Hallal-Calleros, and R.T. Damian. 2002. Taenia crassiceps: androgen reconstitution of the host leads to protection during cysticercosis. *Experimental parasitology* 100:209-216.

Morales-Montor, J., S. Baig, R. Mitchell, K. Deway, C. Hallal-Calleros, and R.T. Damian. 2001. Immunoendocrine interactions during chronic cysticercosis determine male mouse feminization: role of IL-6. *Journal of immunology* 167:4527-4533.

Morales-Montor, J., and C. Larralde. 2005. The role of sex steroids in the complex physiology of the host-parasite relationship: the case of the larval cestode of Taenia crassiceps. *Parasitology* 131:287-294.

Mulvenna, J., L. Moertel, M.K. Jones, S. Nawaratna, E.M. Lovas, G.N. Gobert, M. Colgrave, A. Jones, A. Loukas, and D.P. McManus. 2010. Exposed proteins of the Schistosoma japonicum tegument. *International journal for parasitology* 40:543-554.

Nagao, K., F. Ginhoux, W.W. Leitner, S. Motegi, C.L. Bennett, B.E. Clausen, M. Merad, and M.C. Udey. 2009. Murine epidermal Langerhans cells and langerin-expressing dermal dendritic cells are unrelated and exhibit distinct functions. *Proceedings of the National Academy of Sciences of the United States of America* 106:3312-3317.

Nandan, D., T. Yi, M. Lopez, C. Lai, and N.E. Reiner. 2002. Leishmania EF-1alpha activates the Src homology 2 domain containing tyrosine phosphatase SHP-1 leading to macrophage deactivation. *The Journal of biological chemistry* 277:50190-50197.

O'Connell, A.E., L.A. Kerepesi, G.L. Vandergrift, D.R. Herbert, V.A.N.W. TJ, D.C. Hooper, E.J. Pearce, and D. Abraham. 2009. IL-4(-/-) mice with lethal Mesocestoides corti infections--reduced Th2 cytokines and alternatively activated macrophages. *Parasite immunology* 31:741-749.

Okano, M., A.R. Satoskar, K. Nishizaki, M. Abe, and D.A. Harn, Jr. 1999. Induction of Th2 responses and IgE is largely due to carbohydrates functioning as adjuvants on Schistosoma mansoni egg antigens. *Journal of immunology* 163:6712-6717.

Okano, M., A.R. Satoskar, K. Nishizaki, and D.A. Harn, Jr. 2001. Lacto-N-fucopentaose III found on Schistosoma mansoni egg antigens functions as adjuvant for proteins by inducing Th2-type response. *Journal of immunology* 167:442-450.

Ortona, E., P. Margutti, S. Vaccari, R. Rigano, E. Profumo, B. Buttari, A. Chersi, A. Teggi, and A. Siracusano. 2001. Elongation factor 1 beta/delta of Echinococcus

granulosus and allergic manifestations in human cystic echinococcosis. *Clinical and experimental immunology* 125:110-116.

Ortona, E., S. Vaccari, P. Margutti, F. Delunardo, R. Rigano, E. Profumo, B. Buttari, O. Rasool, A. Teggi, and A. Siracusano. 2002. Immunological characterization of Echinococcus granulosus cyclophilin, an allergen reactive with IgE and IgG4 from patients with cystic echinococcosis. *Clinical and experimental immunology* 128:124-130.

Palm, N.W., R.K. Rosenstein, and R. Medzhitov. 2012. Allergic host defences. *Nature* 484:465-472.

Pearce, E.J., M.K. C, J. Sun, J.T. J, A.S. McKee, and L. Cervi. 2004. Th2 response polarization during infection with the helminth parasite Schistosoma mansoni. *Immunological reviews* 201:117-126.

Perrigoue, J.G., S.A. Saenz, M.C. Siracusa, E.J. Allenspach, B.C. Taylor, P.R. Giacomin, M.G. Nair, Y. Du, C. Zaph, N. van Rooijen, M.R. Comeau, E.J. Pearce, T.M. Laufer, and D. Artis. 2009. MHC class II-dependent basophil-CD4+ T cell interactions promote T(H)2 cytokine-dependent immunity. *Nature immunology* 10:697-705.

Perse, M., and A. Cerar. 2012. Dextran sodium sulphate colitis mouse model: traps and tricks. *Journal of biomedicine & biotechnology* 2012:718617.

Phythian-Adams, A.T., P.C. Cook, R.J. Lundie, L.H. Jones, K.A. Smith, T.A. Barr, K. Hochweller, S.M. Anderton, G.J. Hammerling, R.M. Maizels, and A.S. MacDonald. 2010. CD11c depletion severely disrupts Th2 induction and development in vivo. *The Journal of experimental medicine* 207:2089-2096.

Poncini, C.V., G. Gimenez, C.A. Pontillo, C.D. Alba-Soto, E.L. de Isola, I. Piazzon, and S.M. Cappa. 2010. Central role of extracellular signal-regulated kinase and Toll-like receptor 4 in IL-10 production in regulatory dendritic cells induced by Trypanosoma cruzi. *Molecular immunology* 47:1981-1988.

Pulendran, B., and D. Artis. 2012. New paradigms in type 2 immunity. *Science* 337:431-435.

Pulendran, B., H. Tang, and T.L. Denning. 2008. Division of labor, plasticity, and crosstalk between dendritic cell subsets. *Current opinion in immunology* 20:61-67.

Reyes, J.L., A.F. Espinoza-Jimenez, M.I. Gonzalez, L. Verdin, and L.I. Terrazas. 2011. Taenia crassiceps infection abrogates experimental autoimmune encephalomyelitis. *Cellular immunology* 267:77-87.

Reyes, J.L., C.A. Terrazas, J. Alonso-Trujillo, N. van Rooijen, A.R. Satoskar, and L.I. Terrazas. 2010. Early removal of alternatively activated macrophages leads to< i> Taenia crassiceps</i> cysticercosis clearance in vivo. *International journal for parasitology* 40:731-742.

Reyes, J.L., and L.I. Terrazas. 2007. The divergent roles of alternatively activated macrophages in helminthic infections. *Parasite immunology* 29:609-619.

Reynolds, L.A., K.J. Filbey, and R.M. Maizels. 2012. Immunity to the model intestinal helminth parasite Heligmosomoides polygyrus. *Seminars in immunopathology* 34:829-846.

Rivas-Caicedo, A., G. Soldevila, T.I. Fortoul, A. Castell-Rodriguez, L. Flores-Romo, and E.A. Garcia-Zepeda. 2009. Jak3 is involved in dendritic cell maturation and CCR7-dependent migration. *PloS one* 4:e7066.

Robinson, M.J., D. Sancho, E.C. Slack, S. LeibundGut-Landmann, and C. Reis e Sousa. 2006. Myeloid C-type lectins in innate immunity. *Nature immunology* 7:1258-1265.

Rodriguez-Sosa, M., J.R. David, R. Bojalil, A.R. Satoskar, and L.I. Terrazas. 2002a. Cutting edge: susceptibility to the larval stage of the helminth parasite Taenia crassiceps is mediated by Th2 response induced via STAT6 signaling. *Journal of immunology* 168:3135-3139.

Rodriguez-Sosa, M., I. Rivera-Montoya, A. Espinoza, M. Romero-Grijalva, R. Lopez-Flores, J. Gonzalez, and L.I. Terrazas. 2006. Acute cysticercosis favours rapid and more severe lesions caused by Leishmania major and Leishmania mexicana infection, a role for alternatively activated macrophages. *Cellular immunology* 242:61-71.

Rodriguez-Sosa, M., R. Saavedra, E.P. Tenorio, L.E. Rosas, A.R. Satoskar, and L.I. Terrazas. 2004. A STAT4-dependent Th1 response is required for resistance to the helminth parasite Taenia crassiceps. *Infection and immunity* 72:4552-4560.

Rodriguez-Sosa, M., A.R. Satoskar, R. Calderon, L. Gomez-Garcia, R. Saavedra, R. Bojalil, and L.I. Terrazas. 2002b. Chronic helminth infection induces alternatively activated macrophages expressing high levels of CCR5 with low interleukin-12 production and Th2-biasing ability. *Infection and immunity* 70:3656-3664.

Rodriguez-Sosa, M., A.R. Satoskar, J.R. David, and L.I. Terrazas. 2003. Altered T helper responses in CD40 and interleukin-12 deficient mice reveal a critical role for Th1 responses in eliminating the helminth parasite Taenia crassiceps. *International journal for parasitology* 33:703-711.

Rodriguez, M., L.I. Terrazas, R. Marquez, and R. Bojalil. 1999. Susceptibility to Trypanosoma cruzi is modified by a previous non-related infection. *Parasite immunology* 21:177-185.

Rook, G.A. 2007. The hygiene hypothesis and the increasing prevalence of chronic inflammatory disorders. *Trans R Soc Trop Med Hyg* 101:1072-1074.

Sasikumar, A.N., W.B. Perez, and T.G. Kinzy. 2012. The many roles of the eukaryotic elongation factor 1 complex. *Wiley interdisciplinary reviews. RNA* 3:543-555.

Sciutto, E., G. Fragoso, M.L. Diaz, F. Valdez, R.M. Montoya, T. Govezensky, C. Lomeli, and C. Larralde. 1991. Murine Taenia crassiceps cysticercosis: H-2 complex and sex influence on susceptibility. *Parasitology research* 77:243-246.

Sciutto, E., G. Fragoso, and C. Larralde. 2011. Taenia crassiceps as a model for Taenia solium and the S3Pvac vaccine. *Parasite immunology* 33:79-80.

Shilling, R.A., B.S. Clay, A.G. Tesciuba, E.L. Berry, T. Lu, T.V. Moore, H.S. Bandukwala, J. Tong, J.V. Weinstock, R.A. Flavell, T. Horan, S.K. Yoshinaga, A.A. Welcher, J.L. Cannon, and A.I. Sperling. 2009. CD28 and ICOS play complementary non-overlapping roles in the development of Th2 immunity in vivo. *Cellular immunology* 259:177-184.

Silverman, J.M., and N.E. Reiner. 2011. Leishmania exosomes deliver preemptive strikes to create an environment permissive for early infection. *Frontiers in cellular and infection microbiology* 1:26.

Skelly, P.J., P.M. Dougan, A. Maule, T.A. Day, and C.B. Shoemaker. 2001. Cloning and characterization of a muscle isoform of a Na,K-ATPase alpha subunit (SNaK1) from Schistosoma mansoni. *Parasitology* 123:277-284.

Smalley, K.S., M. Xiao, J. Villanueva, T.K. Nguyen, K.T. Flaherty, R. Letrero, P. Van Belle, D.E. Elder, Y. Wang, K.L. Nathanson, and M. Herlyn. 2009. CRAF inhibition induces apoptosis in melanoma cells with non-V600E BRAF mutations. *Oncogene* 28:85-94.

Soares, H., H. Waechter, N. Glaichenhaus, E. Mougneau, H. Yagita, O. Mizenina, D. Dudziak, M.C. Nussenzweig, and R.M. Steinman. 2007. A subset of dendritic cells induces CD4+ T cells to produce IFN-gamma by an IL-12-independent but CD70-dependent mechanism in vivo. *The Journal of experimental medicine* 204:1095-1106.

Spolski, R.J., J. Corson, P.G. Thomas, and R.E. Kuhn. 2000. Parasite-secreted products regulate the host response to larval Taenia crassiceps. *Parasite immunology* 22:297-305.

Steinman, R.M., G. Kaplan, M.D. Witmer, and Z.A. Cohn. 1979. Identification of a novel cell type in peripheral lymphoid organs of mice. V. Purification of spleen dendritic cells, new surface markers, and maintenance in vitro. *The Journal of experimental medicine* 149:1-16.

Sun, J., M. Walsh, A.V. Villarino, L. Cervi, C.A. Hunter, Y. Choi, and E.J. Pearce. 2005. TLR ligands can activate dendritic cells to provide a MyD88-dependent negative signal for Th2 cell development. *Journal of immunology* 174:742-751.

Sun, S.C. 2011. Non-canonical NF-kappaB signaling pathway. *Cell research* 21:71-85.

Sutterwala, F.S., Y. Ogura, and R.A. Flavell. 2007. The inflammasome in pathogen recognition and inflammation. *Journal of leukocyte biology* 82:259-264.

Takeda, K., and S. Akira. 2004. TLR signaling pathways. *Seminars in immunology* 16:3-9.

Taylor, B.C., C. Zaph, A.E. Troy, Y. Du, K.J. Guild, M.R. Comeau, and D. Artis. 2009. TSLP regulates intestinal immunity and inflammation in mouse models of helminth infection and colitis. *The Journal of experimental medicine* 206:655-667.

Terrazas, C.A., E.K. Huitron, A. Vazquez, I. Juarez, G.M. Camacho, E.A. Calleja, and M. Rodriguez-Sosa. 2011. MIF Synergizes with Trypanosoma cruzi Antigens to Promote Efficient Dendritic Cell Maturation and IL-12 Production via p38 MAPK. *International journal of biological sciences* 7:1298.

Terrazas, C.A., L.I. Terrazas, and L. Gomez-Garcia. 2010. Modulation of dendritic cell responses by parasites: a common strategy to survive. *Journal of biomedicine & biotechnology* 2010:357106.

Terrazas, L.I. 2008. The complex role of pro- and anti-inflammatory cytokines in cysticercosis: immunological lessons from experimental and natural hosts. *Current topics in medicinal chemistry* 8:383-392.

Terrazas, L.I., R. Bojalil, T. Govezensky, and C. Larralde. 1994. A role for 17-beta-estradiol in immunoendocrine regulation of murine cysticercosis (Taenia crassiceps). *The Journal of parasitology* 80:563-568.

Terrazas, L.I., R. Bojalil, T. Govezensky, and C. Larralde. 1998. Shift from an early protective Th1-type immune response to a late permissive Th2-type response in murine cysticercosis (Taenia crassiceps). *The Journal of parasitology* 84:74-81.

Terrazas, L.I., M. Cruz, M. Rodriguez-Sosa, R. Bojalil, F. Garcia-Tamayo, and C. Larralde. 1999. Th1-type cytokines improve resistance to murine cysticercosis caused by Taenia crassiceps. *Parasitology research* 85:135-141.

Terrazas, L.I., D. Montero, C.A. Terrazas, J.L. Reyes, and M. Rodriguez-Sosa. 2005a. Role of the programmed Death-1 pathway in the suppressive activity of alternatively activated macrophages in experimental cysticercosis. *Int J Parasitol* 35:1349-1358.

Terrazas, L.I., D. Montero, C.A. Terrazas, J.L. Reyes, and M. Rodríguez-Sosa. 2005b. Role of the programmed Death-1 pathway in the suppressive activity of alternatively activated macrophages in experimental cysticercosis. *International journal for parasitology* 35:1349.

Thomas, P.G., M.R. Carter, A.A. Da'dara, T.M. DeSimone, and D.A. Harn. 2005. A helminth glycan induces APC maturation via alternative NF-kappa B activation independent of I kappa B alpha degradation. *Journal of immunology* 175:2082-2090.

Toledo, A., G. Fragoso, G. Rosas, M. Hernandez, G. Gevorkian, F. Lopez-Casillas, B. Hernandez, G. Acero, M. Huerta, C. Larralde, and E. Sciutto. 2001. Two epitopes shared by Taenia crassiceps and Taenia solium confer protection against murine T. crassiceps cysticercosis along with a prominent T1 response. *Infection and immunity* 69:1766-1773.

Toledo, A., C. Larralde, G. Fragoso, G. Gevorkian, K. Manoutcharian, M. Hernandez, G. Acero, G. Rosas, F. Lopez-Casillas, C.K. Garfias, R. Vazquez, I. Terrazas, and E. Sciutto. 1999. Towards a Taenia solium cysticercosis vaccine: an epitope shared by Taenia crassiceps and Taenia solium protects mice against experimental cysticercosis. *Infection and immunity* 67:2522-2530.

van Die, I., S.J. van Vliet, A.K. Nyame, R.D. Cummings, C.M. Bank, B. Appelmelk, T.B. Geijtenbeek, and Y. van Kooyk. 2003. The dendritic cell-specific C-type lectin DC-SIGN is a receptor for Schistosoma mansoni egg antigens and recognizes the glycan antigen Lewis x. *Glycobiology* 13:471-478.

van Vliet, S.J., S. Bay, I.M. Vuist, H. Kalay, J.J. Garcia-Vallejo, C. Leclerc, and Y. van Kooyk. 2013. MGL signaling augments TLR2-mediated responses for enhanced IL-10 and TNF-alpha secretion. *Journal of leukocyte biology*

Villa, O.F., and R.E. Kuhn. 1996. Mice infected with the larvae of Taenia crassiceps exhibit a Th2-like immune response with concomitant anergy and downregulation of Th1-associated phenomena. *Parasitology* 112 (Pt 6):561-570.

Virginio, V.G., K.M. Monteiro, F. Drumond, M.O. de Carvalho, D.M. Vargas, A. Zaha, and H.B. Ferreira. 2012. Excretory/secretory products from in vitro-cultured Echinococcus granulosus protoscoleces. *Molecular and biochemical parasitology* 183:15-22.

Voehringer, D., K. Shinkai, and R.M. Locksley. 2004. Type 2 immunity reflects orchestrated recruitment of cells committed to IL-4 production. *Immunity* 20:267-277.

Whelan, M., M.M. Harnett, K.M. Houston, V. Patel, W. Harnett, and K.P. Rigley. 2000. A filarial nematode-secreted product signals dendritic cells to acquire a phenotype that drives development of Th2 cells. *Journal of immunology* 164:6453-6460.

Willms, K., C.B. Shoemaker, P.J. Skelly, and A. Landa. 2004. Cloning and expression of a Na(+), K(+)-ATPase alpha-subunit from Taenia solium (TNaK1alpha). *Molecular and biochemical parasitology* 138:79-82.

Wills-Karp, M., R. Rani, K. Dienger, I. Lewkowich, J.G. Fox, C. Perkins, L. Lewis, F.D. Finkelman, D.E. Smith, P.J. Bryce, E.A. Kurt-Jones, T.C. Wang, U. Sivaprasad, G.K. Hershey, and D.R. Herbert. 2012. Trefoil factor 2 rapidly induces interleukin 33 to promote type 2 immunity during allergic asthma and hookworm infection. *The Journal of experimental medicine* 209:607-622.

Zepeda, N., S. Solano, N. Copitin, A.M. Fernandez, L. Hernandez, P. Tato, and J.L. Molinari. 2010. Decrease of peritoneal inflammatory CD4(+), CD8(+), CD19(+) lymphocytes and apoptosis of eosinophils in a murine Taenia crassiceps infection. *Parasitology research* 107:1129-1135.

Apéndice I

Artículos Publicados

Reyes JL, Terrazas CA, Vera-Arias L, Terrazas LI. Differential response of antigen presenting cells from susceptible and resistant strains of mice to Taenia crassiceps infection. Infect Genet Evol. 2009 Dec;9(6):1115-27. doi: 10.1016/j.meegid.2009.05.011. Epub 2009 May 22. PubMed PMID: 19465163.

Terrazas CA, Gómez-García L, Terrazas LI. Impaired pro-inflammatory cytokine production and increased Th2-biasing ability of dendritic cells exposed to Taenia excreted/secreted antigens: A critical role for carbohydrates but not for STAT6 signaling. Int J Parasitol. 2010 Aug 1;40(9):1051-62. doi:10.1016/j.ijpara.2010.02.016. Epub 2010 Mar 30. PubMed PMID: 20361966.

Terrazas CA, Sánchez-Muñoz F, Mejía-Domínguez AM, Amezcua-Guerra LM, Terrazas LI, Bojalil R, Gómez-García L. Cestode antigens induce a tolerogenic-like phenotype and inhibit LPS inflammatory responses in human dendritic cells. Int J Biol Sci.2011;7(9):1391-400. Epub 2011 Nov 1. PubMed PMID: 22110390; PubMed Central PMCID: PMC3221946.

César A. Terrazas, Steve Oghumu, Marcela Hernández, Laura Bonifaz, Luis I. Terrazas andAbhay R. Satoskar. Helminth-excreted/secreted products are recognized by multiple receptors on DCs to block the TLRresponse and bias Th2 polarization in a cRAF dependent pathway.FASEB J, En revisión.

Terrazas CA, Terrazas LI, Gómez-García L. Modulation of dendritic cell responses by parasites: a common strategy to survive. J Biomed Biotechnol. 2010;2010:357106. doi: 10.1155/2010/357106. Epub 2010 Feb 24. Review. PubMed PMID: 20204070; PubMed Central PMCID: PMC2829630.

Capítulo de libro:

CA Terrazas, M Rodríguez-Sosa, LI Terrazas. Cestoda: Tapeworm InfectionImmunity to Parasitic Infection, 307-322

Infection, Genetics and Evolution 9 (2009) 1115–1127

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Differential response of antigen presenting cells from susceptible and resistant strains of mice to *Taenia crassiceps* infection

José L. Reyes, César A. Terrazas, Laura Vera-Arias, Luis I. Terrazas *

Unidad de Biomedicina, Facultad de Estudios Superiores-Iztacala, Universidad Nacional Autónoma de México, Mexico

ARTICLE INFO

Article history:
Received 30 January 2009
Received in revised form 30 April 2009
Accepted 3 May 2009
Available online 22 May 2009

Keywords:
Taenia crassiceps
Dendritic cells
Alternatively activated macrophages

ABSTRACT

Antigen presenting cells (APCs) are critically involved in the interaction between pathogens and the host immune system. Here, we examined two different populations of APCs in mice that are susceptible (BALB/c) or resistant (C57BL/6) to *Taenia crassiceps* cysticercosis. Bone marrow-derived dendritic cells (BMDCs) from both strains of mice were exposed to *T. crassiceps* excreted/secreted antigens (TcES) and, at the same time, to the Toll-like receptor (TLR) ligand LPS. BMDCs from BALB/c mice underwent a partial maturation when incubated with TcES and displayed decreased responses to TLR-dependent stimuli associated with low CD80, CD86, CD40 and CCR7 expression and impaired IL-15 production. These BMDCs-induced impaired allogenic responses. In contrast, BMDCs from C57BL/6 mice displayed normal maturation and induced strong allogenic responses. Moreover, the exposure to TcES resulted in a lower production of IL-12 and TNF-α by LPS-activated DCs from BALB/c mice compared to C57BL/6 DCs. Three parameters of macrophage activation were assessed during *Taenia* infection: LPS + IFN-γ-induced production of IL-12, TNF-α and nitric oxide (NO) in vitro; infection-induced markers for alternatively activated macrophages (Arginase-1, RELM-α, Ym-1 and TREM-2 expression) and suppressive activity. The maximum response to LPS + IFN-γ-induced TNF-α, IL-12 and NO production by macrophages from both strains of mice occurred 2 wk post-infection. However, as infection progressed, the production of these molecules by BALB/c macrophages declined. While the BALB/c macrophages displayed impaired pro-inflammatory responses, these macrophages showed strong Arginase-1, Ym-1, RELM-α and TREM-2 expression. By contrast, C57BL/6 macrophages maintained a pro-inflammatory profile and low transcripts for alternative activation markers. Macrophages from *T. crassiceps*-infected BALB/c mice showed stronger suppressive activity than those from C57BL/6 mice. These findings suggest that APC activation at both early and late time points during *T. crassiceps* infection is a possible mechanism that underlies the differential susceptibility to *T. crassiceps* infection displayed by these mouse strains.

1. Introduction

Genetic background influences the outcome of many parasitic diseases. For example, in *Leishmania major* infection, C57BL/6 mice mount a strong Th1 response and resolve the infection (Sacks and Noben-Trauth, 2002). In contrast, BALB/c mice mount a Th2-dominant response and develop chronic lesions (Sacks and Noben-Trauth, 2002). In other protozoan infections, such as toxoplasmosis, an opposite finding it has been observed: C57BL/6 mice succumb to *Toxoplasma gondii* infection despite a Th1 response, whereas BALB/c mice display resistance and survive for a longer period of time to similar challenges (Fux et al., 2003).

In helminthic infections, the genetic background of the host also plays an important role in the outcome of the infection. Intestinal nematodes induce different responses: *Heligmosomoides polygyrus* establishes long-term infections in most strains of mice and induces Th2-polarized responses (Gause et al., 2003). Furthermore, AKR mice clear the parasite faster than BALB/c mice, whereas C57BL/6 mice remain more susceptible to similar infections (Maizels and Yazdanbakhsh, 2003). *Nippostrongylus brasiliensis* infection is relatively short-term (6–10 days) under standard high-dose infections and induces a powerful and protective Th2 response; however, low-dose infections are typically chronic (Finkelman et al., 2004). Susceptibility to *Trichuris muris*, another intestinal nematode, is also linked to the development of a Th1 response; mice that fail to mount a protective Th2 response develop chronic infections (Patel et al., 2009). Furthermore, conflicting data on nematodes that are not strictly gastrointestinal, such as *Litomosoides sigmodontis*, have been reported. C57BL/6 mice are resistant to *L. sigmodontis* in an IL-4-dependent manner,

* Corresponding author at: Unidad de Biomedicina, FES-Iztacala, UNAM, Av. De los Barrios 1, Los Reyes Iztacala, Tlalnepantla, Edo. de Mexico, Mexico 54090, Mexico. Tel.: +52 55 5623 1292x438; fax: +52 55 5623 1138.
E-mail address: literrazas@campus.iztacala.unam.mx (L.I. Terrazas).

1567-1348/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.meegid.2009.05.011

while BALB/c mice are susceptible to the same infection, despite their high levels of IL-4 (Le Goff et al., 2002). Moreover, BALB/c IL-$4^{-/-}$ mice develop similar parasite loads to WT mice. Surprisingly, infection of IL-4 receptor (IL-4Rα)-deficient BALB/c mice, which cannot respond to either IL-4 or IL-13, led to the accelerated death of the adult stage of the parasite, rather than enhanced parasite survival (Volkmann et al., 2003; Maizels et al., 2004). This was associated with a switch to a type 1 response, suggesting that the adult parasite can be killed by Th1 inflammatory responses (Saeftel et al., 2003). Thus, type 2 responses are a critical determinant of the outcome of nematode infection but with very different dynamics depending on the stage of the parasite and strain of the host. In contrast to the well-described adaptive immunity against these helminth infections, the role of antigen presenting cells (APCs) is still unclear. There have only been limited studies on the macrophage and DC response to helminth antigens and the impact of these responses on the outcome of the infection is not known. Neurocysticercosis (NCC) is the most frequent parasitic disease affecting the central nervous system. It is a life-threatening helminth infection caused by the ingestion of eggs or oncospheres of the cestode *Taenia solium*, which after being activated in the intestines, rapidly migrate to the blood and frequently reside in the brain. The infection of the intermediate hosts by the metacestode stage of cestode species, especially *Taenia crassiceps* and *Mesocestoides corti*, appear to be very good models to unveil some of the mechanisms of the host–parasite interplay in cysticercosis and neurocysticercosis (Terrazas, 2008). Cysticercosis, which is caused by *T. crassiceps*, naturally infects rodents and the final hosts are canines. Nevertheless, there are reports demonstrating that immuno-compromised humans can develop *T. crassiceps*-cysticercosis (Heldwein et al., 2006). Additionally, antigenic similarities have been very well established between *T. solium* and *T. crassiceps* metacestodes (Suzuki et al., 2007). Therefore, sera from human patients suffering from NCC can recognize *T. crassiceps* antigens. Similarly, sera from mice infected with *T. crassiceps* are able to recognize *T. solium* antigens.

In experimental cysticercosis caused by *T. crassiceps* the immune response in BALB/c mice is initially Th1-like but becomes highly Th2-polarized 3 or 4 wk after infection (Terrazas, 2008). Resistance to this parasite has been associated with an early Th1-type response, as well as with the genetic background (Sciutto et al., 1991, 1995; Terrazas et al., 1998). Thus, BALB/c (MHC haplotype $H2^d$) mice are susceptible to infection, whereas C57BL/6 mice (MHC haplotype $H2^b$) are relatively resistant to a similar challenge with *T. crassiceps*. However, the immunological mechanisms associated with the differential susceptibility to *T. crassiceps* of these two strains of mice have been largely undefined. Even a comparative evolution of the immune response of BALB/c and C57BL/6 mice in response to *T. crassiceps* infection has not been evaluated. Therefore, we explored the mechanisms underlying the differences in susceptibility of these strains of mice. We evaluated and compared the response of APCs, such as dendritic cells and macrophages. Furthermore, we analyzed the antibody levels in the serum and cytokine production by splenocytes from both strains of mice to polyclonal and antigen-specific stimuli and associated our findings with the kinetics of parasite growth.

2. Materials and methods

2.1. Mice

6–8-wk-old female BALB/cAnN mice and C57BL/6 mice were purchased from Harlan Laboratories (México) and were maintained in a pathogen free environment at the FES-Iztacala, U.N.A.M. animal facility in accordance with Institutional and National guidelines.

2.2. Parasites, infection protocol and antigens

Metacestodes of *T. crassiceps* (ORF) were harvested in sterile conditions from the peritoneal cavity of female BALB/c mice after 2–4 months of infection. The cysticerci were washed four times in phosphate-buffered saline (PBS; 0.15 M NaCl, 0.01 M sodium phosphate buffer, pH 7.2) and used for mouse infection. Female BALB/c and C57BL/6 mice were infected with an intraperitoneal (i.p.) injection of 10 small non-budding cysticerci of *T. crassiceps* suspended in 0.3 ml PBS. The infected mice were sacrificed at weeks 2, 4 and 8 post-infection and the parasites harvested from their peritoneal cavity were counted. To obtain *T. crassiceps* excreted/secreted products (TcES), metacestodes were maintained in culture in PBS at 37 °C, the supernatant was collected after 24–48 h and centrifuged for 10 min at 5000 rpm. The protein was concentrated using Amicon Ultra Filters with a 50 and 100 kDA membrane cutoff (Millipore). The protein concentration was determined using a commercially available Bradford assay, treated with proteases inhibitors and stored at −70 °C until further use.

2.3. Cell preparations and culture conditions

The spleen was removed in sterile conditions from infected mice. Single cell suspensions were prepared by gently teasing apart the spleen in RPMI-1640 supplemented with 10% fetal bovine serum, 100 units of penicillin/streptomycin, 2 mM glutamine, 25 mM HEPES buffer and 1% non-essential amino acids (all from GIBCO, BRL Grand Island, New York). The cells were centrifuged and the erythrocytes were lysed by resuspending cells in Boyle's solution (0.17 M Tris and 0.16 M ammonium chloride). Following two washes, the viable cells were counted by trypan blue exclusion with a Neubauer hemocytometer and the splenocytes were adjusted to 3×10^6 cells/ml in the same medium. Aliquots (100 μl) of the adjusted cell suspensions were placed into 96-well flat bottom culture plates (Costar, Cambridge, MA) and stimulated with a soluble extract of *T. crassiceps* (25 μg/ml) or with plate-bound anti-CD3 antibody (1 μg/ml) at 37 °C for 96 or 72 h, respectively.

2.4. Evaluation of cytokine production in vitro

Cell suspensions of lymphoid cells prepared as described above were diluted in supplemented RPMI-1640 to 3×10^6 cells/ml. The cell suspensions (1 ml) were placed in each well of a 24-well plate (Costar) and incubated with 1 μg/ml of anti-CD3 for 72 h under similar conditions. After centrifugation, the supernatants were collected, aliquoted and stored at −20 °C until used. The IFN-γ, IL-4 and IL-13 levels were measured using a sandwich ELISA according to the manufacturer's instructions (Peprotech-México, México, D.F.).

2.5. Isolation and activation of peritoneal macrophages

Peritoneal exudate cells (PECs) were obtained from the peritoneal cavity of 2-, 4- and 8-wk-*T. crassiceps* infected mice (BALB/c or C57BL/6 mice). The cells were washed twice with cold PBS and the red blood cells were lysed by resuspending the cells in Boyle's solution. Following two washes, the viable cells were counted by trypan blue exclusion with a Neubauer hemocytometer. The PECs were adjusted to 5×10^6 cells/ml in supplemented and cultured in 6-well plates (Costar). After 2 h at 37 °C and 5% CO_2, the non-adherent cells were removed by washing with warm supplemented RPMI medium. The adherent cells were removed using EDTA and readjusted to 1×10^6 cells/ml. The viability was checked at this point again (>90%). The cells (1 ml) were plated in 24-well plates (Costar) and the cells were activated

by the addition of LPS (1 μg/ml, *Escherichia coli* 111:B4; Sigma, St. Louis, MO) plus IFN-γ (20 ng/ml, Peprotech). The cells were incubated at 37 °C and 5% CO_2 for 24 h. After this time, some of the cultured macrophages were processed for cytokine production. The cells were >90% macrophages, as determined by flow cytometry (data not shown).

2.6. RT-PCR assay to evaluate the macrophage activation status

The level of Arginase-1 (Arg-1), triggering receptor expressed on myeloid cells 2 (TREM-2), inducible nitric oxide synthase (iNOS), tumor necrosis factor-α (TNF-α), interferon gamma (IFN-γ), macrophage migration inhibitory factor (MIF) and Resistin-like molecule-α (RELM-α) mRNA transcripts in peritoneal macrophages was determined by reverse transcription (RT)-PCR. At the indicated time points, adherent peritoneal macrophages from *T. crassiceps*-infected BALB/c and C57BL/6 mice were aseptically removed and without any further stimulation were processed for RNA extraction using the TRIzol reagent (Invitrogen, Carlsbad, CA) and a propanol–chloroform technique. The RNA was quantified and 3 μg of RNA were reverse transcribed using the Superscript II First Strand Synthesis Kit (Invitrogen) and an oligo dT primer, as recommended by the manufacturer.

Once cDNA was obtained conventional PCR was performed. The PCR reactions contained (in a 25 μl final volume) 5× PCR buffer blue, 10 mM dNTP, 40 nM each forward and reverse primer (Table 1), 1 unit of Taq DNA polymerase (Sacace Biotechnologies, Italy) and 2 μl of the cDNA.

The program used for the amplification of each gene was an initial denaturation at 95 °C for 5 min, 35 cycles of 95 °C for 40 s, the indicated melting temperature (Table 1) for 50 s and 72 °C for 40 s and a final extension step of 72 °C for 4 min. All reactions were carried out in a thermal cycler (Corbett Research, Australia). Finally, to observe the amplified products, a 1.5% agarose gel was prepared and samples were loaded with blue juice buffer containing SYBR Green (Invitrogen). The gels were visualized using a Fujifilm FLA 5000 scanner (Fuji, Japan) with FLA 5000 image reader V2.1 software to capture the shown images. The specificity of the PCR was verified by the absence of signal in the no-template controls of macrophage samples. The sequences of the primers used are available in Table 1.

2.7. Densitometry analysis

For the different time points each sample expression levels were normalized against the Housekeeping gene GAPDH and values are presented as arbitrary units (an average of 2–3 animals is shown).

2.8. Detection of nitric oxide production

The nitric oxide level in the supernatants of the cultured macrophages were assayed by determining the increase in nitrite concentration (Migliorini et al., 1991) using the Griess reaction adapted to microwell plates (Costar). Briefly, 50 μl of culture supernatant was mixed with an equal volume of Griess reagent, incubated for 10 min at room temperature in the dark and the absorbance was measured at 570 nm in an automatic microplate reader (Multiskan Ascent, Thermolab Systems). The values were quantified using serial dilutions of sodium nitrite.

2.9. Analysis of cell surface markers in macrophages

The F_c receptors on the peritoneal macrophages were blocked with anti-mouse CD16/CD32 (Biolegend, CA, USA) and stained with a FITC-conjugated monoclonal antibody against F4/80 (Biolegend, CA, USA) and PE-conjugated antibodies against PD-L1 and PD-L2 (Biolegend). The stained cells were analyzed on a FACsCalibur flow cytometer using Cell Quest software (Becton Dickinson).

2.10. Co-culture of macrophages-CD90 cells

Co-culture of macrophages obtained from infected mice with naive CD90 cells was performed as previously reported (Terrazas et al., 2005). Briefly, macrophages were obtained as described. Splenocytes were prepared from naive mice, and enriched for $CD90^+$ cells (95% by FACs analysis) using CD90 magnetic cell sorter beads (MACS, Miltenyi Biotec). CD90 cells were plated in 96 well flat bottom plates which were pre-coated with anti-CD3 and anti-CD28 antibodies (Biolegend) at 1 μg/ml. Three hours later macrophages were added to CD90 T cells at ratios of 1:4, 1:8 and 1:16 (macrophages:CD90). Co-cultures were maintained at

Table 1
Primers sequences used to amplify AAMΦ's markers and cytokines.

Gene	Sequence	MT (°C)	Cycles	Prod.	Reference
GAPDH	F—CTC ATg ACC ACA gTC CAT gC R—CAC ATT ggg ggT Agg AAC AC	56	35	201	Renshaw et al. (2002)
Ym-1	F—TCA CAg gTC Tgg CAA TTC TTC Tg R—TTT gTC CTT Agg Agg gCT TCC TC	56	35	436	Nair et al. (2003)
Relm-α	F—ggT CCC AgT gCA TAT ggA TgA gAC R—CAC CTC TTC ACT CgA ggg ACA gTT	65	35	290	Nair et al. (2003)
Arginase-1	F—CAg AAg AAT ggA AgA gTC Ag R—CAg ATA TgC Agg gAg TCA CC	54	35	250	Nair et al. (2003)
iNOS	F—CTg gAg gAg CTC CTg CCT CATg R—gCA gCA TCC CCT CTg ATg gTg	62	35	449	Yoshida et al. (2000)
TREM-2	F—TCC CAA gCC CTC AAC ACC A R—TTC CAg CAA ggg TgT CAT CTg CgA	56	35	230	Kim et al. (2005)
IFN-γ	F—AgC ggC TgA CTg AAC TCA gAT TgT Ag R—GTC ACA gTT TTC AgC TgT ATA ggg	57	35	243	Ulett et al. (2000)
TNF-α	F—ggC Agg TCT ACT TTg gAg TCA TTg C R—ACA TTC gAg gCT CCA gTg AAT TCg	59	35	307	Ulett et al. (2000)
MIF	F—gCC AgA ggg gTT TCT gTC g R—gTT CgT gCC gCT AAA AgT CA	58	35	118	Tuncman et al. (2006)

MT, Melt temperature; MIF, macrophage migration inhibitory factor; F, forward primer; R, reverse primer.

37.8 °C and 5% CO_2 for 72 h, and then [^{3}H] thymidine (185 GBb/mmol activity, Amersham, England) 0.5 μCi/well was added and incubated for a further 18 h. Cells were harvested on a 96-well harvester (Tomtec, Finland) then counted using a 1450 micro β-plate counter (Trilux, Finland). Values are represented as counts per minute (CPM) from triplicate wells.

2.11. In vitro bone marrow-derived dendritic cell maturation and cytokine production

Dendritic cells (DCs) were obtained as previously described (Lutz et al., 1999). Briefly, femurs and tibias were aseptically harvested from naïve BALB/c or C57BL/6 mice and the bone marrow was flushed. The cells were washed and adjusted to 5×10^5 cells/ml and cultured with 20 ng/ml of recombinant murine GM-CSF (Peprotech, México). Five days later, the BMDCs (10^5) were cultured in 200 μl in 96-well flat-bottom culture plates and stimulated with medium alone, 1 μg/ml LPS (*E. coli* 0111:B4, Sigma) or 20 μg/ml TcES. Some BMDC cultures were first incubated with 20 μg/ml and immediately stimulated with 1 μg/ml LPS. After 24 h, the supernatants of the cell cultures were collected and the IL-12, IL-15 and TNF-α levels were determined using commercially available ELISA kits (Peprotech, México).

2.12. Analysis of cell surface markers in BMDCs

The surface expression of DC maturation markers was analyzed using multicolor flow cytometry. DCs (either untreated or stimulated for 24 h with LPS, LPS + TcES or TcES) were harvested, washed and suspended in cold PBS containing 5% FCS and 0.05% NaN_3. The F_c receptors were blocked with anti-mouse CD16/CD32 for 20 min at 4 °C. The cells were washed and triple stained with an APC-conjugated antibody against CD11c, FITC-conjugated monoclonal antibodies against CD40 or MHC-II and PE-conjugated antibodies against CCR7, CD86 or CD80 (all antibodies were from Biolegend). The stained cells were analyzed on a FACsCalibur flow cytometer using Cell Quest software (Becton Dickinson).

2.13. DC-T cell allogeneic co-cultures

Allogeneic $CD4^+$ T cells were enriched using a CD4 T cell Isolation Kit (Miltenyi Biotec, Germany) according to the manufacturer's instructions. The DCs were activated with LPS, TcES or both for 24 h, washed and co-cultured with allogeneic naïve $CD4^+$ T cells (10^5 cells/well) in complete RPMI 1640 culture medium. The proliferation was quantified by pulsing the cells for 18 h with 0.5 μCi of [^{3}H] thymidine (Amersham Biosciences). The cells were then harvested and the incorporation of radioactivity was assessed.

2.14. Antibody ELISA

Peripheral blood was collected 2, 4 and 8 wk after infection following tail snips of the *T. crassiceps*-infected BALB/c and C57BL/6 mice. *T. crassiceps*-specific IgG1 and IgG2a levels were determined by ELISA, as previously described. The results are expressed as the maximum serum dilution (endpoint titer) in which antibody was still detected. Total IgE production was detected using the Opt-ELISA from Biolegend.

2.15. Statistical analysis

The comparisons between the BALB/c and C57BL/6 groups were made using Student's unpaired *t* test. A $p < 0.05$ was considered significant. The statistical significance of the serum titer was determined by non-parametric tests using the Mann-Whitney *U*-Wilcoxon Rank test.

3. Results

3.1. Time course of T. crassiceps infection in BALB/c and C57BL/6 mice

Following i.p. inoculation of ten *T. crassiceps* metacestodes, BALB/c mice developed large parasite loads 8 wk post-infection, whereas similarly infected C57BL/6 mice developed either no or small parasite burdens with significantly fewer parasites per mouse (Fig. 1A). Interestingly, the differences in parasite burden were evident as early as 2 wk post-infection when no parasite was detectable in 75% of the C57BL/6 mice, while 80% of BALB/c mice contained at least 2 larvae. These differences became greater as the infection progressed. After 4 wk of infection, 100% of the BALB/c mice-harbored different amounts of parasites in their peritoneal cavity, while only 20% of the C57BL/6 mice harbored a few parasites. The parasite loads increased at 8 wk post-infection. Similarly, antibody responses to *T. crassiceps* infection showed important differences between strains, the BALB/c mice had higher titers of anti-*Taenia* IgG1 and Total IgE (Fig. 1B and C) but lower levels of Ag-specific IgG2a when compared with the infected C57BL/6 mice (Fig. 1D).

3.2. Kinetics of in vitro cell proliferation and cytokine production by Ag-stimulated splenocytes from BALB/c and C57BL/6 mice

Splenocytes were isolated from both strains of mice at different time points after infection. The splenocytes (3×10^6 ml^{-1}) were stimulated with either 25 μg/ml of soluble *T. crassiceps* antigen (AgTc) or 1 μg/ml of plate-bound anti-CD3 antibody and cultured for 4 or 3 days, respectively. The proliferative response was measured using ^{3}H-thymidine uptake. Two wk post-infection, the splenocytes from BALB/c mice displayed an increased proliferative response compared to C57BL/6 splenocytes to both types of stimuli. However, as the infection became more chronic, the proliferative response to both AgTc and anti-CD3 in the BALB/c mice declined, whereas the C57BL/6 mice maintained a more consistent response to both stimuli as the infection progressed (Fig. 2A and B).

The culture supernatants from Ag-stimulated proliferation assays were analyzed using an ELISA for the presence of the Th1-associated cytokine IFN-γ and the Th2-associated cytokines IL-4 and IL-13. Ag-stimulated splenocytes from both strains produced similar levels of IFN-γ 2 wk post-infection (Fig. 2C). However, 4 and 8 wk post-infection, the antigen-specific IFN-γ production by the spleen cells from BALB/c decreased and did not reach the level produced by the C57BL/6 splenocytes (Fig. 2C). In contrast, as early as 2 wk post-infection, the splenocytes from the BALB/c mice produced significantly more IL-13 than the C57BL/6 splenocytes. However, both strains of mice displayed comparable levels of IL-4 production. As the infection progressed, the BALB/c mice produced significantly greater levels of both IL-4 and IL-13 compared to splenocytes from *T. crassiceps*-infected C57BL/6 mice (Fig. 2D and E).

3.3. Cytokine production by macrophages

Macrophages were isolated from the peritoneal cavities of both strains of infected mice and either unstimulated (basal) or stimulated for 24 h with LPS (1 μg/ml) and IFN-γ (20 ng/ml). The supernatants were collected and analyzed for IL-12, TNF-α and NO production. As seen in Fig. 3, the macrophages from *T. crassiceps*-infected mice produced lower levels of IL-12, TNF-α and NO than the macrophages isolated from C57BL/6 mice, which maintained a higher pro-inflammatory response throughout the infection (Fig. 3A–C).

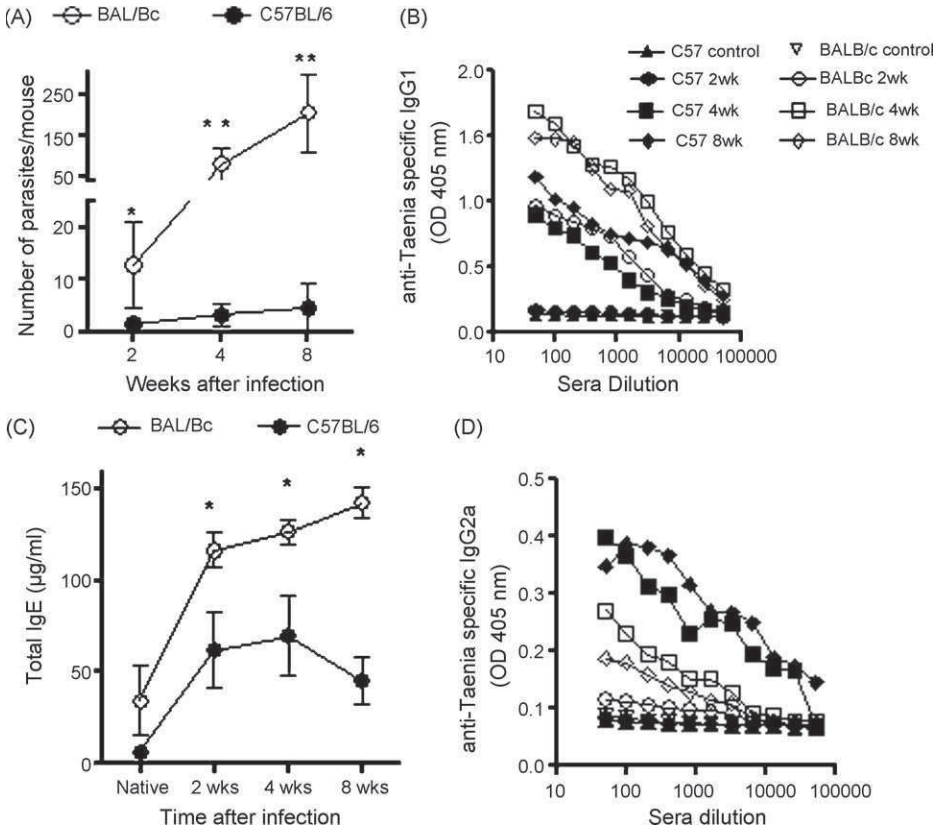


Fig. 1. Time course of *T. crassiceps* infection and antibody response in BALB/c and C57BL/6 mice. (A) Parasite load: Animals were infected i.p. with ten cysticerci and the parasite load was monitored 2, 4 and 8 wk post-infection. The data are expressed as the mean number of parasites per mouse ± SD. The data are representative of two independent experiments ($n = 10$ mice per group). $^{*}p < 0.05$; $^{**}p < 0.01$. Antibody responses: The mice were bled by tail snipping at different time points following *T. crassiceps* infection and the serum level of the Th2-associated IgG1 (B) and total IgE (C), as well as the Th1-associated IgG2a (D) was measured by ELISA.

3.4. Dynamics of macrophage gene expression at site of infection

Next, we analyzed whether *T. crassiceps* infection of BALB/c or C57BL/6 mice affected the expression of different genes in macrophages at the site of infection. Macrophages were isolated from the peritoneal cavities of both strains of infected mice 2, 4 and 8 wk post-infection. RNA was isolated from the adherent cells (>90% positive for F4/80) without any stimulation. Consistent with our own findings, and in agreement with previous studies (Terrazas et al., 2005; Ghassabeh et al., 2006), the macrophages from BALB/c mice rapidly (2 wk post-infection) and strongly up-regulated genes encoding proteins that are associated with alternative activation, such as Arg-1, RELM-α, Ym-1 and triggering receptor expressed on myeloid cells 2 (TREM-2). Additionally, at this time point, there was weak expression of iNOS. However, as infection progressed some changes were observed in these markers, whereas Ym-1 transcripts decreased by week 8 p.i. and Arg-1 expression was maintained without changes on weeks 4 and 8; the transcripts of RELM-α and TREM-2 were more elevated by week 8 after infection on macrophages from *T. crassiceps*-infected BALB/c mice, in contrast, transcripts for iNOS were inhibited (Fig. 4A). On the other hand, macrophages from the C57BL/6 mice displayed a weak and transient expression of Arg-1, Ym-1 and TREM-2 early after infection, as well as increased transcripts of iNOS (Fig. 4A and B). While the expression of iNOS was sustained at week 4, the expression of Arg-1, TREM-2, Ym-1 and RELM-α was down-regulated as the infection progressed in this strain of mice (Fig. 4A and B). Additionally, the macrophages isolated from the C57BL/6 mice expressed a similar mRNA level for the pro-inflammatory cytokines TNF-α, and MIF throughout the infection compared to the macrophages from BALB/c mice, however, transcripts for IFN-γ were higher at week 8 post-infection in C57BL/6 mice. In fact, by week 8 post-infection, the macrophages from C57BL/6 mice expressed a clear pro-inflammatory profile, whereas the BALB/c macrophages displayed mixed transcripts of alternative activation and pro-inflammatory markers (Fig. 4A). A semi-quantitative densitometry analysis using 2–3 samples per time point is presented in Fig. 4B, samples were normalized against their own housekeeping gene. These data further support differences in gene expression between macrophages obtained from BALB/c mice versus macrophages from C57BL/6 mice after infection with *T. crassiceps* metacestodes.

T. crassiceps infection induces greater PD-L2 expression as well as suppressive activity on BALB/c macrophages than C57BL/6 macrophages. To investigate whether the different markers that were altered on the macrophages during *T. crassiceps* infection could also reflect other activities, we analyzed the expression of surface markers on macrophages from both strains and determined their previously reported suppressive activity. The surface expression of PD-L1 and PD-L2 has been associated with alternatively activated macrophages (AAMϕ) (Smith et al., 2004; Terrazas et al., 2005) and differences in the expression of these molecules during *T. crassiceps* infection were observed between the two strains. Interestingly, PD-L1 expression was similarly increased on macrophages from both strains of mice 2 and 4 wk post-infection. However, in chronic infections, the macrophages from BALB/c mice expressed twice as much PD-L1 than macrophages from C57BL/6 mice, which down-regulated PD-L1 expression to levels observed at 2 wk post-infection. A clear contrast was evident in the expression of PD-L2, 2 wk post-infection the expression was low and similar between the two strains; however, by week 4 post-infection an increase in the expression of PD-L2

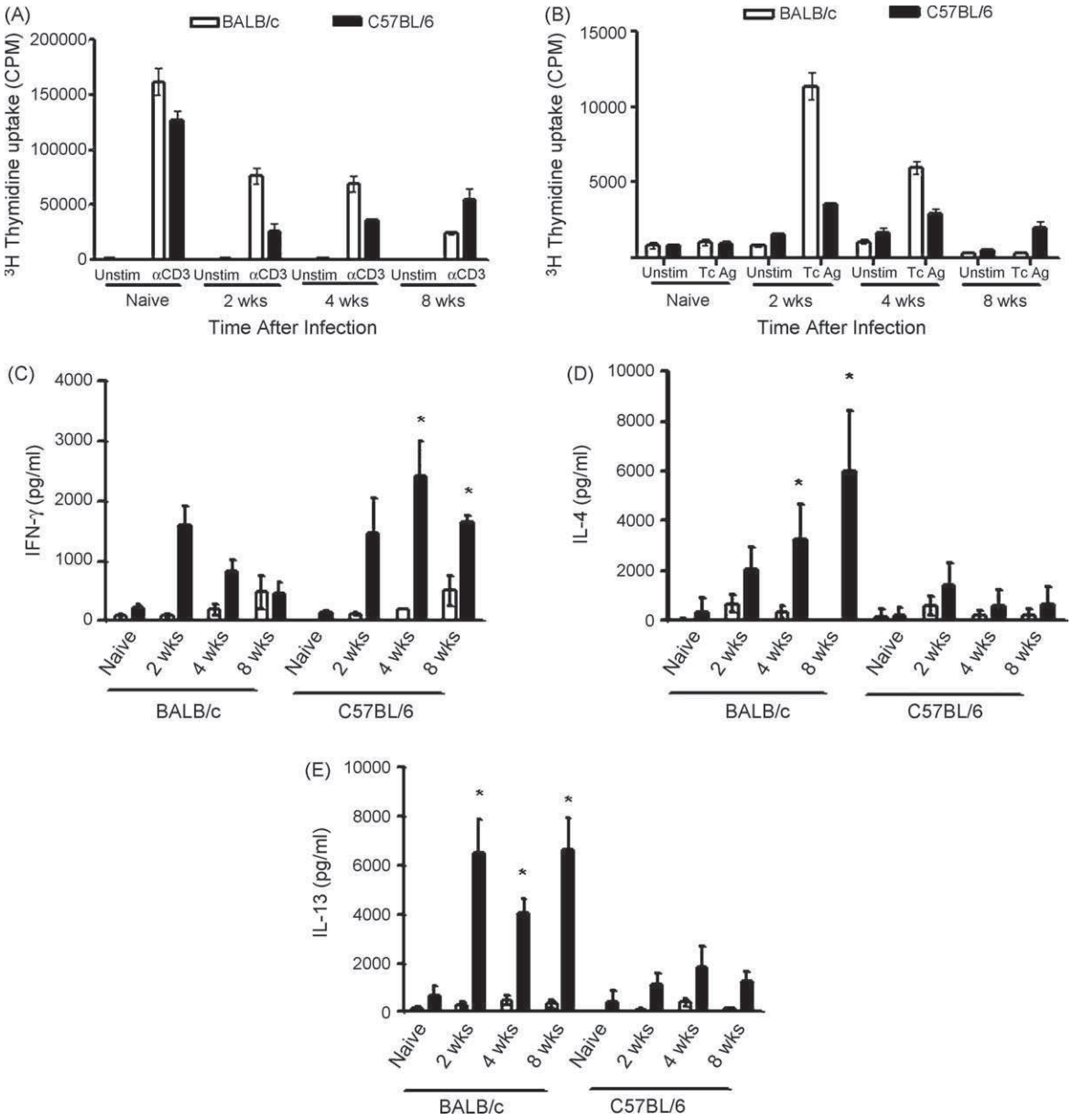


Fig. 2. Kinetics of the adaptive immune response. Spleen cell proliferation in response to anti-CD3 stimuli (A) and to *T. crassiceps* Ag (B). IFN-γ (C), IL-4 (D) and IL-13 (E) production in response to *T. crassiceps* Ag-stimulated splenocytes from *T. crassiceps*-infected BALB/c and C57BL/6 mice as well as from naïve mice. The data are the mean of two independent experiments at each time point. An asterisk indicates statistically significant ($p < 0.05$) differences between the groups.

was detected on BALB/c macrophages and the highest expression was detected 8 wk post-infection. In contrast, PD-L2 expression on the macrophages of C57BL/6 mice remained low throughout the infection (Fig. 5A).

