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INMUNOREGULACIÓN POR EL CÉSTODO *Taenia crassiceps* Y SU EFECTO SOBRE EL DESARROLLO DE LA ENCEFALOMIELITIS AUTOINMUNE EXPERIMENTAL

TESIS

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ABREVIATURAS

AAMΦ´s (Alternatively Activated Macrophages)- Macrófagos alternativamente activados

Ag- Antígeno

APC (Antigen Presenting Cell)- Célula presentadora de Antígeno

CAMΦ´s (Clasically Activated Macrophages)- Macrófagos clásicamente activados

ConA- Concanavalina A

CD- Células dendriticas

EAE(Experimental Autoimmune Encephalomyelitis)-Encefalomielitis autoinmune experimental

ES-62- Glicoproteina excretada secretada de 62 KDa de peso de *Acantochilonema vitae*

i.p. - intraperitoneal

IFN- Interferon

Ig- Inmunoglobulina

IL- Interleucina

iNOS (Inducible Nitric Oxide Synthase)- Sintasa de oxido nítrico inducible

MOG 35-55- (Myelin Oligodendrocyte Glycoprotein) - Peptido de la glicoproteina de mielina de los oligodendrocitos

MΦ´s - Macrófagos

PD1 (Programmed Death receptor 1)- Receptor de muerte programada 1

PDL1- Ligando 1 del receptor PD1

PDL2- Ligando 2 del receptor PD1

PECs (Peritoneal exudate cells)- células totales de exudado peritoneal

PMN (Polimorfonuclear cells) - Células polimorfonucleares

RI- Respuesta Inmune

Relm α (Resistin-like molecule alfạ-Molécula similar a resistina ò Fizz-1

s.c. - subcutánea

SEA (Schistosoma Soluble Egg Antigen)- Antigeno soluble de huevo de Schistosoma

SNC- Sistema nervioso central

TcAg- Antigeno total soluble de *Taenia crassiceps*

Th1 (T helper 1) - Células T cooperadoras tipo 1

Th2 (T helper 2) - Células T cooperadoras tipo 2

TNF (Tumor Necrosis Factor) - Factor de necrósis tumoral

1. RESUMEN

Los helmintos parásitos causan millones de infecciones en todo el mundo, sobretodo en países en desarrollo. El exitoso estilo de vida de estos helmintos se debe a que han desarrollado variados y eficientes mecanismos de evasión sobre el sistema inmune de sus respectivos hospederos. La polarización hacia respuestas tipo Th2 y la generación de subpoblaciones reguladoras destacan como 2 de los principales mecanismos utilizados por los helmintos para poder sobrevivir dentro de su hospedero. El cestodo *Taenia crassiceps* se ha utilizado para tratar de comprender mejor las interacciones hospedero-parásito; al igual que otras infecciones por helmintos, tiene la capacidad de llevar la respuesta inmune hacia Th2 concomitantemente con la colonización de su hospedero. También la presencia de este parásito induce un aumento significativo de macrófagos alternativamente activados (AAMΦ`s) con capacidad supresora. En este trabajo intentamos definir la relevancia de estos macrófagos (MΦ`s) en la infección experimental por *T. crassiceps*. Primero, investigamos si la presencia de los AAMΦ`s podía asociarse con la susceptibilidad diferencial en 2 cepas de ratones. Se observó que a diferencia de los ratones de la cepa C57BL/6, la cual es altamente resistente a la infección por *T. crassiceps,* los ratones BALB/c presentan en la cavidad peritoneal una gran población de células adherentes con abundante expresión de los marcadores de AAMΦ`s (Relm α, Ym-1, Arginasa1, PDL1, PDL2), mientras que estos mismos marcadores no se sobrexpresaron en los ratones C57BL/6. Interesantemente, sólo los MΦ`s de la cepa susceptible conservaron la capacidad de suprimir la proliferación de células T *in vitro*, sugiriendo que el reclutamiento de esta población está asociado al establecimiento de *T. crassiceps.* Posteriormente, con el objetivo de demostrar la posible participación de los AAMΦ`s durante la cisticercosis experimental llevamos a cabo experimentos de eliminación de MΦ`s con clodronato encapsulado en liposomas. Se observó que la administración de liposomas disminuyó efectivamente a los AAMΦ`s (células $F480^{+}$ $MMR^{+}$) y que esto ocasionó una disminución significativa en el número de parásitos presentes en la cavidad peritoneal de estos ratones. Mas aún, la ausencia de estos MΦ`s durante las 3 primeras semanas fue suficiente para impedir que *T. crassiceps* se estableciera de manera normal en su hospedero, lo que se demostró por la baja carga parasitaria encontrada. Por el contrario, la eliminación de esta población después de la 5ª semana de infección no tuvo efecto significativo en el número de

parásitos, confirmando así que la aparición temprana de los AAMΦ`s es necesaria para que este cestodo se establezca. Se ha demostrado que todos estos fenómenos de inmuno-regulación en conjunto aumentan la susceptibilidad a infecciones subsecuentes por protozoarios intracelulares, en las que el estado de activación de los MΦ`s es determinante, ya sea para restringir o para permitir su crecimiento.

Finalmente, en este trabajo se demostró que *T. crassiceps* puede disminuir la gravedad de la Encefalomielitis Autoinmune Experimental (EAE). Este modelo es ampliamente estudiado en ratones C57BL/6, ya que son susceptibles a dicha enfermedad; sin embargo son considerados resistentes a la infección con *T. crassiceps*. Por lo tanto, utilizamos una dosis alta de *T. crassiceps* (40 metacestodos) para romper esta resistencia. Encontramos que con esta dosis los ratones C57BL/6 desarrollan una fuerte respuesta Th2 y, a diferencia de la infección con 20 metacestodos, se reclutan AAMΦ`s de manera abundante. Así, después de 8 semanas de infección, los ratones C57BL/6 se inmunizaron subcutáneamente con 300 μg del péptido MOG $_{35-55}$ para inducir EAE. Encontramos una gravedad casi nula de los signos de EAE, asociados a una baja infiltración celular en la médula espinal, un microambiente anti-inflamatorio en el cerebro y una respuesta mixta Th2/Th17. Por el contrario, los ratones no infectados presentaron parálisis total de ambas patas traseras, una clara infiltración celular en la médula espinal y una respuesta mixta de tipo Th1/Th17. En conclusión los AAMΦ`s son importantes para el establecimiento de *T. crassiceps,* ya que sólo las cepas susceptibles los desarrollan y la ausencia de éstos ocasiona un crecimiento disminuido del parásito. Esta población posiblemente afecta el desarrollo de reacciones autoinmunes/inflamatorias exacerbadas en el hospedero. La generación de AAMΦ`s durante la infección crónica con *T. crassiceps* podría ser el mecanismo a través del cual puede inhibirse el desarrollo de la EAE, ya que no se detectaron células T reguladoras. El estudio más detallado de los mecanismos que inducen a los AAMΦ`s, por ejemplo los antígenos secretados del parásito que los favorecen, podrá dar información útil para el desarrollo de tratamientos alternativos ó complementarios en enfermedades autoinmunes donde se presenta un alto grado de inflamación.

1.1 ABSTRACT

Helminth parasites cause millions of infections worldwide, overall in developing countries. This succesful life style is due to these organisms have developed a broad range of efficient mechanisms of immune evasion/regulation in their hosts. Thus, Th2 skewing ability and regulatory/suppresor cell populations recruitment are the main hallmarks of helminth infections. *Taenia crassiceps,* which is a cestode, has been very helpful to understand host-parasite interplay and similar to nematode and trematode parasites posses the ability to direct host´s immune response towards a Th2-type response concomitantly with parasite growth. Further, this parasite induces significant increase of alternatively activated macrophages (AAMΦ`s) with suppresive potential. In this study we attempted better understand the role of macrophages (MΦ`s) in *T. crassiceps* infection. First, we decided to seek an association between different mouse strain susceptibility and the presence of AAMΦ`s. We observed that in contrast to C57BL/6 mouse strain, considered as resistant to *T. crassiceps*, infected BALB/c mice recruited a high population of adherent peritoneal cells with abundant expression of AAMΦ`s gene markers (Relm α, Ym-1, Arginase1, PDL1, PDL2), whereas these same markers were not overexppressed in infected C57BL/6 mice. Interestingly, only AAMΦ`s from the susceptible strain remained with in vitro suppressive ability over T cells. These data suggested that AAMΦ`s recruitment could be necessary for *T. crassiceps* establishment. Thereafter, in order to demonstrate a role for AAMΦ`s during experimental cysticercosis we achieved macrophage-depleting experiments using clodronate-loaded liposomes. We confirmed that clodronate-liposomes administration effectively depleted AAMΦ`s ($F480^+$ MMR^+ cells) and this phenomenom caused a significant decrease in parasite load. Moreover, AAMΦ`s depletion during the first weeks (3) of infection was enough to dampen *T. crassiceps* proliferation in the host as showed with a low parasite burden. In sharp contrast, late clodronate-liposomes treatment had almost null effect in parasite load, confirming that early appearance of AAMΦ`s is needed to mantain *T. crassiceps* infection. It has been shown that all these immuneregulation phenomena increase host susceptibility to intracellular pathogens, where macrophage activation status determines the infection outcome. Finally, in this work we showed that *T. crassiceps* infection could ablate clinical signs and severity in Experimental Autoimmune Encephalomyelitis (EAE) a mouse model for multiple

sclerosis (MS). This model has been largely studied in C57BL/6 mouse strain given these mice are highly susceptible to such disease; however, they are considered resistant to *T. crassiceps* infection. We found that 40 metacestodes dose turns C57BL/6 mice susceptible to this infection and induces a strong Th2 immune response, contrary to 20 metacestodes dose. Next, we aimed to evaluate the ability of *T. crassiceps* infection to reduce the severity course of EAE. Only 50% of *T. crassiceps* –infected mice displayed EAE symptoms, which were significantly less severe than those observed in uninfected mice. This effect was associated with both decreased MOG $_{35-55}$-specific splenocyte proliferation and IL-17 production as wells as with a limited leukocyte infiltration into the spinal cord. Infection with *T. crassiceps* induced an anti-inflammatory cytokine microenvironment, including decreased TNF-α production and high MOG $_{35-55}$-specific production of IL-4 and IL-10. Also, markers for AAMΦ`s were detected and these AAMΦ`s were highly suppressive in *T. crassiceps*-infected and EAE induced mice there was a reduction in the entry of $CD3^+$ $Foxp3^-$ cells into the brain. Therefore, *T. crassiceps*-induced immune regulation decreased EAE severity by dampening T cell activation, proliferation and migration to the CNS. A deeper and more detailed study of mechanisms underlying AAMΦ`s generation could lead to the identification of molecules from this parasite involved in this regulation. Such identification will be helpful to develop either alternative or complementary treatments targeted to inflammatory diseases.

2. INTRODUCCIÓN

2.1 Diversidad de helmintos parásitos

Los helmintos son un grupo diverso de metazoarios en los que se puede encontrar distintas formas, de las que sobresalen los gusanos redondos (Nematodos) y los gusanos aplanados (Platihelmintos). Además, estos organismos han adoptado diferentes estilos de vida, entre ellos el parasitismo. Los helmintos parásitos causan altas tasas de morbilidad en todo el mundo y se estima que millones de personas están infectados actualmente. Este grupo de parásitos generalmente no causa la muerte de su hospedero; sin embargo, afecta su desarrollo debido a que consume diferentes nutrientes [2].

Estos parásitos poseen variadas estrategias para concluir sus respectivos ciclos de vida y pasar de un hospedero a otro. Por ejemplo presentan estadios de latencia como los huevos, que son resistentes y permiten una fácil infección. La transmisión también se favorece al alterar el comportamiento del hospedero en el que se encuentran y facilitar tanto el establecimiento de la infección como el hecho de que algún depredador consuma presas infectadas. Otra forma de transmisión que se lleva a cabo es de forma activa, es decir, etapas larvarias de algunos parásitos helmintos tienen la capacidad de atravesar la piel y penetrar a su hospedero [3].

Estos parásitos tienen diferentes nichos en donde alojarse, que van desde el tracto gastrointestinal, los músculos, los pulmones y el hígado, hasta lugares tan complejos y delicados como el SNC y los ojos.

Otra característica de los helmintos parásitos es que una vez que infectan a su hospedero pueden permanecer en este durante un tiempo muy prolongado sin ser eliminados, es decir, causan infecciones de tipo crónico. Para esto, los helmintos han logrado desarrollar diferentes estrategias de sobrevida. Una de éstas, posiblemente la más importante, es la capacidad de modular el sistema inmune de sus hospederos para evitar ser eliminados. A las estrategias para evadir o suprimir el sistema inmune utilizadas por los helmintos y otros patógenos, actualmente se les conoce como fenómenos de inmunomodulación e inmunosupresión, respectivamente [3].

3. ANTECEDENTES

3.1 Inmunoregulación por helmintos parásitos

Como ya se mencionó, los helmintos parásitos son muy diversos, ya sea morfológicamente, como en mecanismos de transmisión y ciclos de vida; sin embargo, gracias al intenso estudio en modelos experimentales y seres humanos parasitados naturalmente, ahora sabemos que los helmintos inducen respuestas inmunes de tipo Th2. Este tipo de respuesta se caracterizan por la detección de altos niveles circulantes de citocinas como IL-4, IL-5, IL-13; más recientemente se han identificado la IL-25 y la linfopoietina tímica estromal (TSLP) como moléculas que se sobreproducen en presencia de helmintos [4-7] . En cuanto a la producción de anticuerpos, predominan IgG1 e IgE ya que estos isotipos son favorecidos por las citocinas Th2, mientras que es baja la producción del isotipo IgG2a que es favorecido por la citocina tipo Th1, Interferón gamma (IFN)-γ. Se ha reportado también que los helmintos intestinales pueden disminuir los movimientos peristálticos, lo que impide que los parásitos se fijen a la mucosa intestinal[4].

Actualmente, no son claros los mecanismos por los cuales los helmintos polarizan la respuesta de su hospedero hacia Th2. Sin embargo, algunos estudios preliminares han demostrado que vías de co-estimulación como OX-40L, están involucradas en dicho proceso[8, 9]. Se ha descrito también el papel fundamental que los antígenos de helmintos tienen, ya que tanto células dendríticas (CDs) como MΦ´s que son expuestos a antígenos (Ag´s) de helmintos tienen la capacidad de modificar a las células T hacia un perfil Th2 [10]. Importantemente, el estudio de Ag´s ya caracterizados de helmintos como el ES-62 (Glicoproteina de los productos de excreción/ secreción de 62 KDa de peso molecular) de *Acantochilonema vitae* y el SEA (Ag´s solubles del huevo de *Schistosoma*) han dado lugar a la identificación de las regiones capaces de modificar a las células presentadoras de antígenos (APCs) para llevar hacia un perfil Th2 la respuesta inmune (RI) del hospedero, por lo que ahora sabemos que la fosfatidilserina de ES-62 y la lisofosfatidilserina de *Schistosoma* son indispensables para dicha función[11].

Además de citocinas y anticuerpos asociados con respuesta tipo Th2, los helmintos inducen anergia en las células T del hospedero ya que éstas no responden a la estimulación con Ag´s del helminto, ni a estímulos policlonales no relacionados como la Concanavalina A ó la estimulación con anti-CD3[4]. Los esfuerzos realizados para determinar la causa de la anergia de células T han llevado a la identificación de subpoblaciones celulares como las células T reguladoras[12, 1311, 1211, 1211, 1211, 1211, 12] y los macrófagos alternativamente activados, que poseen la capacidad de suprimir la RI. [11, 12,14, 15]

3.2 Células T reguladoras (T regs) en infecciones causadas por helmintos parásitos

Las T regs se identificaron desde la década de los 60´s, pero ha sido recientemente cuando ha surgido más interés por esta población celular. Se han podido identificar diferentes subtipos de ellas, su importante papel en la regulación de tolerancia periférica y hasta las probables vías de desarrollo de las T regs. Se han identificado 3 diferentes subtipos de T Regs que son: las células Th3 que regulan la RI a través de la secreción de TGF β, las células Tr 1 encargadas de secretar IL-10 y las células T regs naturales $CD4^+$ $CD25^+$ Foxp 3^+ que aparentemente inhiben la proliferación celular y producción de citocinas con un mecanismo dependiente de contacto celular. Estos grupos de T regs, a su vez, se subdividen en células reguladoras naturales y adaptativas, las primeras son generadas en el timo por lo que se consideran Ag-independiente, mientras que las adaptativas se generan en la periferia y ante la presencia de algún estímulo antigénico [16, 17].

Debido a que estas poblaciones de T regs poseen la capacidad de regular la respuesta inmune, algunos parásitos (incluyendo los helmintos), han explotado esta población como vía de escape para poder llevar a cabo una infección exitosa.

Algunos reportes recientes han descrito la presencia de células T reguladoras en infecciones causadas por helmintos y su papel como posible mecanismo de evasión. Por ejemplo, se ha observado que tanto la fase de larva como el estadio adulto de la microfilaria *Brugia malayi* inducen el reclutamiento de células reguladoras $CD4^+$ $Foxp3^+$ $CD\ 103^+$ a los 7 días posteriores a su implantación en la cavidad peritoneal. Los autores de este estudio demostraron que estas células reguladoras recuperadas de animales quirúrgicamente implantados con *Brugia malayi* se generaban independientemente de la presencia de la respuesta Th2 y AAMΦ´s, y además, que suprimían la proliferación *in vitro* de células vírgenes estimuladas con APC´s ó anti-CD3[13]. También se sabe que la secreción de TGF β por parte de las células $Foxp3^+$ inducidas por *Brugia malayi* es capaz de apagar las repuestas Th1 o Th2 de manera indistinta [18].

Baumgart et. al. han demostrado que la presencia de *Schistosoma mansoni* incrementa de manera significativa el porcentaje de células $CD4^+$ $Foxp3^+$ en granulomas hepáticos así como en ganglios, y que la eliminación de estas células ocasiona una mayor inflamación[19]. El grupo de Watanabe demostró que un alto porcentaje de las células T

que forman parte del granuloma causado por el huevo de este parásito en ratones CBA/J infectados crónicamente, tienen un fenotipo regulador debido a la expresión del factor de transcripción Foxp3, y que cuentan con la capacidad de suprimir la proliferación de células estimuladas con anti-CD3 *in vitro*. [20] De manera interesante, las células T reguladoras inducidas por este trematodo afectan el desarrollo de patologías no relacionadas como la diabetes en ratones genéticamente propensos a desarrollarla[21]. De manera similar el nematodo intestinal *Heligmosomoides polygyrus* induce un aumento en la población de células T $CD25^+$ Foxp3+ con un alto nivel de TGF β. También Finney y sus colegas han demostrado la presencia de 2 subpoblaciones reguladoras $CD25^+$ $Foxp3^+$ y $CD25^+$ $Foxp3^-$, observando una mayor expresión de TGF β en la subpoblación $Foxp3^-$ sorpresivamente[22]. El papel de estas células reguladoras en la infección causada por *Heligmosomoides polygyrus* aun no esta claro, ya que el grupo de Hartmann realizó la transferencia de células $CD25^+$ $CD103^+$ $Foxp3^+$ y no encontró diferencia en la carga parasitaria de los ratones receptores[23]. Interesantemente, al igual que con *Schistosoma mansoni*, el nematodo *Heligmosomoides polygyrus* altera reacciones inmunológicas no relacionadas, como las alérgicas[24]. El modelo de infección experimental con el nematodo parásito *Trichinella spiralis* también se ha utilizado para estudiar los mecanismos de inmuno-regulación inducidos por helmintos. También se ha descrito que este parásito induce una población de células T CD4+CD25+Foxp3+ que secreta altos niveles de IL-10 y TGF β . Mas aún, esta población celular ocasionó una disminución significativa de IL-5 y como consecuencia, disminuyó la reacción alérgica ocasionada por la sensibilización con OVA [25].

En conclusión, estos datos sugieren que los helmintos parásitos podrían estar explotando la generación de células T reguladoras como una posible vía de evasión de la RI del hospedero para establecer infecciones de tipo crónico.

Sin embargo, la mayor parte de la investigación de este tema se concentra en modelos de infecciones causadas por nematodos y trematodos y muy poco se sabe del impacto que causan las infecciones causadas por cestodos en enfermedades autoinmunes y alérgicas.

3.3 Identificación reciente de células B reguladoras (B regs) en infecciones causadas por helminthos parásitos

En los últimos años, se ha identificado una población de células B con capacidad reguladora, que además de estar presente en infecciones experimentales causadas por protozoarios como *Leishmania*[26], también se ha encontrado en murinos infectados por el nematodo *Heligmosomoides polygyrus* y el trematodo *Schistosoma mansoni*. En el caso de *H. polygyrus* Wilson y colaboradores describieron un aumento significativo de una población $CD4^{-}CD19^{+}$ $CD23^{hi}$ en ganglio mesentérico después de la infección. Esta población tenía una fuerte función reguladora ya que su transferencia fue capaz de inhibir la eosinofilia y la producción de IL-5, que a su vez conllevó a la reducción de una respuesta alérgica en las vías respiratorias. De igual manera, la trasferencia de esta misma población celular redujo la severidad de la EAE, en ambos casos de manera independiente de IL-10[27]. Adicionalmente, en el modelo murino de esquistosomosis Mangan et.al. describieron el aumento de una población de células B con la capacidad de producir altos niveles de IL-10 después de recibir nueva estimulación con LPS. Estas células B productoras de IL-10 suprimieron la anafilaxia ocasionada en los ratones debido a la administración de Penicilina (Pen)-v; sin embargo, cuando las células B se eliminaron dicho efecto se perdió[28]. Este mismo grupo posteriormente demostró que la infección con *Schistosoma mansoni* genera una población de células B $CD1d^{hi}$ que inhibe la reacción alérgica inducida por la sensibilización con el péptido OVA en las vías respiratorias a través de un mecanismo dependiente de IL-10[29].

El interés por los mecanismos de inmunomodulación inducidos por helmintos, ha llevado a la identificación de otra población con capacidad reguladora como las B regs. Debido al gran interés que ha generado esta población celular y el reciente estudio de la misma, ahora es identificada como células $CD19^{+}CD5^{+}CD1d^{hi}$. Se requieren más estudios para identificar marcadores de superficie que faciliten su análisis, comprender si existen subpoblaciones, y si la producción de IL-10 es un mecanismo en común, y finalmente estudiar la presencia y probable participación de las B regs en infecciones causadas por cestodos, ya que no existe algún estudio sobre las células B regs en infecciones por esta clase de parásitos.

3.4 Células Dendríticas (CD) en infecciones causadas por helmintos

Las células dendríticas (CD) coordinan el desarrollo de la RI adaptativa debido a la gran capacidad que poseen de integrar señales provenientes del ambiente y de entregar esta información a las células T. Durante las infecciones las CD son criticas para controlar y eliminar a los patógenos. De manera importante, estos mismos parásitos inducen la maduración y actividad de las CD. La maduración y activación de las CD involucra el aumento en la expresión de moléculas esenciales para el proceso de co-estimulación y presentación de antígeno tales como: CD80, CD86, CD40 y MHCII. Junto con lo antes mencionado, la secreción de citocinas influencia el tipo y la intensidad de la RI adaptativa. Se requieren diferentes interacciones de moléculas derivadas de parásitos con receptores presentes en las CD, como los receptores tipo toll (TLRs) y las lectinas tipo C (CLRs)[30].

Los helmintos parásitos al igual que parásitos intracelulares modulan la actividad de las CD como una de las principales maneras para generar un ambiente que les permita sobrevivir dentro de su hospedero. Aunque no se conocen por completo los mecanismos involucrados en la modulación de las CD por parte de estos helmintos, se esta generando información de suma importancia describiendo la interacción entre moléculas derivadas de los helmintos y diversos receptores.

Interacción CD-Nemátodos

Se sabe que CD expuestas a los antígenos secretados de *Brugia malayi* inducen una respuesta por parte de las células T mas débil comparada con CD no expuestas a estos antígenos. Además, los antígenos de este mismo parásito disminuyen la producción de citocinas pro-inflamatorias en respuesta a estimulación con ligandos de TLRs.

Nippostrongylus brasiliensis es otro nemátodo parásito que secreta una glicoproteína llamada NES. La exposición de las CD ante este NES ocasiona una polarización de las células T hacia el perfil Th2. En este caso, se ha descrito que en dicho fenómeno de polarización están involucradas las moléculas de co-estimulación CD86 y OX40L. Otro ejemplo de modulación de la actividad de las CD por moléculas de helmintos parásitos proviene de los componentes de alto peso molecular de el adulto del nemátodo *Ascaris suum*. Las CD expuestas a estos antígenos pierden la capacidad de aumentar MHC-II en

respuesta al adyuvante completo de Freund (CFA). También se identificó que la fosforilcolina derivada delos esfingolípidos de *A. Suum* disminuyen la secreción de IL-12 y TNF-α así como la inhibición de moléculas co-estimuladoras como CD40, CD80, CD86 y CD54 en respuesta al LPS. Un caso sobresaliente es el del antígeno ES-62 de *Achantochilonema vitae* ya que este antígeno indujo una fuerte expresión de CD40, CD80 y CD86 además de niveles bajos pero significativos de IL-12 y TNF-α de manera MyD 88-dependiente. Las moleculas derivadas de el adulto de *Heligmosomoides polygyrus* no poseen la capacidad de madurar a las CD[30].

Interacción CD-Trematodos

Toda la información en lo que concierne a la interacción entre CD y Trematodos proviene del estudio del modelo experimental de Schistosomiasis. Actualmente se sabe que el antígeno soluble del huevo (SEA) contiene antígenos que polarizan la respuesta de las células T hacia un perfil tipo Th2. Se han descrito antígenos como el alfa-3 fucosa, beta 2 xylosa y Lewis X que inducen importantes cambios en la actividad de las CD. Lípidos derivados de *Schistosoma mansoni* que contienen lisofosfatidilserina actúan como factores promotores de una respuesta Th2 a través del bloqueo de la producción de IL-12 por parte de las CD.El antígeno LNFP III de *S. mansoni* le confiere a las CD la capacidad de inducir respuestas Th2 y dicho proceso depende de la fosforilación de ERK1/2[30].

Interacción CD-Céstodos

Los estudios que describen la interacción entre esta clase de helmintos y las CD son menores comparados con lo que se conoce en los nematodos y los trematodos. Se sabe que el antígeno B y los antígenos del liquido hydatidico de *Echinococcus granulosus* afectan la diferenciación de los monocitos hacia CD, ya que se encuentra un menor número de células. Además, estos mismos antígenos disminuyen la expresión en membrana de la molécula CD1d. También se ha descrito que los antígenos secretados por *E. granulosus* inducen una muy baja expresión de CD80 y CD86, mientras que la producción de TNF-α e IL-12p70 se vió abatida.

En el caso de *Taenia crassiceps* se ha descrito que los carbohidratos presentes en antígenos solubles son los responsables de polarizar la respuesta hacia Th2 y que los

antígenos de alto peso molecular secretados por los metacestodos de este parásito disminuyen la expresión de CD80, CD86 y CD40 en la superficie de las CD. Sin embargo se ha observado que esta disminución no está presente la cepa C57BL/6, la cual es altamente resistente a la infección con *T. crassiceps* indicando que la modulación de las CD por parte de los antígenos de este cestodo es un elemento clave en el curso de la infección[30].

Por lo tanto, como observamos en esta parte las CD son una población celular blanco de los helmintos parásitos y que la modulación de la actividad de estas CD, sobre todo en su capacidad de presentación de antígeno, pueden determinar el curso de las diferentes infecciones.

3.5 Macrófagos alternativamente activados en infecciones causadas por helmintos parásitos

Los macrófagos son una estirpe celular que se genera en la médula ósea, contenida en los huesos largos de los mamíferos ó en la bolsa de Fabricio en las aves. A los precursores en médula ósea, los monocitos circulantes y los macrófagos maduros diferenciados residentes de los diferentes órganos en conjunto, actualmente se les conoce como el "Sistema de fagocitos mononucleares"[31].

Este sistema ha sido ampliamente conservado durante la evolución debido a que realiza importantes funciones dentro de los organismos. Estas células participan en diferentes procesos para el mantenimiento de la homeostasis de los cuales destacan la eliminación de células apoptóticas, la producción de citocinas reguladoras y de factores de crecimiento, que en conjunto permiten mantener a los organismos funcionales. Sin embargo, las funciones más ampliamente estudiadas de los macrófagos son aquellas que realizan como parte fundamental del sistema inmune. Estas células tienen la capacidad de detectar la presencia de un amplio rango de patógenos (desde virus hasta helmintos parásitos), ya que poseen receptores especializados en el reconocimiento de patrones moleculares asociados a patógenos (PAMPs), tales como receptores tipo toll (TLRs), receptores tipo lectina y NODs. Además, constan con la maquinaria necesaria para tomar, procesar y presentar antígenos por lo que son consideradas como el "puente" que une la inmunidad innata con la adaptativa. En adición a estas 2 funciones, los macrófagos son células efectoras, es decir, tienen la capacidad de eliminar patógenos fagocitados a través de la activación de mecanismos microbicidas tales como la producción de moléculas tóxicas para éstos, y en ocasiones para el mismo hospedero. Esta producción es mediada por diferentes enzimas, entre las que destacan iNOS y NADPH oxidasa, a través del fenómeno conocido como estallido respiratorio. Para que los macrófagos lleven a cabo todas estas funciones requieren de un proceso de activación. Dicho proceso implica que diferentes moléculas como PAMP´s y citocinas actúen sobre los macrófagos a través de sus respectivos receptores, iniciando así una cascada de señalización, mediada principalmente por cinasas que tiene como objetivo final la translocación de factores de transcripción y el inicio de la expresión de diversos genes induciendo así la activación de los macrófagos[1].

Los macrófagos son células del sistema inmune innato con funciones conocidas durante la respuesta primaria a patógenos, para el mantenimiento de homeostasis en diferentes

tejidos, coordinación de la respuesta inmune adaptativa, en inflamación y reparación. Es una población celular muy dinámica con la capacidad de adaptarse al microambiente cambiante en el que se desarrollan. De manera similar a la dicotomía de las células T los macrófagos pueden presentar diferentes estados de activación[1, 31, 32].

En los últimos años la intensa investigación sobre la inmunobiología de los macrófagos ha llevado a la identificación de diferentes estados de activación de estas células, que depende del microambiente en el que se encuentren. Actualmente, son reconocidos tres estados de activación de los macrófagos. Primero, los macrófagos clásicamente activados (CAMΦ´s ó M1), los macrófagos con este fenotipo se desarrollan en respuesta a estímulos inflamatorios endógenos tales como el IFNγ, principal citocina Th1, ó estímulos exógenos de la misma naturaleza que el Lipopolisacárido (LPS) bacteriano. El IFNγ puede convertir a los macrófagos de un estado basal hacia células con potente actividad de presentadora de antígenos, mayor producción de citocinas pro-inflamatorias (IL-12, TNFα e IL-1β), o de moléculas tóxicas como óxido nítrico (ON) que depende de la expresión de iNOS, y la fagocitosis mediada por complemento o anticuerpos[1] (ver figura 1). iNOS utiliza como sustrato L-Arginina para producir ON, molécula tóxica para los patógenos. Por lo tanto estos CAMΦ´s se asocian con citocinas de tipo Th1 y con la erradicación de patógenos intracelulares.

Desde el trabajo de Mosmann se identificó a IL-4 como citocina con funciones antagónicas a las del IFN γ; en línea con ésto, ahora se sabe que los macrófagos estimulados con IL-4 presentan un estado de activación distinto a los CAMΦ´s, caracterizado por la disminución en la producción de IL-12 e IL-1β. Importantemente, se encontró que la IL-13 induce efectos redundantes a los de IL-4 sobre los macrófagos. En 1992, debido al hallazgo del aumento del receptor de manosa como principal marcador de macrófagos estimulados con IL-4, junto con la inducción de moléculas de MHC II, se propuso el concepto de macrófagos alternativamente activados (AAMΦ´s)[33]. Este estado de activación cuenta con alta expresión de Arginasa-1, contraparte de iNOS y cuyos productos finales son urea y prolinas, en lugar de óxido nítrico. Además, los AAMΦ´s sobreexpresan genes como el de molécula parecida a la resistina (Relm α), la Ym-1 y las moléculas parecidas a quitinasas (Chaff´s)[32] (ver figura 1).

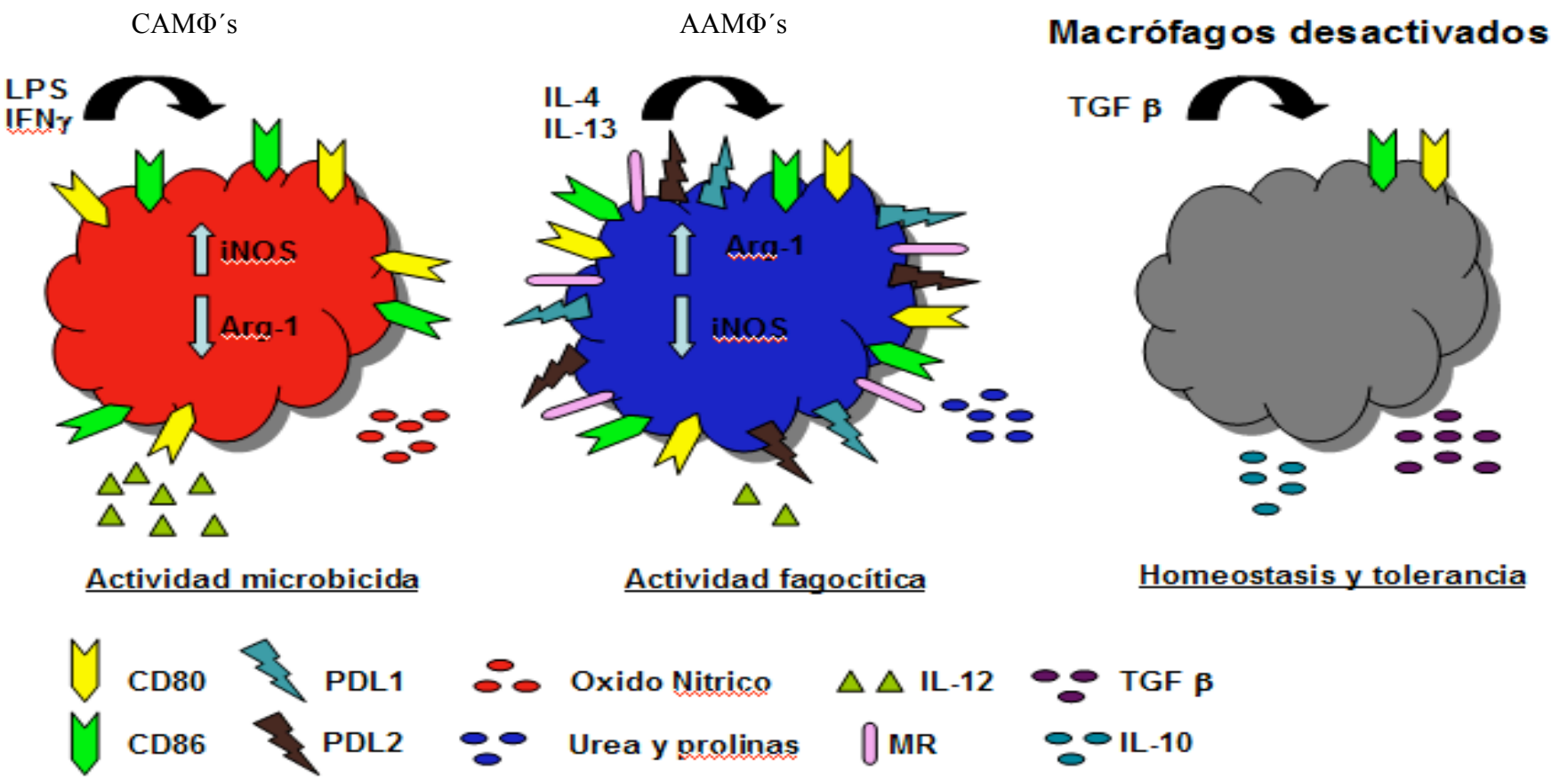
Otra característica de los AAMΦ´s es que tienen la capacidad de fusionarse y formar las llamadas células gigantes multinucleadas, y dicho fenómeno es altamente dependiente de la interacción entre la molécula de adhesión E-Cadherina y la catenina, que se

pueden encontrar en diversas condiciones patológicas como granulomas causados por parásitos como *Mycobacterium spp,Schistosoma*[34, 35] y en el cerebro de pacientes con SIDA[33,34, 35,36]. La función de estas células gigantes multinucleadas hasta el momento se desconoce.

Los estímulos que promueven ya sea CAMΦ´s ó AAMΦ´s tambien pueden influir sobre la capacidad y especificidad de migración de las distintas poblaciones ya que también regulan la expresión diferencial de quimiocinas y receptores de quimiocinas[37].