Recently, macrophages in helminthic infections have been associated with suppressive activities (Rodriguez-Sosa et al., 2006; Taylor et al., 2006). Given that we detected a higher expression of PDL-2, a molecule with inhibitory activity when bind to its receptor PD-1 on T cells (Sharpe et al., 2007); we decided to determine whether these macrophages recruited to the site of infection were able to suppress T cell proliferation. Peritoneal macrophages were isolated from BALB/c and C57BL/6 mice 8 wk post-infection and were co-cultured with splenocytes from naïve mice previously stimulated with anti-CD3/CD28. Three days later, the T cell proliferation was measured by ^{3}H-thymidine uptake. As shown in Fig. 5B, the macrophages from *T. crassiceps* infected-BALB/c mice, in a ratio-dependent manner significantly suppressed T cell proliferation. However, the macrophages from *T. crassiceps*-infected C57BL/6 mice were unable to suppress this response.

TcES induce partial up-regulation of MHC class II and co stimulatory-molecules expression on DC and impair full maturation after LPS-mediated stimuli in DCs from BALB/c but not C57BL/6 mice. The ability of excreted-secreted *Taenia* products to up-regulate the expression of MHC-II and costimulatory molecules on BMDC was tested comparing it versus LPS, which was chosen for its known ability to maturate and activate DC to produce pro-inflammatory cytokines. DCs were cultured in medium alone (RPMI) or exposed to TcES, LPS, or TcES plus LPS for 24 h prior to analysis of the surface phenotype. As shown in Table 2, the expression of maturation markers, such as CD86, CD80, CD40 and CCR7, on BMDCs from BALB/c mice was affected by the exposure to TcES antigens, whereas MHC-II expression remained unaltered. However, the BMDCs from C57BL/6 mice exposed to the same concentration of TcES did not show alteration in their response compared to LPS-induced maturation.

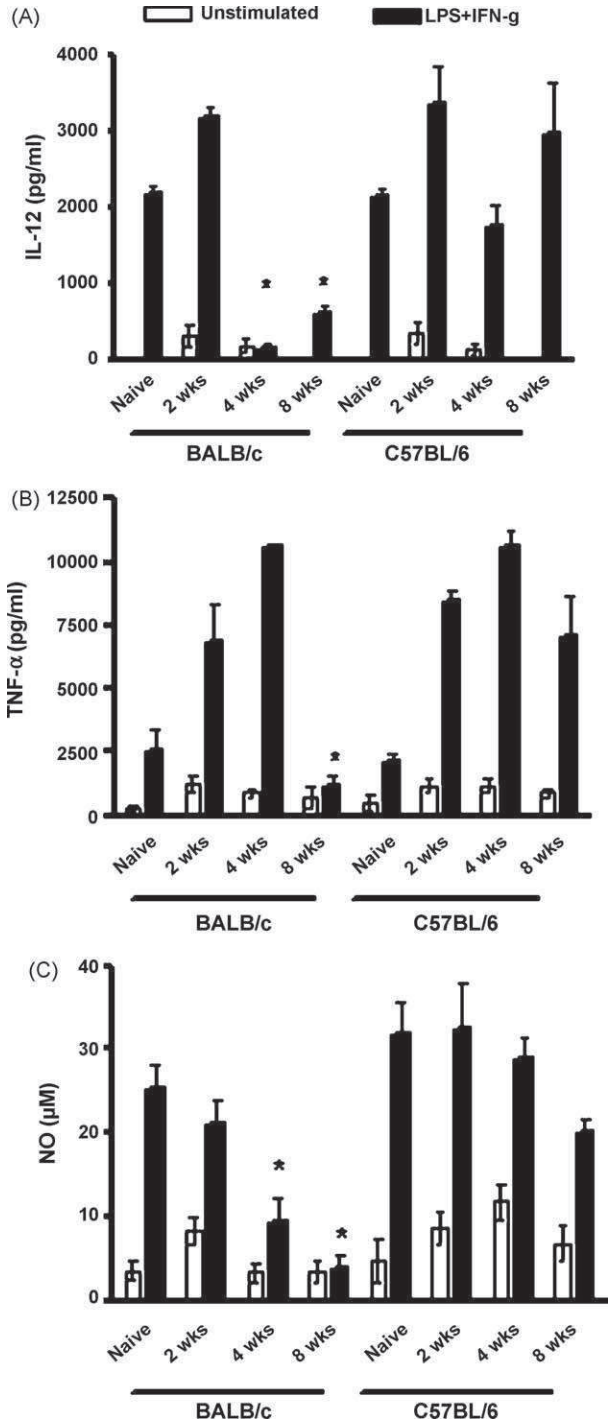


Fig. 3. LPS and IFN-γ activation of macrophages isolated from *T. crassiceps*-infected BALB/c and C57BL/6 mice. Peritoneal macrophages were isolated as described at different time points post-infection and stimulated with LPS (1 μg/ml) plus IFN-γ (20 ng/ml). Supernatants were collected 24 h later and cytokine production IL-12 (A), TNF-α (B) and NO (C) was measured using an ELISA (A and B) and Griess reaction (C). The data are the mean of two independent experiments at each time point. An asterisk indicates statistically significant ($p < 0.05$) differences between the groups.

3.5. Allogenic DC activation is inhibited in BALB/c DCs exposed to Taenia antigens

In order to know whether the alterations in the expression of the surface maturation markers affects the ability of BMDCs exposed to TcES antigens to induce allogenic proliferation in a mixed lymphocyte reaction, BMDCs from BALB/c mice were exposed to TcES, LPS or LPS plus TcES for 24 h and immediately co-cultured for 3 days with magnetically purified $CD4^+$ cells isolated from healthy C57BL/6 mice. The proliferative response was analyzed using ^{3}H-thymidine uptake. Similar experiments were performed using BMDCs from C57BL/6 mice and $CD4^+$ cells from BALB/c mice. As seen in Fig. 6A, the exposure of BALB/c BMDCs to TcES significantly affected their ability to induce an allogenic response (the proliferation was inhibited more than 50%). Interestingly, the C57BL/6 BMDCs were much less affected by the TcES and the allogenic stimulation was significantly better than that induced by BALB/c BMDCs exposed to *Taenia* antigens (Fig. 6B).

3.6. DC activation is modified in BALB/c but not in C57BL/6 mice after exposure to TcES

Finally, to further evaluate whether innate immune responses can be altered directly by *Taenia* antigens, we generated bone marrow-derived DCs from BALB/c and C57BL/6 mice that were similarly exposed to *T. crassiceps* excreted/secreted antigens (TcES), LPS or both and the inflammatory cytokine production was evaluated. The production of pro-inflammatory cytokines showed important differences, as the BALB/c BMDCs produced less IL-15, IL-12 and TNF-α in response to LPS when they were also exposed to TcES. However, the BMDCs from C57BL/6 mice maintained their ability to respond to LPS stimulation (exception for IL-15) with high levels of these pro-inflammatory cytokines even in the presence of *Taenia* antigens (Fig. 7A–C).

4. Discussion

Based on early observations and looking only for the number of parasites after 4 wk of infection with *T. crassiceps*, Sciutto et al. (1991) concluded that C57BL/6 mice were resistant to this parasite, whereas BALB/c mice were susceptible. These results were attributed to the different MHC-II haplotypes of these strains ($H2^b$ and $H2^d$, respectively). However, no immunological analyses were done. In this study, we have provided, for the first time, a comparative study of the immune response to this helminth in both susceptible and resistant strain of mice. We evaluated the kinetics of parasite growth in both strains of mice. We found that, as early as 2 wk post-infection, BALB/c ($H2^d$) mice harbored viable parasites, whereas few parasites were found in C57BL/6 ($H2^b$) mice. As the infection progressed, these differences became more evident and, by week 8 post-infection, the BALB/c mice harbored five- to ten-fold more parasites than the C57BL/6 mice, 80% of which cleared the infection. Interestingly, the susceptible phenotype is seen even though BALB/c splenocytes displayed an increased proliferative response and produced IFN-γ-levels similar to C57BL/6 splenocytes early after infection. However, at later time points, these levels decreased in BALB/c splenocytes, whereas C57BL/6 splenocytes maintained a consistent IFN-γ production and cell proliferation throughout the course of infection. In contrast, the production of the Th2-associated cytokines IL-4 and IL-13 was significantly elevated in BALB/c splenocytes compared to C57BL/6 splenocytes. Furthermore, the IgG1 and IgE levels were significantly higher in BALB/c mice. These data confirm that a Th2-type response is not associated with protection in experimental cysticercosis, as unlike has been observed in other helminthic infections (McKay and Khan, 2003; Patel et al., 2009).

We have also analyzed the response of two types of APCs, macrophages and DCs. APCs play a central role in the activation and differentiation of T lymphocytes into Th1 cells, Th2 cells or Tregs. Both macrophages and DCs take up antigens, become activated and migrate to present the antigenic peptides on MHC

Table 2
Expression of surface MHC-II, CD80, CD86, CD40 and CCR7 on CD11c$^+$ BMDCs following incubation with TcES, LPS, LPS + TcES or unprimed control (RPMI).

Strain	Treatment	MHCII	CD80	CD86	CD40	CCR7
C57BL/6	RPMI MFI (a)	69.2 ± 7.1	33.8 ± 5.6	58.9 ± 11.9	21.3 ± 2.8	20.7 ± 3.6
	% (b)	50.8 ± 1.8	18.5 ± 2.9	41.4 ± 4.4	43.5 ± 0.5	30.3 ± 10.4
	TcES	229 ± 6.1	42.5 ± 4.7	91.2 ± 15.9	47 ± 0.1	54.8 ± 16.7
		54.8 ± 6.7	71.6 ± 11.6	53.9 ± 3.9	49.9 ± 15.4	45.6 ± 2.2
	LPS	270.8 ± 12	56.1 ± 2.2	157.6 ± 14.8	72.2 ± 1.8	88.9 ± 0.7
		72.1 ± 1.8	90.5 ± 2	79.6 ± 3.4	68.6 ± 3.3	71.3 ± 20.3
	LPS/TcES	317.6 ± 23.9	60.5 ± 4.1	121.9 ± 18	45.1 ± 11.7	42.4 ± 12.9
		66.8 ± 2.6	65.3 ± 14.6	73.93 ± 3.1	68.7 ± 2.5	52.8 ± 14.8
BALB/c	RPMI	101.7 ± 6.3	29.7 ± 2.5	40.4 ± 2.5	27.3 ± 1.3	24.3 ± 1.3
		56.2 ± 1.8	28.9 ± 3.1	52.3 ± 2.3	31.2 ± 4.8	24 ± 3.1
	TcES	163.7 ± 35.2	38.9 ± 2.6	47.6 ± 2.9	35 ± 4.6	20.7 ± 0.13
		71.7 ± 1.6	40 ± 6	58.3 ± 3	48.4 ± 4.7	35.5 ± 4.7
	LPS	251.2 ± 54	72.3 ± 2.7	118.6 ± 8.3	82.2 ± 3.4	50.4 ± 5.5
		80.8 ± 3.6	80.7 ± 17.8	79.3 ± 5.3	79.4 ± 2.3	69.8 ± 4.7
	LPS/TcES	192.2 ± 46.8	**47.6 ± 4.6 (*p < 0.01)**	**85.1 ± 6.4 (*p < 0.01)**	**41.7 ± 6.5 (*p < 0.01)**	**30 ± 3.8 (*p < 0.02)**
		79.4 ± 2.4	**44.9 ± 1 (*p < 0.01)**	61.1 ± 10.3	**57.7 ± 8.4 (*p < 0.03)**	**34.5 ± 5.7 (*p < 0.01)**

Values are the cumulative results of 3 sets of experiments. (a) Data are expressed as mean ± standard deviation of mean geometric fluorescence intensities (MFI) by flow cytometric analysis. (b) These data are expressed as percentage of positive cells for the respective surface marker.
* *p*-value versus corresponding LPS stimulation (Student's *t* test).

molecules. This process involves (among others) phagocytosis, up-regulation of costimulatory molecules, such as CD80, CD86 and CD40 as well as MHC molecules (Lee and Iwasaki, 2007). Failures in this process could modify the outcome of the immune response and the course of the infection. APCs are the first line of contact between parasites and the immune system, thus this interaction is crucial for the relationship between host and parasite.

Macrophages play a key role in directing the host immune response to parasites and they can also function as effector cells. The recruitment and activation of macrophages by microbial products (e.g., LPS or helminth-derived molecules) results in the release or inhibition of several key cytokines, such as IL-12, IL-6, TNF-α, IL-10 and NO (Rodriguez-Sosa et al., 2002a,b; Goodridge et al., 2004; Smith et al., 2004). These immune mediators play crucial roles in the development of immunity against a variety of pathogens, but their role in helminthic infections is less well understood (Allen and Loke, 2001; Goodridge et al., 2001). In this study, we demonstrated that macrophages from *T. crassiceps*-

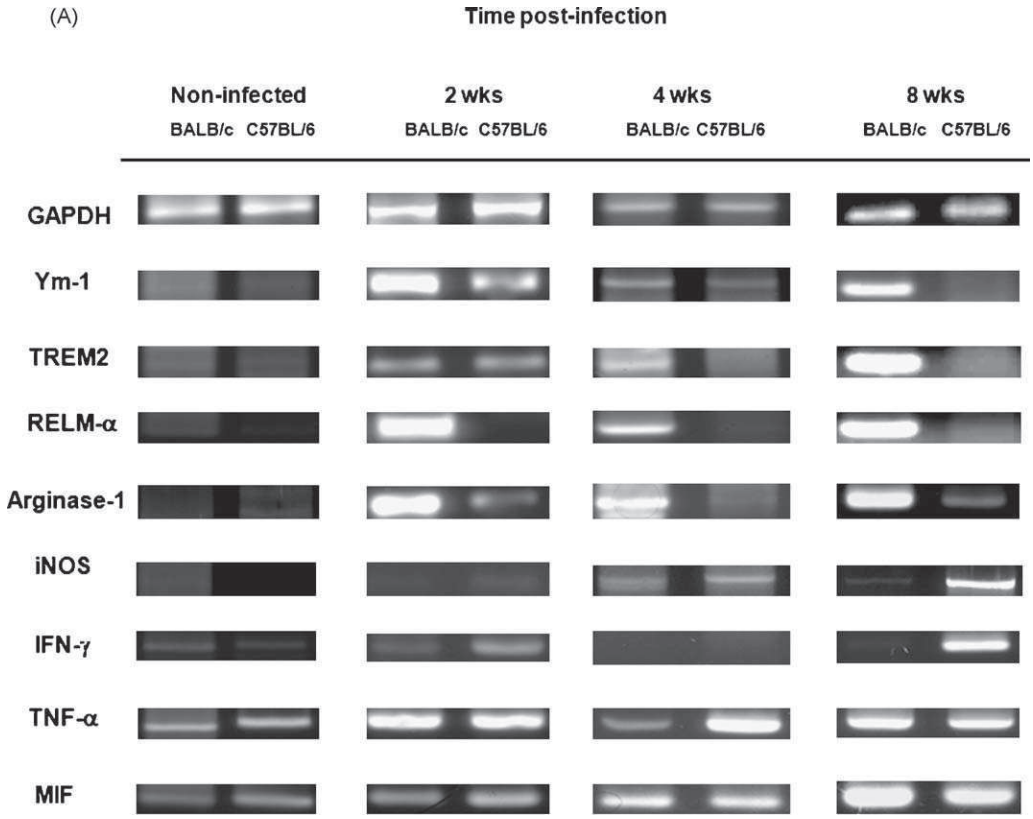


Fig. 4. Kinetics of macrophage activation at site of infection during *T. crassiceps* infection. (A) Macrophages were isolated from the peritoneal cavity of BALB/c and C57BL/6 mice at different time points following infection with ten cysticerci. Without any additional stimulation, RNA was extracted and RT-PCR was performed for GAPDH, RELM-α, Ym-1, Arg-1, TREM-2, IFN-γ, TNF-α and MIF. (B) Densitometry analysis of 2–3 mice per group per time of infection was performed in order to give a semi-quantitative observation. Values represent arbitrary units showing higher or lower density with respect to their own housekeeping gene transcript.

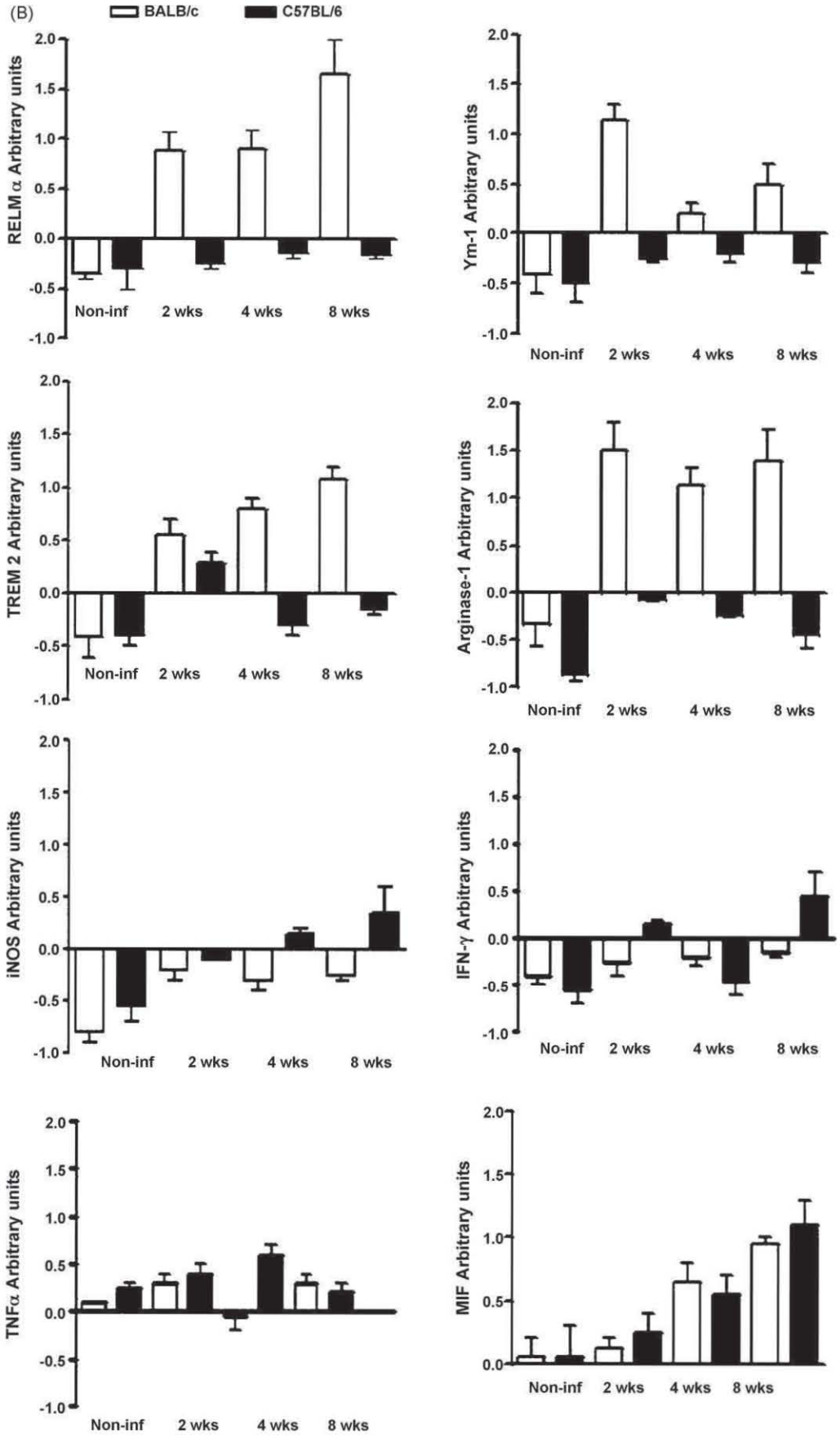


Fig. 4. (*Continued*).

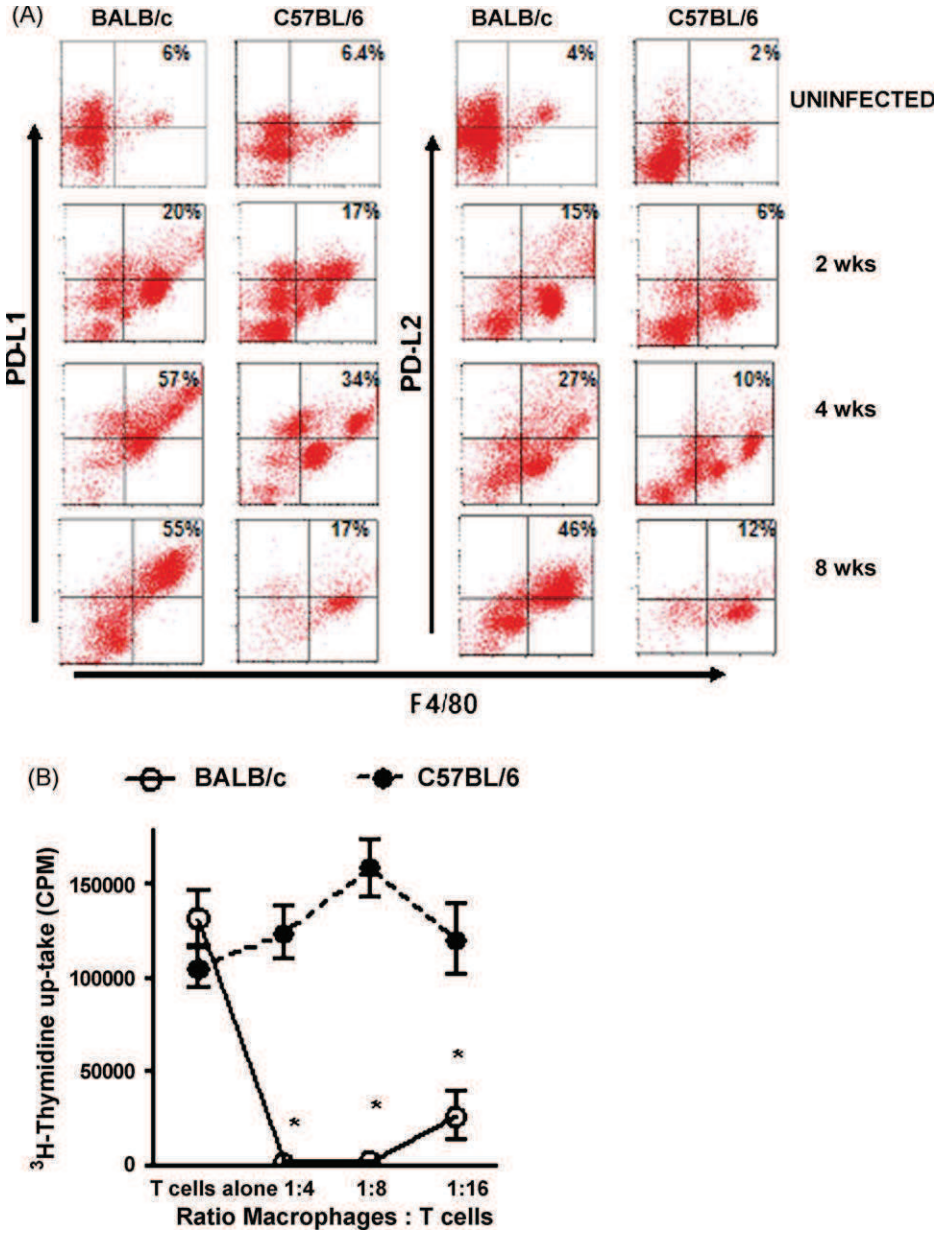


Fig. 5. Kinetics of PD-L1 and PD-L2 expression and suppressive activity of peritoneal macrophages after *T. crassiceps* infection. (A) Peritoneal cells were isolated from BALB/c and C57BL/6 mice at different time points after infection with ten cysticerci. Without any additional stimulation, the cells were processed for flow cytometry and analyzed for the expression of F4/80, PD-L1 and PD-L2. (B) The suppressive activity of the macrophages was assayed in a co-culture with $CD90^+$ naïve cells stimulated with plate-bound anti-CD3/CD28 antibodies. An asterisk indicates statistically significant ($p < 0.05$) differences between the groups.

infected BALB/c mice recruited to the site of infection transiently produced high levels of IL-12, TNF-α and NO in response to pro-inflammatory stimuli, such as LPS + IFN-γ. However, as the infection progressed, these cells showed a substantially reduced capacity to produce these cytokines after similar stimulation. Interestingly, macrophages from C57BL/6 mice maintained the capacity to produce pro-inflammatory cytokines. These data are consistent with the susceptible phenotype observed in IL-12 KO mice (Rodriguez-Sosa et al., 2003) and suggest a major role for macrophages in cysticercosis. The mechanism underlying the inhibition of LPS + IFN-γ-induced pro-inflammatory cytokine production in our system remains to be elucidated, however, as we have previously found high IFN-γ receptor expression in these macrophages (Rodriguez-Sosa et al., 2006), it may be associated with an impaired intracellular signaling in BALB/c mice but not in C57BL/6 mice.

The relevance of these observations is highlighted by the finding that macrophages from BALB/c mice became rapidly alternatively activated after *T. crassiceps* infection, whereas macrophages from C57BL/6 mice presented a transient and incomplete alternate activation. As the infection progressed, the macrophages from BALB/c mice displayed higher transcripts of four genes that are associated with alternative activation (Arg-1, Ym-1, TREM-2 and RELM-α), whereas discrete changes on the expression of pro-inflammatory cytokines could be detected. This suggests a mixed macrophage population. In contrast, the macrophages from C57BL/6 mice mainly over-expressed transcripts for pro-inflammatory cytokines and not for alternative activation markers. Thus, the presence and may be the persistence of AAMϕ is another striking difference between the susceptible and resistant strains of mice to *T. crassiceps* infection. Interestingly, AAMϕ have been documented in all three classes of parasitic

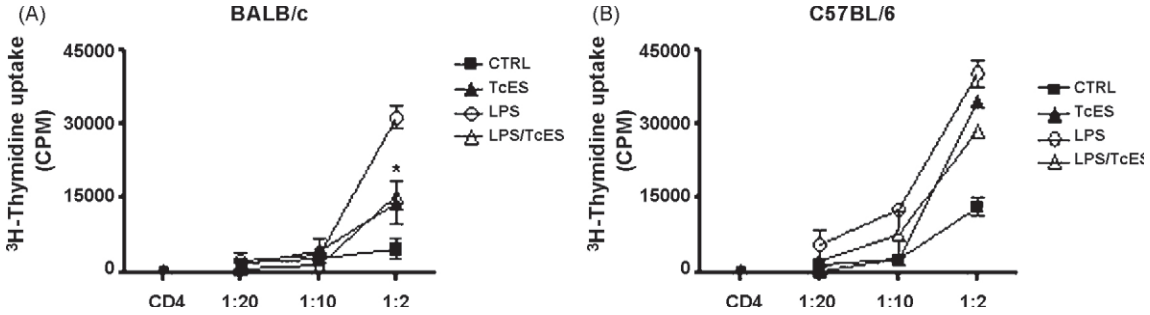


Fig. 6. Co-stimulation of DCs from BALB/c, but not in C57BL/6 mice, with LPS plus TcES decreases DC allogenic stimulation (A) BMDCs from BALB/c mice were stimulated for 24 h with medium alone (CTRL), TcES, LPS or TcES + LPS and co-cultured with $CD4^+$ cells from naïve C57BL/6 mice. (B) BMDCs from C57BL/6 mice were stimulated for 24 h with medium alone (CTRL), TcES, LPS or TcES + LPS and co-cultured with $CD4^+$ cells from naïve BALB/c mice. Allogenic stimulation capacity was tested using different numbers of BMDCs cultured with 10^5 allogenic $CD4^+$ T cells for 3 days and ^{3}H-thymidine was added for the last 18 h of culture. Data are representative of two independent experiments, $^*p < 0.05$ (Student's t test).

helminths, such as nematodes, trematodes and cestodes (Reyes and Terrazas, 2007). However, divergent roles have been reported for these types of regulatory cells (Reyes and Terrazas, 2007). For example, lung fibrosis caused by *N. brasiliensis* infection has been associated with the presence of AAMϕ (Marsland et al., 2008), whereas in *H. polygyrus* infection a protective role has been proposed (Anthony et al., 2007). In contrast, in filariasis (Taylor et al., 2006) and *Hymenolepis diminuta* infection (Persaud et al.,

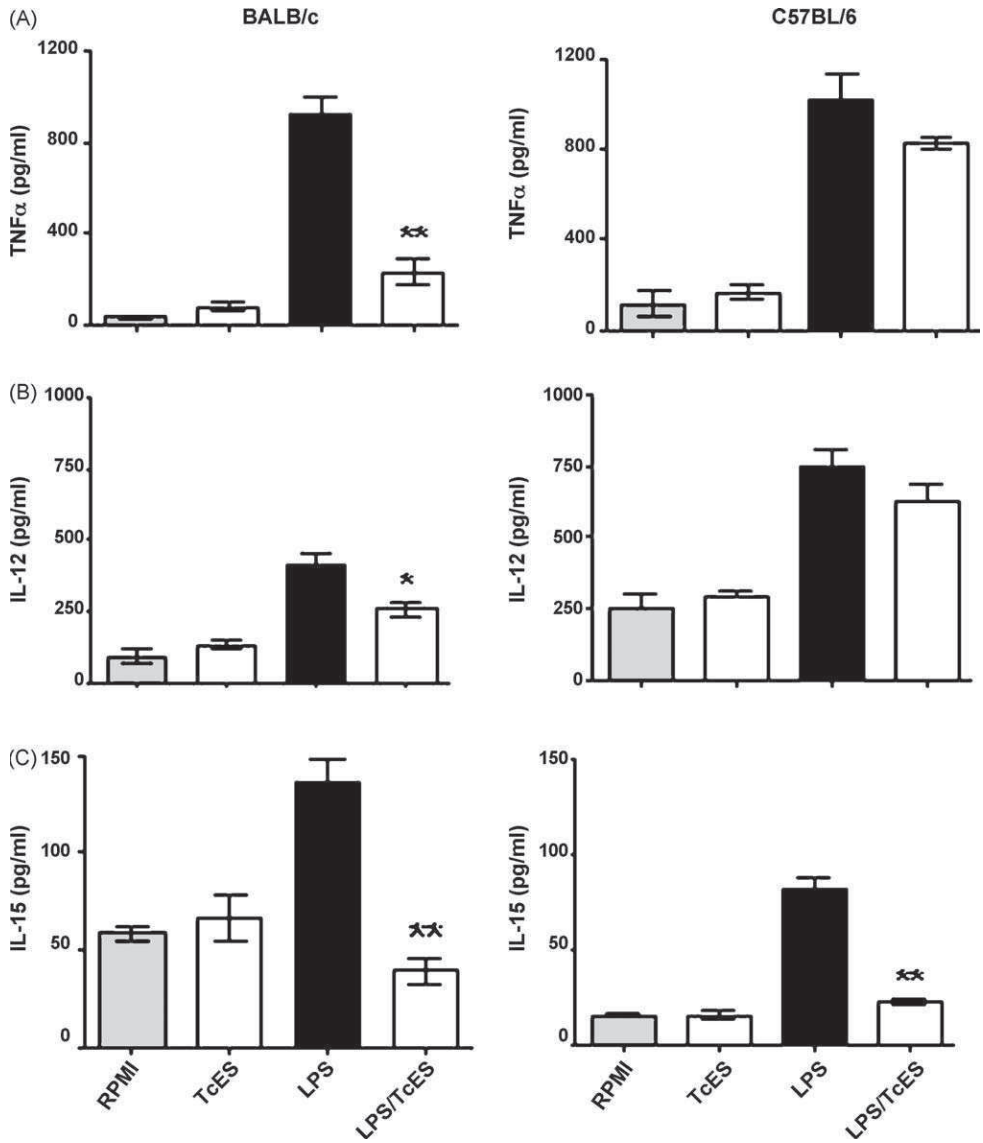


Fig. 7. Differential effects of TcES exposure on the cytokine production by LPS-stimulated DCs. Effect of TcES (20 μg/ml) on the production of TNF-α (A), IL-12 (B) and IL-15 (C) by C57BL/6 and BALB/c BMDCs stimulated with 1 μg/ml LPS. The data are representative of two independent experiments. An asterisk indicates a statistically significant differences ($p < 0.05$).

2007) the functional role for AAMϕ is still not fully understood, but AAMϕ have been associated with suppressive activity. Furthermore, AAMϕ are necessary to avoid exacerbated pathology in experimental schistosomiasis (Herbert et al., 2004). In this study, the suppressive activity of AAMϕ from *T. crassiceps*-infected BALB/c mice suggests an important role for these regulatory cells in experimental cysticercosis, such suppressive activity was associated with a higher expression of PD-L1 and PD-L2 which is in line with recent observations in this model (Terrazas et al., 2005) and in *N. brasiliensis* infection (Siracusa et al., 2008). In contrast, macrophages from C57BL/6 mice did not show suppressive activity, even though they generated higher levels of NO, which has been proposed as a molecule involved in the down-regulation of T cell proliferation in other helminthic infections (Atochina et al., 2001). Our finding suggest that *T. crassiceps* infection leads to the modulation of classically activated macrophages (CAM) in C57BL/6 mice, and alternatively activated macrophages in BALB/c mice, seems that the early predominant macrophage population, AAMϕ or CAM could be important in determining the outcome of *T. crassiceps* infection.

Here we also compared the ability of DCs from BALB/c and C57BL/6 mice to up-regulate maturation markers upon exposure to *Taenia* antigens and LPS activation. We found that the response of C57BL/6 DCs to TcES + LPS was associated with an increase in the production of TNF-α and IL-12 and normal maturation. However, the activation of BALB/c DCs in the presence of TcES was associated with a decrease in the expression of CD86, CD80, and CD40 as well as a lower production of IL-12, TNF-α and IL-15. It is known that for an optimal immune response DCs should not only effectively load up and process antigen and produce cytokines, but they also need to migrate to lymph nodes to stimulate T cells, interestingly, after exposure to TcES LPS-stimulated BALB/c DCs displayed a down-regulated expression of CCR7, a chemokine receptor that favors cell migration (Ohl et al., 2004). This may impair the ability of DCs to migrate from the peripheral tissue to the draining lymph nodes or spleen in order to activate T cells (Ohl et al., 2004). Furthermore, this combination of low costimulatory molecule expression, the low pro-inflammatory cytokine production and may be the decreased ability to migrate of the DCs may favor parasite persistence in BALB/c mice, probably by biasing towards Th2 responses given the low production of IL-12 or by inducing a state of "tolerance" given the impaired maturation observed. Moreover, the fact that BALB/c DCs exposed to TcES + LPS displayed a low ability to induce an allogenic response implies that *Taenia* antigens appear to directly induce a state of DC hyporesponsiveness in BALB/c mice, but not in C57BL/6 mice. Whether all these findings are exclusively a result of the H2 haplotype differences is unknown. Taken together our data on APCs suggest that defective macrophage and DC activation may contribute to *T. crassiceps* susceptibility.

Even though previous studies have associated the MHC-II haplotype H-2^d with susceptibility and the haplotype H-2^b with resistance to cysticercosis (Sciutto et al., 1991), the correlation between resistance, DC activity, Th1 development and the absence of AAMϕ suggests that inflammatory responses are an important component of the effector mechanisms that limit larval growth, which is difficult to associate with a given H2 haplotype. In support of this, susceptible BALB/c mice lacking the STAT6 gene were resistant to this infection (Rodriguez-Sosa et al., 2002a,b), despite the presence of the H-2^d haplotype. Furthermore, C57BL/6 mice lacking the STAT4 gene were highly susceptible to *T. crassiceps* infection (Rodriguez-Sosa et al., 2004). In further support, the neutralization of IFN-γ and the inhibition of iNOS *in vivo* results in greater susceptibility to *T. crassiceps* infection (Terrazas et al., 1999; Alonso-Trujillo et al., 2007). Therefore, a more complex relationship among several factors, such as host genes, the site of infection, the immune response, the parasite's genome (Hinojosa-Juarez et al., 2008) and co-evolution, appears to be involved in the outcome of this particular parasitic infection.

In summary, the results presented here demonstrate that the immune response to *T. crassiceps* differs between susceptible and resistant strains of mice. C57BL/6 mice predominantly produced a more sustained Th1-associated IFN-γ response, their DCs were refractory to modulation by *Taenia* antigens, recruited CAMϕ to the site of infection and effectively controlled the parasite growth following infection with *T. crassiceps*. On the other hand, BALB/c mice mounted an IL-13-associated Th2-type response, their DC activity was highly altered by exposure to TcES, recruited high numbers of AAMϕ to the site of infection and developed larger parasite loads.

Acknowledgments

This work was supported by grant # 60956-M from CONACYT and grant IN208706 from PAPIIT-UNAM, and it is part of the requirements to obtain the PhD degree in the Postgraduate Program in Biomedical Sciences, Facultad de Medicina, UNAM, for J.L.R. and C.A.T. who contributed equally to this work and were supported by a fellowship from CONACYT-Mexico. We thank to MVZ Leticia Flores and Tomas Villamar for their excellent care of animals, as well as Irma Rivera-Montoya for her technical assistance.

References

Allen, J.E., Loke, P., 2001. Divergent roles for macrophages in lymphatic filariasis. Parasite Immunol. 23, 345–352.

Alonso-Trujillo, J., Rivera-Montoya, I., Rodriguez-Sosa, M., Terrazas, L.I., 2007. Nitric oxide contributes to host resistance against experimental Taenia crassiceps cysticercosis. Parasitol. Res. 100, 1341–1350.

Anthony, R.M., Rutitzky, L.I., Urban Jr., J.F., Stadecker, M.J., Gause, W.C., 2007. Protective immune mechanisms in helminth infection. Nat. Rev. Immunol. 7, 975–987.

Atochina, O., Daly-Engel, T., Piskorska, D., McGuire, E., Harn, D.A., 2001. A schistosome-expressed immunomodulatory glycoconjugate expands peritoneal Gr1(+) macrophages that suppress naive CD4(+) T cell proliferation via an IFN-gamma and nitric oxide-dependent mechanism. J. Immunol. 167, 4293–4302.

Finkelman, F.D., Shea-Donohue, T., Morris, S.C., Gildea, L., Strait, R., Madden, K.B., Schopf, L., Urban Jr., J.F., 2004. Interleukin-4- and interleukin-13-mediated host protection against intestinal nematode parasites. Immunol. Rev. 201, 139–155.

Fux, B., Rodrigues, C.V., Portela, R.W., Silva, N.M., Su, C., Sibley, D., Vitor, R.W., Gazzinelli, R.T., 2003. Role of cytokines and major histocompatibility complex restriction in mouse resistance to infection with a natural recombinant strain (type I-III) of *Toxoplasma gondii*. Infect. Immun. 71, 6392–6401.

Gause, W.C., Urban Jr., J.F., Stadecker, M.J., 2003. The immune response to parasitic helminths: insights from murine models. Trends Immunol. 24, 269–277.

Ghassabeh, G.H., De Baetselier, P., Brys, L., Noel, W., Van Ginderachter, J.A., Meerschaut, S., Beschin, A., Brombacher, F., Raes, G., 2006. Identification of a common gene signature for type II cytokine-associated myeloid cells elicited in vivo in different pathologic conditions. Blood 108, 575–583.

Goodridge, H.S., Marshall, F.A., Wilson, E.H., Houston, K.M., Liew, F.Y., Harnett, M.M., Harnett, W., 2004. In vivo exposure of murine dendritic cell and macrophage bone marrow progenitors to the phosphorylcholine-containing filarial nematode glycoprotein ES-62 polarizes their differentiation to an anti-inflammatory phenotype. Immunology 113, 491–498.

Goodridge, H.S., Wilson, E.H., Harnett, W., Campbell, C.C., Harnett, M.M., Liew, F.Y., 2001. Modulation of macrophage cytokine production by ES-62, a secreted product of the filarial nematode Acanthocheilonema viteae. J. Immunol. 167, 940–945.

Heldwein, K., Biedermann, H.G., Hamperl, W.D., Bretzel, G., Loscher, T., Laregina, D., Frosch, M., Buttner, D.W., Tappe, D., 2006. Subcutaneous *Taenia crassiceps* infection in a patient with non-Hodgkin's lymphoma. Am. J. Trop. Med. Hyg. 75, 108–111.

Herbert, D.R., Holscher, C., Mohrs, M., Arendse, B., Schwegmann, A., Radwanska, M., Leeto, M., Kirsch, R., Hall, P., Mossmann, H., Claussen, B., Forster, I., Brombacher, F., 2004. Alternative macrophage activation is essential for survival during schistosomiasis and downmodulates T helper 1 responses and immunopathology. Immunity 20, 623–635.

Hinojosa-Juarez, A.C., Sandoval-Balanzario, M., McManus, D.P., Monroy-Ostria, A., 2008. Genetic similarity between cysticerci of *Taenia solium* isolated from human brain and from pigs. Infect. Genet. Evol. 8, 653–656.

Kim, Y., Sato, K., Asagiri, M., Morita, I., Soma, K., Takayanagi, H., 2005. Vontribution of nuclear factor of activated T cells c1 to the transcriptional control of immunoreceptor osteoclast-associated receptor but not triggering receptor expressed by myeloid cells-2 during osteoclastogenesis. J. Biol. Chem. 280, 32905–32913.

Le Goff, L., Lamb, T.J., Graham, A.L., Harcus, Y., Allen, J.E., 2002. IL-4 is required to prevent filarial nematode development in resistant but not susceptible strains of mice. Int. J. Parasitol. 32, 1277–1284.

Lee, H.K., Iwasaki, A., 2007. Innate control of adaptive immunity: dendritic cells and beyond. Semin. Immunol. 19, 48–55.

Lutz, M.B., Kukutsch, N., Ogilvie, A.L., Rössner, S., Koch, F., Romani, N., Schuler, G., 1999. An advanced culture method for generating large quantities of highly pure dendritic cells from mouse bone marrow. J. Immunol. Methods. 223, 77–92.

Maizels, R.M., Balic, A., Gomez-Escobar, N., Nair, M., Taylor, M.D., Allen, J.E., 2004. Helminth parasites—masters of regulation. Immunol. Rev. 201, 89–116.

Maizels, R.M., Yazdanbakhsh, M., 2003. Immune regulation by helminth parasites: cellular and molecular mechanisms. Nat. Rev. Immunol. 3, 733–744.

Marsland, B.J., Kurrer, M., Reissmann, R., Harris, N.L., Kopf, M., 2008. *Nippostrongylus brasiliensis* infection leads to the development of emphysema associated with the induction of alternatively activated macrophages. Eur. J. Immunol. 38, 479–488.

McKay, D.M., Khan, W.I., 2003. STAT-6 is an absolute requirement for murine rejection of *Hymenolepis diminuta*. J. Parasitol. 89, 188–189.

Migliorini, P., Corradin, G., Corradin, S.B., 1991. Macrophage NO_2-production as a sensitive and rapid assay for the quantitation of murine IFN-gamma. J. Immunol. Methods 139, 107–114.

Ohl, L., Mohaupt, M., Czeloth, N., Hintzen, G., Kiafard, Z., Zwirner, J., Blankenstein, T., Henning, G., Forster, R., 2004. CCR7 governs skin dendritic cell migration under inflammatory and steady-state conditions. Immunity 21, 279–288.

Patel, N., Kreider, T., Urban Jr., J.F., Gause, W.C., 2009. Characterisation of effector mechanisms at the host:parasite interface during the immune response to tissue-dwelling intestinal nematode parasites. Int. J. Parasitol. 39, 13–21.

Persaud, R., Wang, A., Reardon, C., McKay, D.M., 2007. Characterization of the immuno-regulatory response to the tapeworm *Hymenolepis diminuta* in the non-permissive mouse host. Int. J. Parasitol. 37, 393–403.

Renshaw, M., Rockwell, J., Engleman, C., Gewirtz, A., Katz, J., Sambhara, S., 2002. Cutting edge: impaired Toll-like receptor expression and function in aging. J. Immunol. 169, 4697–4701.

Reyes, J.L., Terrazas, L.I., 2007. The divergent roles of alternatively activated macrophages in helminthic infections. Parasite Immunol. 29, 609–619.

Rodriguez-Sosa, M., Rivera-Montoya, I., Espinoza, A., Romero-Grijalva, M., Lopez-Flores, R., Gonzalez, J., Terrazas, L.I., 2006. Acute cysticercosis favours rapid and more severe lesions caused by *Leishmania major* and *Leishmania mexicana* infection, a role for alternatively activated macrophages. Cell Immunol. 242, 61–71.

Rodriguez-Sosa, M., David, J.R., Bojalil, R., Satoskar, A.R., Terrazas, L.I., 2002a. Cutting edge: susceptibility to the larval stage of the helminth parasite Taenia crassiceps is mediated by Th2 response induced via STAT6 signaling. J. Immunol. 168, 3135–3139.

Rodriguez-Sosa, M., Saavedra, R., Tenorio, E.P., Rosas, L.E., Satoskar, A.R., Terrazas, L.I., 2004. A STAT4-dependent Th1 response is required for resistance to the helminth parasite *Taenia crassiceps*. Infect. Immun. 72, 4552–4560.

Rodriguez-Sosa, M., Satoskar, A.R., Calderon, R., Gomez-Garcia, L., Saavedra, R., Bojalil, R., Terrazas, L.I., 2002b. Chronic helminth infection induces alternatively activated macrophages expressing high levels of CCR5 with low interleukin-12 production and Th2-biasing ability. Infect. Immun. 70, 3656–3664.

Rodriguez-Sosa, M., Satoskar, A.R., David, J.R., Terrazas, L.I., 2003. Altered T helper responses in CD40 and interleukin-12 deficient mice reveal a critical role for Th1 responses in eliminating the helminth parasite *Taenia crassiceps*. Int. J. Parasitol. 33, 703–711.

Sacks, D., Noben-Trauth, N., 2002. The immunology of susceptibility and resistance to *Leishmania major* in mice. Nat. Rev. Immunol. 2, 845–858.

Saeftel, M., Arndt, M., Specht, S., Volkmann, L., Hoerauf, A., 2003. Synergism of gamma interferon and interleukin-5 in the control of murine filariasis. Infect. Immun. 71, 6978–6985.

Sciutto, E., Fragoso, G., Baca, M., De la Cruz, V., Lemus, L., Lamoyi, E., 1995. Depressed T-cell proliferation associated with susceptibility to experimental *Taenia crassiceps* infection. Infect. Immun. 63, 2277–2281.

Sciutto, E., Fragoso, G., Diaz, M.L., Valdez, F., Montoya, R.M., Govezensky, T., Lomeli, C., Larralde, C., 1991. Murine *Taenia crassiceps* cysticercosis: H-2 complex and sex influence on susceptibility. Parasitol. Res. 77, 243–246.

Sharpe, A.H., Wherry, E.J., Ahmed, R., Freeman, G.J., 2007. The function of programmed cell death 1 and its ligands in regulating autoimmunity and infection. Nat. Immunol. 8, 239–245.

Siracusa, M.C., Reece, J.J., Urban Jr., J.F., Scott, A.L., 2008. Dynamics of lung macrophage activation in response to helminth infection. J. Leukoc. Biol. 84, 1422–1433.

Smith, P., Walsh, C.M., Mangan, N.E., Fallon, R.E., Sayers, J.R., McKenzie, A.N., Fallon, P.G., 2004. *Schistosoma mansoni* worms induce anergy of T cells via selective up-regulation of programmed death ligand 1 on macrophages. J. Immunol. 173, 1240–1248.

Suzuki, L.A., Arruda, G.C., Quagliato, E.M., Rossi, Q.L., 2007. Evaluation of *Taenia solium* and *Taenia crassiceps* cysticercal antigens for immunodiagnosis of neurocysticercosis using ELISA on cerebrospinal fluid samples. Rev. Soc. Bras. Med. Trop. 40, 152–155.

Taylor, M.D., Harris, A., Nair, M.G., Maizels, R.M., Allen, J.E., 2006. F4/80+ alternatively activated macrophages control CD4+ T cell hyporesponsiveness at sites peripheral to filarial infection. J. Immunol. 176, 6918–6927.

Terrazas, L.I., Bojalil, R., Govezensky, T., Larralde, C., 1998. Shift from an early protective Th1-type immune response to a late permissive Th2-type response in murine cysticercosis (*Taenia crassiceps*). J. Parasitol. 84, 74–81.

Terrazas, L.I., Cruz, M., Rodriguez-Sosa, M., Bojalil, R., Garcia-Tamayo, F., Larralde, C., 1999. Th1-type cytokines improve resistance to murine cysticercosis caused by *Taenia crassiceps*. Parasitol. Res. 85, 135–141.

Terrazas, L.I., Montero, D., Terrazas, C.A., Reyes, J.L., Rodriguez-Sosa, M., 2005. Role of the programmed Death-1 pathway in the suppressive activity of alternatively activated macrophages in experimental cysticercosis. Int. J. Parasitol. 35, 1349–1358.

Terrazas, L.I., 2008. The complex role of pro- and anti-inflammatory cytokines in cysticercosis: immunological lessons from experimental and natural hosts. Curr. Top. Med. Chem. 8, 383–392.

Tuncman, G., Hirosumi, J., Solinas, G., Chang, L., Karin, M., Hotamisligil, G.S., 2006. Functional in vivo interactions between JNK1 and JNK2 isoforms in obesity and insulin resistance. Proc. Natl. Acad. Sci. U S A. 103, 10741–10746.

Ulett, G.C., Ketheesan, N., Hirst, R.G., 2000. Cytokine gene expression in innately susceptible BALB/c mice and relatively resistant C57BL/6 mice during infection with virulent Burkholderia pseudomallei. Infect. Immun. 68, 2034–2042.

Volkmann, L., Bain, O., Saeftel, M., Specht, S., Fischer, K., Brombacher, F., Matthaei, K.I., Hoerauf, A., 2003. Murine filariasis: interleukin 4 and interleukin 5 lead to containment of different worm developmental stages. Med. Microbiol. Immunol. 192, 23–31.

Yoshida, A., Maruyama, H., Kumagai, T., Amano, T., Kobayashi, F., Zhang, M., Himeno, K., Ohta, N., 2000. Schistosoma mansoni infection cancels the susceptibility to Plasmodium chabaudi through induction of type 1 immune responses in A/J mice. Int. Immunol. 12, 1117–1125.

International Journal for Parasitology 40 (2010) 1051–1062

Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

Impaired pro-inflammatory cytokine production and increased Th2-biasing ability of dendritic cells exposed to *Taenia* excreted/secreted antigens: A critical role for carbohydrates but not for STAT6 signaling

César A. Terrazas [a], Lorena Gómez-García [b], Luis I. Terrazas [a,*]

[a] Unidad de Biomedicina, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México, Mexico
[b] Departamento de Inmunología, Instituto Nacional de Cardiología, México, D.F., Mexico

ARTICLE INFO

Article history:
Received 2 January 2010
Received in revised form 23 February 2010
Accepted 24 February 2010

Keywords:
Taenia crassiceps
Dendritic cells
Excreted/secreted helminth antigens
Modulation of immune response

ABSTRACT

In cysticercosis, a parasitic disease caused by cestodes, the details of early interactions between parasite antigens and innate cells from the host are not well understood. In this study, the role of cestode-conditioned dendritic cells (DCs) in priming Th1 versus Th2 responses to bystander antigen was examined by using $CD11c^+$ DCs as antigen-presenting cells and naive $CD4^+$ DO11.10 lymphocytes specific to ovalbumin (OVA) as responding cells. No conventional maturation was induced in DCs exposed to *Taenia crassiceps* excreted/secreted antigens (*Tc*ES). The ability of *Tc*ES to affect Toll-like receptor (TLR)-mediated maturation and the pro-inflammatory response was analyzed by co-pulsing DCs with *Tc*ES and TLR ligands. DCs exposed to *Tc*ES blocked TLR4, TLR9 and *Toxoplasma* soluble antigen-induced phenotypic maturation. *Tc*ES-exposed DCs also blocked secretion of pro-inflammatory cytokines and alloreactive T cell proliferation, while preserving IL-10 production. DCs pulsed with *Tc*ES + OVA suppressed IFN-γ, whereas they induced greater IL-4 production by $CD4^+$ DO11.10 cells. *Tc*ES with chemically-altered glycans failed to modulate TLR-mediated activation of DCs and their Th1-inhibitng ability, which was STAT6-independent. Our results reflect the capacity of *Tc*ES glyco-antigens to modulate Th1-type and inflammatory responses mediated through DC activation.