En tercer lugar, se ha reconocido el impacto que la IL-10 y el TGF β, citocinas consideradas reguladoras, tienen sobre los macrófagos; debido a que los macrófagos estimulados con IL-10 presentan una notable disminución en la expresión de moléculas co-estimuladoras y por lo tanto una pobre capacidad de presentación de antígenos, se les han llamado macrófagos en estado de "verdadera desactivación"[38]. Debido a que los AAMΦ´s se describieron *in vitro* después de haber sido estimulados con citocinas como la IL-4 y la IL-13, es decir, en respuesta a citocinas Th2[39] , no pasó mucho tiempo antes de que se demostrara que los helmintos parásitos, conocidos por polarizar fuertemente la respuesta inmune en sus hospederos hacia un perfil Th2, en realidad poseían la capacidad de inducir AAMΦ´s, y es de hecho de modelos experimentales con diferentes helmintiasis de donde más información se ha generado con respecto a las propiedades de los AAMΦ´s (ver figura 1).

Figura 1.- Activación diferencial de los macrófagos.



Abreviaturas: CAMΦ´s;macrófagos clásicamente activados, AAMΦ´s;macrófagos alternativamente activados,iNOS;sintasa de óxido nítrico inducible , Arg-1;arginasa 1, MR; receptor de manosa, PDL1;ligando 1 del receptor de muerte programada (PD) [1].

AAMΦ´s en infecciones causadas por Nemátodos

Los nematodos son gusanos redondos que causan diversas enfermedades en humanos, así como en roedores, de donde se ha podido generar información sobre los AAMΦ´s.

Brugia malayies una filaria que no infecta a los roedores de manera natural; sin embargo, la implantación intraperitoneal de este parásito ha sido utilizada durante los últimos años para comprender aspectos básicos de inmunomodulación. La infección intraperitoneal con *B. malayi* induce el reclutamiento de un alto porcentaje de macrófagos $F4/80^{+}$ que expresan Arginasa-1 preferencialmente en lugar de iNOS y que además poseen la capacidad de inhibir la proliferación de células T activadas de forma policlonal *in vitro*[40, 41]. En este modelo fue en el que por primera vez se describió que estos macrófagos sobreexpresan genes que ahora sabemos definen al estado de activación alternativa: estos genes son Relm α y Ym1[42] ambos codifican para quitinasas, de las cuales aún se desconocen sus funciones . Se demostró que la actividad inhibitoria de los AAMΦ´s dependía del contacto celular entre éstas y las células T, aunque las moléculas de membrana involucradas no se definieron[43].

Litomosoides sigmodontises otro nematodo que a diferencia de *B. malayi* es unparásito natural de roedores. Se demostró que también la presencia de este parásito inducía AAMΦ´s y que además los clásicos marcadores Relm α y Ym-1 se podían detectar fácilmente en sitios por donde migraba el parásito y en la cavidad pleural, que es el sitio de residencia[44, 45]. Cuando se estudió la capacidad supresora de los AAMΦ´s se encontró que estas células suprimían la actividad proliferativa y que TGF β podría estar involucrada. De manera interesante, este estudio sugiere que los AAMΦ´s se pueden diseminar a los ganglios linfáticos sólo cuando la microfilaria salía de la cavidad pleural[46].

Nippostrongylus brasiliensis es un nematodo que ha sido ampliamente utilizado para comprender la inmunomodulación e infecciones en el tracto gastrointestinal, que presenta un ciclo de vida complejo que implica una infección subcutánea; conforme el parásito madura y migra, induce una fuerte respuesta Th2 en los pulmones y los ganglios asociados a éstos[47]. Aparte de las citocinas Th2, se pueden observar altamente expresados los genes Relm α, Ym-1 y AMCase en los pulmones, sugiriendo la presencia de AAMΦ´s. En este modelo se identificó por primera vez que aparte de las citocinas clásicas Th2 IL-4 e IL-13, la IL-21 puede aumentar la actividad de los AAMΦ´s y por el contrario en ratones deficientes en IL-21 hay menor cantidadde estos macrófagos, y por lo tanto menos fibrosis pulmonar. Debido a que los AAMΦ´s

dependen de citocinas Th2[48], generalmente se asocian a la etapa adaptativa de la respuesta inmune; sin embargo, en este estudio por primera vez se demostró que los AAMΦ´s aparecen a los 4 días posteriores a la infección, aún en ratones con inmunodeficiencia combinada severa (SCID) en los cuales no hay células T. De manera sobresaliente se notó que los AAMΦ´s decaen después a partir de los 8 días post-infección, demostrando que una vez resuelto el daño mecánico y la inflamación generados por la migración de *N. brasiliensis* esta población regresa a niveles similares a los encontrados en ratones no infectados[49]. Recientemente, Siracusa y sus colaboradores realizaron una cinética de aparición y funcionalidad de AAMΦ´s en el pulmón de ratones infectados con *N. brasiliensis*. Los macrófagos recuperados de un lavado broncoalveolar a los 2 y 3 días post-infección presentan el fenotipo F4/80^{+}, MHCII ^{hi}, CD40^{+}, CD80^{+}, CD86^{+} y secretaron IL-12. Conforme avanza el tiempo de infección, es decir, a los 8 y 13 días estos macrófagos comienzan a expresar PDL1 y PDL2 en la membrana y tienen la capacidad de suprimir la proliferación de células T. A pesar de que hay moléculas que aparecen más tarde, los marcadores Arginasa1, Relm α, Ym-1 y Ym-2 se pueden detectar tan temprano como a los 3 días post-infección[50]. En otro estudio reciente se demostró que el daño ocasionado por la presencia de *N. brasiliensis* en los pulmones es tan grave que se puede desarrollar enfisema y una enfermedad crónica obstructiva pulmonar que se asoció a la prolongada presencia de AAMΦ´s, productores de MMP12[51].

Por lo tanto, los AAMΦ´s se pueden generar en ausencia de células Th2 en tiempos tan cortos como 2 y 3 días, dejando abierta la posibilidad de que directamente los antígenos de *N. brasiliensis* los inducen o que otra fuente innata y temprana de IL-4 e IL-3 lleve a cabo esta importante función.

Heligmosomoides polygyrus es un nematodo parásito natural del ratón. Los ratones son infectados con el estadio L3 y estas larvas salen del estómago, migrando después de 36 h hacia el intestino delgado donde penetran la mucosa y se mudan hacia la muscularis interna, enquistándose en la pared muscular[14]. Al igual que los demás helmintos *H. polygyrus* polariza la respuesta hacia Th2 y causa infecciones de tipo crónico. Los AAMΦ´s en esta infección se encuentran únicamente en el sitio de infección y dependen de las citocinas Th2 ya que en ratones STAT 6-/- e IL-4-/- el reclutamiento de AAMΦ´s se vió significativamente afectado. Más aún, la menor cantidad de AAMΦ´s se asoció con alta parasitemia y alta producción de huevos sugiriendo que éstos restringen el crecimiento del parásito. Lo anterior fue demostrado porque en infecciones

secundarias los ratones son más resistentes porque poseen células Th2 de memoria y por lo tanto más AAMΦ´s y porque cuando estos últimos se eliminaban con liposomas que contenían clodronato, se reducía la expulsión de *H. polygyrus*. Ésta es por lo tanto la primera evidencia de que los AAMΦ´s juegan un papel importante como efectores en la infección causada por un nematodo intestinal promoviendo su expulsión[52].

AAMΦ´s en infecciones causadas por Tremátodos

El modelo murino de esquistosomiasis ha sido de gran utilidad para comprender diferentes aspectos de la relación hospedero-parásito como protección, susceptibilidad y patología[53]. La primera evidencia de la capacidad moduladora de los macrófagos en la infección con *Schistosoma mansoni* surgió en la década de los 90´s, cuando el grupo de investigación de Miguel Stadecker describió que los macrófagos aislados del granuloma hepático tenían la capacidad de anergizar las células T activadas por estímulos policlonales[54]. Este grupo tambien describió que el efecto supresor de los macrófagos era reversible si se incluía IL-2 y que si se utilizaban éstos como CPAs, se polarizaban las células T hacia Th2. A pesar de estos importantes hallazgos no se describieron más funciones ni características de estos macrófagos, y no fue hasta hace pocos años que se identificaron como AAMΦ´s y que la supresión ejercida era contacto-dependiente. Actualmente se sabe que en la infección con *S. mansoni* estos AAMΦ´s inhiben la proliferación de las células T por un mecanismo que involucra principalmente a la molécula de co-estimulación negativa PDL1. Esto último se lleva a cabo porque *S. mansoni* induce la sobre expresión de PDL1 en la membrana de los macrófagos, que al unirse a su receptor PD-1 en las células T activadas disminuye la proliferación celular[55]. En estudios más recientes se demostró que los AAMΦ´s dependen de IL-4, ya que los ratones deficientes de IL-4Rα no generan AAMΦ´s y sucumben ante la infección. El hecho de que los ratones IL-4Rα -/- sucumben ante la infección por *S. mansoni* se debe a que se desarrolla una inflamación exacerbada y no controlada mediada por Th17. Por lo tanto, en el caso de *S. mansoni* la inducción de AAMΦ´s es dependiente de IL-4/IL-13 y esencial para evitar que el daño inmuno-patológico comprometa la sobrevida del hospedero, mostrando así otra faceta desconocida de los AAMΦ´s[56].

Con el propósito de identificar algunos antígenos importantes para la inducción de AAMΦ´s, el intenso trabajo ha llevado a la identificación de la peroxiredoxina como un antígeno secretado de *S. mansoni*,capaz de inducir Relm α y Ym-1 independientemente

de IL-4 e IL-13 en los macrófagos de la cavidad peritoneal. Además, en presencia de estos macrófagos las células T presentan un fenotipo Th2[57]. El carbohidrato inmunomodulador Lacto-N-fucopentosa III (LNFP III) es un antígeno soluble del huevo de *S. mansoni.* Atochina y colaboradores demostraron que la activación *in vivo* de macrófagos peritoneales con LNFP III aumenta la expresión de Arginasa 1 y Ym-1, independientemente de IL-4 e IL-13, pero no otros marcadores como Relm α y MGL1. A pesar de esto, utilizando el modelo de presentación de antígenos con células T DO.11.10 específicas para ovalbumina, demostraron que aún sin los otros marcadores los AAMΦ´s influyen sobre las células T para producir IL-10 e IL-13[58]. Otro aspecto importante para comprender la función de los AAMΦ´s en las diversas infecciones, es la naturaleza de los receptores encargados de modular la activación alternativa. Utilizando la inyección intravenosa de huevos de *S. mansoni* para generar un granuloma pulmonar, Joshi et. al. demostraron que en ausencia de TLR-3 se desarrolló un granuloma de mayor tamaño, concomitante a una mayor deposición de colágena y presencia de macrófagos que expresaban Arginasa-1 y CCL2, marcadores de AAMΦ´s[35].

En conclusión los AAMΦ´s generados durante la infección con *S. mansoni* tienen capacidad supresora a través de la vía PD1-PDL1, pueden controlar la inflamación exacerbada prolongando la sobrevida del hospedero y como presentadoras de antígeno llevan la respuesta hacia Th2.

Fasciola hepaticatiene un ciclo de vida complejo que involucra hospederos intermediarios (caracoles) y definitivo (ganado vacuno). Como otros helmintos *F. hepatica* induce una respuesta Th2. También se ha descrito reclutamiento de AAMΦ´s en etapas tempranas de la enfermedad o de la administración de antígenos excretados/secretados de este parásito. Recientemente, se ha identificado a la Tioredoxin peroxidasa de *F. hepatica* como un antígeno capaz de reclutar AAMΦ´s en la cavidad peritoneal e inducir la expresión de marcadores de activación alternativa *in vitro* en macrófagos de la línea celular RAW264.7[59]. Estos AAMΦ´s producen altos niveles de IL-10 y prostaglandina E2, mientras que la producción de IL-12 es mínima. Otro antígeno de *F. hepatica* ya identificado y aislado es la 2-Cys peroxiredoxina. Recientemente se demostró que este antígeno es capaz de generar AAMΦ´s y que éstos inducen la producción de IL-4 por las células T . El avance ha sido notorio respecto a la identificación de antígenos específicos y recombinantes con capacidad moduladora.

AAMΦ´s en infecciones causadas por Céstodos

Taenia crassiceps es un céstodo que no tiene la capacidad de infectar al humano, a menos que este se encuentre inmuno comprometido. En la naturaleza *T. crassiceps* utiliza a los cánidos como hospederos definitivos y los roedores como hospederos intermediarios. La infección de roedores con la etapa larval de *T. crassiceps* ha sido tomada como modelo para comprender las interacciones hospedero-parásito en la cisticercosis, así como para estudiar moléculas antigénicas como candidatos posibles para vacunas y para inmunodiagnóstico en humanos por infectados por *T. solium*, principalmente por la alta similitud antigénica. Después de la infección inicial con los metacestodos de *T. crassiceps* se puede detectar una rápida y transitoria respuesta de tipo Th1 que posteriormente se polariza a Th2[60]. Debido al cambio en la producción de citocinas, también cambia la función de células como los macrófagos y el reflejo de este cambio es el hecho de que los macrófagos de la etapa aguda, donde predomina la producción de IFN γ, producen altos niveles de IL-12 y ON, es decir son CAMΦ´s. En la etapa crónica, en la que predomina la respuesta Th2, predominan los AAMΦ´s, los cuales tiene alta expresión de moléculas como CCR5, CD23 en la membrana y más recientemente, receptor de manosa (MR), lectinas tipo C (MGL1 y MGL2), Ym1, Arginasa-1, Relm α y TREM2[61-63]. Al igual que en los nematodos, estos AAMΦ´s dependen de STAT6 e IL-4 ya que en ratones STAT6 -/- o IL4Rα -/- no se pueden generar AAMΦ´s en respuesta a la infección con *T. crassiceps*. En cuanto al papel desempeñado por estos macrófagos durante la infección, se encontró que tienen la capacidad de inhibir la respuesta proliferativa de las células T, lo cual es dependiente del contacto célula-célula. Posteriormente, se identificó que este efecto correlacionaba con la expresión de las moléculas de co-estimulación negativa PDL1 y PDL2. La participación de esta vía se estudió a través del uso se anticuerpos para bloquear el efecto de estas moléculas. Interesantemente, se demostró que PDL1 y PDL2 participan de manera directa en la actividad supresora de los AAMΦ´s generados durante la infección con *T. crassiceps*[64]. Debido a la actividad supresora de los AAMΦ´s y al paso de Th1 a Th2 en la respuesta inmune, se hipotetizó que la generación de estos macrófagos fuera necesaria para el establecimiento de los metacestodos de *T. crassiceps*. En nuestro laboratorio más recientemente se encontró que la eliminación de estos AAMΦ´s es un paso limitante para el crecimiento de *T. crassiceps.* Más aún se

observó que estos AAMΦ´s aparecen antes de las 4 semanas previamente estimadas, por lo que la eliminación de estos AAMΦ´s durante las 3 primeras semanas de infección son suficientes para impedir el crecimiento normal[65].

Hymenolepis diminuta es un parásito helminto que recientemente se ha utilizado para estudiar la respuesta intestinal ante un cestodo. Los ratones infectados con *H. diminuta* presentan sobre expresión de marcadores para AAMΦ´s y células T reguladoras en el intestino, lo que pone en evidencia la fuerte regulación inducida por este helminto. La aparición de estos AAMΦ´s pudo ser detectada a tiempos tan tempranos como 8 días después de la infección. Nada se sabe en cuanto a la participación de los AAMΦ´s, sin embargo los autores correlacionaron la aparición de los AAMΦ´s con el tiempo en el que se expulsó a *H. diminuta*[66].

Echinococcus multilocularis es un parásito de cánidos que en ciertas condiciones puede infectar al humano y ocasionar equinococosis alveolar, debido al alojamiento del huevos en los pulmones. La infección experimental intraperitoneal de *E. multilocularis* se utiliza para estudiar los mecanismos de inmunomodulación inducidos por este helminto parásito. Los macrófagos obtenidos a las 6 semanas post-infección de la cavidad peritoneal de animales infectados con *E. multilocularis* tienen la capacidad de inhibir la proliferación de células T ante un estímulo no relacionado, como es el péptido de OVA, y ejercen el mismo efecto sobre células T estimuladas con Con A; este último efecto fue contacto dependiente. A pesar de tener identificada la función supresora de estos macrófagos no se determinó si presentaban los marcadores de AAMΦ´s, por lo tanto se requieren más experimentos para determinar si la infección experimental con *E. multilocularis* efectivamenterecluta AAMΦ´s con capacidad supresora[67].

Mesocestoides corties un cestodo que invade órganos vitales y al igual que con otros helmintos, la protección depende de la producción de IL-4. A mediados de la década de los 90 se demostró por primera vez, en este modelo de infección, que los macrófagos recuperados de cavidad peritoneal de ratones infectados con *M. corti* tienen una pobre capacidad estimuladora sobre células T delos ganglios mesentéricos; sin embargo, no se analizó si estos macrófagos en realidad eran AAMΦ´s.

Recientemente se caracterizó la respuesta inmune en ratones deficientes de IL-4 (IL-4 -/-) y se encontró que estos ratones tuvieron 100% de mortalidad a los 32 días post-

infección mientras que los ratones silvestres sobrevivieron hasta 1 año. La deficiencia de IL-4 ocasionó un menor reclutamiento de monocitos y macrófagos; además, la carga parasitaria en hígado, cavidad peritoneal y torácica fue mayor. Como era de esperar, los ratones IL-4 -/- presentaron mayor producción de citocinas inflamatorias como IFN γ y TNF α[68]. Más importante fue el hecho de que la ausencia de IL-4 provocó menor cantidad de AAMΦ´s en el granuloma hepático. En conclusión, este reporte sugirió que *M. corti* induce AAMΦ´s para evitar que una respuesta inflamatoria exacerbada dañe al hospedero, de manera similar al caso de *S. mansoni*.

En el apéndice de publicaciones obtenidas en el artículo 1, se muestran otros marcadores de membrana de los AAMΦ´s encontrados en los diferentes modelos de infecciones con helmintos .

3.6 Inmunomodulación por *Taenia crassiceps*

Taenia crassiceps es un céstodo parásito natural de cánidos y roedores. En el intestino de cánidos silvestres se encuentra el estadio adulto, el cual es hermafrodita y se encarga de la producción de huevos, que posteriormente serán excretados con las heces. Los huevos liberados al ambiente pueden ser consumidos por roedores, en los cuales después de la eclosión se encontrará la fase de metacestodo, principalmente en tejido subcutaneo ó cavidades. Finalmente los cánidos pueden adquirir nuevamente la infección si se alimentan de roedores infectados[69].

Durante años se ha utilizado la infección experimental de ratones de laboratorio con metacestodos de *T. crassiceps* como modelo de estudio de la infección en humanos causada por *T. solium* y para comprender los fenómenos de inmunomodulación. Los estudios realizados han permitido descifrar diferentes eventos reguladores inducidos por *T. crassiceps*, que le permitencrecer de manera exitosa en la cavidad peritoneal de los ratones. Es bien sabido que las hembras son más susceptibles que los machos a esta infección, por lo que se reveló un importante papel de los esteroides sexuales[70]. También se sabe que durante la etapa aguda de la infección predomina la producción de IFN-γ, es decir una respuesta Th1. Mientras que conforme avanza el tiempo de infección esta respuesta Th1 comienza a disminuir de manera concomitante con el aumento de una respuesta Th2, caracterizada por la alta producción de IL-4, IL-13 y PGE2 a la par con el crecimiento de *T. crassiceps*, sugiriendo así que el ambiente de citocinas Th2 podría favorecer el establecimiento de los metacestodos[60, 71]. Algunos estudios posteriores, utilizando anticuerpos para bloquear el efecto del IFN-γ, o ratones deficientes en moléculas como CD40, STAT-4 o STAT-6, demostraron que la respuesta Th1 impide el crecimiento de este céstodo, mientras que cuando predomina una respuesta Th2 éste parásito puede colonizar a su hospedero sin algún problema[72, 73].

Además de la producción de citocinas Th2 durante la etapa crónica de la infección con *T. crassiceps,* también se pueden detectar altos niveles de anticuerpos de los isotipos IgG1 e IgE, mientras que la producción de IgG2a es mínima[74]. Se ha observado un marcado estado de anergia de las células T de ratones infectados crónicamente (>4 semanas) ante la estimulación con Ag´s del helminto, así como ante activadores policlonales, tales como la con A ó anti CD3.

Nuestro grupo identificó una subpoblación de AAMΦ´s con sobreexpresión de CCR5 y CD23 en la membrana celular, reclutados al sitio de infección de *T. crassiceps*, que si son utilizados como APC´s polarizan la respuesta hacia Th2. Estos AAMΦ´s producen bajos niveles de IL-12 y altos niveles de IL-10, demostrando así su potencial anti-inflamatorio[63]. El interés por estos macrófagos llevó, en estudios posteriores, a generar más conocimiento sobre los AAMΦ´s en la infección por *T. crassiceps*. Se demostró que también expresan los genes característicos de los AAMΦ´s descritos en otras infecciones, tales como Arginasa-1, Relm α y Ym-1 pero además que tienen la capacidad de inhibir la proliferación de células $CD90^{+}$ activadas con anti CD3. Dicho proceso de inhibición es mediado por las moléculas PDL1 y PDL2, sobre-expresadas en los AAMΦ´s[64]. En conjunto estos datos demostraron los fuertes fenómenos de inmunomodulación inducidos por *T. crassiceps* para llevar a cabo una infección exitosa, incluso alteran la RI a retos posteriores no relacionados, como la infección con patógenos intracelulares. Así, se observó que la infección crónica con *T. crassiceps* permite un crecimiento mayor de *Salmonella typhimurium* aún después de la vacunación con antígenos de *S. typhimurium*, y dicho fenómeno se asoció con la incapacidad de los animales infectados con *T. crassiceps* para producir anticuerpos heterólogos contra *S. Typhimurium*[75]. Posteriormente, se demostró la infección crónica con *T. crassiceps* también aumenta la susceptibilidad a la infección posterior con *Trypanosoma cruzi*[76] y *Leishmania*. En este último estudio se encontró que a pesar de que los ratones previamente infectados con *T. crassiceps* tenían una respuestaTh1, , los macrófagos provenientes de estos animales mostraban una estado de activación alternativo, hecho que explicó la susceptibilidad a la segunda infección[77].

Sin embargo, no se ha estudiado si los mecanismos de regulación inmune inducidos por *T. crassiceps* que modifican el curso de enfermedades parasitarias pueden ó no afectar el curso de enfermedades autoinmunes o alérgicas.

3.7 Hipótesis de la higiene

En 1989 David Strachan publicó un estudio sobre la fiebre del heno, en el cual reportó que las familias con más habitantes por casa y con sistemas de saneamiento e higiene deficientes, entre otros factores, eran menos propensas a desarrollar alergia contra el heno. Por lo tanto, éste fue el primer estudio que relacionó la falta de higiene con menor probabilidad de desarrollar reacciones de hipersensibilidad, lo que llevó a Strachan a proponer esto como la hipótesis de la higiene[78].

En los últimos años el interés acerca de los factores implicados en el desarrollo y control de las enfermedades de tipo alérgica y autoinmunes ha crecido, ya que los factores genéticos no parecen ser los únicos responsables ó no explican del todo dichas patologías, como se ha demostrado en estudios realizados en gemelos. Además, algunas correlaciones geográficas muestran que hay una coexistencia muy baja entre parásitosis y enfermedades alérgicas o autoinmunes[79]. Por todo esto, la hipótesis de la higiene ha sido retomada y ahora se estudia tanto en modelos experimentales como en seres humanos, el impacto que tienen las infecciones sobre otros parásitos u otros tipos de enfermedades.

Los helmintos han desarrollado una gran variedad de estrategias para evadir la RI generada por sus respectivos hospederos con el propósito de eliminarlos. De esta forma, desde hace tiempo se sospechó y en los últimos años se ha podido comprobar, que tanto las infecciones por helmintos como patógenos unicelulares alteran la RI de una manera tan importante que comprometen al hospedero ante subsecuentes infecciones y además pueden disminuir la severidad o en algunos casos impedir el desarrollo de reacciones alérgicas y enfermedades mediadas por inflamación exacerbadas[80].

Así, se ha demostrado que la infección con diferentes helmintos modula el desarrollo de enfermedades autoinmunes experimentales, tales como la diabetes tipo 1 (T1D), la encefalomielitis autoinmune experimental (EAE) y artritis inducida por colagenasa (CIA). Ver tabla 1.

Tabla 1.- Efecto de la infección por diferentes helmintos parásitos sobre enfermedades de tipo autoinmune.

HELMINTO	HOSPEDERO	VIA DE INFECCIÓN	NUMERO DE PARÁSITOS INOCULADOS	EFECTO OBSERVADO	MECANISMO RESPONSABLE DEL EFECTO OBSERVADO	REF.
NEMATODOS					IFN γ IL-4 ↓ IL-17 ↑ IL-10	81
Trichinella spiralis	Ratas Dark Agouti	Inoculación gástrica	500 Larvas 1	Disminución de EAE		
	Ratones NOD	Inoculación gástrica Suspendidas en agarosa	400 Larvas	Abrogación de Diabetes	↓ IFN γ↑ IgE IL-4 IL-10	82
Heligmosomoides polygyrus	Ratones NOD	Inoculación gástrica Suspendidas en agarosa	300 Larvas	Abrogación de Diabetes	↓ IFN γ↑ IgE IL-4 IL-10	82
TREMÁTODOS						
Schistosoma mansoni	Ratón C57BL/6J	Percutánea	70 cercarias	Disminución de EAE	IFN γ ↓ ON TNF α	83
	Ratón DBA/1	Inmersión de la cola en agua con cercarias	40 cercarias	Disminución de CIA	IgG2a ↓ IFN γ↑ IL-4 IL-17 TNF α	84
	Ratones NOD	Percutánea	30 cercarias	Disminución de diabetes mellitus	↓ IgG ↑ IgM	85
Fasciola hepática	Ratón C57BL/6	Oral	10 metacercarias	Disminución de EAE	↓ IFN γ↑ TGF β IL-17	86
CÉSTODOS						
Taenia crassiceps	Ratón BALB/c	i.p.	20 metacestodos	Disminución de T1D	↓ TNF α↑ IL-4	87

Abreviaturas : EAE; Encefalomielitis Autoinmne Experimental. CIA; Artritis inducida por colagenasa. T1D; Diabetes tipo 1. i.p. Intraperitoneal. NOD; Diabéticos no obesos

3.8 Encefalomielitis Autoinmune Experimental (EAE) como modelo murino para la Esclerosis Multiple (MS) humana

El modelo de Encefalomielitis Autoinmune Experimental (EAE) se generó en 1933 en la universidad de Rockefeller para estudiar los episodios de parálisis que algunas veces se observaban en individuos vacunados. Por lo tanto, el modelo de EAE inicialmente se construyó para estudiar la encefalomielitis aguda diseminada. Ahora, después de más de 75 años de mejoramientos y variaciones, se obtuvo un modelo que reproduce tanto la reacción inflamatoria como la desmielinización en el SNC[88].

El intenso estudio de este modelo ha llevado a la identificación de los mecanismos inmunes que inician y mantienen los signos de la EAE/MS, por lo que ahora conocemos 3 pasos indispensables para que un organismo la desarrolle. El primero consiste en la activación de células T $CD4^+$ periféricas autoreactivas contra diferentes regiones de lipoproteínas que forman la cubierta de mielina. El segundo paso consiste en la proliferación y polarización, generalmente hacia el perfil Th1 y Th17, de estas células autoreactivas en órganos inmunes periféricos, como el bazo. El tercer paso es la migración de estas células hacia el SNC, el posterior ingreso hacia el parénquima y el inicio de una reacción inflamatoria debido a la producción de citocinas como TNF-α , IL-1-β e IL-12 junto con la producción de quimiocinas como CCL5 y CXCL10 que ocasionan la llegada de diversas poblaciones celulares como células T $CD8^+$, neutrófilos, células B y macrófagos; estos últimos son los responsables de fagocitar a la mielina y dejar a los axones ¨desnudos¨, lo cual ocasiona las fallas en la transmisión de impulsos eléctricos[89, 90]. También se ha descrito que la reacción inflamatoria tan exacerbada tiene un efecto sobre poblaciones celulares residentes tales como microglia, astroglia, oligodendroglia y neuronas. Así, ahora la EAE no es reconocida sólo como una enfermedad inflamatoria desmielinizante, sino también como un desorden neurodegenerativo debido a la apoptosis ocasionada por TNF-α sobre los oligodendrocitos y la muerte neuronal después de la pérdida axonal.

El interés en este modelo ha llevado a la identificación de las moléculas blanco para las células T presentes en la mielina. Entres dichas moléculas destacan la proteína básica de mielina (MBP), la proteína proteolipídica de la mielina (PLP) y la glicoproteína de mielina de los oligodendrocitos (MOG). Por lo tanto, ahora existen 2 diferentes maneras de inducir la EAE: la primera es generar de manera *in vitro* clonas de células T de perfil

Th1 específicas para cualquiera de estas 3 proteínas, y la segunda inyectando péptidos de estas proteínas en adyuvante completo de Freund (ACF) junto con toxina de *Bordetella pertussis*. De estas diferentes maneras de inducir EAE, el modelo más estudiado es la administración de el péptido MOG_{35-55} en ACF y toxina de *B. pertussis* en ratones de la cepa C57BL/6[89].

De manera interesante, se sabe que tanto en humanos como en ratones, las hembras son más susceptibles a EAE y MS en una proporción de 2 a 1. El fondo genético también determina la susceptibilidad a la EAE ya que por ejemplo, en el modelo inducido por la administración con $MOG_{35-55,}$los ratones de la cepa C57BL/6 desarrollan signos más graves que los ratones de la cepa BALB/c[91]. Ahora se sabe que el inicio de la EAE/MS es un evento multifactorial. Otros factores que se asocian al desarrollo de esta enfermedad son los polimorfismos a nivel de las moléculas del MHC II, ya que existen individuos con células CD4+ circulantes capaces de reconocer antígenos de la cubierta de mielina, sin embargo, estos individuos no desarrollaron MS[88]. Los factores ambientales tales como infecciones con diferentes patógenos también parecen afectar el curso de la EAE y, más importantemente, existen correlaciones geográficas en las cuales se muestra un patrón inverso entre la presencia de enfermedades autoinmunes y lugares endémicos de enfermedades parasitarias[92]. Recientemente otro factor que se ha relacionado al desarrollo de enfermedades autoinmunes como la EAE/MS es el estado nutricional de los individuos, ya que se ha observado que un nivel bajo de vitamina D en los individuos facilita el desarrollo de esta enfermedad autoinmune[93].

Finalmente, como se puede observar en la tabla 1 el modelo de la EAE se ha utilizado para demostrar el impacto de infecciones causadas por helmintos en enfermedades autoinmunes, seguramente a través de la secreción de antígenos ya que ninguno de estos parásitos reside o migra por el SNC. Más importante; el modelo de EAE ha sido útil para desarrollar los tratamientos terapéuticos actuales aplicados a pacientes con MS como el Betaferon, Natalizumab y Copaxona, que son la administración de IFN-β , un anticuerpo dirigido contra la integrina α-4-β1 que impide la entrada de leucocitos hacia el SNC y un péptido que genera respuestas tipo Th2 hacia la mielina, respectivamente[89].

4. HIPÓTESIS

La inmunomodulación inducida por la infección con *T. crassiceps,*disminuye la gravedad de la Encefalomielitis Autoinmune Experimental (EAE), la cual depende de una respuesta inflamatoria exacerbada en el SNC.

5. OBJETIVO GENERAL

-Demostrar que la infección previa con *T. crassiceps* favorece el desarrollo de AAMΦ´s con actividad supresora en diferentes cepas de ratones, lo que causará un curso menos grave de la EAE.

5.1 Objetivos particulares

- Analizar la expresión de genes marcadores de AAMΦ´s por RT-PCR en células peritoneales adherentes obtenidas de ratones susceptibles y resistentes (BALB/c vs. C57BL/6, respectivamente) igualmente infectados con *T. crassiceps.*

-Analizar por FACS la presencia en membrana de moléculas características de AAMΦ´s comparando cepas susceptibles vs. resistentes.

-Determinar la producción diferencial de citocinas y anticuerpos específicos en la cepa susceptible y la cepa resistente a la infección con *T. crassiceps*.

-Evaluar el desarrollo de los AAMΦ´s.

-Determinar la importancia de los AAMΦ´s en las fases temprana y tardía de la infección con *T. crassiceps*.

-Determinar la dosis de metacestodos de *T. crassiceps* para romper la resistencia a este parásito en la cepa C57BL/6.

-Establecer el modelo de EAE inmunizando subcutáneamente con el péptido MOG $_{35\text{-}55}$.

-Determinar el efecto de la infección previa de *T. crassiceps* en el desarrollo de la EAE.

-Cuantificar y comparar la producción de citocinas en los ratones con y sin infección.

6. MATERIALES Y MÉTODOS

Estos pueden consultarse con detalle en cada una de las publicaciones ubicadas en el anexo de publicaciones obtenidas.

Ratones, parásitos e infección

Se utilizaron ratones hembra de 6 a 8 semanas de edad de la cepa BALB/c y de la cepa C57BL/6. Los ratones fueron infectados i.p. con 20 (BALB/c) y 40 (C57BL/6) metacestodos de *Taenia crassiceps* cepa ORF.

ELISA para cuantificar citocinas en suero

Los ratones fuero sangrados a los tiempos indicados y la sangre obtenida se centrifugó a 3500 rpm durante 10 min. El suero obtenido se congeló a -70 ºC y se utilizó para cuantificar el nivel de citocinas circulantes, siguiendo las recomendaciones del fabricante. Los pares de anticuerpos para las diferentes citocinas fueron comprados de Peprotech, México.

Se utilizaron placas de 96 pozos (Nunc, maxisorp) que se cubrieron con el anticuerpo de captura (1µg/ml) utilizando 100µl/pozo. Después de 12 hr de incubación a 4 ºC se lavaron 5 veces las placas y se realizó una incubación con solución de bloqueo (PBS/tween 0.05%) 2 hr 37ºC para evitar pegado inespecífico. Posteriormente, se realizaron 5 lavados más y las placas se dejaron incubar toda la noche a 4 ºC con una curva patrón de cada una de las citocinas recombinantes y con los sueros obtenidos de los ratones. Después, las placas se lavaron 5veces más y se adicionó el anticuerpo acoplado a biotina (1µg/ml) para finalmente revelar las placas adicionando avidina-peroxidasa y ABTS/H_2O_2 como sustrato. Las placas se leyeron a 405 nm y los valores se expresaron como pg/ml.

ELISA para cuantificar anticuerpos en suero

Se cubrieron placas de 96 pozos (Nunc, Polysorp) con 10µg/ml de antígeno total soluble de *T. crassiceps* ó el péptido MOG $_{35-55}$ donde se indica. Después de 12 hr de incubación a 4 ºC se lavaron 5 veces las placas y se colocaron los sueros obtenidos de los animales iniciando a una dilución 1:25. Después, las placas se lavaron 5veces más y

se adicionó el anticuerpo anti ratón-IgG1 (Zymed) y anti ratón-IgG2a (Zymed) ambos acoplados a biotina y streptavidina. Las reacciones se revelaron adicionando ABTS/H_2O_2 como sustrato. Las placas se leyeron a 405 nm y los valores se expresaron como densidad óptica detectada en la dilución 1:25.

RT-PCR

Se obtuvieron células totales de peritoneo, se ajustaron a $5x10^6$ células/ml y se sembraron 1ml/pozo en placas de 12 pozos (Nunc). Después de dejar adherir por 2 hr a 37ºC se removieron las células no adherentes con lavados con medio DMEM tibio. A las células adherentes se les adicionó trizol (Invitrogen) y se extrajo el RNA total de estas células adherentes con cloroformo. En el caso del análisis de RT-PCR en el cerebro, éste se extrajo en condiciones asépticas y se adicionó el trizol (Invitrogen) para extraer el RNA total del cerebro.

La cantidad de RNA total se cuantificó utilizando una dilución 1:200 en un espectrofotómetro (1 D.O.=40µg), se ajustó la cantidad de RNA total a una concentración de 5µg y se llevó a cabo la transcripción reversa utilizando el kit First strand synthesis (Invitrogen). Una vez que se obtuvo el cDNA se realizaron los ensayos de PCR en un termociclador (Corbett research) utilizando las siguientes concentraciones para amplificar cada uno de los genes de nuestro interés:

Agua	9.25 µl
Buffer 10x	2.5 µl
MgCl2	0.75 µl
DNTP mix	0.5 µl
Primer F	2.0 µl
Primer R	2.0 µl
DNA Taq polimerasa	1.0 µl
Muestra DNA	1.25 µl

Posteriormente, se realizó una electroforesis para observar los productos en un gel de agarosa. Se utilizó un gel de agarosa (ICN Biochemicals) al 1.5% en Buffer TBE 1x

(Apéndice I) sobre una cámara molde y un peine de 12 pozos, que se mantuvo a temperatura ambiente hasta solidificarse.

Una vez hecho el gel, se hizo una dilución 1:4 de DNA y se colocaron por pozo, 2 µl de la muestra previamente diluida a 8 µl de buffer de carga blue juice (Invitrogen), a partir del segundo pozo ya que en el primero se colocaron 3 µl del marcador de peso molecular 100 pb (Invitrogen).