1. Introduction

Helminths and their antigens are now recognized as possessing important immunomodulatory activities, but how helminth infections cause these immuno-regulatory events remains unclear (Maizels and Yazdanbakhsh, 2003). A key feature of helminth infections is the induction of Th2-biased immune responses in their hosts (Maizels and Yazdanbakhsh, 2003; Maizels et al., 2004), where antigen-presenting cells (APCs) may play an important role in this process (MacDonald and Maizels, 2008). Pattern recognition receptors (PRRs) of dendritic cells (DCs) recognize and bind to conserved pathogen motifs such as lipopolysaccharide (LPS) in Gram-negative bacteria, flagellin, RNA of viruses and several molecules in intracellular protozoa (Akira and Takeda, 2004), including cyclophilin from *Toxoplasma gondii* (Aliberti et al., 2003) and glycoinositolphospholipids (GIPL) from *Trypanosoma cruzi* (Brodskyn et al., 2002). Among PRRs, the Toll-like receptor (TLR) family is the main group of receptors known to be involved in maturation and induction of inflammatory cytokines in DCs (Akira and Takeda, 2004). Most of the currently known pathogen molecules recognized by TLRs are primarily found in intracellular pathogens capable of instructing DCs to release pro-inflammatory cytokines and drive Th1 responses (Akira and Takeda, 2004). In contrast, the results of interactions between helminths and the innate immune system are not well understood. However, some advances in this area indicating that helminth extracts may instruct Th2 responses have been reached through studies on nematodes (Holland et al., 2000; Whelan et al., 2000; Pinelli et al., 2007) and trematodes (Pearce et al., 2004), whereas the interactions of cestode antigens with the innate immune system and their role in Th2 polarization has been largely neglected (Maizels, 2009).

We have investigated the immunomodulatory activities of the cestode *Taenia crassiceps*, which as in most helminth infections induces Th2-type biased immune responses (Villa and Kuhn, 1996; Terrazas et al., 1998; Toenjes et al., 1999) as well as an important anergy of splenocytes in response to both antigen-specific and polyclonal stimuli (Sciutto et al., 1995; Villa and Kuhn, 1996; Rodriguez-Sosa et al., 2003, 2004). Moreover, altered susceptibility has been observed when *T. crassiceps*-infected mice were co-infected with *T. cruzi* (Rodriguez et al., 1999), a virus (Spolski

* Corresponding author. Address: Unidad de Biomedicina, FES-Iztacala, Universidad Nacional Autónoma de México, Av. De los Barrios # 1, Los Reyes Iztacala, 54090 Tlalnepantla, Edo. de México, Mexico. Tel.: +52 55 5623 1333x39794; fax: +52 55 5623 1138.
E-mail address: literrazas@campus.iztacala.unam.mx (L.I. Terrazas).

0020-7519/$36.00
doi:10.1016/j.ijpara.2010.02.016

et al., 2002) or *Leishmania* (Rodriguez-Sosa et al., 2006). Thus, in addition to activating Th2-type biased responses, infection with *T. crassiceps* can regulate other lymphocyte functions in vivo, suggesting that this infection may induce a strong modulation of the immune response.

In an attempt to dissect mechanisms that lead to immune modulation in experimental murine cysticercosis, we examined the role of DCs as APCs in response to *T. crassiceps* excreted/secreted (*Tc*ES) antigens as well as their role in the modulation of DC responses to pro-inflammatory stimuli. In this study we found that *T. crassiceps*-derived antigens had the ability to suppress DC production of pro-inflammatory cytokines such as TNF-α, IL-12 and IL-15 in response to several inflammatory molecules. Furthermore, when *T. crassiceps*-exposed DCs were used as APCs, they suppressed IFN-γ production and increased IL-4 levels on $CD4^+$ DO11.10 cells in response to OVA peptide. Interestingly, these activities were associated with intact glycan structures but were STAT6-independent.

Our results reflect the potential ability of *T. crassiceps*-derived molecules to modulate inflammatory responses mediated through DC activation to non-related pro-inflammatory antigens. We believe this is the first time that *Taenia*-derived antigens have been shown to modulate DC activation.

2. Materials and methods

2.1. Mice

Six- to 8-week-old female BALB/cAnN and C57BL/6 mice were purchased from Harlan Laboratories (México), and DO11.10 mice transgenic for $OVA_{323–339}$-specific αβ-TCR on a BALB/c background (Jackson Labs, USA) were used as a source of antigen-specific T cells. In some experiments $STAT6^{-/-}$ mice on a BALB/c background (Jackson Labs) were used as a source of DCs.

2.2. Ethics statement

Mice were housed in clean cages (four per case) and they were maintained under pathogen-free conditions at Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México, México animal facility. They received food and water ad libitum. No pain was induced given that most of the experiments were ex vivo. Mice were sacrificed using a CO_2 chamber. Facultad de Estudios Superiores Iztacala from the Universidad Nacional Autónoma de México approved this study. These institutions were aware that the study was conducted, adhering to the institution's guidelines for animal husbandry.

2.3. Parasites and antigens

Metacestodes of *T. crassiceps* were harvested under sterile conditions from the peritoneal cavity of female BALB/c mice after 2–4 months of infection. The cysticerci were washed four times in sterile PBS and maintained in culture in PBS at 37 °C for 24 h. *Tc*ES were recovered from the supernatant and centrifuged for 10 min at 1000*g*. Next, the protein was concentrated by using Amicon Ultra Filter with a 50 kDa membrane (Millipore), protease inhibitors were added and samples were stored at −70 °C until further use. In some experiments *Tc*ES was deglycosilated using metaperiodate treatment and tested for the presence of glycans as previously described (Gomez-Garcia et al., 2005). Briefly, SDS–PAGE and lectin blotting were performed using standard techniques. Antigen extracts in non-reducing sample buffer were boiled for 5 min at 95 °C and separated on 7.5% polyacrylamide gel at a concentration of 10 μg/well. Separated proteins were transferred to nitrocellulose membrane (Amersham, Piscataway, NJ, USA) by using a Western blotting unit (Bio-Rad). The membrane was blocked overnight at 4 °C with 2% (w/v) BSA in PBS pH 7.2, washed thoroughly with PBS/Tween 0.1% and incubated with Con A-Peroxidase (SIGMA) for 3 h. After washing, peroxidase bound on the membrane was developed with 1:1000 PBS/H_2O_2 and diaminobenzidine at a concentration of 2 mg/mL.

2.4. Heat-inactivation of TcES products

In some experiments, and in order to negate the ability of intact proteins to activate DC, *Tc*ES products were deposited in an Eppendorf tube (0.5 ml) and induced through heat inactivation by heating on of the surface of a water bath at 100 °C for 15 min. Denaturation of proteins was confirmed by running PAGE under native conditions.

2.5. Generation of bone marrow-derived DCs (BMDCs)

DCs were obtained as previously described (Lutz et al., 1999). Briefly, bone marrow cells were isolated by flushing femurs and tibia with culture medium. Red blood cells were lysed with 0.83% ammonium chloride. $CD11c^+$ $CD11b^+$ DCs were generated using granulocyte macrophage colony stimulating factor (GM-CSF) as described by Lutz et al. (1999). In brief, bone marrow cells were plated at 1×10^6 cells/ml in medium supplemented with recombinant GM-CSF (Peprotech-México) at a final concentration of 20 ng/ml in 100 mm Petri dishes in a volume of 10 ml. On day 3, fresh medium containing GM-CSF was added. On day 5, non-adherent cells were collected, centrifuged, resuspended in fresh medium and cultured for an additional 24 h in 12-well plates with the appropriate stimuli.

2.6. In vitro stimulation of DCs and quantification of cytokine production

BMDCs were positively selected by incubation with anti-$CD11c^+$ (N418) microbeads and flowed through a Magnetic Column (Miltenyi Biotec). Cells sorted (85%) as $CD11c^+$ (5×10^5) were cultured in 500 μl medium in 12-well plates and activated with 1 μg/ml *Escherichia coli* LPS (Sigma–Aldrich), 5 μg/ml *Toxoplasma* soluble antigen (TSA) from *T. gondii* RH strain (kindly provided by Dr. Rafael Saavedra, Universidad Nacional Autónoma de México, Mexico) or 10 μg/ml of synthetic oligonucleotides containing unmethylated CpG sequences (CpG) (InvivoGen, San Diego, CA, USA). All cytokines, parasite antigens and TLR ligands, except LPS, were shown to be endotoxin-free as determined by a Limulus-amebocyte assay (Sigma). Viability of $CD11c^+$ cells was tested using 7-Amino-actinomycin D (via-probe, BD-Pharmingen, USA). Viability was above 90% after 24 h of treatment. In some experiments, 10 μg/ml rat anti-mouse IL-10 antibodies (clone 1B1.3A) were used to block IL-10 (Biolegend, San Diego, CA, USA). DCs exposed to *T. crassiceps* antigens were first incubated for less than 5 min with 20 μg/ml of ES antigens with either intact or oxidized carbohydrates with sodium periodate as previously described (Gomez-Garcia et al., 2005). After 24 h, supernatants were collected and IL-12p40, IL-12p70, IL-15 and TNF-α levels were determined using commercially available ELISA kits (Peprotech, México).

2.7. Analysis of cell surface markers in BMDCs

Surface expression of DC maturation markers was analyzed using multicolor flow cytometry. DCs (either untreated or stimulated for 24 h with LPS, LPS + *Tc*ES, CpG, CpG + *Tc*ES, TSA, TSA + *Tc*ES or *Tc*ES alone) were harvested, washed and suspended in cold PBS containing 5% FCS and 0.05% NaN_3. F_c receptors were blocked with

anti-mouse CD16/CD32 for 20 min at 4 °C. Cells were washed and triple stained with an APC-conjugated antibody against CD11c, FITC-conjugated monoclonal antibodies against CD80, CD40 or Major Histocompatibility Complex-II (MHC-II) and phycoeritrin-conjugated antibodies against CCR7, OX40L or CD86 (all antibodies from Biolegend, USA). Cells were analyzed on a FACsCalibur flow cytometer using Cell Quest software (Becton Dickinson).

2.8. Allostimulation assay

BMDCs were activated with LPS, LPS + *Tc*ES or CpG, CpG + *Tc*ES for 24 h, washed and co-cultured at DC:T cell ratios of 1:2, 1:10 and 1:20 with 5×10^5 $CD4^+$ cells enriched using a CD4-T cell isolation kit (Miltenyi Biotec, Germany) from naïve C57BL/6 mice. At 72 h, 0.5 μCi/well of [^{3}H] Thymidine (185 GBb/mmol activity, Amersham, England) was added to the co-cultures; 18 h later the cells were harvested and the incorporation of radioactivity was assessed using a 1450 microβ-plate counter (Trilux, Finland). Values are represented as counts per minute (CPM) from triplicate wells.

2.9. Stimulation of transgenic $CD4^+$ T cells for cytokine production

Naïve $CD4^+$ T cells (10^5 cells/well) were positively isolated from spleens of DO11.10 TCR transgenic mice (BALB/c) using MACS technology (Miltenyi Biotec, Germany) and were co-cultured with purified CD11c DCs (2×10^4 cells/well) in a total volume of 1 ml in 24-well plates. DCs were previously exposed to *T. crassiceps* antigens (20 μg/ml), LPS (1 μg/ml), both or unexposed. HPLC-purified OVA peptide (323–339) with the sequence ISQAVHAAHAEINEAGR-COOH was synthesized by Invitrogen, USA, added and then left for 4 days to co-cultures at 10 μg/ml, at which point supernatants were analyzed for cytokine detection.

2.10. Statistical analysis

The statistical significance of differences between treated and control DCs in cytokine release assays were calculated using the Student's *t*-test of repeated measures. The statistical significance of differences between mean fluorescence intensity expression levels detected by flow cytometry was calculated using a Mann–Whitney two-tailed test. Values were considered significant when $P < 0.05$. GraphPad Prism software was used for all statistical analyses.

3. Results

3.1. Larvae of T. crassiceps cultured in vitro secrete a range of low and high molecular weight glycoproteins

Metacestodes of *T. crassiceps* were obtained from mice at 6–8 weeks p.i. Larvae were washed extensively with endotoxin-free PBS and parasites were cultured for 24–48 h in saline. Supernatants containing ES products were collected, centrifuged and concentrated using a cut-off membrane of 50 kDa (>50 kDa). Remaining proteins were further concentrated using a 10 kDa cut-off membrane (10 to <50 kDa). Protein concentrations were determined and PAGE was performed on the two fractions. Given that the low molecular fraction did not alter DC function in response to LPS (Supplementary Fig. S1) we focused on the high molecular fraction of ES products. A lectin blot analysis was performed to determine whether ES products >50 kDa were glycoproteins. Fig. 1 shows the PAGE and lectin blot analysis of *Taenia* ES products. *T. crassiceps* secreted at least five different proteins with molecular weights ranging from 56 to 170 kDa. The ES products tested positive for lectin, indicating that they were glycoproteins.

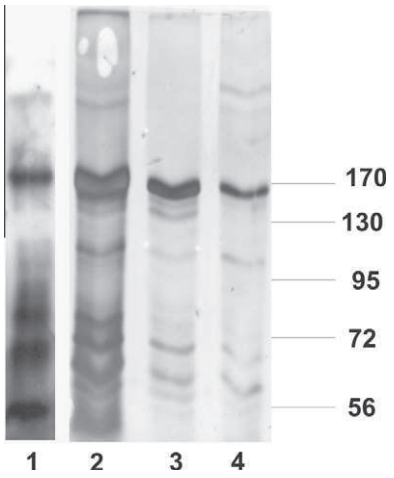


Fig. 1. Electrophoretic and lectin blot analysis of *Taenia crassiceps* excreted/secreted products (*Tc*ES). Freshly obtained cysticerci of *T. crassiceps* were cultured in the absence of sera for 24–48 h, supernatants were collected, centrifuged as described in Section 2, and processed for PAGE analysis. The same supernatants were processed for lectin blot revealed with Concanavalin-A. Most of the products detected were glycoproteins. Lane1, *Tc*ES lectin blot. Lane 2, *Tc*ES PAGE. Lane 3, *Tc*ES PAGE mock treatment. Lane 4, *Tc*ES PAGE with metaperiodate treatment. Numbers on the right indicate molecular weight.

3.2. TcES modify DC activation by partial up-regulation of MHC class II and co-stimulatory molecule expression after TLR-mediated stimuli

To evaluate the capacity of ES *Taenia* products to induce maturation of DCs, we pulsed BMDCs with different amounts of *Tc*ES (ranging from 10 to 60 μg/ml, Supplementary Fig. S2) and chose a dose of 20 μg/ml because it the minimal dose which had a significant effect on DC cytokine production. Up-regulation of MHC-II and co-stimulatory molecule expression by BMDCs was evaluated by comparison with the microbe-derived stimuli LPS, CpG and TSA which were chosen for their known ability to mature and activate DCs (Manickasingham et al., 2003; Akira and Takeda, 2004). *Tc*ES alone did not induce DC maturation, as none of the maturation markers (CD86, CD80, CD40 and CCR7) were significantly up-regulated in BMDCs (Fig. 2) and MHC-II expression was unchanged. Next, we investigated whether *Tc*ES could inhibit the maturation of DCs induced by the TLR4 and TLR9 ligands LPS and CpG, respectively. DCs were cultured in medium alone (DC-medium) or exposed to *Tc*ES, LPS, CpG or *Tc*ES + LPS, *Tc*ES + CpG for 24 h prior to analysis of maturation marker expression. As expected, LPS and CpG induced a mature phenotype in DCs with increased expression of CD40, CD86, CD80 and CCR7. However, when *Tc*ES was added at the same time as TLR-mediated stimuli the results showed that both the LPS and the CpG-induced up-regulation of CD40, CD80 and CCR7 were inhibited (30–45%), as expressed in mean fluorescence intensity (MFI), compared with DCs activated by LPS or CpG alone (Fig. 2A and B).

3.3. Allogenic DC activation is down-regulated in DCs exposed to TcES

We tested whether the alterations in expression of the surface maturation markers affected the ability of BMDCs exposed to *Tc*ES antigens to induce allogenic proliferation in a mixed lymphocyte reaction. BMDCs from BALB/c mice were exposed to *Tc*ES, CpG or CpG + *Tc*ES for 24 h and immediately co-cultured for 3 days with magnetically-purified $CD4^+$ cells isolated from spleens of healthy C57BL/6 mice. The proliferative response was analyzed using ^{3}H-thymidine uptakes. Exposure of BALB/c BMDCs to *Tc*ES significantly affected their ability to induce allogenic responses. The proliferation of $CD4^+$ cells was inhibited more than 50% compared with CpG treated BMDCs (Fig. 2C). An independent experiment was

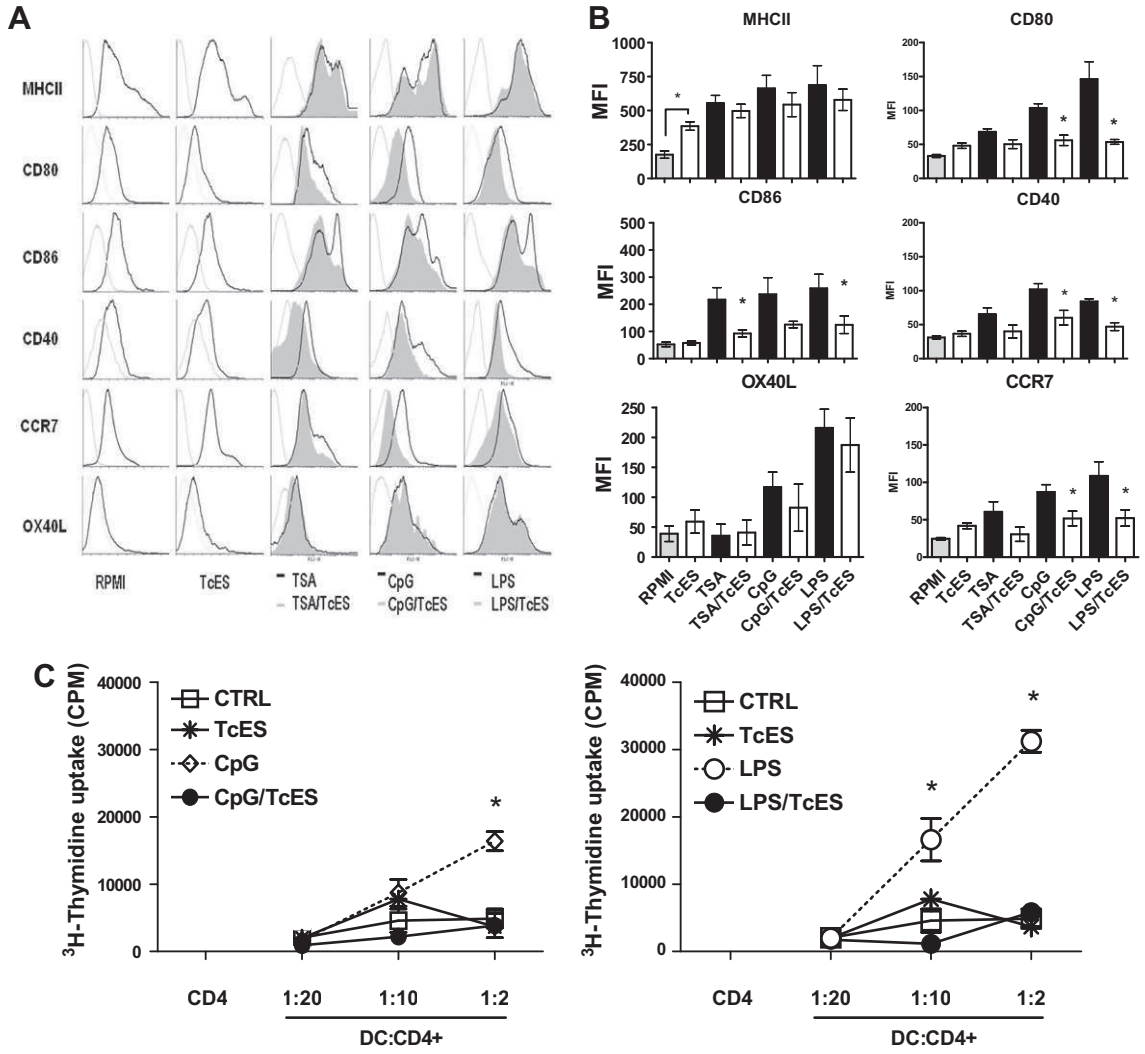


Fig. 2. *Taenia crassiceps* excreted/secreted antigens (*Tc*ES) inhibit *Toxoplasma* soluble antigen (TSA), CpG or lipopolysaccharide (LPS)-induced dendritic cell (DC) maturation and allogenic stimulation. (A) *Tc*ES does not induce maturation of immature bone marrow-derived dendritic cells (BMDCs). Immature DCs were incubated with TSA, CpG, LPS or *Tc*ES for 24 h and activation was determined by measuring the expression of CD80, CD86, CD40, OX40L and CCR7. The dotted line represents isotype controls; the dark line represents immature DCs treated with either TSA (5 μg/ml), LPS (1 μg/ml), *Tc*ES (20 μg/ml) or CpG (2 μg/ml). Filled histogram represents DCs treated with *Tc*ES plus corresponding Toll-like receptor (TLR) stimuli. One representative experiment out of three is shown. (B) *Tc*ES inhibits TSA, CpG or LPS-induced maturation as shown by lower mean fluorescence intensity (MFI). Immature DCs were treated with LPS (1 μg/ml), CpG (2 μg/ml) or TSA (5 μg/ml) in the presence or absence of *Tc*ES (20 μg/ml). Maturation was determined by measuring CD80, CD86, CD40, OX40L and CCR7expression as MFI. Error bars represent the mean ± SD of at least four independent experiments. $^{*}P < 0.05$ using Mann–Whitney two-tailed test. (C) $CD4^{+}$ spleen cells from C57BL/6 mice were co-cultured with DCs (BALB/c) exposed to *Tc*ES and TLR ligands. The proliferation of allogenic T cells was lower in the presence of immature DCs cultured with medium alone (□, CTRL) and was higher in the presence of DCs which had received a maturation stimulus such as CpG (◇) or LPS (○). DCs which were differentiated in the presence of *Tc*ES showed poor ability to induce proliferation (⁎). However, DCs stimulated with *Tc*ES + CpG or *Tc*ES + LPS demonstrated significantly reduced proliferation compared with that induced by CpG or LPS alone (●). $^{*}P < 0.05$. Data are representative of two independent experiments. CPM, counts per minute.

similarly performed using *Tc*ES, LPS or both to determine cell proliferation induced by stimulated DCs. In Fig. 2C it can be observed that DCs exposed to *Tc*ES displayed a lower allostimulation ability and significantly reduced the proliferation rate of $CD4^{+}$ cells induced by LPS-matured DCs.

3.4. TcES-pulsed BMDCs does not induce pro-inflammatory cytokines but inhibits DC activation induced by TLR ligands

We analyzed whether exposure of DCs to *Tc*ES may alter the pro-inflammatory response of those cells to TLR-mediated stimuli. The production of IL-12, TNF-α and IL-15, three key cytokines associated with inflammatory responses, was assayed. Exposure of DCs to *Tc*ES (20 μg/ml) for 24 h did not induce significant secretion of IL-12p40, IL-12p70, TNF-α or IL-15 compared with unstimulated DCs (Fig. 3). As expected, we found that BMDCs stimulated with LPS (1 μg/ml), CpG (10 μg/ml) or TSA (5 μg/ml) induced high production of the pro-inflammatory cytokines IL-12, IL-15 and TNF-α (Fig. 3). We then analyzed whether acute exposure of DCs to *Tc*ES may influence the response to TLR-mediated stimuli. BMDCs were exposed to *Tc*ES and immediately stimulated with LPS, CpG or TSA for 24 h. Exposure to *Tc*ES down-regulated the production of all pro-inflammatory cytokines tested and inhibited the response to most of the TLR ligand stimuli (Fig. 3). Interestingly, IL-10 production was unaltered in the same cultures (Fig. 3).

3.5. IL-10 apparently is not involved in down-regulation of the pro-inflammatory response of DCs exposed to TcES

Previous studies with other helminth products have proposed that IL-10 is one of the main molecules involved in the suppressive mechanism observed after helminth-DC encounters (Segura et al.,

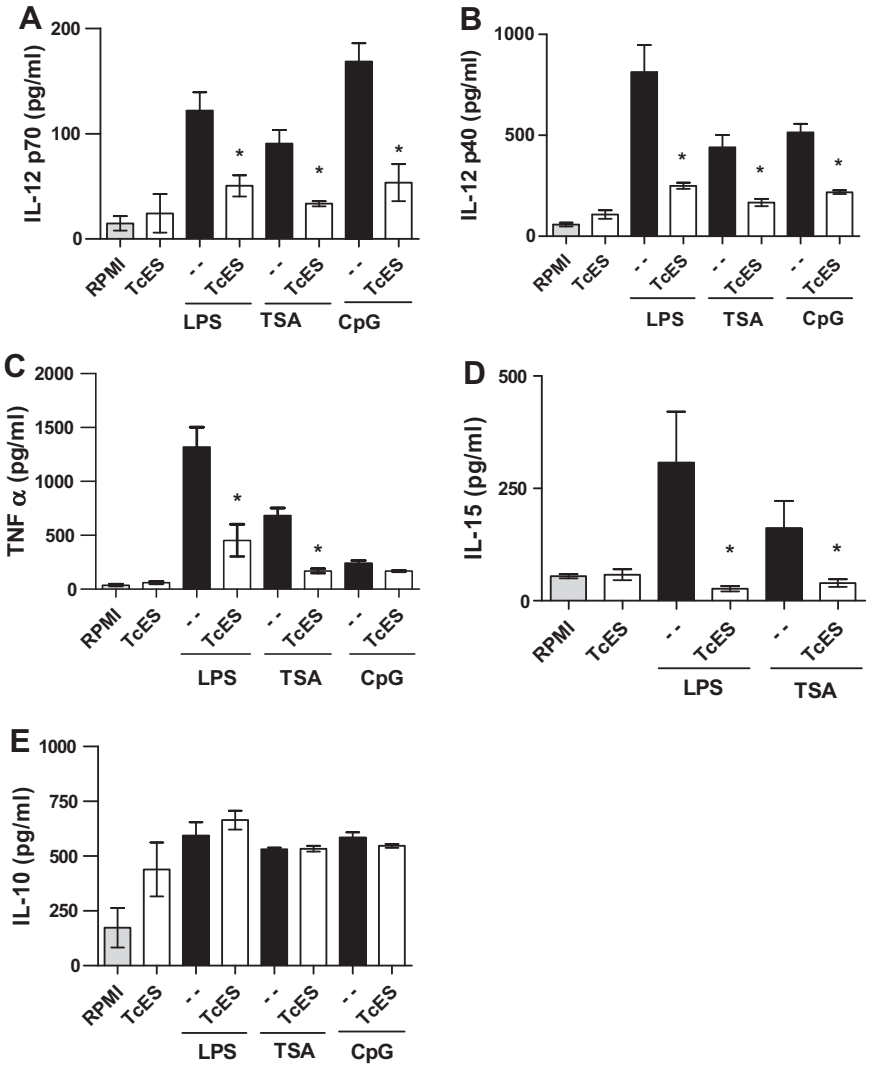


Fig. 3. Bone marrow-derived dendritic cells BMDCs exposed to *Taenia crassiceps* excretory/secretory products (*Tc*ES) show an impaired response to Toll-like receptor (TLR) stimuli. After 5 days in granulocyte macrophage colony stimulating factor (GM-CSF) enriched media BMDCs were exposed to lipopolysaccharide (LPS) (1 μg/ml), CpG (10 μg/ml), *Toxoplasma* soluble antigen (TSA) (5 μg/ml) and/or *Tc*ES (20 μg/ml) for 24 h. Supernatants were collected and analyzed by ELISA. (A) IL-12p70 production. (B) IL-12p40 production. (C) TNF-α production. (D) IL-15 production. (E) IL-10 production. *Tc*ES exposure suppressed the secretion of TNF-α, IL-12p40, IL-12p70 and IL-15 induced by different pro-inflammatory stimuli. Data are representative of three independent experiments performed in triplicate. $^{*}P < 0.05$. RPMI medium was the control. - - indicates the absence of *Tc*ES on the culture.

2007). Here we detected that IL-10 expression was maintained at a similar level under different treatments (Fig. 3E); therefore, in order to determine the role of this cytokine we developed similar experiments adding anti-IL-10 blocking antibodies (10 μg/ml) to the DC cultures. When DCs were exposed to *Tc*ES plus LPS and IL-10 was blocked by monoclonal antibodies the down-regulation of IL-12 and IL-15 production was not reversed (Table 1), indicating that IL-10 secretion might not mediate the main effects of *Tc*ES on DC activation.

3.6. Heat-inactivated TcES maintain the ability of modulate BMDC responses

Previous work on other helminths has suggested that proteins secreted by these parasites may be involved in immunomodulation (Balic et al., 2004). Thus, we sought some putative molecules involved in antigen modulation of DCs. We heated *Tc*ES (h*Tc*ES) to 100 °C for 15 min in order to denaturize the protein content. Then, the effect of h*Tc*ES was tested for down-regulation after LPS + h*Tc*ES treatment in both membrane markers and in cytokine production. As observed in Fig. 4, h*Tc*ES still maintained the ability to modulate TLR-induced maturation of DCs. Similarly, pro-inflammatory cytokines such as IL-12 and TNF-α were significantly down-regulated by the addition of h*Tc*ES to BMDC cultures

Table 1
Effect of IL-10 blockade on cytokine response of bone marrow-derived dendritic cells (BMDCs) exposed to *Taenia crassiceps* excreted/secreted glycoproteins (*Tc*ES).

Treatment	IL-12p40		IL-15	
	Isotype	α IL-10	Isotype	α IL-10
RPMI	66 ± 5	62 ± 3	55 ± 5	39 ± 9
*Tc*ES	107 ± 21	69 ± 33	58 ± 12	46 ± 10
LPS	813 ± 134	759 ± 81	308 ± 113	128 ± 15
LPS/*Tc*ES	250 ± 15[a]	72 ± 41[a]	28 ± 6[a]	41 ± 5[a]
TSA	440 ± 61	410 ± 38	192 ± 52	101 ± 9
TSA/*Tc*ES	167 ± 18[a]	280 ± 15	39 ± 8[a]	50 ± 22

Data are given as mean (pg ± SD) of triplicate cultures and are representative of two independent experiments ([a]$P < 0.05$). Similar results were obtained using anti-IL-10R antibodies (data not shown). LPS, lipopolysaccharide; TSA, *Toxoplasma* soluble antigen.

(Fig. 4). Thus, it appears that intact proteins on *Tc*ES were not essential for its immunomodulatory activity.

3.7. A critical role for intact glycans on TcES-mediated BMDC modulation in response to TLR ligands and to biased Th2 responses in vitro

Antigens from *T. crassiceps* carrying glycans have been shown to be necessary for inducing Th2 responses in vivo (Gomez-Garcia et al., 2006). This type of activity has also been observed in other helminth-derived molecules such as soluble extracts of *Brugia malayi* (Tawill et al., 2004) and schistosome egg antigen (SEA) of *Schistosoma mansoni* (Okano et al., 1999). It is probable that these glycan structures are recognized through DCs and confer the ability to DCs to prime Th2 responses or to inhibit pro-inflammatory responses mediated by TLR stimulus (Geijtenbeek and Gringhuis, 2009). To address the possibility that glycans in *Tc*ES may modify the TLR-mediated response in DCs as well as biasing a Th2 response in vitro, we pulsed DCs with *Tc*ES or with this antigen previously treated with metaperiodate (p*Tc*ES) and DCs were immediately stimulated with LPS or TSA. We found that neither *Tc*ES nor p*Tc*ES were able to induce IL-12 or TNF-α in DCs (Fig. 5). When DCs were incubated with p*Tc*ES plus LPS or TSA, BMDCs maintained their ability to respond to these TLR ligands with high IL-12 and TNF-α production (Fig. 5A–D), but BMDCs exposed to intact *Tc*ES or their control mock-treated *Tc*ES again had reduced production of these pro-inflammatory cytokines (Fig. 5A–D). Therefore, the ability of *Tc*ES to decrease the response to TLR ligands on DCs was abrogated in the absence of intact glycans.

To test whether the carbohydrates present in *Tc*ES glycoproteins were required in an in vitro-induced Th2-type response, BMDCs were loaded with OVA in the presence of *Tc*ES, p*Tc*ES and/or LPS or TSA for 24 h and then co-cultured with naive $CD4^+$ from naïve DO11.10 mice. After 4 days, culture supernatants were collected and cytokines analyzed by ELISA. Interestingly, DCs pulsed with intact *Tc*ES were able to prime IL-4 (Fig. 6) and IL-5 (data not shown) production by $CD4^+$ in response to OVA. Conversely, DCs stimulated with metaperiodate treated *Tc*ES were poor IL-4 and IL-5 inducers. In contrast, intact *Tc*ES abrogated LPS and TSA-induced IFN-γ production in $CD4^+$ DO11.10 cells, and this effect was not observed in the absence of intact glycans in p*Tc*ES (Fig. 6).

3.8. Absence of signal transducer and activator of transcription 6 (STAT6) does not alter the effect of TcES on DCs

Recently it has been demonstrated that other cell types exist besides T cells or DCs, such as basophils and mast cells (Kuroda

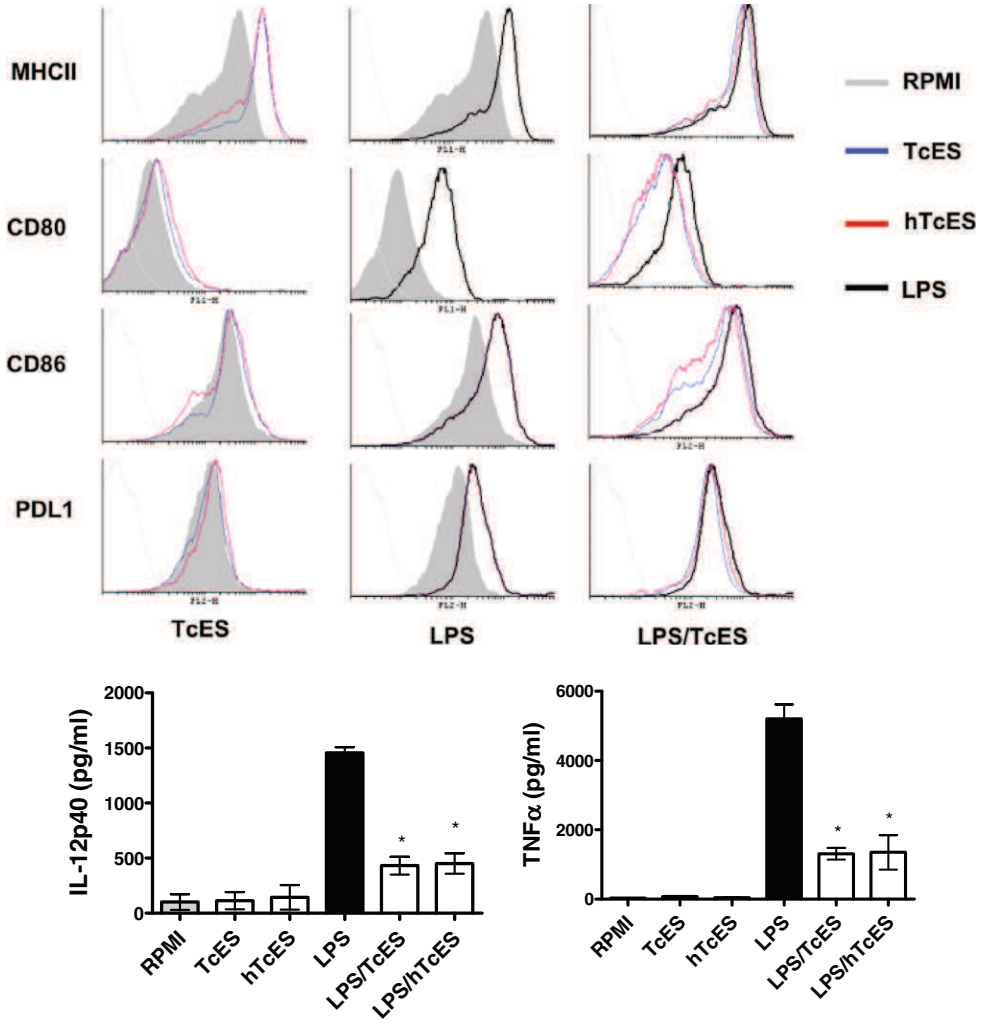


Fig. 4. Heat-inactivated *Taenia crassiceps* excretory/secretory product (*Tc*ES) maintain the ability of modulate bone marrow-derived dendritic cells (BMDCs) responses. *Tc*ES was heated for 15 min at 100 °C (h*Tc*ES) and their ability to modulate surface markers on BMDCs (flow cytometry) as well as IL-12 and TNF-α production (ELISA) in response to lipopolysaccharide (LPS) was analyzed. Data are representative of two independent experiments performed in triplicate. $^*P < 0.05$. RPMI medium was the non-stimulated control culture. MHC-II, major histocompatibility complex-II; PDL1, pathway death ligand-1.

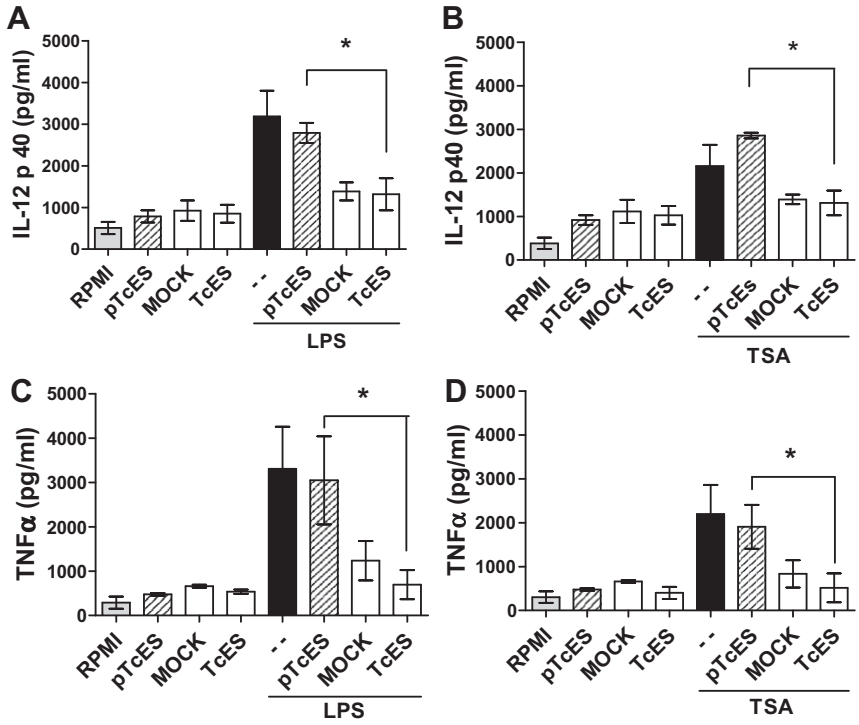


Fig. 5. Carbohydrates present in *Taenia crassiceps* excretory/secretory products (*Tc*ES) are necessary to decrease lipopolysaccharide (LPS) and *Toxoplasma* soluble antigen (TSA)-induced IL-12 and TNF-α production. *Tc*ES was treated with metaperiodate to oxidize the carbohydrates (p*Tc*ES). Dendritic cells (DCs) were exposed to the respective Toll-like receptor (TLR) stimuli plus *Tc*ES or p*Tc*ES. While *Tc*ES down-regulated IL-12p40 (A) and (B), as well as TNF-α (C) and (D) production, periodate-treated *Tc*ES were incapable of down-modulating TLR responses. $^{*}P < 0.05$ using Student's *t*-test. RPMI medium was the non-stimulated control. - - Indicates the absence of *Tc*ES on the culture.

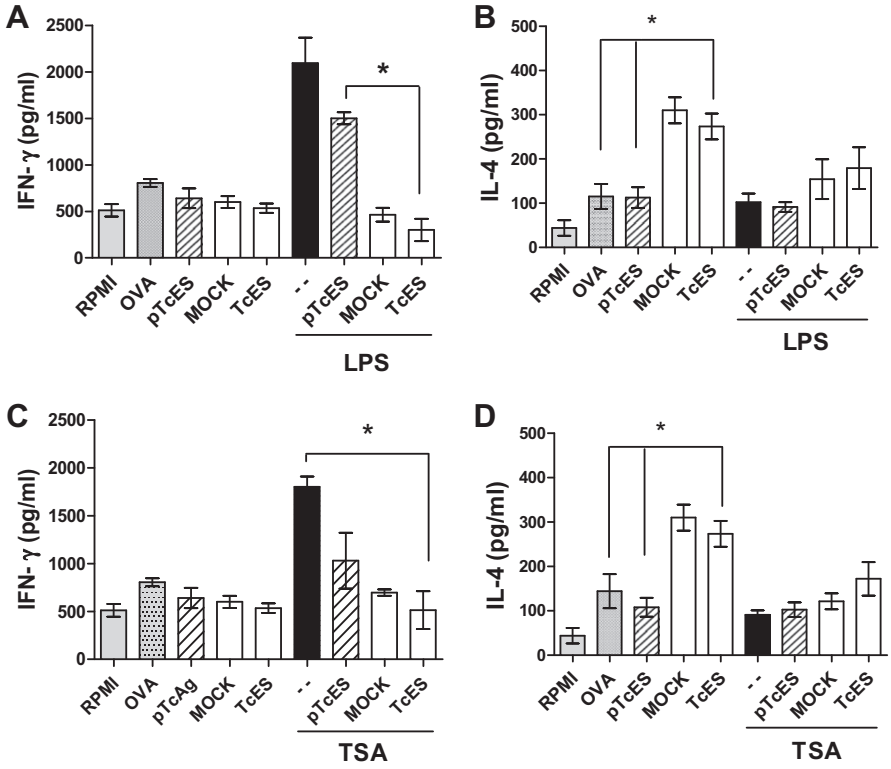


Fig. 6. Defective ability for Th2 cell differentiation of *Taenia crassiceps* excretory/secretory products (*Tc*ES) with altered glycans. (A) DO11.10 $CD4^{+}$ cells were isolated and stimulated with bone marrow-derived dendritic cells (BMDCs) pulsed with ovalbumin peptide (OVA) + *Tc*ES, OVA + lipopolysaccharide (LPS), OVA + periodate-treated *Tc*ES (p*Tc*ES) for 3 days. Supernatants were collected and assayed by ELISA for IFN-γ and IL-4 (B) production. Similarly, BMDCs were pulsed with OVA + *Tc*ES, OVA + *Toxoplasma* soluble antigen (TSA), OVA + *Tc*ES + TSA, OVA + p*Tc*ES + TSA for 3 days and IFN-γ (C) as well IL-4 (D) was detected in supernatants. Results are expressed as the mean ± SEM of three to four replicates per group and are representative of two independent experiments, $^{*}P < 0.05$. RPMI medium was the non-stimulated control culture. - - indicates the absence of *Tc*ES on the culture.

et al., 2009), that may be an early source of IL-4 and this situation may influence the activity of BMDCs. On the other hand, it is known that certain highly conserved cytokine genes are present in helminths, for example, *B. malayi* expresses homologues of macrophage migration inhibitory factor (MIF) which have biological activity in host cells (Prieto-Lafuente et al., 2009). Another homologue, TGF-β, is secreted by this parasite as well as by *Heligmosomoides polygyrus* (Hewitson et al., 2009). In addition, cysticerci were collected from mice with a biased Th2 immune microenvironment that may carry some IL-4 or IL-13 contamination. Thus, in order to explore whether an early source of IL-4, IL-4 contamination or IL-4-like molecules was influencing the effect of *Tc*ES on BMDC activity, we decided to use BMDCs from STAT6-deficient ($STAT6^{-/-}$) mice. Given that STAT6 is required for IL-4 and IL-13 mediated activity and as an early source of IL-4 may be implicated in DC modulation by *Tc*ES, we next assessed the response of BMDCs lacking STAT6 to LPS, TSA and *Tc*ES. A strong reduction (>75% decrease) of both IL-12p40 and IL-15 production induced by the selected TLR ligand combinations was observed in BMDCs from $STAT6^{-/-}$ mice that were exposed to *T. crassiceps*-derived glycoproteins (Fig. 7A). As expected, $STAT6^{-/-}$ BMDCs responded strongly to LPS and TSA as evidenced by augmentation of the IL-12p40 and IL-15 production (Fig. 7A). These data showed that BMDCs from $STAT6^{-/-}$ mice exposed to *Tc*ES behaved essentially as cells derived from $STAT6^{+/+}$ mice.

Notably, $STAT6^{-/-}$ BMDCs exposed to *Tc*ES retained the ability to prime Th2 cytokine production in vitro while down-regulating OVA-specific IFN-γ secretion in $CD4^+$ DO11.10 cells (Fig. 7B). Taken together with the defective OVA-specific Th2 response detailed above, these results demonstrate that intact glycan structure is critical for DCs to drive Th2 responses favored by *Tc*ES antigens, and this effect is STAT6-independent.

3.9. Live cysticerci modulate DC maturation/activation in vitro

To demonstrate that the ex vivo release of soluble factors by *T. crassiceps* cysticerci can directly modulate DC activation, we developed an experiment using freshly isolated cysticerci, extensively washed and seeded into transwell cell-culture plates with BMDCs plus LPS, CpG or RPMI medium on the bottom and cysticerci seeded on top of the transwell (0.4 μm pore size). Supernatants were harvested after 24 h and processed for ELISA, and DCs for flow cytometry. Interestingly, the co-culture with cysticerci did not induce DC maturation, as the activation markers CD80, OX40L, MHC-II or CCR7 were not up-regulated. In contrast, LPS- and CpG-matured DCs in the absence of live cysticerci showed the normal mature phenotype as expected (Fig. 8A), but the combination of live cysticerci plus TLR4 and/or TLR9 ligands inhibited the mature phenotype in a non-contact-dependent manner where up-regulation of CD80, OX40L and CCR7 was inhibited (25–53%) compared with LPS- or CpG-activated DCs in the absence of cysticerci (Fig. 8A). This effect was also reflected by reduced IL-12p40 and TNF-α production (Fig. 8B).

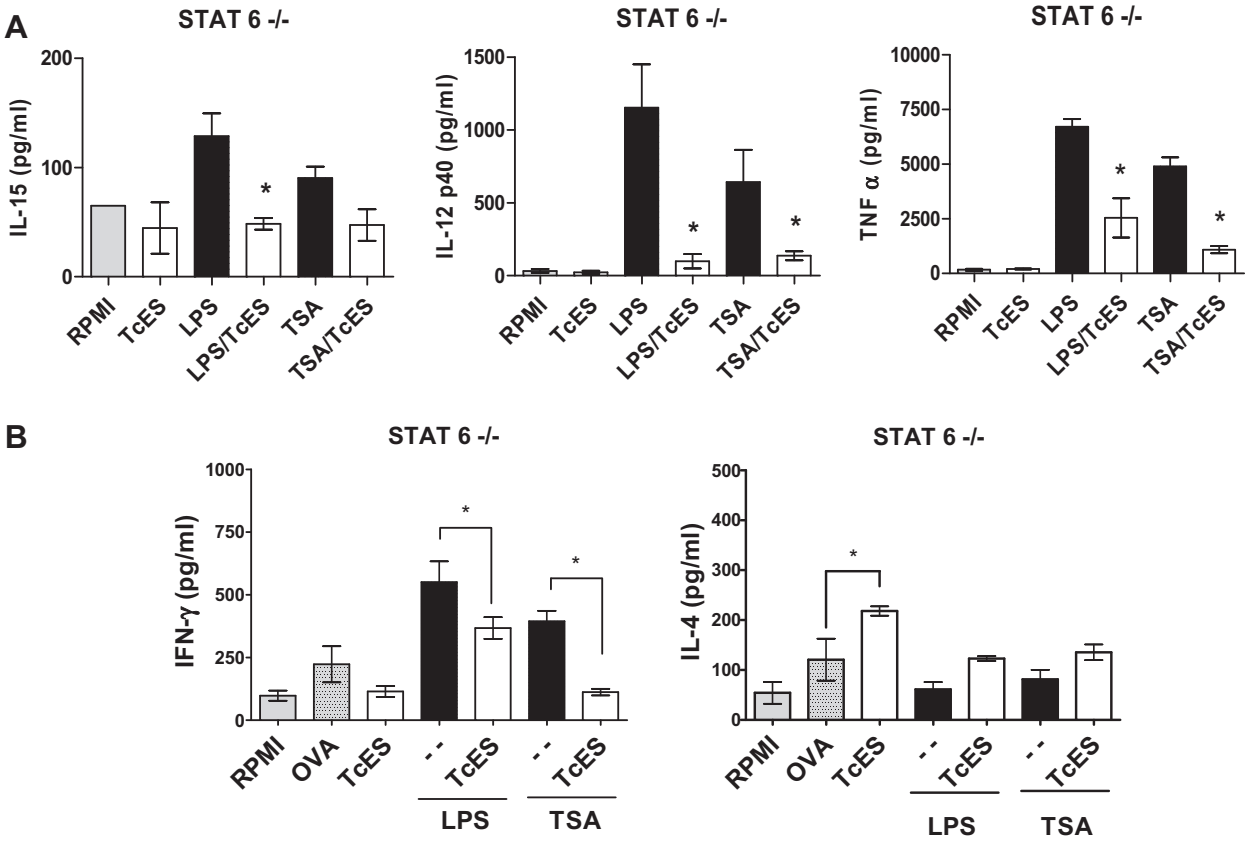


Fig. 7. Signal transducer and activator of transcription 6 (STAT6) signaling is not involved in *Taenia crassiceps* excretory/secretory product (*Tc*ES) activity on dendritic cells (DCs). (A) Bone marrow-derived dendritic cells (BMDCs) were derived from $STAT6^{-/-}$ mice and treated as described in Section 2. $STAT6^{-/-}$ DCs pulsed with *Tc*ES were incapable of secreting IL-12p40, TNF-α or IL-15 in response to either lipopolysaccharide (LPS) or *Toxoplasma* soluble antigen (TSA). (B) $STAT6^{-/-}$ BMDCs stimulated in the presence of 1 μg/ml ovalbumin peptide (OVA), 1 μg/ml LPS, 5 μg TSA and/or 20 μg/ml *Tc*ES, were washed and then co-cultured with DO11.10 $CD4^+$ T cells in a 1:10 DC:$CD4^+$ ratio for 72 h. Supernatants were analyzed by ELISA for IFN-γ and IL-4 production. Both the biasing-IL-4 and the IFN-γ suppression effects of *Tc*ES were maintained in $STAT6^{-/-}$ DCs. Results are expressed as the mean ± SEM of three to four replicates per group and are representative of two independent experiments, $^*P < 0.05$. RPMI medium was the unstimulated control culture. - - Indicates the absence of *Tc*ES on the culture.

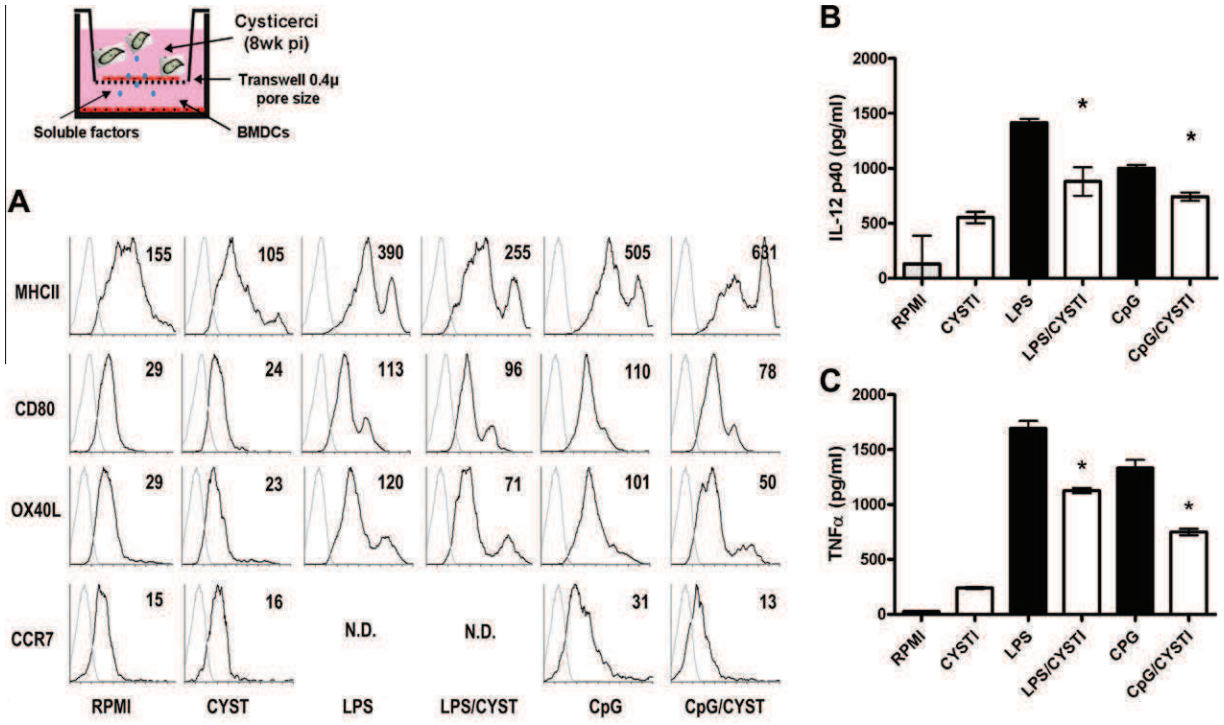


Fig. 8. Live *Taenia crassiceps* cysticerci mimics *T. crassiceps* excretory/secretory product (*Tc*ES) effects on dendritic cells (DCs). Cysticerci were obtained from 8 weeks infected mice, washed and seeded in six-well plates separated from DCs using a transwell (0.40 µm size pore). (A) Cysticerci alone did not induce co-stimulatory molecules in DCs, however, co-stimulatory molecules were up-regulated by CpG or lipopolysaccharide (LPS) but down-regulated in the presence of live cysticerci plus Toll-like receptor (TLR) ligands. (B) Products released by cysticerci did not lead to IL-12p40 or TNF-α production. However, IL-12p40 and TNF-α were decreased when DCs were stimulated with LPS or CpG in the presence of live cysticerci (B) and (C). RPMI medium was the non-stimulated control culture.