Se colocaron las muestras en la cámara hacia el polo positivo y se corrió a 100 Volts, 45 Amperes durante 30 minutos. Se observó en un transiluminador con luz UV (FujiFilm FLA-5000) y se capturó la foto utilizando el programa ImageReader FLA-5000 V2.1.

De esta manera se analizaron los marcadores típicos de los AAMΦ´s.

Inducción y evaluación de EAE

Los ratones fueron inyectados de manera s.c. con 300 µg del péptido $MOG3_{5-55}$emulsificado en adyuvante completo de Freund (CFA) en un volumen final de 200 µl. En este mismo día y 2 días después también se les administró de manera i.p. a los ratones 200 ng de toxina de Pertussis.

Para evaluar la gravedad durante el curso de la EAE los ratones fueron observados para asignar el puntaje de acuerdo a la siguiente escala utilizada:

0; sin signos, 0.5 flacidez en la cola, 1; parálisis de la cola, 1.5; parálisis en la cola y falla parcial en una pata trasera, 2; parálisis en la cola y una pata trasera, 2.5;parálisis en cola, una pata trasera y parcial falla en la otra pata trasera, 3; parálisis en la cola y ambas patas traseras, 4; moribundo y 5; muerto.

Cultivo de células de bazo

En condiciones asépticas, dentro de una campana de flujo (VECO) se extrajo el bazo de ratones infectados, colocándolo en una caja petri, posteriormente se separaron las células del resto del tejido utilizando separadores celulares (B. D. Falcon, cell strainer) y se depositaron las células en tubos estériles de 10 ml (Nalge, Nunc Int).

Para concentrarlas se centrifugó a 1500 rpm por 10 minutos y posteriormente fueron tratadas con 5 ml de solución hemolizante (Apéndice I) durante 15 minutos para eliminar los eritrocitos restantes. Se centrifugaron por 10 minutos a 1500 rpm y se decantaron. A las células libres de eritrocitos se agregó 5 ml de medio D-MEM (Gibco, suplementado con D-glucosa y libre de L-Glutamina, 10% SFB y streptomicina) para homogenizar y contar las células viables utilizando la prueba de exclusión con azul tripano, tomando 20 µl de muestra y 20 µl de azul tripano (Sigma Chemicals co). En la región de linfocitos de la cámara de Neubauer se colocaron 10 µl con micropipeta estéril del homogenizado y se contaron 5 cuadrantes para obtener el número de leucocitos por mililitro, para después ajustar a $3X10^6$ células/ml con medio D-MEM. Se colocaron 100 µl por pozo en una placa de cultivo celular de 96 pozos (B. D. Falcon), para obtener una concentración final de $3X10^5$ células por pozo. Una vez colocadas las células en la placa, se estimularon con 25 µg por ml de antígeno total de *T. crassiceps.* Esta re-estimulación se llevó a cabo por 5 días en un incubador a 37ºC con 5% de CO_2 y terminado el tiempo indicado, se recolectaron los sobrenadantes y congelaron a -70ºC hasta su uso para cuantificar la producción de citocinas por ELISA.

Citometría de flujo

Los ratones infectados con *T. crassiseps* se sacrificaron en la campana de flujo, posteriormente se inyectaron i.p. con 10 ml de solución salina estéril (Pisa) para realizar un lavado peritoneal y con la jeringa se recuperaron células totales del peritoneo, que se colocaron en tubos de 15 ml. Los tubos con las células se centrifugaron durante 10 minutos a 2000 rpm para concentrarlas, se decantó el sobrenadante, dentro de la campana, se resuspendió el botón de células y se le adicionó a cada tubo 3 ml de buffer para FACS (Apéndice I). Se homogenizó la suspensión para realizar el conteo en la cámara de Neubauer, ajustándolas con el buffer de FACS a 1 millón de células por ml y se colocaron en tubos para análisis de citometría (B. D. Falcon).

A las células ajustadas y concentradas se les agregó 300 µl del mismo buffer donde primero se incubaron 15 min con anti-CD16/32 y se tiñeron con 1 µl de los anticuerpos conjugados con los distintos marcadores de fluorescencia y un anticuerpo isotipo (IgG2a) como control para pegado inespecífico, a una concentración de 0.2 µg combinados de la siguiente manera:

F480 (APC, BioLegend) – MMR (FITC, BioLegend)
F480 (APC, BioLegend) – MAC 3(FITC, BioLegend)
F480 (APC, BioLegend) – PDL1 (PE, BioLegend)
F480 (APC, BioLegend) – PDL2 (PE, BioLegend)
F480 (APC, BioLegend) – OX40L (PE, BioLegend)
F480 (APC, BioLegend) – Siglec F (PE, BioLegend)
F480 (APC, BioLegend) – Gr1(PE, BioLegend)

Una vez agregados los anticuerpos se incubó durante 30 minutos a 4ºC, se lavaron las células dos veces más con buffer de FACS a 2000 rpm por 10 minutos para eliminar los anticuerpos no adheridos, finalmente se les agrego 500 µl para su lectura utilizando el programa Cell Quest y el citometro FACSCalibur (Becton Dickinson) para la captura de cada muestra.

7. RESULTADOS

7.1 Análisis de la capacidad de activación de APC´s en cepa susceptible (BALB/c) y resistente (C57BL/6) a la infección experimental con T. crassiceps.

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**.Infection Genetics and Evolution. Dec 9 (6); 1115-27.

En este trabajo se analizó la respuesta inmune global así como el estado de activación y respuesta de macrófagos y DC durante la infección con *T. crassiceps* comparando una cepa clásicamente considerada como susceptible (BALB/c) con una cepa resistente (C57BL/6) de ratones.

-Los ratones BALB/c son más susceptibles a la infección con *T. crassiceps* y presentan una respuesta inmune predominantemente tipo Th2, por el contrario los ratones C57BL/6 son más resistentes y de manera concomitante muestran una respuesta inmune de tipo Th1.

Es bien sabido que en el caso de la cisticercosis experimental (*T. crassiceps*) la cepa de ratones C57BL/6 es altamente resistente a dicha infección, mientras que la cepa BALB/c es altamente susceptible [94]. Sin embargo, muy poco se sabe de los mecanismos inmunológicos que determinan esta susceptibilidad diferencial, por lo que se analizó si había alguna diferencia en cuanto a la activación de macrófagos y DC durante esta infección.

En este trabajo se infectaron ratones hembra de la cepa susceptible (BALB/c) y cepa resistente (C57BL/6) con 10 metacestodos de *T. crassiceps* por vía i.p., y se cuantificó la carga parasitaria a lo largo de 8 semanas. Como se esperaba, los ratones BALB/c presentaron una carga parasitaria más alta comparada con la de los ratones C57BL/6 (Fig. 1 A en artículo 2) confirmando las observaciones previas; de igual manera, los niveles de IgG1 e IgE específicos contra Ag´s de *T. crassiceps* se encontraron significativamente más altos en ratones BALB/c comparados con los C57BL/6 igualmente infectados (ver Fig. 1B y Fig. 1C artículo 2).

En cuanto a la respuesta de los esplenocitos en los diferentes tiempos post-infección, se observó que los ratones BALB/c disminuyen la respuesta proliferativa ante el re-estímulo con Ag de *Taenia* y con anti-CD3 conforme avanza el tiempo de infección, mientras que los ratones C57BL/6 mantuvieron dicha respuesta (Fig. 2A, Fig 2B artículo 2). Se analizó la producción de citocinas de células totales de bazo y se determinó que los ratones BALB/c predominantemente secretaban IL-4 e IL-13 sobre el IFNγ mientras que los ratones C57BL/6 presentaron el patrón inverso de producción de citocinas (ver Fig 2C, Fig 2D y Fig 2E artículo 2).

-Los ratones BALB/c presentan AAMΦ´s con poca producción de citocinas pro-inflamatorias y alta capacidad supresora, mientras que los ratones C57BL/6 no generan esta población.

Posteriormente se estudió la respuesta *in vitro* de macrófagos recuperados a diferentes tiempos de infección de la cavidad peritoneal de ambas cepas de ratones, ante estímulos pro-inflamatorios como LPS e IFN-γ. En esta parte del trabajo se demostró que los macrófagos provenientes de ratones BALB/c infectados tienen una capacidad muy limitada para producir citocinas pro-inflamatorias como IL-12 y TNF-αasí como ON a las 8 semanas después de la infección. Por el contrario, los macrófagos de ratones C57BL/6 mantuvieron el perfil pro-inflamatorio durante las 8 semanas (Fig 3A, Fig 3B, Fig 3C artículo 2). Además, se obtuvieron macrófagos a diferentes tiempos de infección y sin estímulo adicional se realizó el ensayo de RT-PCR para determinar la expresión de genes típicamente asociados a activación alternativa.

Lo que se puede observar en la Fig 4A es que desde la segunda semana de infección los ratones BALB/c sobre-expresan los principales marcadores de AAMΦ´s (Ym-1, Relm α, TREM2 y Arginasa-1) mientras que los ratones C57BL/6 presentan un ligero aumento de Ym1 y Arginasa-1; mas aún, conforme avanzó el tiempo de infección los ratones C57BL/6 dejaron de expresar estos marcadores mientras que los BALB/c mantuvieron ó en algunos casos los sobre-expresaron. Estos datos se confirmaron con el análisis densitométrico presentado en la figura 4B (artículo 2). Además de determinar el estado de activación de los macrófagos por RT-PCR también se analizó la presencia en membrana de moléculas involucradas con la capacidad supresora de estos AAMΦ´s previamente descrita [64]. La figura 5A (artículo 2) muestra el ensayo de citometría de flujo que se llevó a cabo para demostrar que los ratones BALB/c reclutaban al sitio de

infección (cavidad peritoneal) una población de macrófagos que sobreexpresan PDL1 y PDL2. Por otro lado, se observó que los ratones C57BL/6 presentaban un menor porcentaje de estos macrófagos. Para determinar la capacidad supresora de estas poblaciones se realizaron co-cultivos de los macrófagos con linfocitos T $CD90^+$ provenientes de animales sanos, en diferentes proporciones, y claramente se observó que sólo los macrófagos provenientes de ratones BALB/c poseían la capacidad de suprimir la proliferación celular. De esta manera se demostró que en los ratones BALB/c se pueden generar AAMΦ´s con capacidad supresora a diferencia de lo que ocurre en los ratones C57BL/6.

-Las CD derivadas de ratones BALB/c expuestas a Ag´s de *T. crassiceps* poseen capacidad supresora y baja producción de citocinas pro-inflamatorias en respuesta a LPS.

En esta parte se estudió la respuesta de CD derivadas de médula ósea ante Ag´s excretados secretados de *T. crassiceps* (TcES) en ambas cepas de ratones (BALB/c y C57BL/6). Primeramente se demostró que las DC provenientes de ratones BALB/c que tuvieron contacto con los TcES tienen la capacidad de inhibir una respuesta proliferativa alogénica cuando se cultivaron en presencia de células $CD4^+$ de ratones C57BL/6 únicamente si sembraban en la proporción 1:2, de células CD:CD4 pero no si se utilizaban en la proporción 1:10 y 1:20. Mas aún, estos TcES disminuyen significativamente el efecto que normalmente tiene el LPS sobre las DC (Fig. 5A, artículo 2). De manera contraria, las CD derivadas de ratones C57BL/6 expuestas a TcES no tienen efecto sobre la proliferación de las células $CD4^+$ (Fig. 6B, artículo 2). Además, las DC´s provenientes de ratones BALB/c expuestas a TcES y LPS indujeron una menor respuesta alogénica debido a que no maduraron de manera óptima, al contrario que las DC´s obtenidas de ratones C57BL/6 que sí fueron capaces de inducir una fuerte respuesta alogénica.

Después se probó la capacidad de producir citocinas de las CD derivadas de ambas cepas, después de exponerlas a LPS, TcES y LPS+TcES, cuantificando TNF-α, IL-12 e IL-15 en el sobrenadante. Se observó que la exposición a LPS indujo una alta producción de TNF-α e IL-12 en CD de ambas cepas, misma que disminuye si además se exponen al mismo tiempo con TcES, pero sólo en las CD derivadas de ratones BALB/c. La producción de IL-15, inducida por el contacto con LPS se disminuyó ante la presencia de TcES en ambas cepas.

7.2 Estudio del papel que tienen los macrófagos durante la infección con *T. crassiceps.*

Reyes J.L., Terrazas C.A., Alonso-Trujillo J., Van Rooijen, Satoskar A.R. & Terrazas L.I. (2010) **Early removal of alternatively activated macrophages leads to *Taenia crassiceps* cysticercosis clearance *in vivo*** Intl. J. Parásitol. 40(6);731-42

Este trabajo se llevó a cabo con el propósito de determinar la relevancia que los macrófagos tienen durante la infección con *T. crassiceps*. Para alcanzar este objetivo se utilizaron liposomas con clodronato para eliminar esta población celular y se observó una importante disminución de parásitos en la cavidad peritoneal cuando se eliminaron los AAMΦ´s

-Los AAMΦ´s aparecen a la segunda semana de infección con *T. crassiceps*.

Hace 7 años se describió por primera vez que *T. crassiceps* induce el reclutamiento de una población con características de AAMΦ´s [63] a la cavidad peritoneal; posteriormente se caracterizó con más profundidad esta población, y se demostró que tiene capacidad supresora sobre células T activadas. Sin embargo, se desconocía en que punto de la infección aparecían estos macrófagos; por lo tanto, en esta primera parte se realizó una cinética utilizando RT-PCR y citometría de flujo para saber cuándo los macrófagos infiltrados presentan el fenotipo de AAMΦ´s, respectivamente. La figura 1A (artículo 3) muestra la cinética de reclutamiento de dicha población utilizando citometría de flujo; se observó que a partir de la primera semana de infección se infiltra un alto porcentaje de células $F4/80^{+}$ pero que no presentan una expresión significativa de los marcadores de membrana de AAMΦ´s, como PDL2, IL-4Rα y MR. Además aparece una población de eosinófilos ($F480^{lo}$ $SiglecF^{+}$) que se infiltran rápidamente a la cavidad peritoneal y aumentan conforme avanza la infección. Sin embargo, estos eosinófilos disminuyeron significativamente en la semana 3 y es a este mismo tiempo cuando predominan los AAMΦ´s. Estos datos se pueden observar en la fig. 1 B (artículo 3): los marcadores de activación alternativa aparecen en la semana 2. De esta manera, se confirmó que la infección con *T. crassiceps* induce una población de AAMΦ´s más rápido de lo que se sabía, es decir a la semana 2 p.i.

Después de que se determinó el tiempo en el que aparecen los AAMΦ´s se utilizó el tratamiento con liposomas de clodronato para evaluar la participación de los macrófagos durante la infección con *T. crassiceps.*

-El tratamiento con liposomas de clodronato aumenta la resistencia a la infección experimental con *T. crassiceps*.

La utilización de liposomas de clodronato es la herramienta que últimamente ha permitido describir las funciones que los macrófagos llevan a cabo durante diversas condiciones patológicas tales como infecciones[95-97], fibrosis[98], autoinmunidad[99] y angiogénesis[100]. En el caso de infecciones causadas por helmintos la información es escasa y recientemente se describió que la eliminación de los macrófagos impide que el nematodo intestinal *Heligmosomoides polygyrus* sea expelido como normalmente lo hace la respuesta Th2 de memoria[52]. Nuestro grupo ha descrito que los macrófagos que se infiltran a la cavidad peritoneal tienen un potencial supresor *in vitro*; sin embargo, se desconocía el papel *in vivo* durante la infección con *T. crassiceps*.

Debido a lo anterior, en este estudio se utilizaron dichos liposomas para dilucidar el papel que los macrófagos desempeñan, tomando en cuenta la premisa de que, debido a su potencial supresor, podrían disminuir la respuesta de las células T y favorecer el establecimiento de *T. crassiceps*.

Los ratones fueron infectados con 20 metacestodos y tratados con 2 mg por semana de liposomas de clodronato, durante las primeras 8 semanas de infección, mientras que sus respectivos controles recibieron 2 mg de liposomas con PBS, ambos grupos fueron sacrificados a las 4 y 8 semanas p.i para determinar la carga parasitaria. La figura 2 A (artículo 3) muestra que los ratones tratados con clodronato presentan una cantidad de parásitos 2 y 9 veces menor que los ratones que recibieron PBS a las 4 y 8 semanas p.i. respectivamente. Por lo tanto, se demostró que los metacestodos de *T. crassiceps*sobreviven más en su hospedero si están presentes estos macrófagos.

Para comprobar que el tratamiento con clodronato era funcional se cuantificaron las células del exudado peritoneal (PECs). En la figura 2 B (artículo 3) se puede observar claramente que durante la infección con *T. crassiceps* las PECs aumentaron conforme el tiempo de infección avanzaba durante las primeras 8 semanas de infección. Por el contrario, los ratones tratados con liposomas de clodronato tienen una disminución

significativa de PECs indicando que este tratamiento disminuye las poblaciones celulares que infiltran al sitio de residencia de *T. crassiceps.*

-Los liposomas de clodronato eliminan específicamente a los macrófagos.

En el ensayo previo se demostró que las PECs de ratones tratados con clodronato se encuentran disminuidas; sin embargo, no se estudió la especificidad del tratamiento, por lo que se llevó a cabo análisis de citometría de flujo utilizando marcadores específicos para macrófagos (F4/80) y eosinófilos (Siglec F) ya que la eosinofilia es una característica de las infecciones por helmintos. De hecho se notó que esta población se infiltra a la cavidad peritoneal durante las primeras 2 semanas(fig. 1 A, artículo 3) por lo que se analizaron ambas poblaciones.

Para discernir entre estas poblaciones actualmente se definen como sigue:

Macrófagos; F4/80 ^{hi} Siglec F ^{lo}

Eosinofilos; F4/80 ^{lo} Siglec F ^{hi}

Partiendo de esta clasificación, en la figura 3A (artículo 3) se puede observar que mientras una abundante población F4/80 ^{lo} Siglec F ^{hi} permanece intacta en ambos grupos experimentales, la población F4/80 ^{hi} Siglec F ^{lo} se detectó significativamente afectada mostrando así que los macrófagos son eliminados de manera específica. La figura 3 B muestra un análisis alternativo de citometría en donde nuevamente se observa que las células F4/80 ^{hi} son las únicas afectadas.

Después de confirmar la eliminación de los macrófagos se decidió analizar cual subpoblación de éstos era la que desaparecía. Uno de los primeros efectos descritos para la acción de IL-4 sobre los macrófagos fue la inducción del receptor de manosa (MR)[33]; desde entonces se ha considerado que los macrófagos que sobre-expresan el MR se encuentran alternativamente activados. Además, es bien sabido que *T. crassiceps* induce AAMΦ´s por lo que se realizó el ensayo de citometría para confirmar la desaparición de dicha población celular.

Como se esperaba, en la figura 3C (artículo 3) se observa que la infección con *T. crassiceps* induce una gran población de células F4/80 ^{hi} MR^{+} (AAMΦ´s) las cuales disminuyen drásticamente después de 4 semanas de tratamiento con clodronato, mientras que la población F4/80 ^{lo} Siglec F ^{hi} permaneció intacta. Finalmente, en la figura 3 D (artículo 3) se muestra una foto representativa del ensayo de cytospin que de igual forma comprueba que el tratamiento con PBS no tiene efecto, ya que se

encontraron células PMN con macrófagos, mientras que el tratamiento con clodronato ocasiona una disminución en los macrófagos pero no en los PMN.

Debido a que la supresión de células T por parte de los AAMΦ´s es la función que nos llevó a proponer que la eliminación de estos AAMΦ´s debería ser importante para el establecimiento de *T. crassiceps*, se analizó el efecto de los liposomas con clodronato sobre los macrófagos que expresan las moléculas involucradas en dicho fenómeno de supresión (PDL1 y PDL2). La figura 4A (artículo 3) muestra el análisis de citometría de flujo realizado a las 4 y 8 semanas p.i. para determinar si los macrófagos con potencial supresor también eran eliminados con el tratamiento de clodronato. Primeramente se analizó el efecto del clodronato sobre la población de macrófagos en general, para lo cual utilizamos 2 marcadores de esta población (F/480 y MAC3). Se observó que tanto a la semana 4 como a la semana 8 p.i. el porcentaje de células $F480^{+}$ $MAC3^{+}$ se disminuye en 60 y 77% después del tratamiento con clodronato. En cuanto a los macrófagos con potencial supresor se observa que a la semana 4 y a la 8 p.i. hay un alto porcentaje de macrófagos peritoneales que expresan PDL1 40±4 y 37±6, respectivamente. Pero nuevamente dichos porcentajes de macrófagos con PDL1 se reducen hasta en un 87 % después del tratamiento con clodronato, permaneciendo sólo 16±4 y 5±2 del porcentaje de los macrófagos a la semana 4 y 8, respectivamente. Se encontraron resultados similares para los macrófagos que expresan PDL2, ya que en este caso el porcentaje de disminución fue de 59 y 79% a las 4 y 8 semanas p.i., respectivamente.

Arginasa-1, Relm α y Ym-1 son los marcadores típicos de los AAMΦ´s. Por lo tanto se realizó un análisis de RT-PCR en células adherentes de peritoneo para determinar si estos genes se estaban sobreexpresando, y corroborar así que la población que disminuye con el tratamiento de clodronato efectivamente es de AAMΦ´s. En la figura 4B (artículo 3) se observa que los ratones tratados con PBS sobre-expresan Arginasa-1, Relm α y Ym-1 como demuestra la banda representativa de la electroforesis en gel de agarosa y el análisis de densitometría mostrado a la semana 4 y 8 p.i.

Debido a que el ensayo se realizó en células peritoneales adherentes quisimos confirmar que estas células son macrófagos en más del 90%, como se ha reportado. Para determinar morfológicamente el tipo de células que se tomaron para el análisis de RT-PCR, sellevaron a cabo ensayos de cytospin.

En la figura 4C (artículo 3) se muestran fotos en donde se puede observar que las células adherentes son efectivamente macrófagos y de hecho se observan unos

macrófagos fusionados, lo cual también está reportado por ser una característica más de los AAMΦ´s; por el contrario las células no adherentes eran predominantemente PMN.

Después de demostrar que los AAMΦ´s son necesarios para que *T. crassiceps* se establezca en los ratones, se decidió estudiar si la eliminación de los macrófagos en diferentes etapas de la infección modifica su curso,. Para cumplir este objetivo los ratones se trataron con clodronato durante la etapa aguda de la infección ó durante la fase crónica.

-La eliminación de los macrófagos aumenta la resistencia a *T. crassiceps* sólo si se administran durante la fase aguda de la infección.

Nuestro grupo ha demostrado que los macrófagos que se infiltran a la cavidad peritoneal durante la infección con *T. crassiceps* modifican sus características funcionales conforme avanza la infección, por lo que en esta parte el objetivo fue determinar si la ausencia de los macrófagos en la etapa temprana o tardía altera la infección. En la figura A (artículo 3) se presenta el curso normal de la infección que alcanza un nivel de 295± 38 parásitos después de 8 semanas de infección; en contraste el grupo de ratones tratados únicamente las 3 primeras semanas con clodronato presentan una reducción significativa en la carga parasitaria (25±9), mientras que en los ratones tratados durante la etapa crónica (a partir de la semana 5) no se encontró diferencia significativa en el número de parásitos con respecto al grupo control (280±33). Así que la presencia de los macrófagos desde el inicio de la infección es crucial para que esta sea exitosa.
En la figura 5B (artículo 3) se muestra el análisis de citometría de flujo de la presencia de macrófagos ($F4/80^+$ $MAC3^+$) y macrófagos potencialmente supresores ($F4/80^+$ $PDL1^+$ y $F4/80^+$ $PDL2^+$) presentes en la cavidad peritoneal de los ratones con los distintos tratamientos. Lo que se puede apreciar es que los ratones que fueron tratados durante las primeras 3 semanas con clodronato no presentaron el mismo número de las diferentes poblaciones analizadas comparados con los ratones tratados con PBS, sobretodo en la población $F4/80^+$, ya que están por debajo de los ratones tratados con PBS en un 60 y 70%. Otro hallazgo importante fue que las 3 poblaciones analizadas presentan prácticamente el mismo número en los ratones tratados con clodronato las últimas 3 semanas, lo que sugiere que el tratamiento no tiene efecto si se aplica en un tiempo en el cual hay millones de células en la cavidad peritoneal.

De igual manera que en los experimentos previos, se analizó el estado de activación de los macrófagos peritoneales adherentes en los ratones tratados con clodronato, en diferentes etapas de la infección, para determinar si expresaban los marcadores de AAMΦ´s (Arginasa-1, Relm αy Ym-1). La figura 5C (artículo 3) muestra el ensayo de RT-PCR en el que se observa que la eliminación temprana de los macrófagos ocasiona una clara disminución de los marcadores de AAMΦ´s, mientras que los ratones tratados con clodronato las últimas 3 semanas presentan el aumento de la expresión esperada de Arginasa-1, Relm α y Ym-1 asociado a los AAMΦ´s; esto confirman que el tratamiento en la fase tardía no eliminó los macrófagos como se esperaba, probablemente debido a el gran número de macrófagos presentes en la cavidad peritoneal.

En la parte final de este estudio se evaluó el efecto que tiene el clodronato sobre la respuesta inmune contra *T. crassiceps* que se genera en el bazo.

-La eliminación de los AAMΦ´s permite que se recupere la respuesta proliferativa y se produzcan citocinas de un perfil mixto (Th1/ Th2) en bazo.

En las infecciones experimentales causadas por helmintos parásitos se conoce muy bien el hecho de que las células T se encuentran anérgicas, ya que no responden a Ag´s del agente infeccioso, ni a estímulos no relacionados como Con A y anticuerpos anti-CD3 [4]. En el caso de la infección experimental con *T. crassiceps* se ha encontrado este mismo fenómeno de anergia. Por lo tanto, en esta última parte llevamos a cabo ensayos de re-estimulación de células totales de bazo con TcAg para analizar la proliferación y la producción de citocinas especificas. La figura 6A (artículo 3) muestra que los ratones tratados con PBS tuvieron una muy baja respuesta proliferativa ante el estímulo de TcAg de acuerdo a lo que ya se sabía. Por el contrario, las células de los ratones tratados con clodronato, que carecen de macrófagos, recuperan la capacidad de proliferar en respuesta al TcAg. En cuanto a la producción de citocinas encontramos que los ratones tratados con PBS producen predominantemente IL-13 (Fig. 6 C artículo 3) lo cual nos indica una polarización hacia Th2 de la RI, mientras que los ratones tratados con clodronato durante 8 semanas, produjeron altas cantidades de las diferentes citocinas cuantificadas IL-4, IL-13 e IFN-γ (Fig. 6 B, 6C y 6D del artículo 3, respectivamente).

Los datos obtenidos en este estudio demuestran que la presencia de AAMΦ´s favorece la sobrevida de *T. crassiceps* mientras que la eliminación de estos AAMΦ´s las primeras 3 semanas ó durante las 8 semanas de infección restringe el crecimiento del parásito asociada a una fuerte respuesta celular en el bazo.

7.3 La infección previa con *Taenia crassiceps* disminuye la gravedad de los signos de la Encefalomielitis Autoinmune Experimental (EAE).

Reyes J.L., Espinoza-Jimenez A.F., Gonzalez M.I., Verdin L. & Terrazas L.I. (2011) ***Taenia crassiceps* infection abrogates experimental autoimmune encephalomyelitis** Cell. Immunol. 267 (2); 77-87.

En esta última parte estábamos interesados en estudiar los mecanismos de inmunoregulación utilizados por *T. crassiceps* para establecerse en su hospedero, y saber si éstos podrían alterar el curso de una enfermedad de tipo autoinmune dependiente de la activación de células T autoreactivas como la EAE.

-Los ratones de la cepa C57BL/6 infectados con 40 metacestodos de *T. crassiceps* fueron susceptibles a la infección y desarrollaron una respuesta tipo Th2.

Es bien conocido que la cepa de ratones C57BL/6 es resistente a la infección con *T. crassiceps,* ya que desarrolla una fuerte respuesta Th1 y no puede generar AAMΦ´s. De manera importante, el modelo de EAE inducido por la administración de el péptido MOG $_{35\text{-}55}$ está establecido en ratones C57BL/6 debido ala fuerte respuesta inflamatoria que estos desarrollan. Debido a esto, se decidió infectar con diferentes dosis de metacestodos para poder obtener una dosis con la cual esta resistencia se revirtiera es decir, una dosis a la cual *T. crassiceps* se estableciera e indujera una respuesta Th2.

Como se puede observar en la figura 1A (artículo 4) esto último se obtuvo infectando a los ratones con 40 metacestodos, ya que se observa a las 8 semanas de infección una carga parasitaria de 850 metacestodos en promedio, lo cual es mayor a lo obtenido con ratones susceptibles (BALB/c) infectados con 20 metacestodos. También se observó una clara respuesta tipo Th2 tanto en el perfil de citocinas como en la respuesta humoral, reflejada por los altos niveles de IL-4 en comparación con los de IFNγ (figura 1B artículo 4), así como el predominio del isotipo IgG1 sobre IgG2a (figura 1 C artículo 4). Además, pudimos detectar que conforme avanza el tiempo de infección se incrementan los niveles de IgE circulantes (figura 1D artículo 4), confirmando lo anterior ya que este Ac también es dependiente de la IL-4. De esta manera se

demostróque una alta dosis de metacestodos de *T. crassiceps* revierte la resistencia previamente observada ya que induce, a diferencia de la dosis baja, una respuesta Th2.

-La infección con 40 metacestodos de *T. crassiceps* en ratones C57BL/6 ocasionó la infiltración de AAMΦ´s y redujo el porcentaje de células $CD4^+Foxp3^+$ en el bazo.

En nuestro laboratorio se ha demostrado que la infección con *T. crassiceps* recluta AAMΦ´s a la cavidad peritoneal. Aquí se observó que la infección con una alta dosis de metacestodos ocasionó que ésta misma población de AAMΦ´s apareciera. Como se muestra en la figura 2A (artículo 4), conforme avanza la infección y más claramente a partir de la semana 8, la expresión de genes como Arginasa-1, TREM2, Relm α, Ym1, PDL1, PDL2 es abundante y, sorpresivamente, también de TNF-α. Además, utilizando citometría se demostró que a las 8 semanas de infección hay células $F480^+$ $PDL1^+$ $PDL2^+$ MR^+ sugiriendo un potencial supresor para los AAMΦ´s (figura 2C artículo 4).

Los diferentes modelos murinos de enfermedades infecciosas han dejado ver que en realidad existen diferentes poblaciones con capacidad reguladora/supresora. La población de células T reguladoras $Foxp3^+$ es una de ellas, que además es una de las más ampliamente estudiadas. Por lo tanto se analizó, la posibilidad de, que al igual que en el caso de los AAMΦ´s, se elevara el porcentaje de células T reguladoras $Foxp3^+$ en ganglios mesentéricos y en bazo, como una parte adicional de los eventos de inmunoregulación inducidos por *Taenia crassiceps.* En la figura 2C (artículo 4), se observa que dicho aumento no se dió; de hecho, la infección causó una disminución en el porcentaje basal de las células T reguladoras, al menos en el bazo.
Estos datos sugieren que este céstodo indujo abundantes AAMΦ´s pero no células T $Foxp3^+$ y que los AAMΦ´s son suficientes para dominar la RI de su hospedero y*T. crassiceps* pueda establecerse.

-La infección crónica (8 semanas) con *T. crassiceps* redujo significativamente la severidad de la EAE, asociada a un menor infiltrado celular hacia la medula espinal.

Una vez que se demostró que la infección con 40 metacestodos indujo una fuerte respuesta tipo Th2 y que ésta se asemeja a la infección de ratones susceptibles (BALB/c), el objetivo fue demostrar que estos mecanismos alteraran el curso del modelo murino de EAE. Primero, fue notorio que la infección crónica con *T. crassiceps* redujo considerablemente los signos clínicos típicos de la EAE. Como se puede ver en la figura 3A (artículo 4) los ratones no infectados presentaron los signos clásicos de la EAE a partir del día 9 posterior a la administración del MOG $_{35-55}$. También se observó que a los 21 días post-inmunización se alcanzó el punto más alto (puntaje de 3) en cuanto a severidad, lo cual significa parálisis en ambas patas traseras. De manera interesante, en esta misma figura se observa que los ratones previamente infectados mostraron signos de EAE al día 9, si bien los signos fueron notablemente menos graves, siendo cuando mucho flacidez de la cola (puntaje de 0.5).
En la figura 3B de este mismo artículo 4 se observó una infiltración celular muy intensa en la médula espinal lumbar en los ratones no infectados, mientras que la presencia de *T. crassiceps* ocasionó nula o en algunos casos muy baja infiltración celular, correlacionando así con los signos clínicos observados.

-La presencia de *T. crassiceps* ocasionó un perfil mixto de citocinas Th2/Th17.

Con el objetivo de comprender mejor cual es el impacto de la infección con *T. crassiceps* en el sistema inmune del hospedero durante el desarrollo de la EAE, se analizó el nivel de citocinas circulantes, así como en sobrenadante de células de bazo re-estimuladas con el péptido encefalitogénico MOG $_{35-55}$. Se observó en la figura 4A (artículo 4), que los ratones no infectados tuvieron un nivel bajo de IL4 circulante, mientras que por el contrario y como era de esperarse, los ratones infectados presentaron altos niveles de esta citocina, mostrando así una clara polarización hacia la respuesta tipo Th2. Como contraparte y de manera sorpresivam se encontró que el nivel de IFN γ (Th1) fue similar (figura 4B artículo 4). Recientemente, se ha observado que en las enfermedades autoinmunes se sobre-produce la IL-17, la cual parece ser muy importante para que este tipo de enfermedades se desarrollen[101-103]; por lo tanto se evaluó la producción de IL-17. De manera inesperada, el grupo de los ratones infectados

que presentó signos muchos menos graves, presentó niveles más altos de IL-17 en suero comparados con los ratones no infectados con EAE (figura 4C artículo 4). Es bien conocido que la citocina TNF α tiene un alto potencial pro-inflamatorio y tiene un papel importante en la EAE, por lo tanto también se analizó la producción de esta citocina. En la figura 4D (artículo 4) se observó una producción significativamente más baja, sobre todo en los primeros 15 días post-inmunización, en los ratones crónicamente infectados comparados con los ratones no infectados. Por lo tanto, los ratones que presentan menos severidad en los signos de la EAE tuvieron altos niveles de IL-17 y de IL-4.

-La infección crónica con *T. crassiceps* ocasionó una baja respuesta proliferativa anti- MOG $_{35-55}$ en bazo, asociada con el aumento de marcadores de AAMΦ´s pero no de células CD4 $^{+}$ Foxp3 $^{+}$

Es bien sabido que la infección con *T. crassiceps* induce anergia en el bazo a Ag´s de este parásito así como a estímulos no relacionados. En esta parte se decidió analizar la generación de clonas anti-MOG$_{35-55}$ mediante la respuesta proliferativa en bazo como una probable causa de menor infiltrado observado en la médula espinal. En el día en el que los signos de la EAE alcanzaron su mayor puntaje (21 d.p.i.) se extrajeron las células totales de bazo y se probó la capacidad proliferativa de 2 maneras. En la primera se re-estimularon con 50 μg de MOG $_{35-55}$ por 3 días y en la segunda se eliminaron las células adherentes mientras que las células no adherentes se sembraron en placas cubiertas con Ac´s anti-CD3. Después, se analizó la incorporación de (^{3}H) timidina en ambos cultivos y encontramos que las células provenientes de ratones infectados no proliferaron ante MOG $_{35-55}$ ni ante el estímulo anti-CD3, como se observa en la figura 5A y 5B respectivamente (artículo 4). Después de que encontramos este defecto en los ratones infectados se decidió buscar, de igual manera en el pico de la EAE, si había algún aumento en el porcentaje de AAMΦ´s ó de células CD4 $^{+}$ Foxp3^{+} mediante la técnica de RT-PCR y de FACS, respectivamente. Primero, se observó que el porcentaje de células CD4^{+} Foxp3^{+} en los ratones no infectados inmunizados casi se triplicó comparado con los ratones sanos (6±3 vs. 16±4, respectivamente), sorpresivamente encontramos que este mismo aumento se dio en los ratones infectados inmunizados comparado con los ratones sanos, sin embargo, este aumento fue similar comparado con los ratones no infectados cursando con EAE (16±4 vs. 17±2 respectivamente). Por lo tanto, la baja respuesta proliferativa no se pudo asociar a un incremento de células CD4^{+} Foxp3^{+} en el bazo (Figura 5C artículo 4). De manera adicional se analizó la capacidad

supresora de los MΦ´s peritoneales y la expresión de genes asociados a AAMΦ´s en células adherentes en el bazo de estos mismos ratones. Como se puede ver en la figura 5D (artículo 4) las células T co-cultivadas con MΦ´s obtenidos de ratones no infectados inmunizados presentan una incorporación de (^{3}H) timidina mucho mas alta que las células T co-cultivadas con MΦ´s obtenidos de ratones infectados inmunizados demostrando así que solo estos últimos poseen capacidad supresora. En el caso del análisis de las células adherentes de bazo se observó sobre-expresión de los principales marcadores de AAMΦ´s (Ym-1, Relm α y Arginasa-1) en las células obtenidas de los ratones infectados (figura 5E artículo 4).

-La presencia de *T. crassiceps* suprimió la respuesta Th17 y promovió una respuesta Th2 reguladora de manera MOG_{35-55}-especifica, ambos fenómenos en el bazo.

Como ya se mencionó la producción de IL-17 es fundamental en las enfermedades autoinmunes inflamatorias, aquí previamente encontramos que los ratones infectados que presentaron menor gravedad tenían un nivel mas alto de IL-17 circulante en comparación con los ratones no infectados. En esta parte se decidió analizar el perfil de las clonas MOG_{35-55}-especificas que se generaron en el bazo en presencia ó ausencia de *T. crassiceps*. En la figura 6 de este artículo mostramos la producción de citocinas en respuesta al péptido encefalitogénico MOG_{35-55}. Se observó una alta producción de IL-4 e IL-10 por parte de los esplenocitos obtenidos de ratones con infección crónica de *T. crassiceps* y con EAE(figura 6A y 6E, respectivamente artículo 4). Este hecho explica parcialmente la menor severidad de la EAE observada en estos ratones ya que estas citocinas no tienen la capacidad de iniciar respuestas inflamatorias exacerbadas, mas bien se relacionan con la etapa de resolución de la EAE. Por el contrario, al analizar la producción de citocinas de esplenocitos de ratones no infectados encontramos un perfil MOG_{35-55}-especifico distinto, caracterizado por una baja producción de IL-4, nula producción de IL-10 y altos niveles de IL-17. Mientras que no hubo diferencias en la producción de IFN-γ y TNF-α importantes citocinas para el desarrollo de la EAE (figura 6B y 6D, respectivamente artículo 4). Así, estos datos nos sugieren que en presencia de *T. crassiceps* las clonas que pueden reconocer al péptido MOG_{35-55} tuvieron un perfil mixto Th2/Th1mientras que la ausencia de la infección generó clonas MOG_{35-55}-

especificas Th17/Th1, ambos tipos de respuesta relacionadas con un curso grave de la EAE.

-Los ratones previamente infectados tienen sobreexpresados los genes marcadores de AAMΦ´s en el cerebro y menor cantidad de células $CD3^+$ $Foxp3^-$.

Finalmente, debido a que la respuesta inflamatoria ocurre en el SNC también se decidió analizar el impacto de la infección en este órgano distante. Para abordar esto nuevamente realizamos ensayos de RT-PCR y citometría de flujo. En la primera parte se observó que en el pico de la EAE, los ratones crónicamente infectados sobre-expresaron los marcadores de AAMΦ´s (Relm α, Ym1, TREM2 y Arginasa-1) que además están relacionados con reparación de tejido, mientras que los ratones no infectados presentaron un nivel similar de expresión a los ratones no inmunizados (figura 7A). También se analizó la expresión de genes de citocinas que generan reacciones inflamatorias exacerbadas (TNF-α y la subunidad p19 de IL-23). En la figura 7B (artículo 4) se puede ver que el TNF-α está sobre-expresado en los ratones no infectados, comparado con los ratones infectados, lo que fue corroborado por PCR en tiempo real (Figura 7C artículo 4). Por el contrario mostró que a pesar de tener menos signos de EAE los ratones infectados presentaron un expresión ligeramente más alta de (p19) IL-23 que los ratones no infectados, igualmente inmunizados. En la última parte de los ensayos de RT-PCR se decidió analizar la expresión de factores de transcripción para subpoblaciones Th1 (T-bet), Th2 (GATA3) y T reguladoras (Foxp3). En la figura 7D (artículo 4) se observó una mayor expresión de T bet en los ratones no infectados, sugiriendo la presencia de células Th1.

Una vez que se observó que los AAMΦ´s generados por la infección de *T. crassiceps* podrían estar aminorando la inflamación en el SNC, se decidió abordar la participación de células $Foxp3^+$ a pesar de que en órganos periféricos no encontramos un aumento significativo de esta población. El análisis de citometría de flujo (figura 7E artículo 4) comprobó lo observado con los cortes de médula espinal, es decir, que los ratones no infectados presentaron un aumento del doble de células $CD3^+$ $Foxp3^-$ (consideradas efectoras) comparados con los ratones sanos (23±3 vs. 10±1, respectivamente), sugiriendo así que infiltraron células efectoras por lo observado con el ensayo de RT-PCR , en lugar de células reguladoras, mientras que los ratones previamente infectados

e inducidos a EAE mostraron un nivel similar de células $CD3^{+}$ $Foxp3^{-}$ comparado con ratones sanos (10±1 vs. 9.3±, respectivamente).

Así se encontró que la infección previa con *T. crassiceps*, que reside en la cavidad peritoneal, puede aminorar reacciones de tipo inflamatorio y que la inmunomodulación inducida por este parásito es tan fuerte que impacta en sitios lejanos del lugar donde reside como sería bazo, médula espinal y cerebro.

8. DISCUSIÓN GENERAL

Los helmintos parásitos son capaces de establecer infecciones de tipo crónico en sus diversos hospederos. En este tipo de infecciones dichos parásitos han desarrollado complejas y eficientes formas para regular/suprimir la R.I. de sus hospederos[3, 4]. Por lo que, la regulación inmune por helmintos se ha convertido actualmente en un área de gran interés. Debido a la intensa investigación, ahora se sabe que los helmintos polarizan la R.I. hacia el perfil Th2 tanto en modelos experimentales como en estudios realizados a pacientes infectados con alguno de estos parásitos. Sin embargo, los mecanismos y moléculas involucrados en tal regulación aún no están completamente entendidos. Además, la relevancia de esta respuesta Th2 en protecciónparece depender del tipo de parásito y del sitio de localización de éste. Recientemente, se ha identificado una población única de macrófagos inducidos por infecciones por helmintos. Se cree que estos macrófagos podrían determinar el curso de las diferentes infecciones en donde se han descrito, sin embargo, el papel que tiene esta población celular parece ser diverso[14, 15].

Taenia crassiceps es un cestodo que polariza la respuesta inmune hacia Th2 e induce el reclutamiento de una población de AAMΦ`s con potencial supresor[64], sin embargo la participación de estos en la susceptibilidad o resistencia a *T. crassiceps*no ha sido explorada.

En esta parte de la tesis intentamos profundizar en cuanto a la participación de estos AAM Φ`s en la infección por *T. crassiceps* y su posible efecto sobre fenómenos inmunes no relacionados como enfermedades de tipo autoinmune.

Primero, se sabe que *T. crassiceps* puede infectar de manera diferencial a diferentes cepas de ratones por lo que estuvimos interesados en conocer si esta susceptibilidad diferencial se podía asociar con la presencia de AAM Φ`s. Es bien conocido que los ratones de la cepa BALB/c son altamente susceptibles a la infección i.p. con *T. crassiceps*comparados con los ratones de la cepa C57BL/6. Así, después de la infección con 10 metacestodos los ratones BALB/c presentaron una alta carga parasitaria y una clara polarización de la R.I. hacia Th2, mientras que los ratones C57BL/6 presentan un número de parásitos significativamente menor y no desarrollaron una respuesta Th2, confirmando hallazgos anteriores. Una vez que observamos los datos mencionados

decidimos analizar, durante el curso de la infección, el genotipo y fenotipo de los macrófagos. De manera interesante encontramos que los macrófagos reclutados en la cavidad peritoneal de la cepa susceptible (BALB/c) fue una población de macrófagos expresando Ym-1^+, Relm α^+, TREM-2^+, Arginasa-1^+ (AAMΦ´s) desde la semana 2 y que dicha población permaneció por lo menos hasta la semana 8 después de la infección. En contraste, los macrófagos reclutados en la cepa resistente (C57BL/6) solo presentaron una débil y transitoria expresión de estos marcadores en la semana 2 ya que no se sostuvo durante el curso de la infección concomitantemente a la eliminación del parásito. Estas células también se analizaron por citometría de flujo y se pudo observar nuevamente que solo los ratones BALB/c sostienen la población de macrófagos con un fenotipo supresor ya que sobrexpresaron las moléculas PDL1 y PDL2, previamente asociadas con la capacidad supresora de esta población de macrófagos. Por otro lado, en la parte final de este trabajo demostramos que los ratones de la cepa C57BL/6 pueden hacerse susceptibles a *T. crassiceps* si se utiliza una dosis alta de metacestodos.

Cuando se aumentó la dosis de 20 a 40 metacestodos se observó que en la semana 8 post-infección estos ratones tenían cargas parasitarias mas altas de lo que usualmente se encuentra en los ratones BALB/c. Junto con lo anterior, se desarrolló una fuerte respuesta tipo Th2 y reclutamiento de AAM Φ`s. Como ya se mencionó, estos eventos de inmunomodulación no se encontraron en ratones de esta misma cepa pero infectados con 10 metacestodos. De esta manera, observamos una vez mas que solo en los hospederos en los cuales *T. crasscieps* puede establecerse (en este caso ratones C57BL/6 infectados con 40 metacestodos) se desarrolla una respuesta Th2 y en consecuencia se recluta un alto número de AAM Φ`s.

Estos datos sugieren que la generación y reclutamiento de los AAM Φ`s es indispensable para el establecimiento de *T. crassiceps* en su hospedero.

Observaciones como el hecho de demostrar que el cambio hacia una respuesta Th2 es necesario para la proliferación de *T. crassiceps* y que los AAM Φ `s se desarrollan solo en cepas susceptibles nos llevo a cuestionar si estos macrófagos podrían fungir como la población celular efectora de las citocinas Th2 en el modelo de infección por *T. crassiceps.* Para resolver esto utilizamos liposomas conteniendo clodronato, que únicamente llegan a células fagociticas ya que no tienen la capacidad de atravesar de forma pasiva las membranas celulares y al acumularse altos niveles de clodronato ocasionan muerte por apoptosis. La administración de estos liposomas demostró que efectivamente los AAMΦ`s eran eliminados, mientras que otras poblaciones como los

eosinófilos (figura articulo 3) y las DC permanecieron en porcentajes similares o aumentaron, respectivamente. Demostrando así la especificidad de los liposomas con clodronato para eliminar a los macrófagos. De manera sobresaliente, demostramos que la eliminación total así como la eliminación de forma temprana de los macrófagos durante la infección con *T. crassiceps* ocasionó una notable disminución en el número de parásitos recuperados de la cavidad peritoneal de los ratones tratados.

En conjunto estos resultados nos confirmaron que efectivamente estos macrófagos son necesarios para que se establezca una infección exitosa por parte de *T. crassiceps*. Recientemente, se demostró que la producción de ON dependiente de la enzima iNOS, predominantemente expresada en los macrófagos, es importante para impedir el crecimiento de *T. crassiceps*[104], por lo que la disminución en la producción de ON debido a la inducción preferentemente de Arginasa-1 en lugar de iNOS, podría ser uno de los principales objetivos, junto con la disminución en la producción de citocinas pro-inflamatorias, por parte de este cestodo para inducir el cambio en el estado de activación de CAMΦ`s hacia AAMΦ`s para poder establecerse en su hospedero.

De igual forma que con *T. crassiceps*, se ha descrito también que parásitos intracelulares podrían utilizar esta polarización de los macrófagos como un mecanismo de evasión/regulación inmune.

El parásito flagelado *Trypanosoma brucei* induce esta población de AAMΦ`s únicamente en hospederos susceptibles a este protozoario en los cuales establece una infección de tipo crónica, mientras que en los hospederos en donde prevalecen los CAMΦ`s el parásito es eliminado junto con un alto nivel de daño colateral en el hospedero[105]. Otros protozoarios parásitos parecen también favorecerse de la inducción de los AAMΦ`s. Por ejemplo, *Leishmania major* y *Leishamnia infantum* causan la disminución en la producción de IL-12 y aumento de la actividad de Arginasa-1 por lo que se diseminan mucho mas en su hospedero[106]. El efecto benéfico de la inducción de AAMΦ`s para *Leishmania* también se observó en ratones deficientes de IL-4Rα en los cuales se retrasó el progreso de la enfermedad a pesar de tener una producción de citocinas tipo Th2 normal[107]. El agente causal de la enfermedad de Chagas, *Trypanosoma cruzi,* también utiliza a los AAMΦ`s como vía de escape ya que una característica de los ratones susceptibles (BALB/c) a este parasito es la sobreexpresión de Arginasa-1 mientras que los ratones resistentes (C57BL/6) expresaron predominantemente iNOS[108]. En dicho fenómeno la cruzipaina, Ag secretado por *T. cruzi,*parece tener una gran relevancia[109]. En años recientes se demostró que

*Toxoplasma gondii*podría también generar AAMΦ`s como mecanismo de evasión inmune[110].

En el caso de *Cryptococcus neoformans* se encontró que este hongo parásito prolifera de manera no restringida cuando estos macrófagos se encuentran en estado de activación alternativo[111]. Fortaleciendo así el modelo propuesto de que la generación de estos AAMΦ`s podría ser un mecanismo de regulación inmune utilizado por algunos parásitos para poder proliferar, al que *T. crassiceps* se ajusta bien.

De manera contraria al caso de *T. crassiceps*, los AAMΦ`s parecen llevar a cabo en otros helmintos parásitos, diversas y en algunos casos opuestas funciones a las de facilitar la colonización del hospedero. La participación de estos AAMΦ`s ha sido estudiada en otros helmintos y a diferencia de nuestros resultados la eliminación de estos macrófagos impide la eliminación del nematodo residente del tracto gastrointestinal *Heligmosomoides polygyrus*[52], mostrando que a pesar de que la inducción de estos AAMΦ`s parece ser un mecanismo intrínseco de los helmintos parásitos, la función de los AAMΦ`s difiere según el parásito del que se trate. En el caso del trematodo *Schistosoma mansoni* los AAMΦ`s son necesarios para contrarrestar la fuerte reacción inflamatoria tipo Th17 inducida por el granuloma hepático que como resultado final ocasiona la muerte del hospedero[14]. En infecciones experimentales causadas por helmintos parásitos tales como *Brugia malayi*, *Litomosoides sigmodontis* y *Nippostrongylus brasiliensis* se sabe que estos AAMΦ`s poseen capacidad supresora sobre células T, sin embargo, la relevancia de estas células en cuanto al curso de la infección o protección del hospedero no ha sido explorada[14].

Finalmente, analizamos si la fuerte inmunomodulación inducida por *T. crassiceps* podría impactar de manera considerable el desarrollo de reacciones inflamatorias exacerbadas no relacionadas. Para este objetivo utilizamos el modelo murino de Encefalomielitis Autoinmune Experimental (EAE). Notamos que los ratones previamente infectados con *T. crassiceps* presentaron un nivel casi nulo de los signos clínicos asociados a la EAE. Este efecto fue dependiente de la cantidad de parásitos encontrados en la cavidad peritoneal, ya que en ratones en los que no logró establecerse abundantemente *T. crassiceps*, es decir, encontramos números tan bajos como 15 parásitos a la semana 8 de infecciónmostraron un curso de la EAE mucho más grave, mientras que en ratones en donde la carga parasitaria fue de 800-1000 metacestodos en el peritoneo, la EAE se encontró casi completamente abrogada. Además, hallazgos

como la sobreexpresión de marcadores de AAMΦ`s en el SNC y la fuerte anergia en células de bazo de ratones infectados e inducidos a EAE sugieren que en el caso de los ratones infectados, estos macrófagos podrían migrar a los sitios en donde se esta llevando acabo una reacción inflamatoria exacerbada que amenace la integridad del hospedero. Por lo tanto es necesario estudiar con mayor profundidad el papel de los AAMΦ`s en el efecto de *T. crassiceps* sobre la EAE.

Reportes previos demostraron que otros helmintos de igual manera afectaron el desarrollo de la EAE, sin embargo, nuestros resultados mostraron que el impacto sobre la EAE es mayor con *T. crassiceps* que con cualquiera de otros helmintos estudiados. Por ejemplo, la infección con *Schistosoma mansoni*redujo la incidencia de la EAE y retrasó el inicio de la misma, pero conforme avanzó el tiempo los ratones infectados presentaron una severidad similar de EAE comparados con los ratones no infectados[83]. Por lo tanto, el efecto de la infección por *Schistosoma* sobre la EAE fue menor y transitorio que el observado en la infección con *T. crassiceps.* A pesar de que en ambos modelos la inducción de la EAE fue en tiempos crónicos de infección (6 semanas con *Schistosoma* y 8 semanas con *T. crassiceps*). Una probable explicación es que en el caso de *Schistosoma* se genera un granuloma hepático por la deposición de huevecillos y se ha demostrado que poblaciones como los AAMΦ`s se reclutan al sitio del granuloma y son necesarios para controlar la inmunopatología y evitar que el hospedero muera. Así, estas poblaciones reguladoras serían menos eficientes y estarían disponibles en menor cantidad al momento en el que la EAE se estuviera iniciando. En contraste, la infección con *T. crassiceps* induce abundantes AAMΦ`s que no tendrían que migrar hacia otro lugar para aminorar otros eventos inflamatorios asociados a la propia infección como los granulomas.

En la infección experimental con*Fasciola hepatica*también se observó un efecto sobre la EAE. En este caso solo se encontró un ligero retraso en el curso de la EAE, podría deberse a que los ratones únicamente tenían un día de infección antes de la inmunización con MOG $_{35\text{-}55}$. Aun así, la infección con este trematodo ocasionó una disminución de las respuestas tipo Th1 y Th17[86].El nematodo*Trichinella spiralis* también tiene la capacidad de reducir la severidad de la EAE en ratas. En la infección con *T. spiralis*se identificó a las células T reguladoras Foxp3^{+}y a la IL-10 como responsables de dicho efecto, en contraste con *T. crassiceps*que parece no inducir células T reguladoras Foxp3^{+}, sino por el contrario suprimió el porcentaje basal de esta población reguladora[81]. Estas T regs tampoco parecen llevar a cabo alguna función

sobre el desarrollo de la EAE, ya que el porcentaje de esta población fue similar en los ratones no infectados con un score clínico mucho mas severo además de que no detectamos la expresión de Foxp3 en el cerebro en el pico más alto de los síntomas de la EAE, en ambos grupos de ratones. Por lo tanto, los mecanismos de regulación generados por los helmintos son diversos. Recientemente se demostró que los factores de transcripción GATA3 y Foxp3 pueden interactuar y bloquearse mutuamente[112], surgiendo así la probable explicación del porqué en el caso del modelo de*T. crassiceps*, en el cual encontramos una fuerte respuesta Th2 sistémica, hay niveles muy bajos de células T $Foxp3^{+}$.

Un hallazgo sobresaliente ha sido el hecho de encontrar niveles más altos de IL-17 circulante y al mismo tiempo, menor producción de esta citocina de manera MOG_{35-55}-específica en los ratones infectados e inmunizados, los cuales desarrollaron menos síntomas de EAE, además de producir la misma cantidad de IFNγ en estas mismas células de bazo. Ya que la disminución de citocinas Th1 (IFNγ) y Th17 (IL-17) hasta el momento ha sido la principal característica encontrada en los modelos en los que se retrasó o redujo la severidad de la EAE. Por lo tanto, en nuestro modelo podría encontrarse una fuente diferente a las células T que produzcan altos niveles de IL-17, que de alguna manera generen un microambiente favorable para *T. crassiceps* (i.e. contra-regule la diferenciación de células Th1). La fuente de IL-17 podrían ser células de la inmunidad innata como linfocitos gamma-delta (γ), células NKT invariantes (iNKT) y células mieloides como recientemente se ha descrito[113] ó probablemente exista una población mixta Th2/Th17 que permita el crecimiento de *T. crassiceps*, ya que la producción de ambas citocinas fue muy similar.

Adicionalmente, investigamos si la administración del Ag soluble total de *T. crassiceps* podía modular la respuesta inmune y alterar el curso de la EAE. Encontramos que la administración de 150 μg de este Ag, distribuida en 3 inyecciones por semana a partir del día de la inmunización con MOG_{35-55} no tuvo efecto significativo sobre el desarrollo de la EAE, demostrando así que se requiere de los metacestodos vivos de *T. crassiceps* ó de los Ags Excretados/Secretados para modificar las reacciones inflamatorias que pudieran surgir en el hospedero. Estos datos coinciden con un reporte previo en el que la infección con el protozoario *Trypanosoma cruzi* disminuyó la EAE, mientras que la administración del Ag total de *T. cruzi* no tuvo el mismo efecto[114].

En conjunto los datos obtenidos en este trabajo y los reportados previamente sugieren que *T. crassiceps* esta dentro del grupo de parásitos que inducen un cambio en la activación de los macrófagos para generar un ambiente permisible para su crecimiento y que el surgimiento de esta población reguladora esta influido también por el fondo genético de los hospederos. Mas aún, estos AAMΦ`s podrían ser los responsables de aminorar reacciones inflamatorias exacerbadas en el caso de hospederos parasitados por*T. crassiceps* mientras que, contrario a otros modelos, las células T reguladoras no parecen tener un papel relevante. Por lo tanto, comprender los mecanismos subyacentes en la generación de estos AAMΦ`s permitirá proponer tratamientos para modular el estado de activación de los macrófagos y poder combatir de mejor manera a las enfermedades parasitarias y/ó autoinmunes.

9. CONCLUSIONES

1- La respuesta inmune tipo Th2 y la población de AAMΦ´s se genera y recluta únicamente en las cepas de ratón susceptibles a *Taenia crassiceps*.

2- La población de AAMΦ´s aparece en la cavidad peritoneal apartir de la semana 3 después de la infección, en ratones BALB/c.

3- La eliminación temprana de los AAMΦ´s restringe el crecimiento de *Taenia crassiceps*.

4-La anergia observada en los esplenocitos dehospederos parasitados por *Taenia crassiceps* se revierte en ausencia de los AAMΦ´s.

5- Es posible revertir la resistencia de la cepa C57BL/6 infectando con 40 metacestodos de *Taenia crassiceps,* lo que generauna respuesta tipo Th2 y el reclutamiento de AAMΦ´s que es un ambiente permisible para este parásito.

6-La infección previa con *Taenia crassiceps* inhibe el desarrollo de la EAE a través de:

- Un menor infiltrado inflamatorio hacia la médula espinal.
- Impedir la generación de clonas tipo Th-17 MOG_{35-55} especificas.
- Inducir una respuesta tipo Th2 MOG_{35-55} especifica, considerada como no encefalitogénica.

7-La infección con*Taenia crassiceps*induce la expresión de marcadores de AAMΦ´s en el cerebro de ratones con EAE.

8-La infección con *Taenia crassiceps* evita la migración de células $CD3^{+}$al cerebro durante la EAE.

10. REFERENCIAS

1. Gordon S, Martinez FO. Alternative activation of macrophages: mechanism and functions. Immunity 2010;32:593-604.
2. Loverde PT, Li C, Maizels RM, Geary TG, Colley DG. Molecular helminthology: an integrated approach. Am J Trop Med Hyg 2002;66:346-7.
3. Maizels RM, Yazdanbakhsh M. Immune regulation by helminth parasites: cellular and molecular mechanisms. Nat Rev Immunol 2003;3:733-44.
4. Maizels RM, Balic A, Gomez-Escobar N, Nair M, Taylor MD, Allen JE. Helminth parasites--masters of regulation. Immunol Rev 2004;201:89-116.
5. Saenz SA, Siracusa MC, Perrigoue JG, Spencer SP, Urban JF, Jr., Tocker JE, Budelsky AL, Kleinschek MA, Kastelein RA, Kambayashi T, Bhandoola A, Artis D. IL25 elicits a multipotent progenitor cell population that promotes T(H)2 cytokine responses. Nature;464:1362-6.
6. Owyang AM, Zaph C, Wilson EH, Guild KJ, McClanahan T, Miller HR, Cua DJ, Goldschmidt M, Hunter CA, Kastelein RA, Artis D. Interleukin 25 regulates type 2 cytokine-dependent immunity and limits chronic inflammation in the gastrointestinal tract. J Exp Med 2006;203:843-9.
7. Taylor BC, Zaph C, Troy AE, Du Y, Guild KJ, Comeau MR, Artis D. TSLP regulates intestinal immunity and inflammation in mouse models of helminth infection and colitis. J Exp Med 2009;206:655-67.
8. Ito T, Wang YH, Duramad O, Hori T, Delespesse GJ, Watanabe N, Qin FX, Yao Z, Cao W, Liu YJ. TSLP-activated dendritic cells induce an inflammatory T helper type 2 cell response through OX40 ligand. J Exp Med 2005;202:1213-23.
9. Ierna MX, Scales HE, Schwarz H, Bunce C, McIlgorm A, Garside P, Lawrence CE. OX40 interactions in gastrointestinal nematode infection. Immunology 2006;117:108-16.
10. Goodridge HS, Marshall FA, Wilson EH, Houston KM, Liew FY, Harnett MM, Harnett W. 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 2004;113:491-8.
11. Steinfelder S, Andersen JF, Cannons JL, Feng CG, Joshi M, Dwyer D, Caspar P, Schwartzberg PL, Sher A, Jankovic D. The major component in schistosome eggs responsible for conditioning dendritic cells for Th2 polarization is a T2 ribonuclease (omega-1). J Exp Med 2009;206:1681-90.
12. Wilson MS, Maizels RM. Regulatory T cells induced by parasites and the modulation of allergic responses. Chem Immunol Allergy 2006;90:176-95.
13. McSorley HJ, Harcus YM, Murray J, Taylor MD, Maizels RM. Expansion of Foxp3+ regulatory T cells in mice infected with the filarial parasite Brugia malayi. J Immunol 2008;181:6456-66.
14. Reyes JL, Terrazas LI. The divergent roles of alternatively activated macrophages in helminthic infections. Parasite Immunol 2007;29:609-19.
15. Jenkins SJ, Allen JE. Similarity and diversity in macrophage activation by nematodes, trematodes, and cestodes. J Biomed Biotechnol;2010:262609.
16. Sakaguchi S, Sakaguchi N. Regulatory T cells in immunologic self-tolerance and autoimmune disease. Int Rev Immunol 2005;24:211-26.

17. Sakaguchi S, Ono M, Setoguchi R, Yagi H, Hori S, Fehervari Z, Shimizu J, Takahashi T, Nomura T. Foxp3+ CD25+ CD4+ natural regulatory T cells in dominant self-tolerance and autoimmune disease. Immunol Rev 2006;212:8-27.
18. Babu S, Blauvelt CP, Kumaraswami V, Nutman TB. Regulatory networks induced by live parasites impair both Th1 and Th2 pathways in patent lymphatic filariasis: implications for parasite persistence. J Immunol 2006;176:3248-56.
19. Baumgart M, Tompkins F, Leng J, Hesse M. Naturally occurring CD4+Foxp3+ regulatory T cells are an essential, IL-10-independent part of the immunoregulatory network in Schistosoma mansoni egg-induced inflammation. J Immunol 2006;176:5374-87.
20. Watanabe K, Carter JM, Neely-Burnam M, Colley DG. Relative imbalance between T regulatory cells and activated T cells in mice with differential morbidity in chronic Schistosoma mansoni infections. Parasite Immunol 2009;31:440-6.
21. Zaccone P, Burton O, Miller N, Jones FM, Dunne DW, Cooke A. Schistosoma mansoni egg antigens induce Treg that participate in diabetes prevention in NOD mice. Eur J Immunol 2009;39:1098-107.
22. Finney CA, Taylor MD, Wilson MS, Maizels RM. Expansion and activation of CD4(+)CD25(+) regulatory T cells in Heligmosomoides polygyrus infection. Eur J Immunol 2007;37:1874-86.
23. Rausch S, Huehn J, Kirchhoff D, Rzepecka J, Schnoeller C, Pillai S, Loddenkemper C, Scheffold A, Hamann A, Lucius R, Hartmann S. Functional analysis of effector and regulatory T cells in a parasitic nematode infection. Infect Immun 2008;76:1908-19.
24. Hartmann S, Schnoeller C, Dahten A, Avagyan A, Rausch S, Lendner M, Bocian C, Pillai S, Loddenkemper C, Lucius R, Worm M, Hamelmann E. Gastrointestinal nematode infection interferes with experimental allergic airway inflammation but not atopic dermatitis. Clin Exp Allergy 2009;39:1585-96.
25. Park HK, Cho MK, Choi SH, Kim YS, Yu HS. Trichinella spiralis: infection reduces airway allergic inflammation in mice. Exp Parasitol 2011;127:539-44.
26. Ronet C, Hauyon-La Torre Y, Revaz-Breton M, Mastelic B, Tacchini-Cottier F, Louis J, Launois P. Regulatory B cells shape the development of Th2 immune responses in BALB/c mice infected with Leishmania major through IL-10 production. J Immunol 2010;184:886-94.
27. Wilson MS, Taylor MD, O'Gorman MT, Balic A, Barr TA, Filbey K, Anderton SM, Maizels RM. Helminth-induced CD19+CD23hi B cells modulate experimental allergic and autoimmune inflammation. Eur J Immunol 2010;40:1682-96.
28. Mangan NE, Fallon RE, Smith P, van Rooijen N, McKenzie AN, Fallon PG. Helminth infection protects mice from anaphylaxis via IL-10-producing B cells. J Immunol 2004;173:6346-56.
29. Amu S, Saunders SP, Kronenberg M, Mangan NE, Atzberger A, Fallon PG. Regulatory B cells prevent and reverse allergic airway inflammation via FoxP3-positive T regulatory cells in a murine model. J Allergy Clin Immunol 2010;125:1114-1124 e8.
30. Terrazas CA, Terrazas LI, Gomez-Garcia L. Modulation of dendritic cell responses by parasites: a common strategy to survive. J Biomed Biotechnol 2010;2010:357106.
31. Gordon S. The macrophage: past, present and future. Eur J Immunol 2007;37 Suppl 1:S9-17.

32. Martinez FO, Helming L, Gordon S. Alternative activation of macrophages: an immunologic functional perspective. Annu Rev Immunol 2009;27:451-83.
33. Gordon S, Fraser I, Nath D, Hughes D, Clarke S. Macrophages in tissues and in vitro. Curr Opin Immunol 1992;4:25-32.
34. Silva-Teixeira DN, Ferreira MG, Nogueira-Machado JA, Doughty BL, Goes AM. Human giant cell formation induced in vitro by Schistosoma mansoni antigens. Braz J Med Biol Res 1993;26:609-13.
35. Joshi AD, Schaller MA, Lukacs NW, Kunkel SL, Hogaboam CM. TLR3 modulates immunopathology during a Schistosoma mansoni egg-driven Th2 response in the lung. Eur J Immunol 2008;38:3436-49.
36. Gupta N, Gupta R, Rajwanshi A, Bakshi J. Multinucleated giant cells in HIV-associated benign lymphoepithelial cyst-like lesions of the parotid gland on FNAC. Diagn Cytopathol 2009;37:203-4.
37. Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. Trends Immunol 2004;25:677-86.
38. Fujiwara N, Kobayashi K. Macrophages in inflammation. Curr Drug Targets Inflamm Allergy 2005;4:281-6.
39. Gordon S. Alternative activation of macrophages. Nat Rev Immunol 2003;3:23-35.
40. Loke P, MacDonald AS, Allen JE. Antigen-presenting cells recruited by Brugia malayi induce Th2 differentiation of naive CD4(+) T cells. Eur J Immunol 2000;30:1127-35.
41. MacDonald AS, Maizels RM, Lawrence RA, Dransfield I, Allen JE. Requirement for in vivo production of IL-4, but not IL-10, in the induction of proliferative suppression by filarial parasites. J Immunol 1998;160:1304-12.
42. Loke P, Nair MG, Parkinson J, Guiliano D, Blaxter M, Allen JE. IL-4 dependent alternatively-activated macrophages have a distinctive in vivo gene expression phenotype. BMC Immunol 2002;3:7.
43. Loke P, MacDonald AS, Robb A, Maizels RM, Allen JE. Alternatively activated macrophages induced by nematode infection inhibit proliferation via cell-to-cell contact. Eur J Immunol 2000;30:2669-78.
44. Nair MG, Cochrane DW, Allen JE. Macrophages in chronic type 2 inflammation have a novel phenotype characterized by the abundant expression of Ym1 and Fizz1 that can be partly replicated in vitro. Immunol Lett 2003;85:173-80.
45. Nair MG, Gallagher IJ, Taylor MD, Loke P, Coulson PS, Wilson RA, Maizels RM, Allen JE. Chitinase and Fizz family members are a generalized feature of nematode infection with selective upregulation of Ym1 and Fizz1 by antigen-presenting cells. Infect Immun 2005;73:385-94.
46. Taylor MD, Harris A, Nair MG, Maizels RM, Allen JE. F4/80+ alternatively activated macrophages control CD4+ T cell hyporesponsiveness at sites peripheral to filarial infection. J Immunol 2006;176:6918-27.
47. Gause WC, Urban JF, Jr., Stadecker MJ. The immune response to parasitic helminths: insights from murine models. Trends Immunol 2003;24:269-77.
48. Pesce JT, Ramalingam TR, Wilson MS, Mentink-Kane MM, Thompson RW, Cheever AW, Urban JF, Jr., Wynn TA. Retnla (relmalpha/fizz1) suppresses helminth-induced Th2-type immunity. PLoS Pathog 2009;5:e1000393.
49. Raes G, Beschin A, Ghassabeh GH, De Baetselier P. Alternatively activated macrophages in protozoan infections. Curr Opin Immunol 2007;19:454-9.

50. Siracusa MC, Reece JJ, Urban JF, Jr., Scott AL. Dynamics of lung macrophage activation in response to helminth infection. J Leukoc Biol 2008;84:1422-33.
51. Marsland BJ, Kurrer M, Reissmann R, Harris NL, Kopf M. Nippostrongylus brasiliensis infection leads to the development of emphysema associated with the induction of alternatively activated macrophages. Eur J Immunol 2008;38:479-88.
52. Anthony RM, Urban JF, Jr., Alem F, Hamed HA, Rozo CT, Boucher JL, Van Rooijen N, Gause WC. Memory T(H)2 cells induce alternatively activated macrophages to mediate protection against nematode parasites. Nat Med 2006;12:955-60.
53. Pearce EJ, MacDonald AS. The immunobiology of schistosomiasis. Nat Rev Immunol 2002;2:499-511.
54. Stadecker MJ, Kamisato JK, Chikunguwo SM. Induction of T helper cell unresponsiveness to antigen by macrophages from schistosomal egg granulomas. A basis for immunomodulation in schistosomiasis? J Immunol 1990;145:2697-700.
55. Smith P, Walsh CM, Mangan NE, Fallon RE, Sayers JR, McKenzie AN, Fallon PG. Schistosoma mansoni worms induce anergy of T cells via selective up-regulation of programmed death ligand 1 on macrophages. J Immunol 2004;173:1240-8.
56. Herbert DR, Holscher C, Mohrs M, Arendse B, Schwegmann A, Radwanska M, Leeto M, Kirsch R, Hall P, Mossmann H, Claussen B, Forster I, Brombacher F. Alternative macrophage activation is essential for survival during schistosomiasis and downmodulates T helper 1 responses and immunopathology. Immunity 2004;20:623-35.
57. Donnelly S, Stack CM, O'Neill SM, Sayed AA, Williams DL, Dalton JP. Helminth 2-Cys peroxiredoxin drives Th2 responses through a mechanism involving alternatively activated macrophages. Faseb J 2008;22:4022-32.
58. Atochina O, Da'dara AA, Walker M, Harn DA. The immunomodulatory glycan LNFPIII initiates alternative activation of murine macrophages in vivo. Immunology 2008;125:111-21.
59. Donnelly S, O'Neill SM, Sekiya M, Mulcahy G, Dalton JP. Thioredoxin peroxidase secreted by Fasciola hepatica induces the alternative activation of macrophages. Infect Immun 2005;73:166-73.
60. Terrazas LI, Bojalil R, Govezensky T, Larralde C. Shift from an early protective Th1-type immune response to a late permissive Th2-type response in murine cysticercosis (Taenia crassiceps). J Parasitol 1998;84:74-81.
61. Raes G, Brys L, Dahal BK, Brandt J, Grooten J, Brombacher F, Vanham G, Noel W, Bogaert P, Boonefaes T, Kindt A, Van den Bergh R, Leenen PJ, De Baetselier P, Ghassabeh GH. Macrophage galactose-type C-type lectins as novel markers for alternatively activated macrophages elicited by parasitic infections and allergic airway inflammation. J Leukoc Biol 2005;77:321-7.
62. Brys L, Beschin A, Raes G, Ghassabeh GH, Noel W, Brandt J, Brombacher F, De Baetselier P. Reactive oxygen species and 12/15-lipoxygenase contribute to the antiproliferative capacity of alternatively activated myeloid cells elicited during helminth infection. J Immunol 2005;174:6095-104.
63. Rodriguez-Sosa M, Satoskar AR, Calderon R, Gomez-Garcia L, Saavedra R, Bojalil R, Terrazas LI. Chronic helminth infection induces alternatively activated macrophages expressing high levels of CCR5 with low interleukin-12 production and Th2-biasing ability. Infect Immun 2002;70:3656-64.