4. Discussion

APCs are the first line of contact between parasites and the immune system, and play a central role in the activation and differentiation of T lymphocytes into Th1, Th2 or Treg cells. DCs take up antigens, become activated and migrate to present the antigenic peptides on MHC molecules. This process involves phagocytosis, up-regulation of co-stimulatory molecules, such as CD80, CD86, CD40, as well as migration and cytokine release (Lee and Iwasaki, 2007). Failures in this process may modify the outcome of the immune response and the course of the infection.

During recent years, considerable attention has been focused on identifying helminth-derived antigens that might be associated with the immunomodulation observed during helminth parasitic diseases (Maizels et al., 2004), with special emphasis on the role of innate immune cells in the recognition of and response to helminth parasites and their antigens (Perrigoue et al., 2008; Carvalho et al., 2009). Most available data are from trematodes such as *S. mansoni* (van Liempt et al., 2007; van Riet et al., 2009) and from nematodes such as *B. malayi* (Semnani et al., 2003, 2008), *Nippostrongylus brasiliensis* (Balic et al., 2004), *Ascaris suum* (Silva et al., 2006), *Necator americanus* (Fujiwara et al., 2009) and recently, *H. polygyrus* (Segura et al., 2007), but there are very few data on cestodes (Hewitson et al., 2009). Even though some of these helminths may share some features in APC modulation, there are important differences in DC activation or inactivation (Carvalho et al., 2009). For example, *S. mansoni* SEA was shown to augment CpG- and LPS-induced IL-10 production (Kane et al., 2004), while exposure of DCs to *B. malayi* microfilariae reduced IL-10 production (Semnani et al., 2003). Exposure to *H. polygyrus*-derived ES antigens suppressed IL-10 secretion by DCs stimulated with CpG or LPS (Segura et al., 2007). Thus, different helminth parasites may trigger distinct and possibly multiple mechanisms to induce modulation of DC functions. Data on the role of *Taenia*-derived antigens in modulating DC activities has not been previously published.

Here we analyzed the ability of DCs to up-regulate maturation markers and pro-inflammatory cytokines after exposure to *T. crassiceps* antigen and TLR ligands such as LPS, TSA and CpG. We found that the activity of *Tc*ES induces limited immunomodulation of BMDCs, leading to a selective maturation as characterized by a moderate expression of co-stimulatory molecules (CD80 and CD86), MHC-II, CCR7 and very low-level release of pro-inflammatory cytokines such as IL-12, TNF-α and IL-15 with maintenance of IL-10 production. This selective maturation might be a mechanism by which *Tc*ES-pulsed DCs promote a Th2-like immune response of naive transgenic CD4-T cells compared with the Th1-like response that is induced by fully-matured DCs after LPS stimulation. Moreover, the down-regulation of pro-inflammatory cytokines such as IL-12p70, a key cytokine involved in initiating Th1-type $CD4^+$ T-cell responses (Romani et al., 1994) which together with TNF-α and IL-15 make an appropriate microenvironment for Th1 promotion in vitro, may favor a Th2-type response by *Tc*ES-pulsed DCs (Mattei et al., 2001; Ohteki et al., 2001; Herring et al., 2002). However, the specific mechanism by which *Tc*ES induces selective maturation of DCs and the subsequent polarization of naive $CD4^+$ T cells towards IL-4 producing cells is the subject of ongoing investigation. Other helminth–innate immunity studies have suggested that TLRs may participate in such early interactions (Thomas et al., 2003; van Riet et al., 2009); Lacto-*N*-fucopentaose III from *S. mansoni* and ES62 from *A. vitae* seem to bind and signal through TLR4 (Thomas et al., 2003; Goodridge et al., 2005). Nevertheless, in our case the putative signaling of *Tc*ES through TLRs is unlikely (at least for TLR4, TLR9 and TLR11), given that the activation of DCs with *Tc*ES in the presence of LPS, CpG or TSA inhibited the maturation of these cells compared with DCs without *Tc*ES. In other works it has been demonstrated that agonist combination of

several TLRs on DCs synergize and trigger full maturation and potent Th1-type polarized responses by increasing pro-inflammatory cytokine production such as IL-12 and IL-23 (Theiner et al., 2008; Zhu et al., 2008). In the present study, DCs pulsed with *Tc*ES plus LPS, CpG or TSA displayed an inhibited pro-inflammatory profile; therefore, it is possible that *Tc*ES may signal through a different receptor or may act as TLR antagonists or even competitors for TLR ligands.

Another likely molecule involved in down-modulating DC activity by *Tc*ES is IL-10, which has been observed to play an important role using other sources of helminth antigens (Segura et al., 2007). However, in our hands, IL-10 can be ruled out given that when we block either the IL-10 receptor (data not shown) or IL-10 in vitro, the effect of *Tc*ES on TLR-mediated pro-inflammatory cytokines remained intact. This was an unexpected result given that IL-10 appears to be a key factor in helminth-derived antigens for DC modulation, suggesting that *Tc*ES may act by directly inhibiting TLR signaling and not through the release of IL-10 that may act in a paracrine pathway. The use of IL-10KO BMDCs will be necessary to confirm this.

Previous studies have shown that $CD4^+$ from IL-$4R^{-/-}$ mice can respond to *N. brasiliensis*-secreted proteins stimulation with IL-4 release (Balic et al., 2006), suggesting that a partial Th2 response can be achieved in the absence of IL-4 signaling; however, IL-4 and IL-10 were necessary for a complete Th2 response. More recently a glycoprotein secreted by *S. mansoni* eggs called omega-1 which induces strong Th2 responses in vitro and in vivo has been identified. In addition, in vivo studies with IL-$4R\alpha^{-/-}$ mice demonstrated that IL-4R signaling is dispensable for the in vivo priming of Th2 responses by omega-1 (Everts et al., 2009). Therefore, our data demonstrating that BMDCs from $STAT6^{-/-}$ mice after *Tc*ES exposure are still able to induce a Th2 response to a bystander antigen reinforce the idea that a pathway other than STAT6 is involved in the signaling induced by *Tc*ES glycans and that this effect is not linked to endogenous IL-4 production. The biological relevance of a STAT6-independent pathway triggered by *T. crassiceps* antigens without clear cytokine release on DCs remains to be defined. Together, these studies support the idea that a Th2 response can begin with an early recognition of inherent signals in this cestode by innate immune cells such as DCs.

Infection with *T. crassiceps* leads to Th2 responses which can be replicated in vivo by simple injection of soluble extract antigens, where the intact structure of glycans plays a key role (Gomez-Garcia et al., 2006). However, the molecular mechanisms by which cestode antigens interact with and influence the immune system are not fully understood. The fact that *Tc*ES are essentially glycoproteins suggest that in our model carbohydrate *T. crassiceps* products can lead DCs to acquire a special phenotype able to instruct Th2 responses, as has been seen in *S. mansoni*, *N. brasiliensis* and *Ancylostoma*-derived products, which can affect the phenotype of DCs and abrogate their capacity to respond to pro-inflammatory stimuli such as LPS (Balic et al., 2004; Kane et al., 2004; van Liempt et al., 2007; Semnani et al., 2008). Here we found that periodate-treated *Tc*ES plus LPS or TSA promoted the production of IFN-γ by $CD4^+$ DO11.10 cells associated with markedly increased DC production of IL-12 and TNF-α. This finding suggests that DCs may recognize glycan structures on *Tc*ES and supports the notion that glycosylated *T. crassiceps* antigens are important in inducing Th2-type responses in vivo (Gomez-Garcia et al., 2006). Therefore, the inhibitory effects of *Tc*ES glycans on DC IL-12p70 production could have consequences on the Th cell biasing during the course of DC–CD4 interaction and support that glycans may participate in *T. crassiceps* immunomodulation by inactivating DC-pro-inflammatory cytokine release. Indeed, some carbohydrate structures on *T. crassiceps* antigens have been described (Lee et al., 2005), and some of those have similarities with those found in *S. mansoni*, which also have immunomodulatory activities. In line with this, a recent study using a purified glycoprotein (omega-1) from *S. mansoni* eggs showed that it was capable of instructing DCs to induce Th2 polarization (Everts et al., 2009). Moreover, an important finding related to host–parasite interaction using two cestodes such as *Mesocestoides corti* and *Taenia solium* was the release of parasite glycoconjugates that were taken up by infiltrating mononuclear cells (Alvarez et al., 2008). In further support of these observations, recent studies have shown that C-type lectins (CLR) are involved in recognizing carbohydrate structures on helminths products (van Liempt et al., 2007). Emerging evidence shows that CLRs can not only facilitate phagocytosis and adhesion as previously suggested, but can also induce intracellular signaling (Geijtenbeek and Gringhuis, 2009). Different roles for CLRs in DC modulation have been described, such as those of Dectin-1, which can synergize with TLRs to boost pro-inflammatory cytokines (Dennehy et al., 2008), and DC-SIGN which can in turn antagonize TLR signaling (Gringhuis et al., 2007). Our data support the idea that intact glycan structures are critically involved in *T. crassiceps*-induced DC modulation; however, the existence of specific receptors for such putative glycans on *Tc*ES need to be further investigated. Another important topic recently addressed by others is to determine which intracellular pathways can be turned on or off directly by helminth-derived products (Goodridge et al., 2005; Segura et al., 2007; Semnani et al., 2008; van Riet et al., 2009). For example it has recently been shown that *Ascaris* and *Schistosoma* products can signal mainly through pERK1/2 instead of the p38 pathway, and if the ratio favors pERK, then DCs instruct a Th2 response (van Riet et al., 2009). Another interesting possibility is that helminth-derived products may induce the expression of molecules with negative regulatory activity on TLR-signaling pathways, such as SOCS-1 (Semnani et al., 2008).

The demonstration that live cysticerci can mimic our data with *Tc*ES on DC maturation and cytokine production provides further support that soluble factors released by *T. crassiceps* affect DC activities. There are few studies analyzing soluble released factors from *Taenia* and their effect on the immune response. Baig et al. (2005) found that *T. crassiceps* cysticerci release small (<25 kDa) proteases which may alter cytokine availability in spleen cell cultures, while Spolski et al. (2000) found that bulk soluble factors suppressed spleen cell proliferation in vitro (Spolski et al., 2000); however, no further studies were performed. Thus, our findings open a new area of study for basic immunology in cysticercosis.

In conclusion we have demonstrated, to our knowledge for the first time, that DCs pulsed with *Tc*ES antigens modulate the pro-inflammatory response to several TLR-mediated stimuli and inhibit a Th1-like immune response in vitro, where glycan structure, but neither proteins nor STAT6-signaling, might have an essential role.

Acknowledgments

This work was supported by Grant # 60956-M from CONACYT (Mexico) and Grant IN212909 from PAPIIT-UNAM (Mexico), and it is part of the requirements to obtain the PhD degree in the Postgraduate Program in Biomedical Sciences, Facultad de Medicina, UNAM, for C.A.T., who was supported by a fellowship from CONACYT-Mexico.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijpara.2010.02.016.

References

Akira, S., Takeda, K., 2004. Toll-like receptor signalling. Nat. Rev. Immunol. 4, 499–511.

Aliberti, J., Valenzuela, J.G., Carruthers, V.B., Hieny, S., Andersen, J., Charest, H., Reis e Sousa, C., Fairlamb, A., Ribeiro, J.M., Sher, A., 2003. Molecular mimicry of a CCR5 binding-domain in the microbial activation of dendritic cells. Nat. Immunol. 4, 485–490.

Alvarez, J.I., Rivera, J., Teale, J.M., 2008. Differential release and phagocytosis of tegument glycoconjugates in neurocysticercosis: implications for immune evasion strategies. PLoS Negl. Trop. Dis. 2, e218.

Baig, S., Damian, R.T., Morales-Montor, J., Olecki, P., Talhouk, J., Hashmey, R., White Jr., A.C., 2005. Characterization of excretory/secretory endopeptidase and metallo-aminopeptidases from *Taenia crassiceps* metacestodes. J. Parasitol. 91, 983–987.

Balic, A., Harcus, Y., Holland, M.J., Maizels, R.M., 2004. Selective maturation of dendritic cells by *Nippostrongylus brasiliensis*-secreted proteins drives Th2 immune responses. Eur. J. Immunol. 34, 3047–3059.

Balic, A., Harcus, Y.M., Taylor, M.D., Brombacher, F., Maizels, R.M., 2006. IL-4R signaling is required to induce IL-10 for the establishment of T(h)2 dominance. Int. Immunol. 18, 1421–1431.

Brodskyn, C., Patricio, J., Oliveira, R., Lobo, L., Arnholdt, A., Mendonca-Previato, L., Barral, A., Barral-Netto, M., 2002. Glycoinositolphospholipids from *Trypanosoma cruzi* interfere with macrophages and dendritic cell responses. Infect. Immun. 70, 3736–3743.

Carvalho, L., Sun, J., Kane, C., Marshall, F., Krawczyk, C., Pearce, E.J., 2009. Review series on helminths, immune modulation and the hygiene hypothesis: mechanisms underlying helminth modulation of dendritic cell function. Immunology 126, 28–34.

Dennehy, K.M., Ferwerda, G., Faro-Trindade, I., Pyz, E., Willment, J.A., Taylor, P.R., Kerrigan, A., Tsoni, S.V., Gordon, S., Meyer-Wentrup, F., Adema, G.J., Kullberg, B.J., Schweighoffer, E., Tybulewicz, V., Mora-Montes, H.M., Gow, N.A., Williams, D.L., Netea, M.G., Brown, G.D., 2008. Syk kinase is required for collaborative cytokine production induced through Dectin-1 and Toll-like receptors. Eur. J. Immunol. 38, 500–506.

Everts, B., Perona-Wright, G., Smits, H.H., Hokke, C.H., van der Ham, A.J., Fitzsimmons, C.M., Doenhoff, M.J., van der Bosch, J., Mohrs, K., Haas, H., Mohrs, M., Yazdanbakhsh, M., Schramm, G., 2009. Omega-1, a glycoprotein secreted by *Schistosoma mansoni* eggs, drives Th2 responses. J. Exp. Med. 206, 1673–1680.

Fujiwara, R.T., Cancado, G.G., Freitas, P.A., Santiago, H.C., Massara, C.L., Dos Santos Carvalho, O., Correa-Oliveira, R., Geiger, S.M., Bethony, J., 2009. *Necator americanus* infection: a possible cause of altered dendritic cell differentiation and eosinophil profile in chronically infected individuals. PLoS Negl. Trop. Dis. 3, e399.

Geijtenbeek, T.B., Gringhuis, S.I., 2009. Signalling through C-type lectin receptors: shaping immune responses. Nat. Rev. Immunol. 9, 465–479.

Gomez-Garcia, L., Lopez-Marin, L.M., Saavedra, R., Reyes, J.L., Rodriguez-Sosa, M., Terrazas, L.I., 2005. Intact glycans from cestode antigens are involved in innate activation of myeloid suppressor cells. Parasite Immunol. 27, 395–405.

Gomez-Garcia, L., Rivera-Montoya, I., Rodriguez-Sosa, M., Terrazas, L.I., 2006. Carbohydrate components of *Taenia crassiceps* metacestodes display Th2-adjuvant and anti-inflammatory properties when co-injected with bystander antigen. Parasitol. Res. 99, 440–448.

Goodridge, H.S., Marshall, F.A., Else, K.J., Houston, K.M., Egan, C., Al-Riyami, L., Liew, F.Y., Harnett, W., Harnett, M.M., 2005. Immunomodulation via novel use of TLR4 by the filarial nematode phosphorylcholine-containing secreted product, ES-62. J. Immunol. 174, 284–293.

Gringhuis, S.I., den Dunnen, J., Litjens, M., van Het Hof, B., van Kooyk, Y., Geijtenbeek, T.B., 2007. C-type lectin DC-SIGN modulates Toll-like receptor signaling via Raf-1 kinase-dependent acetylation of transcription factor NF-kappaB. Immunity 26, 605–616.

Herring, A.C., Lee, J., McDonald, R.A., Toews, G.B., Huffnagle, G.B., 2002. Induction of interleukin-12 and gamma interferon requires tumor necrosis factor alpha for protective T1-cell-mediated immunity to pulmonary *Cryptococcus neoformans* infection. Infect. Immun. 70, 2959–2964.

Hewitson, J.P., Grainger, J.R., Maizels, R.M., 2009. Helminth immunoregulation: the role of parasite secreted proteins in modulating host immunity. Mol. Biochem. Parasitol. 167, 1–11.

Holland, M.J., Harcus, Y.M., Riches, P.L., Maizels, R.M., 2000. Proteins secreted by the parasitic nematode *Nippostrongylus brasiliensis* act as adjuvants for Th2 responses. Eur. J. Immunol. 30, 1977–1987.

Kane, C.M., Cervi, L., Sun, J., McKee, A.S., Masek, K.S., Shapira, S., Hunter, C.A., Pearce, E.J., 2004. Helminth antigens modulate TLR-initiated dendritic cell activation. J. Immunol. 173, 7454–7461.

Kuroda, E., Ho, V., Ruschmann, J., Antignano, F., Hamilton, M., Rauh, M.J., Antov, A., Flavell, R.A., Sly, L.M., Krystal, G., 2009. SHIP represses the generation of IL-3-induced M2 macrophages by inhibiting IL-4 production from basophils. J. Immunol. 183, 3652–3660.

Lee, H.K., Iwasaki, A., 2007. Innate control of adaptive immunity: dendritic cells and beyond. Semin. Immunol. 19, 48–55.

Lee, J.J., Dissanayake, S., Panico, M., Morris, H.R., Dell, A., Haslam, S.M., 2005. Mass spectrometric characterisation of *Taenia crassiceps* metacestode *N*-glycans. Mol. Biochem. Parasitol. 143, 245–249.

Lutz, M.B., Kukutsch, N., Ogilvie, A.L., Rossner, S., Koch, F., Romani, N., Schuler, G., 1999. An advanced culture method for generating large quantities of highly pure dendritic cells from mouse bone marrow. J. Immunol. Methods 223, 77–92.

MacDonald, A.S., Maizels, R.M., 2008. Alarming dendritic cells for Th2 induction. J. Exp. Med. 205, 13–17.

Maizels, R.M., 2009. Exploring the immunology of parasitism – from surface antigens to the hygiene hypothesis. Parasitology 1, 16.

Maizels, R.M., Yazdanbakhsh, M., 2003. Immune regulation by helminth parasites: cellular and molecular mechanisms. Nat. Rev. Immunol. 3, 733–744.

Maizels, R.M., Balic, A., Gomez-Escobar, N., Nair, M., Taylor, M.D., Allen, J.E., 2004. Helminth parasites – masters of regulation. Immunol. Rev. 201, 89–116.

Manickasingham, S.P., Edwards, A.D., Schulz, O., Reis e Sousa, C., 2003. The ability of murine dendritic cell subsets to direct T helper cell differentiation is dependent on microbial signals. Eur. J. Immunol. 33, 101–107.

Mattei, F., Schiavoni, G., Belardelli, F., Tough, D.F., 2001. IL-15 is expressed by dendritic cells in response to type I IFN, double-stranded RNA, or lipopolysaccharide and promotes dendritic cell activation. J. Immunol. 167, 1179–1187.

Ohteki, T., Suzue, K., Maki, C., Ota, T., Koyasu, S., 2001. Critical role of IL-15–IL-15R for antigen-presenting cell functions in the innate immune response. Nat. Immunol. 2, 1138–1143.

Okano, M., Satoskar, A.R., Nishizaki, K., Abe, M., Harn Jr., D.A., 1999. Induction of Th2 responses and IgE is largely due to carbohydrates functioning as adjuvants on *Schistosoma mansoni* egg antigens. J. Immunol. 163, 6712–6717.

Pearce, E.J., Kane, C.M., Sun, J., Taylor, J.J., McKee, A.S., Cervi, L., 2004. Th2 response polarization during infection with the helminth parasite *Schistosoma mansoni*. Immunol. Rev. 201, 117–126.

Perrigoue, J.G., Marshall, F.A., Artis, D., 2008. On the hunt for helminths: innate immune cells in the recognition and response to helminth parasites. Cell. Microbiol. 10, 1757–1764.

Pinelli, E., Brandes, S., Dormans, J., Fonville, M., Hamilton, C.M., der Giessen, J., 2007. *Toxocara canis:* effect of inoculum size on pulmonary pathology and cytokine expression in BALB/c mice. Exp. Parasitol. 115, 76–82.

Prieto-Lafuente, L., Gregory, W.F., Allen, J.E., Maizels, R.M., 2009. MIF homologues from a filarial nematode parasite synergize with IL-4 to induce alternative activation of host macrophages. J. Leukoc. Biol. 85, 844–854.

Rodriguez, M., Terrazas, L.I., Marquez, R., Bojalil, R., 1999. Susceptibility to *Trypanosoma cruzi* is modified by a previous non-related infection. Parasite Immunol. 21, 177–185.

Rodriguez-Sosa, M., Satoskar, A.R., David, J.R., Terrazas, L.I., 2003. Altered T helper responses in CD40 and interleukin-12 deficient mice reveal a critical role for Th1 responses in eliminating the helminth parasite *Taenia crassiceps*. Int. J. Parasitol. 33, 703–711.

Rodriguez-Sosa, M., Saavedra, R., Tenorio, E.P., Rosas, L.E., Satoskar, A.R., Terrazas, L.I., 2004. A STAT4-dependent Th1 response is required for resistance to the helminth parasite *Taenia crassiceps*. Infect. Immun. 72, 4552–4560.

Rodriguez-Sosa, M., Rivera-Montoya, I., Espinoza, A., Romero-Grijalva, M., Lopez-Flores, R., Gonzalez, J., Terrazas, L.I., 2006. Acute cysticercosis favours rapid and more severe lesions caused by *Leishmania major* and *Leishmania mexicana* infection, a role for alternatively activated macrophages. Cell. Immunol. 242, 61–71.

Romani, L., Mencacci, A., Tonnetti, L., Spaccapelo, R., Cenci, E., Wolf, S., Puccetti, P., Bistoni, F., 1994. Interleukin-12 but not interferon-gamma production correlates with induction of T helper type-1 phenotype in murine candidiasis. Eur. J. Immunol. 24, 909–915.

Sciutto, E., Fragoso, G., Baca, M., De la Cruz, V., Lemus, L., Lamoyi, E., 1995. Depressed T-cell proliferation associated with susceptibility to experimental *Taenia crassiceps* infection. Infect. Immun. 63, 2277–2281.

Segura, M., Su, Z., Piccirillo, C., Stevenson, M.M., 2007. Impairment of dendritic cell function by excretory–secretory products: a potential mechanism for nematode-induced immunosuppression. Eur. J. Immunol. 37, 1887–1904.

Semnani, R.T., Liu, A.Y., Sabzevari, H., Kubofcik, J., Zhou, J., Gilden, J.K., Nutman, T.B., 2003. *Brugia malayi* microfilariae induce cell death in human dendritic cells, inhibit their ability to make IL-12 and IL-10, and reduce their capacity to activate $CD4^+$ T cells. J. Immunol. 171, 1950–1960.

Semnani, R.T., Venugopal, P.G., Leifer, C.A., Mostbock, S., Sabzevari, H., Nutman, T.B., 2008. Inhibition of TLR3 and TLR4 function and expression in human dendritic cells by helminth parasites. Blood 112, 1290–1298.

Silva, S.R., Jacysyn, J.F., Macedo, M.S., Faquim-Mauro, E.L., 2006. Immunosuppressive components of *Ascaris suum* down-regulate expression of costimulatory molecules and function of antigen-presenting cells via an IL-10-mediated mechanism. Eur. J. Immunol. 36, 3227–3237.

Spolski, R.J., Corson, J., Thomas, P.G., Kuhn, R.E., 2000. Parasite-secreted products regulate the host response to larval *Taenia crassiceps*. Parasite Immunol. 22, 297–305.

Spolski, R.J., Alexander-Miller, M.A., Kuhn, R.E., 2002. Suppressed cytotoxic T lymphocyte responses in experimental cysticercosis. Vet. Parasitol. 106, 325–330.

Tawill, S., Le Goff, L., Ali, F., Blaxter, M., Allen, J.E., 2004. Both free-living and parasitic nematodes induce a characteristic Th2 response that is dependent on the presence of intact glycans. Infect. Immun. 72, 398–407.

Terrazas, L.I., Bojalil, R., Govezensky, T., Larralde, C., 1998. Shift from an early protective Th1-type immune response to a late permissive Th2-type response in murine cysticercosis (*Taenia crassiceps*). J. Parasitol. 84, 74–81.

Theiner, G., Rossner, S., Dalpke, A., Bode, K., Berger, T., Gessner, A., Lutz, M.B., 2008. TLR9 cooperates with TLR4 to increase IL-12 release by murine dendritic cells. Mol. Immunol. 45, 244–252.

Thomas, P.G., Carter, M.R., Atochina, O., Da'Dara, A.A., Piskorska, D., McGuire, E., Harn, D.A., 2003. Maturation of dendritic cell 2 phenotype by a helminth glycan uses a Toll-like receptor 4-dependent mechanism. J. Immunol. 171, 5837–5841.

Toenjes, S.A., Spolski, R.J., Mooney, K.A., Kuhn, R.E., 1999. The systemic immune response of BALB/c mice infected with larval *Taenia crassiceps* is a mixed Th1/Th2-type response. Parasitology 118 (Pt. 6), 623–633.

van Liempt, E., van Vliet, S.J., Engering, A., Garcia Vallejo, J.J., Bank, C.M., Sanchez-Hernandez, M., van Kooyk, Y., van Die, I., 2007. *Schistosoma mansoni* soluble egg antigens are internalized by human dendritic cells through multiple C-type lectins and suppress TLR-induced dendritic cell activation. Mol. Immunol. 44, 2605–2615.

van Riet, E., Everts, B., Retra, K., Phylipsen, M., van Hellemond, J.J., Tielens, A.G., van der Kleij, D., Hartgers, F.C., Yazdanbakhsh, M., 2009. Combined TLR2 and TLR4 ligation in the context of bacterial or helminth extracts in human monocyte derived dendritic cells: molecular correlates for Th1/Th2 polarization. BMC Immunol. 10, 9.

Villa, O.F., Kuhn, R.E., 1996. Mice infected with the larvae of *Taenia crassiceps* exhibit a Th2-like immune response with concomitant anergy and downregulation of Th1-associated phenomena. Parasitology 112 (Pt. 6), 561–570.

Whelan, M., Harnett, M.M., Houston, K.M., Patel, V., Harnett, W., Rigley, K.P., 2000. A filarial nematode-secreted product signals dendritic cells to acquire a phenotype that drives development of Th2 cells. J. Immunol. 164, 6453–6460.

Zhu, Q., Egelston, C., Vivekanandhan, A., Uematsu, S., Akira, S., Klinman, D.M., Belyakov, I.M., Berzofsky, J.A., 2008. Toll-like receptor ligands synergize through distinct dendritic cell pathways to induce T cell responses: implications for vaccines. Proc. Natl. Acad. Sci. USA 105, 16260–16265.

IVYSPRING INTERNATIONAL PUBLISHER

International Journal of Biological Sciences

2011; 7(9):1391-1400

Research Paper

Cestode Antigens Induce a Tolerogenic-Like Phenotype and Inhibit LPS Inflammatory Responses in Human Dendritic Cells

César A. Terrazas[3*], Fausto Sánchez-Muñoz[1*], Ana M. Mejía-Domínguez[2], Luis M. Amezcua-Guerra[1], Luis I. Terrazas[3], Rafael Bojalil [1,4], Lorena Gómez-García[1]✉

1. Department of Immunology, Instituto Nacional de Cardiología Ignacio Chávez, México City, México
2. Blood Bank, Instituto Nacional de Cardiología Ignacio Chávez, México City, México
3. Biomedicine Unit, Facultad de Estudios Superiores Iztacala, UNAM, México City, México
4. Department of Health Care, Universidad Autónoma Metropolitana Xochimilco, México City, México

* These two authors contributed equally to this work.

✉ Corresponding author: lorena.gomez@cardiologia.org.mx

Received: 2011.09.01; Accepted: 2011.10.01; Published: 2011.11.01

Abstract

Pathogens have developed strategies to modify Dendritic Cells (DCs) phenotypes and impair their functions in order to create a safer environment for their survival. DCs responses to helminths and their derivatives vary among different studies. Here we show that excretory/secretory products of the cestode *Taenia crassiceps* (TcES) do not induce the maturation of human DCs judged by a lack of increment in the expression of CD83, HLA-DR, CD80 and CD86 molecules but enhanced the production of IL-10 and positively modulated the expression of the C-type lectin receptor *MGL* and negatively modulated the expression of *DC-SIGN*. Additionally, these antigens were capable of down-modulating the inflammatory response induced by LPS in these cells by reducing the expression of the maturation markers and the production of the inflammatory cytokines IL-1β, TNF, IL-12 and IL-6. The effects of TcES upon the DCs responses to LPS were stronger if cells were exposed during their differentiation to the helminth antigens. All together, these findings suggest the ability of TcES to induce the differentiation of human DCs into a tolerogenic-like phenotype and to inhibit the effects of inflammatory stimuli.

Key words: Dendritic cells, tolerogenic phenotype, *Taenia crassiceps*.

Introduction

Dendritic cells fulfill a major role in the immune system directing the kind and the intensity of the response mounted against the diverse antigens and autoantigens encountered [1]. Thus, these cells can determine whether immunity or tolerance is exerted among the plethora of possible stimuli [2]. Different DCs subsets [3-4], phenotypes [5-6] and even microenvironments to which these cells are exposed [7-8], may determine the type of immune response induced by them. However, in general terms, immature DCs (iDCs) that face a pathogenic stimulus will enter in a process characterized by the expression of maturation and co-stimulation molecules as well as the production of cytokines that promote and modulate inflammation and effector cell functions, including IL-12, IL-1β, IL-6, TNF and IL-8 [1]. All these features allow DCs to trigger full lymphocyte responses [1]. On the contrary in a steady state, DCs will not display an enhanced expression of maturation markers and they will rather show an increased secretion of regulatory cytokines such as IL-10 and TGF-β [9-11]. Interestingly, these DCs have been described as cells capable

of inducing tolerance instead of immunoreactivity [12]. DCs express a wide repertory of receptors that allow them to interact with pathogens, tissue-derived danger signals and self-antigens [1]. Among these, Toll-like receptors (TLRs) and C-type lectin receptors (CLRs) are differentially expressed by subsets [13] and perhaps phenotypes [14] of DCs. The cross-talk known to occur between these receptors can either inhibit or enhance specific signals in DCs, thereby modulating their phenotype and function and accounting for the different pathways to tolerance or immune activation [13, 15-17]. Since changes in the expression or activity of CLRs and TLRs can influence crucial aspects of the immune response, targeting these receptors is not surprisingly one of the mechanisms that parasites presumably use to impair DCs function and improve their survival within the host [18-19].

Helminth antigens have been a matter of intense research during the last years due to their capacity to modulate immune responses [20]. Described modulatory properties range from suppression of antigen-specific and unspecific T cell responses [21-22] to induction of specific cells responses such as alternatively activated macrophages and regulatory T cells [23-24]. In recent years, it has been shown that helminth antigens can also affect the response of DCs, presumably as a strategy to divert the immune response elicited against them [25]. Nevertheless it has been demonstrated that different helminth-derived molecules can affect DCs responses, it has not been possible to define a general phenotype induced by these parasites since distinct effects upon these cells have been observed. For instance, while some studies have shown that helminth antigens derived from *Ascaris suum* [26], *Schistosoma mansoni* [27], *Nippostrongylus brasiliensis* [28] or *Fasciola hepatica* [29] induce costimulatory molecule expression on DCs and production of inflammatory cytokines as IL-12p40, TNF or IL-6, other studies using *Heligmosomoides polygyrus* [24] and once again *S. mansoni* [30], have reported immature phenotypes with no cytokine secretion, indicating that different helminths and even different molecules derived from the same helminth can affect DCs activity in various ways.

To date most studies have addressed the activity that nematode and trematode-derived antigens exert upon DCs while the effects of cestode antigens on these cells are much less well explored. Most importantly, most of these studies have been done using mouse-derived DCs limiting our knowledge regarding the modulatory activity of helminth-derived antigens on human DCs. *Taenia crassiceps* is a cestode parasite of rodents and canines that shares a remarkable antigenic resemblance with the cestode parasite of humans *Taenia solium* [31-32]; thus, this parasite has been used as a model for the study of cysticercosis for many years [33-34]. Recently, we described that *Taenia crassiceps* excreted-secreted antigens (TcES) impair murine bone marrow-derived DCs (BMDCs) function down-modulating their TLR-mediated pro-inflammatory activity and inducing Th2 responses in allogenic assays [35]. However, the activity of these modulatory antigens on human DCs has not been addressed to date.

Here we explored the capacity of TcES to modulate the phenotype of human DCs responses. We found that despite the source of these antigens is not a human parasite, they can indeed modify human DCs phenotype and function. TcES did not induce maturation of DCs but instead down-modulated the expression of maturation and co-stimulatory molecules in response to the pro-inflammatory stimulus LPS. Even more, TcES enhanced production of IL-10 by these DCs but not IL-12, IL-1β, TNF and IL-6. Also, TcES dramatically reduced the production of these pro-inflammatory cytokines after LPS stimulation and this effect was more pronounced when DCs were exposed to the antigens when they were being differentiated from monocytes. Moreover, TcES modulated the expression of PRRs involved on key functions of DCs such as *DC-SIGN* and *MGL*. All together, these results indicate that TcES induce a tolerogenic-like phenotype in human DCs and down-modulate the inflammatory response that LPS induces in these cells.

Materials and Methods

Parasites and antigens

Metacestodes of *T. crassiceps* were harvested under sterile conditions from the peritoneal cavity of female Balb/c mice after 2-4 months of infection. The cysticerci were washed four times with sterile PBS and maintained in culture in PBS at 37°C for 24h. TcES were recovered from the supernatant and centrifuged for 10 min at 1000g. The upper fraction was concentrated using 50kDa Amicon Ultra Filter (Millipore). Protease inhibitors were added to the ≥50kDa fraction and samples were stored at -70°C until further use.

Monocyte-derived dendritic cells

Human peripheral blood mononuclear cells (PBMC) were obtained from buffy coats of 15 healthy blood donors from the Instituto Nacional de Cardiología Ignacio Chávez´s blood bank. Informed consent was obtained for the use of blood samples according to the declaration of Helsinki and the local scientific and ethics committees approved the proto-

col. PBMC were isolated by Ficoll-gradient centrifugation (GE Healthcare), analyzed in a Coulter AcT for cellular types (Beckman Coulter) and 3X10^6 monocytes were left to adhere in 6-well culture plates for 2h. After this period, non-adherent cells were washed away and adherent cells were cultured in RPMI medium supplemented with 10% SFB and penicillin/streptomycin in presence of 400 U/ml of IL-4 and 800 U/ml of GM-CSF during 6 days with replacement of medium and cytokines at day 3. For experiments assessing the effects of TcES during DC differentiation cells received 20ug/ml of TcES at day 0 and 3 (pre-stimulation). At day 6 non-adherent cells were recovered and placed for 24h in fresh medium. At this point we determined by flow cytometry the percentage of CD11c+ cells and for all experiments this was ≥ 80%. At day 7, both pre-stimulated and not pre-stimulated cells were challenged with 20ug/ml TcES, 1ug/ml LPS or a combination of them for 24h. For the real-time PCR analysis cells were stimulated for a period of 3h using the same stimuli. DCs derived from donors numbered 1-6 were used for the analysis of cell surface markers. DCs derived from donors numbered 1-9 were used for the analysis of cytokine production by ELISA. DCs derived from donors 10-15 were used for the analysis of mRNA expression. 1 X 10^6 DCs were used for each assay.

Quantification of cytokine production

Supernatants were recovered after the 24h stimulation period and production of the cytokines TNF, IL-6, IL-12, IL-10 (Peprotech), IL-23, IL-1β and TGF-β (R&D) was measured by ELISA kits in the supernatants of DCs cultures. For the analysis of cytokine mRNAs levels cells were recovered from cultures 3h post-stimulation.

Analysis of cell surface markers

Surface expression of maturation and co-stimulatory molecules was analyzed using multicolor flow cytometry. Human DCs were harvested post-stimulation, washed and suspended in FACS buffer (PBS/FBS 5%/0.05% NaN_3). Fc receptors were blocked with 1µg/µl of human IgG for 30 min at 4°C. Cells were washed and triple stained with an APC-conjugated antibody against CD11c, FITC conjugated antibodies against Human Leukocyte Antigen-DR (HLA-DR) and CD80 and PE-conjugated antibodies against CD83 and CD86 (all antibodies from Biolegend). Cells were analyzed on a FACsCalibur flow cytometer using Cell Quest software (Beckton Dickinson).

Quantitative real-time PCR

Cells (10^6) were lysed in 1000 µl TRIzol (Invitrogen) and incubated for 5 min at RT in presence of 1ul of Glycogen (20ug/ul stock) (Invitrogen). RNA was subsequently isolated from the water phase according to the manufacturer's instruction. The cDNA was generated using 250 ng of total RNA, random hexamers and the Transcriptor first strand cDNA synthesis kit (ROCHE). RT-qPCR analysis was performed using the LightCycler 2.0 system (ROCHE) with LNA hydrolysis probes from the Universal Probe Library Roche (UPL), and intron spanning primers (Table 1) from Invitrogen. One µl of cDNA was amplified with 400nM of primers, 100 nM of UPL probe, with the LighCycler TaqMan® Master (ROCHE) followed by 45 cycles of 95° 10 sec. 60° 30 sec., and 72° 1 sec. Reference genes ACTB and GAPDH transcripts were used for relative quantification. The mRNA relative quantification of target genes was conducted using the LightCycler software 4.1, according to the 2-ΔΔCt method. The calibrator sample employed was the non-stimulated DCs.

Table 1 - Primers for RT-qPCR.

Gene	GENEBANK	PRIMERS (5´-3´)	Amplicon Size (bp)	PROBE UPL
TLR2	NM_003264.3	CGTTCTCTCAGGTGACTGCTC	66	#14
		TCTCCTTTGGATCCTGCTTG		
TLR4	NM_138554.2	CTGCGTGAGACCAGAAAGC	75	#33
		TTCAGCTCCATGCATTGATAA		
CD209 (DC SIGN)	NM_021155.3 NM_001144893.1 NM_001144894.1 NM_001144895.1 NM_001144896.1 NM_001144897.1 NM_001144899.1	CCCAGCTCGTCGTAATCAA CCAGGTGAAGCGGTTACTTC	80	#73
M6PR (Manose Receptor)	NM_002355.2	GCTGGAGGACTGGACTGCTA	62	#76
		TGTCTGCCAGGATTCTCTCAC		
CLEC10A (Clec)	NM_006344.2	GAATCACACCCTCCAGACCTC	83	#16

	NM_182906.2	TCTGAGGTTGTCACAGCTGAA		
IL23	NM_016584.2	TGTTCCCCATATCCAGTGTG	77	#76
		TCCTTTGCAAGCAGAACTGA		
TGFB1	NM_000660.3	GCAGCACGTGGAGCTGTA	76	#72
		CAGCCGGTTGCTGAGGTA		
ACTB	NM_001101.3	CCAACCGCGAGAAGATGA	97	#64
		CCAGAGGCGTACAGGGATAG		
GADPH	NM_002046.3	AGCCACATCGCTCAGACAC	66	#60
		GCCCAATACGACCAAATCC		

RT-qPCR assays were intron spaning. DC SIGN and CLEC isoforms mRNAs enlisted were detected by the assays.

Statistics

Data are expressed as mean and standard errors. Comparisons were performed by the Wilcoxon signed rank (matched pairs) test. Significance was set on a p value<0.05. All analyses were performed with the GraphPad Prism v. 5 statistical software.

Results

Human DCs exposed to *Tc*ES show an immature-like phenotype

The maturation status of DCs can shape the balance to tolerance or immunity. Interestingly, different pathogens can interfere with this process on DCs hence affecting the infection outcome [2, 25].To determine the maturation status of human DCs exposed to TcES, LPS or both, we performed three-color cytometric analysis using the molecules HLA-DR (MHCII), CD83, CD80 and CD86 as markers.

We found that TcES, the excreted-secreted antigens of the cestode *T. crassiceps*, did not induce maturation of human DCs as judged by the absence of up-regulation (compared to those that only received medium) of any of the maturation and co-stimulation molecules tested in this study (Fig.1A). As expected, DCs that received LPS expressed HLA-DR molecules and showed a significant increment in CD83, CD80 and CD86 expression compared to non-stimulated cells, indicating they were fully mature (Fig 1A). However, when these cells were exposed to a combination of LPS plus TcES, the presence of CD83, CD80 and CD86 dropped, suggesting that TcES interfere with the typical maturation process induced by pro-inflammatory stimuli such as LPS.

Others have reported that monocytes exposed to helminth antigens have an impaired capability to differentiate into DCs [36-37]. In addition, infection with metacestodes is accompanied by antigens in circulation [38-39] that might affect the activity of monocytes. To assess if the differentiation of monocytes into DCs was affected by TcES we added these antigens to our cultures at day 0 and 3. We found that there was no difference in the proportion of CD11c+ cells between cultures that received TcES during their differentiation and the ones that received it only as a final challenge, indicating that TcES did not affect the capability of human monocytes to differentiate into DCs (Fig 1B). Additionally, DCs that received TcES during their differentiation and were re-challenged at day 7 with TcES, LPS or the combination of them, responded in the same way as the cells that received the antigens only when they were mature; this is, those exposed to TcES showed an immature phenotype and the ones exposed to LPS plus TcES expressed significantly less maturation and co-stimulation molecules compared to those that received only LPS. An exception was found for CD83, where early exposure to TcES affected significantly its expression after LPS stimulation when compared to DCs that were not exposed to such antigens during differentiation (Fig 1B).

TcES induce production of IL-10 by human DCs and impaired their LPS-inflammatory cytokine response

It is well known that soluble factors released by DCs after pathogen exposure are considered the third signal needed for lymphocyte activation and polarization of immune responses [40]. In contrast to those related to exposure to virus, bacteria or fungi, the soluble factors released by DCs after exposure to helminths are less well characterized. Human DCs phenotype in response to TcES, LPS and LPS/TcES was also assessed by determining their cytokine profile.

In our experiments, TcES failed to induce production of the cytokines IL-12, TNF, IL-1β and IL-6 by human DCs but interestingly these antigens induced the production of the regulatory cytokine IL-10. When DCs were stimulated with LPS, levels of all the cytokines were enhanced (Fig. 2) however; combination of LPS and TcES diminished cytokine LPS response of IL-12, TNF, IL-1β and IL-6, leaving IL-10 production unchanged.

The levels of IL-23 and TGF-β detected by ELISA were too low to make a proper quantification of them, so we decided to determine the transcription of mRNA of these cytokines by RT-qPCR. We found that as expected, LPS enhanced the transcripts of the inflammatory cytokine IL-23 and the addition of TcES did not influence this increment. TcES by itself did not induce an up-regulation of this messenger confirming its non pro-inflammatory activity (Fig. 2B). Even when the expression of TGF-β was somewhat higher in response to TcES, it was not statistically significant (Fig. 2B)

Interestingly, when human DCs were exposed to TcES during their differentiation, the effect of these antigens on LPS-induced cytokine production was more pronounced. In this scenario, LPS-induced production of IL-12, TNF and IL-6 was significantly down-regulated in cells that received TcES during their differentiation compared to those cells that did not. The addition of TcES plus LPS reduced even further the production of IL-12 and TNF (Fig. 3).

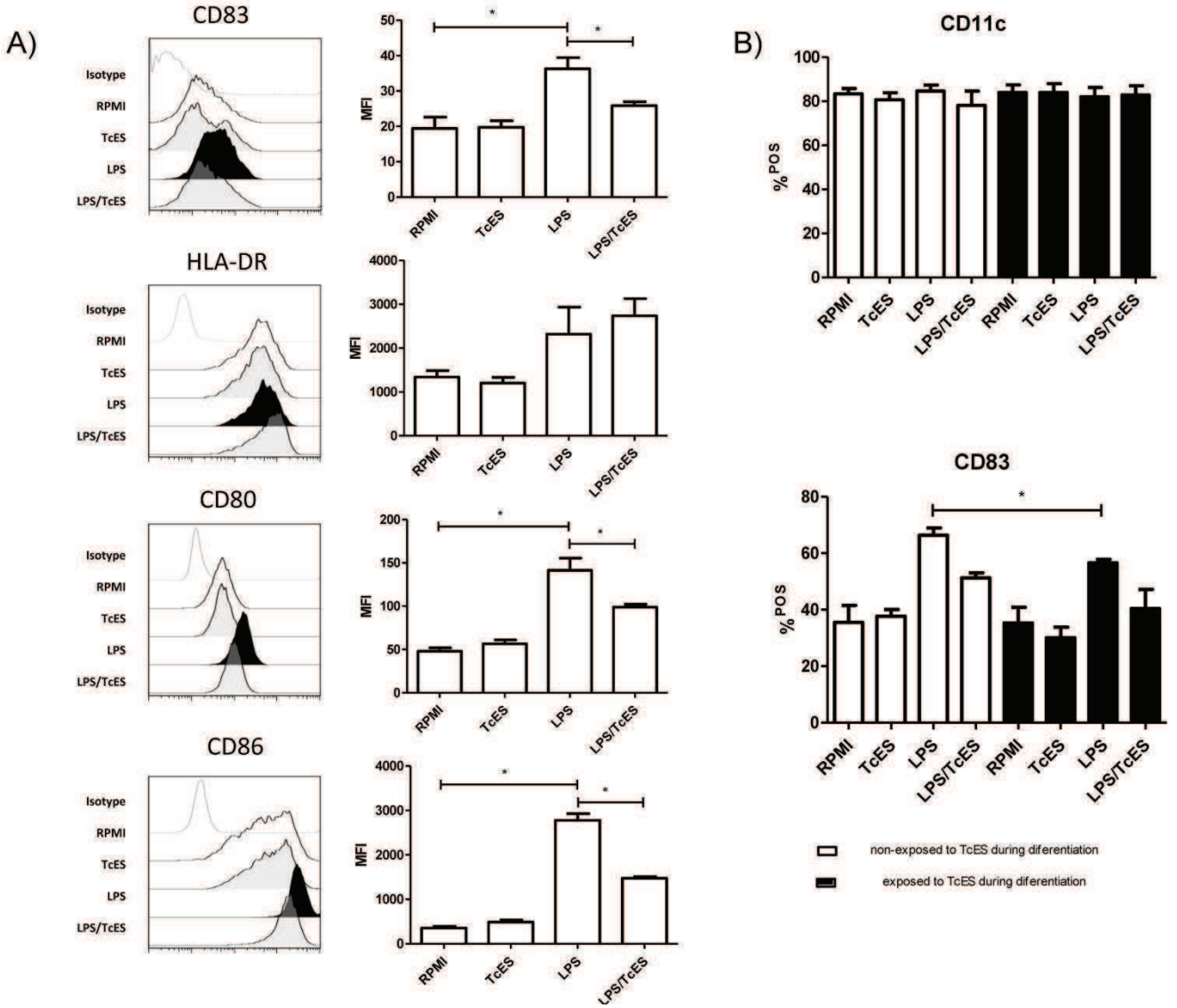


Figure 1. Expression of maturation markers in human DCs induced by TcES. A) iDCs were stimulated with 20μg of TcES, 1μg of LPS or a combination of them and 24h post-stimulation were stained with anti-CD83, anti-CD80, anti-CD86 and anti-HLA-DR and analyzed by flow-cytometry. B) iDCs stimulated with 20μg of TcES, 1μg of LPS or a combination of them *vs* human monocytes that were exposed to 20ug/ml of TcES at day 0 and 3 of their differentiation to DCs. Once differentiated in iDCs they were stimulated with LPS, LPS+TcES or TcES alone. DCs derived from donors 1-6 (six donors) were used for this analysis. Assays were performed once for each donor. Data represents means ± standard error of six independent experiments. *P<0.05 was deemed significant.

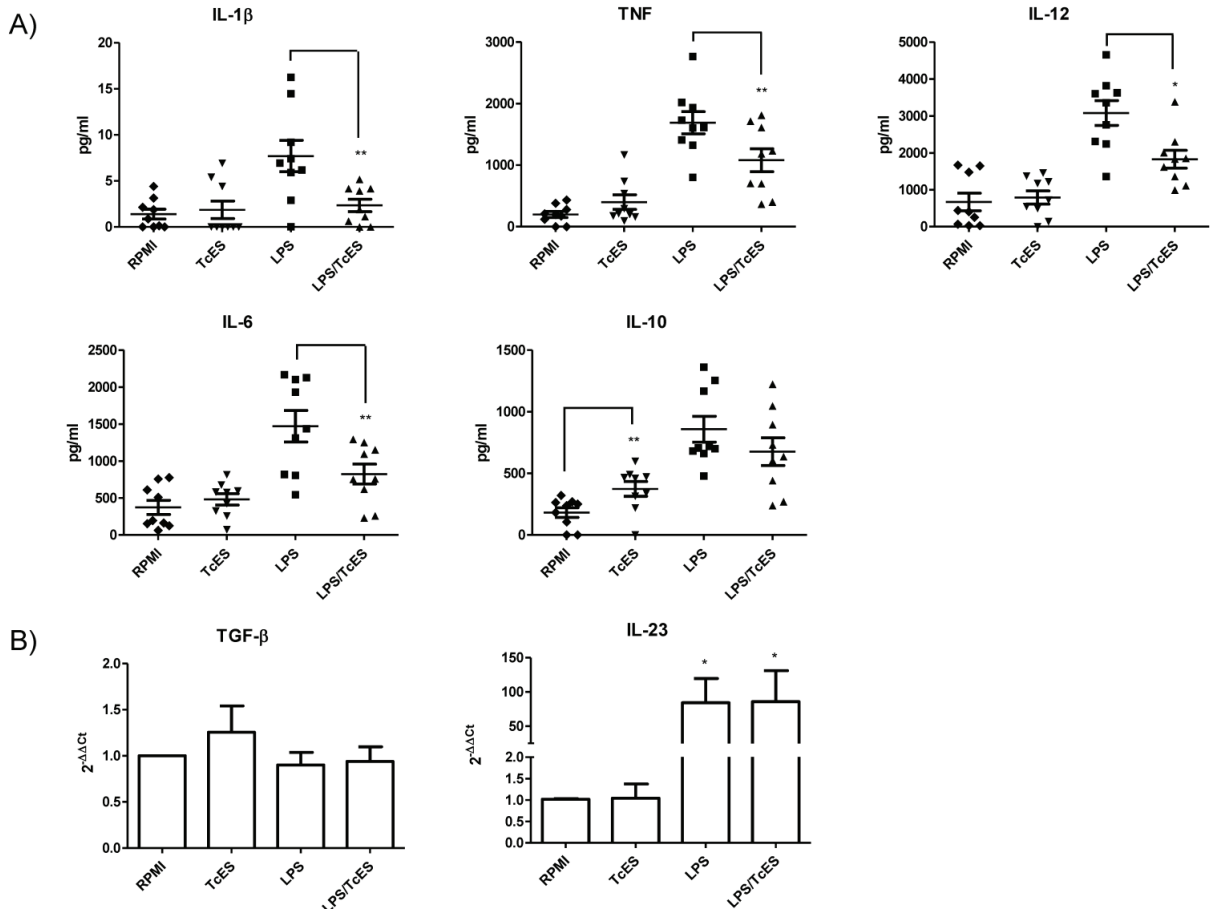


Figure 2. Cytokine response of human DCs exposed to TcES. Monocyte-derived DCs were stimulated with 20µg of TcES, 1µg of LPS or a combination of them. A) Supernatants were collected 24h post-stimulation and cytokine response was measured using commercial ELISA kits (Peprotech and R&D). DCs derived from donors 1-9 (nine donors) were used for this analysis. Assays were performed in duplicate. Data represents means ± standard error of nine independent experiments. * P<0.05 was deemed significant. LPS *vs* RPMI was significantly different for all cytokines B) Cells were collected 3h post-stimulation and RNA was extracted with TRIzol reagent and analysis of RT-qPCR was performed according to M&M. DCs derived from donors 10-15 (six donors) were used for this analysis. Assays were performed in duplicate. Data represents means ± standard error of six independent experiments. *P<0.05 was deemed significant.

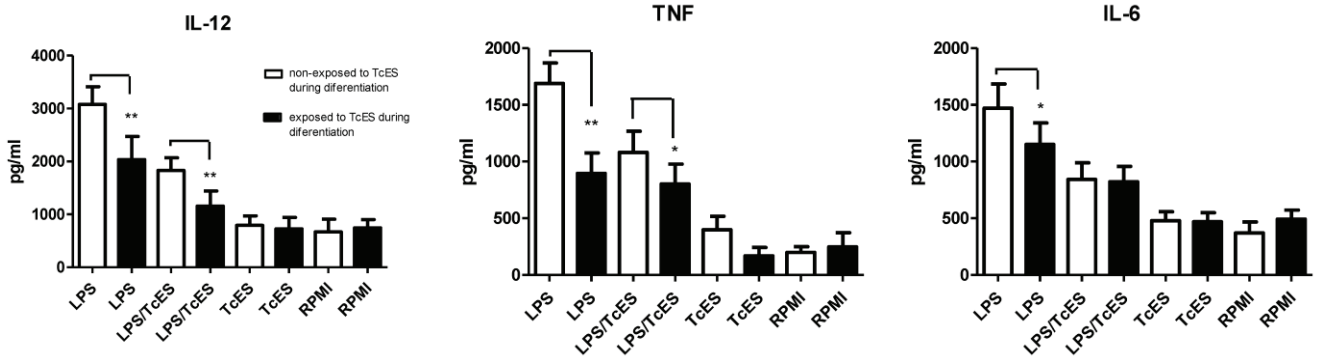


Figure 3. Comparison of cytokine response of human DCs exposed during differentiation from monocytes or at the end of their differentiation to TcES. Human monocytes were exposed to 20ug/ml of TcES at day 0 and 3 of their differentiation to DCs. Once differentiated in iDCs they were stimulated with LPS, LPS+TcES or TcES alone. Supernatants were collected 24h post-stimulation and production of cytokines measured using ELISA kits. DCs derived from donors 1-9 (nine donors) were used for this analysis. Assays were performed in duplicate. Data represents means ± standard error of nine independent experiments. *P<0.05 was deemed significant.

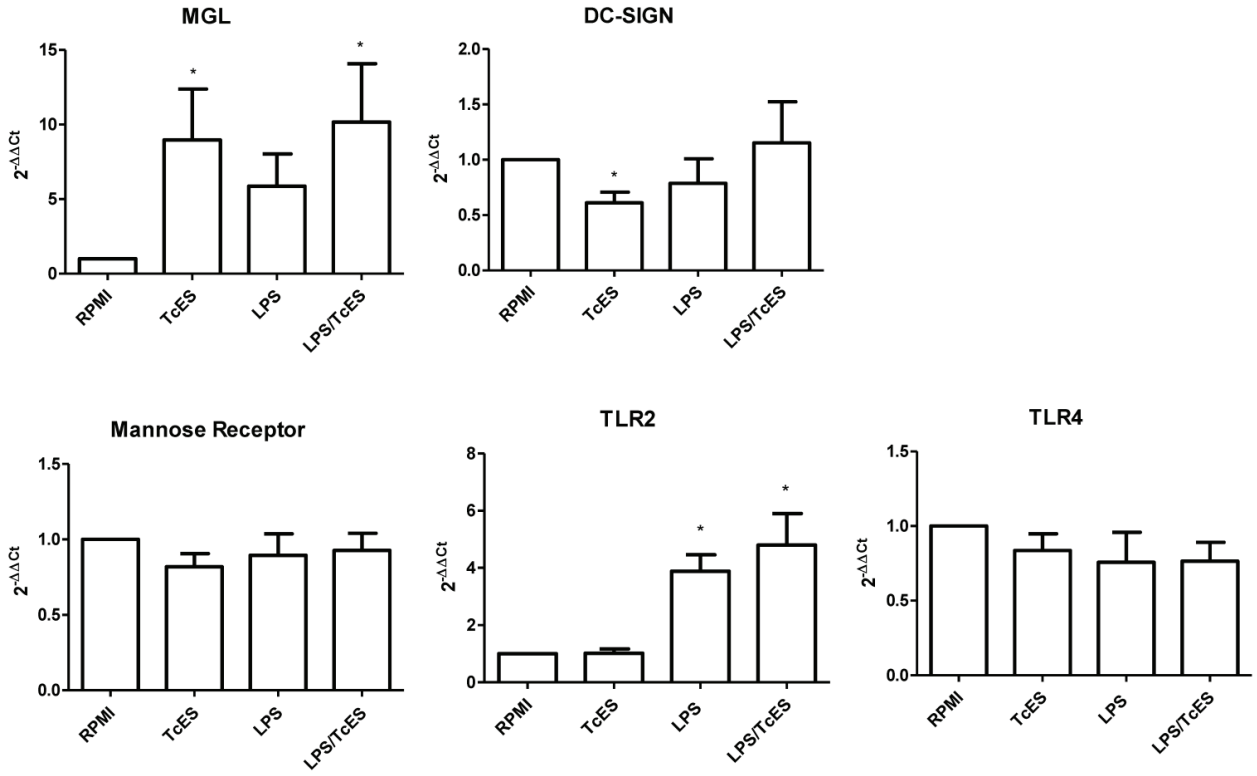


Figure 4. CLR and TLR expression in human DCs exposed to TcES. mRNA levels of MGL, DC-SIGN, MR, TLR4 and TLR2 were determined by RT-qPCR according to M&M in human DC exposed during 3h to 20μg of TcES, 1μg of LPS or a combination of them. DCs derived from donors 10-15 (six donors) were used for analysis. Assays were performed in duplicate. Data represents means ± standard error of six independent experiments. *P<0.05 was deemed significant.

TcES modulates expression of CLRs but not TLRs in human DCs

In order to assess if changes induced in DC phenotype by TcES was extended to CLRs and TLRs previously reported as important in the responses to helminth antigens [25], we performed RT-qPCR analysis to human DCs exposed to TcES, LPS and the combination of them.

We found that TcES modulate positively the transcription of the C-type lectin receptor MGL while LPS failed to do so (Fig 4). When the stimulus was a combination of TcES and LPS the MGL up-regulation was again observed, indicating that TcES is able to induce its transcriptional enhancement even in the presence of a complete different stimulus such as LPS. On the contrary, the transcription of another C-type lectin receptor, DC-SIGN, was diminished only in presence of TcES but not of LPS or LPS plus TcES. The mRNA expression of the mannose receptor was not modified under any stimuli tested in this study. Similarly, when we explored the activity of TcES on the expression of the two TLRs involved in LPS recognition, TLR2 and TL4, we did not observe any modulation (Fig 4).

Discussion

In this work we demonstrated that the excreted-secreted antigens of the cestode *T. crassiceps* (TcES) modify the phenotype of human DCs. We found that when exposed to TcES, these cells acquired a phenotype compatible with tolerogenic DCs judged by their low levels of maturation markers such as CD83, CD80 and CD86 and the lack of secretion of inflammatory cytokines but instead an enhanced production of IL-10. The effects of TcES upon DCs were not limited to their capacity to induce a tolerogenic-like phenotype, since these antigens also down-modulated the pro-maturation and pro-inflammatory activities that LPS exerts on DCs, by reducing the expression of CD83, CD80, CD86 and the production of IL-12, TNF, IL-1β and IL-6 but interestingly, not the one of IL-10.

Our results are in accordance with other reports describing that DCs show an "immature" phenotype after exposure to helminth antigens and where such exposure can also impair the subsequent TLR-mediated response induced in these cells [30, 41]. However, IL-10 production by DCs exposed to helminth antigens has not been a common observation.

For instance, others have found that DCs exposed to *Echinococcus granulosus* or *Trichuris muris*-derived antigens had an enhanced production of IL-10, however these cells also produced significant amounts of TNF and/or IL-6 [36, 42]. The induction of IL-10 by TcES may also possibly explain the apparent selective down-modulation of pro-inflammatory cytokines by TcES in DCs challenged with LPS. To our knowledge, this is the first study to report an enhanced production of the regulatory IL-10 by human DCs in response to helminth antigens, with no concomitant secretion of pro-inflammatory cytokines, reinforcing the idea of the induction of a tolerogenic-like phenotype in these cells by TcES. It will be crucial to address if these DCs, besides its tolerogenic phenotype, are consequently able of inducing T regulatory responses.

As we stated earlier, most of the tolerogenic DCs described to date display an immature like-phenotype characterized by a lower expression of co-stimulatory molecules, a decreased production of pro-inflammatory key cytokines such as IL-12 and a preferential production of regulatory cytokines such as IL-10 and TGF-β [9-11]. Treatment of DCs with agents as IL-10 or corticoids stimulates their differentiation into a tolerogenic type [2, 7-8, 43]. Particularly, tolerogenic DCs that receive dexamethasone show a differential expression of the CLRs DC-SIGN, MR and MGL, where only the last one is up-regulated [14]. Interestingly, we found that human DCs exposed to TcES express a *MGL* transcriptional up-regulation, suggesting once again an induction of a tolerogenic phenotype in these cells. DC-SIGN is another CLR with important functions in DCs. This receptor not only supports recognition and internalization of pathogens for a later antigen presentation but it is also involved in shaping dendritic and T cell responses [44]. Interestingly, we observed that TcES but not LPS, down-modulated transcriptional expression of *DC-SIGN* in human DCs. In accordance with our findings, it has been shown that the human herpesvirus 6 [45], *Leishmania donovani*, *L. major* [46], and the helminth *Brugia malayi* [47] are able to reduce the expression of this CLR in DCs, presumably interfering with their function. Considering that the balance between the TLR and the CLR repertoire and their functional cooperation participate in the fine-tuning of regulatory mechanisms to allow appropriate immune responses, the over-expression of *MGL* in DCs exposed to TcES and the simultaneous down-regulation of *DC-SIGN*, may be part of the possible mechanisms used by *T. crassiceps* to modify the phenotype and hence the function of human DCs, directing the balance towards immune suppression and allowing in this way its own survival. We have previously shown that *T. crassiceps* antigens are heavily glycosilated [18] and that the carbohydrates present in these antigens are critical for their modulatory activities [18, 35, 48]. For these reasons, it is not surprising that TcES could modulate the expression of receptors such as *MGL* and *DC-SIGN*, which recognize sugar moieties. In fact, *T. crassiceps* antigens are glycosilated [49] with the specific carbohydrates known to be recognized at least by DC-SIGN. More studies are necessary to address if the mRNA pattern of expression of CLRs observed in this study in DCs exposed to TcES is also observed at the protein level, but yet more important is to determine if these receptors are involved in the recognition of and in the modulatory activity that *T. crassiceps*-antigens exert on human DCs.

Our results indicate that DCs challenged with TcES can impair their response to LPS by inhibiting the maturation process and reducing the secretion of pro-inflammatory cytokines such as IL-12, IL-1β, TNF and IL-6, suggesting that TcES render these DCs unable to mature and probably incapable to support an inflammatory response after challenged with to a TLR stimulus. In this study we also examined the response of monocytes to TcES during their differentiation into DCs. In contrast with other studies that have shown that helminths or their products can affect the differentiation of monocytes into DCs [36-37], we found that exposure of monocytes to TcES did not affect the final percentage of CD11c+ cells, suggesting that differentiation of monocytes into DCs is not affected by TcES. However, when stimulated with LPS, TcES-differentiated DCs showed a significant reduction in the percentage of CD83+ cells and a more pronounced dropping in their production of IL-1β, TNF and IL-6 when compared with DCs that were not exposed to TcES during their differentiation, suggesting that if monocytes encounter excreted/secreted antigens of *T. crassiceps* in their differentiation process to DCs, this could greatly affect their ability to mature and to respond to subsequent TLR stimulation. This is a plausible scenario to happen also in vivo since it has been reported that infections with metacestodes are accompanied with the release of antigens to circulation [38-39].

There has been a lot of speculation regarding the use of helminth antigens as modulators in autoimmune disorders [50-51]. Recent findings indicate that the cestode *T. crassiceps* is capable of ameliorating autoimmune pathologies in animal models [52]. Importantly, despite this cestode is not a human parasite in this work we demonstrate that its molecules have a modulatory activity upon human cells, opening the possibility to use these antigens as modulators in human disorders. A possible explanation for the con-

served activity of TcES across species may simply rely on the immunogenic resemblance between the human cestode parasite *T. solium* and *T. crassiceps* [32, 53].

In summary, here we show that TcES induce a tolerogenic-like phenotype in human DCs as judged by the lack of enhancement of maturation and co-stimulatory molecules, an elevated production of IL-10 and the up-regulation of *MGL* expression. Additionally, these antigens are capable of impair the LPS pro-inflammatory activity in the same cells, down-modulating their maturation and secretion of inflammatory cytokines.

Acknowledgments

This work was supported by Instituto Nacional de CardiologÍa Ignacio Chávez. To laboratory technician Angeles Soster Contreras for her assistance to obtain blood samples.

Conflict of Interests

The authors have declared that no conflict of interest exists.

References

1. Banchereau J, et al. Immunobiology of dendritic cells. Annu Rev Immunol, 2000. 18: 767-811.
2. Steinman R.M, Hawiger D, and Nussenzweig M.C. Tolerogenic dendritic cells. Annu Rev Immunol, 2003. 21: 685-711.
3. Goubier A, et al. Plasmacytoid dendritic cells mediate oral tolerance. Immunity, 2008. 29(3): 464-75.
4. Pulendran B, et al. Modulating the immune response with dendritic cells and their growth factors. Trends Immunol, 2001. 22(1): 41-7.
5. Jonuleit H, et al. Induction of interleukin 10-producing, non-proliferating CD4(+) T cells with regulatory properties by repetitive stimulation with allogeneic immature human dendritic cells. J Exp Med, 2000. 192(9): 1213-22.
6. Dhodapkar M.V, et al. Antigen-specific inhibition of effector T cell function in humans after injection of immature dendritic cells. J Exp Med, 2001. 193(2): 233-8.
7. Piemonti L, et al. Glucocorticoids affect human dendritic cell differentiation and maturation. Immunol J, 1999. 162(11): 6473-81.
8. Steinbrink K, et al. Induction of tolerance by IL-10-treated dendritic cells. Immunol J, 1997. 159(10): 4772-80.
9. Coombes J.L, et al. A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. J Exp Med, 2007. 204(8): 1757-64.
10. Akbari O, DeKruyff R.H, and Umetsu D.T. Pulmonary dendritic cells producing IL-10 mediate tolerance induced by respiratory exposure to antigen. Nat Immunol, 2001. 2(8): 725-31.
11. Roncarolo M.G, Levings M.K, and Traversari C. Differentiation of T regulatory cells by immature dendritic cells. J Exp Med, 2001. 193(2): F5-9.
12. Ghiringhelli F, et al. Tumor cells convert immature myeloid dendritic cells into TGF-beta-secreting cells inducing CD4+CD25+ regulatory T cell proliferation. J Exp Med, 2005. 202(7): 919-29.
13. Geijtenbeek T.B, et al. Self- and nonself-recognition by C-type lectins on dendritic cells. Annu Rev Immunol, 2004. 22: 33-54.
14. van Vliet S.J, et al. Differential regulation of C-type lectin expression on tolerogenic dendritic cell subsets. Immunobiology, 2006. 211(6-8): 577-85.
15. Appelmelk B.J, et al. Cutting edge: carbohydrate profiling identifies new pathogens that interact with dendritic cell-specific ICAM-3-grabbing nonintegrin on dendritic cells. Immunol J, 2003. 170(4): 1635-9.
16. Geijtenbeek T.B, et al. Mycobacteria target DC-SIGN to suppress dendritic cell function. J Exp Med, 2003. 197(1): 7-17.
17. Gantner B.N, et al. Collaborative induction of inflammatory responses by dectin-1 and Toll-like receptor 2. J Exp Med, 2003. 197(9): 1107-17.
18. Gomez-Garcia L, et al. Intact glycans from cestode antigens are involved in innate activation of myeloid suppressor cells. Parasite Immunol, 2005. 27(10-11): 395-405.
19. Geijtenbeek T.B and Gringhuis S.I. Signalling through C-type lectin receptors: shaping immune responses. Nat Rev Immunol, 2009. 9(7): 465-79.
20. Maizels R.M, et al. Regulation of pathogenesis and immunity in helminth infections. J Exp Med, 2009. 206(10): 2059-66.
21. Semnani R.T, et al. Filaria-induced immune evasion: suppression by the infective stage of Brugia malayi at the earliest host-parasite interface. Immunol J, 2004. 172(10): 6229-38.
22. Sciutto E, et al. Depressed T-cell proliferation associated with susceptibility to experimental Taenia crassiceps infection. Infect Immun, 1995. 63(6): 2277-81.
23. Reyes J.L and Terrazas L.I. The divergent roles of alternatively activated macrophages in helminthic infections. Parasite Immunol, 2007. 29(12): 609-19.
24. Segura M, et al. Impairment of dendritic cell function by excretory-secretory products: a potential mechanism for nematode-induced immunosuppression. Eur Immunol J, 2007. 37(7): 1887-904.
25. Terrazas C.A, Terrazas L.I, and Gomez-Garcia L. Modulation of dendritic cell responses by parasites: a common strategy to survive. J Biomed Biotechnol. 2010;: 357106.
26. Kean D.E, et al. Dissecting Ascaris glycosphingolipids for immunomodulatory moieties--the use of synthetic structural glycosphingolipid analogues. Parasite Immunol, 2006. 28(3): 69-76.
27. Jenkins S.J and Mountford A.P. Dendritic cells activated with products released by schistosome larvae drive Th2-type immune responses, which can be inhibited by manipulation of CD40 costimulation. Infect Immun, 2005. 73(1): 395-402.
28. Balic A, et al. Selective maturation of dendritic cells by Nippostrongylus brasiliensis-secreted proteins drives Th2 immune responses. Eur Immunol J, 2004. 34(11): 3047-59.
29. Dowling D.J, et al. Major secretory antigens of the helminth Fasciola hepatica activate a suppressive dendritic cell phenotype that attenuates Th17 cells but fails to activate Th2 immune responses. Infect Immun. 2010;78(2): 793-801.
30. van Liempt E, et al. Schistosoma mansoni soluble egg antigens are internalized by human dendritic cells through multiple C-type lectins and suppress TLR-induced dendritic cell activation. Mol Immunol, 2007. 44(10): 2605-15.
31. Espindola N.M, et al. Cysticercosis immunodiagnosis using 18- and 14-kilodalton proteins from Taenia crassiceps cysticercus antigens obtained by immunoaffinity chromatography. J Clin Microbiol, 2005. 43(7): 3178-84.
32. Toledo A, et al. Two epitopes shared by Taenia crassiceps and Taenia solium confer protection against murine T. crassiceps cysticercosis along with a prominent T1 response. Infect Immun, 2001. 69(3): 1766-73.
33. Villa O.F and Kuhn R.E. Mice infected with the larvae of Taenia crassiceps exhibit a Th2-like immune response with concomitant anergy and downregulation of Th1-associated phenomena. Parasitology, 1996. 112 (Pt 6): 561-70.

34. Rodriguez-Sosa M, et al. A STAT4-dependent Th1 response is required for resistance to the helminth parasite Taenia crassiceps. Infect Immun, 2004. 72(8): 4552-60.
35. Terrazas C.A, Gomez-Garcia L, and Terrazas L.I. Impaired pro-inflammatory cytokine production and increased Th2-biasing ability of dendritic cells exposed to Taenia excreted/secreted antigens: A critical role for carbohydrates but not for STAT6 signaling. Int J Parasitol. 2010;40(9): 1051-62.
36. Rigano R, et al. Echinococcus granulosus antigen B impairs human dendritic cell differentiation and polarizes immature dendritic cell maturation towards a Th2 cell response. Infect Immun, 2007. 75(4): 1667-78.
37. Fujiwara R.T, et al. Necator americanus infection: a possible cause of altered dendritic cell differentiation and eosinophil profile in chronically infected individuals. PLoS Negl Trop Dis, 2009. 3(3): e399.
38. Nguekam A, et al. Kinetics of circulating antigens in pigs experimentally infected with Taenia solium eggs. Vet Parasitol, 2003. 111(4): 323-32.
39. Alexander A.M, et al. Long-term clinical evaluation of asymptomatic subjects positive for circulating Taenia solium antigens. Trans R Soc Trop Med Hyg. 2010;104(12): 809-10.
40. Cronin S.J and Penninger J.M. From T-cell activation signals to signaling control of anti-cancer immunity. Immunol Rev, 2007. 220: 151-68.
41. Hamilton C.M, et al. The Fasciola hepatica tegumental antigen suppresses dendritic cell maturation and function. Infect Immun, 2009. 77(6): 2488-98.
42. D'Elia R and Else K.J. In vitro antigen presenting cell-derived IL-10 and IL-6 correlate with Trichuris muris isolate-specific survival. Parasite Immunol, 2009. 31(3): 123-31.
43. Moore K.W, et al. Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol, 2001. 19: 683-765.
44. Svajger U, et al. C-type lectin DC-SIGN: an adhesion, signalling and antigen-uptake molecule that guides dendritic cells in immunity. Cell Signal. 2010;22(10): 1397-405.
45. Niiya H, et al. Transcriptional downregulation of DC-SIGN in human herpesvirus 6-infected dendritic cells. J Gen Virol, 2004. 85(Pt 9): 2639-42.
46. Revest M, et al. Comparison of the immunomodulatory effects of donovani L and L. major excreted-secreted antigens, particulate and soluble extracts and viable parasites on human dendritic cells. Vaccine, 2008. 26(48): 6119-23.
47. Talaat K.R, et al. Preexposure to live Brugia malayi microfilariae alters the innate response of human dendritic cells to Mycobacterium tuberculosis. J Infect Dis, 2006. 193(2): 196-204.
48. Gomez-Garcia L, et al. Carbohydrate components of Taenia crassiceps metacestodes display Th2-adjuvant and anti-inflammatory properties when co-injected with bystander antigen. Parasitol Res, 2006. 99(4): 440-8.
49. Lee J.J, et al. Mass spectrometric characterisation of Taenia crassiceps metacestode N-glycans. Mol Biochem Parasitol, 2005. 143(2): 245-9.
50. McKay D.M. The therapeutic helminth? Trends Parasitol, 2009. 25(3): 109-14.
51. Wilson M.S and Maizels R.M. Regulation of allergy and autoimmunity in helminth infection. Clin Rev Allergy Immunol, 2004. 26(1): 35-50.
52. Argueta-Donohue J, et al. Leishmania mexicana: participation of NF-kappaB in the differential production of IL-12 in dendritic cells and monocytes induced by lipophosphoglycan (LPG). Exp Parasitol, 2008. 120(1): 1-9.
53. Peralta R.H, et al. Taenia crassiceps cysticerci: Characterization of the 14-kDa glycoprotein with homologies to antigens from Taenia solium cysticerci. Exp Parasitol, 2010 Mar;124(3):295-300.

The FASEB Journal article fj.13-228932. Published online August 1, 2013.

The FASEB Journal • Research Communication

Helminth-excreted/secreted products are recognized by multiple receptors on DCs to block the TLR response and bias Th2 polarization in a cRAF dependent pathway

César A. Terrazas,[*,†,‡] Marcela Alcántara-Hernández,[§] Laura Bonifaz,[§] Luis I. Terrazas,[*,1] and Abhay R. Satoskar[†,‡,1]

*Unidad de Biomedicina, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México, Mexico City, Mexico; [†]Department of Microbiology and [‡]Department of Pathology, The Ohio State University Medical Center, Columbus, Ohio, USA; and [§]Centro Médico Siglo XXI, Unidad de Investigación Médica en Inmunoquímica, Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico

ABSTRACT **Dendritic cells (DCs) recognize pathogens and initiate the T-cell response. The DC-helminth interaction induces an immature phenotype in DCs; as a result, these DCs display impaired responses to TLR stimulation and prime Th2-type responses. However, the DC receptors and intracellular pathways targeted by helminth molecules and their importance in the initiation of the Th2 response are poorly understood. In this report, we found that products excreted/secreted by *Taenia crassiceps* (TcES) triggered cRAF phosphorylation through MGL, MR, and TLR2. TcES interfered with the LPS-induced NFκB p65 and p38 MAPK signaling pathways. In addition, TcES-induced cRAF signaling pathway was critical for down-regulation of the TLR-mediated DC maturation and secretion of IL-12 and TNF-α. Finally, we show for the first time that blocking cRAF in DCs abolishes their ability to induce Th2 polarization *in vitro* after TcES exposure. Our data demonstrate a new mechanism by which helminths target intracellular pathways to block DC maturation and efficiently program Th2 polarization.—Terrazas, C. A., Alcántara-Hernández, M., Bonifaz, L., Terrazas, L. I., Satoskar, A. R. Helminth-excreted/secreted products are recognized by multiple receptors on DCs to block the TLR response and bias Th2 polarization in a cRAF dependent pathway. *FASEB J.* 27, 000–000 (2013). www.fasebj.org**

Key Words: CLRs · dendritic cell · mannose receptor · immunomodulation · MGL

HELMINTH PARASITES ARE a major public health problem in developing countries, and they can establish chronic infection in their hosts, owing to their ability to escape or modulate the host's immune system *via* multiple mechanisms. These parasites skew the immune response toward a T-helper 2 (Th2) and/or a regulatory environment associated with high levels of interleukin (IL)-4, IL-13, IL-5, and IL-10. In addition, helminth infections impair immunity against other unrelated infections and may affect vaccine efficacy (1). One potential immune evasion strategy involves the modulation of early responses by dendritic cells (DCs; refs. 2, 3). DCs sample the environment and recognize pathogen-associated molecular patterns (PAMPs) as well as the host's danger signals. When DCs are activated by viral, bacterial, or protozoan-derived molecules that bind Toll-like receptors (TLRs), intracellular signaling through the mitogen-activated protein kinase (MAPK) family (*e.g.*, p38, JNK, ERK), the adapter molecule MyD88, and the transcription factor nuclear factor κB (NFκB) is initiated, leading to the up-regulation of costimulatory molecules (*e.g.*, CD80, CD86, CD40) and chemokine receptors, as well as the release of proinflammatory cytokines, such as IL-12 and tumor necrosis factor α (TNF-α) (4). After pathogen recognition, DCs migrate from the peripheral tissue, armored with a variety of signals to activate T cells in the lymph nodes. T-cell polarization depends on signals delivered by DCs. For example, while IL-12 secretion activates T

Abbreviations: BMDC, bone marrow-derived dendritic cell; CLR, C-type lectin receptor; DC, dendritic cell; IL, interleukin; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MFI, mean fluorescence intensity, MGL, macrophage galactose C-type lectin; MR, mannose receptor; NFκB, nuclear factor κB; OVA, ovalbumin; SEA, soluble egg antigen; TcES, *Taenia crassiceps*-excreted/secreted molecules; Th, T helper; TNFα, tumor necrosis factor alpha; TLR, Toll-like receptor; WT, wild type

[1] Correspondence: A.S., Department of Pathology, The Ohio State University, Columbus, OH 43210, USA. E-mail: abhay.satoskar@osumc.edu; L.I.T., Unidad de Biomedicina, FES-Iztacala, UNAM, Av. De los Barrios 1, Los Reyes Iztacala, Tlalnepantla, Edo. de Mexico, Mexico 54090. E-mail: literrazas@campus.iztacala.unam.mx

doi: 10.1096/fj.13-228932

This article includes supplemental data. Please visit *http://www.fasebj.org* to obtain this information.

cells toward a Th1 profile, IL-10 and TGF-β are required to prime T_{reg} cells. However, the signals delivered by DCs necessary to induce a Th2 response have not yet been clearly defined (3, 5, 6).

In contrast to classical DC activation, mounting evidence indicates that DCs exposed to different helminth-derived molecules display a conserved phenotype similar to immature DCs, but, unlike immature DCs, they show an impaired response to several TLR agonists, although they are still efficient Th2 and/or T_{reg} inducers (2, 3). Despite the growing interest in understanding DC-helminth interactions, not much is known about the ligands, receptors, and intracellular signaling pathways involved. Glycosylated molecules derived from helminths are suspected to play a role in the induction of Th2 responses (2, 6–9). The main surface receptors on DCs that recognize glycosylated structures are the C-type lectin receptors (CLRs). Although some helminths have been shown to bind CLRs, it is still unknown whether this interaction triggers signaling in DCs (10, 11).

Taenia crassiceps is a helminth parasite that has been used in the study of parasite-host interactions in cysticercosis (12). As the infection progresses, the immune response elicited by this parasite is polarized to Th2 (13, 14). Like other helminth derivatives, the *T. crassiceps*-excreted/secreted molecules (TcES) impair the ability of bone marrow-derived DCs (BMDCs) and human-derived DCs to respond to different proinflammatory ligands, such as lipopolysaccharide (LPS), CpG, and *Toxoplasma gondii* antigen. Moreover, TcES-treated DCs prime the Th2 response *in vitro* in a carbohydrate-dependent fashion (15, 16).

To investigate the mechanisms involved in helminth-DC immune modulation and identify molecules targeted by TcES, we used blocking antibodies against C-type lectins as well as MAPK inhibitors to block intracellular pathways. We found that TcES bound preferentially to mannose receptor (MR) and macrophage galactose C-type lectin (MGL), and $TLR2^{-/-}$ and $MGL^{-/-}$ DCs displayed a reduced ability to bind TcES. Exposure of DCs to TcES *in vitro* resulted in the enhanced localization of RAS to the cytoplasm and phosphorylation of cRAF, but not ERK1/2, p38, or NFκB p65. We also showed that cRAF phosphorylation was mediated by multiple-receptor engagement, including MR, MGL, and TLR2. On the other hand, TcES inhibited LPS-mediated phosphorylation of p38 and NFκB p65, DC maturation, cytokine production, and the ability of LPS-treated DCs to prime Th1 responses *in vitro*. Using a selective cRAF inhibitor (GW5074) or cRAF-specific siRNA, we found that DCs exposed to TcES were unable to impair the up-regulation of IL-12, TNF-α, CD80, and CD86 in response to LPS. Moreover, the ability of TcES-exposed DCs to efficiently promote the production of IL-4 and IL-13 by T cells was abolished in DCs pretreated with cRAF inhibitor. Taken together, our results demonstrate for the first time that the cRAF pathway is triggered by helminth molecules through multiple receptors and that cRAF is essential for the blockage of TLR mediated inflammatory signaling in DCs, the attenuation of Th1 polarization and the promotion of Th2 polarization *in vitro*. These findings provide new information on the mechanism by which helminths induce intracellular signaling to block the TLR pathway in DCs and direct a Th2 polarized immune response. This knowledge can be exploited in the identification of novel therapeutic strategies against parasitic worms, autoimmune diseases, and allergic reactions.

MATERIALS AND METHODS

Mice

Wild-type (WT) C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME, USA), and MGL-deficient mice were obtained from The Scripps Research Institute (La Jolla, CA, USA). The mice were maintained in a pathogen free animal facility at The Ohio State University (Columbus, OH, USA) and Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México (Mexico City, Mexico) in accordance with U.S. National Institutes of Health and institutional guidelines.

Parasites and TcES

Metacestodes of *T. crassiceps* were harvested under sterile conditions from the peritoneal cavity of female BALB/c mice after 6–8 wk of infection. The cysticerci were washed 4 times in sterile PBS and cultured in PBS at 37°C for 24 h. TcES were recovered from the supernatant and centrifuged for 10 min at 5000 rpm. Next, the proteins were concentrated using an Amicon Ultrafilter with a 50-kDa cutoff membrane (Millipore, Billerica, MA, USA). The high-molecular-mass molecules were collected, and protease inhibitors were added. The samples were stored at −70°C until further use.

DC stimulation and coculture assays

BMDCs were obtained as described previously (16). DCs were incubated with TcES (40 μg/ml) or LPS (1 μg/ml). After 24 h, DCs were analyzed by flow cytometry, and supernatants were recovered for cytokine detection by ELISA. For cRAF inhibition experiments, DCs were preincubated with cRAF inhibitor GW5074 (Merck KGaA, Darmstadt, Germany) or DMSO for 2 h. cRAF RNA knockdown experiments were performed using SmartPool On-Target plus RAF1 siRNA or nontargeting control siRNA along with Dharmafect transfection medium (ThermoFisher Scientific, Lafayette, CO, USA), as described previously (17). For cocultures, DCs were preloaded with ovalbumin (OVA) peptide (2 μg/ml) for 2 h and incubated with GW5074 for an additional 2 h, then stimulated with TcES and/or LPS. After 24 h, DCs were washed 3 times with PBS and coincubated with purified OTII $CD4^+$ T cells at a 1:2 ratio. Supernatants were recovered 7 d later, and cytokines produced were analyzed by ELISA. For *in vivo* studies, DCs were stimulated 24 h *in vitro*, then injected into the footpad of C57BL/6 mice that had previously been adoptively transferred with CFSE-labeled OTII $CD4^+$ T cells. After 7 d, lymph node cells were obtained and stimulated with PMA/ionomycin for 4 h. Intracellular IL-4 production was measured by flow cytometry using APC anti-IL-4 antibody

(BioLegend, San Diego, CA, USA) on cells gated on $CD4^+$ and $CFSE^+$ populations.

Labeling of TcES with FITC

TcES were labeled with FITC (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer's protocol. Briefly, 1 mg/ml solution of TcES was prepared in 0.1 M sodium bicarbonate buffer (pH 9). FITC was dissolved in DMSO at 1 mg/ml, and 50 μl of FITC solution was then slowly added to the TcES solution in 5-μl aliquots with gentle stirring. The resulting TcES-FITC solution was incubated for 8 h at 4°C in the dark, and then washed 3 times with PBS using a 50-kDa cutoff membrane (Amicon Ultrafilter; Millipore) and centrifugation at 4000 *g* for 20 min. The proteins were quantified by Bradford assay and maintained at 4°C.

Antibody blocking assays

Different glycans were used for the competition assays, as described by van Vliet *et al.* (10). Laminarin, mannan, and GalNac (all 100 μg/ml) were used to block CLRs (Sigma-Aldrich). EGTA (10 mM) was used to disrupt the structure of the C-type lectins. Blocking antibodies anti-MR, anti-TLR2 (BioLegend, San Diego, CA, USA) and anti-MGL (Hycult Biotech, Plymouth Meeting, PA, USA) were used at a concentration of 10 μg/ml. DCs were cultured with or without blocking reagents and/or antibodies for 30 min at 37°C, then incubated with TcES-FITC for 30 min at 37°C. The cells were washed 3 times with FACS buffer, and TcES fluorescence was detected by flow cytometry. The role of individual receptors or receptor combinations in TcES-mediated inhibition of IL-12 and TNF-α induction after stimulation by LPS was analyzed by ELISA. In addition, the ability of TcES to induce Th2 responses in coculture experiments was evaluated.

Analysis of cell surface markers on BMDCs

Surface expression of DC maturation markers was analyzed using multicolor flow cytometry. After 24 h of stimulation, BMDCs were harvested, washed, and resuspended in cold PBS containing 5% FCS and 0.05% NaN_3. Fc receptors were blocked with mouse serum for 20 min at 4°C. The cells were washed and stained with APC-conjugated anti-CD11c, FITC-conjugated anti-CD80, and PE-conjugated anti-CD86 antibodies (BioLegend). The cells were analyzed on a FACSCalibur flow cytometer using Cell Quest software (BD Biosciences, San Jose, CA, USA). The data are represented as mean fluorescence intensity (MFI).

TLRs and NFκB reporter assay

HEK cells transfected with TLR4/CD14, TLR2/CD14, or TLR2/TLR6 were kindly donated by Dr. Laura Bonifaz (Instituto Mexicano del Seguro Social, Mexico City, Mexico). The HEK cells were plated in 12-well plates at 2×10^5 cells/well and stimulated with TcES. LPS and zymosan were used as positive controls for the TLR4- and TLR2-transfected cells, respectively. After stimulation for 24 h, the supernatants were recovered, and the production of human IL-8 was analyzed by ELISA. For NFκB reporter assays, RAW-blue cells were purchased from InvivoGen (San Diego, CA, USA). Briefly, 100,000 cells were plated in a 96-well plate and stimulated with LPS, TcES, or both. After 24 h of incubation, 50 μl of supernatant was recovered, to which 150 μl of Quanti-Blue substrate (InvivoGen) was added. Levels of secreted embryonic alkaline phosphatase indicative of NFκB activation were analyzed, measuring the absorbance at 655 nm.

Phospho-flow cytometry/immunofluorescence

BMDCs obtained after 7 d of culture were washed and plated in RPMI 1640 medium without GM-CSF for 24 h. Then 2×10^5 cells were stimulated for 30 min with the different treatments as described above. After stimulation, the cells were incubated for 15 min with anti-CD16/32, and then stained with anti-CD11c antibody (BioLegend). After incubation, the cells were fixed with 2% paraformaldehyde for 10 min at room temperature, washed with staining buffer, and permeabilized with ice-cold methanol for 10 min at 4°C. Cells were then washed twice with staining buffer, then incubated with a primary rabbit anti-mouse antibody against phospho-cRAF or phospho-SYK (1:100; Cell Signaling, Danvers MA, USA) for 20 min in the dark at room temperature. Finally, the cells were labeled with a secondary antibody, donkey anti-rabbit DyLight 488 (BioLegend) at a concentration of 0.25 $\mu g/10^6$ cells for 20 min at room temperature in the dark. The cells were then analyzed by flow cytometry or by fluorescence microscopy. Secondary antibody alone was used as a negative control.

Immunoblot

DCs were differentiated as described above. On d 7, the DCs were harvested and plated at 2.5×10^6 cells/ml in 6-well plates. The cells were used 24 h after plating to eliminate any residual effects from GM-CSF. DCs were stimulated with different doses of TcES at the indicated times. In some experiments, inhibitors were added 2 h before stimulation. After stimulation, the cells were centrifuged at 1500 rpm for 5 min and washed with PBS. The cells were then lysed with lysis buffer for 15 min and lysates were quantified using the BCA assay (ThermoFisher Scientific) and then frozen at −80°C until further use. The proteins were resolved using 10% SDS-PAGE and blotted onto a PDVF membrane for 2 h. The membrane was blocked using TBS supplemented with 10% nonfat milk for 2 h, incubated overnight at 4°C on a rotary platform with primary antibodies against RAS or p-MEK1/2 (Millipore), p-cRAF Ser338, p-p38, p-ERK1/2 or p-NFκB p65 Ser536 (Cell Signaling) at a dilution of 1:1000. Membranes were washed 3 times for 5 min with PBS containing 0.5% Tween-20 and incubated with the secondary antibody anti-rabbit-HRP (Cell Signaling) for 2 h. Finally, chemiluminescence was developed using an Amersham ECL chemiluminescent kit (GE Healthcare Biosciences, Pittsburgh, PA, USA) and acquired using FluorChem HD2 Imager (ProteinSimple, Santa Clara, CA, USA).

DC and T-cell cocultures

DCs were obtained as described above, and $CD4^+$ T cells were obtained from OTII mice by negative selection using magnetic microbeads. The DCs were plated at 5×10^4 cells/well in 96-well plates and then stimulated as follows. First, 2 μg/ml OVA peptide 323–339 was added to the cultures. After 2 h, cRAF inhibitor was added at the indicated concentrations. After another 2 h, TcES and/or LPS were added to the DCs. After 24 h, the DCs were harvested and centrifuged, and supernatants were removed. The DCs were then washed 3 times with PBS to avoid any inhibitory action on T cells. Finally, T cells were added to the DC cultures at a DC:T-cell ratio of 1:2 and maintained for 7 d. The supernatants from the cocultures were analyzed for IL-10, IL-4, IL-13, and IFN-γ production using a sandwich ELISA (BioLegend).

Adoptive transfer

OTII $CD4^+$ T lymphocytes were isolated from spleens using negative magnetic separation (Miltenyi Biotec, Auburn, CA, USA). After negative selection, the cells were analyzed by flow cytometry; 90% of the isolated cells were $CD4^+$. $CD4^+$ cells were labeled with 10 μM CFSE. Then, 5×10^6 cells were adoptively transferred intravenously into naive C57BL/6 mice. At 24 h after transfer, BMDCs previously loaded with 2 μg/ml OVA peptide and stimulated with TcES in the presence or absence of the cRAF inhibitor GW5074 were injected into the right footpad of adoptively transferred mice. Control mice received BMDCs only loaded with OVA. After 7 d, the LNs and the spleen were recovered, homogenized using 70-μM strainers, and stimulated with PMA and ionomycin in the presence of brefeldin A for 5 h. Cells were then stained with phycoerythrin-labeled CD4 antibody (BioLegend), and intracellular IL-4 production was measured by flow cytometry using APC-conjugated anti-IL-4 antibody (BioLegend) on cells gated on $CD4^+$ and $CFSE^+$ populations.

Statistical analysis

The statistical significance of differences between treated and control DCs in the cytokine release assays was calculated using a paired Student's *t* test. The statistical significance of differences between mean fluorescence intensities detected by flow cytometry was calculated using a 2-tailed Mann-Whitney test. Differences were considered significant when $P < 0.05$. GraphPad Prism software (GraphPad, San Diego, CA, USA) was used for all statistical analyses. Error bars represent SEM.

RESULTS

TcES bind MR and MGL

The innate recognition of pathogen associated molecules by DCs is crucial to the initiation of the immune response. Different studies have shown that helminth derived molecules can be recognized by C-type lectins (10, 11). In our model, we previously found that TcES inhibit LPS triggered immune responses and skew T-cell polarization toward a Th2 phenotype *in vitro* in a carbohydrate-dependent fashion. We reported that most of the TcES were bound to concanavalin A, indicating that TcES are glycosylated with glucose, mannose, or galactose (16). In another study, Lee *et al.* (18) reported that the main N-terminal glycosylation structures in TcES were mannose, fucose, galactose, and GlcNAc. The researchers also found a rare Fucα1 → 3GlcNAc antenna on *T. crassi*ceps molecules (18). The natural candidates for carbohydrate recognition are CLRs, such as MR, MGL, and DC-SIGN. Among CLRs, it has been shown that DC-SIGN and Dectin-1 crosstalk with TLR intracellular signaling, thereby affecting the response of the affected DCs to TLR ligands (19, 20). Thus, we hypothesized that the receptors that bind the glycomolecules on TcES initiate crosstalk with TLR signaling and block the proinflammatory response of DCs. To investigate the roles of different CLRs in TcES recognition, TcES molecules were labeled with FITC to perform binding assays. TcES-FITC bound BMDCs at 37°C, indicating that TcES-FITC can be recognized by DCs; however, incubation of DCs and TcES at 4°C had reduced fluorescence (**Fig. 1*A*, *B***). Next, we used EGTA as a calcium chelator to impair the binding function of the CLRs. EGTA pretreatment significantly reduced the MFI of TcES-FITC on DCs, indicating that EGTA-treated cells bind fewer TcES and suggesting a role for CLRs in TcES recognition. To identify the CLRs involved in TcES recognition, BMDCs were preincubated with different carbohydrates, such as mannan, N-acetylgalactosamide, or laminarin, or with a blocking antibody against MR or MGL. TcES-FITC fluorescence was reduced when BMDCs were preincubated with N-acetylgalactosamide or mannan, suggesting a role for MGL or MR, respectively (Fig. 1). These findings were confirmed using blocking antibodies against MGL and MR, which significantly reduced the MFI of TcES-FITC compared to DCs treated with isotype antibody controls (Fig. 1*B*). In addition, $MGL^{-/-}$ BMDCs and splenic $CD11c^+$ cells had reduced TcES-binding ability (Fig. 1*A–C*). In contrast, laminarin, a ligand of Dectin-1, did not significantly decrease TcES-FITC binding to DCs (Fig. 1*A*, *B*). These data suggest that TcES can bind to BMDCs *via* multiple receptors including MR and MGL.

TcES inhibit TNF-α and IL-12 production in $MGL^{-/-}$ or $TLR2^{-/-}$ DCs

Previously, we showed that BMDCs from C57BL/6 mice are refractory to TcES modulation compared with BMDCs derived from Balb/c mice. This phenomenon is associated with resistance of C57BL/6 mice to *T. crassiceps* infection (21). However, it is possible to induce successful *T. crassiceps* infection in C57BL/6 mice by injecting 4 times more parasites that those used to infect Balb/c mice (22). With this in mind, we tested whether C57BL/6 BMDCs could be modulated by TcES in a dose-dependent fashion. We found that the minimal dose required to inhibit the up-regulation of costimulatory molecules CD80 and CD86, and cytokines IL-12 and TNF-α, after LPS stimulation, was 40 μg of TcES (**Fig. 2*A*, *B***). Next, we examined whether the receptors that we identified to be involved in TcES recognition had a role in modulating the activities of TcES. Having demonstrated that $MGL^{-/-}$ BMDCs, as well as WT BMDCs cultured with MGL–blocking antibody or N-acetylgalactosamide, had impaired TcES-FITC binding, we determined whether MGL mediates the TcES inhibition of TLR-induced DC maturation and proinflammatory cytokine release. After TcES stimulation, we did not observe any differences in the modulation of BMDCs of WT and $MGL^{-/-}$ mice, as the IL-12 and TNF-α induced by LPS were similarly inhibited by TcES. However, $MGL^{-/-}$ BMDCs exhibited reduced production of TNF-α and enhanced production of IL-10 in response to LPS compared to WT BMDCs (Fig. 2*C*). Since TLR2 is involved in the resistance to *T. crassiceps* infection (23), TcES may include molecules that can bind TLR2. To test this hypothesis, we performed binding assays with TcES-FITC using BMDCs and splenic $CD11c^+$

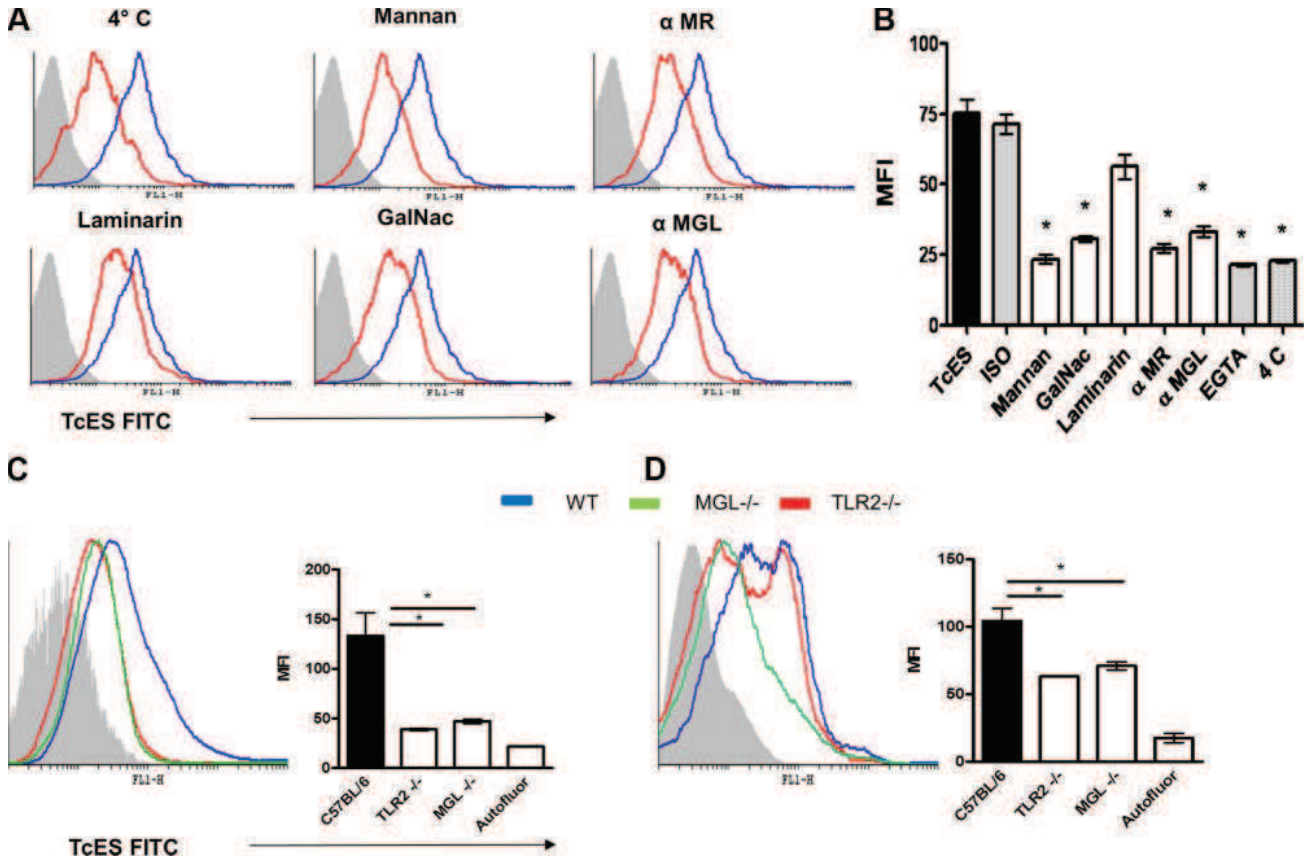


Figure 1. BMDCs recognize TcES through MR, MGL, and TLR2. DCs were preincubated with mannan to block mannose receptor, GalNac to block MGL, laminarin to block Dectin-1, or antibodies against MR or MGL. Cells were subsequently incubated with fluorescently labeled TcES, and fluorescence was analyzed by flow cytometry. *A*) Blue histograms indicate DCs incubated 30 min with TcES alone at 37°C. Red histograms indicate DCs incubated 30 min with the indicated treatment before TcES incubation. *B*) Data represented as MFI of DCs with different treatments and untreated DCs exposed to TcES-FITC. Basal levels of autofluorescence were subtracted from all treatments. Data are representative of 3 independent experiments. $*P < 0.05$. *C*) BMDCs from TLR2- or MGL-deficient mice were incubated for 30 min with TcES-FITC, and binding was analyzed by flow cytometry. Histograms depicting MFIs are representative of 3 independent experiments. $*P < 0.05$. *D*) Total splenocytes from WT, $TLR2^{-/-}$, or $MGL^{-/-}$ mice were incubated with TcES-FITC for 30 min and analyzed by flow cytometry. Cells are gated on $CD11c^{+}$ populations. Histograms represent MFIs. $*P < 0.05$.

DCs from TLR2-deficient mice. We observed that BMDCs and splenic $CD11c^{+}$ DCs from $TLR2^{-/-}$ mice displayed an impaired ability to recognize TcES-FITC compared with cells from WT mice (Fig. 1*C*, *D*). To determine whether TcES recognition by TLR2 blocks TLR-induced proinflammatory responses, we incubated $TLR2^{-/-}$ and WT BMDCs with TcES. As expected, TcES-exposed WT BMDCs blocked IL-12 and TNF-α secretion induced by LPS; interestingly, this TcES-mediated impairment of IL-12 and TNF-α production was not changed in $TLR2^{-/-}$ BMDCs (Fig. 2*D*). Taken together, our results demonstrate that although both TLR2 and MGL bind TcES, it appears that these molecules alone do not play a role in the impairment of the DC response to LPS.

TcES are unable to activate TLR2 or TLR4 on reporter cell lines

The inhibitory activity of TcES on BMDCs is carbohydrate dependent (16). Some glycosylated molecules derived from parasites are recognized by TLR4 or TLR2 on DCs (24–26). Further, TLR2 binds TcES (Fig. 1*C*), and TLR2-deficient mice exhibit an enhanced parasitic load in *T. crassiceps* infection (23). To investigate whether TcES mediates TLR2 and TLR4 activation, we used IL-8 reporter HEK cell lines transfected with TLR2/CD14, TLR2/TLR6, or TLR4/CD14, which produce IL-8 on TLR2 or TLR4 activation and subsequent NFκB signaling. Addition of TcES to HEK cells transfected with TLR2/CD14, TLR2/TLR6, or TLR4/CD14 did not result in any differences in IL-8 production compared to untreated cells (Supplemental Fig. S1). This data shows that TcES do no activate the more common TLR2 heterodimers or the heterodimer TLR4/CD14.

TcES impair the LPS-induced phosphorylation of p38 MAPK and NFκB p65

DCs detect pathogens through TLRs, which play a major role in the increased production of proinflammatory cytokines by activating the MAPK and MyD88-NFκB pathways (4). Because TcES-exposed BMDCs had impaired responses to LPS stimulation, we hy-

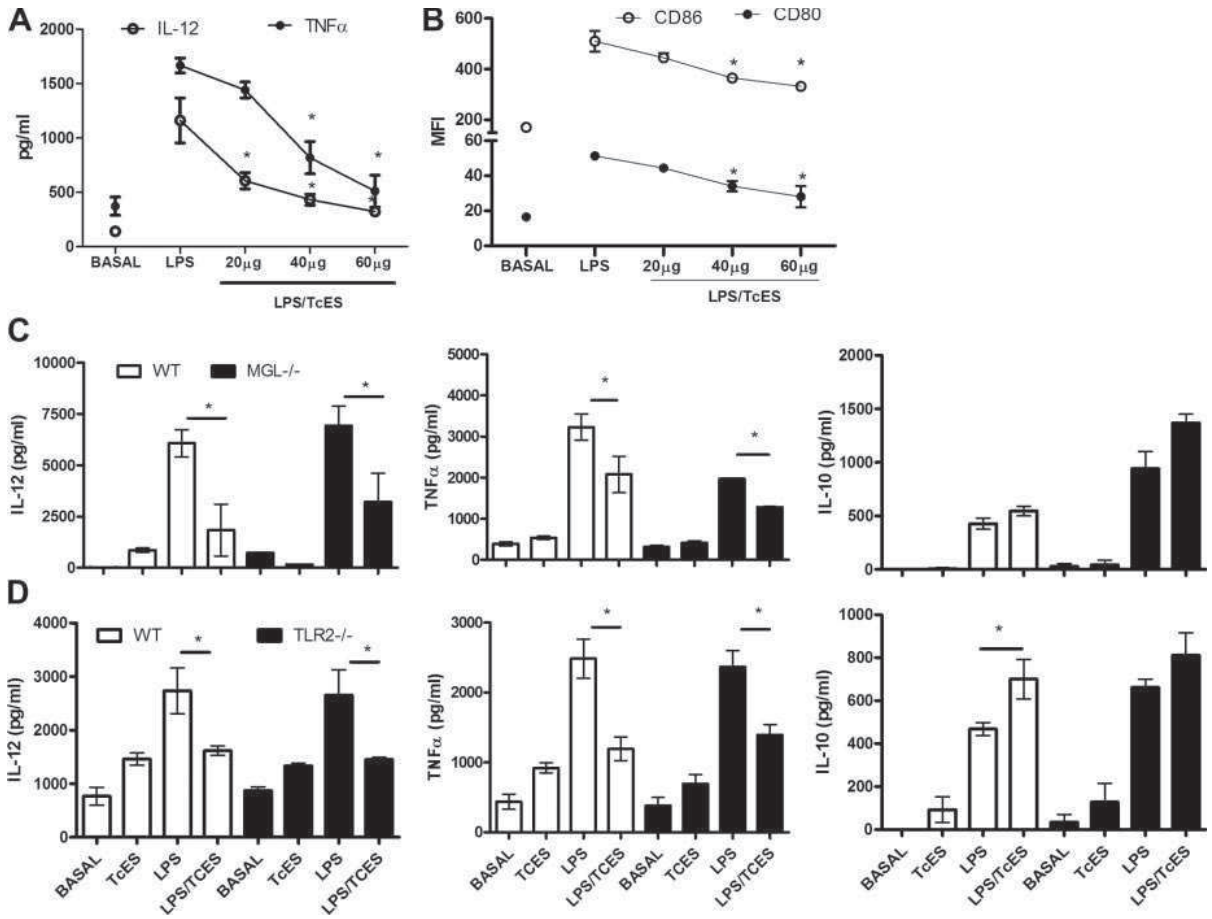


Figure 2. TcES inhibit TNF-α and IL-12 production in $MGL^{-/-}$ and $TLR2^{-/-}$ BMDCs. *A*) BMDCs derived from C57BL/6 mice were treated with LPS and different doses of TcES. After 24 h of stimuli, production of IL-12 and TNF-α was evaluated in supernatants (*A*), and cell surface costimulatory markers CD80 and CD86 were evaluated by flow cytometry (*B*). BMDCs from WT and $MGL^{-/-}$ (*C*) or $TLR2^{-/-}$ (*D*) mice were exposed to 1 μg LPS, 40 μg TcES, or both. After 24 h incubation, production of IL-12, TNF-α, and IL-10 in culture supernatants was determined using ELISA. $n = 3$. $^*P < 0.05$.

pothesized that TcES could affect the intracellular signaling initiated by LPS. We therefore investigated whether the main MAPKs implicated in TLR signaling are affected by TcES, using Western blot detection of their phosphorylated products. In agreement with several studies, we found increased p38, MEK, and ERK1/2 phosphorylation in BMDCs after 30 min of LPS stimulation. In contrast, p38 and MEK were not phosphorylated in BMDCs exposed to TcES alone (**Fig. 3*A***). Interestingly, BMDCs exposed to TcES and LPS showed decreased p38 phosphorylation, but MEK and ERK1/2 were phosphorylated at the same level as in BMDCs exposed to LPS alone (Fig. 3*A*). To investigate whether the exposure of DCs to TcES alters the final step in TLR signaling, we analyzed the phosphorylation of NFκB p65 and observed that exposure of DCs to TcES alone did not result in NFκB p65 phosphorylation. Further, while LPS alone induced strong phosphorylation of NFκB p65, BMDCs exposed to both TcES and LPS resulted in significantly attenuated NFκB p65 phosphorylation (Fig. 3*A*, *B*). Together, these data strongly suggest that TcES modulate intracellular signaling that affects the TLR pathway.