64. Terrazas LI, Montero D, Terrazas CA, Reyes JL, Rodriguez-Sosa M. Role of the programmed Death-1 pathway in the suppressive activity of alternatively activated macrophages in experimental cysticercosis. Int J Parasitol 2005;35:1349-58.
65. 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;40:731-42.
66. Persaud R, Wang A, Reardon C, McKay DM. Characterization of the immuno-regulatory response to the tapeworm Hymenolepis diminuta in the non-permissive mouse host. Int J Parasitol 2007;37:393-403.
67. Mejri N, Gottstein B. Intraperitoneal Echinococcus multilocularis infection in C57BL/6 mice affects CD40 and B7 costimulator expression on peritoneal macrophages and impairs peritoneal T cell activation. Parasite Immunol 2006;28:373-85.
68. O'Connell AE, Kerepesi LA, Vandergrift GL, Herbert DR, TJ VANW, Hooper DC, Pearce EJ, Abraham D. IL-4(-/-) mice with lethal Mesocestoides corti infections--reduced Th2 cytokines and alternatively activated macrophages. Parasite Immunol 2009;31:741-9.
69. Willms K, Zurabian R. Taenia crassiceps: in vivo and in vitro models. Parasitology 2010;137:335-46.
70. Morales-Montor J, Escobedo G, Vargas-Villavicencio JA, Larralde C. The neuroimmunoendocrine network in the complex host-parasite relationship during murine cysticercosis. Curr Top Med Chem 2008;8:400-7.
71. Terrazas LI, Bojalil R, Rodriguez-Sosa M, Govezensky T, Larralde C. Taenia crassiceps cysticercosis: a role for prostaglandin E2 in susceptibility. Parasitol Res 1999;85:1025-31.
72. Rodriguez-Sosa M, Satoskar AR, David JR, Terrazas LI. 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 2003;33:703-11.
73. Rodriguez-Sosa M, Saavedra R, Tenorio EP, Rosas LE, Satoskar AR, Terrazas LI. A STAT4-dependent Th1 response is required for resistance to the helminth parasite Taenia crassiceps. Infect Immun 2004;72:4552-60.
74. Rodriguez-Sosa M, Rosas LE, David JR, Bojalil R, Satoskar AR, Terrazas LI. Macrophage migration inhibitory factor plays a critical role in mediating protection against the helminth parasite Taenia crassiceps. Infect Immun 2003;71:1247-54.
75. Rubio M, Tato P, Govezensky T, Molinari JL. Depressed immunity to a Salmonella typhimurium vaccine in mice experimentally parasitized by Taenia crassiceps. Vet Parasitol 1998;74:179-89.
76. Rodriguez M, Terrazas LI, Marquez R, Bojalil R. Susceptibility to Trypanosoma cruzi is modified by a previous non-related infection. Parasite Immunol 1999;21:177-85.
77. Rodriguez-Sosa M, Rivera-Montoya I, Espinoza A, Romero-Grijalva M, Lopez-Flores R, Gonzalez J, Terrazas LI. Acute cysticercosis favours rapid and more severe lesions caused by Leishmania major and Leishmania mexicana infection, a role for alternatively activated macrophages. Cell Immunol 2006;242:61-71.
78. Strachan DP. Hay fever, hygiene, and household size. BMJ 1989;299:1259-60.
79. Zaccone P, Fehervari Z, Phillips JM, Dunne DW, Cooke A. Parasitic worms and inflammatory diseases. Parasite Immunol 2006;28:515-23.

80. Yazdanbakhsh M, Wahyuni S. The role of helminth infections in protection from atopic disorders. Curr Opin Allergy Clin Immunol 2005;5:386-91.
81. Gruden-Movsesijan A, Ilic N, Mostarica-Stojkovic M, Stosic-Grujicic S, Milic M, Sofronic-Milosavljevic L. Mechanisms of modulation of experimental autoimmune encephalomyelitis by chronic Trichinella spiralis infection in Dark Agouti rats. Parasite Immunol 2010;32:450-9.
82. Saunders KA, Raine T, Cooke A, Lawrence CE. Inhibition of autoimmune type 1 diabetes by gastrointestinal helminth infection. Infect Immun 2007;75:397-407.
83. La Flamme AC, Ruddenklau K, Backstrom BT. Schistosomiasis decreases central nervous system inflammation and alters the progression of experimental autoimmune encephalomyelitis. Infect Immun 2003;71:4996-5004.
84. Osada Y, Shimizu S, Kumagai T, Yamada S, Kanazawa T. Schistosoma mansoni infection reduces severity of collagen-induced arthritis via down-regulation of pro-inflammatory mediators. Int J Parasitol 2009;39:457-64.
85. Araujo MI, Hoppe BS, Medeiros M, Jr., Carvalho EM. Schistosoma mansoni infection modulates the immune response against allergic and auto-immune diseases. Mem Inst Oswaldo Cruz 2004;99:27-32.
86. Walsh KP, Brady MT, Finlay CM, Boon L, Mills KH. Infection with a helminth parasite attenuates autoimmunity through TGF-beta-mediated suppression of Th17 and Th1 responses. J Immunol 2009;183:1577-86.
87. Espinoza-Jimenez A, Rivera-Montoya I, Cardenas-Arreola R, Moran L, Terrazas LI. Taenia crassiceps infection attenuates multiple low-dose streptozotocin-induced diabetes. J Biomed Biotechnol 2010;2010:850541.
88. Steinman L, Zamvil SS. How to successfully apply animal studies in experimental allergic encephalomyelitis to research on multiple sclerosis. Ann Neurol 2006;60:12-21.
89. Sospedra M, Martin R. Immunology of multiple sclerosis. Annu Rev Immunol 2005;23:683-747.
90. Langrish CL, Chen Y, Blumenschein WM, Mattson J, Basham B, Sedgwick JD, McClanahan T, Kastelein RA, Cua DJ. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. J Exp Med 2005;201:233-40.
91. Baxter AG. The origin and application of experimental autoimmune encephalomyelitis. Nat Rev Immunol 2007;7:904-12.
92. Fleming JO, Cook TD. Multiple sclerosis and the hygiene hypothesis. Neurology 2006;67:2085-6.
93. Niino M. Vitamin D and its immunoregulatory role in multiple sclerosis. Drugs Today (Barc) 2010;46:279-90.
94. Sciutto E, Fragoso G, Diaz ML, Valdez F, Montoya RM, Govezensky T, Lomeli C, Larralde C. Murine Taenia crassiceps cysticercosis: H-2 complex and sex influence on susceptibility. Parasitol Res 1991;77:243-6.
95. Fink K, Ng C, Nkenfou C, Vasudevan SG, van Rooijen N, Schul W. Depletion of macrophages in mice results in higher dengue virus titers and highlights the role of macrophages for virus control. Eur J Immunol 2009;39:2809-21.
96. Leendertse M, Willems RJ, Giebelen IA, Roelofs JJ, van Rooijen N, Bonten MJ, van der Poll T. Peritoneal macrophages are important for the early containment of Enterococcus faecium peritonitis in mice. Innate Immun 2009;15:3-12.
97. Bhatia S, Fei M, Yarlagadda M, Qi Z, Akira S, Saijo S, Iwakura Y, van Rooijen N, Gibson GA, St Croix CM, Ray A, Ray P. Rapid host defense against

Aspergillus fumigatus involves alveolar macrophages with a predominance of alternatively activated phenotype. PLoS One 2011;6:e15943.
98. Kitamoto K, Machida Y, Uchida J, Izumi Y, Shiota M, Nakao T, Iwao H, Yukimura T, Nakatani T, Miura K. Effects of liposome clodronate on renal leukocyte populations and renal fibrosis in murine obstructive nephropathy. J Pharmacol Sci 2009;111:285-92.
99. Nikolic T, Geutskens SB, van Rooijen N, Drexhage HA, Leenen PJ. Dendritic cells and macrophages are essential for the retention of lymphocytes in (peri)-insulitis of the nonobese diabetic mouse: a phagocyte depletion study. Lab Invest 2005;85:487-501.
100. Kataoka K, Nishiguchi KM, Kaneko H, van Rooijen N, Kachi S, Terasaki H. The roles of vitreal macrophages and circulating leukocytes in retinal neovascularization. Invest Ophthalmol Vis Sci 2011;52:1431-8.
101. Nakae S, Nambu A, Sudo K, Iwakura Y. Suppression of immune induction of collagen-induced arthritis in IL-17-deficient mice. J Immunol 2003;171:6173-7.
102. Chen Y, Langrish CL, McKenzie B, Joyce-Shaikh B, Stumhofer JS, McClanahan T, Blumenschein W, Churakovsa T, Low J, Presta L, Hunter CA, Kastelein RA, Cua DJ. Anti-IL-23 therapy inhibits multiple inflammatory pathways and ameliorates autoimmune encephalomyelitis. J Clin Invest 2006;116:1317-26.
103. Hu Y, Shen F, Crellin NK, Ouyang W. The IL-17 pathway as a major therapeutic target in autoimmune diseases. Ann N Y Acad Sci 2011;1217:60-76.
104. Alonso-Trujillo J, Rivera-Montoya I, Rodriguez-Sosa M, Terrazas LI. Nitric oxide contributes to host resistance against experimental Taenia crassiceps cysticercosis. Parasitol Res 2007;100:1341-50.
105. Baetselier PD, Namangala B, Noel W, Brys L, Pays E, Beschin A. Alternative versus classical macrophage activation during experimental African trypanosomosis. Int J Parasitol 2001;31:575-87.
106. Stempin CC, Dulgerian LR, Garrido VV, Cerban FM. Arginase in parasitic infections: macrophage activation, immunosuppression, and intracellular signals. J Biomed Biotechnol 2010;2010:683485.
107. Mohrs M, Holscher C, Brombacher F. Interleukin-4 receptor alpha-deficient BALB/c mice show an unimpaired T helper 2 polarization in response to Leishmania major infection. Infect Immun 2000;68:1773-80.
108. Cuervo H, Pineda MA, Aoki MP, Gea S, Fresno M, Girones N. Inducible nitric oxide synthase and arginase expression in heart tissue during acute Trypanosoma cruzi infection in mice: arginase I is expressed in infiltrating CD68+ macrophages. J Infect Dis 2008;197:1772-82.
109. Stempin CC, Garrido VV, Dulgerian LR, Cerban FM. Cruzipain and SP600125 induce p38 activation, alter NO/arginase balance and favor the survival of Trypanosoma cruzi in macrophages. Acta Trop 2008;106:119-27.
110. El Kasmi KC, Qualls JE, Pesce JT, Smith AM, Thompson RW, Henao-Tamayo M, Basaraba RJ, Konig T, Schleicher U, Koo MS, Kaplan G, Fitzgerald KA, Tuomanen EI, Orme IM, Kanneganti TD, Bogdan C, Wynn TA, Murray PJ. Toll-like receptor-induced arginase 1 in macrophages thwarts effective immunity against intracellular pathogens. Nat Immunol 2008;9:1399-406.
111. Muller U, Stenzel W, Kohler G, Werner C, Polte T, Hansen G, Schutze N, Straubinger RK, Blessing M, McKenzie AN, Brombacher F, Alber G. IL-13 induces disease-promoting type 2 cytokines, alternatively activated macrophages

and allergic inflammation during pulmonary infection of mice with Cryptococcus neoformans. J Immunol 2007;179:5367-77.
112. Wei J, Duramad O, Perng OA, Reiner SL, Liu YJ, Qin FX. Antagonistic nature of T helper 1/2 developmental programs in opposing peripheral induction of Foxp3+ regulatory T cells. Proc Natl Acad Sci U S A 2007;104:18169-74.
113. Cua DJ, Tato CM. Innate IL-17-producing cells: the sentinels of the immune system. Nat Rev Immunol 2010;10:479-89.
114. Tadokoro CE, Vallochi AL, Rios LS, Martins GA, Schlesinger D, Mosca T, Kuchroo VK, Rizzo LV, Abrahamsohn IA. Experimental autoimmune encephalomyelitis can be prevented and cured by infection with Trypanosoma cruzi. J Autoimmun 2004;23:103-15.

11. PUBLICACIONES OBTENIDAS

11.1 Artículo 1.- Reyes J.L. & Terrazas L.I. (2007) **The divergent roles of alternatively activated macrophages in helminthic infections.** Parasite Immunol. 29(12); 609-619.

11.2 Artículo 2.- Reyes J.L., Terrazas C.A., Vera-Arias L. & 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(6);1115-27.

11.3 Artículo 3.- Reyes J.L., Terrazas C.A., Alonso-Trujillo J., Van Rooijen, Satoskar A.R. & Terrazas L.I. (2010) **Early removal of alternatively activated macrophages leads to Taenia crassiceps cysticercosis clearance in vivo** Intl. J. Parasitol. 40(6);731-42.

11.4 Artículo 4.- Reyes J.L., Espinoza-Jimenez A.F., Gonzalez M.I., Verdin L. & Terrazas L.I. (2011)***Taenia crassiceps*** **infection abrogates experimental autoimmune encephalomyelitis** Cell. Immunol. 267 (2); 77-87.

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Review Article

The divergent roles of alternatively activated macrophages in helminthic infections

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SUMMARY

Macrophages play crucial roles in the immune response, as they can initiate, modulate and also be final effector cells during immune responses to infections. Macrophages are derived from myeloid precursor cells in bone marrow and are widely distributed in every tissue of the body. Over the past 10 years, the concepts about macrophage activation have clearly changed; macrophages are not called activated or inactivated as they used to be. These changes in the concept of macrophage response is the result of many in vitro *and* in vivo *studies, but the major support for the current concept of alternatively activated macrophages (AAMϕ) comes from parasitic helminth infections. Parasitic helminths have developed complex mechanisms to evade and modulate host immunity. Infections with these parasites induce strong polarized Th2-type immune responses frequently associated with impaired T-cell proliferative responses to parasitic or unrelated antigens. Given the recent advances in understanding the immunoregulatory capabilities of helminthic infections, it has been suggested that macrophages can be a target for immunomodulation. Furthermore, they become altered when a host experiences chronic exposure to helminth parasites or their by-products, which favour the induction of AAMϕ. How AAMϕ participate in modulating host immunity during helminth infections and what their roles are in clearing or favouring parasite survival remains elusive. Here we review the most recent advances in the literature on AAMϕ at the host–parasite interface, including three classes of helminths: nematodes (*Brugia, Nippostrongylus, Litomosoides, Heligmosomoides*), trematodes (*Schistosoma, Fasciola*) and cestodes (*Taenia, Echinococcus, Hymenolepis*).*

Keywords *alternatively activated macrophages cestodes, trematodes, nematodes*

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PARASITE HELMINTH DIVERSITY

Parasitic helminths are a highly diverse group of organisms which display very different morphologies, accessory structures, sexual and feeding behaviours as well as life cycle stages. Likewise, they also generate a variety of diseases and allocate into a variety of niches in their hosts, ranging from spending their entire life in a specific organ or tissue, such as the gastrointestinal tract, to travelling into different organs and systems in the host (skin, bladder, muscle, liver, lung and brain), to finally establish in a specific organ where they may cause disease. Helminth parasites also appear to follow extremely varied and complicated routes of infection of host tissues. Infections mainly originate following ingestion of eggs/larvae (oral route) or through active penetration of the skin by parasite larvae or the bite of their vectors (cutaneous route).

After oral infection, some parasitic nematodes remain in the gastrointestinal tract for the rest of their life. The infective larvae of some nematode parasites, however, penetrate the intestinal wall and are transported by blood flow to the liver or other organs, transiently passing through the lungs or heart.

The migration profiles of the major trematode parasites differ significantly from those of gastrointestinal nematodes. The blood flukes (schistosomes) also follow the percutaneous route to the lungs, but are then carried to and settle in the mesenteric veins (*Schistosoma mansoni*). The liver flukes (*Fasciola hepatica* and *F. gigantica*) do not take advantage

of the host's circulatory system for their transport. Rather, recently ingested larvae actively penetrate the intestinal wall of the host and then migrate through the peritoneum towards the liver.

The life cycle of cestodes (tapeworms) involves definitive and one or more intermediate hosts. If the intermediate host is a mammal – and this may include man as an accidental host – the hooked larva penetrates the gut wall and is distributed throughout the body via the blood and the lymphatic system. In different sites of the intermediate host, it develops into an infective cyst (muscle, peritoneal cavity and brain). The cyst may be ingested with the raw flesh of the intermediate host by the final host (man, dog and cat). In the intestinal tract of the final host, the scolex becomes exposed and attaches to the intestinal mucosa where the tapeworm develops into adult form.

Thus, based on such diversity in helminth parasites and on such diversity in the diseases they cause, one could expect a wide range of different helminth-induced immune responses. Despite these differences, most of these organisms induce very similar immune responses in their host. We can collectively term this reaction as the characteristic immune response to a 'helminth infection', keeping in mind that this immune response is not necessarily the only reaction of the host immune system to a specific infection. Immune response to a helminth infection is typified by the profile of cytokines they induce, such as high levels of IL-4, IL-5, IL-10 and IL-13, and also high levels of characteristic antibodies such as IgG1 and IgE, as well as increased numbers of particular cell populations such as eosinophils, goblet cells and mast cells (1). Low T cell proliferative responses to polyclonal stimuli and to specific parasite antigens as well as 'bystander' decreased T cell proliferation are frequently observed (2). Two more shared features of helminth infections have recently been identified, including regulatory cell subpopulations such as T regulatory cells (3) and alternatively activated macrophages (AAMϕ) (4,5). Noteworthy is that besides experimental infections, these responses are features in human parasitosis caused by helminths.

This review will focus on the role of AAMϕ, which have been documented in all three classes of parasitic helminths such as nematodes, trematodes and cestodes. However, they appear to play differential roles according to the type and momentum of a specific infection. In this review, we put together different reports, arguing that AAMϕ may attack the parasite, may favour host colonization or may avoid immunopathological damage in the host.

MACROPHAGE DIVERSITY

Macrophages are dedicated phagocytic cells and are one of the most ubiquitous types of cells in many organs and tissues. They are also versatile cells that are known to play three key roles in the immune response. First, macrophages serve as early detectors for invading pathogens through pattern recognition receptors, such as toll-like receptors and C-type lectins. Second, macrophages function as antigen-presenting cells (APC) which initiate host immune responses. Until recently, the third and final role that macrophages played was as effector cells when they directly kill a pathogen (6). A fourth role has been highlighted by recent reports showing that macrophages can play a role as regulatory and suppressor cells as evidenced in parasitic infections and in tumour-bearing hosts (7,8).

In contrast to 15 years ago, when macrophages were classified only as activated or deactivated macrophages, current advances in molecular immunology and the study of macrophage participation in different pathological conditions in human and mouse models have been useful in discovering that macrophages have many facets. Presently, different sophisticated classifications of macrophages have been proposed. These include a simple dual classification such as classical activation (CAMϕ) vs. AAMϕ, a concept originally introduced in the early 90s by Gordon's research group who observed a direct *in vitro* effect of IL-4 on macrophage expression of the mannose receptor (MR) (6). Such a key observation gave rise to a completely new field of study, macrophage polarization, which depends on proinflammatory (also termed M1 macrophages) or anti-inflammatory (also termed M2 macrophages) situations and that at least in mice have been suggested to be strain dependent (9). Besides the CAMϕ (M1) and AAMϕ (M2) subpopulations described below, a more complex but not less interesting classification is that proposed by Mantovani *et al*. (10). They maintain the use of M1 for macrophages with proinflammatory abilities and use the term M2 in a more generic sense and subdivide the M2 population into three distinct subpopulations according to best defined routes of activation and response of these macrophages. Based on such criteria, M2 macrophages are classified into subpopulations M2a (which reflects the AAMϕ); M2b, which are activated by immune complexes and release high levels of IL-10; and M2c, which are induced by IL-10 and represent the original version of deactivated macrophages. This last classification has been proposed mainly based on the type of stimuli that made the macrophage subpopulations distinguishable and, on the other hand, by the type of chemokines that the macrophages release (10).

All of these types of macrophages can be found in a variety of immunological conditions, such as immune responses to intracellular pathogens, parasitic protozoa, tumours, autoimmunity and, of course, in helminthic infections. Recent excellent reviews have addressed the

role of macrophages in cancer and in parasitic protozoan infections (7,8,11), but this review is the first to our knowledge that is focused on alternative macrophage activation and responses to all three classes of helminths.

As a matter of convention, we will use the dichotomy CAMϕ and AAMϕ. Facing such diversity in the states of macrophage activation, we hypothesize that such activation is microenvironment-dependent (including structural and secreted/excreted molecules of the parasites), meaning that macrophages have high plasticity to respond to the changing environment in the host. Thus, macrophages are able to respond to the microbial and cytokine milieu, driving the expression of polarized functional properties. In general, the best characterized subpopulation of macrophages are the CAMϕ which are induced by IFN-γ, TNF-α and microbial products such as lipopolysaccharides (LPS). CAMϕ can be identified by their high ability to produce proinflammatory cytokines and chemokines such as IL-1β, IL-12, IL-23, TNF-α, and CXCL-9, -10, -11 and -16, while producing low amounts of IL-10; they also produce high levels of reactive oxygen and nitrogen intermediates such as nitric oxide (NO) and reactive oxygen species (ROS) (9,10,12). CAMϕ have been identified as central players in mediating resistance against intracellular parasites and tumours. Conversely, AAMϕ are generally characterized by low production of IL-1β, IL-12, and IL-23, but with discrete IL-10 production (6). They also are greater producers of urea and sometimes of regulatory cytokines such as TGF-β. AAMϕ are frequently associated with polarized Th2-type responses, and indeed most reports agree that AAMϕ are IL-4- and IL-13 induced and play a central role in tissue repair (13). In this classification, the pathway of activation plays a critical role and determines the mechanism by which macrophages metabolize the amino acid L-arginine. Activation by IFN-γ and TNF-α in CAMϕ favours the production of NO, which is mediated by increasing activity of nitric-oxide synthase 2 (NOS2). In contrast, an increasing arginase-1 (Arg-1) activity is observed in AAMϕ by IL-4 and IL-13, favouring L-arginine metabolism towards proline, polyamines and urea production (12). More specific markers for AAMϕ have been recently identified from *in vivo* parasitic infections as well as from mice bearing tumours (14). The most relevant signature markers besides Arg-1 and MR are Fizz1, Ym1, TREM-2, AMCase, mMGL1 and mMGL2 (see Table 1 for abbreviations) (14).

There is a growing interest in the role and function of AAMϕ, as this subpopulation of macrophages has been associated with a diversity of immunological situations ranging from individuals bearing tumours, where strong suppressive activity has been observed, to parasitic infections where a less defined role has been probed.

ROLE OF AAMϕ IN HELMINTHIC INFECTIONS

Nematodes

Brugia malayi

Filariasis is an important human nematode infection affecting thousands of people around the world. A mouse model of infection with *B. malayi* has been used in the past few years to understand basic aspects of immunomodulation during filariasis. Intraperitoneal infection of mice with *B. malayi* adults induces the recruitment of high numbers of F4/80$^+$ macrophages along with a strong Th2-type response in a couple of weeks (15,16). When the peritoneal macrophages isolated by adherence were co-cultured with antigen-specific or naïve polyclonally stimulated T cells, a profound inhibition in the proliferative response was observed. These macrophages displayed high Arg-1 activity instead of NOS2 activity. Further studies performed by the same group showed an up-regulation in these macrophages of two more characteristic genes, Fizz1 and Ym1, considered since then as markers for AAMϕ in several nematode infections (4). Similarly, increased IL-10 and TGF-β production in this infection were associated with AAMϕ. Such inhibitory activity and gene expression were determined to be IL-4-dependent, given that mice lacking IL-4 were unable to induce these AAMϕ, whereas IL-10$^{-/-}$ mice sustained both AAMϕ induction and suppressive activity during *B. malayi* infection (4,15). In this specific type of infection, the AAMϕ elicited were named NeMϕ, and their ability to suppress T cell proliferative responses was found to be contact-dependent and only partially TGF-β-dependent (16,17). However, molecules involved in the cell-to-cell contact were undefined. Nevertheless, these NeMϕ induced by *B. malayi* are considered to play an anti-inflammatory role at the site of infection.

Litomosoides sigmodontis

Another model to study the immunosuppressive effect of filarial infections has been *L. sigmodontis* that, in contrast to *B. malayi*, can follow its natural course of infection in mice. Thus, it was also demonstrated that *L. sigmodontis* infection induces F4/80$^+$ AAMϕ (NeMϕ) where Fizz1 and Ym1 were up-regulated at the sites of parasite migration and residence in the pleural cavity. A similar macrophage-dependent suppressive activity as well as a similar pattern of expression of Arg-1, Fizz1 and Ym1 were also detected during *L. sigmodontis* infection, reminiscent of *B. malayi* studies (18,19). Again, *L. sigmodontis*-induced NeMϕ suppressive activity was contact-dependent and minimally associated with TGF-β. In contrast, blockade of the IL-10 receptor and CTLA-4 did not have any effect on NeMϕ suppressive activity. NeMϕ were most often found close to the parasite, and they appear

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Table 1 Helminths belonging to phylogenetically separated groups induce AAMϕ whose function remains debatable. List of the main molecules reported to be associated with AAMϕ on helminth parasitic infections

Parasite	Increased gene expression of AAMϕ markers	APC function	Suppressive activity	Role in disease	Reference
Nematodes					
Brugia malayi	Fizz1, Ym1, Arg-1	Th2-biasing ability	Yes (contact dependent/TGF β-partially dependent)	Unknown	(4,15–17)
Litomosoides sigmodontis	Fizz1, Ym1, Arg-1	Unknown	Yes (contact dependent/TGF-β partially dependent)	Unknown	(20)
Nippostrongylus brasiliensis	Fizz1, Fizz2, Ym1, Arg-1, MMR, AMCase IL-21R	Unknown	Unknown	Lung homeostasis after acute injury	(18,19,36)
Heligmosomoides polygyrus	Fizz1, Ym1, MR, AMCase, IL-4R	Unknown	Unknown	Host protection, arginase-mediated parasite clearance	(27)
Trematodes					
Schistosoma mansoni	Fizz1, Ym1, Arg-1 AMCase, MR, IL-21R	Th2-biasing ability	Yes (PD/PDL1 pathway dependent)	Divergent role: host protection down-modulate Th1-mediated immunopathology and progressive pathology in lung and liver granuloma formation	(30,33)
Fasciola hepatica	Fizz1, Ym1, Arg-1	Unknown	Unknown	Unknown	(34,35)
Cestodes					
Taenia crassiceps	Fizz1, Ym1, Arg-1, MR, 12/15 LOX, mMGL, CD23, CCR5, OX40L, SLAM, TREM2	Th2-biasing ability	Yes (PD1–PDL pathway dependent and ROS partially dependent)	May favour parasite installation	(5,43,44,63)
Hymenolepis diminuta	Fizz1, Ym1, Arg-1	Unknown	Unknown	Unknown	(52)
Echinococcus multilocularis	ND	Down-regulated	Yes (ND)	Unknown	(51)

Fizz1, found in inflammatory zone 1; AMCase, acidic mammalian chitinase; LOX, lipoxygenase; MR, macrophage mannose receptor; Arg-1, arginase-1; PD, programmed death receptor; PDL, programmed death ligand; mMGL, mouse macrophage galactose-type-C-type lectin, CCR5, CC chemokine receptor 5, ND, not determined; TREM, triggering receptor expressed on myeloid cells.

to disseminate to lymph node (LN) only when microfilaraemia exit in the pleural cavity (20). The functional role for AAMϕ in filariasis is still not fully clear, but evidently induces suppressive activity.

Nippostrongylus brasiliensis

This parasite has been widely used as a model for intestinal nematode infections. Infection is performed subcutaneously, and as the parasites mature, they migrate and enter the lungs where *N. brasiliensis* induce strong, polarized Th2-type responses in the lungs and in lung-associated LNs (21). Besides the high levels of IL-4 and IL-13 expressions, other genes were also highly expressed in the lungs of infected mice, such as AMCase, Fizz1 and Ym1, suggesting the presence of AAMϕ (Table 1). This set of AAMϕ markers has recently been associated with the expression of IL-21R during *N. brasiliensis* infection, and this receptor was shown to be involved in augmenting AAMϕ activity, mainly by increasing IL-4Rα and IL-13Rα expressions. Furthermore, lung fibrosis caused by *N. brasiliensis* infection was associated with the presence of AAMϕ (22), given that mice lacking IL-21R induced less AAMϕ and therefore pulmonary fibrosis was reduced. Thus, a strong Th2 response together with the presence of AAMϕ is associated with the pathology observed in lungs of *N. brasiliensis*-infected mice. In contrast, other recent observations indicate that AAMϕ expressing Arg-1, Ym1, Fizz1 and MR can be induced in the lungs in the absence of CD4 T cells in response to *N. brasiliensis* infection (23). In this study, GSL-I$^+$ AAMϕ were detected *in situ* (lungs) as early as 4 days post infection (p.i.) in either severe combined immunodeficiency (SCID) or wild type (WT) mice. Nevertheless, these populations remained just past 8 days p.i. in WT mice which displayed a remarkably fast resolution of both mechanical damage and inflammation caused by *N. brasiliensis*. Conversely, inflammation and damage in the lungs of SCID mice persisted after 8 days p.i. (23). From these results, two important conclusions can be highlighted. First, AAMϕ can be elicited in innate immune responses when Th2-specific responses cannot be found, suggesting that *N. brasiliensis* may directly induce AAMϕ or that an early source of IL-4 from innate cells (e.g. mast cells and NK cells) may be enough to induce AAMϕ. Second, AAMϕ can also play a role as regulatory cells by avoiding excessive lung inflammation as well as remodelling function. A more recent report showed that AAMϕ induction during *N. brasiliensis* infection is strictly dependent on the STAT6-signalling pathway (24). Together these findings imply a clear IL-4/IL-13-dependency for AAMϕ to merge during this nematode infection.

Heligmosomoides polygyrus

This is a natural mouse gastrointestinal nematode parasite. Mice became infected with the L_3 infective larvae. These larvae exsheath in the stomach and then, after 36 h, move to the small intestine where they can penetrate the mucosa and migrate down to the muscularis interna where they encyst within the muscular wall. Larvae that reach this state mature and develop to adults to emerge from the cysts into the intestinal lumen (25,26). As much as other classes of helminths, *H. polygyrus* triggers polarized Th2-type responses and can induce chronic infections. If parasites are eliminated after a primary infection, the Th2 memory response protects the host against re-infection. Intestinal AAMϕ have recently been found surrounding developing *H. polygyrus* larvae following a secondary infection (27). As seen in *B. malayi*, *L. sigmodontis* and *N. brasiliensis* infections, these AAMϕ were scarce in STAT6$^{-/-}$ and IL-4$^{-/-}$ mice which displayed higher parasite and egg burdens. When WT mice were depleted of either CD4 cells using a specific antibody *in vivo* or were depleted of AAMϕ by using clodronate-loaded liposomes, a defective worm expulsion was observed in parallel with increased egg recoveries (27). Surprisingly, these data suggested for the first time a protective role for AAMϕ in a helminth infection besides the classical involvement of eosinophils. As mentioned above, arginase activity is one of the characteristic features of AAMϕ. In this study, when arginase was chemically blocked in resistant mice, an enhanced larval recovery was observed, suggesting a role for arginase in host protective responses against the intestinal phase of *H. polygyrus*. Until that work, no role for arginase had been associated with protective responses in infectious diseases. Notably, AAMϕ in this infection were only detected at the site of infection, as also seen with *B. malayi*, *L. sigmodontis* and *N. brasiliensis*.

Trematodes

Schistosoma mansoni

The mouse model for studying the host–parasite relationship in schistosomiasis has been of great utility to understand mechanisms involved in protection, susceptibility and pathology of this important trematode disease that affects millions of people. Early in the 90s, Stadecker's research group found that macrophages isolated from liver granulomas of *S. mansoni*-infected mice were able to anergize T cell responses to specific and polyclonal stimuli (28). Granulomas in murine schistosomiasis peak 7 or 8 weeks after infection, a time period in which hosts have developed already dominant Th2-type responses (29). Stadecker also found this suppressive activity was IL-2 reversible and demonstrated that these macrophages were able to drive Th2 responses. Nevertheless, mechanisms participating in both Th2-driven and suppressive activities were undefined. These macrophages isolated from liver granulomas were not defined as AAMϕ at that time. However, almost 15 years later, these macrophages

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have been better defined. It is now known that T cell (both CD4 and CD8) anergy induced by these macrophages during experimental schistosomiasis are cell contact-dependent and a clear participation of the PD-1/PDL pathway has been demonstrated, mainly via selective up-regulation of PD-L1 in these macrophages (30). PD-L1 and PD-L2 are relatively new negative signalling accessory molecules that act through their receptor PD-1 expressed in activated T cells, thereby down-regulating their proliferation (31,32). These suppressive macrophages were also determined to be AAMϕ (30). On the other hand, mice lacking the IL-4Rα chain specifically on their macrophages (LysM-^{Cre}IL-4Rα$^{-/flox}$ mice) succumbed to acute schistosomiasis. This result was associated with the impaired ability of LysM^{Cre}IL-4Rα$^{-/flox}$ mice to recruit AAMϕ to granuloma tissue despite having a normal Th2-type response. In contrast, elevated numbers of CAMϕ were detected in liver granulomas of these mice (33). Therefore, induction of IL-4/IL-13-dependent AAMϕ was essential to avoid immunopathological damage and to survive acute schistosomiasis.

Fasciola hepatica

This trematode has a complex life cycle that includes intermediate (snails) and definitive hosts (cattle or human). As with other helminths, *F. hepatica* induces Th2-type responses. Recently, the induction of markers for AAMϕ at early stages of infection has been reported (Table 1). This induction can be mimicked in mice by injecting excreted/secreted products of *F. hepatica* (34). Notwithstanding that regulatory cytokines were augmented in these macrophages, the immunomodulatory (suppressive or Th2 biasing) activity of this population was not achieved. However, in other experimental model using cattle infection, it was suggested that AAMϕ can mask tuberculosis detection by impairing delayed type hypersensitivity (DTH) responses to BCG (35).

Cestodes

Taenia crassiceps

Excellent reviews have recently been published regarding the immune response during trematode and nematode infections (2,21,36). However, the class cestoda has been largely neglected, even though these parasites produce important threatening diseases around the world. The infection of the intermediate hosts by the metacestode stage of *Taenia* species, and especially *T. crassiceps*, appears to be a very good model to unveil some of the mechanisms of the host–parasite interplay in cysticercosis. *Taenia crassiceps* cysticercosis naturally affects rodents and the final hosts are canines. Nevertheless, there are reports demonstrating that immunocompromised humans can develop *T. crassiceps* cysticercosis (37,38). The metacestode stage of *T. crassiceps* has the advantage of an asexual budding reproduction (39). This biological phenomenon has been useful in generating long-lasting infections in laboratory mice, where regularly the parasite is inoculated i.p., and after a few weeks, hundreds of macroscopic parasites can be reached from the peritoneal cavity. Additionally, antigenic similarities have been very well established between *T. solium* and *T. crassiceps* metacestodes (40). Thus, sera from human patients suffering from neurocysticercosis positively recognize *T. crassiceps* antigens. After initial infection with *T. crassiceps* cysticerci, a rapid but transient Th1 response is observed in the host (41), and the immune response is sequentially biased to a Th2 response following a period of a mixed Th1/Th2 response (42). Along with this dynamic immune response, macrophages recruited in the peritoneal cavity change as infection progresses. Peritoneal macrophages (F4/80$^+$) isolated at different times after challenge with *T. crassiceps* have been used as APC and tested for their ability to regulate Th1/Th2 differentiation. Macrophages from acute infections produced high levels of IL-12 and NO, paralleled with low levels of IL-6 and have the ability to induce strong antigen-specific CD4$^+$ T cell proliferation in response to unrelated antigens (5). In contrast, macrophages from chronic infections produced a different pattern of cytokines and chemokines, associated with a poor ability to induce antigen-specific proliferations in CD4$^+$ T cells. Failure to induce proliferation was not due to deficiencies in the expression of accessory molecules, since MHC-II, CD40 and B7-2 were up-regulated, together with CD23 and CCR5 as infection progressed (5). Besides these molecules, another set of markers has recently been found elevated in the peritoneal macrophages from *T. crassiceps*-infected mice (Table 1), such as high expression of MR, the C-type lectins (mMGL1 and mMGL2), Arg-1, Fizz1, Ym1 and TREM-2, confirming that these macrophages are alternatively activated (43,44). Macrophages from chronic infections were able to bias CD4$^+$ T cells to produce IL-4, but not IFN-γ, contrary to macrophages from acute infections, just like those described previously in nematode infections. Furthermore, studies using STAT6$^{-/-}$ mice revealed that the STAT6-mediated signalling pathway was essential for the expansion of AAMϕ in murine cysticercosis (5,45). More recently, it has been shown that IL-4Rα$^{-/-}$ mice were also unable to induce AAMϕ after *T. crassiceps* infection (14). Together, these studies performed by independent groups agree with the early observation of IL-4 dependency for *Brugia* and *Schistosoma* AAMϕ induction.