TcES induce cRAF phosphorylation in BMDCs

The fact that TcES bind CLRs (Fig. 1) and affect signaling pathways in DCs (Fig. 3*A*) led us to investigate the intracellular pathways that are affected by the activation of the CLRs. Recent advances in the understanding of the intracellular signaling induced by CLRs have highlighted cRAF and SYK as molecules that modify of TLR signaling (19, 20, 27). To test whether TcES can activate these pathways, we exposed BMDCs to TcES for 5–60 min and found that cRAF phosphorylation was significantly enhanced; interestingly, cRAF phosphorylation was detected at an early time point in TcES-exposed BMDCs and remained phosphorylated for ≥1 h (Fig. 3*A*, *C*). Further detection of cRAF by fluorescence microscopy demonstrated that cRAF was localized in the cytoplasm after 30 min of exposure to TcES (Fig. 3*D*). Moreover, in BMDCs treated with TcES and LPS, cRAF phosphorylation was detected, but at a lower level than in BMDCs treated with TcES alone (Fig. 3*A*). In contrast, SYK phosphorylation was absent in TcES treated DCs (Supplemental Fig. S1*D*). The canonical cRAF signaling pathway begins with RAS activation (28). Interestingly, we found enhanced amounts of

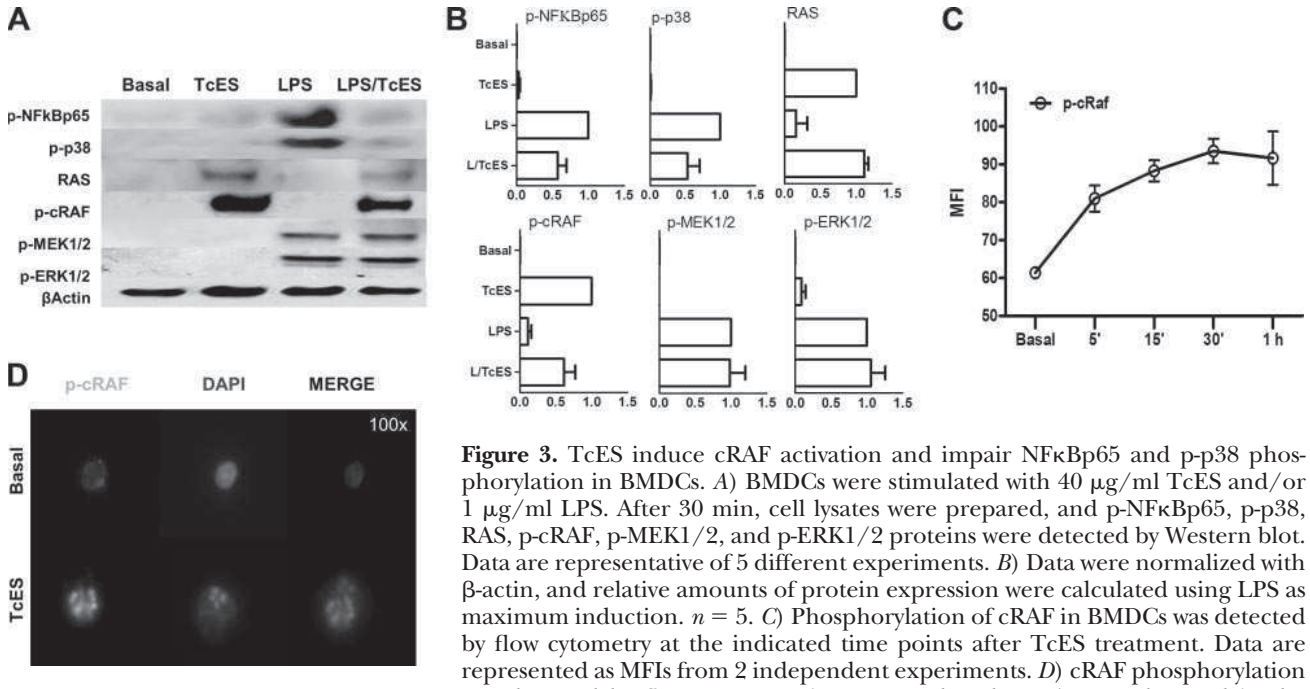


Figure 3. TcES induce cRAF activation and impair NFκBp65 and p-p38 phosphorylation in BMDCs. *A*) BMDCs were stimulated with 40 μg/ml TcES and/or 1 μg/ml LPS. After 30 min, cell lysates were prepared, and p-NFκBp65, p-p38, RAS, p-cRAF, p-MEK1/2, and p-ERK1/2 proteins were detected by Western blot. Data are representative of 5 different experiments. *B*) Data were normalized with β-actin, and relative amounts of protein expression were calculated using LPS as maximum induction. $n = 5$. *C*) Phosphorylation of cRAF in BMDCs was detected by flow cytometry at the indicated time points after TcES treatment. Data are represented as MFIs from 2 independent experiments. *D*) cRAF phosphorylation was detected by fluorescence microscopy. Phospho-cRAF was detected in the cytoplasm after 30 min of TcES exposure.

RAS in the cytoplasm of TcES treated DCs. However, we did not detect the phosphorylation of MEK or ERK1/2, which are downstream targets of cRAF (Fig. 3*A*). RAS also participates in the initiation of the PI3K signaling pathway. We therefore investigated the role of this pathway using PI3Kγ-deficient DCs. We found that these were modulated by TcES in a similar fashion as in WT DCs. This result eliminates the possibility that DC modulation by TcES involves PI3Kγ (Supplemental Fig. S2).

TcES impair TLR signaling and DC maturation in a cRAF-dependent pathway

Because TcES impair DC maturation in response to TLR stimulation (Fig. 2*A*) and (16), we investigated the possible role of cRAF in the modulation of DC responses. As we showed earlier, after TLR4 stimulation using LPS as an agonist, IL-12, TNF-α, CD80, and CD86 were up regulated, but these responses were impaired by exposure to TcES (**Fig. 4*A*, *B***). However, when BMDCs were pretreated with both the cRAF inhibitor GW5074 and TcES, cRAF phosphorylation was impaired (Fig. 4*C*). In addition, the production of IL-12 and TNF-α and the up-regulation of CD80 and CD86 were restored to levels that are typically observed after LPS stimulation. Since TcES diminished NFκB phosphorylation in LPS treated cells, we determined the effect of cRAF inhibition on NFκB p65 using the NFκB p65 RAW blue reporter cell line. As in BMDCs, LPS enhanced NFκB activation, and the presence of TcES reduced the levels of LPS induced NFκB activation, although to a lesser degree. Chemical inhibition of cRAF restored NFκB activity induced by LPS on TcES treated RAW blue reporter cells (Fig. 4*B*). To reinforce our data obtained using the chemical inhibitor GW5074; we knocked down cRAF in DCs using siRNA. DCs treated with cRAF siRNA, reduced cRAF protein levels by 50% compared with those treated with nontargeting control siRNA (Fig. 4*D*). Consistent with our cRAF chemical inhibition results, siRNA knockdown of cRAF on DCs sufficiently blocked the immunomodulatory activity of TcES, and restored IL-12 and TNF-α production in LPS stimulated cells. (Fig. 4*E*). These findings demonstrate that TcES elicit an intracellular signal through a cRAF-dependent pathway that impairs both the expression of CD80 and CD86 and the release of IL-12 and TNF-α that are triggered by TLR4 stimulation. To our knowledge, this is the first report showing that helminth molecules trigger cRAF to modulate DC responses.

cRAF phosphorylation in TcES-treated DCs enhances Th2 but impairs Th1 responses *in vitro* and *in vivo*

DCs exposed to helminth-derived molecules polarize Th2 responses *in vitro* and *in vivo* (2, 3). However, the mechanisms in DCs that instruct the induction of the Th2 response are incompletely understood (3, 5). Since we found that TcES induce cRAF phosphorylation, it is possible that signaling through this pathway directs DCs to deliver signals that prime Th2 responses. To address this possibility, we loaded DCs with OVA peptide and incubated with transgenic $CD4^+$ T cells from OTII mice *in vitro*, then analyzed T-cell polarization via production of cytokines. After seven d of coculture, exposure to TcES conditioned the DCs to induce the release of IL-13 and IL-4 by the $CD4^+$ T cells; however, IFN-γ was not detected (**Fig. 5*A***). To demonstrate a role for cRAF in mediating the produc-

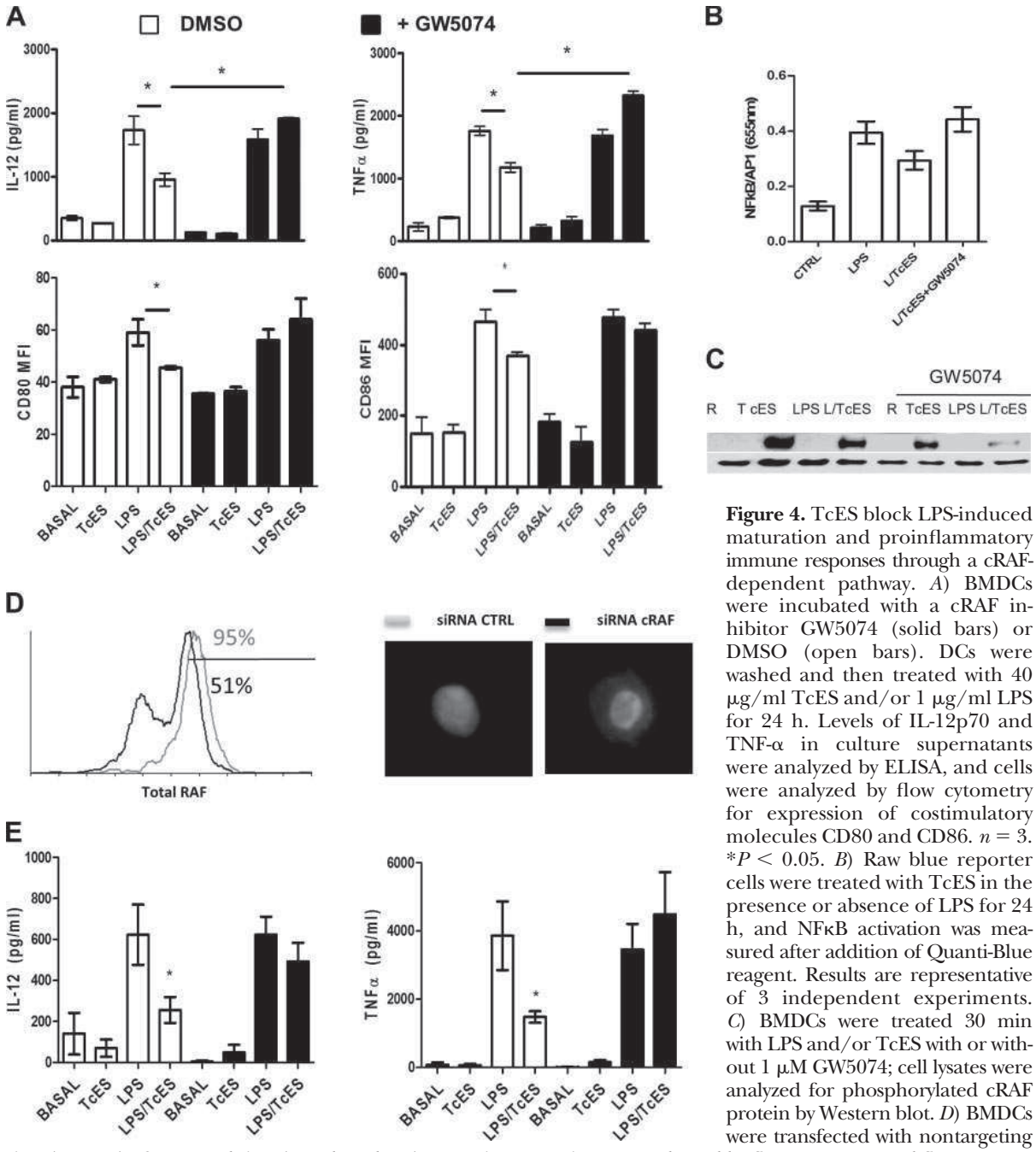


Figure 4. TcES block LPS-induced maturation and proinflammatory immune responses through a cRAF-dependent pathway. *A*) BMDCs were incubated with a cRAF inhibitor GW5074 (solid bars) or DMSO (open bars). DCs were washed and then treated with 40 μg/ml TcES and/or 1 μg/ml LPS for 24 h. Levels of IL-12p70 and TNF-α in culture supernatants were analyzed by ELISA, and cells were analyzed by flow cytometry for expression of costimulatory molecules CD80 and CD86. $n = 3$. $^*P < 0.05$. *B*) Raw blue reporter cells were treated with TcES in the presence or absence of LPS for 24 h, and NFκB activation was measured after addition of Quanti-Blue reagent. Results are representative of 3 independent experiments. *C*) BMDCs were treated 30 min with LPS and/or TcES with or without 1 μM GW5074; cell lysates were analyzed for phosphorylated cRAF protein by Western blot. *D*) BMDCs were transfected with nontargeting siRNA or cRAF SmartPool siRNA, and total cRAF protein expression was evaluated by flow cytometry and fluorescence microscopy. *E*) BMDCs transfected with nontargeting siRNA or cRAF siRNA were stimulated as above for 24 h, and production of IL-12 and TNF-α was evaluated by ELISA. Data are representative of 3 independent experiments. $^*P < 0.05$.

tion of IL-13 and IL-4 by DCs exposed to TcES, we treated DCs for 2 h with different concentrations of cRAF inhibitor prior to TcES stimulation. BMDCs exposed to TcES plus 1 μM or 10 μM cRAF inhibitor exhibited an impaired ability to induce OVA-specific IL-13 and IL-4 secretion by T cells compared with BMDCs exposed to TcES alone (Fig. 5*A*). When OVA peptide loaded BMDCs were stimulated with LPS, they induced Th1 cytokines in *in vitro* cocultures, but when the DCs were treated with TcES and LPS, IFN-γ levels were reduced (Fig. 5*B*). Given that TcES exposure reduced the maturation of LPS-stimulated BMDCs, and this phenotype resulted in impaired IFN-γ production in $CD4^+$ T cells, we tested whether the blockage of cRAF in DCs would restore IFN-γ production in $CD4^+$ T cells. Consistent with the recovery of IL-12 production after cRAF inhibitor pretreatment (Fig. 4*A*), BMDCs preincubated with cRAF inhibitor and exposed to TcES and LPS were efficient Th1 inducers (Fig. 5*B*). These data demonstrate that TcES promote Th2 cytokine secretion in a cRAF-dependent manner and that cRAF phosphorylation is required for impairing the Th1-inducing ability of LPS-treated DCs.

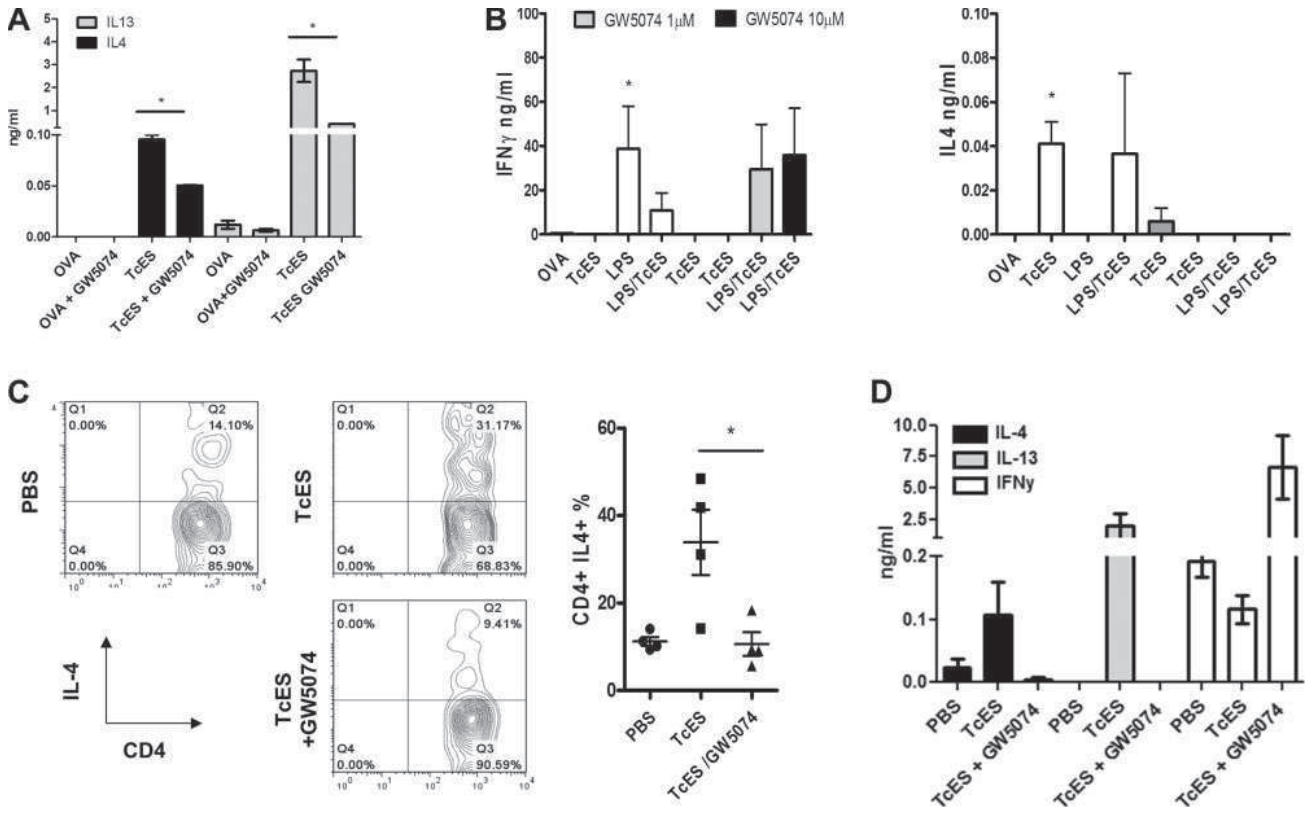


Figure 5. Th2 induction by TcES-exposed DCs is cRAF dependent. *A*) DCs were loaded with OVA and treated with TcES in the presence or absence of cRAF inhibitor. Following treatment, DCs were washed and cocultured with OTII $CD4^+$ T cells for 7 d. Production of IL-4 and IL-13 in culture supernatants was analyzed by ELISA. *B*) Cocultures were also carried out in the presence of LPS, and production of IFN-γ and IL-4 in culture supernatants was determined by ELISA. *C*) OVA-loaded BMDCs stimulated with TcES with or without cRAF inhibitor were injected into the footpad of WT mice previously adoptively transferred with CFSE-labeled $CD4^+$ OTII cells. Production of IL-4 in adoptively transferred T cells in popliteal and inguinal lymph nodes were analyzed at d 7 postinjection by flow cytometry. Plots represent cells gated for $CFSE^+$ and $CD4^+$ populations. Graph shows percentage of IL-4 producing $CD4^+$ cells; $n = 4$. $^*P < 0.05$. *D*) Popliteal lymph node cells from the above experiment were restimulated with OVA peptide for 4 d, and supernatants were analyzed for IL-4, IL-13, and IFNγ production by ELISA. Data are representative of 2 independent experiments; $n = 4$. $^*P < 0.05$.

Finally, we addressed whether cRAF is critical for the induction of Th2 responses by TcES-treated DCs *in vivo*. To this end, BMDCs were loaded with OVA and pretreated with cRAF inhibitor before exposure to TcES. The DCs were then transferred into the footpad of WT mice that had previously received CFSE-labeled $CD4^+$ T cells purified from OTII transgenic mice. At 7 d after transfer, draining lymph nodes were recovered, and IL-4 production was evaluated by intracellular flow cytometry. We observed that the mice which received OVA loaded DCs exposed to TcES alone showed enhanced IL-4 production in the transgenic OTII $CD4^+$ cells. In contrast, DCs pretreated with cRAF inhibitor before TcES exposure showed similar IL-4 production by OTII $CD4^+$ cells comparable to untreated DCs (Fig. 5*C*). We observed similar decreased production of IL-4 and IL-13 as well as increased production of IFNγ in popliteal lymph node cultures of adoptively transferred mice receiving TcES exposed DCs pretreated with cRAF inhibitor. (Fig. 5*D*). Taken together, these results show that cRAF phosphorylation in TcES-treated DCs enhances Th2 and impairs Th1 responses *in vitro* and *in vivo*.

Combined TcES recognition by MR, MGL, and TLR2 is necessary to block DC maturation and induce Th2 responses

To determine the link between the receptors involved in TcES recognition and the down-regulation of TLR responses, we examined whether the combination of MR, MGL, and TLR2 was necessary to modulate the responses of DCs to further TLR4 stimuli. Consistent with our data using MGL- and TLR2-deficient DCs, blocking antibodies against MGL or TLR2 did not significantly affect the impairment of cytokine production by TcES treated C57BL/6 DCs (**Fig. 6*A***). Blocking MR restored the production of TNF-α but not IL-12 in TcES treated DCs and stimulated by LPS (Fig. 6*A*, *B*). Simultaneously blocking MR, MGL, and TLR2 completely restored the ability of TcES exposed DCs to respond efficiently to LPS stimulation, producing IL-12 and TNF-α at levels similar to DCs stimulated with LPS alone (Fig. 6*A*). Following the same strategy, we tested whether these receptors are important in directing Th2 responses in DCs due to TcES exposure. In coculture assays, we observed a partial inhibition of IL-4 and IL-13

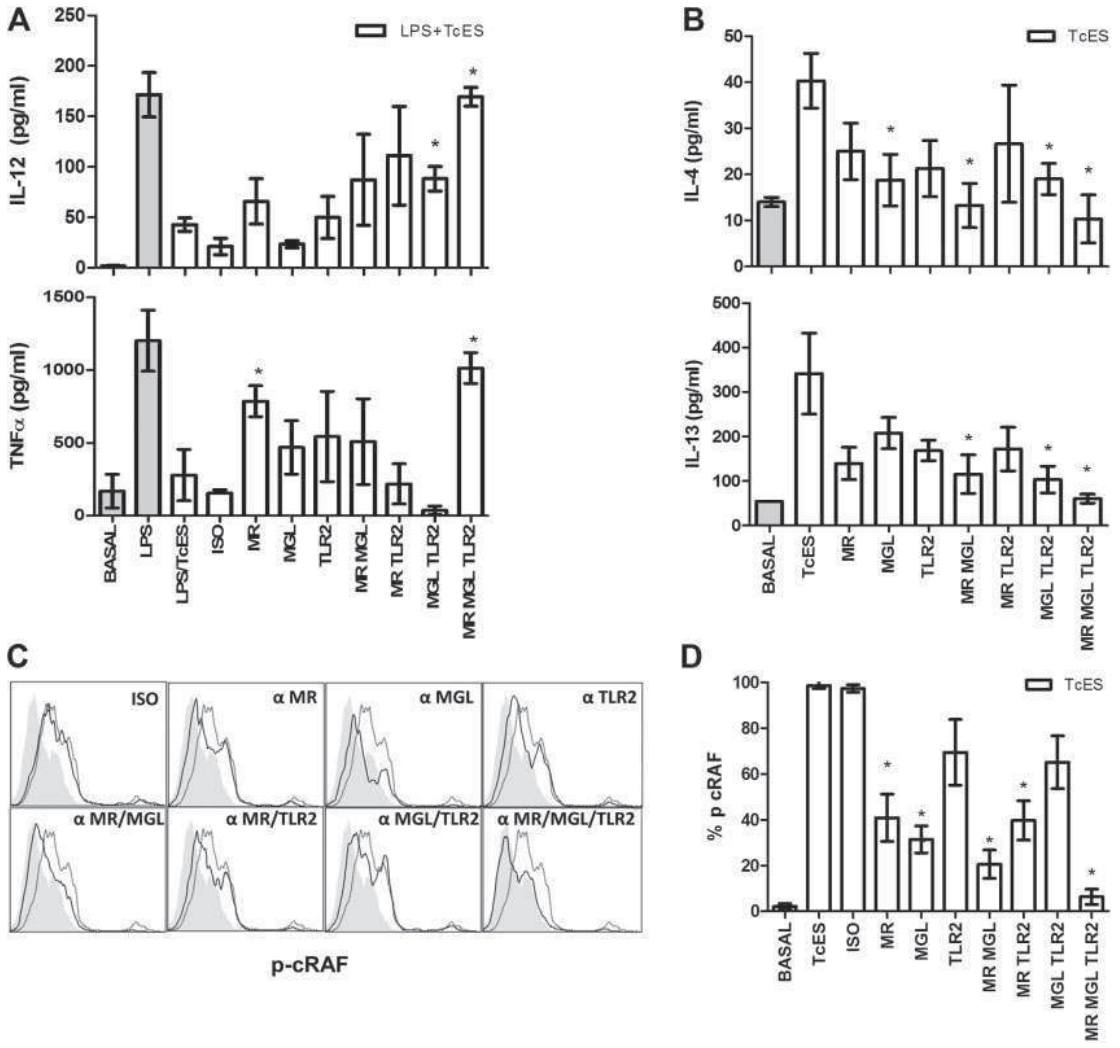


Figure 6. TcES trigger cRAF phosphorylation through combined recognition by MR, MGL, and TLR2. *A*) BMDCs were pretreated with the indicated blocking antibodies for 30 min, and then stimulated with LPS and/or TcES for 24 h. Production of IL-12 and TNF-α was evaluated by ELISA. Indicated significance compared with LPS/TcES; $n = 3$. $^*P < 0.05$. *B*) BMDCs were treated as in *A*, and exposed to TcES for 24 h, then cocultured with OTII-purified T cells for 6 d. Production of IL-4 and IL-13 was evaluated by ELISA. Significance compared with TcES alone; $n = 3$. $^*P < 0.05$. *C*) MR, MGL, and/or TLR2 were blocked as in *A*, and BMDCs were stimulated with 40 μg/ml of TcES for 30 min. Intracellular cRAF phosphorylation was analyzed by flow cytometry. Dotted histograms indicate DCs incubated 30 min with TcES alone; black histograms indicate DCs incubated 30 min with the indicated blocking antibody before TcES incubation; $n = 2$. *D*) Levels of cRAF phosphorylation in DCs subjected to blocking treatments as in *C*. $^*P < 0.05$ *vs.* TcES.

production after blocking MR, MGL, or TLR2 individually. However, simultaneously blocking MR, MGL, and TLR2 completely inhibited IL-4 and IL-13 production induced by TcES (Fig. 6*B*). These results show that the MR, MGL, and TLR2 receptor combination is required to mediate the effects of TcES in inhibiting the production of IL-12 and TNF-α in DCs and subsequent Th2 polarization. Finally, since TcES induced modulation of DCs occurs through a cRAF-dependent signaling pathway, we determined whether blocking one or multiple receptors could reduce cRAF phosphorylation. We found that blocking MR or MGL significantly reduced cRAF phosphorylation, while blocking TLR2 slightly reduced cRAF phosphorylation, but this was not significant. However, when MR, MGL, and TLR2 were blocked simultaneously, cRAF phosphorylation elicited by TcES was completely abrogated (Fig. 6*C, D*). These results demonstrate a link between MR, MGL, TLR2 and cRAF as critical players in a signaling pathway exploited by *T. crassiceps* to inhibit DC maturation and direct Th2 responses (**Fig. 7**).

DISCUSSION

Our study demonstrates that helminth-derived molecules are recognized by multiple receptors, such as MR, MGL, and TLR2, on DCs, and together they trigger a cRAF-mediated intracellular signaling pathway that negatively affects LPS-induced DC activation and affects eventual T-cell responses. DCs play a major role in the initiation of Th2 polarization in response to helminth pathogens because depletion of DCs impairs the Th2 response elicited by worm infection (29). In several

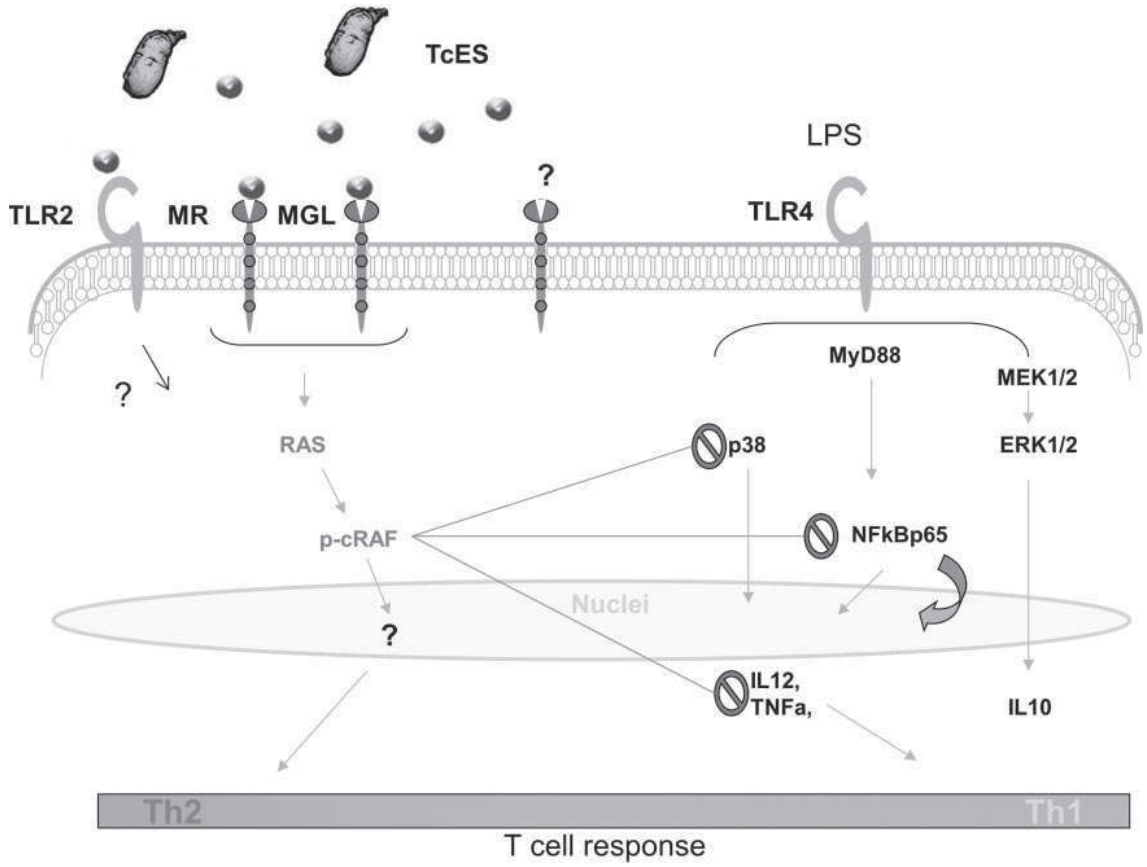


Figure 7. Model showing the cRAF-dependent modulation of DC responses by TcES. Helminth parasites release excreted/secreted products that can alter immune responses. TcES are recognized by mannose receptor, MGL, and TLR2. Receptors act in conjunction to mediate TcES signaling. Signaling could be initiated by RAS, a molecule found in the cytoplasm of TcES-treated DCs. Ultimately, TcES signaling results in the phosphorylation of cRAF. cRAF-mediated signaling is able to inhibit the maturation of DCs and subsequent cytokine release induced by TLR4. In addition, TcES down-regulates the p38 and NFκB pathway, possibly in a cRAF-dependent manner. On the other hand, TLR4 signaling attenuates cRAF phosphorylation and Th2 response induced by TcES, indicating a cross regulation between both pathways. Finally, cRAF activation conditions DCs to induce Th2 responses, *via* a mechanism that is incompletely understood.

experimental conditions, DCs remain in an immature state after exposure to helminth derivatives, but in contrast to the immature phenotype, DCs are irresponsive to further TLR stimulation and exhibit an enhanced ability to prime Th2 responses *in vivo* and *in vitro* (30–34). These data indicate that DCs do not remain immature after helminth exposure but they are primed in a nonclassical manner.

In this study, we investigated the mechanisms of DC regulation by TcES. The fact that the carbohydrates in TcES were important for their modulatory activities (16), lead us to investigate the role of receptors on DCs with the ability to recognize glycosylated structures as well as the intracellular signaling pathways they trigger. The lack of recognition of TcES by EGTA-treated DCs strongly suggested a role for C-type lectins, whose function is dependent on calcium. TcES are rich in structures recognized by concanavalin A, and the main glycosylation moieties in *T. crassiceps* are mannose, galactose, and fucose (16, 18). The content of carbohydrates present in TcES correlated with the ability of MR and MGL to recognize such molecules. In addition, TLR2 had a role in TcES recognition. The multiple receptors involved in TcES recognition were not surprising, since TcES are a complex mixture of molecules that remain to be identified. In support of our findings, other helminth-derived molecules bind to different receptors, including MR, MGL, and TLR2 (10). These receptors have been associated with the regulation of DCs responses. For example, MR mediates the internalization of ω-1, a ribonuclease present in *Schistosoma mansoni* soluble egg antigens (SEAs) that is capable of down-regulating proinflammatory cytokine production and skewing toward Th2 responses (35). On the other hand, MGL has been associated with Th2-promoting ability (36). Moreover, we did not see significant changes in DCs responses to TcES after blockade of MGL, but MR blockage resulted in a recovery of TNF-α production. Other receptors that can bind glycoconjugates and that are also involved in helminth recognition are TLR2 and TLR4 (24–26). Although TcES recognition was impaired in TLR2$^{-/-}$ BMDCs, TLR2 deficiency did not impact the modulatory activities of TcES on DCs. Because TLR2$^{-/-}$ mice are more susceptible to *T. crassiceps* infection, it is likely that TLR2 enhances the protective immune response against *T.*

crassiceps (23), probably by binding other molecules not present in TcES. TLR4 has been associated with the induction of Th2 polarization by DCs after the recognition of LFNIII, a carbohydrate that is present in SEA and ES62 (24, 33). However, we observed that TcES did not activate TLR4-transfected HEK cells, and we have reported that *T. crassiceps* molecules polarize cells toward a Th2 profile even in C3H/HeJ mice, which have a TLR4 mutation (8). These results suggest that *T. crassiceps* molecules may enhance Th2 responses in a TLR4-independent fashion.

Several reports have shown the cooperation between receptors to recognize pathogens and modulate intracellular signaling affecting the outcome of responses. For example, TLR2 can form heterodimers with TLR1 or TLR6, and can act in synergism with CLRs such as MGL and Dectin-1 (37, 38). We tested whether combinations of blocking antibodies could affect the phenotype of DCs after TcES exposure. We found that when MR, MGL, and TLR2 were collectively blocked, the response to LPS stimulation was restored in DCs treated with TcES. Moreover, the ability of TcES to induce Th2 responses was completely abolished with collective blockage of all three receptors, while blockage of individual receptors MR, MGL, or TLR2 resulted in reduced levels of Th2 cytokines. Although the various molecules that comprise of TcES remain to be fully characterized, our data suggests that some redundancy potentially exists in receptor recognition of TcES. This could further explain why other reports show that blockage of one receptor does not alter the resulting DC phenotype after exposure to helminth molecules (26, 39). Future experiments will be required to demonstrate whether a combination of these receptors recognizes a single TcES molecule, or multiple molecules bind to different receptors on DCs.

Our results showed that exposure of BMDCs to TcES diminished p38 MAPK and NFκB p65 phosphorylation induced by LPS, events associated with IL-12 and TNF-α production (4, 40, 41). On the other hand, TcES did not block the LPS-induced MEK1/2 and ERK1/2 phosphorylation. Because ERK1/2 is associated with IL-10 production, this mechanism can explain why TcES does not affect IL-10 production (42, 43) and suggests that TcES specifically impair the inflammatory pathway. Few studies have addressed the intracellular signaling elicited by helminth derivatives and their ability to prime Th2 responses. SEA stimulation results in slight phosphorylation of ERK1/2, which leads to cFOS stabilization; these events impair IL-12 production by DCs and are correlated with Th2 polarization (44). In addition, NFκB1 was necessary to develop Th2 responses after SEA treatment (45). In our study, we did not find any ERK1/2, p38, or NFκB p65 phosphorylation in response to TcES alone. In contrast, we found that TcES increased cRAF phosphorylation. Different studies have shown that cRAF phosphorylation is triggered by other pathogens, such as HIV, *Mycobacterium tuberculosis*, and *Helicobacter pylori*, in a DC-SIGN dependent pathway (19, 20). However, there are no data on the activation of these pathways by helminth derivatives. We found that TcES increased cRAF phosphorylation very early after stimulation and maintained this phosphorylation for at least 1 h. This result suggests that early cRAF phosphorylation is triggered directly by TcES and not by an autocrine feedback of soluble factors produced by DCs. Analyzing the cRAF canonical signaling pathway, we found that the upstream signaling molecule RAS was localized in high amounts in the cytoplasm, which could indicate receptor internalization (28). In contrast, the downstream molecules MEK or ERK1/2 were not phosphorylated in response to TcES stimulation. Previous findings showed that phosphorylation of cRAF but not MEK and ERK1/2 occurs in response to ManLAM (20). In other study, the tick saliva molecule Salp15 was recognized by DC-SIGN and impaired the production of TNF-α and IL-12 by decreasing messenger stability and nuclear remodeling, respectively. Interestingly, TNF-α regulation was mediated through a cRAF- and MEK-dependent pathway, but IL-12 regulation was only cRAF dependent (27). The fact that TcES were able to down-regulate TLR responses in a cRAF dependent pathway, and that other pathogens trigger this pathway resulting in altered TLR responses (20, 27, 46) suggest that cRAF might be a common target used by pathogens to impair DC function. Our findings showed that TcES induced a cRAF dependent pathway triggered by the combination of the receptors MR, MGL, and TLR2. Although crosslinking of MGL has been shown to lead to ERK1/2 phosphorylation, a downstream target of cRAF (37, 47), ours is the first report to show that MR, MGL, and TLR2 are directly associated with cRAF signaling. In addition, the MR elicited response can be amplified by TLR2 (48), supporting the fact that MR, MGL, and TLR2 can act together to induce intracellular signaling.

In our model, how cRAF affects the TLR signaling pathway remains to be elucidated. One possibility is that cRAF is not activated completely, thereby lacking the ability to induce the canonical MEK-ERK1/2 pathway. It has been shown that cRAF can bind directly to IκB (49), and perhaps, this association could impair NFκB release and subsequent DC activation observed in our study. Another possibility is that cRAF could preferentially induce the activation of the heterodimer RelB/p52, commonly known as the alternative NFκB pathway. In line with this hypothesis, the Th2 inducer LNFPIII, a carbohydrate derived from *S. mansoni*, has been shown to activate the alternative NFκB pathway on DCs (45). It should be noted, however, that cRAF phosphorylation induced by TcES stimulation of DCs was necessary for the priming of Th2 responses. This supports the idea that helminths activate different pathways that compete with or block TLR responses and are simultaneously involved in skewing the T-cell response toward a Th2 profile rather than the default DC activation pathway. However, it is still not clear whether cRAF induces costimulatory molecules or soluble factors that are able to induce Th2 polarization, and future experiments are necessary to address which

Th2-inducing factors could be modulated by cRAF. In contrast with our finding associating cRAF to Th2 responses, Dectin-1 triggers the cRAF pathway on DCs. leading to enhanced cytokine production and positive regulation of the Th1 and Th17 responses (19). This discrepancy could be the result of differences in receptors or receptor combination engaged on DCs that triggers the cRAF pathway. Additional studies are necessary to understand how cRAF can be involved in anti-inflammatory (27) or inflammatory DC responses (19). We also found that cRAF inhibition in TcES treated DCs enhanced IFNγ production *in vivo* but not *in vitro*. Since primed DCs can be instructed by other signals coming from T cells (50), such as CD40, it is likely that TcES-treated DCs can be refractory to those signals in a cRAF dependent fashion. However the fact that IFNγ production was detected *in vivo*, but not *in vitro*, suggests that cells other than $CD4^+$ T cells can provide such signals.

We further observed that a mutual negative regulation between the signals elicited by TcES and those triggered by LPS on DCs that affected the T-cell outcome. These data suggest that DC responses could be governed by the ratio between the inhibitory and stimulatory signals presented by the pathogen or microenvironment. In accordance with this idea, an early Th1 response is achieved in *T. crassiceps*-infected mice, which correlates with disrupted parasites in the peritoneal cavity and, possibly, the release of vesicular fluid, which in turn can activate the immune system to secrete early IFNγ. However, as the infection progresses, there is a switch from an early protective Th1 response to a permissive Th2 response (13). Our studies indicate that this shift is potentially caused by TcES, probably blocking the Th1 responses triggered by the vesicular fluid and inducing Th2 immune responses. This hypothesis can be supported by a recent study showing that only low-molecular-mass molecules contained in the vesicular fluid of *Taenia solium* were able to induce proliferation as well as IL-1β, TNF-α, and IL-2 production by human lymphocytes (51). Our findings provide evidence that DC responses are orchestrated by the engagement of multiple receptors and the interaction of their downstream intracellular pathways. Certainly, the number of receptors as well as their interactions expands the ability to recognize and respond to different pathogens. FJ

The authors thank Dr. Steve Oghumu for his help with experiments and critical reading of the paper. This work was supported by grant 167799 from Consejo Nacional de Ciencia y Tecnología (CONACYT; Mexico) and Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (PAPIIT)-Universidad Nacional Autónoma de México (UNAM) IN213512, and is part of the requirements to obtain the Ph.D. degree in the Postgraduate Program in Biomedical Sciences, Facultad de Medicina, UNAM, for C.A.T., who was supported by a fellowship from CONACYT-Mexico, Programa de Doctorado en Ciencias Biomédicas (PDCB) UNAM, and Fulbright–Mexico-U.S. Commission for Educational and. Cultural Exchange (COMEXUS). The research in the A.R.S laboratory is supported by the grants from the U.S. National Institutes of Health. The authors declare no conflicts of interest.

REFERENCES

1. Maizels, R. M., Balic, A., Gomez-Escobar, N., Nair, M., Taylor, M. D., and Allen, J. E. (2004) Helminth parasites–masters of regulation. *Immunol. Rev.* **201,** 89–116
2. Terrazas, C. A., Terrazas, L. I., and Gomez-Garcia, L. (2010) Modulation of dendritic cell responses by parasites: a common strategy to survive. *J. Biomed. Bio/Technol.* **2010,** 357106 doi: 10.1155/2010/357106
3. MacDonald, A. S., and Maizels, R. M. (2008) Alarming dendritic cells for Th2 induction. *J. Exp. Med.* **205,** 13–17
4. Kawai, T., and Akira, S. (2007) TLR signaling. *Sem. Immunol.* **19,** 24–32
5. Pulendran, B., and Artis, D. (2012) New paradigms in type 2 immunity. *Science* **337,** 431–435
6. Tundup, S., Srivastava, L., and Harn, D. A., Jr. (2012) Polarization of host immune responses by helminth-expressed glycans. *Ann. N. Y. Acad. Sci.* **1253,** E1–E13
7. Okano, M., Satoskar, A. R., Nishizaki, K., Abe, M., and Harn, D. A., Jr. (1999) Induction of Th2 responses and IgE is largely due to carbohydrates functioning as adjuvants on *Schistosoma mansoni* egg antigens. *J. Immunol.* **163,** 6712–6717
8. Gomez-Garcia, L., Rivera-Montoya, I., Rodriguez-Sosa, M., and Terrazas, L. I. (2006) Carbohydrate components of *Taenia crassiceps* metacestodes display Th2-adjuvant and anti-inflammatory properties when co-injected with bystander antigen. *Parasitol. Res.* **99,** 440–448
9. Okano, M., Satoskar, A. R., Nishizaki, K., and Harn, D. A., Jr. (2001) Lacto-N-fucopentaose III found on *Schistosoma mansoni* egg antigens functions as adjuvant for proteins by inducing Th2-type response. *J. Immunol.* **167,** 442–450
10. Van Liempt, E., van Vliet, S. J., Engering, A., Garcia Vallejo, J. J., Bank, C. M., Sanchez-Hernandez, M., van Kooyk, Y., and van Die, I. (2007) *Schistosoma mansoni* soluble egg antigens are internalized by human dendritic cells through multiple C-type lectins and suppress TLR-induced dendritic cell activation. *Mol. Immunol.* **44,** 2605–2615
11. Guasconi, L., Serradell, M. C., Garro, A. P., Iacobelli, L., and Masih, D. T. (2011) C-type lectins on macrophages participate in the immunomodulatory response to *Fasciola hepatica* products. *Immunology* **133,** 386–396
12. Terrazas, L. I. (2008) The complex role of pro- and anti-inflammatory cytokines in cysticercosis: immunological lessons from experimental and natural hosts. *Curr. Topics Med. Chem.* **8,** 383–392
13. Terrazas, L. I., Bojalil, R., Govezensky, T., and Larralde, C. (1998) Shift from an early protective Th1-type immune response to a late permissive Th2-type response in murine cysticercosis (*Taenia crassiceps*). *J. Parasitol.* **84,** 74–81
14. Villa, O. F., and Kuhn, R. E. (1996) Mice infected with the larvae of *Taenia crassiceps* exhibit a Th2-like immune response with concomitant anergy and downregulation of Th1-associated phenomena. *Parasitology* **112**(Pt. 6), 561–570
15. Terrazas, C. A., Sanchez-Munoz, F., Mejia-Dominguez, A. M., Amezcua-Guerra, L. M., Terrazas, L. I., Bojalil, R., and Gomez-Garcia, L. (2011) Cestode antigens induce a tolerogenic-like phenotype and inhibit LPS inflammatory responses in human dendritic cells. *Int. J. Biol. Sci.* **7,** 1391–1400
16. Terrazas, C. A., Gomez-Garcia, L., and Terrazas, L. I. (2010) Impaired pro-inflammatory cytokine production and increased Th2-biasing ability of dendritic cells exposed to *Taenia* excreted/secreted antigens: A critical role for carbohydrates but not for STAT6 signaling. *Int. J. Parasitol.* **40,** 1051–1062
17. Gringhuis, S. I., and Geijtenbeek, T. B. (2010) Carbohydrate signaling by C-type lectin DC-SIGN affects NF-kappaB activity. *Methods Enzymol.* **480,** 151–164
18. Lee, J. J., Dissanayake, S., Panico, M., Morris, H. R., Dell, A., and Haslam, S. M. (2005) Mass spectrometric characterisation of *Taenia crassiceps* metacestode N-glycans. *Mol. Biochem. Parasitol.* **143,** 245–249
19. Gringhuis, S. I., den Dunnen, J., Litjens, M., van der Vlist, M., Wevers, B., Bruijns, S. C., and Geijtenbeek, T. B. (2009) Dectin-1 directs T helper cell differentiation by controlling noncanonical NF-kappaB activation through Raf-1 and Syk. *Nat. Immunol.* **10,** 203–213

20. Gringhuis, S. I., den Dunnen, J., Litjens, M., van Het Hof, B., van Kooyk, Y., and Geijtenbeek, T. B. (2007) C-type lectin DC-SIGN modulates Toll-like receptor signaling via Raf-1 kinase-dependent acetylation of transcription factor NF-kappaB. *Immunity* **26,** 605–616
21. Reyes, J. L., Terrazas, C. A., Vera-Arias, L., and Terrazas, L. I. (2009) Differential response of antigen presenting cells from susceptible and resistant strains of mice to *Taenia crassiceps* infection. *Infect. Genet. Evol.* **9,** 1115–1127
22. Reyes, J. L., Espinoza-Jimenez, A. F., Gonzalez, M. I., Verdin, L., and Terrazas, L. I. (2011) *Taenia crassiceps* infection abrogates experimental autoimmune encephalomyelitis. *Cell. Immunol.* **267,** 77–87
23. Reyes, J. L., Gonzalez, M. I., Ledesma-Soto, Y., Satoskar, A. R., and Terrazas, L. I. (2011) TLR2 mediates immunity to experimental cysticercosis. *Int. J. Biol. Sci.* **7,** 1323–1333
24. Goodridge, H. S., Marshall, F. A., Else, K. J., Houston, K. M., Egan, C., Al-Riyami, L., Liew, F. Y., Harnett, W., and Harnett, M. M. (2005) Immunomodulation via novel use of TLR4 by the filarial nematode phosphorylcholine-containing secreted product, ES-62. *J. Immunol.* **174,** 284–293
25. van der Kleij, D., Latz, E., Brouwers, J. F., Kruize, Y. C., Schmitz, M., Kurt-Jones, E. A., Espevik, T., de Jong, E. C., Kapsenberg, M. L., Golenbock, D. T., Tielens, A. G., and Yazdanbakhsh, M. (2002) A novel host-parasite lipid cross-talk. Schistosomal lysophosphatidylserine activates toll-like receptor 2 and affects immune polarization. *J. Biol. Chem.* **277,** 48122–48129
26. Vanhoutte, F., Breuilh, L., Fontaine, J., Zouain, C. S., Mallevaey, T., Vasseur, V., Capron, M., Goriely, S., Faveeuw, C., Ryffel, B., and Trottein, F. (2007) Toll-like receptor (TLR)2 and TLR3 sensing is required for dendritic cell activation, but dispensable to control *Schistosoma mansoni* infection and pathology. *Microbes Infect.* **9,** 1606–1613
27. Hovius, J. W., de Jong, M. A., den Dunnen, J., Litjens, M., Fikrig, E., van der Poll, T., Gringhuis, S. I., and Geijtenbeek, T. B. (2008) Salp15 binding to DC-SIGN inhibits cytokine expression by impairing both nucleosome remodeling and mRNA stabilization. *PLoS Pathogens* **4,** e31
28. Roy, S., Wyse, B., and Hancock, J. F. (2002) H-Ras signaling and K-Ras signaling are differentially dependent on endocytosis. *Mol. Cell. Biol.* **22,** 5128–5140
29. Phythian-Adams, A. T., Cook, P. C., Lundie, R. J., Jones, L. H., Smith, K. A., Barr, T. A., Hochweller, K., Anderton, S. M., Hammerling, G. J., Maizels, R. M., and MacDonald, A. S. (2010) CD11c depletion severely disrupts Th2 induction and development in vivo. *J. Exp. Med.* **207,** 2089–2096
30. Whelan, M., Harnett, M. M., Houston, K. M., Patel, V., Harnett, W., and Rigley, K. P. (2000) A filarial nematode-secreted product signals dendritic cells to acquire a phenotype that drives development of Th2 cells. *J. Immunol.* **164,** 6453–6460
31. Balic, A., Harcus, Y., Holland, M. J., and Maizels, R. M. (2004) Selective maturation of dendritic cells by *Nippostrongylus brasiliensis*-secreted proteins drives Th2 immune responses. *Eur. J. Immunol.* **34,** 3047–3059
32. Kane, C. M., Cervi, L., Sun, J., McKee, A. S., Masek, K. S., Shapira, S., Hunter, C. A., and Pearce, E. J. (2004) Helminth antigens modulate TLR-initiated dendritic cell activation. *J. Immunol.* **173,** 7454–7461
33. Thomas, P. G., Carter, M. R., Atochina, O., Da'Dara, A. A., Piskorska, D., McGuire, E., and Harn, D. A. (2003) Maturation of dendritic cell 2 phenotype by a helminth glycan uses a Toll-like receptor 4-dependent mechanism. *J. Immunol.* **171,** 5837–5841
34. Segura, M., Su, Z., Piccirillo, C., and Stevenson, M. M. (2007) Impairment of dendritic cell function by excretory-secretory products: a potential mechanism for nematode-induced immunosuppression. *Eur. J. Immunol.* **37,** 1887–1904
35. Everts, B., Hussaarts, L., Driessen, N. N., Meevissen, M. H., Schramm, G., van der Ham, A. J., van der Hoeven, B., Scholzen, T., Burgdorf, S., Mohrs, M., Pearce, E. J., Hokke, C. H., Haas, H., Smits, H. H., and Yazdanbakhsh, M. (2012) Schistosome-derived omega-1 drives Th2 polarization by suppressing protein synthesis following internalization by the mannose receptor. *J. Exp. Med.* **209,** 1753–1767, S1751
36. Van Vliet, S. J., Steeghs, L., Bruijns, S. C., Vaezirad, M. M., Snijders Blok, C., Arenas Busto, J. A., Deken, M., van Putten, J. P., and van Kooyk, Y. (2009) Variation of *Neisseria gonorrhoeae* lipooligosaccharide directs dendritic cell-induced T helper responses. *PLoS Pathogens* **5,** e1000625
37. Van Vliet, S. J., Bay, S., Vuist, I. M., Kalay, H., Garcia-Vallejo, J. J., Leclerc, C., and van Kooyk, Y. (2013) MGL signaling augments TLR2-mediated responses for enhanced IL-10 and TNF-alpha secretion. [E-pub ahead of print] *J. Leukoc. Biol.* doi: 10.1189/jlb.1012520
38. Robinson, M. J., Sancho, D., Slack, E. C., LeibundGut-Landmann, S., and Reis e Sousa, C. (2006) Myeloid C-type lectins in innate immunity. *Nat. Immunol.* **7,** 1258–1265
39. Saunders, S. P., Walsh, C. M., Barlow, J. L., Mangan, N. E., Taylor, P. R., McKenzie, A. N., Smith, P., and Fallon, P. G. (2009) The C-type lectin SIGNR1 binds *Schistosoma mansoni* antigens in vitro, but SIGNR1-deficient mice have normal responses during schistosome infection. *Infect. Immun.* **77,** 399–404
40. Zhang, S., and Kaplan, M. H. (2000) The p38 mitogen-activated protein kinase is required for IL-12-induced IFN-gamma expression. *J. Immunol.* **165,** 1374–1380
41. Terrazas, C. A., Huitron, E., Vazquez, A., Juarez, I., Camacho, G. M., Calleja, E. A., and Rodriguez-Sosa, M. (2011) MIF synergizes with *Trypanosoma cruzi* antigens to promote efficient dendritic cell maturation and IL-12 production via p38 MAPK. *Int. J. Biol. Sci.* **7,** 1298–1310
42. Qian, C., Jiang, X., An, H., Yu, Y., Guo, Z., Liu, S., Xu, H., and Cao, X. (2006) TLR agonists promote ERK-mediated preferential IL-10 production of regulatory dendritic cells (diffDCs), leading to NK-cell activation. *Blood* **108,** 2307–2315
43. Poncini, C. V., Gimenez, G., Pontillo, C. A., Alba-Soto, C. D., de Isola, E. L., Piazzon, I., and Cappa, S. M. (2010) Central role of extracellular signal-regulated kinase and Toll-like receptor 4 in IL-10 production in regulatory dendritic cells induced by *Trypanosoma cruzi*. *Mol. Immunol.* **47,** 1981–1988
44. Agrawal, S., Agrawal, A., Doughty, B., Gerwitz, A., Blenis, J., Van Dyke, T., and Pulendran, B. (2003) Cutting edge: different Toll-like receptor agonists instruct dendritic cells to induce distinct Th responses via differential modulation of extracellular signal-regulated kinase-mitogen-activated protein kinase and c-Fos. *J. Immunol.* **171,** 4984–4989
45. Thomas, P. G., Carter, M. R., Da'dara, A. A., DeSimone, T. M., and Harn, D. A. (2005) A helminth glycan induces APC maturation via alternative NF-kappa B activation independent of I kappa B alpha degradation. *J. Immunol.* **175,** 2082–2090
46. Gringhuis, S. I., van der Vlist, M., van den Berg, L. M., den Dunnen, J., Litjens, M., and Geijtenbeek, T. B. (2010) HIV-1 exploits innate signaling by TLR8 and DC-SIGN for productive infection of dendritic cells. *Nat. Immunol.* **11,** 419–426
47. Napoletano, C., Zizzari, I. G., Rughetti, A., Rahimi, H., Irimura, T., Clausen, H., Wandall, H. H., Belleudi, F., Bellati, F., Pierelli, L., Frati, L., and Nuti, M. (2012) Targeting of macrophage galactose-type C-type lectin (MGL) induces DC signaling and activation. *Eur. J. Immunol.* **42,** 936–945
48. Van de Veerdonk, F. L., Marijnissen, R. J., Kullberg, B. J., Koenen, H. J., Cheng, S. C., Joosten, I., van den Berg, W. B., Williams, D. L., van der Meer, J. W., Joosten, L. A., and Netea, M. G. (2009) The macrophage mannose receptor induces IL-17 in response to *Candida albicans*. *Cell. Host. Microbe* **5,** 329–340
49. Li, S., and Sedivy, J. M. (1993) Raf-1 protein kinase activates the NF-kappa B transcription factor by dissociating the cytoplasmic NF-kappa B-I kappa B complex. *Proc. Natl. Acad. Sci. U. S. A.* **90,** 9247–9251
50. Abdi, K., Singh, N. J., and Matzinger, P. (2012) Lipopolysaccharide-activated dendritic cells: "exhausted" or alert and waiting? *J. Immunol.* **188,** 5981–5989
51. Amit, P., Prasad, K. N., Kumar, G. R., Shweta, T., Sanjeev, J., Kumar, P. V., and Mukesh, T. (2011) Immune response to different fractions of *Taenia solium* cyst fluid antigens in patients with neurocysticercosis. *Exp. Parasitol.* **127,** 687–692

Received for publication February 06, 2013.
Accepted for publication July 22, 2013.