Another striking observation has been the ability of AAMϕ isolated from *T. crassiceps*-infected mice to inhibit the proliferative response of naïve T cells (44). Apparently, this effect involves a cell contact-dependent pathway. Supporting evidence for cell contact-dependent involvement was associated with increased expression of PD-L1 and

PD-L2 in AAMϕ. The participation of the PD-1 pathway was tested by blocking PD-L1 and PD-L2, or PD-1 by adding mAbs to co-cultures of naïve T cells with AAMϕ from *T. crassiceps*-infected mice. Blockade of the PD-1 pathway significantly reduced AAMϕ suppressive activity and therefore T cells proliferated normally. These data indicate that PD-L1 and PD-L2 are directly involved in the cell contact suppressive activity of AAMϕ from *T. crassiceps*-infected mice. Whether this will also be true for the contact-dependent suppression seen in nematode infection is yet to be tested.

AAMϕ induced by *T. crassiceps* infection were also demonstrated to suppress the specific response of $CD4^+$ DO11·10 cells to OVA peptide stimulation when normal macrophages were used as APC. Again, the blockade of PD-1 re-established the peptide-specific proliferative response of $CD4^+$ DO11·10 cells. Therefore, AAMϕ can participate as a third party suppressive cell. Similarly, the presence of AAMϕ in a DC-mediated mixed lymphocyte reaction was enough to inhibit the response of CD4 cells from a different genetic background (44). Thus, AAMϕ induced during *T. crassiceps* infection suppress immunological events mediated through distinct molecular mechanisms that potentially may induce strong proinflammatory responses. It was also demonstrated that the PD-1/PD-L pathway participates in modulating anti-*Taenia*-specific cell proliferative response.

Initial support for *in vivo* AAMϕ induction by *Taenia* glycoproteins has recently been reported (46). Together with thioredoxin from *F. hepatica*, it seems that helminth-derived molecules can induce these types of cells in wild-type mice; however, the role for IL-4 in the antigen induction of AAMϕ needs to be clarified.

Translating these series of results to the immune balance in neurocysticercosis, it is therefore possible that the presence of AAMϕ with suppressive activity and low proinflammatory profile may be necessary to turn off possible dangerous inflammatory responses in the brain. In fact, a series of reports suggest that active inflammatory responses in neurocysticercosis leads to pathological symptoms (47), whereas a silent (anti-inflammatory) immune response has been associated with asymptomatic neurocysticercosis (48,49).

Echinococcus multilocularis

Alveolar echinococcosis is a chronic cestode infection caused by the metacestode of *E. multilocularis*. In experimental conditions, infection is achieved by i.p. inoculation of the metacestodes. Similar to the *T. crassiceps* model, the early immune response is transiently dominated by a Th1 type, but progress of the infection leads to biased Th2-type responses (50). Macrophages appear to play a familiar role in the host–parasite interface. Macrophages isolated from the peritoneal cavity (close to the parasite) 6 weeks p.i. induced a significant reduction in T cell response to an unrelated antigen (OVA peptide) compared to peritoneal macrophages from naïve mice (51). These *E. multilocularis*-induced macrophages also suppressed Con-A-stimulated lymphocyte proliferation, in which the effect was cell contact dependent (51), even though previous data sustained that soluble factors (NO) were involved in the low proliferative response during echinococcosis (50). Further experiments are necessary to clarify whether or not macrophages induced by *E. multilocularis* infection belong to the AAMϕ subpopulation and whether the cell contact suppressive activity is PD-1 mediated as observed in both schistosomiasis and cysticercosis.

Hymenolepis diminuta

This tapeworm has been recently used to study the immune response evoked against cestodes at the intestinal level. Infection with *H. diminuta* in mice rapidly drives mesenteric LN cells to produce IL-10, TGF-β and IL-4. In the intestine of *H. diminuta*-infected mice, markers were detected for natural regulatory cells as well as markers for AAMϕ by RT-PCR (Table 1). These AAMϕ arrive at day 8 p.i (52). Although direct regulatory activity was not tested, the authors correlate the appearance of AAMϕ with the peak time of worm expulsion, suggesting that AAMϕ may participate in protection against this cestode infection.

As noticed here, AAMϕ have been consistently identified during infections with all the three classes of helminth parasites. Nonetheless, the definition of what constitutes a macrophage varies from study to study based on the panel of cell surface markers used (Table 2). Likewise, the state of alternative activation has also been defined using a diversity of techniques (Table 2). Together, these data support the idea that the induction of AAMϕ is another common feature in helminth infections.

ANOTHER DIVERGENT ROLE FOR AAMϕ

AAMϕ induced by helminth infections may have another role. It is well established that previous helminth infection can modulate the immune response in a 'bystander' fashion. Therefore, given that concomitant infections are common in developing countries, secondary infections of nonrelated pathogens such as protozoan parasites or mycobacterium may be facilitated. Many concomitant infections are documented in experimental models as well as in human beings living in endemic areas (36,53). Most of the protozoan parasites and mycobacterium (malaria, trypanosomiasis, leishmaniasis and mycobacterium) share one thing, which is the requirement for macrophage internalization where the pathogens proliferate. Taking this into account, at the time when the helminth parasite has subverted the host immune

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Table 2 Specific membrane macrophage markers and isolation tissues for AAMϕ induced by diverse helminthic infections

Parasite	Macrophage specific markers	AAMϕ isolation tissue	AAMϕ characterization technique	Reference
Nematodes				
Brugia malayi	$F4/80^+$	Adherent PECs mediastinal and parathymic LN[a]	EST/RT-PCR/WB	(4,19)
Litomosoides sigmodontis	$F4/80^+$	Thoracic/pleural cavity mediastinal and parathymic LN[a]	RT-PCR	(19,20)
Nippostrongylus brasiliensis	$F4/80^+/CD11b^+$	Lung and small intestine[b]	RT-PCR/IHC/WB	(19,22,23)
Heligmosomoides polygyrus	$F4/80^+$	Small intestine[a,b]	IHC	(27)
Trematodes				
Schistosoma mansoni	$F4/80^+/CD11b^+$	Schistosoma egg-induced liver granulomas and adherence[a,b]	FC/IHC	(33)
Fasciola hepatica	$F4/80^+/CD14^+$	Adherent PECs and PBMC[a]	RT-PCR/UQ	(34,35)
Cestodes				
Taenia crassiceps	$F4/80^+/Mac3^+/CD11b^+/CD14^+$	Adherent PECs[a]	RT-PCR/UQ/FC	(5,43,44,63)
Hymenolepis diminuta	ND	Small and large intestine[b]	RT-PCR	(52)
Echinococcus multilocularis	$Mac1^+/CD11b^+$	Adherent PECs[a]	ND	(51)

LN, lymph node; ND, not determined; PECs, peritoneal exudate cells; PBMC, peripheral blood mononuclear cells; EST, expressing sequence tag; IHC, immunohistochemistry; UQ, urea quantification; WB, Western blot; FC, flow cytometry.
[a]Enrichment > 85%; [b]*in situ* detected.

system and macrophages have reached an alternative state of activation characterized by enhanced phagocytic ability and decreased microbicidal/toxic activities, intracellular pathogens can replicate freely. Some examples are the co-infections *Schistosoma*/leishmaniasis (54), *Litomosoides*/leishmaniasis (55), *Taenia*/leishmaniasis (56), *Taenia*/trypanosomiasis (57), filaria/malaria (58) or in general helminth/tuberculosis (59). In all cases, AAMϕ were unable to fully eliminate the second pathogen. Together these data suggest that previous helminth infections have modulatory effects on the immune response elicited against a second challenge mainly by affecting macrophage activity rather than completely inhibiting Th1-type responses.

On the other hand, many experimental as well as geographical correlations in humans indicate that helminth infections can down-regulate allergic as well as autoimmune inflammatory disorders (60,61). Although these disorders are adaptive immune response dependent, a role for innate immunity is well recognized; hence, macrophages and other APC populations that initiate and condition the adaptive response are critical participants. If a macrophage population is conditioned by the alternative state-inducer microenvironment, polarization of the autoantigen response towards anti-inflammatory effector cells can occur. In addition to their function as Th2-biasing APC, AAMϕ posses a suppressor ability and, given that this one is nonspecific, the course of autoimmune and allergic diseases may be less severe. As noted here, one mechanism used by parasite helminths to establish successful infections can explain why the course of aetiologically different (i.e. infectious, autoimmune or allergic) disorders can be altered in helminth-bearing hosts.

CONCLUDING REMARKS

We have reviewed here the available evidence from experimental *in vivo* data suggesting that AAMϕ are widely induced by all the three classes of helminths and that they play different roles in those infections. AAMϕ can be identified as a subpopulation of macrophages with immunomodulatory activities that can be protective for the host by limiting unwanted exacerbated inflammatory responses. However, they may have a role as effector cells eliminating at least one intestinal helminthic infection. AAMϕ induced by helminths can also perform tissue repair and remodelling after infection but, on the other hand, they are part of an immunomodulatory strategy to successfully colonize the host. Experimental evidence continues to support a role for AAMϕ in the immunoregulation by diverse helminth parasitic diseases as stated here. Yet, many questions remain unanswered. Why are AAMϕ localized close to the parasites most of the time? Can these AAMϕ migrate to other LN where a new stimulus is delivered? Presently, there is no clear evidence for AAMϕ migration. Do they respond to chemokines? How? Are they refractory to proinflammatory cytokine stimuli, even when they express the right receptors? Until now, AAMϕ stimulated with inflammatory cytokines or/and LPS do not secrete the expected molecules (62–64).

Do helminth-derived molecules induce AAMϕ directly? Is it really strictly necessary for an IL-4-dependent signalling pathway for AAMϕ to merge in a helminth infection? Until now, STAT6$^{-/-}$, IL-4$^{-/-}$ and IL-4R$^{-/-}$ mice have consistently been reported for their inability to induce AAMϕ *in vivo* in response to the three different classes of helminths. Can we use AAMϕ (transfer them) to treat inflammatory diseases to promote and sustain a Th2-polarized immune response associated with a more favourable anti-inflammatory and host-protective environment?

Deciphering these mechanisms will give us new valuable information to better understand helminth immunomodulation as well as to take advantage of their properties.

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REFERENCES

1 Maizels RM & Yazdanbakhsh M. Immune regulation by helminth parasites: cellular and molecular mechanisms. *Nat Rev Immunol* 2003; **3**: 733–744.
2 Maizels RM, Balic A, Gomez-Escobar N, Nair M, Taylor MD & Allen JE. Helminth parasites – masters of regulation. *Immunol Rev* 2004; **201**: 89–116.
3 Wilson MS & Maizels RM. Regulatory T cells induced by parasites and the modulation of allergic responses. *Chem Immunol Allergy* 2006; **90**: 176–195.
4 Loke P, Nair MG, Parkinson J, Guiliano D, Blaxter M & Allen JE. IL-4 dependent alternatively-activated macrophages have a distinctive *in vivo* gene expression phenotype. *BMC Immunol* 2002; **3**: 7.
5 Rodriguez-Sosa M, Satoskar AR, Calderon R, *et al*. Chronic helminth infection induces alternatively activated macrophages expressing high levels of CCR5 with low interleukin-12 production and Th2-biasing ability. *Infect Immun* 2002; **70**: 3656–3664.
6 Gordon S. Alternative activation of macrophages. *Nat Rev Immunol* 2003; **3**: 23–35.
7 Noel W, Raes G, Hassanzadeh GG, De Baetselier P & Beschin A. Alternatively activated macrophages during parasite infections. *Trends Parasitol* 2004; **20**: 126–133.
8 Sica A & Bronte V. Altered macrophage differentiation and immune dysfunction in tumor development. *J Clin Invest* 2007; **117**: 1155–1166.
9 Mills CD, Kincaid K, Alt JM, Heilman MJ & Hill AM. M-1/M-2 macrophages and the Th1/Th2 paradigm. *J Immunol* 2000; **164**: 6166–6173.
10 Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A & Locati M. The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol* 2004; **25**: 677–686.
11 William GHC & Brombacher F. Alternatively activated macrophages in experimental parasitic infections. In Terrazas LI (ed.): *Advances in the Immunobiology of Parasitic Diseases*. Research Sign Post, India, 2007; in press.
12 Munder M, Eichmann K & Modolell M. Alternative metabolic states in murine macrophages reflected by the nitric oxide synthase/arginase balance: competitive regulation by CD4$^+$ T cells correlates with Th1/Th2 phenotype. *J Immunol* 1998; **160**: 5347–5354.
13 Gordon S & Taylor PR. Monocyte and macrophage heterogeneity. *Nat Rev Immunol* 2005; **5**: 953–964.
14 Ghassabeh GH, De Baetselier P, Brys L, *et al*. Identification of a common gene signature for type II cytokine-associated myeloid cells elicited *in vivo* in different pathologic conditions. *Blood* 2006; **108**: 575–583.
15 MacDonald AS, Maizels RM, Lawrence RA, Dransfield I & Allen JE. Requirement for *in vivo* production of IL-4, but not IL-10, in the induction of proliferative suppression by filarial parasites. *J Immunol* 1998; **160**: 1304–1312.
16 Loke P, MacDonald AS & Allen JE. Antigen-presenting cells recruited by *Brugia malayi* induce Th2 differentiation of naive CD4(+) T cells. *Eur J Immunol* 2000; **30**: 1127–1135.
17 Loke P, MacDonald AS, Robb A, Maizels RM & Allen JE. Alternatively activated macrophages induced by nematode infection inhibit proliferation via cell-to-cell contact. *Eur J Immunol* 2000; **30**: 2669–2678.
18 Nair MG, Cochrane DW & Allen JE. Macrophages in chronic type 2 inflammation have a novel phenotype characterized by the abundant expression of Ym1 and Fizz1 that can be partly replicated in vitro. *Immunol Lett* 2003; **85**: 173–180.
19 Nair MG, Gallagher IJ, Taylor MD, *et al*. Chitinase and Fizz family members are a generalized feature of nematode infection with selective upregulation of Ym1 and Fizz1 by antigen-presenting cells. *Infect Immun* 2005; **73**: 385–394.
20 Taylor MD, Harris A, Nair MG, Maizels RM & Allen JE. F4/80$^+$ alternatively activated macrophages control CD4$^+$ T cell hyporesponsiveness at sites peripheral to filarial infection. *J Immunol* 2006; **176**: 6918–6927.
21 Gause WC, Urban JF Jr & Stadecker MJ. The immune response to parasitic helminths: insights from murine models. *Trends Immunol* 2003; **24**: 269–277.
22 Pesce J, Kaviratne M, Ramalingam TR, *et al*. The IL-21 receptor augments Th2 effector function and alternative macrophage activation. *J Clin Invest* 2006; **116**: 2044–2055.
23 Reece JJ, Siracusa MC & Scott AL. Innate immune responses to lung-stage helminth infection induce alternatively activated alveolar macrophages. *Infect Immun* 2006; **74**: 4970–4981.
24 Reese TA, Liang HE, Tager AM, *et al*. Chitin induces accumulation in tissue of innate immune cells associated with allergy. *Nature* 2007; **447**: 92–96.
25 Sukhdeo MV, O'Grady RT & Hsu SC. The site selected by the larvae of *Heligmosomoides polygyrus*. *J Helminthol* 1984; **58**: 19–23.
26 Cypess RH, Lucia HL, Dunsford HA & Enriquez FJ. The tissue reactions of mice to infection with *Heligmosomoides polygyrus*. *J Helminthol* 1988; **62**: 69–76.
27 Anthony RM, Urban JF Jr, Alem F, *et al*. Memory T (H) 2 cells induce alternatively activated macrophages to mediate protection against nematode parasites. *Nat Med* 2006; **12**: 955–960.
28 Stadecker MJ, Kamisato JK & Chikunguwo SM. Induction of T helper cell unresponsiveness to antigen by macrophages from schistosomal egg granulomas. A basis for immunomodulation in schistosomiasis? *J Immunol* 1990; **145**: 2697–2700.

29 Pearce EJ & MacDonald AS. The immunobiology of schistosomiasis. *Nat Rev Immunol* 2002; **2**: 499–511.
30 Smith P, Walsh CM, Mangan NE, *et al*. *Schistosoma mansoni* worms induce anergy of T cells via selective up-regulation of programmed death ligand 1 on macrophages. *J Immunol* 2004; **173**: 1240–1248.
31 Latchman Y, Wood CR, Chernova T, *et al*. PD-L2 is a second ligand for PD-1 and inhibits T cell activation. *Nat Immunol* 2001; **2**: 261–268.
32 Sharpe AH, Wherry EJ, Ahmed R & Freeman GJ. The function of programmed cell death 1 and its ligands in regulating autoimmunity and infection. *Nat Immunol* 2007; **8**: 239–245.
33 Herbert DR, Holscher C, Mohrs M, *et al*. Alternative macrophage activation is essential for survival during schistosomiasis and downmodulates T helper 1 responses and immunopathology. *Immunity* 2004; **20**: 623–635.
34 Donnelly S, O'Neill SM, Sekiya M, Mulcahy G & Dalton JP. Thioredoxin peroxidase secreted by *Fasciola hepatica* induces the alternative activation of macrophages. *Infect Immun* 2005; **73**: 166–173.
35 Flynn RJ, Mannion C, Golden O, Hacariz O & Mulcahy G. Experimental *Fasciola hepatica* infection alters responses to tests used for diagnosis of bovine tuberculosis. *Infect Immun* 2007; **75**: 1373–1381.
36 Nair MG, Guild KJ & Artis D. Novel effector molecules in type 2 inflammation: lessons drawn from helminth infection and allergy. *J Immunol* 2006; **177**: 1393–1399.
37 Francois A, Favennec L, Cambon-Michot C, *et al*. *Taenia crassiceps* invasive cysticercosis: a new human pathogen in acquired immunodeficiency syndrome? *Am J Surg Pathol* 1998; **22**: 488–492.
38 Heldwein K, Biedermann HG, Hamperl WD, *et al*. Subcutaneous *Taenia crassiceps* infection in a patient with non-Hodgkin's lymphoma. *Am J Trop Med Hygiene* 2006; **75**: 108–111.
39 Chau CY & Freeman RS. Intraperitoneal passage of *Taenia crassiceps* in rats. *J Parasitol* 1976; **62**: 837–839.
40 Ishida MM, Rubinsky-Elefant G, Ferreira AW, Hoshino-Shimizu S & Vaz AJ. Helminth antigens (*Taenia solium*, *Taenia crassiceps*, *Toxocara canis*, *Schistosoma mansoni* and *Echinococcus granulosus*) and cross-reactivities in human infections and immunized animals. *Acta Trop* 2003; **89**: 73–84.
41 Terrazas LI, Bojalil R, Govezensky T & Larralde C. Shift from an early protective Th1-type immune response to a late permissive Th2-type response in murine cysticercosis (*Taenia crassiceps*) *J Parasitol* 1998; **84**: 74–81.
42 Toenjes SA, Spolski RJ, Mooney KA & Kuhn RE. The systemic immune response of BALB/c mice infected with larval *Taenia crassiceps* is a mixed Th1/Th2-type response. *Parasitology* 1999; **118**: 623–633.
43 Raes G, Brys L, Dahal BK *et al*. Macrophage galactose-type C-type lectins as novel markers for alternatively activated macrophages elicited by parasitic infections and allergic airway inflammation. *J Leukoc Biol* 2005; **77**: 321–327.
44 Terrazas LI, Montero D, Terrazas CA, Reyes JL & Rodriguez-Sosa M. Role of the programmed death-1 pathway in the suppressive activity of alternatively activated macrophages in experimental cysticercosis. *Int J Parasitol* 2005; **35**: 1349–1358.
45 Rodriguez-Sosa M, David JR, Bojalil R, Satoskar AR & Terrazas LI. Cutting edge: susceptibility to the larval stage of the helminth parasite *Taenia crassiceps* is mediated by Th2 response induced via STAT6 signaling. *J Immunol* 2002; **168**: 3135–3139.
46 Gomez-Garcia L, Rivera-Montoya I, Rodriguez-Sosa M & Terrazas LI. Carbohydrate components of *Taenia crassiceps* metacestodes display Th2-adjuvant and anti-inflammatory properties when co-injected with bystander antigen. *Parasitol Res* 2006; **99**: 440–448.
47 Chavarria A, Roger B, Fragoso G, *et al*. TH2 profile in asymptomatic *Taenia solium* human neurocysticercosis. *Microbes Infect* 2003; **5**: 1109–1115.
48 Bueno EC, dos Ramos Machado L, Livramento JA & Vaz AJ. Cellular immune response of patients with neurocysticercosis (inflammatory and non-inflammatory phases). *Acta Trop* 2004; **91**: 205–213.
49 Chavarria A, Fleury A, Garcia E, Marquez C, Fragoso G & Sciutto E. Relationship between the clinical heterogeneity of neurocysticercosis and the immune-inflammatory profiles. *Clin Immunol* 2005; **116**: 271–278.
50 Vuitton DA. The ambiguous role of immunity in echinococcosis: protection of the host or of the parasite? *Acta Trop* 2003; **85**: 119–132.
51 Mejri N & Gottstein B. Intraperitoneal *Echinococcus multilocularis* infection in C57BL/6 mice affects CD40 and B7 costimulator expression on peritoneal macrophages and impairs peritoneal T cell activation. *Parasite Immunol* 2006; **28**: 373–385.
52 Persaud R, Wang A, Reardon C & McKay DM. Characterization of the immuno-regulatory response to the tapeworm *Hymenolepis diminuta* in the non-permissive mouse host. *Int J Parasitol* 2007; **37**: 393–403.
53 O'Neal SE, Guimaraes LH, Machado PR, *et al*. Influence of helminth infections on the clinical course of and immune response to *Leishmania braziliensis* cutaneous leishmaniasis. *J Infect Dis* 2007; **195**: 142–148.
54 La Flamme AC, Scott P & Pearce EJ. Schistosomiasis delays lesion resolution during Leishmania major infection by impairing parasite killing by macrophages. *Parasite Immunol* 2002; **24**: 339–345.
55 Lamb TJ, Graham AL, Le Goff L & Allen JE. Co-infected C57BL/6 mice mount appropriately polarized and compartmentalized cytokine responses to *Litomosoides sigmodontis* and *Leishmania major* but disease progression is altered. *Parasite Immunol* 2005; **27**: 317–324.
56 Rodriguez-Sosa M, Rivera-Montoya I, Espinoza A, *et al*. Acute cysticercosis favours rapid and more severe lesions caused by *Leishmania major* and *Leishmania mexicana* infection, a role for alternatively activated macrophages. *Cell Immunol* 2006; **242**: 61–71.
57 Rodriguez M, Terrazas LI, Marquez R & Bojalil R. Susceptibility to *Trypanosoma cruzi* is modified by a previous non-related infection. *Parasite Immunol* 1999; **21**: 177–185.
58 Graham AL, Lamb TJ, Read AF & Allen JE. Malaria–filaria coinfection in mice makes malarial disease more severe unless filarial infection achieves patency. *J Infect Dis* 2005; **191**: 410–421.
59 Kahnert A, Seiler P, Stein M, *et al*. Alternative activation deprives macrophages of a coordinated defense program to *Mycobacterium tuberculosis*. *Eur J Immunol* 2006; **36**: 631–647.
60 Elston DM. The hygiene hypothesis and atopy: bring back the parasites? *J Am Acad Dermatol* 2006; **54**: 172–179.
61 McKay DM. The beneficial helminth parasite? *Parasitology* 2006; **132**: 1–12.

62 Kuroda E, Yoshida Y, En Shan B & Yamashita U. Suppression of macrophage interleukin-12 and tumour necrosis factor-α production in mice infected with *Toxocara canis*. *Parasite Immunol* 2001; **23**: 305–311.
63 Brys L, Beschin A, Raes G, *et al*. Reactive oxygen species and 12/15-lipoxygenase contribute to the antiproliferative capacity of alternatively activated myeloid cells elicited during helminth infection. *J Immunol* 2005; **174**: 6095–6104.
64 Rodriguez-Sosa M, Saavedra R, Tenorio EP, Rosas LE, Satoskar AR & Terrazas LI. A STAT4-dependent Th1 response is required for resistance to the helminth parasite *Taenia crassiceps*. *Infect Immun* 2004; **72**: 4552–4560.

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

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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,

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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).

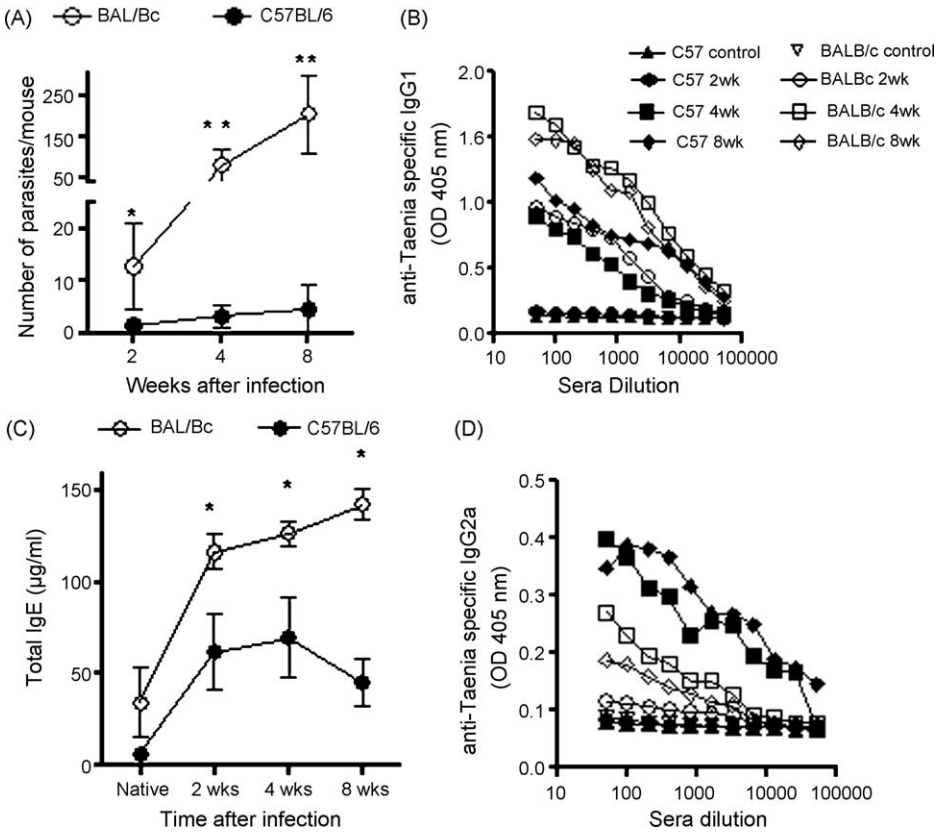

Fig. 1. Time course of *T. crassiceps* infection and antibody response in BALB/c and C57BL/6 mice. (A) Parasite load: Animals were infected i.p. with ten cysticerci and the parasite load was monitored 2, 4 and 8 wk post-infection. The data are expressed as the mean number of parasites per mouse ± SD. 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

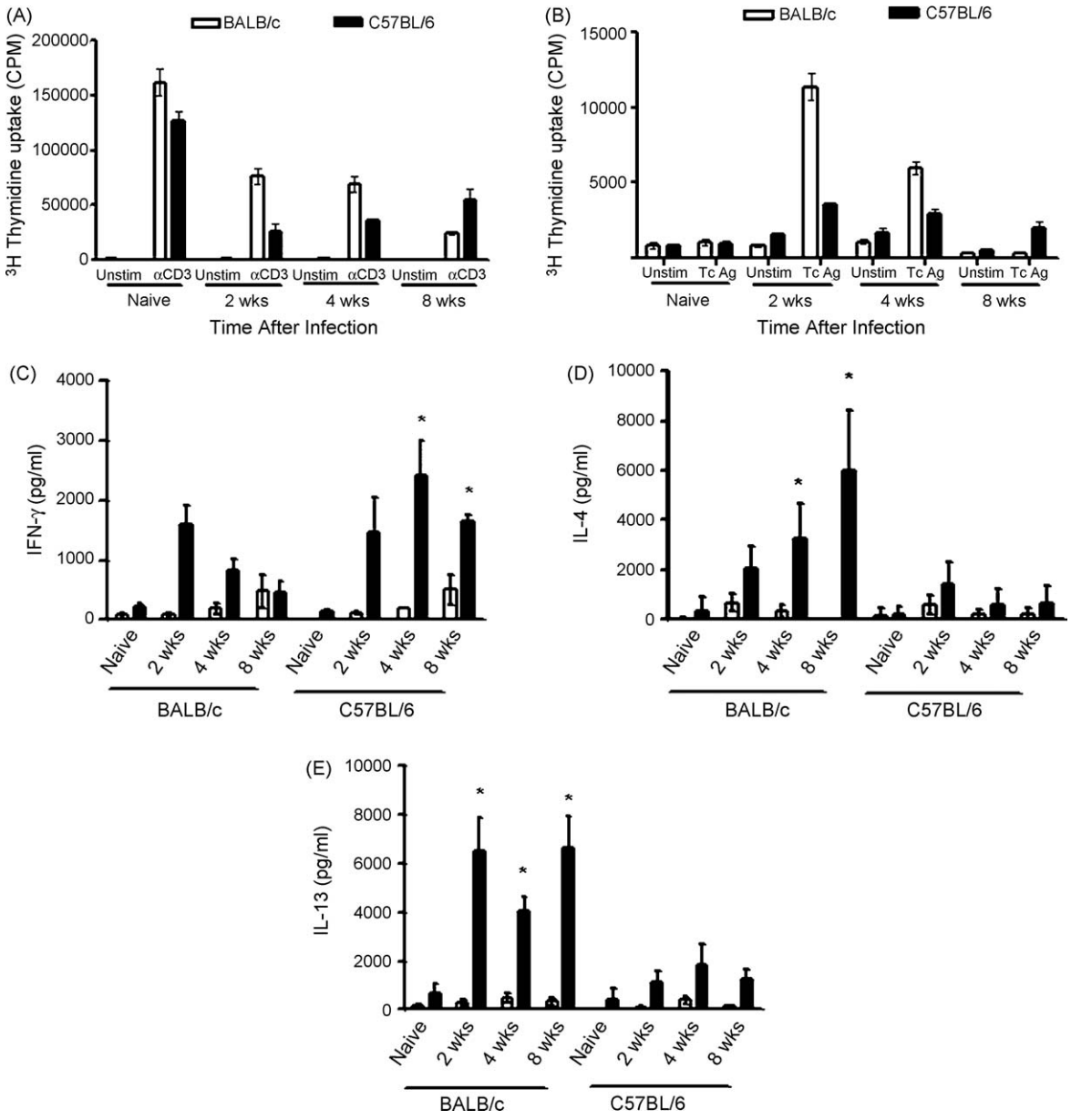

Fig. 2. Kinetics of the adaptive immune response. Spleen cell proliferation in response to anti-CD3 stimuli (A) and to *T. crassiceps* Ag (B). IFN-γ (C), IL-4 (D) and IL-13 (E) production in response to *T. crassiceps* Ag-stimulated splenocytes from *T. crassiceps*-infected BALB/c and C57BL/6 mice as 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.

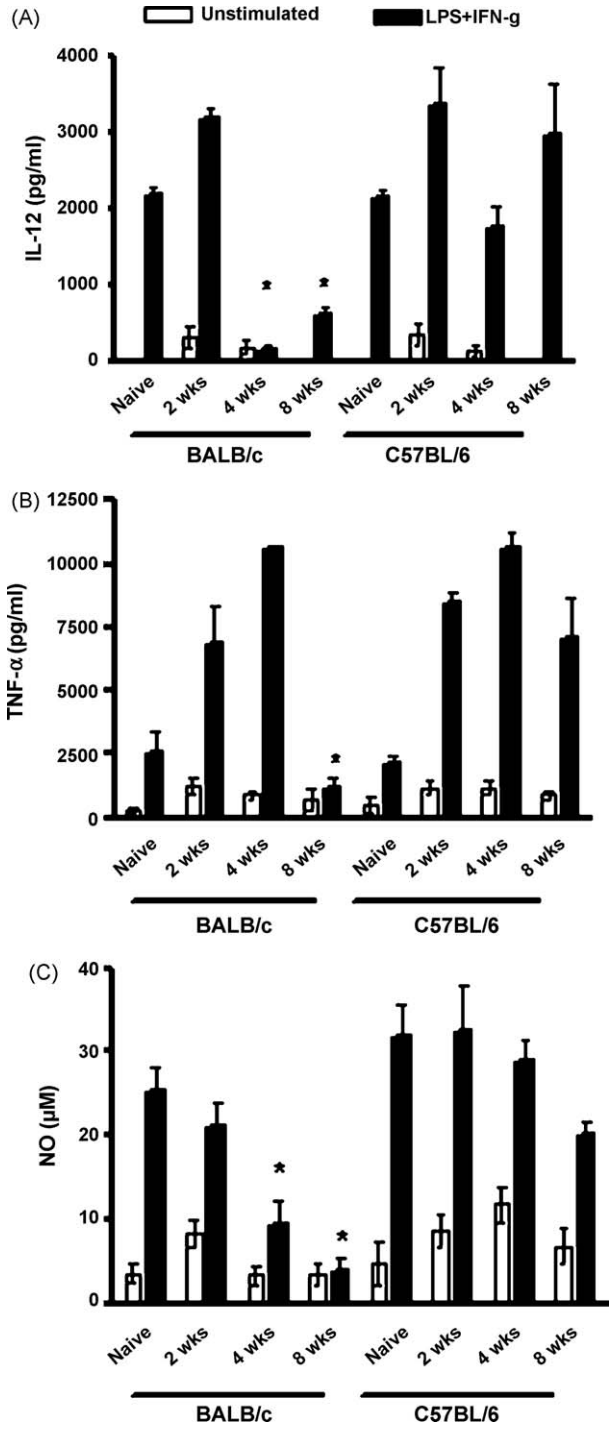

Fig. 3. LPS and IFN-γ activation of macrophages isolated from *T. crassiceps*-infected BALB/c and C57BL/6 mice. Peritoneal macrophages were isolated as described at different time points post-infection and stimulated with LPS (1 μg/ml) plus IFN-γ (20 ng/ml). Supernatants were collected 24 h later and 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*-

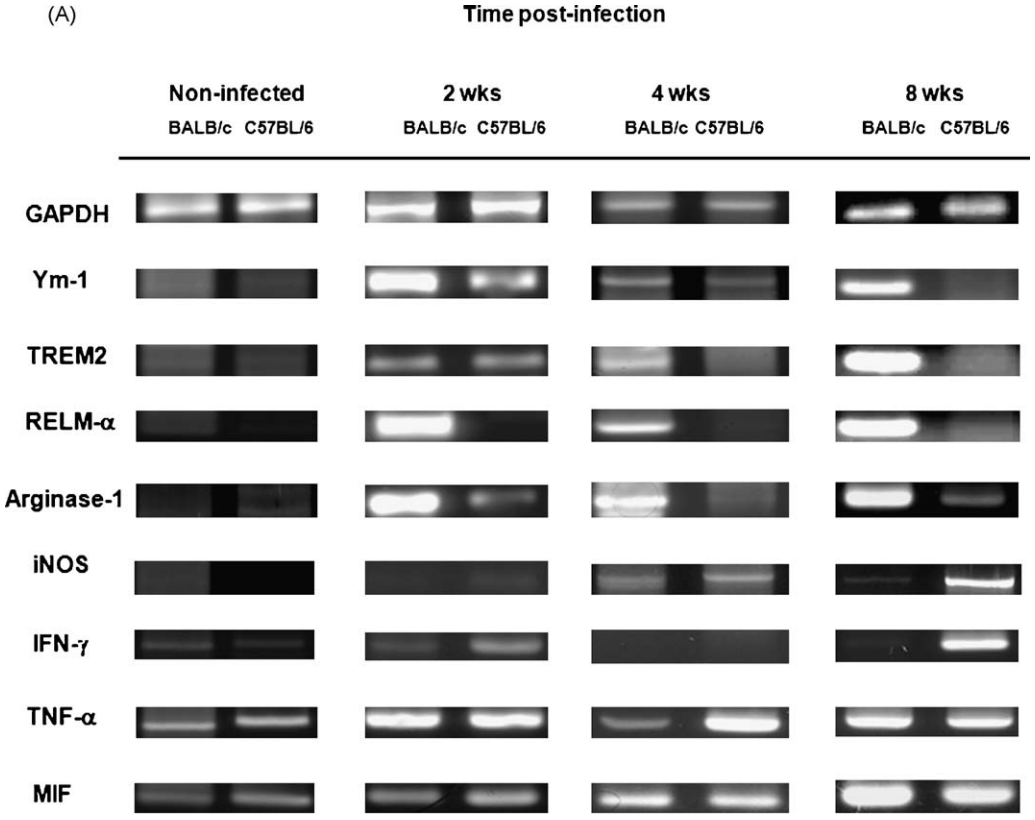

Fig. 4. Kinetics of macrophage activation at site of infection during *T. crassiceps* infection. (A) Macrophages were isolated from the peritoneal cavity of BALB/c and C57BL/6 mice at different time points following infection with ten cysticerci. Without any additional stimulation, RNA was extracted 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.