Hindawi Publishing Corporation
Journal of Biomedicine and Biotechnology
Volume 2010, Article ID 357106, 19 pages
doi:10.1155/2010/357106

Review Article

Modulation of Dendritic Cell Responses by Parasites: A Common Strategy to Survive

César A. Terrazas,[1] Luis I. Terrazas,[1] and Lorena Gómez-García[2]

[1] *Unidad de Biomedicina, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México (UNAM), 54090 Tlalnepantla, Estado de México, Mexico*
[2] *Department of Immunology, Instituto Nacional de Cardiología "Ignacio Chávez", Secretaria de Salud, Juan Badiano no. 1, Col Sección XVI, 14080 Tlalpan, México D.F., Mexico*

Correspondence should be addressed to Lorena Gómez-García, lorena.gomez@cardiologia.org.mx

Received 21 August 2009; Accepted 18 November 2009

Academic Editor: Abhay R. Satoskar

Parasitic infections are one of the most important causes of morbidity and mortality in our planet and the immune responses triggered by these organisms are critical to determine their outcome. Dendritic cells are key elements for the development of immunity against parasites; they control the responses required to eliminate these pathogens while maintaining host homeostasis. However, there is evidence showing that parasites can influence and regulate dendritic cell function in order to promote a more permissive environment for their survival. In this review we will focus on the strategies protozoan and helminth parasites have developed to interfere with dendritic cell activities as well as in the possible mechanisms involved.

1. Introduction

Dendritic cells (DCs) control the development of adaptive immune responses due to their remarkable ability to integrate signals coming from the environment, deliver this information to naive T cells, and in turn activate them inducing the appropriate response for the initial stimuli [1, 2]. During infection, the responses induced by DC are critical to control and eliminate the invading agent, the infection itself being the inducer of DC activity. DC maturation and activation involves upregulation of several molecules that play main roles in costimulation and antigen presentation to T cells such as CD80, CD86, CD40, and major histocompatibility complex II (MHC-II), along with the release of cytokines that influence the type and intensity of the immune response [2–4]. Mature DCs are fully potent antigen-presenting cells (APCs) that will prime naïve T cells inducing their differentiation and proliferation [1, 5]. Protozoan parasites can activate and induce the maturation of different DC subsets and in most cases the activity of these cells leads to a response that is effective in controlling the infection [6–8]. The case of helminthes is more complex since most reports show a partial maturation of DC in response to these parasites or their antigens that does not contribute with parasite elimination [9–11]. In any case, it has been shown that both, helminthes and protozoan, are capable to interfere with DC activity promoting a more permissive environment for their own survival inside their host [11–14]. Different interactions of parasite-derived molecules with receptors on DC such as Toll-like receptors (TLRs), C-type lectin receptors (CLRs), and others seem to be the key event in the mechanisms that ultimately will lead to the altered function in these cells [15–18]. In this review we will focus on some important protozoan and helminth parasites and their ability to modify DC function, the implications of such modulation, and the possible mechanisms involved. We will discuss the differences and similarities in the interference with DC activity between these two distinct parasite groups.

2. Protozoan Parasites

2.1. Plasmodium. It has been shown that different species of *Plasmodium sp.* can modulate the response of DC

[6, 13, 19–22]. While infection by some nonlethal strains seems to induce the activation, maturation, and secretion of important proinflammatory cytokines such as IL-12 by DC [6, 22], infection not only with lethal strains such as *P. yoelii* YM or *P. berghei* but also with the nonlethal *P. vivax* can impair DC function. Modified DC function by *Plasmodium* species can be achieved through varied mechanisms. Some of these mechanisms involve a decrement in total DC numbers as well as an altered ratio of myeloid versus plasmacytoid cell subsets, the latter being probably involved in the induction of a regulatory phenotype mediated by regulatory T cells (Tregs) and IL-10 production [12]. Other studies have reported null or reduced capacity of *Plasmodium*-exposed DCs to prime T cells [6, 21–24], situation that could be related with the inability of these DCs to establish prolonged interactions with naïve CD4+ T cells [23]. Interestingly, DCs from infected mice with lethal or no lethal strains show an impairment in their response to TLR stimulation, as a marked reduction in IL-12 secretion is observed upon stimulation with LPS, CpG, or poly:IC, resulting in some cases, in the inversion of the IL-12/IL-10 secretion pattern displayed by DC [6, 21, 24]. Since these changes can occur as malaria infection progresses [21], altogether these data suggest that *Plasmodium* has the ability to switch an aggressive immune environment into a more permissive one for its survival. Other effects that *Plasmodium* has on the function of DC have been reported. These effects can be attributed to the invading species, the parasite life stages, or the products they release. For example, a preferential increment in $CD8^-$ DC in spleen is observed in the acute phase of *P. chabaudi* infection. Importantly, only $CD8^-$ dendritic cells induce proliferation of merozoite surface protein- (MSP1-) specific T cells, and since these T cells produce considerable levels of IL-4 and IL-10, it is suggested that the change from a Th1 to a Th2 response that takes place in *P. chabaudi* infection [25] can be attributed to these already modified DC populations [13].

Maturation of human monocyte-derived DC (MDC) through CD40L can be also prevented by *P. falciparum* merozoites. These DCs show a diminished IL-12p70 secretion but enhance IL-10 production and prime CD4+ naive T cells to produce higher levels of IL-10 and lower levels of IFN-γ. These effects by merozoites on DC seem to involve the activation of the extracellular-signal regulated kinase (ERK) pathway [26]. In contrast, in the same study, DC exposed to *P. falciparum* infected erythrocytes respond to CD40 signaling secret proinflammatory cytokines that lead to a proinflammatory response by naive CD4+ T cells, and this time, p38 mitogen-activated protein (MAP) kinase plays an essential role. These evidences indicate that *P. falciparum* uses distinct kinase pathways to modulate the activity of DC [26]. Moreover, those findings also indicate a different activity on DC function between the whole parasite and its products.

DC maturation can also be compromised in vivo during the last stage of *P. yoelii* infection and in vitro when DC are cultured in the presence of *P. yoelii*-infected erythrocytes [27]. Interestingly, the presence of the complete parasite is not necessary to interfere with DCs function and by-products of hemoglobin degradation mediated by *P. falciparum* such as hemozoin can also modulate the activity of DC. This malarial pigment inhibits the differentiation and maturation of MDC, reducing the expression of major MHC-II and the costimulatory molecules CD83, CD80, CD54 and CD40 [28, 29]. T cells activated in the spleen by hemozoin-containing DC, are not completely functional since they can not secrete cytokines or migrate to B-cells follicles [19].

More, recently it was shown that soluble factors released by *P. yoelii* or *P. falciparum*-infected erythrocytes can inhibit LPS induced maturation of DC and change their cytokine secretion profile resulting in a failure to produce IL-12 [27]. Induction of regulatory DC that in turn promotes a Treg response is another mechanism by which malaria parasites may subvert host immune systems. Surprisingly, this effect appears to be mediated by recognition of the parasite's molecules by TLR9 [12, 30, 31].

Considering the importance of IL-12 and the Th1 response along with the full activation of DC to prime efficiently T cells in the immune response against *Plasmodium* species, the modulatory effects over DC function discussed above, seem to be an effective evasion strategy actively induced by *Plasmodium* parasites that certainly can influence the outcome of the infection.

There is evidence supporting the interaction of *Plasmodium* parasites with several receptors expressed by DC. For example, among the different surface antigens expressed on *P. falciparum,* the *Plasmodium falciparum* erythrocyte membrane protein 1 (PfRBC-1) seems to play a main role [32]. This protein can mediate adherence to DC through the interaction with the Scavenger receptor CD36 [33] but also through the proteoglycan molecule, chondroitin sulfate A (CSA) [34, 35]. However, the interactions of PfRBC -1 seem not to be related with the interference of *P. falciparum* with DC function, in fact; in vitro studies have shown that modulation of DC responses might not need contact between these cells and the infected erythrocytes [35], opening the possibility for parasite-soluble factors playing a major role. This is well exemplified by the interaction of *Plasmodium* parasites and TLR2, TLR4 and TLR9 which can indeed recognize infected erythrocytes-derived products [36–38]. Soluble extracts from *P. falciparum* and hemozoin (by presenting malaria DNA) can activate DC in a TLR9 dependent fashion, inducing up-regulation of costimulatory molecules and proinflammatory cytokines and chemokines, some of them involved in host resistance against *P. falciparum* [36, 37, 39]. Interestingly, it was recently shown that *P. yoelii* uses the interaction with the same receptor on DC to in turn induce Treg cells, a response that is associated with *P.yoelii*-immune evasion [30]. This finding indicates that different *Plasmodium* species may induce different responses through their interaction with TLR9. However, there is still little information about the receptors and ligands involved in the DC downregulation consistently observed in *Plasmodium* infections or in response to their products. See Table 1.

2.2. Leishmania. Clearance and resistance to *Leishmania* infections is dependent on the development of a Th1 response and the production of IL-12 [40–42], and in this regard, DCs play a critical role [43]. It has been shown that during *Leishmania* infections, engulfment of amastigotes and promastigotes by DCs leads to their activation and a consequent IL-12 and TNF-α production that can contribute with host resistance [44, 45]. However, more accurate is the fact that the effect of *Leishmania* infection on DC maturation and cytokine production could vary for the different *Leishmania* species and strains; for example, while the uptake of *L. major* promastigotes and amastigotes results in DC maturation and IL-12 production [46, 47], promastigotes of *L. amazonensis* can interfere with DC responses affecting their differentiation and decreasing their ability as APC [48]. Both stages of this specie can reduce CD40 and CD83 surface expression and IL-12p40 production by bone-marrow-derived dendritic cells (BMDCs) in a mechanism that requires the activation of the MAP kinase ERK [7, 49]. Additionally, IL-12p40, IL-12p70, and IL-6 are downregulated and IL-10 secretion increased when infected DC are treated with LPS [7]. Lack of activation and a reduced IL-12 production by DCs also observed after *L. mexicana* infection along with a delay in their apoptotic process [50, 51]. *L. donovani* can also inhibit DCs maturation and modify their CCR7 expression, interfering with their migration to the periarteriolar lymphoid sheath [52, 53]. Interestingly, some of the regulatory effects that *L. donovani* has on DC depend on the phosphoglycans present in the parasite [52]. In fact, several *Leishmania* products can also impair DC activity as it has been shown that parasite culture media or lipophosphoglycans (LPGs) from *L. major* inhibit the motility of murine splenic DC [54] or the migration of Langerhans cells [55], suggesting that *Leishmania* products may interfere with antigen transportation. *L. major* phosphoglycans (PGs) are involved in downregulation of IL-12p40 production by DC and in the ability of these cells to induce a Th1 response since these molecules favor IL-4 and IL-10 but not IFN-γ production [56]. Moreover, excreted-secreted antigens of *L. donovani* or *L. major* induce a slight decrease in the surface expression of CD40, CD86, human leukocyte antigen-DR (HLA-DR), and cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN) on DC concomitant with a downregulation on IL-10 and IL-12p70 secretion [57]. In the case *of L. mexicana*, the activity of LPGs on DC function also includes a reduction in IL-12 production that may depend on the impairment on NF-κB nuclear translocation induced by parasite's antigens [51]. Since the maturation state of DC seems to determine the type of immune response induced in *Leishmania* infections, where an immature state promotes a Th2 response which does not control the infection and a mature phenotype induces a Th1 response with the subsequent host resistance [58], the interference of these parasites and their products in the maturation process of DC may be critical for parasite survival.

Some studies have shown that activation and production of IL-12 and IFN-α/β by myeloid DC (mDC) and plasmacytoid DC (pDC) after stimulation with *L. major*, *L. brasilienzis,* or *L. infantum* is strictly dependent on TLR9 signaling [59, 60] and the maturation of splenic DC is improved by the myeloid differentiation primary response gene 88 (MyD88)-signaling pathway, indicating an important role of TLRs in activation of DC by *Leishmania* [61]. In addition, others found that TLRs such as TLR2 and TLR4 are involved in the response to *Leishmania* parasites and their products, like LPGs that might signal through TLR2 inducing a TNF-α response [62–64]. Interestingly, a noncharacterized soluble product from *L. brasilienzis* activates DC in a MyD88-independent fashion, suggesting that receptors other than TLRs may play a role in the responses induced by *Leishmania* products on these cells [65]. But even when interaction of *Leishmania* sp. parasites or their products with TLRs and probably other receptors can induce the activation of DC, it is still unknown which kinds of molecules, receptors, and pathways are involved in the impairment of function that has been observed in DC exposed to *Leishmania* parasites, *Leishmania* LPGs, or *Leishmania* excreted-secreted products. Therefore, deeper studies in this field should improve our knowledge on *Leishmania*'s strategies to survive the immune response. See Table 1.

2.3. Trypanosoma. Infection of MDC with *Trypanosoma cruzi* induces functional changes in these cells. For example, secretion of IL-12 and TNF-α is impaired and the maturation process induced by LPS is affected, observing a marked reduction in proinflammatory cytokines secretion as well as on the expression of HLA-DR and CD40. Moreover, the same effects are observed when DCs are cultured in the presence of *T. cruzi*-conditioned medium, indicating a modulatory activity of the molecules released by these parasites [66]; in fact, the exposure of DC to *T. cruzi*-derived GIPL (glycoinositolphospholipids) can also affect the LPS-induced cytokine secretion and expression of costimulatory molecules in similar way that the whole parasite does [67]. Using also in vitro studies, Poncini and colleagues showed that trypomastigote stage of *T. cruzi* fails to activate DCs, these cells become in TGF-β and IL-10 producers and are not efficient as lymphocyte stimulators. For these reasons, the authors classify these cells as regulatory DCs [68]. Importantly, in vivo experiments also robustly show the interference of *T. cruzi* with DC function. At day 14 of an active infection, there is a progressive fall in the number of splenic DCs and this is accompanied with a low expression of the costimulatory molecule CD86, therefore suggesting an immature phenotype. These DCs are unable to migrate to the T cell area in the spleen or undergo maturation upon LPS stimulation [69]. Interestingly, only the infection with *T. cruzi*-virulent strain RA but not the low-virulence strain K98 affects MHC-II expression on splenic DCs and their capacity to prime T cells [70], indicating a relation between virulence and a more effective strategy to avoid immunity. This is further supported by the evidence that the infection of the susceptible strain of mice (BALB/c) with *T. cruzi* induces a downregulation of splenic DC activity. These cells display a diminished CD86 and CD40 expression and a reduced potential to present alloantigens. Importantly, these changes are not observed

Table 1: Protozoan interactions and their regulatory effects on DC.

Parasite	Product	Putative DC Receptor ¶	Effect on DC	Effect on DC upon TLR stimuli	Ref.
Plasmodium					
P. berghei	Infection	N.D.	↓ Tcell priming	↓ IL-12p70, endocytic activity	[22]
	Infection	TLR9	Immature	↑ IL-10	[6, 23, 24, 30]
			↓ T cell priming, shorter DC/T cell interactions	↓ CD40, CD86, MHCII, IL-12p70	
				↑ IL-10	
P. yoelii			↑ Treg*		[21, 31]
	Soluble Factors	N.D.	↓ IL-12	↓ CD40, CD86, MHCII, IL-12	[27]
P. vivax	Infection	N.D.	↓ mDC:pDC	N.D.	[6, 12]
			↑ Treg*		
P. chabaudi	Infection	N.D.	↑ CD8- DC Th2*	N.D.	[13]
P. falciparum	Merozoite	N.D.	↓ IL-12p70, IFNγ*	N.D.	[2, 20]
			↑ ERK,IL-10, IL-10*		
	Hemozoin	N.D.	↓ Immature Differentiation Migration	N.D.	[19, 28]
	Soluble extracts	N.D.	Immature	↓ IL-12	[27]
			↓ IL-12		
Leishmania					
L. major	Promastigote Amastigote	N.D.	Immature	N.D.	[55]
			↓ IL-10		
	LPGs	TLR2	↓ Motility Migration	N.D.	[54]
	PGs		↓ IL-12p40, IFNγ*	↓ IL-12p40	[56]
			↑ IL-4*, IL-10*		
	ES	N.D.	Immature	N.D.	[57]
			↓ DC-SIGN, IL-10, IL-12p70		
L. amazonensis	Promastigote	N.D.	Immature	IL-12p40, IL-10	[45, 48, 49, 65]
			↑ ERK	↓ IL-12p70,	
			↓ IL-12p40	↑ IL-6	
L. mexicana	Promastigote	N.D.	Immature	N.D.	[50]
			↓ IL-12 Apoptosis		

Table 1: Continued.

Parasite	Product	Putative DC Receptor ¶	Effect on DC	Effect on DC upon TLR stimuli	Ref.
	LPG	N.D.	IL-12	N.D.	
			↓ NFkB		[51]
L. donovani	Promastigote	N.D.	Immature	N.D.	
			Migration, CCR7,		
			↓ CD11b, CD51,		[52, 53]
			CD86, IL-12p70		
	ES	N.D.	Immature	N.D.	
			↓ DC-SIGN, IL-10, IL-12p70		[57]
Trypanosoma					
T. cruzi	Infection and Soluble Factors	N.D.	Immature	Migration, IL-12, TNFα,	
			DC number,	↓ IL-6, HLA-DR,	[66, 69, 70]
			↓ T cell priming,	CD40,CD86	
			IL-12, TNF-α		
	Trypomastigote	N.D.	Immature	TNF-α,IL-12p70, IL-6,	
			↑ TGF-β, IL-10	↓ CD40, HLA-DR,	[68, 71]
				↑ IL-10	
	GIPLs	N.D.	IL-12, TNFα,	TNF-α,IL-12, IL-10,	
			↓ IL-10	↓ CD83, CD80, CD86,	[67]
				CD40, HLA-DR	
	Sialyted structures	Siglec-E	N.D.	↓ IL-12p40, IL12p70	
				↑ IL-10	[17]
Toxoplasma					
T. gondii	Infection	N.D.	N.D.	↓ IL-12p40	[87, 91]
	Tachyzoites	N.D.	Immature	IL-12p40,	
			↑ Motility Migration	↓ TNF-α, MHCII	[87, 89, 92]
			↓ T cell priming		
	Soluble Factors	N.D.	Immature	N.D.	[91, 93]
			↑ DC attraction		
	Endogenous LXA_4 parasite-induced	FRPL-1	↑ SOCS2	N.D.	[16]
		AhR	↓ IL-12		
Giardia					
Giardia lamblia	parasite extracts	N.D.	CD80, CD86,	↑ IL-10	[94]
			↑ CD40	↓ IL-12, MHCII,	
			IL-12, TNF-α,	CD80,CD86	
			↓ IL-10		

¶ Involved in modulatory effects on DC.
*T cell response.

in the C57Bl/6 mice, a strain capable to survive the acute phase of this infection [71]. Together, these two studies indicate that modulation of certain DC features by *T. cruzi* depends on both host and parasite genetic backgrounds. They also remind us that even when targeting of DC functions by parasites is an important strategy for immune evasion, it can also unbalance the delicate host/parasite relationship in such a way that infection could be lethal for both.

There are different ways for an intracellular pathogen such as *T. cruzi* interacts with elements of the innate immune system. Macrophages and DCs are cells that

can be infected by this parasite; nevertheless; when these cells become activated by TLR signaling, through either the adaptor molecules MyD88 or TIR-domain-containing adapter-inducing interferon-β (TRIF), both cell types are less prone to allow parasite replication with IFN-β playing an important role in parasite clearance [72]. Different molecules derived from *T. cruzi* can be recognized by TLRs. Glycosylphosphatidylinositol (GPIL) anchors from this protozoan parasite trigger TLR2-dependent antiparasite responses in macrophages [73] while *T. cruzi* DNA can induce their activation by interaction with TLR9 [74]. Indeed, the combination of TLR2 and TLR9 seems to account for all the protective IFN-γ production generated by MyD88-dependent signaling pathways in macrophages exposed to live trypomastigotes in vitro [74]. More importantly for this review's topic, the Tc52-released protein from *T. cruzi* signals via TLR2 and induces DC maturation and production of the inflammatory chemokines IL-8, MCP-1, and MIP-1α [75]. On the other hand, the bradykinin B_2 receptors (B_2R) in DC might be also involved in the activation and maturation of these cells, and in turn in the generation of protective responses such as IL-12 production and the development of Th1 immunity [76]. As for other protozoan parasites, the interaction between DC receptors and *T. cruzi* molecules that lead to downregulated responses in these cells remain poorly understood; however, recent evidence has brought some insights to this phenomena. Erdmann and colleagues demonstrated that sialylated structures from *T. cruzi* pathogenic Tulahuen and to a less extent the less pathogenic Tehuantepec strain interact with the inhibitory sialic acid-binding protein Siglec-E [17]. This lectin receptor is expressed on DC and interestingly its ligation suppresses the LPS-induced production of IL-12 by these cells. The subsequent T cell response is also affected since a marked decrease of IFN-γ production is observed when Siglec-E-ligated DCs are used as APCs. This response could play a role in the immunosuppression observed during *T. cruzi* infection as well as determining the distinct pathogenicity that these two different strains display [17]. In addition, this finding suggests that the interference with DC function induced by *T. cruzi* could be achieved through the interaction of lectin receptors with parasite products. See Table 1.

2.4. Toxoplasma. Either live tachyzoites or a soluble tachyzoite extract from *Toxoplasma gondii* injected into mice induces a rapid and strong production of IL-12 from splenic DC [77, 78]. This IL-12 response is unusually potent since not even stimuli like LPS or CpG can reach the IL-12 levels induced by *T. gondii* antigens [79]. Such reaction is mediated by the specific recognition of *Toxoplasma*-derived profilin by TLR11 expressed specifically on CD8α+ DC [80], although other molecules and other TLRs could be also involved [81, 82]. Nevertheless, other reports indicate that the potency of the *T. gondii* to induce IL-12 can be achieved thanks to the engagement of CCR5 by cyclophylin-18 (C18), a chemokine mimic of MIP-1β derived from the soluble extract of the same parasite [83]. It is, however, important to highlight that the production of high levels of IL-12 secreted by DC in the presence of *T. gondii* antigens quickly reaches baseline level (24 h post stimulation) and DCs become refractory to any further exposure to *T. gondii* in a phenomenon called "DC paralysis" [84]. It is believed that these dramatic changes in the DC response are due to a parasite strategy that assures the control of a potentially lethal parasite growth and prevents a cytokine-mediated immunopathology, as both scenarios could be deleterious for the host and in turn to the parasite [79]. A line of evidence has further identified a sophisticated *T. gondii*-induced modulation of the immune response. The parasite-induced production of the eicosanoid lipoxin A_4 (LXA_4) has been linked to the downregulation of IL-12 production by DC [85] in a mechanism that seems to involve the binding of LXA_4 to the receptors formyl peptide receptor-like 1 (FRPL-1) or aryl hydrocarbon receptor (AhR) on the same cells [16]. This interaction triggers the expression of the suppressor of cytokine signaling-2 (SOCS-2) that in turn helps to control the proinflammatory and otherwise potentially lethal immune response against *T. gondii* [16]. In line with this idea, in the absence of the AhR receptor (AhR knock out) mice succumb rapidly to ME49 *T. gondii* infection, a situation that is associated with the higher proinflammatory response observed in these animals [86].

In contrast with the evidence discussed above, there are some reports that show that invasion of immature DC by *T. gondii*-living parasites does not induce their activation and renders them also resistant to activation by TLR ligands or CD40L [87, 88]. The differences in DC responses upon *T. gondii* exposure could be explained by the identification of different DC subpopulations that are indeed involved in the initial response to *T. gondii;* among those are CD8α+, CD11b+, and, surprisingly, pDC [8]. It is noteworthy to consider the findings from Diana and colleagues who reported that soluble factors released from low-virulent strains of *T. gondii* can inhibit DC maturation [89] while other reports have demonstrated that *T. gondii*-soluble extract can induce a marked IL-12 production by DC [77]. Despite the fact that all these apparently contrasting findings may suggest that different soluble molecules from this parasite can provoke varied responses in DC, this is probably due to different soluble antigens interacting with distinct types of receptors.

T. gondii is capable of exploiting parasitism in a remarkable way by using DC to invade other tissues [88, 90] or other immune cells [91], promoting in turn the spreading and establishment of the infection. This may be achieved by the early target of these cells by *T. gondii* tachyzoites, an interaction that seems to modify the DC motility, trafficking, and migration properties as well as their cytokine effector function following TLR stimulation [88–90, 92]; the last one probably renders them unable to activate direct and indirect killing pathways. In addition, *T. gondii* may use the NK-cell mediating targeting of infected DC to disseminate, allowing viable parasites to enter in NKs that later appeared not to be efficiently targeted by other NK cells [91].

Different mechanisms could be related with the change in trafficking and migration behavior of DC exposed to *T. gondii*; however, it seems that most of these parasite-induced alterations are mediated by G-protein signaling pathways [83, 89, 92]. It has been shown that soluble

factors in excreted-secreted *T. gondii* antigens appear to possess a chemokine-like activity that attracts immature human [93] and mouse DCs [83] while other factors from a parasite extract can even trigger DC migration towards the chemokine MIP-3β [93]. In both human and mouse cells, the chemokine-like activity might be achieved by the *T. gondii*-derived chemokine mimic C-18, although other parasite factors could be involved [83, 93]. In any case, it is likely that this DC attraction might enhance the chances for DC infection while the alteration in DC trafficking and migration properties could participate in parasite dissemination. See Table 1.

2.5. Other Protozoan Parasites. There are several other species of protozoan parasites including *Trichomonas sp*., *Giardia sp.*, *Entamoeba sp*., and *Cryptosporidium sp*. Unfortunately, these parasites have generally been poorly studied as far as their interactions with DC concern probably due to the fact that they have less morbidity and mortality among human populations. Nonetheless, recently it was shown that DCs incubated with *Giardia lamblia* live parasites or its extracts, display enhanced levels of CD40, CD80, and CD86 indicating their mature state. These cells produce small amounts of IL-6 and TNF-α and no IL-10 or IL-12 is detected [94]. Importantly, coincubation of DC with parasite extracts and TLR ligands only enhance IL-10 production while markedly reducing IL-12 secretion and MHC-II, CD80, and CD86 levels. The mechanism of DC inhibition involves the activity of the phosphoinositide 3-Kinase (PI3K) since specific inhibition of this enzyme restores IL-12 production by these cells [94]. It is hypothesized that the ability of *Giardia lamblia* to inhibit IL-12 and enhance IL-10 production may contribute the maintenance of an antiinflammatory environment in the gut.

It is also known that lipopeptidophosphoglycan (LPPG) molecules from *Entamoeba histolytica* establish interactions with TLR2, and TLR4 leading to the release of IL-10, IL-12, and TNF-α [95]; and additionally, DNA from this parasite can be recognized by macrophages through TLR9 triggering TNF-α production [96]. With regard to DC, LPPG and also the parasite's surface Gal-lectin can activate these cells and induce their maturation along with IL-12 production, resulting in the induction of Th1-type responses [97, 98], although no findings regarding DC downregulation are available for this parasite.

TLR receptors are also involved in recognition of *Cryptosporidium parvum* and *Trichomonas vaginalis* but these interactions are normally between the parasites and host cells other than DC [99–102]. See Table 1.

3. Helminth Parasites

In marked contrast to protozoan parasites, helminthes are much larger hence they do not occupy intracellular spaces or are engulfed, having their interactions with immune system through the attachment of some immune cells to their surface or mainly by the recognition of diverse antigens released from the parasite. However, as for any parasite, their survival will depend on their capacity to cope with immune system's attacks. In this sense, interfering with DC activity is again one of the main ways to induce a permissive environment that allows their development inside the host. As noted earlier, during development and differentiation DC can be exposed to different stimuli which significantly influence their function and maturation, and helminth-derived antigens are not the exception to this rule.

3.1. Nematodes. Semnani and coworkers demonstrated that when monocytes undergoing differentiation to DC engulfed *B. malayi* microfilariae antigens they display an inhibited production of IL-12p40, IL-12p70, and IL-10 in response to *Staphylococcus aureus* Cowan antigen (SAC) and SAC plus IFN-γ, suggesting an interference with TLR and IFN-γ signaling induced by this nematode [10]. Cell viability or expression of costimulatory molecules, including MHC-I and MHC-II is not altered; however, DC exposed to these filarial antigens induce lymphocyte activation to a lesser degree than DC that were not exposed [10]. The same group recently reported a reduced TLR4, TLR3, and MyD88 mRNA expression in HMDC, as well as an impaired cytokine response to poly:IC and LPS upon exposure to live *B. malayi* microfilaria (mf) [103]. This indicates that this parasite can alter not just DC maturation but also their TLR responses affecting in turn T cell activation. In addition, MDC exposure to mf led to an enhancement in SOCS1 and SOCS3 mRNA transcripts [103], which are molecules known for their modulatory activity on cytokine production [104]. Furthermore, mf induces apoptosis in HMDC but not in macrophages [103]. Thus, the interaction of this helminth parasite and its antigens with DC clearly interferes with the function of these cells at different levels, from downregulating proinflammatory receptors to the induction of molecules involved in the suppression of cytokine production.

Another nematode, *Nippostrongylus brasiliensis,* secretes in vitro diverse glycoprotein named excretory-secretory products (NES). These antigens polarize the immune response towards a Th2 type without requiring live infection of the mice. Interestingly, BMDC pulsed with NES can upon transfer to naive recipients prime them to a Th2 response [105], suggesting that the Th2 driving properties of this extract occur through DC. In fact, NES upregulates DC markers associated with Th2 promotion, including CD86 and OX40L. In addition, the high levels of IL-12p70 induced by LPS are suppressed in DC that have been preincubated with NES [105]. This situation may contribute to the Th2 polarized response observed either during the infection with *N. brasiliensis* or in response to its antigens.

In in vivo studies, adult *Ascaris suum* high-molecular-weight components (PI) inhibit MHC-II and costimulatory molecules in CD11c+ LN cells from OVA-PI-immunized mice compared with those immunized with OVA-CFA [9]. In line with an immature phenotype, these CD11c+ are poor inducers of proliferation, phenomen that is IL-10 dependent. Interestingly, OVA+PI was administered in CFA (Freund's complete adjuvant), suggesting that PI could inhibit the inflammatory effect of this adjuvant [9]. In addition,

peritoneal CD11c+ cells recruited by pseudocoelomic fluid (PCF) from *A. suum* show basal levels in CD86 expression while BMDCs exposed to the same extract exhibit low increase in CD40 expression and are refractory to LPS stimulus, displaying once again an immature phenotype and a dose-dependent reduction in IL-12 production. [106]. Moreover, glycosphingolipids from *A. suum* containing phosphorylcholine (PC) downregulate IL-12p40 and TNF-α secretion from DC, as well as a MHC-II, CD40, CD80, CD86, and CD54 in response to LPS. This modulation is due to native glycosphingolipids and PC-removed glycosphingolipids, suggesting that a molecule other than PC possesses immunomodulatory properties over DC, although this one has not yet been elucidated [107].

ES-62 is another molecule that contains PC; it is derived from excreted/secreted products of the nematode *Acanthocheilonema vitae* and has been shown to display a variety of immunomodulatory activities [108]. ES-62 exerts its immunomodulatory effects on an array of cells of the murine immune system, including macrophages and DC [108–110]. In particular, exposure of immature DC to ES-62 lead to an unexpected increment in the expression of costimulatory molecules such as CD40, CD80, and CD86 and also, unlike other nematode molecules, ES-62 induce low but significant levels of IL-12p40 and TNF-α production in a MyD88-dependent manner. Interestingly when DC are stimulated with ES-62, there is an up-regulation of TLR4 expression; however, ES-62 had overall inhibitory effects on IL-12 and TNF-α production induced by TLR ligation. This suppressive effect is abrogated in TLR4 knockout but not in C3H/HeJ mice-derived BMDC, suggesting the use of a coreceptor in this ES-62-TLR4-dependent signaling [108]. ES-62 is also capable of shifting BMDC into the "DC2" activation state [108]; when these DC2 cells present an ovoalbumin peptide to naïve CD4+ T cells from OVA TCR transgenic mice, an increase of IL-4 and a decrease of IFN-γ production by lymphocytes are observed. In addition, the switch to a Th2 response is not affected by differential regulation of CD80 or CD86 and it is achieved even in the presence of IL-12 [110]. Importantly, despite the fact that ES-62 is a high Th2 inductor, this molecule is incapable to overcome and bias the immune response to a Th2 type in the context of some inflammatory Th1 conditions such as *T. gondii* infection and *O. volvulus* synthetic protein [111, 112].

Other important data regarding DC activity modulation have come from studies with *Heligmosomoides polygyrus* excreted-secreted-derived products (HpES) and the adult worm homogenate (AWH). None of these antigens induce BMDC maturation and IL-12, TNF-α or IL-10 is not found in culture supernatants [113]. Alike other helminth-derived extracts, HpES alters TLR-induced cytokines and chemokines, since limited IL-12, TNF-α, MCP-1, and RANTES production is elicited by stimulation with CpG, LPS, and poly:IC. Interestingly, the production of the immunoregulatory cytokine IL-10 is also impaired. In addition, costimulatory molecules including CD40, CD86, and MHC-II but not CD80 are drastically downregulated also in response to TLR stimuli [113]. Even more, studies in vitro with a model of CD4+ OVA restricted activation, show that DC treated with HpES not only attenuate but also IL-4 and IFN-γ production by CD4+ T cells but enhance IL-10 leading to Treg induction [113]. The findings for HpES correlate with an in vivo study where spleen DC isolated from *H. polygyrus* infected mice have an increased IL-10 production and a moderate up-regulation of CD80 and CD40. These DC are poor inducers of CD4+ T cell proliferation and have the ability to decrease IFN-γ and enhance IL-4 production [114]. Thus, it is likely that the responses induced by *H. polygyrus* in DC, participate in providing a safer environment for parasite establishment and survival. Importantly, the impaired cytokine effect observed in DC exposed to *H. polygyrus* antigens is TLR2 and TLR4-independent [113] while data are available showing that these same receptors recognize molecules from other helminthes such as ES-62 from *A. vitae* [11, 15, 108, 115]. These findings imply that the recognition and activity of helminth-derived molecules occurs through different receptors. This hypothesis has been further supported by a study that shows that calreticulin (CRT) present in *H. polygyrus* antigens lead to a Th2 response in vivo and this same molecule can be recognized by BMDC through Scavenger receptor A (SR-A) [116]. However; more studies are required to determine the phenotype that such recognition may confer to DC, and whether this phenotype is involved in the polarization toward Th2-type responses observed in the previous studies. Importantly, since CRT is preserved across the helminth parasites [86, 117–121], it is likely that this molecule might represent a potential pathogen-associated molecular pattern (PAMP) with a Th2-polarizing activity. See Table 2.

3.2. Trematodes. The most studied helminthes by far have been *Schistosomes* and in particular *S. mansoni*. They produce a great variety of glycosilated proteins and lipids to which mainly humoral immune responses are directed. In the case of *S. mansoni*, proteins, glycans and lipoconjugates can induce Th2-type responses. Several groups have shown that schistosome soluble egg antigens (SEA) contain molecules that drive the polarized CD4+ Th2 response [122, 123]. Many involved mechanisms have emerged and in this regard DC seem to have a critical role. For example, some glycans found in SEA such as core α-3-fucose, β2-xylose and Lewis X, have been shown to play an important role in the changes observed in DC activity [18]. DC pulsed with fractions containing a motif of α3-fucosylation of a GlcNac or of β-xylosilated core sugar drive strong Th2-cell responses in mice [124]. When egg-derived glycoconjugates are captured, processed, and presented to naïve T lymphocytes by DC, the immune response is again skewed to a Th2-type response. Periodate treatment reverses this effect and CD1d is apparently crucial to this phenomenon, indicating that SEA glycolipids may be involved in the Th2 polarization by DC [124]. SEA activity on DC is even more profound, for instance; immature DC pulsed with this antigenic extract do not show an increase in expression of costimulatory molecules or cytokines while their LPS-induced activation, including expression of MHC-I and costimulatory molecules as well as IL-12 production is also suppressed. In addition, SEA inhibits the ability of CpG, poly:IC and hyaluronic

Table 2: Helminth products interactions and their regulatory effects on DC

Parasite	Product	Putative DC Receptor¶	Effect on DC	Effect on DC upon TLR stimuli	Ref.
Nematodes					
B. malayi	Microfilarie alive	N.D.	Immature ↓	MIP-1, IL-12p70, IL-1α, IFN-α, IL-12p40, MyD88, NFkB (p50-p65)	
			TLR4,TLR3 ↓ IL-8, RANTES, ↑ TNF- α, IL-1 α, IL-1β, SOCS1, SOCS3 DC apoptosis		[103]
	Microfilarie Ag	N.D.	↑ CD80, CD40, MHCI	↓ IL-12p40, IL-12p70, IL-10	[10]
N. brasiliensis	NES	N.D.	↑ OX40L, CD86Th2*	↓ IL-12p70	[105]
A. suum	PI	N.D.	Immature	↓ IL-12p40	[9]
	PCF	N.D.	Immature	IL-12, ↓ CD40, CD86	[106]
	Glycosphingolipids (PC cointained but PC independent)	N.D.	Immature	↓ IL-12p40, TNFα, CD86	[107]
A. vitae	ES-62 (PC contain)	TLR4	TLR4, CD80 * ↑ IL-12p40 (low), TNFα (low) Th2*	IL-12, TNFα ↓↑ IL-10	[108, 110]
H. polygyrus	ES	N.D.	Immature IFNγ*, IL-4* ↓↑ IL-10(Treg)*	IL-12, TNFα MCP1, RANTES, ↓ MHCII, CD40, CD86	[113, 114]
	Calreticulin	SR-A	N.D.	N.D.	[116]
Trematodes					
S. mansoni	SEA	DC-SIGN, MR, MGL	Immature DC-SIGN,MR, ↑ DCIR, MGL, Jagged 2,TLR4 Th2*	IL-12p40,IL-12p70, ↓ TNFα, IL-6, MHCII, CD80, CD86 ↑ IL-10	[18, 124–126]
	LNFPIII	TLR4	Immature ↑ ERK, NFkB, Th2*	N.D.	[15, 128]
	Lysophospahtidyl-serine	TLR2	↑ ERK, c-fos, Th2*, Treg*	↓ IFNγ*	[11, 115]
Cestodes					
E. granulosus	AgB	N.D.	IRAKp, NF-kB, ↑ TNF-α^{low}, IL-10^{low}, IL-6^{low}, Th2*	IL-12p70, ↓ TNFα, IL-6, IL-10, HLA-DR CD80, CD86	[14]
T. crassiceps	ES (carbohydrate dependent)	N.D.	↑ MHCII, Th2*	IL-15, IL-12p40, ↓ IL-12p70, TNFα, CD80, CD86, CD40, CCR7, IFNγ*	

¶ Involved in modulatory effects on DC.
* T cell response.

acid (HA) to induce production of IL-12 and up-regulation of MHC-II, CD80, and CD86 on DC. Even though IL-10 production is augmented in the presence of SEA, not all the SEA effects on DC depend on this cytokine [125, 126]. SEA also suppresses the LPS-induced expression of 46 genes in DC, many of which are proinflammatory, and it also prevents the LPS-induced downregulation of 37 genes that may be involved in the changes observed in DC function upon SEA exposure [125]. Thus, SEA appears to have a profound effect on TLR ligand-induced DC maturation/activation, suppressing inflammatory events associated with development of Th1-type responses. SEA probably also affects the antigen-processing pathway as DC exposed to SEA show a difference in antigen processing by segregating SEA to a different compartment when compared with a bacterial antigen (form *Propionibacterium acnes).* Since these two antigens are handled very differently, this could explain the contrasting responses they induce in these cells [125].

In contrast with other helminthes, molecules from *S. mansoni* have been more fully characterized and important data regarding them have been reported (reviewed in [127]). Lipids from *S. mansoni,* particularly those containing phosphatidylserine, act as Th2-promoting factors through the blockade of IL-12 production by DC while promoting the development of IL-10-producing Treg cells [11]. It has been shown that these molecules can be recognized through TLR2 and interestingly antibody blockade of TLR2 diminishes their ability to induce a Treg response but not the Th2 polarization [11], suggesting another pathway and probably another receptor involved in the DC Th2-driving activity. New data show that these lysophosphatidylserine molecules can increase ERK but not p38 phosphorylation on DC and induce an up-regulation in c-Fos transcription; in contrast, Delta4 (Notch ligand) is strongly downregulated. These combined factors may ultimately lead to the development of a Th2 response [115]. According to this report, the glycoconjugate LNFPIII (also *S. mansoni* egg derived) leads to ERK1/2 phosphorylation but not p38 in DC and posses Th2-biasing ability through the induction of DC2 in a TLR4-dependent manner [15]. NF-kB alternative activation through p105 degradation is necessary for these LNFPIII-mediated effects [128]. However, recent evidence show that the Th2 polarizing effect of SEA is maintained in TLR2 or TLR4 knockout mice and MyD88 is also unnecessary [126]. This finding may rule out the participation of TLR in the effects induced by the molecules present in SEA. These data also open the possibility of the involvement of many others receptors such as CLRs, other lectin or Notch receptors in the recognition and modulatory activity of the *S. mansoni* antigens over DC. According to this idea, DC can recognize carbohydrates present in SEA through CLRs like mannose receptor (MR), macrophage galactose-type lectin (MGL) and DC-SIGN while exposure of the same cells to SEA can upregulate the expression of these same CLRs and the one of DC-immunoreceptor (DCIR) [18]. In addition, there is recent evidence showing that DC may recognize helminth products through molecule-grabbing nonintegrin receptor 1 (SINGR1). This CLR has been shown to bind SEA in vitro, however studies using SINGR1 knock-out mice have not found any significant differences in the kinetic of immune responses after *S. mansoni* infection [129]. SEA can also enhance expression of Jagged 2 in DC, but downregulation of Jagged 2 using RNAi does not affect the ability to prime Th2 responses [130]. This situation could reflect the fact that different receptors present in DC may have similar effects [131]. Due to the diverse molecules found in SEA and indeed in other different parasite products (crude extracts or excreted/secreted products), many receptors could be involved in the recognition of the different molecules present in these extracts, in such a way, that the lack of one receptor might not affect the immune response induced by these antigens. Therefore, helminth parasites probably posses redundant molecules to escape from immunity, unfortunately so far, only few of them have been characterized and their interaction with DC is still far from being understood. Even when CLRs or Notch receptors could be involved in DC recognition of helminth products, more studies are necessary to establish whether these interactions participate in the changes observed in these cells and in turn in the systemic effects they induce**.** See Table 2.

3.3. Cestodes. Studies regarding the interaction of this class of helminthes and DC are far less extensive when compared with trematodes and nematodes. Nevertheless, most of them point to similar pathways of alteration of DC function by cestode-derived molecules. For example, Rigano and colleagues demonstrated that *Echinococcus granulosus* hydatid cyst antigens affect DC in different stages of maturation [14]. Purified antigen B (AgB) and sheep hydatid fluid (SHF) affect host DC differentiation from monocyte precursors by reducing the number of cells that differentiate into immature DCs (iDCs) inhibiting as well the up-regulation of CD1a while increasing CD86 expression. When these cells are stimulated with LPS, there is a significantly lower expression of CD80, CD86, and HLA-DR and lower quantities of TNF-α and IL-12p70. In addition, *E. granulosus* antigens interfere in the maturation process of these iDCs, inducing only a slight up-regulation of CD80, CD86 and a small TNF-α, IL-10, and IL-6 production ablating IL-12p70 completely. In response to LPS, DC previously exposed to *E. granulosus* antigens, express fewer CD80 and CD86 molecules and show reduced TNF-α and IL-12p70 production. When DC matured in the presence of *E. granulosus* antigens are used as APCs, the majority of naïve T lymphocytes differentiate into IL-4-producing cells, suggesting the potential of these antigens to skew the response to a Th2 [14]. However, neither the receptors on DC nor the type of molecules derived from *Echinoccocus* that are involved in DC modulation has been identified in this model.

Another cestode that has recently been proved to interfere with DC activity is *Taenia crassiceps*. Carbohydrates present in soluble antigens of this parasite are responsible for Th2 polarization in vivo [132] and induction of myeloid suppressor innate cells [133]. We have found that DC exposure to excreted/secreted products of high molecular weight from *T. crassiceps* metacestodes (TcES) induce an increment in MHCII expression but not in the costimulatory

molecules CD80, CD86 or CD40 or in cytokine production. In line with findings for other helminthes, DC exposure to TcES results in an abrogated response to TLR ligands such as CpG, LPS and *Toxoplasma* soluble antigens; these include the inhibition of IL-15, IL12p40, IL12p70, TNF-α secretion and costimulatory molecules but no MHC-II expression. In addition, the chemokine receptor CCR7 is also downregulated, suggesting the reduced ability of DC to migrate to rich T cell areas as another possible mechanism used by *T. crassiceps* to evade the immune response. When TcES-stimulated DC are tested in an allostimulation assay, these cells are weak inducers of T cell proliferation and the presence of TcES also downregulate the strong proliferation expected by addition of LPS or CpG. Of particular interest is that periodate-treated TcES are unable to inhibit the production of proinflammatory cytokines elicited by different TLR ligands and in the same way, carbohydrates in TcES are necessary to polarize the immune response towards a Th2 type in a CD4+ OVA transgenic model. This situation opens the possibility for a role of carbohydrate receptors in the modulation of DC function by *T. crassiceps*-antigens *(C, Terrazas, manuscript under review)*. Interestingly, the limited responses observed in DC of BALB/c mice (a susceptible strain for *T. crassiceps* infection) upon TLR stimulation is not observed when DC derived from the resistant mice C57Bl/6 are exposed to TcES [134], indicating that modulation of DC response by *T. crassiceps*-products is a key element in the outcome of this infection. See Table 2.

4. Parasites and the Interference with DC Function: Is There a Common Pathway in a Common Strategy?

The evidence discussed in this review clearly show that by targeting the principal sentinels and directors of the immune system, DC, parasites actively evade immune attack. DC pleiotropy allows protozoan and helminth parasites to interfere with different aspects of the immune response. In most cases, either protozoan or helminth parasites, are able to impair the maturation process and the proinflammatory cytokine production of DC [9, 14, 19, 52, 66, 113], but other effects like a modified migration or an inability to fully differentiate are also found on DC exposed to these parasites or their products [14, 28, 55, 92]. The consequences of these different effects on DC function are diverse, but ultimately it is believed that they provide a less aggressive environment for parasite development inside their host. Several findings indicate the recognition of parasites and their molecules through TLRs [11, 36, 65, 108]. However, it has been more complicated to define whether these interactions lead to the altered phenotype and function that is observed in DC exposed to protozoan and helminth parasites. For the protozoan parasites, most data indicate that recognition and signaling of the whole parasites or their products through TLRs trigger activation pathways in DC that ultimately confers resistance against infection [36, 75, 82]. However, it is important to point out that some of the same molecules that act as TLR ligands and induce a protective response, can also interfere with the DC function by inhibiting their maturation and production of the proinflammatory cytokines associated with parasite clearance [28, 37, 51, 62]. The mechanisms involved in DC "inactivation" are far from being completely understood. One possible explanation for this is the recognition of protozoan-derived molecules by other receptors than TLRs that in turn might interfere with TLR function. According to this rationale, in the last years new evidence has implicated CLRs as important players in intracellular signalling rather than only phagocyte receptors and more importantly, these receptors are also capable of inhibit TLR-mediated signalling [131]. Of special interest is to note, that impairment of TLR-induced response has turned out to be a common and a main feature in the modulation of DC induced not just by protozoan, but also by helminth parasites [66, 87, 103, 113]. Interference with TLR signalling may explain most of the downregulatory effects that these parasites have on DC, since MyD88-dependent pathways are involved in DC maturation and secretion of key cytokines such as IL-12 [61, 135]. However, evidence shows that other receptors and hence other signalling pathways may be involved in DC downregulation. An example of this is the phenomenon of "DC-paralysis" observed after the peak of IL-12 production in response to *T. gondii* parasites or its antigens. This is achieved by the parasite-induced production of lipoxin A_4(LXA_4) [85, 136], which in turn is recognized by the FRPL-1 or AhR receptors on DC [16], triggering SOCS2 and turning off IL-12 production [137]. Likely, similar mechanisms could promote the low response observed on DC exposed to other protozoa and even helminthes since B. malayi microfilariae can also up-regulate expression of SOCS1 and SOCS3 [103]. Alternatively, TLR-stimulation could trigger different responses from DC, probably by becoming tolerogenic [30] or exhausted [84, 138, 139] (Figure 1).