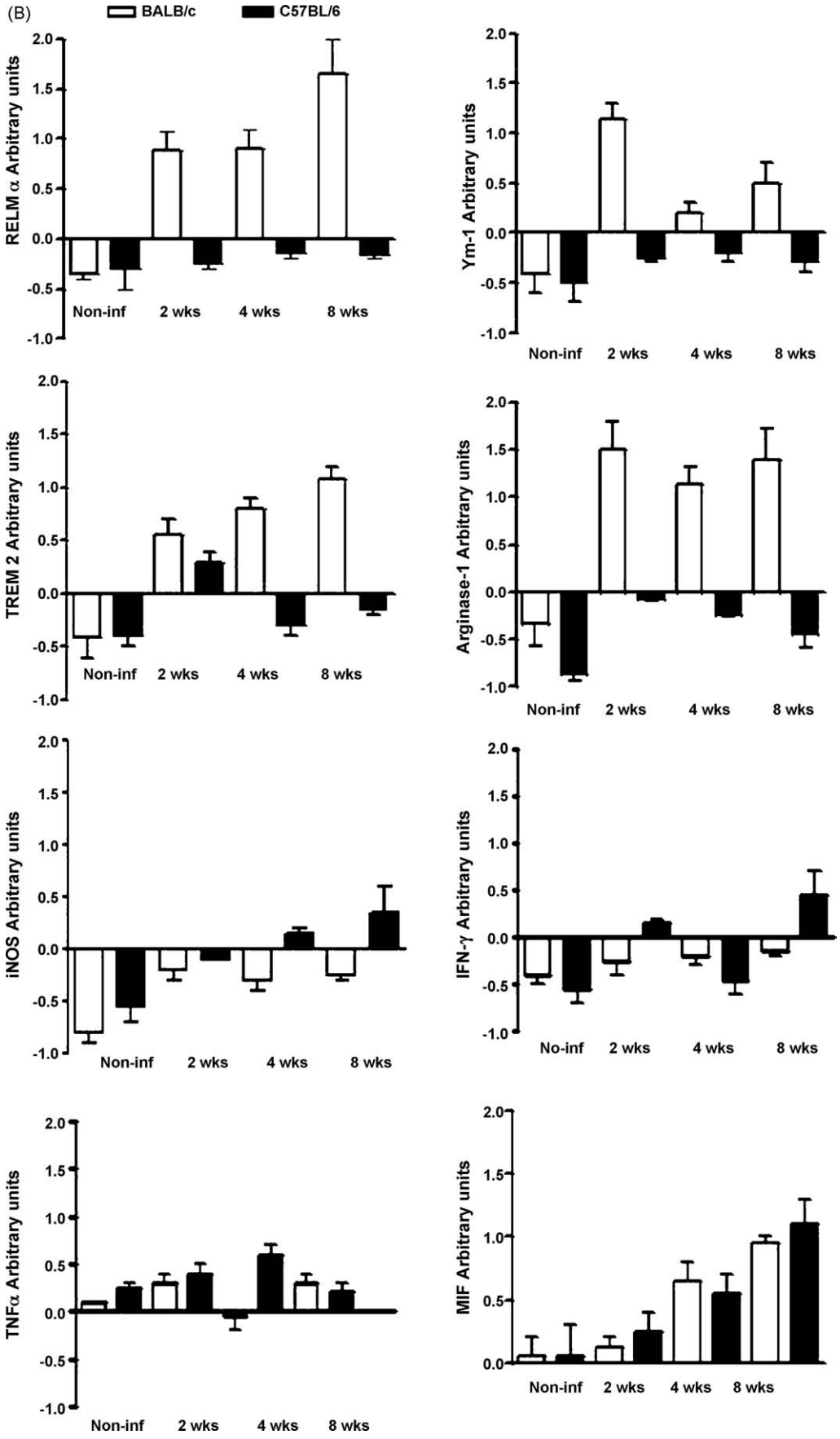

Fig. 4. (*Continued*).

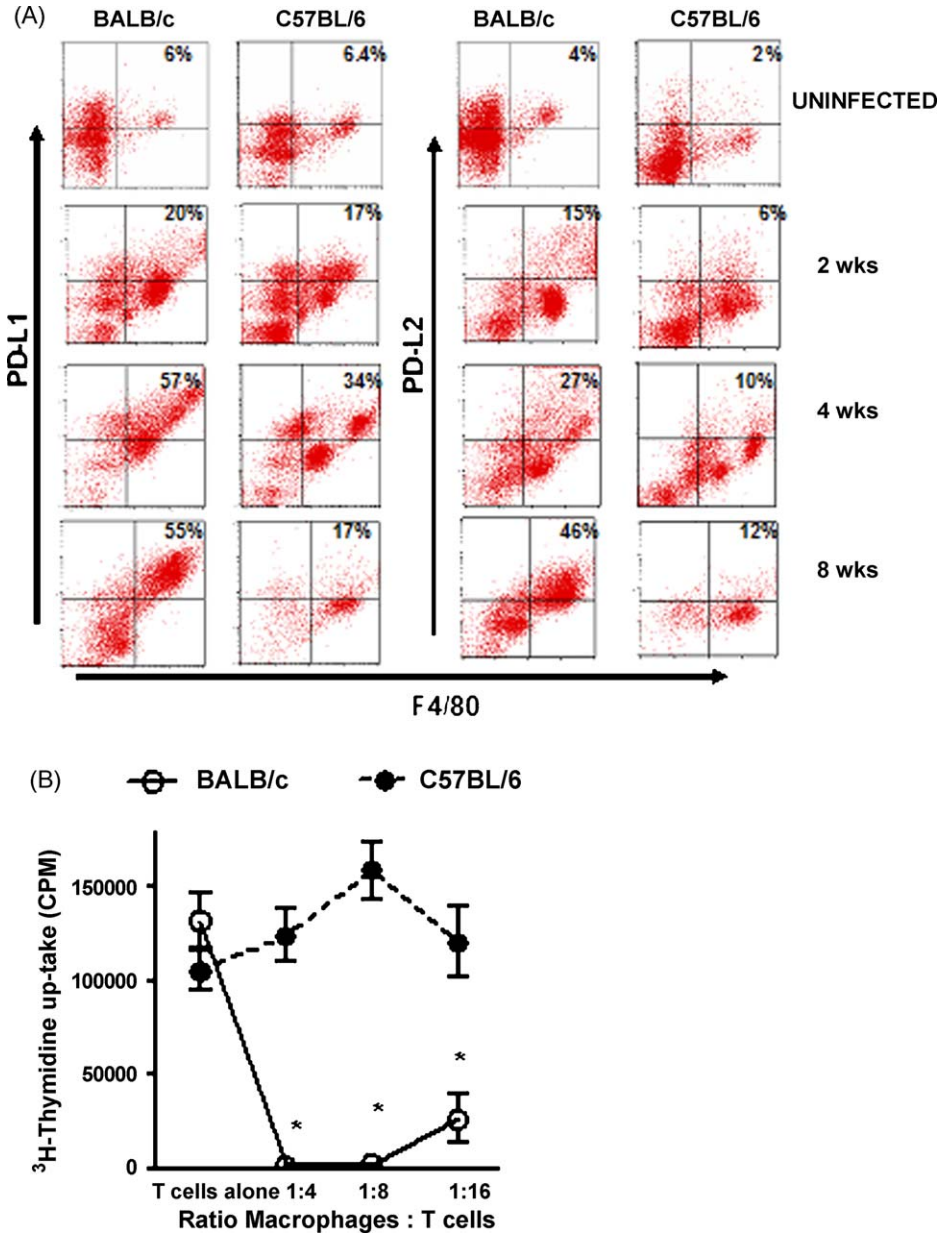

Fig. 5. Kinetics of PD-L1 and PD-L2 expression and suppressive activity of peritoneal macrophages after *T. crassiceps* infection. (A) Peritoneal cells were isolated from BALB/c and C57BL/6 mice at different time points after infection with ten cysticerci. Without any additional stimulation, the cells 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

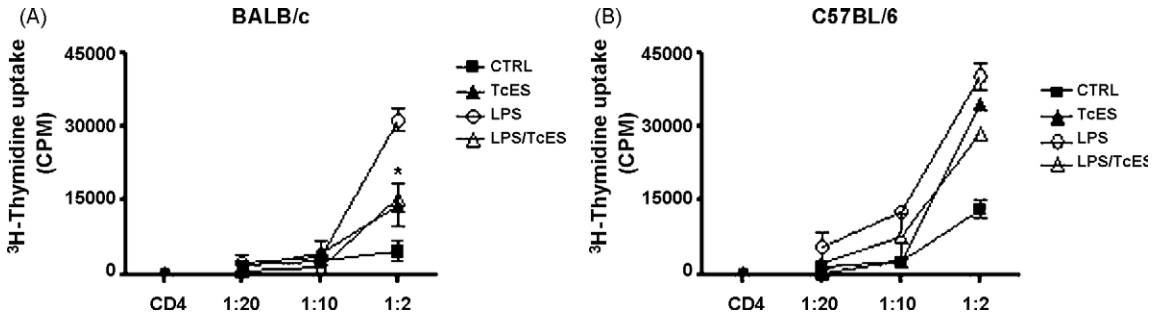

Fig. 6. Co-stimulation of DCs from BALB/c, but not in C57BL/6 mice, with LPS plus TcES decreases DC allogenic stimulation (A) BMDCs from BALB/c mice were stimulated for 24 h with medium alone (CTRL), TcES, LPS or TcES + LPS and co-cultured with $CD4^+$ cells from naïve C57BL/6 mice. (B) BMDCs from C57BL/6 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.,

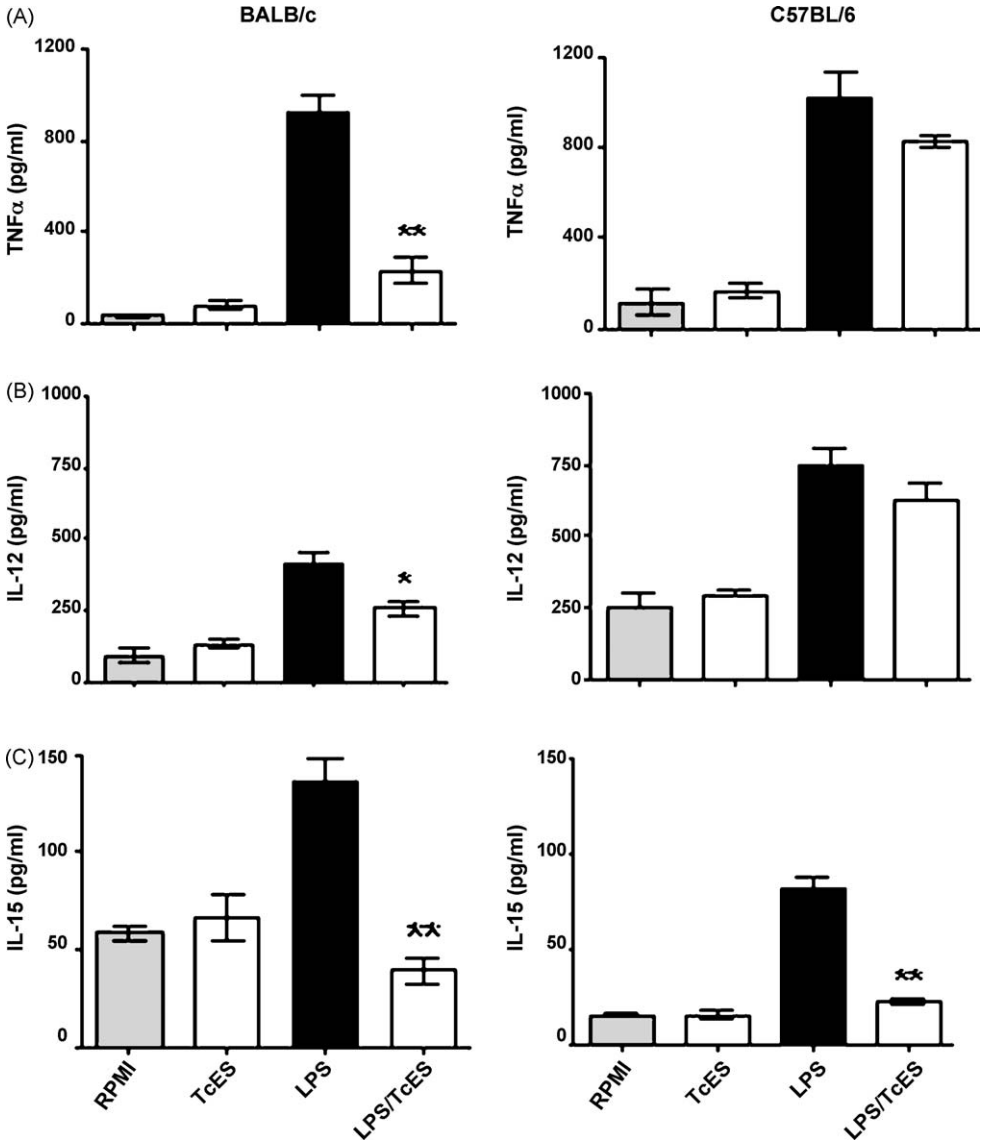

Fig. 7. Differential effects of TcES exposure on the cytokine production by LPS-stimulated DCs. Effect of TcES (20 μg/ml) on the production of TNF-α (A), IL-12 (B) and IL-15 (C) by C57BL/6 and BALB/c BMDCs stimulated with 1 μg/ml LPS. The data are representative of two independent experiments. An asterisk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

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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.

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Early removal of alternatively activated macrophages leads to *Taenia crassiceps* cysticercosis clearance in vivo

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ABSTRACT

To determine the role of alternatively activated macrophages in modulating the outcome of experimental cysticercosis caused by *Taenia crassiceps*, we investigated the effect of removal of alternatively activated macrophage by injecting clodronate-loaded liposomes into susceptible BALB/c mice. Following *T. crassiceps* infection, mice receiving PBS-loaded liposomes developed a dominant Th2-type response associated with the presence of alternatively activated macrophages together with antigen-specific hyporesponsiveness and high parasite burden. In contrast, similarly infected mice treated with clodronate-loaded liposomes mounted a mixed Th1/Th2-type response, reversed antigen-specific hyporesponsiveness and did not carry notable alternatively activated macrophage populations. These factors were associated with increased resistance to *T. crassiceps* cysticercosis. Interestingly, early AAMϕ depletion was enough to limit parasite growth. However, if macrophages were depleted late in the infection, no effect on parasite burden was observed. These findings demonstrate that alternatively activated macrophages play a critical role in mediating susceptibility to experimental cysticercosis in which their early recruitment may favor parasite survival.

1. Introduction

Helminth infections exert profound regulatory effects on the immune systems of their hosts; one common feature of these infections is a strong Th2-biased immune response. However, during the last few years new regulatory mechanisms have been discovered to play a role during helminth infections. One such mechanism is the induction of T-regulatory cells (Tregs) which are now recognized to be involved in pathogen susceptibility as well as in controlling inflammation in some helminth infections caused by *Litomosoides sigmodontis* (D'Elia et al., 2009), *Trichuris muris* (Taylor et al., 2009), *Brugia malayi* (McSorley et al., 2008), *Trichinella spiralis* (Beiting et al., 2007) and *Heligmosomoides polygyrus* (Rausch et al., 2008). Dendritic cells (DCs) have also been reported to be affected by helminth-derived products (Carvalho et al., 2009; Hewitson et al., 2009) and recently a new cell population of macrophages called alternatively activated macrophages (AAMϕs) has been consistently observed in several worm infections (Kreider et al., 2007; Reyes and Terrazas, 2007).

It is well known that macrophage differentiation is plastic, allowing them to adapt and acquire many phenotypes depending on their cytokine microenvironment. Macrophages stimulated with pro-inflammatory cytokines such as IFN-γ and bacterial stimuli such as lipopolysacharides (LPS) are highly activated, capable of killing phagocytosed pathogens. This activation phenotype has prominent features, such as production of inflammatory cytokines and inducible nitric oxide synthase (iNOS)-mediated reactive oxygen species (ROS). These macrophages are now recognized as classically activated macrophages (CAMϕs). In contrast, AAMϕs were originally demonstrated to respond to IL-4 in vitro by increasing mannose receptor (MR) expression (Gordon, 2003) with no evident role in the immune response. Interestingly, similar populations are now known to arise in response to diverse pathological conditions including tumors (Weigert and Brune, 2008; Ostrand-Rosenberg and Sinha, 2009) and other parasitic infections (Noel et al., 2004; Raes et al., 2007). These types of macrophages preserve the ability to phagocytose, present antigen and up-regulate co-stimulatory molecules, but their metabolism of L-arginine is altered due to an increased expression of arginase-1 concomitant with decreased expression of iNOS.

The presence of AAMϕs has been well documented during infection with all three classes of parasitic helminths such as nematodes, trematodes and cestodes, but divergent roles have been re-

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ported for these types of regulatory cells (Reyes and Terrazas, 2007). For example, lung emphysema caused by *Nippostrongylus brasiliensis* infection has been associated with the presence of AAMϕs (Marsland et al., 2008), while in *H. polygyrus* intestinal infection, AAMϕs showed effector functions involved in the clearance of this nematode parasite (Anthony et al., 2006). In contrast, in filariasis (Taylor et al., 2006) and *Hymenolepis diminuta* infection (Persaud et al., 2007), the functional role for AAMϕs is still not fully understood, but here AAMϕs were associated with suppression of the immune response. Another feature of AAMϕs is their anti-inflammatory activity, which serves to dampen immunopathology in experimental schistosomiasis (Herbert et al., 2004). Moreover, during *Taenia crassiceps* infection, we and others (Brys et al., 2005; Terrazas et al., 2005) have found AAMϕs that display suppressive activity associated with high expression of PD-L1 and PD-L2 on their membranes, suggesting an important role for these regulatory cells in experimental cysticercosis. However, a specific role for AAMϕs in *T. crassiceps* infection has not yet been elucidated.

The purpose of this study was to determine the role of AAMϕs in the outcome of murine cysticercosis caused by the helminth *T. crassiceps*. To address this question, we compared the course of *T. crassiceps* infection in BALB/c mice treated with clodronate-loaded liposomes at various time points after infection. In addition, we analyzed antibody profiles in sera, cellular responses and cytokine profiles in spleen cells and peritoneal macrophages as well as dendritic cell activity. Our data demonstrated that AAMϕs represent a key cell population involved in favoring experimental cysticercosis. Here we present the first report, to our knowledge, demonstrating that AAMϕs are deleterious in *T. crassiceps* infection.

2. Materials and methods

2.1. Mice, parasites and infection

Six- to 8-week-old female BALB/cAnN mice were purchased from Harlan Laboratories (México) and were maintained in a pathogen-free environment at the Facultad de Estudios Superiores (FES)-Iztacala, Universidad Nacional Autonoma de Mexico (UNAM), Mexico, animal facility in accordance with institutional and national guidelines. All experiments using mice were approved by the Committee of Bioethics at FES-Iztacala. Mice were injected i.p. with 10 metacestodes of the *T. crassiceps* ORF strain.

2.2. Liposome treatment

Given that clodronate-loaded liposomes are the most effective and widely used method to deplete macrophages in a highly specific way (van Rooijen et al., 1996), we decided to treat mice i.p. with 2 mg of either clodronate-loaded or PBS-loaded liposomes on a weekly basis. Our group has previously shown that in *T. crassiceps* infection, macrophages undergo a phenotypic and functional switch from the acute (2 weeks p.i.) to chronic stage (8 weeks p.i.) (Rodriguez-Sosa et al., 2002b). Taking this into account, we had four different experimental groups: group 1: mice infected with 10 metacestodes and treated during the full 8 weeks with PBS liposomes (control), group 2: mice infected with 10 metacestodes and treated during the full 8 weeks with clodronate-liposomes, group 3: mice infected with 10 metacestodes and treated during the first 3 weeks with clodronate-liposomes and the last 5 weeks with PBS liposomes, and lastly group 4: mice infected with 10 metacestodes and treated during the first 5 weeks with PBS liposomes and the last 3 weeks with clodronate-liposomes. After 8 weeks of infection, mice from the experimental groups were sacrificed and peritoneal lavage was performed under aseptic conditions to obtain and count parasites.

2.3. Flow cytometry analysis

In order to determine drug treatment efficacy, total peritoneal exudate cells (PECs) were analyzed by fluorescence activated cell sorting (FACS) at 4 and 8 weeks p.i. A gate including high forward light scatter (FSC)/high side light scatter (SSC) cells was generated and in that gate the different markers were analyzed. Briefly, 1×10^6 PECs were incubated with anti-CD16 and anti-CD32 antibodies (Biolegend, San Diego, CA, USA) to block non-specific binding. Next, cells were stained with specific anti-F4/80, anti-MAC 3, anti-PD-L1, anti-PD-L2 (all from Biolegend) and anti-Siglec-F (BD-Pharmingen) and incubated for 30 min at 4 °C. Cells were washed twice with 1 ml of FACS buffer (containing 1% FBS and 0.5% of sodium azide in PBS). Analyses of cells were performed using the FACSCalibur system and Cell Quest software (Becton Dickinson).

2.4. Spleen cell cultures

At the indicated time points, spleens were aseptically removed from different groups of mice. Single-cell suspensions were prepared by gently teasing apart the spleen in RPMI-1640 media supplemented with 10% FBS, 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, USA). The cells were centrifuged at 1000 *g* in an Eppendorf centrifuge (5702) and erythrocytes were lysed by resuspending the cells in Boyle's solution (0.17 M Tris and 0.16 M ammonium chloride). Following two washes with PBS, 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. One hundred microliters of cells per well were seeded in 96-well plates (Costar, Cambridge, Massachusetts, USA) and stimulated either with *T. crassiceps* total soluble antigen (TcAg, 25 μg/ml) or concanavalin A (5 μg/ml) as a non-specific stimulus. After incubation

Table 1
Genes and their respective sequences used to determine alternative activation of macrophages.

Gene	Sequence	Melting temperature (°C)	Reference
GAPDH	F- CTC ATG ACC ACA GTC CAT GC R-CAC ATT GGG GGT AGG AAC AC	54	Renshaw et al. (2002)
iNOS	F-CTGGAG GAG CTC CTG CCT CATG R-GCA GCA TCC CCT CTG ATG GTG	65	Yoshida et al. (2000)
Arg-1	F-CAG AAG AAT GGA AGA GTC AG R-CAG ATA TGC AGG GAG TCA CC	54	Nair et al. (2003)
Relm-α	F-GGTCCCAGTGCATATGGATGAGACCATAGA R-CACCTCTTCACTCGAGGGACAGTTGGCAGC	62	Nair et al. (2003)
Ym-1	F-TCACAGGTCTGGCAATTCTTCTG R-TTTGTCCTTAGGAGGGCTTCCTC	56	Nair et al. (2003)

F, forward primer; R, reverse primer.

for 72 h at 37 °C and 5% CO_2, [^{3}H]-thymidine (Amersham) was added and supernatants were frozen and stored at −80 °C until used.

2.5. Cytokine quantization by ELISA sandwich

IFN-γ, IL-4 and IL-13 levels in supernatants from spleen cell cultures were measured using a sandwich ELISA, according to the manufacturer's instructions (Peprotech-México, México, D.F.).

2.6. Reverse transcription (RT)-PCR analyses

The levels of arginase 1 (Arg-1), iNOS, Ym-1 and Resistin-like molecule-α (RELM-α) mRNA transcripts in adherent peritoneal macrophages (which were led to adhere 2 h at 37 °C and 5% CO_2) were determined using RT-PCR. At the indicated time points, adherent peritoneal macrophages from *T. crassiceps*-infected BALB/c and healthy mice were aseptically removed and without any further stimulation were processed for RNA extraction using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and the 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) 5X PCR Buffer blue, 10 mM dNTP, 40 nM each forward and reverse primers (Table 1), 1 U Taq DNA polymerase (Sacace Biotechnologies, Italy) and 2 μl of the cDNA. The program used for the amplification of each gene contained 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 at 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

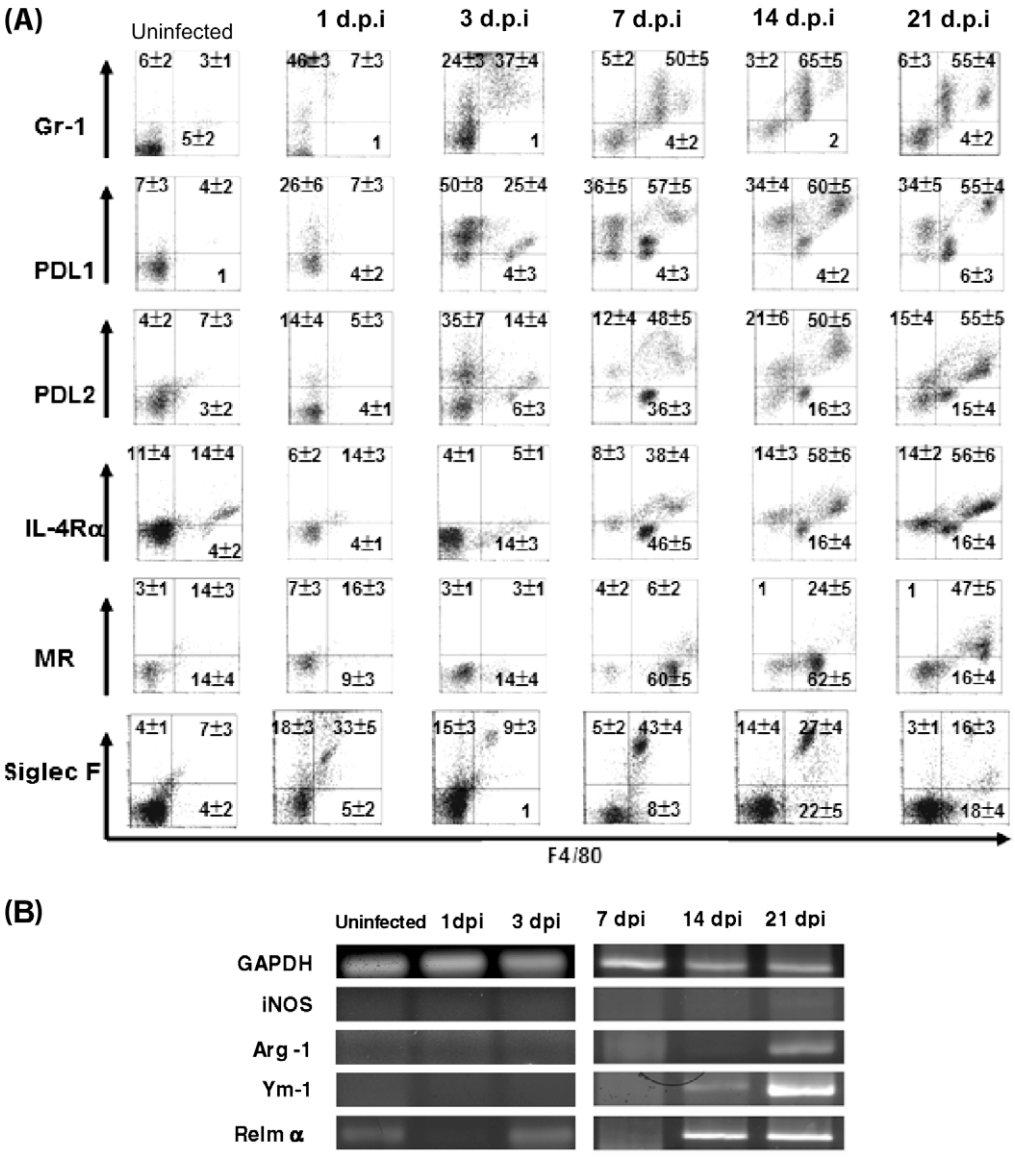

Fig. 1. Kinetics of alternatively activated macrophages during *Taenia crassiceps* acute infection. Peritoneal exudate cells (PECs) were recovered after infection with 10 metacestodes at indicated time points during the first 3 weeks of infection. (A) Flow cytometry analysis shows that macrophages (F4/80^{hi}) are recruited within 3 days p.i. (d.p.i.) and increased numbers are detected as infection progresses. In contrast, neutrophils (Gr-1$^+$) and eosinophils (F4/80^{lo} Siglec-F$^+$) are rapidly recruited. (B) Reverse transcription-PCR assays were performed in adherent peritoneal cells without any additional stimulus in order to determine the activation status of macrophages. mRNA expression shows that macrophages with alternative activation markers are clearly detected within 2 weeks p.i. The data shown are representative of two independent experiments ($n = 4$). MR, mannose receptor.

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 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

At the different time points expression levels for each sample were normalized against the Housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (*gapdh*) using a Fujifilm FLA 5000 scanner to capture the images. Values are presented as arbitrary units (an average of four animals is shown).

2.8. Cytospin

PECs were harvested from different mice, adjusted to 300,000 cells/300 ul and centrifuged at 60*g* for 10 min using a Hermle Z300 (Germany) centrifuge with cytospin adapters. Images were captured using AxioVision Rel 4.6 and an AxioCam ICc3 connected to an Axiostar (Zeiss) Microscope to record the images.

2.9. Statistical analyses

The one-way ANOVA test and 'honestly significant difference' (HSD) Tukey's test were applied to identify significant differences between the observations of multiple groups. The level of significance was established at $P < 0.05$.

3. Results

3.1. Early presence of AAMϕ during T. crassiceps infection

We previously demonstrated that *T. crassiceps* infection induces AAMϕs (Rodriguez-Sosa et al., 2002b; Terrazas et al., 2005); however, the earliest time point that we tested was 4 weeks p.i. Thus, in order to detect the presence of AAMϕs during early infection, we analyzed the population of PECs recruited to the site of infection at different time points, including day 1, after infection with 10 cysticerci. On day 1 p.i., we did not detect the presence of AAMϕs at the site of inoculation, as measured using flow cytometry (Fig. 1A) and RT-PCR (Fig. 1B). At this point, we could detect primarily $Gr1^+/F4/80^-$ cells and Siglec-$F^+/F4/80^{low}$ cells, indicating that at this early time there was no recruitment of macrophages at the site of infection, but the expression of some surface markers, such as PD-L1 and PD-L2, was moderately increased in other cell populations (Fig. 1A). At this time, PECs did not express transcripts for some genes associated with AAMϕ such as Arg-1, Ym-1 and RELM-α (Fig. 1B). The next time points we tested were 3 and 7 days p.i. when some changes started to appear, as shown in Fig. 1A. The population of $Gr1^+$ cells started to express F4/80 by day 3 p.i. ($F4/80^+/Gr1^+$ cells, approx. 37%) and by day 7 p.i. this population increased to 50% plus 4% of $Gr1^-/F4/80^+$. Interestingly, these cells displayed higher expression of PD-L1 and PD-L2, and started to express IL-4Rα as well as MR (or CD206). Nevertheless, at this time point these PECs still did not show any transcripts of alternate activation markers (Fig. 1B). The population of Siglec-F^+ cells decreased by this time point. By day 14 p.i., we detected increasing expression of PD-L1, PD-L2, IL-4Rα and stable expression of MR in F4/80 cells (Fig. 1A) by flow cytometry, whereas Siglec-F^+ cells reached 14%. Also, at this time point, accumulation of Ym-1 and RELM-α transcripts in adherent PECs was detectable by RT-PCR (Fig. 1B), suggesting the initial presence of AAMϕs.

At day 21 p.i., significant expression levels of IL-4Rα MR, PD- L1 and PD-L2 in most $F4/80^+$ cells were detected (Fig. 1A) together with an increase in transcripts for AAMϕ markers (Fig. 1B). Also at this time point we found increasing percentages of single Siglec-F^+ and $F4/80^{hi}$/Siglec-F^{lo}. Thus, the phenotype for macrophages at this time point was $F4/80^+$, IL-4Rα$^+$, MR^+, PD-$L2^+$, PD-$L1^+$, Siglec-F^{lo}, Arg-1^+, $iNOS^-$, RELM-α$^+$, $Ym1^+$, indicating the presence of AAMϕs at the site of infection (Fig. 1A and B).

3.2. Depletion of AAMϕ favors resistance to T. crassiceps infection

The role of AAMϕs in helminth infections in terms of susceptibility or resistance is poorly understood. We decided to directly examine whether AAMϕs play an important role in the outcome of *T. crassiceps* infection. Female mice were infected with 10 cysticerci. The next day, they started to receive i.p. injections every other day with clodronate-loaded liposomes carrying a total of 2 mg of clodronate per week per mouse. Control infected mice received PBS-loaded liposomes. After 4 or 8 weeks p.i., mice were killed and parasite loads were evaluated. As seen in Fig. 2A, mice receiving clodronate-loaded liposomes displayed a significant reduction in parasite loads (>90%, $P < 0.01$, compared with PBS-treated mice). However, mice receiving PBS-loaded liposomes

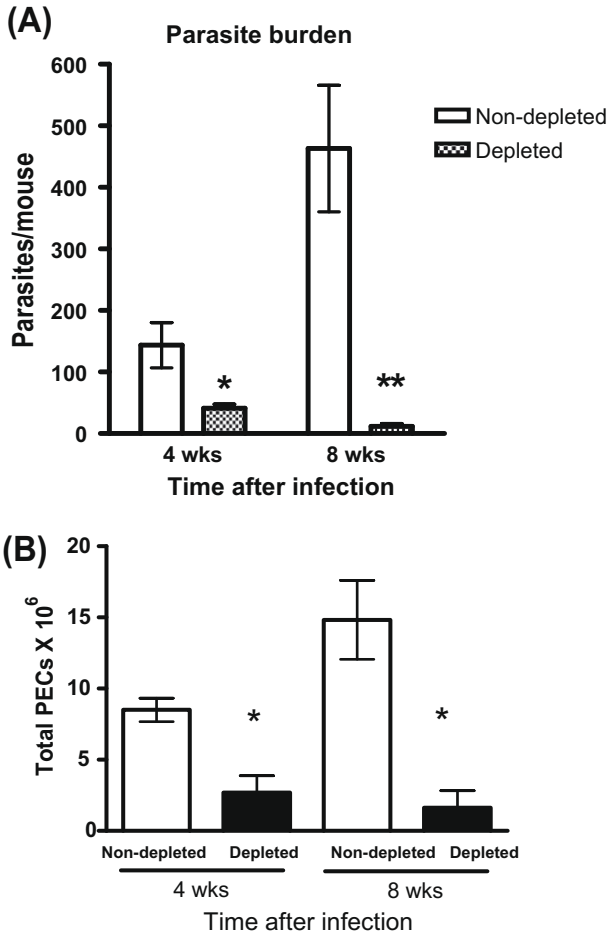

Fig. 2. Depletion of macrophages results in enhanced resistance to *Taenia crassiceps* cysticercosis. (A) Mice were infected with 10 metacestodes of *T. crassiceps* and treated with either PBS- or clodronate-loaded liposomes for 4 or 8 weeks. Parasite load was evaluated at 4 and 8 weeks p.i. and mean + S.D. of parasites in peritoneums are presented ($n = 7$). (B) Numbers of total peritoneal exudate cells (PECs) recruited during infection are depicted. $^*P < 0.05$ compared with non-depleted mice; $^{**}P < 0.01$.

showed a heavy parasite burden similar to that found in untreated-infected mice (data not shown), indicating that liposomes alone did not impair parasite growth.

The effectiveness of clodronate-loaded liposome treatment was evaluated by counting the total population of PECs recruited at different times after infection (Fig. 2B) and by analyzing total PECs stained with anti-F4/80 antibodies and anti-Siglec-F. We observed that mice receiving PBS-liposomes (non-depleted) recruited a higher number of cells (Fig. 2B), as well as a high percentage of F4/80^{hi} cells in the peritoneal cavity (Fig. 3A and B); in contrast, mice treated with clodronate-liposomes displayed a significant reduction of these cells (Fig. 3A and B). Further staining demonstrated the efficacy of clodronate treatment, as PECs from PBS-treated mice showing F4/80^{hi} also displayed high expression of MR, a marker associated with alternative activation (Fig. 3C), whereas the F4/80^{lo} Siglec-F^{+} population remained unchanged after clodronate treatment (Fig. 3A–C); the absence of macrophages was illustrated by cytospin in Fig. 3D.

PECs from animals receiving clodronate-liposomes contained significantly reduced populations of F4/80^{+}PD-L1^{+} PD-L2^{+} MAC 3^{+} cells, while control mice displayed F4/80^{+} cells expressing PD-L1, PD-L2 and MAC 3 (Fig. 4A). These PECs were led to 2 h adherence and RT-PCR showed that they were AAMϕs as determined by detection of transcripts of Arg-1, Ym-1 and RELM-α, but not iNOS (Fig. 4B). In order to show that adherent cells were mainly macrophages we performed cytospin from adherent and non-adherent PECs (Fig. 4C). Together, these data indicated that clodronate-loaded liposomes effectively depleted most of peritoneal macrophages.