For helminthes, the interaction with TLRs on DC lead, in general, to a "immature state" characterized by an absence or a moderate expression of costimulatory molecules together with no proinflammatory cytokines secretion, features that in turn seem to induce the development of a Th2 immune response [140]. Once again, the exact mechanism implicated in DC proinflammatory cytokine downregulation in response to TLR stimulus and the signals elicited by helminth products that condition these same cells to induce a Th2 response still remain unclear [140, 141]. Interestingly, not all the costimulatory molecules are downregulated in DC exposed to helminth products. For OX40L, a molecule important for an optimal Th2 response, there is an up-regulation in presence of some helminth products [105, 142]. In this regard, it would be important to determine if other molecules can be expressed selectively after helminth antigen recognition and this knowledge could lead to a definition of a truly Th2 polarizing DC phenotype. Other features have been investigated in an effort to define the phenotype and characteristics of helminth pulsed DC. While most of the evidence shows these DC are in an "immature state", recent data indicate that SEA can induce phagosome maturation, a characteristic that is associated with DC maturation. In this

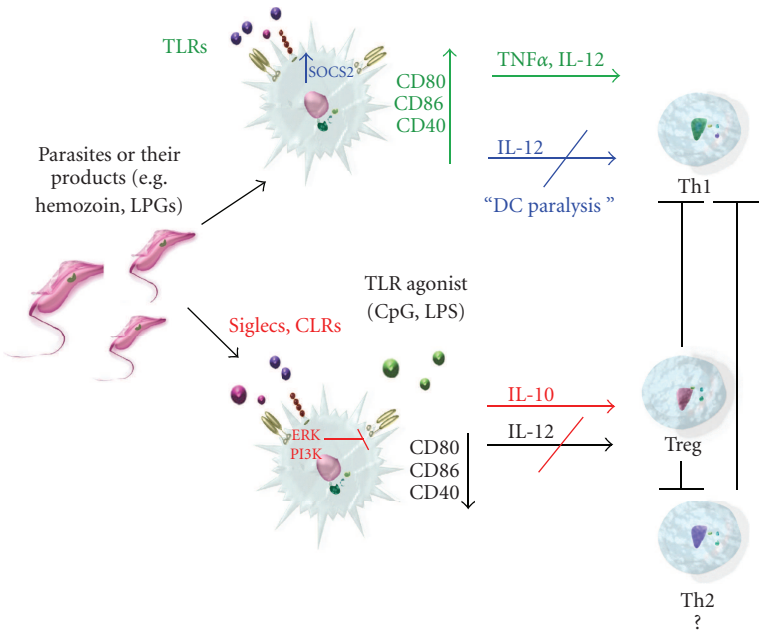


Figure 1: *Overview to protozoan and DC interactions*. Protozoan parasites and their products interact with TLRs on DC leading to their activation and release of proinflammatory cytokines and up-regulation of costimulatory molecules promoting a Th1 responses and the control of the infection. However, in some cases (*T. gondii* infection), this response can be later impaired by the same parasites through mechanisms that involve enhancement of SOCS proteins expression and downregulation of IL-12 production. In addition, interactions of parasite molecules with Siglecs and CLRs may be responsible of maintaining DC in an immature state and refractory to TLR stimuli, diminishing their proinflammatory response likely by using ERK and PI3K-dependent pathways. These DC may lead to activation of Treg responses that presumably favour parasite survival.

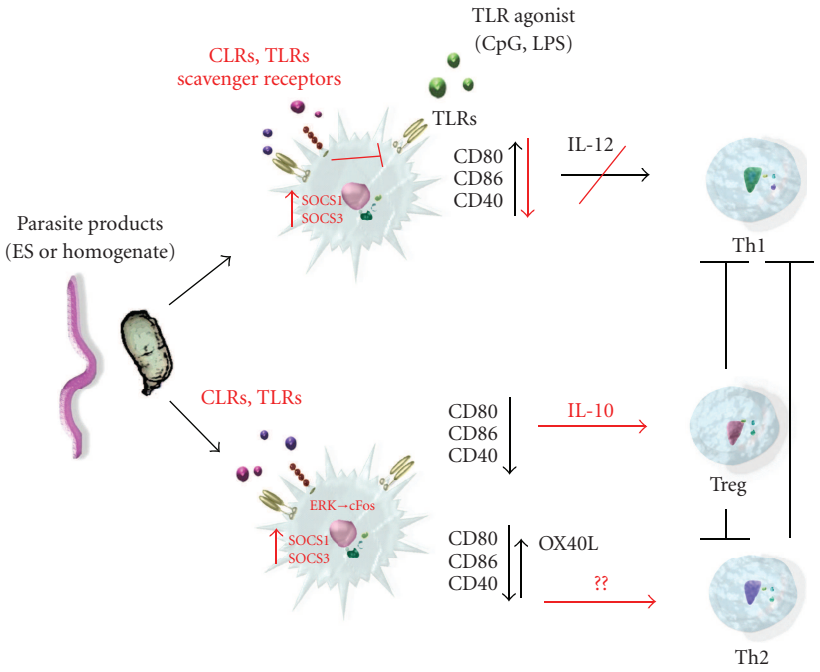


Figure 2: *Overview to helminth and DC interactions.* Helminth products are recognized by receptors such as TLRs, CLRs or Scavenger receptors. These cells remain in an immature state and unresponsive to further TLR stimuli probably due to interaction and signalling of parasite molecules through CLRs. Signalling pathways that implicate ERK phosphorylation, c-Fos up-regulation and expression of SOCS proteins may play a role in downregulation of DC responses particularly by suppressing IL-12 production. Once again, interactions of helminth molecules with CLRs but also with TLRs may be involved in these inhibitory effects. Finally, these helminth-conditioned DC induce a Th2 or Treg lymphocyte responses.

study, LPS and SEA-stimulated DC have similar phagosome acidification and proteolytic activity and this effect is MyD88 independent disregarding the activity by most TLRs [143]. Thus DC exposed to helminth products could exhibit an immature phenotype along with some features of a mature phenotype (Figure 2).

As mentioned earlier in this review, ligation of some CLRs might lead to the impairment of TLR-mediated signalling [131]. For instance, ligation of DC-SIGN on the DC can preferentially induce IL-10 production upon LPS addition [144] and the interaction of this receptor with pathogens such as *Mycobacterium sp*., HIV-1 and *Candida albicans*, affects TLR-4-mediated immune responses by the same cells [145, 146]. Interestingly, DC-SIGN binds SEA and this antigen extract and other glycoconjugates derived from *S. mansoni*, can alter DC responses after TLR-stimulation [18].

Other lectin receptors might also posses the ability to interfere with TLR-induced response in DC. Recently, it has been shown that sialylated structures from *T.* cruzi bind the inhibitory lectin, Siglec-E. Of special interest, is that this interaction likely account for the suppression of LPS-induced IL-12 production and the enhanced levels of IL-10 observed in DC exposed to *T. cruzi* parasites [17]. Supporting this idea, other studies have shown a downregulation of immune cells responses after ligation of Siglecs [147, 148].

Signalling pathways involving activation of the kinases ERK and PI3K have been implicated in the negative regulation of DC maturation and IL-12 synthesis as well as in the enhanced production of IL-10. It is believed that pathogens use these signals to preferentially evoke Th2-type immune responses or at least impaired their opposing party [141, 144, 149–151]. In this regard, it is important to note, that among protozoan and helminth parasites, activation of ERK and PIK3 seem to play indeed, a main role in the interference of DC function. Different examples of ERK activation implicated in impaired DC maturation, downregulation of IL-12 production or polarization of the immune response to Th2 have been reported for both types of parasites [26, 49, 115, 128], whereas the protozoos *G. lamblia* and *L. major* can also inhibit IL-12 synthesis in a PI3K-dependent pathway [94, 151]. Interestingly, pathways ERK and PI3K-dependent can be activated by engagement of CLRs [144, 152],

We believe that interaction of protozoan and helminth molecules with CLRs and probably other lectin receptors, along with the consequent activation of ERK and PI3K-dependent pathways, are key events that might determine the way a DC respond, either to direct parasite stimuli or to heterologous TLR ligands. This mechanism may explain, the induced immature phenotypes observed in DC exposed to these parasites and their products as well as the decreased proinflammatory cytokine production (IL-12 mainly). The enhanced IL-10 synthesis and Treg or Th2 polarization activity of DC exposed to protozoan and helminthes antigens, may also be a consequence of such interactions. Therefore, even when more studies are necessary to determine the exact mechanisms involved in downregulation of DC function by parasites, after all, there is maybe a common pathway that helminth and protozoan parasites use to achieve it (Figures 1 and 2).

5. Concluding Remarks

During evolution, parasites have developed several sophisticated strategies in order to avoid or deal with the attacks of immune system. These studies have shown that interfering with the activity of DC can be one of the most effective ways to induce a safer environment to parasite development. Interestingly, despite the phylogenic distance between protozoa and helminthes and even among helminthes, there seems to be a remarkable similarity in the way all these parasites modulate DC responses. Interaction with different receptors, particularly CLRs, may induce the downregulation in DC function although several other molecules like, Scavenger, lectins or G-coupled signaling receptors could also be involved. Understanding the different mechanisms that these parasites use to interfere with DC will certainly answer important questions that will increase our understanding of these pleiotropic cells and help us to design targeted therapeutic strategies based on this knowledge.

Acknowledgments

The authors would like to thank Dr. Jose Ignacio Saldaña (Imperial College London) for his helpful comments to this article. CONACYT-Mexico supported PhD fellowship for the first author and this review is a requirement to obtain his degree in Biomedical Sciences, UNAM. This work was supported by grant 60956-M from CONACYT-México.

References

[1] A. Lanzavecchia and F. Sallusto, "The instructive role of dendritic cells on T cell responses: lineages, plasticity and kinetics," *Current Opinion in Immunology*, vol. 13, no. 3, pp. 291–298, 2001.

[2] J. Banchereau and R. M. Steinman, "Dendritic cells and the control of immunity," *Nature*, vol. 392, no. 6673, pp. 245–252, 1998.

[3] J. Banchereau, F. Briere, C. Caux, et al., "Immunobiology of dendritic cells," *Annual Review of Immunology*, vol. 18, pp. 767–811, 2000.

[4] P. Guermonprez, J. Valladeau, L. Zitvogel, C. Thery, and S. Amigorena, "Antigen presentation and T cell stimulation by dendritic cells," *Annual Review of Immunology*, vol. 20, pp. 621–667, 2002.

[5] A. Lanzavecchia and F. Sallusto, "Dynamics of T lymphocyte responses: intermediates, effectors, and memory cells," *Science*, vol. 290, no. 5489, pp. 92–97, 2000.

[6] M. N. Wykes, X. Q. Liu, L. Beattie, et al., "Plasmodium strain determines dendritic cell function essential for survival from malaria," *PLoS Pathogens*, vol. 3, no. 7, article e96, 2007.

[7] L. Xin, Y. Li, and L. Soong, "Role of interleukin-1β in activating the CD11chigh CD45RB- dendritic cell subset and priming Leishmania amazonensis- specific $CD4^+$ T cells in vitro and in vivo," *Infection and Immunity*, vol. 75, no. 10, pp. 5018–5026, 2007.

[8] M. Pepper, F. Dzierszinski, E. Wilson, et al., "Plasmacytoid dendritic cells are activated by Toxoplasma gondii to present antigen and produce cytokines," *Journal of Immunology*, vol. 180, no. 9, pp. 6229–6236, 2008.

[9] S. R. Silva, J. F. Jacysyn, M. S. Macedo, and E. L. Faquin-Mauro, "Immunosuppressive components of Ascaris suum down-regulate expression of costimulatory molecules and function of antigen-presenting cells via an IL-10-mediated mechanism," *European Journal of Immunology*, vol. 36, no. 12, pp. 3227–3237, 2006.

[10] R. T. Semnani, H. Sabzevari, R. Iyer, and T. B. Nutman, "Filarial antigens impair the function of human dendritic cells during differentiation," *Infection and Immunity*, vol. 69, no. 9, pp. 5813–5822, 2001.

[11] D. van der Kleij, E. Latz, J. F. Brouwers, et al., "A novel host-parasite lipid cross-talk. Schistosomal lyso-phosphatidylserine activates Toll-like receptor 2 and affects immune polarization," *Journal of Biological Chemistry*, vol. 277, no. 50, pp. 48122–48129, 2002.

[12] K. Jangpatarapongsa, P. Chootong, J. Sattabongkot, et al., "Plasmodium vivax parasites alter the balance of myeloid and plasmacytoid dendritic cells and the induction of regulatory T cells," *European Journal of Immunology*, vol. 38, no. 10, pp. 2697–2705, 2008.

[13] A.-M. Sponaas, E. T. Cadman, C. Voisine, et al., "Malaria infection changes the ability of splenic dendritic cell populations to stimulate antigen-specific T cells," *Journal of Experimental Medicine*, vol. 203, no. 6, pp. 1427–1433, 2006.

[14] R. Rigano, B. Buttari, E. Profumo, et al., "Echinococcus granulosus antigen B impairs human dendritic cell differentiation and polarizes immature dendritic cell maturation towards a Th2 cell response," *Infection and Immunity*, vol. 75, no. 4, pp. 1667–1678, 2007.

[15] P. G. Thomas, M. R. Carter, O. Atochina, et al., "Maturation of dendritic cell 2 phenotype by a helminth glycan uses a Toll-like receptor 4-dependent mechanism," *Journal of Immunology*, vol. 171, no. 11, pp. 5837–5841, 2003.

[16] F. S. Machado, J. E. Johndrow, L. Esper, et al., "Anti-inflammatory actions of lipoxin A4 and aspirin-triggered lipoxin are SOCS-2 dependent," *Nature Medicine*, vol. 12, no. 3, pp. 330–334, 2006.

[17] H. Erdmann, C. Steeg, F. Koch-Nolte, B. Fleischer, and T. Jacobs, "Sialylated ligands on pathogenic Trypanosoma cruzi interact with Siglec-E (sialic acid-binding Ig-like lectin-E)," *Cellular Microbiology*, vol. 11, no. 11, pp. 1600–1611, 2009.

[18] E. van Liempt, S. J. van Vliet, A. Engering, et al., "Schistosoma mansoni soluble egg antigens are internalized by human dendritic cells through multiple C-type lectins and suppress TLR-induced dendritic cell activation," *Molecular Immunology*, vol. 44, no. 10, pp. 2605–2615, 2007.

[19] O. R. Millington, C. Di Lorenzo, R. S. Phillips, P. Garside, and J. M. Brewer, "Suppression of adaptive immunity to heterologous antigens during Plasmodium infection through hemozoin-induced failure of dendritic cell function," *Journal of Biology*, vol. 5, article 5, 2006.

[20] B. C. Urban, D. J. P. Ferguson, A. Pain, et al., "Plasmodium falciparuminfected erythrocytes modulate the maturation of dendritic cells," *Nature*, vol. 400, no. 6739, pp. 73–77, 1999.

[21] J. A. Perry, C. S. Olver, R. C. Burnett, and A. C. Avery, "Cutting edge: the acquisition of TLR tolerance during malaria infection impacts T cell activation," *Journal of Immunology*, vol. 174, no. 10, pp. 5921–5925, 2005.

[22] M. N. Wykes, X. Q. Liu, S. Jiang, C. Hirunpetcharat, and M. F. Good, "Systemic tumor necrosis factor generated during lethal Plasmodium infections impairs dendritic cell function," *Journal of Immunology*, vol. 179, no. 6, pp. 3982–3987, 2007.

[23] C. Ocana-Morgner, K. A. Wong, and A. Rodriguez, "Interactions between dendritic cells and $CD4^+$ T cells during Plasmodium infection," *Malaria Journal*, vol. 7, article 88, 2008.

[24] C. Ocana-Morgner, M. M. Mota, and A. Rodriguez, "Malaria blood stage suppression of liver stage immunity by dendritic cells," *Journal of Experimental Medicine*, vol. 197, no. 2, pp. 143–151, 2003.

[25] J. Langhorne, S. J. Quin, and L. A. Sanni, "Mouse models of blood-stage malaria infections: immune responses and cytokines involved in protection and pathology," *Chemical Immunology*, vol. 80, pp. 204–228, 2002.

[26] P. Mukherjee and V. S. Chauhan, "Plasmodium falciparum-free merozoites and infected RBCs distinctly affect soluble CD40 ligand-mediated maturation of immature monocyte-derived dendritic cells," *Journal of Leukocyte Biology*, vol. 84, no. 1, pp. 244–254, 2008.

[27] J. M. Orengo, K. A. Wong, C. Ocana-Morgner, and A. Rodriguez, "A Plasmodium yoelii soluble factor inhibits the phenotypic maturation of dendritic cells," *Malaria Journal*, vol. 7, article 254, 2008.

[28] O. A. Skorokhod, M. Alessio, B. Mordmuller, P. Arese, and E. Schwarzer, "Hemozoin (malarial pigment) inhibits differentiation and maturation of human monocyte-derived dendritic cells: a peroxisome proliferator-activated receptor-γ-mediated effect," *Journal of Immunology*, vol. 173, no. 6, pp. 4066–4074, 2004.

[29] O. Skorokhod, E. Schwarzer, T. Grune, and P. Arese, "Role of 4-hydroxynonenal in the hemozoin-mediated inhibition of differentiation of human monocytes to dendritic cells induced by GM-CSF/IL-4," *BioFactors*, vol. 24, no. 1–4, pp. 283–289, 2005.

[30] H. Hisaeda, K. Tetsutani, T. Imai, et al., "Malaria parasites require TLR9 signaling for immune evasion by activating regulatory T cells," *Journal of Immunology*, vol. 180, no. 4, pp. 2496–2503, 2008.

[31] K. A. Wong and A. Rodriguez, "Plasmodium infection and endotoxic shock induce the expansion of regulatory dendritic cells," *Journal of Immunology*, vol. 180, no. 2, pp. 716–726, 2008.

[32] P. C. Bull and K. Marsh, "The role of antibodies to Plasmodium falciparum-infected-erythrocyte surface antigens in naturally acquired immunity to malaria," *Trends in Microbiology*, vol. 10, no. 2, pp. 55–58, 2002.

[33] D. I. Baruch, J. A. Gormley, C. Ma, R. J. Howard, and B. L. Pasloske, "Plasmodium falciparum erythrocyte membrane protein 1 is a parasitized erythrocyte receptor for adherence to CD36, thrombospondin, and intercellular adhesion molecule 1," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 93, no. 8, pp. 3497–3502, 1996.

[34] M. Fried and P. E. Duffy, "Adherence of Plasmodium falciparum to chondroitin sulfate A in the human placenta," *Science*, vol. 272, no. 5267, pp. 1502–1504, 1996.

[35] S. R. Elliott, T. P. Spurck, J. M. Dodin, et al., "Inhibition of dendritic cell maturation by malaria is dose dependent and does not require Plasmodium falciparum erythrocyte membrane protein 1," *Infection and Immunity*, vol. 75, no. 7, pp. 3621–3632, 2007.

[36] C. Coban, K. J. Ishii, T. Kawai, et al., "Toll-like receptor 9 mediates innate immune activation by the malaria pigment

hemozoin," *Journal of Experimental Medicine*, vol. 201, no. 1, pp. 19–25, 2005.

[37] S. Pichyangkul, K. Yongvanitchit, U. Kum-arb, et al., "Malaria blood stage parasites activate human plasmacytoid dendritic cells and murine dendritic cells through a Toll-like receptor 9-dependent pathway," *Journal of Immunology*, vol. 172, no. 8, pp. 4926–4933, 2004.

[38] J. Zhu, G. Krishnegowda, and D. C. Gowda, "Induction of proinflammatory responses in macrophages by the glycosylphosphatidylinositols of Plasmodium falciparum: the requirement of extracellular signal-regulated kinase, p38, c-Jun N-terminal kinase and NF-κB pathways for the expression of proinflammatory cytokines and nitric oxide," *Journal of Biological Chemistry*, vol. 280, no. 9, pp. 8617–8627, 2005.

[39] P. Parroche, F. N. Lauw, N. Goutagny, et al., "Malaria hemozoin is immunologically inert but radically enhances innate responses by presenting malaria DNA to Toll-like receptor 9," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 6, pp. 1919–1924, 2007.

[40] S. L. Reiner and R. M. Locksley, "The regulation of immunity to Leishmania major," *Annual Review of Immunology*, vol. 13, pp. 151–177, 1995.

[41] F. Mattner, J. Magram, J. Ferrante, et al., "Genetically resistant mice lacking interleukin-12 are susceptible to infection with Leishmania major and mount a polarized Th2 cell response," *European Journal of Immunology*, vol. 26, no. 7, pp. 1553–1559, 1996.

[42] H. Chakir, A. Campos-Neto, M. Mojibian, and J. R. Webb, "IL-12Rβ2-deficient mice of a genetically resistant background are susceptible to Leishmania major infection and develop a parasite-specific Th2 immune response," *Microbes and Infection*, vol. 5, no. 4, pp. 241–249, 2003.

[43] K. Suzue, S. Kobayashi, T. Takeuchi, M. Suzuki, and S. Koyasu, "Critical role of dendritic cells in determining the Th 1/Th2 balance upon Leishmania major infection," *International Immunology*, vol. 20, no. 3, pp. 337–343, 2008.

[44] E. von Stebut, Y. Belkaid, B. V. Nguyen, M. Cushing, D. L. Sacks, and M. C. Udey, "Leishmania major-infected murine Langerhans cell-like dendritic cells from susceptible mice release IL-12 after infection and vaccinate against experimental cutaneous Leishmaniasis," *European Journal of Immunology*, vol. 30, no. 12, pp. 3498–3506, 2000.

[45] H. Qi, V. Popov, and L. Soong, "Leishmania amazonensis-dendritic cell interactions in vitro and the priming of parasite-specific $CD4^+$ T cells in vivo," *Journal of Immunology*, vol. 167, no. 8, pp. 4534–4542, 2001.

[46] P. Konecny, A. J. Stagg, H. Jebbari, N. English, R. N. Davidson, and S. C. Knight, "Murine dendritic cells internalize Leishmania major promastigotes, produce IL-12 p40 and stimulate primary T cell proliferation in vitro," *European Journal of Immunology*, vol. 29, no. 6, pp. 1803–1811, 1999.

[47] E. von Stebut, Y. Belkaid, T. Jakob, D. L. Sacks, and M. C. Udey, "Uptake of Leishmania major amastigotes results in activation and interleukin 12 release from murine skin-derived dendritic cells: implications for the initiation of anti-Leishmania immunity," *Journal of Experimental Medicine*, vol. 188, no. 8, pp. 1547–1552, 1998.

[48] C. Favali, N. Tavares, J. Clarencio, A. Barral, M. Barral-Netto, and C. Brodskyn, "Leishmania amazonensis infection impairs differentiation and function of human dendritic cells," *Journal of Leukocyte Biology*, vol. 82, no. 6, pp. 1401–1406, 2007.

[49] P. M. Boggiatto, F. Jie, M. Ghosh, et al., "Altered dendritic cell phenotype in response to Leishmania amazonensis amastigote infection is mediated by MAP kinase, ERK," *American Journal of Pathology*, vol. 174, no. 5, pp. 1818–1826, 2009.

[50] C. L. Bennett, A. Misslitz, L. Colledge, T. Aebischer, and C. C. Blackburn, "Silent infection of bone marrow-derived dendritic cells by Leishmania mexicana amastigotes," *European Journal of Immunology*, vol. 31, no. 3, pp. 876–883, 2001.

[51] J. Argueta-Donohue, N. Carrillo, L. Valdes-Reyes, et al., "Leishmania mexicana: participation of NF-κB in the differential production of IL-12 in dendritic cells and monocytes induced by lipophosphoglycan (LPG)," *Experimental Parasitology*, vol. 120, no. 1, pp. 1–9, 2008.

[52] K. Tejle, M. Lindroth, K.-E. Magnusson, and B. Rasmusson, "Wild-type Leishmania donovani promastigotes block maturation, increase integrin expression and inhibit detachment of human monocyte-derived dendritic cells—the influence of phosphoglycans," *FEMS Microbiology Letters*, vol. 279, no. 1, pp. 92–102, 2008.

[53] M. Ato, S. Stager, C. R. Engwerda, and P. M. Kaye, "Defective CCR7 expression on dendritic cells contributes to the development of visceral leishmaniasis," *Nature Immunology*, vol. 3, no. 12, pp. 1185–1191, 2002.

[54] H. Jebbari, A. J. Stagg, R. N. Davidson, and S. C. Knight, "Leishmania major promastigotes inhibit dendritic cell motility in vitro," *Infection and Immunity*, vol. 70, no. 2, pp. 1023–1026, 2002.

[55] A. Ponte-Sucre, D. Heise, and H. Moll, "Leishmania major lipophosphoglycan modulates the phenotype and inhibits migration of murine Langerhans cells," *Immunology*, vol. 104, no. 4, pp. 462–467, 2001.

[56] D. Liu, C. Kebaier, N. Pakpour, et al., "Leishmania major phosphoglycans influence the host early immune response by modulating dendritic cell functions," *Infection and Immunity*, vol. 77, no. 8, pp. 3272–3283, 2009.

[57] M. Revest, L. Donaghy, F. Cabillic, C. Guiguen, and J.-P. Gangneux, "Comparison of the immunomodulatory effects of L. donovani and L. major excreted-secreted antigens, particulate and soluble extracts and viable parasites on human dendritic cells," *Vaccine*, vol. 26, no. 48, pp. 6119–6123, 2008.

[58] C. Wiethe, A. Debus, M. Mohrs, A. Steinkasserer, M. Lutz, and A. Gessner, "Dendritic cell differentiation state and their interaction with NKT cells determine Th1/Th2 differentiation in the murine model of Leishmania major infection," *Journal of Immunology*, vol. 180, no. 7, pp. 4371–4381, 2008.

[59] J. Liese, U. Schleicher, and C. Bogdan, "TLR9 signaling is essential for the innate NK cell response in murine cutaneous leishmaniasis," *European Journal of Immunology*, vol. 37, no. 12, pp. 3424–3434, 2007.

[60] U. Schleicher, J. Liese, I. Knippertz, et al., "NK cell activation in visceral leishmaniasis requires TLR9, myeloid DCs, and IL-12, but is independent of plasmacytoid DCs," *Journal of Experimental Medicine*, vol. 204, no. 4, pp. 893–906, 2007.

[61] C. De Trez, M. Brait, O. Leo, et al., "Myd88-dependent in vivo maturation of splenic dendritic cells induced by Leishmania donovani and other Leishmania species," *Infection and Immunity*, vol. 72, no. 2, pp. 824–832, 2004.

[62] I. Becker, N. Salaiza, M. Aguirre, et al., "Leishmania lipophosphoglycan (LPG) activates NK cells through Toll-like receptor-2," *Molecular and Biochemical Parasitology*, vol. 130, no. 2, pp. 65–74, 2003.

[63] J.-F. Flandin, F. Chano, and A. Descoteaux, "RNA interference reveals a role for TLR2 and TLR3 in the recognition of

Leishmania donovani promastigotes by interferon-γ-primed macrophages," *European Journal of Immunology*, vol. 36, no. 2, pp. 411–420, 2006.

[64] M. J. de Veer, J. M. Curtis, T. M. Baldwin, et al., "MyD88 is essential for clearance of Leishmania major: possible role for lipophosphoglycan and Toll-like receptor 2 signaling," *European Journal of Immunology*, vol. 33, no. 10, pp. 2822–2831, 2003.

[65] L. P. Carvalho, E. J. Pearce, and P. Scott, "Functional dichotomy of dendritic cells following interaction with Leishmania braziliensis: infected cells produce high levels of TNF-α, whereas bystander dendritic cells are activated to promote T cell responses," *Journal of Immunology*, vol. 181, no. 9, pp. 6473–6480, 2008.

[66] L. Van Overtvelt, N. Vanderheyde, V. Verhasselt, et al., "Trypanosoma cruzi infects human dendritic cells and prevents their maturation: inhibition of cytokines, HLA-DR, and costimulatory molecules," *Infection and Immunity*, vol. 67, no. 8, pp. 4033–4040, 1999.

[67] C. Brodskyn, J. Patricio, R. Oliveira, et al., "Glycoinositolphospholipids from Trypanosoma cruzi interfere with macrophages and dendritic cell responses," *Infection and Immunity*, vol. 70, no. 7, pp. 3736–3743, 2002.

[68] C. V. Poncini, C. D. Alba Soto, E. Batalla, M. E. Solana, and S. M. Gonzalez Cappa, "Trypanosoma cruzi induces regulatory dendritic cells in vitro," *Infection and Immunity*, vol. 76, no. 6, pp. 2633–2641, 2008.

[69] D. Chaussabel, B. Pajak, V. Vercruysse, et al., "Alteration of migration and maturation of dendritic cells and T-cell depletion in the course of experimental Trypanosoma cruzi infection," *Laboratory Investigation*, vol. 83, no. 9, pp. 1373–1382, 2003.

[70] C. D. Alba Soto, G. A. Mirkin, M. E. Solana, and S. M. Gonzalez Cappa, "Trypanosoma cruzi infection modulates in vivo expression of major histocompatibility complex class II molecules on antigen-presenting cells and T-cell stimulatory activity of dendritic cells in a strain-dependent manner," *Infection and Immunity*, vol. 71, no. 3, pp. 1194–1199, 2003.

[71] L. Planelles, M. C. Thomas, C. Maranon, M. Morell, and M. C. Lopez, "Differential CD86 and CD40 co-stimulatory molecules and cytokine expression pattern induced by Trypanosoma cruzi in APCs from resistant or susceptible mice," *Clinical and Experimental Immunology*, vol. 131, no. 1, pp. 41–47, 2003.

[72] R. Koga, S. Hamano, H. Kuwata, et al., "TLR-dependent induction of IFN-β mediates host defense against Trypanosoma cruzi," *Journal of Immunology*, vol. 177, no. 10, pp. 7059–7066, 2006.

[73] M. A. S. Campos, I. C. Almeida, O. Takeuchi, et al., "Activation of Toll-like receptor-2 by glycosylphosphatidylinositol anchors from a protozoan parasite," *Journal of Immunology*, vol. 167, no. 1, pp. 416–423, 2001.

[74] A. Bafica, H. C. Santiago, R. Goldszmid, C. Ropert, R. T. Gazzinelli, and A. Sher, "Cutting edge: TLR9 and TLR2 signaling together account for MyD88-dependent control of parasitemia in Trypanosoma cruzi infection," *Journal of Immunology*, vol. 177, no. 6, pp. 3515–3519, 2006.

[75] A. Ouaissi, E. Guilvard, Y. Delneste, et al., "The Trypanosoma cruzi Tc52-released protein induces human dendritic cell maturation, signals via Toll-like receptor 2, and confers protection against lethal infection," *Journal of Immunology*, vol. 168, no. 12, pp. 6366–6374, 2002.

[76] A. C. Monteiro, V. Schmitz, A. Morrot, et al., "Bradykinin B2 Receptors of dendritic cells, acting as sensors of kinins proteolytically released by Trypanosoma cruzi, are critical for the development of protective type-1 responses," *PLoS Pathogens*, vol. 3, no. 11, article e185, 2007.

[77] C. Reis e Sousa, S. Hieny, T. Scharton-Kersten, et al., "In vivo microbial stimulation induces rapid CD40 ligand-independent production of interleukin 12 by dendritic cells and their redistribution to T cell areas," *Journal of Experimental Medicine*, vol. 186, no. 11, pp. 1819–1829, 1997.

[78] O. Schulz, A. D. Edwards, M. Schito, et al., "CD40 triggering of heterodimeric IL-12 p70 production by dendritic cells in vivo requires a microbial priming signal," *Immunity*, vol. 13, no. 4, pp. 453–462, 2000.

[79] J. Aliberti, D. Jankovic, and A. Sher, "Turning it on and off: regulation of dendritic cell function in Toxoplasma gondii infection," *Immunological Reviews*, vol. 201, pp. 26–34, 2004.

[80] F. Yarovinsky, D. Zhang, J. F. Andersen, et al., "Immunology: TLR11 activation of dendritic cells by a protozoan profilin-like protein," *Science*, vol. 308, no. 5728, pp. 1626–1629, 2005.

[81] C. M. Miller, N. R. Boulter, R. J. Ikin, and N. C. Smith, "The immunobiology of the innate response to Toxoplasma gondii," *International Journal for Parasitology*, vol. 39, no. 1, pp. 23–39, 2009.

[82] F. Yarovinsky, "Toll-like receptors and their role in host resistance to Toxoplasma gondii," *Immunology Letters*, vol. 119, no. 1-2, pp. 17–21, 2008.

[83] J. Aliberti, J. G. Valenzuela, V. B. Carruthers, et al., "Molecular mimicry of a CCR5 binding-domain in the microbial activation of dendritic cells," *Nature Immunology*, vol. 4, no. 5, pp. 485–490, 2003.

[84] C. Reis e Sousa, G. Yap, O. Schulz, et al., "Paralysis of dendritic cell IL-12 production by microbial products prevents infection-induced immunopathology," *Immunity*, vol. 11, no. 5, pp. 637–647, 1999.

[85] J. Aliberti and A. Sher, "Role of G-protein-coupled signaling in the induction and regulation of dendritic cell function by Toxoplasma gondii," *Microbes and Infection*, vol. 4, no. 9, pp. 991–997, 2002.

[86] S. Suchitra, K. A. Anbu, D. K. Rathore, M. Mahawar, B. P. Singh, and P. Joshi, "Haemonchus contortus calreticulin binds to C-reactive protein of its host, a novel survival strategy of the parasite," *Parasite Immunology*, vol. 30, no. 6-7, pp. 371–374, 2008.

[87] A. S. McKee, F. Dzierszinski, M. Boes, D. S. Roos, and E. J. Pearce, "Functional inactivation of immature dendritic cells by the intracellular parasite Toxoplasma gondii," *Journal of Immunology*, vol. 173, no. 4, pp. 2632–2640, 2004.

[88] A. L. Bierly, W. J. Shufesky, W. Sukhumavasi, A. E. Morelli, and E. Y. Denkers, "Dendritic cells expressing plasmacytoid marker PDCA-1 are Trojan horses during Toxoplasma gondii infection," *Journal of Immunology*, vol. 181, no. 12, pp. 8485–8491, 2008.

[89] J. Diana, F. Persat, M.-J. Staquet, et al., "Migration and maturation of human dendritic cells infected with Toxoplasma gondii depend on parasite strain type," *FEMS Immunology and Medical Microbiology*, vol. 42, no. 3, pp. 321–331, 2004.

[90] N. Courret, S. Darche, P. Sonigo, G. Milon, D. Buzoni-Gatel, and I. Tardieux, "CD11c- and CD11b-expressing mouse leukocytes transport single Toxoplasma gondii tachyzoites to the brain," *Blood*, vol. 107, no. 1, pp. 309–316, 2006.

[91] C. M. Persson, H. Lambert, P. P. Vutova, et al., "Transmission of Toxoplasma gondii from infected dendritic cells to natural killer cells," *Infection and Immunity*, vol. 77, no. 3, pp. 970–976, 2009.

[92] H. Lambert, N. Hitziger, I. Dellacasa, M. Svensson, and A. Barragan, "Induction of dendritic cell migration upon Toxoplasma gondii infection potentiates parasite dissemination," *Cellular Microbiology*, vol. 8, no. 10, pp. 1611–1623, 2006.

[93] J. Diana, C. Vincent, F. Peyron, S. Picot, D. Schmitt, and F. Persat, "Toxoplasma gondii regulates recruitment and migration of human dendritic cells via different soluble secreted factors," *Clinical and Experimental Immunology*, vol. 141, no. 3, pp. 475–484, 2005.

[94] J. D. Kamda and S. M. Singer, "Phosphoinositide 3-kinase-dependent inhibition of dendritic cell interleukin-12 production by Giardia lamblia," *Infection and Immunity*, vol. 77, no. 2, pp. 685–693, 2009.

[95] C. Maldonado-Bernal, C. J. Kirschning, Y. Rosenstein, et al., "The innate immune response to Entamoeba histolytica lipopeptidophosphoglycan is mediated by Toll-like receptors 2 and 4," *Parasite Immunology*, vol. 27, no. 4, pp. 127–137, 2005.

[96] C. P. A. Ivory, M. Prystajecky, C. Jobin, and K. Chadee, "Toll-like receptor 9-dependent macrophage activation by Entamoeba histolytica DNA," *Infection and Immunity*, vol. 76, no. 1, pp. 289–297, 2008.

[97] H. Vivanco-Cid, C. Alpuche-Aranda, I. Wong-Baeza, et al., "Lipopopeptidephosphoglycan from Entamoeba histolytica activates human macrophages and dendritic cells and reaches their late endosomes," *Parasite Immunology*, vol. 29, no. 9, pp. 467–474, 2007.

[98] C. P. A. Ivory and K. Chadee, "Activation of dendritic cells by the Gal-lectin of Entamoeba histolytica drives Th1 responses in vitro and in vivo," *European Journal of Immunology*, vol. 37, no. 2, pp. 385–394, 2007.

[99] K. A. Rogers, A. B. Rogers, B. A. Leav, et al., "MyD88-dependent pathways mediate resistance to Cryptosporidium parvum infection in mice," *Infection and Immunity*, vol. 74, no. 1, pp. 549–556, 2006.

[100] X.-M. Chen, S. P. O'Hara, J. B. Nelson, et al., "Multiple TLRs are expressed in human cholangiocytes and mediate host epithelial defense responses to Cryptosporidium parvum via activation of NF-κB," *Journal of Immunology*, vol. 175, no. 11, pp. 7447–7456, 2005.

[101] J.-H. Chang, J.-Y. Park, and S.-K. Kim, "Dependence on p38 MAPK signalling in the up-regulation of TLR2, TLR4 and TLR9 gene expression in Trichomonas vaginalis-treated HeLa cells," *Immunology*, vol. 118, no. 2, pp. 164–170, 2006.

[102] M. R. Zariffard, S. Harwani, R. M. Novak, P. J. Graham, X. Ji, and G. T. Spear, "Trichomonas vaginalis infection activates cells through Toll-like receptor 4," *Clinical Immunology*, vol. 111, no. 1, pp. 103–107, 2004.

[103] R. T. Semnani, P. G. Venugopal, C. A. Leifer, S. Mostbock, H. Sabzevari, and T. B. Nutman, "Inhibition of TLR3 and TLR4 function and expression in human dendritic cells by helminth parasites," *Blood*, vol. 112, no. 4, pp. 1290–1298, 2008.

[104] A. Yoshimura, T. Naka, and M. Kubo, "SOCS proteins, cytokine signalling and immune regulation," *Nature Reviews Immunology*, vol. 7, no. 6, pp. 454–465, 2007.

[105] A. Balic, Y. Harcus, M. J. Holland, and R. M. Maizels, "Selective maturation of dendritic cells by Nippostrongylus brasiliensis-secreted proteins drives Th2 immune responses," *European Journal of Immunology*, vol. 34, no. 11, pp. 3047–3059, 2004.

[106] B. W. McConchie, H. H. Norris, V. G. Bundoc, et al., "Ascaris suum-derived products suppress mucosal allergic inflammation in an interleukin-10-independent manner via interference with dendritic cell function," *Infection and Immunity*, vol. 74, no. 12, pp. 6632–6641, 2006.

[107] D. E. Kean, I. Ohtsuka, K. Sato, et al., "Dissecting Ascaris glycosphingolipids for immunomodulatory moieties—the use of synthetic structural glycosphingolipid analogues," *Parasite Immunology*, vol. 28, no. 3, pp. 69–76, 2006.

[108] H. S. Goodridge, F. A. Marshall, K. J. Else, et al., "Immunomodulation via novel use of TLR4 by the filarial nematode phosphorylcholine-containing secreted product, ES-62," *Journal of Immunology*, vol. 174, no. 1, pp. 284–293, 2005.

[109] W. Harnett, M. R. Deehan, K. M. Houston, and M. M. Harnett, "Immunomodulatory properties of a phosphorylcholine-containing secreted filarial glycoprotein," *Parasite Immunology*, vol. 21, no. 12, pp. 601–608, 1999.

[110] M. Whelan, M. M. Harnett, K. M. Houston, V. Patel, W. Harnett, and K. P. Rigley, "A filarial nematode-secreted product signals dendritic cells to acquire a phenotype that drives development of Th2 cells," *Journal of Immunology*, vol. 164, no. 12, pp. 6453–6460, 2000.

[111] K. N. Couper, W. Chen, K. M. Houston, W. Harnett, and L. L. Johnson, "ES-62 is unable to modulate Toxoplasma gondii-driven Th1 responses and pathology," *Parasite Immunology*, vol. 27, no. 4, pp. 147–150, 2005.

[112] L. Al-Riyami, C. A. Egan, J. E. Bradley, S. Lustigman, and W. Harnett, "Failure of ES-62 to inhibit T-helper type 1 responses to other filarial nematode antigens," *Parasite Immunology*, vol. 30, no. 5, pp. 304–308, 2008.

[113] M. Segura, Z. Su, C. Piccirillo, and M. M. Stevenson, "Impairment of dendritic cell function by excretory-secretory products: a potential mechanism for nematode-induced immunosuppression," *European Journal of Immunology*, vol. 37, no. 7, pp. 1887–1904, 2007.

[114] C.-C. Chen, S. Louie, B. A. McCormick, W. A. Walker, and H. N. Shi, "Helminth-primed dendritic cells alter the host response to enteric bacterial infection," *Journal of Immunology*, vol. 176, no. 1, pp. 472–483, 2006.

[115] E. van Riet, B. Everts, K. Retra, et al., "Combined TLR2 and TLR4 ligation in the context of bacterial or helminth extracts in human monocyte derived dendritic cells: molecular correlates for Th1/Th2 polarization," *BMC Immunology*, vol. 10, article 9, 2009.

[116] J. Rzepecka, S. Rausch, C. Klotz, et al., "Calreticulin from the intestinal nematode Heligmosomoides polygyrus is a Th2-skewing protein and interacts with murine scavenger receptor-A," *Molecular Immunology*, vol. 46, no. 6, pp. 1109–1119, 2009.

[117] C. Cabezon, G. Cabrera, R. Paredes, A. Ferreira, and N. Galanti, "Echinococcus granulosus calreticulin: molecular characterization and hydatid cyst localization," *Molecular Immunology*, vol. 45, no. 5, pp. 1431–1438, 2008.

[118] F. Guillou, E. Roger, Y. Mone, et al., "Excretory-secretory proteome of larval Schistosoma mansoni and Echinostoma caproni, two parasites of Biomphalaria glabrata," *Molecular and Biochemical Parasitology*, vol. 155, no. 1, pp. 45–56, 2007.

[119] F. Mendlovic, J. Carrillo-Farga, J. Torres, J. P. Laclette, and A. Flisser, "Differential expression of calreticulin in developmental stages of Taenia solium," *Journal of Parasitology*, vol. 92, no. 4, pp. 789–795, 2006.

[120] J. A. Winter, O. R. Davies, A. P. Brown, M. C. Garnett, S. Stolnik, and D. Pritchard, "The assessment of hookworm calreticulin as a potential vaccine for necatoriasis," *Parasite Immunology*, vol. 27, no. 4, pp. 139–146, 2005.

[121] D. I. Pritchard, A. Brown, G. Kasper, et al., "A hookworm allergen which strongly resembles calreticulin," *Parasite Immunology*, vol. 21, no. 9, pp. 439–450, 1999.

[122] A. T. Vella, M. D. Hulsebosch, and E. J. Pearce, "Schistosoma mansoni eggs induce antigen-responsive CD44-hi T helper 2 cells and IL-4-secreting CD44-lo cells: potential for T helper 2 subset differentiation is evident at the precursor level," *Journal of Immunology*, vol. 149, no. 5, pp. 1714–1722, 1992.

[123] G. A. Cook, A. Metwali, A. Blum, R. Mathew, and J. V. Weinstock, "Lymphokine expression in granulomas of Schistosoma mansoni-infected mice," *Cellular Immunology*, vol. 152, no. 1, pp. 49–58, 1993.

[124] C. Faveeuw, T. Mallevaey, K. Paschinger, et al., "Schistosome N-glycans containing core α3-fucose and core β2-xylose epitopes are strong inducers of Th2 responses in mice," *European Journal of Immunology*, vol. 33, no. 5, pp. 1271–1281, 2003.

[125] C. M. Kane, L. Cervi, J. Sun, et al., "Helminth antigens modulate TLR-initiated dendritic cell activation," *Journal of Immunology*, vol. 173, no. 12, pp. 7454–7461, 2004.

[126] C. M. Kane, E. Jung, and E. J. Pearce, "Schistosoma mansoni egg antigen-mediated modulation of Toll-like receptor (TLR)-induced activation occurs independently of TLR2, TLR4, and MyD88," *Infection and Immunity*, vol. 76, no. 12, pp. 5754–5759, 2008.

[127] J. P. Hewitson, J. R. Grainger, and R. M. Maizels, "Helminth immunoregulation: the role of parasite secreted proteins in modulating host immunity," *Molecular and Biochemical Parasitology*, vol. 167, no. 1, pp. 1–11, 2009.

[128] P. G. Thomas, M. R. Carter, A. A. Da'Dara, T. M. DeSimone, and D. A. Harn, "A helminth glycan induces APC maturation via alternative NF-κB activation independent of IκBα degradation," *Journal of Immunology*, vol. 175, no. 4, pp. 2082–2090, 2005.

[129] S. P. Saunders, C. M. Walsh, J. L. Barlow, et al., "The C-type lectin SIGNR1 binds Schistosoma mansoni antigens in vitro, but SIGNR1-deficient mice have normal responses during schistosome infection," *Infection and Immunity*, vol. 77, no. 1, pp. 399–404, 2009.

[130] C. M. Krawczyk, J. Sun, and E. J. Pearce, "Th2 differentiation is unaffected by jagged2 expression on dendritic cells," *Journal of Immunology*, vol. 180, no. 12, pp. 7931–7937, 2008.

[131] T. B. H. Geijtenbeek and S. I. Gringhuis, "Signalling through C-type lectin receptors: shaping immune responses," *Nature Reviews Immunology*, vol. 9, no. 7, pp. 465–479, 2009.

[132] L. Gomez-Garcia, I. Rivera-Montoya, M. Rodriguez-Sosa, and L. I. Terrazas, "Carbohydrate components of Taenia crassiceps metacestodes display Th2-adjuvant and anti-inflammatory properties when co-injected with bystander antigen," *Parasitology Research*, vol. 99, no. 4, pp. 440–448, 2006.

[133] L. Gomez-Garcia, L. M. Lopez-Marin, R. Saavedra, J. L. Reyes, M. Rodriguez-Sosa, and L. I. Terrazas, "Intact glycans from cestode antigens are involved in innate activation of myeloid suppressor cells," *Parasite Immunology*, vol. 27, no. 10-11, pp. 395–405, 2005.

[134] J. L. Reyes, C. A. Terrazas, L. Vera-Arias, and L. I. Terrazas, "Differential response of antigen presenting cells from susceptible and resistant strains of mice to Taenia crassiceps infection," *Infection, Genetics and Evolution*, vol. 9, no. 6, pp. 1115–1127, 2009.

[135] E. Muraille, C. De Trez, M. Brait, P. De Baetselier, O. Leo, and Y. Carlier, "Genetically resistant mice lacking MyD88-adapter protein display a high susceptibility to Leishmania major infection associated with a polarized Th2 response," *Journal of Immunology*, vol. 170, no. 8, pp. 4237–4241, 2003.

[136] J. Aliberti, "Host persistence: exploitation of anti-inflammatory pathways by Toxoplasma gondii," *Nature Reviews Immunology*, vol. 5, no. 2, pp. 162–170, 2005.

[137] F. S. Machado, L. Esper, A. Dias, et al., "Native and aspirin-triggered lipoxins control innate immunity by inducing proteasomal degradation of TRAF6," *Journal of Experimental Medicine*, vol. 205, no. 5, pp. 1077–1086, 2008.

[138] A. Langenkamp, M. Messi, A. Lanzavecchia, and F. Sallusto, "Kinetics of dendritic cell activation: impact on priming of TH1,TH2 and nonpolarized T cells," *Nature Immunology*, vol. 1, no. 4, pp. 311–316, 2000.

[139] P. Kalinski, J. H. N. Schuitemaker, C. M. U. Hilkens, E. A. Wierenga, and M. L. Kapsenberg, "Final maturation of dendritic cells is associated with impaired responsiveness to IFN-γ and to bacterial IL-12 inducers: decreased ability of mature dendritic cells to produce IL-12 during the interaction with Th cells," *Journal of Immunology*, vol. 162, no. 6, pp. 3231–3236, 1999.

[140] A. S. MacDonald and R. M. Maizels, "Alarming dendritic cells for Th2 induction," *Journal of Experimental Medicine*, vol. 205, no. 1, pp. 13–17, 2008.

[141] L. Carvalho, J. Sun, C. Kane, F. Marshall, C. Krawczyk, and E. J. Pearce, "Review series on helminths, immune modulation and the hygiene hypothesis: mechanisms underlying helminth modulation of dendritic cell function," *Immunology*, vol. 126, no. 1, pp. 28–34, 2009.

[142] S. J. Jenkins, G. Perona-Wright, A. G. F. Worsley, N. Ishii, and A. S. MacDonald, "Dendritic cell expression of OX40 ligand acts as a costimulatory, not polarizing, signal for optimal Th2 priming and memory induction in vivo," *Journal of Immunology*, vol. 179, no. 6, pp. 3515–3523, 2007.

[143] F. A. Marshall and E. J. Pearce, "Uncoupling of induced protein processing from maturation in dendritic cells exposed to a highly antigenic preparation from a helminth parasite," *Journal of Immunology*, vol. 181, no. 11, pp. 7562–7570, 2008.

[144] E. Caparros, P. Munoz, E. Sierra-Filardi, et al., "DC-SIGN ligation on dendritic cells results in ERK and PI3K activation and modulates cytokine production," *Blood*, vol. 107, no. 10, pp. 3950–3958, 2006.

[145] S. I. Gringhuis, J. den Dunnen, M. Litjens, B. van het Hof, Y. van Kooyk, and T. H. Geijtenbeek, "C-type lectin DC-SIGN modulates Toll-like receptor signaling via Raf-1 kinase-dependent acetylation of transcription factor NF-κB," *Immunity*, vol. 26, no. 5, pp. 605–616, 2007.

[146] T. B. H. Geijtenbeek, S. J. Van Vliet, E. A. Koppel, et al., "Mycobacteria target DC-SIGN to suppress dendritic cell function," *Journal of Experimental Medicine*, vol. 197, no. 1, pp. 7–17, 2003.

[147] M. Ando, W. Tu, K.-I. Nishijima, and S. Iijima, "Siglec-9 enhances IL-10 production in macrophages via tyrosine-based motifs," *Biochemical and Biophysical Research Communications*, vol. 369, no. 3, pp. 878–883, 2008.

[148] A. F. Carlin, S. Uchiyama, Y.-C. Chang, A. L. Lewis, V. Nizet, and A. Varki, "Molecular mimicry of host sialylated glycans allows a bacterial pathogen to engage neutrophil Siglec-9 and dampen the innate immune response," *Blood*, vol. 113, no. 14, pp. 3333–3336, 2009.

[149] K. M. Ardeshna, A. R. Pizzey, S. Devereux, and A. Khwaja, "The PI3 kinase, p38 SAP kinase, and NF-κB signal transduction pathways are involved in the survival and maturation of lipopolysaccharide-stimulated human monocyte-derived dendritic cells," *Blood*, vol. 96, no. 3, pp. 1039–1046, 2000.

[150] A. Puig-Kroger, M. Relloso, O. Fernandez-Capetillo, et al., "Extracellular signal-regulated protein kinase signaling pathway negatively regulates the phenotypic and functional maturation of monocyte-derived human dendritic cells," *Blood*, vol. 98, no. 7, pp. 2175–2182, 2001.

[151] T. Fukao, M. Tanabe, Y. Terauchi, et al., "P13K-mediated negative feedback regulation of IL-12 production in DCs," *Nature Immunology*, vol. 3, no. 9, pp. 875–881, 2002.

[152] C.-H. Chen, H. Floyd, N. E. Olson, et al., "Dendritic-cell-associated C-type lectin 2 (DCAL-2) alters dendritic-cell maturation and cytokine production," *Blood*, vol. 107, no. 4, pp. 1459–1467, 2006.

[153] S. Suchitra, K. A. Anbu, D. K. Rathore, M. Mahawar, B. P. Singh, and P. Joshi, "Haemonchus contortus calreticulin binds to C-reactive protein of its host, a novel survival strategy of the parasite," *Parasite Immunology*, vol. 30, no. 6-7, pp. 371–374, 2008.

[154] T. B. H. Geijtenbeek and S. I. Gringhuis, "Signalling through C-type lectin receptors: shaping immune responses," *Nature Reviews Immunology*, vol. 9, no. 7, pp. 465–479, 2009.

Otros artículos publicados durante el doctorado

Oghumu S, Dong R, Varikuti S, Shawler T, Kampfrath T, Terrazas CA, Lezama-Davila C, Ahmer BM, Whitacre CC, Rajagopalan S, Locksley R, Sharpe AH, Satoskar AR. Distinct Populations of Innate CD8+ T Cells Revealed in a CXCR3 Reporter Mouse.J Immunol.2013 Jan 21. [Epub ahead of print] PubMed PMID: 23338236.

Pan L, Terrazas C, Lezama-Davila CM, Rege N, Gallucci JC, Satoskar AR, Kinghorn AD. Cordifolide A, a sulfur-containing clerodane diterpene glycoside from Tinospora cordifolia. Org Lett. 2012 Apr 20;14(8):2118-21. doi: 10.1021/ol300657h. Epub 2012 Apr 12. PubMed PMID: 22497272; PubMed Central PMCID:
PMC3348544.

López-Flores R, Bojalil R, Benítez JC, Ledesma-Soto Y, Terrazas CA, Rodríguez-Sosa M, Terrazas LI. Consecutive low doses of cyclosporine A induce pro-inflammatory cytokines and accelerate allograft skin rejection. Molecules. 2011 May 11;16(5):3969-84. doi: 10.3390/molecules16053969. PubMed PMID: 21562466.

Reyes JL, Terrazas CA, Alonso-Trujillo J, van Rooijen N, Satoskar AR, Terrazas LI. Early removal of alternatively activated macrophages leads to Taenia crassiceps cysticercosis clearance in vivo. Int J Parasitol. 2010 May;40(6):731-42. doi: 10.1016/j.ijpara.2009.11.014. Epub 2010 Jan 5. PubMed PMID: 20045000.

Terrazas CA, Huitron E, Vazquez A, Juarez I, Camacho GM, Calleja EA, Rodriguez-Sosa M. MIF synergizes with Trypanosoma cruzi antigens to promote efficient dendritic cell maturation and IL-12 production via p38 MAPK. Int J Biol Sci. 2011;7(9):1298-310. Epub 2011 Oct 25. PubMed PMID: 22110382; PubMed Central PMCID: PMC3221366.

Terrazas CA, Juarez I, Terrazas LI, Saavedra R, Calleja EA, Rodriguez-Sosa M. Toxoplasma gondii: impaired maturation and pro-inflammatory response of dendritic cells in MIF-deficient mice favors susceptibility to infection. Exp Parasitol. 2010 Nov;126(3):348-58. doi: 10.1016/j.exppara.2010.03.009. Epub 2010 Mar 21. PubMed PMID: 20331989.