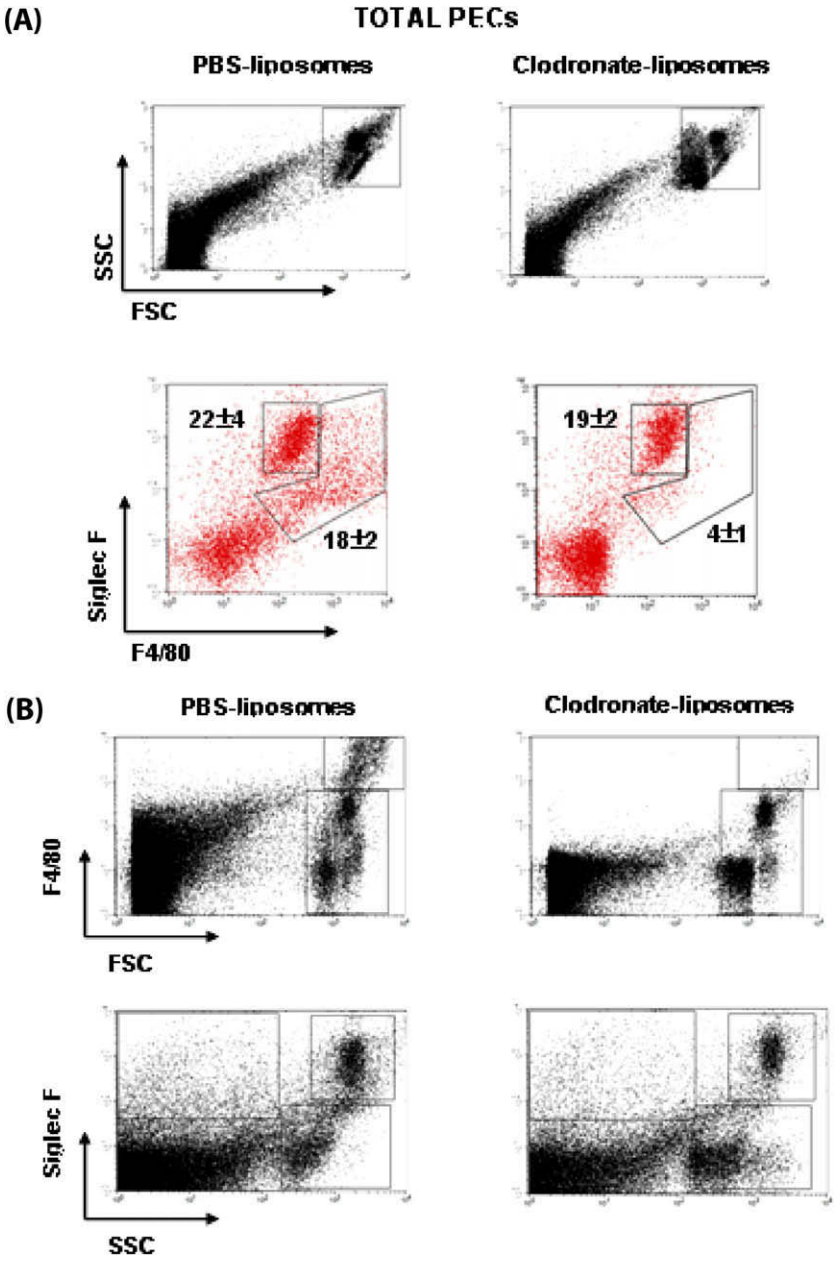

Fig. 3. Clodronate treatment efficiently depletes F4/80^{hi} cells. To determine whether clodronate-liposome treatment targeted the expected population, Flow cytometry analysis was performed on total peritoneal exudate cells (PECs) (A), and evaluation of macrophage and eosinophil survival was performed at 4 weeks after parasite inoculation from PECs (gate selected, square). A clear decrease in the F4/80^{hi} cell population was observed together with the presence of F4/80^{lo}/Siglec-F^{+} (eosinophils). (B) Dot plots showing the effect of clodronate treatment on F4/80^{hi} cells and on Siglec-F^{+} cells. (C) F4/80^{hi} cells also express mannose receptor, which is associated with alternative activation (this population is eliminated after clodronate treatment) whereas F4/80^{lo}Cells express Siglec-F (this population is not affected by treatment). (D) Cytospin of total PECs from PBS- or clodronate-loaded liposomes treated mice (100×). SSC, side light scatter; FSC, forward light scatter; MR, mannose receptor.

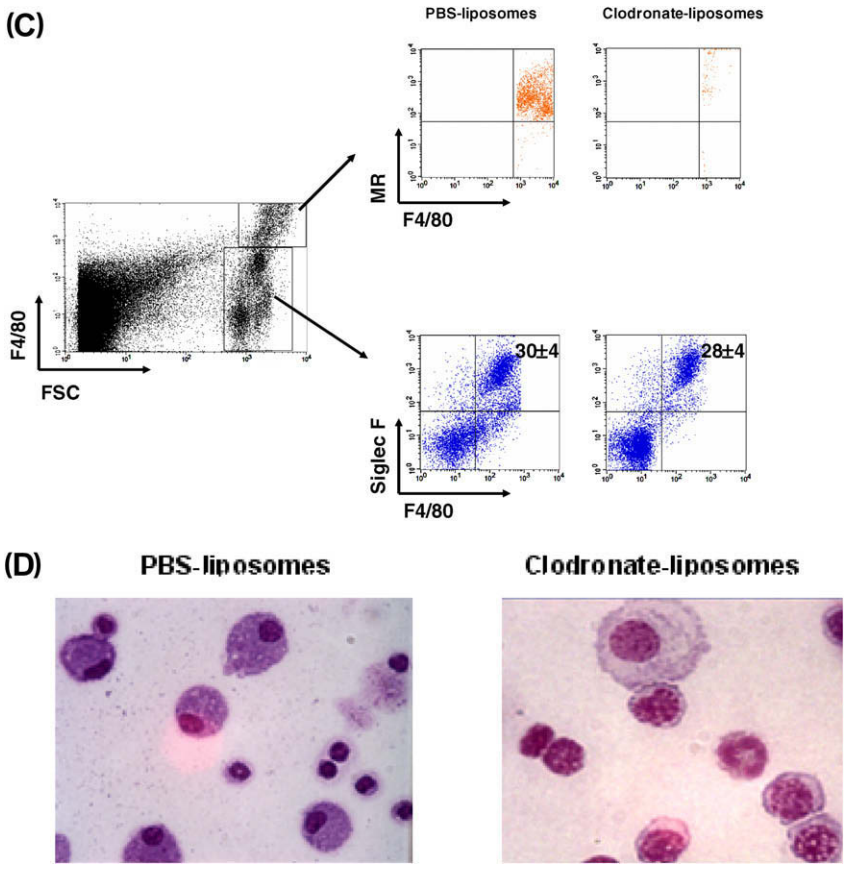

Fig. 3 (*continued*)

3.3. Early, but not late, depletion of AAMϕ is effective in restricting T. crassiceps growth

As we illustrated in Fig. 1, AAMϕs began to be recruited as early as week 2 p.i. and clearly appeared by week 3 p.i. Thus, we decided to determine whether the timing of macrophage depletion may be important to the outcome of this infection. A group of infected mice were treated with clodronate-liposomes during the first 3 weeks of infection and then treatment was stopped. Another group of mice infected at the same time was treated with clodronate-liposomes during the last 3 weeks of infection. Their respective controls were treated with PBS-liposomes during the full course of the experiment and the experiments were finalized at week 8 p.i. *Taenia crassiceps*-infected mice receiving late treatment with clodronate-liposomes were unable to clear the parasite and displayed parasite burdens similar to those obtained from PBS-liposome-treated mice (Fig. 5A). In contrast, parasite burdens were markedly reduced when clodronate-liposome treatment was started early in the infection (Fig. 5A, $P < 0.01$ compared with PBS-liposome treatment). Despite not finding a significant reduction in F4/80$^+$ cells (Fig. 5B) our data on RT-PCR analysis did show that early depletion of macrophages prevented the increase of AAMϕ markers, while macrophages in the late-depleted group still displayed the markers Arg-1, Ym-1, and RELM-α (Fig. 5C).

3.4. The immune response to T. crassiceps is slightly modified after AAMϕ depletion

It is known that *T. crassiceps* infection induces a strong Th2-type response and that this type of response leads to successful infection (Terrazas et al., 1998). We asked whether macrophage depletion may affect the development of a Th2 response and if this could be the reason why parasite growth was impaired. Thus, we analyzed specific antibody response to *T. crassiceps* antigens. As expected, mice treated with PBS-liposomes displayed increasing IgG1 levels as the infections progressed (data not shown). Similar responses were observed in mice undergoing full macrophage depletion which at the end of infection showed indistinguishable levels of IgG1 compared with all the other groups. Likewise, levels of anti-*Taenia* specific IgG2a were not modified by any treatment (data not shown).

When we analyzed the proliferative response of spleen cells 8 weeks p,i., we found that AAMϕ depletion, in all cases, significantly improved the response to Taenia antigens compared with the non-depleted group, which displayed poor antigen-specific cell proliferation (Fig. 6A).

After analysis of cytokine profiles, differences in IL-4 and IL-13 production were found. Splenocytes from fully depleted mice displayed the highest IL-4 and IL-13 production in response to Taenia antigens (Fig. 6B and C). However, splenocytes from mice undergoing early macrophage depletion showed a lower production of these cytokines, while mice undergoing late macrophage depletion produced similar levels to those obtained from non-depleted animals (Fig. 6B and C). Th1-associated IFN-γ production was also increased in the fully-depleted group compared with other treatments (Fig. 6D). Again, mice undergoing early depletion of macrophages showed the lowest IFN-γ production. This observation was not due to poor cell viability, given that the same spleen cell populations produced IFN-γ in response to Con-A (data not shown).

3.5. DCs in spleens are not altered after macrophage depletion

In order to show that treatment with clodronate did not alter the development, function or presence of other antigen-presenting

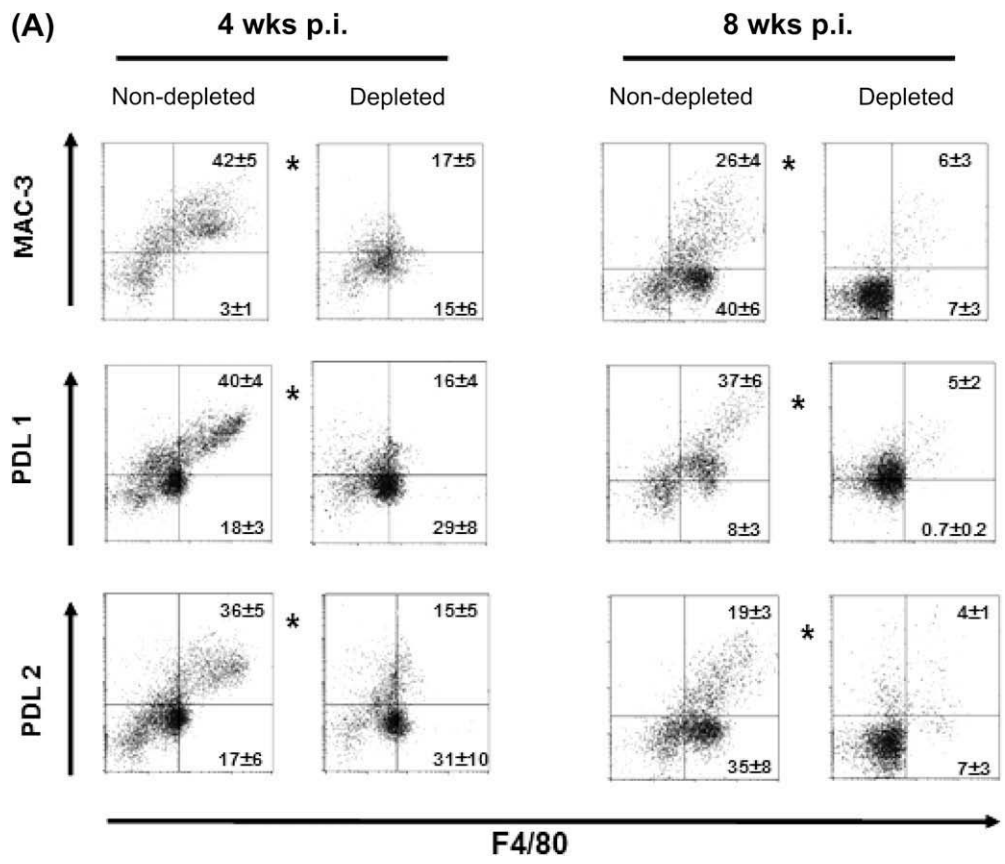

Fig. 4. Clodronate treatment alters other markers of alternative activation. (A) A decrease in populations expressing MAC 3, PD-L1 and PD-L2 is observed after 4 or 8 weeks (wks) treatment with clodronate-liposomes. (B) Analyses of mRNA transcripts for alternatively activated macrophage (AAMϕ) markers were performed and no expression of AAMϕ markers was detected in the remaining cells obtained from animals treated with clodronate-liposomes. Data shown are representative of four to five animals assayed in two separate experiments. mRNA came from peritoneal adherent cells. $^{*}P < 0.05$ compared with non-depleted mice. (C) Cytospin preparation from adherent and non-adherent peritoneal exudate cells (PECs) (100×).

cells such as DCs, we performed a bone marrow-derived DC (BMDC) culture from infected mice receiving the clodronate treatment for 8 weeks. Clodronate-treated mice were able to develop mature DCs after LPS stimulation for 24 h (data not shown). Interestingly, the population of DCs in the spleens of *T. crassiceps*-infected mice treated with clodronate-liposomes was unaltered compared with infected, non-depleted mice (data not shown). In contrast, F4/80^{+} cells were reduced by clodronate treatment (from 11.5 ± 2% in PBS-treated mice to 4.8 ± 1% in clodronate-treated mice). Thus, the observed higher antigen-specific proliferative response of splenocytes may be associated with both normal percentages of DCs and the lower presence of macrophages in the spleen.

4. Discussion

Helminth-induced immune regulation is currently an area of great interest. Indeed, several studies have identified a unique population of macrophages during diverse parasitic helminth infections that adopt an alternatively activated phenotype, expressing IL-4Rα^{hi}, CD206^{+}, Arg-1^{+}, iNOS^{-}, RELM-α^{+}, Ym-1^{+} (Kreider et al., 2007; Reyes and Terrazas, 2007). These macrophages are thought to influence the outcome of infections. However, their definitive role in protection or susceptibility, as well as their effector or regulatory activities, remain debatable and seem to be variable in such infections (Reyes and Terrazas, 2007). AAMϕs have been found as effector cells in *H. polygyrus* secondary infections, where macrophage depletion by clodronate treatment or arginase inhibition abrogated protective responses and favor survival of this intestinal parasite (Anthony et al., 2006). More recently, it was also demonstrated that treatment with either clodronate-liposomes or arginase inhibitors resulted in impaired expulsion of the nematode *N. brasiliensis* (Zhao et al., 2008) by an indirect effect that was blocking smooth muscle hypercontractility. Also, it has been shown that AAMϕs are present during *H. diminuta* infections at the time of intestinal expulsion, but their participation in the clearance of this cestode has not been definitively demonstrated (Persaud et al., 2007). On the other hand, trematode infections also induce AAMϕs but no clear role has been defined relative to effector functions (Donnelly et al., 2005, 2008). In experimental cysticercosis caused by *T. crassiceps*, we and others have investigated different immunological mechanisms involved in protection and susceptibility such as STAT6, STAT4, MIF, iNOS, parasite-release factors, protease activity, etc. (Rodriguez-Sosa et al., 2002a, 2003, 2004; Baig et al., 2005), and have reported the presence of AAMϕs in this infection (Rodriguez-Sosa et al., 2002b; Terrazas et al., 2005). Despite of a considerable body of work on the immunology of *T. crassiceps* infection, the roles of AAMϕs in cysticerci persistence and in the control of immune response have not previously been evaluated.

To experimentally determine the functional role of AAMϕs on *T. crassiceps* infection, we treated infected BALB/c mice with clodronate-loaded liposomes to deplete macrophages. Our data demonstrated a functional association between the presence of AAMϕs and the survival of *T. crassiceps*. We report three novel findings. First, we showed that the recruitment of AAMϕs starts as early as 2 weeks p.i. and that these cell populations increase as the

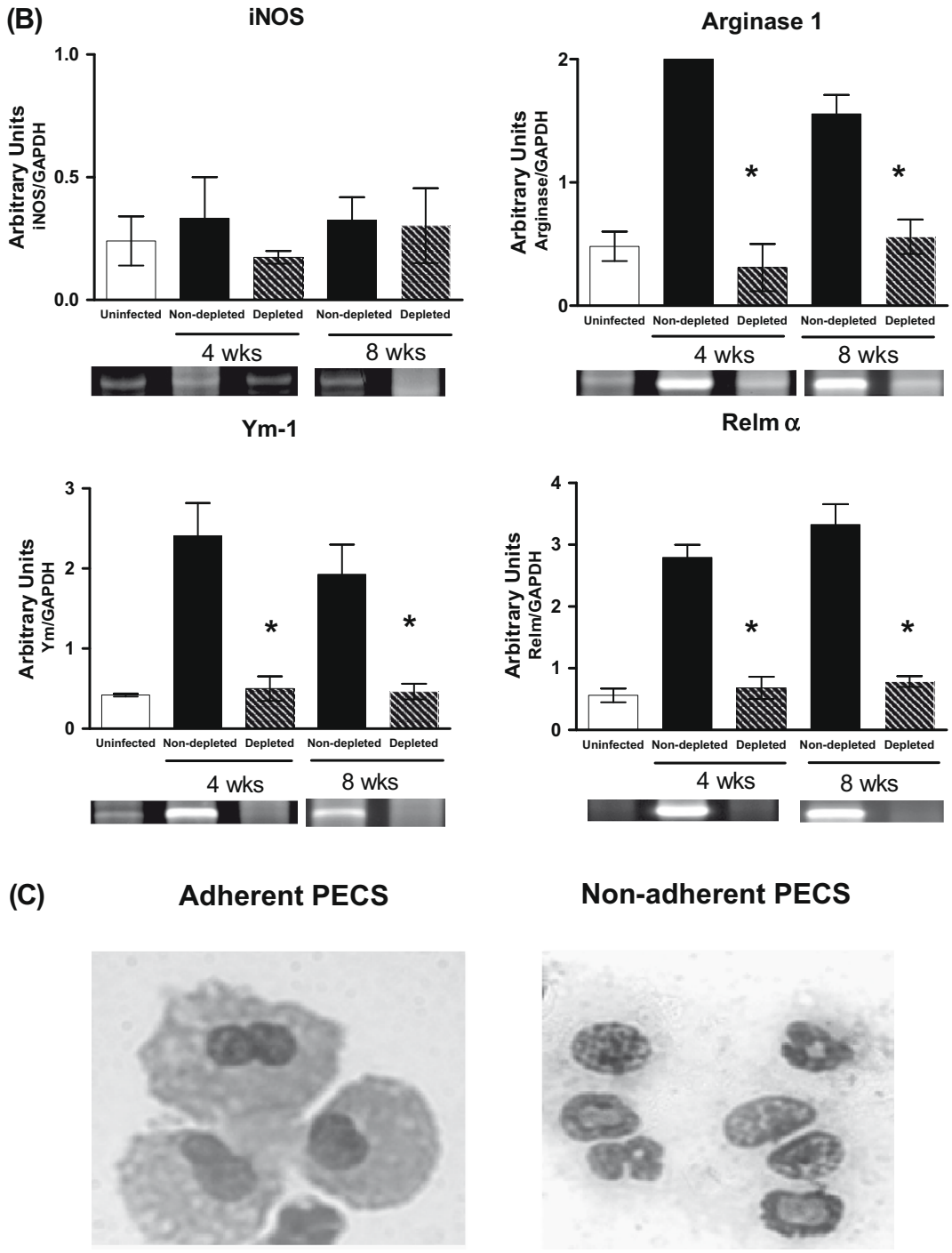

Fig. 4 (*continued*)

infection progresses. Second, complete as well as early depletion of macrophages following clodronate treatment resulted in increased resistance to *T. crassiceps* infection. Third, in vivo removal of AAMϕs reversed the classical antigen-specific cell hyporesponsiveness in experimental cysticercosis.

Consistent with previous studies, we have shown that *T. crassiceps* infection induced the increased recruitment of AAMϕs which displayed up-regulation of F4/80, MR, IL-4Rα, PD-L1 and PD-L2 together with increased transcription of Arg-1, RELM-α and Ym-1. Recruitment of this cell type started after 2 weeks of infection with 10 non-budding metacestodes of *T. crassiceps*. As the infection progressed, increased expression of all these markers was observed. Similarly, there was an increase in parasite load, suggesting that AAMϕs do not participate in controlling parasite growth in this infection. In line with this idea, the outcome of the infection was dramatically affected by macrophage depletion since, unlike other helminth infections (Anthony et al., 2006; Zhao et al., 2008), the absence of AAMϕ favored resistance to *T. crassiceps*. This outcome is in contrast with recent descriptions of the prominent role of AAMϕs in other helminth infection models, in which Th2 responses were protective. Examples of these include *N. brasiliensis* and *H. polygyrus* infections, in which AAMϕ and arginase were the pivotal mediators induced by Th2-type responses that acted to favor a protective response (Anthony et al., 2006; Zhao et al.,

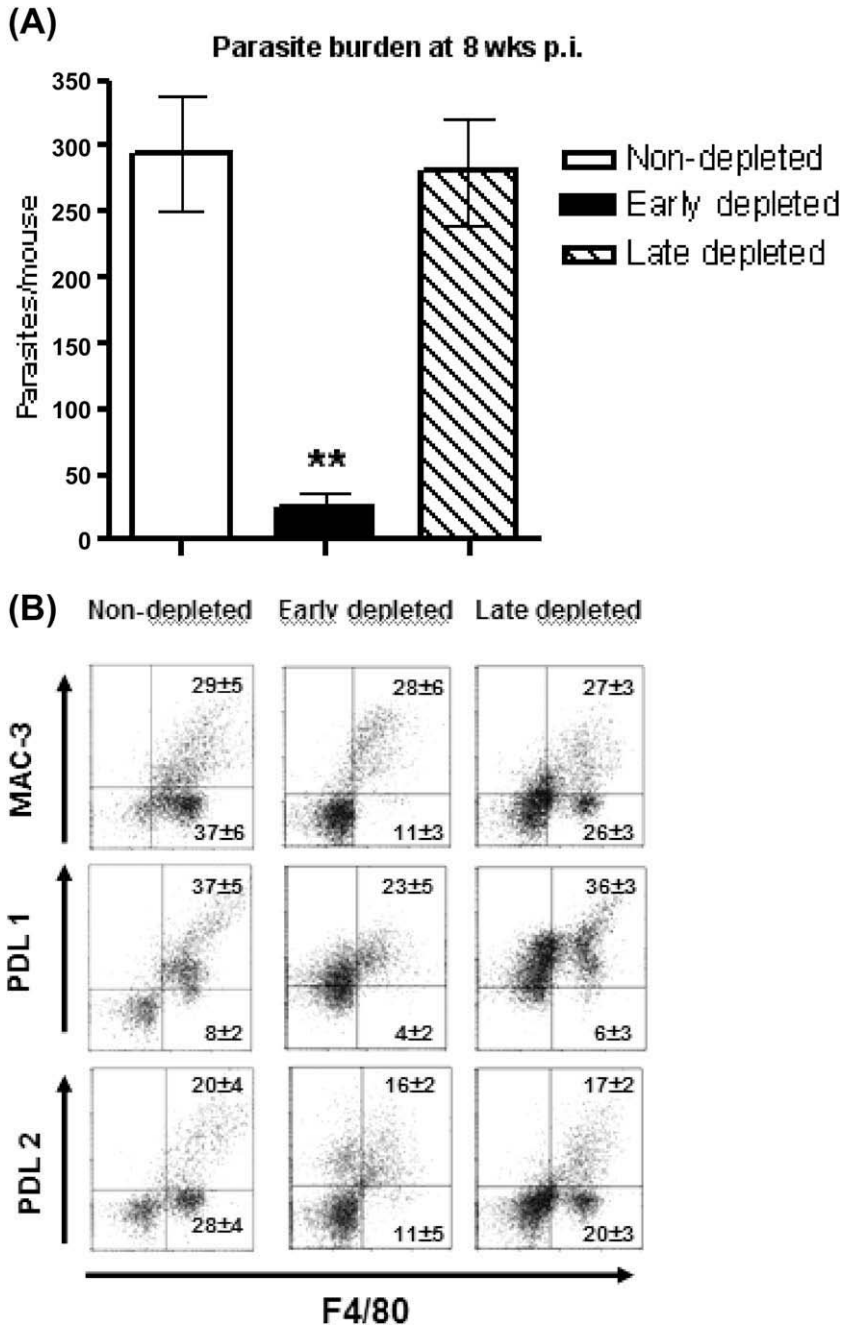

Fig. 5. Time of removal of macrophages is important for *Taenia crassiceps* susceptibility. (A) To evaluate the participation of macrophages in susceptibility to *T. crassiceps*, infected mice were treated with clodronate-loaded liposomes during the first 3 weeks of infection and thereafter mice received PBS liposomes. Late participation of macrophages was determined by injection of PBS-liposomes during 5 weeks of acute infection and clodronate-liposomes were given during the last 3 weeks. $^{**}P < 0.01$ compared with PBS-treated mice at 8 weeks p.i. (B) Macrophage depletion was tested by flow cytometry. (C) Reverse transcription-PCR assays on adherent peritoneal exudate cells. Data shown are representative of two separate experiments ($n = 7$). $^{*}P < 0.05$ compared with infected non-depleted or late-depleted groups.

2008). This discrepancy can be explained by the site where the parasites live. While *N. brasiliensis* and *H. polygyrus* are mainly intestinal parasites (Patel et al., 2009), *T. crassiceps* infection occurs in the peritoneal cavity, where different effector mechanisms may be necessary to eliminate this parasite. These data suggest a divergent role for AAMϕs in different helminth infections.

In the current study, significant changes were also observed in the antigen-specific proliferative response in clodronate-liposome treated mice. The most characteristic change was the increased proliferative response observed in each group that received treatment. This observation agrees with the suppressive activity of AAMϕs previously reported in distinct helminth infections, such as *Brugia* (Loke et al., 2000), *Litomosoides* (Nair et al., 2005) and *Taenia* (Terrazas et al., 2005). However, parasite clearance did not necessarily correlate with cell proliferation, since mice receiving clodronate in the late phase of the infection displayed better proliferative responses than mice receiving empty liposomes, despite the fact that the parasite loads of each group were similar. These data suggest that while AAMϕs suppressed cell proliferation, other elements of the immune response were untouched by clodronate treatment. For example, we did not observe a change in the Th1/Th2 profile or in antibody production during infection, neither in

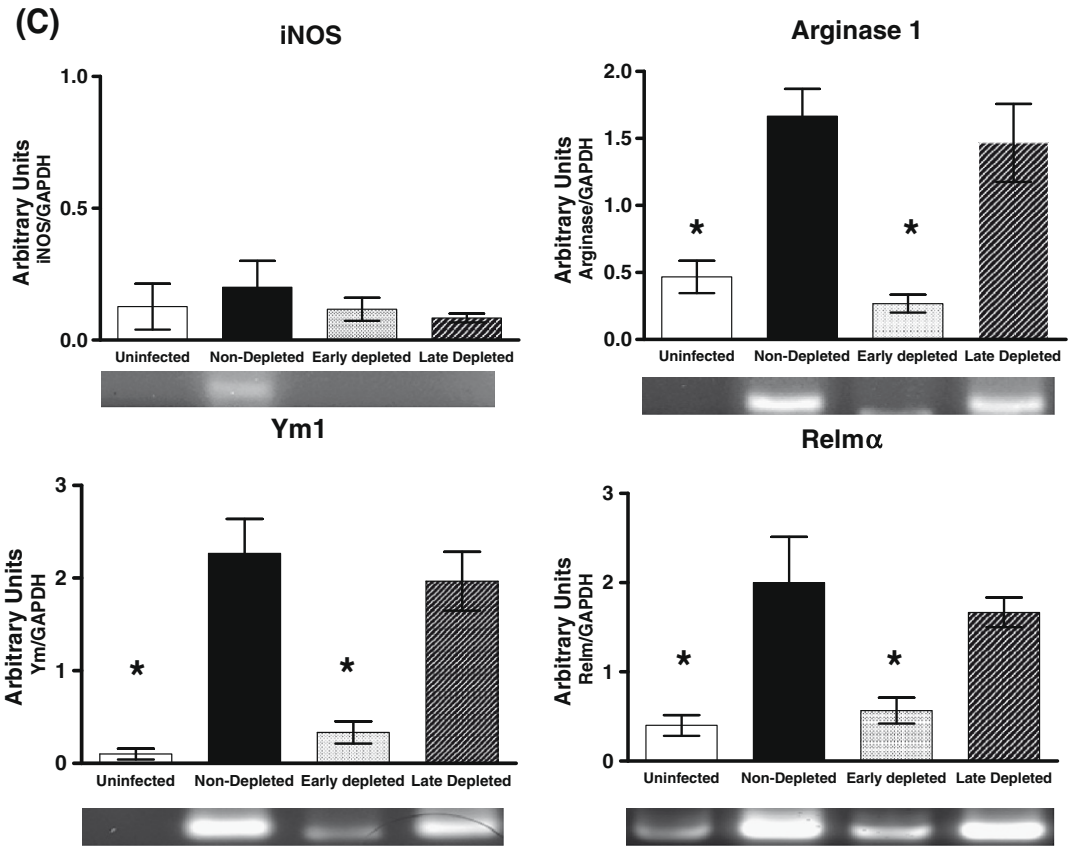

Fig. 5 (*continued*)

the percentage of eosinophils (at 4 weeks p.i.), suggesting that more cell types are also involved in regulating immunity to *T. crassiceps*. The development of DCs from the bone marrow of mice treated with clodronate further supports this idea, given that we could find normal percentages of DCs in the spleens. Interestingly, fully depleted mice displayed increased secretion of both Th1 type cytokines (IFN-γ) and Th2-type cytokines (IL-4 and IL-13), which suggests that AAMϕs are able to indistinctly affect Th1- and Th2-type responses. Another interesting finding was the fact that in chronic infections (8 weeks), treatment with clodronate also led to the disappearance of the F4/80^{lo} populations in the PECs. We do not know at this moment whether long-term clodronate treatment may also eliminate eosinophils or if the absence of parasites as well as AAMϕs may influence the attraction of these cells, given that it has been reported that alternatively activated macrophages contribute substantially to tissue recruitment of eosinophils (Voehringer et al., 2007). Interestingly, there are no reports on long-term treatment with clodronate-liposomes since most of the studies using this treatment have been performed over a short time (Schmidt-Weber et al., 1996; Leendertse et al., 2009). On the other hand, the contrasting effects of full, early or late depletion of macrophages on T cell responses need more detailed analysis. With the present data it is evident that only clodronate treatment throughout the whole time of infection displayed consistent results, improving antigen-specific proliferative responses, Th1 as well as Th2 responses, and may be more effective for AAMϕ elimination. More detailed experiments will be necessary to define the effects of the other treatments regarding antigen-specific responses.

One of the main difficulties in precisely delineating the major roles of AAMϕs in helminth infections is that many of the associated AAMϕ molecules are not restricted to macrophages (Jenkins and Allen, 2010). For example, RELM-α appears to be produced by eosinophils rather than macrophages in liver granulomas of *Schistosoma mansoni*-infected mice (Pesce et al., 2009), whilst in lung granulomas induced by schistosome eggs, RELM-α^{+} cells include macrophages, eosinophils and airway epithelial cells (Nair et al., 2009). In *B. malayi* infection, both macrophages (F4/80^{hi} Siglec-F^{-}) and eosinophils (Siglec-F^{hi} F4/80^{lo}) are able to produce this protein (Jenkins and Allen, 2010). However, in our model the infection is restricted to the peritoneal cavity; therefore, we do not need to deal with granuloma development involving many distinct cells, nor the noise of epithelial cells producing RELM-α. Moreover, the adherent peritoneal population was demonstrated to be mainly composed of macrophages and therefore the RT-PCR detection of RELM-α, Ym-1 and Arg-1 may come mostly from macrophages. Nevertheless, as we did not analyze the expression of these markers specifically in eosinophils, we cannot rule out that these as well as other cell types recruited during this infection may express the same markers. But, interestingly, when macrophages were depleted all of these markers were down-regulated, even though a high level of eosinophils was still present.

In the current paradigm, Th2 cells play a central role in the development of resistance to intestinal helminth infections, likely through the production of IL-4 and IL-13 which have been shown to modulate the participation of eosinophils, goblet cells and, recently, AAMϕs (Khan et al., 2001; Marillier et al., 2008; Fabre et al., 2009). However, the study presented here, using an extraintestinal phase of helminth infection, revealed early and sustained presence of AAMϕs associated with the progression of infection and with low spleen cell proliferative responses, suggesting that AAMϕs are capable of inhibiting T cell responses. In line with this idea, we recently demonstrated that strains of mice resistant to *T. crassiceps* infection did not develop AAMϕs (Reyes et al., 2009). On

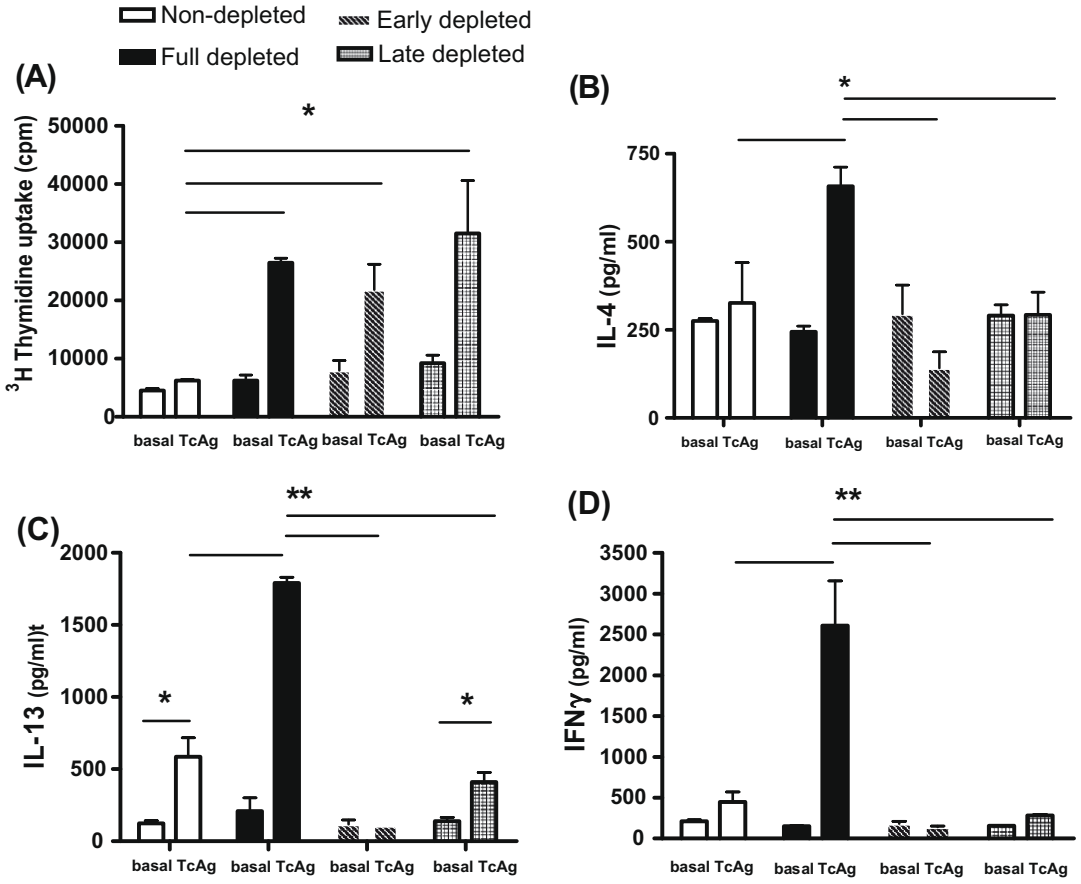

Fig. 6. Depletion of macrophages allows increased anti-*Taenia*-specific proliferation and cytokine production. Spleen cells obtained from mice treated with clodronate-liposomes throughout the full 8 week infection or at early, or late time points during infection, were cultured either in the presence of *Taenia crassiceps* total antigen (TcAg) or in medium (basal) for 96 h. Proliferation and cytokine production were evaluated, showing that the absence of macrophages enhanced proliferation (A) and representative Th2 and Th1 cytokines such as IL-4 and IL-13 (B and C) and IFN-γ (D) are produced. Data shown are representative of two separate experiments ($n = 8$). $^{*}P < 0.05$; $^{**}P < 0.01$.

the other hand, the lung phase of *N. brasiliensis* infection also recruits AAMϕs which in this case shows suppressive activity (Reece et al., 2006). Taken together, these results suggest that there may be fundamentally different mechanisms and/or regulatory pathways that dictate how the T cell response to helminth infections is generated, depending on the type of helminth, phase of the infection and genetic background of the host. Understanding the mechanics of these events would help in the design of optimal strategies for immune-based therapies designed to enhance or modulate responses to helminth infections.

In conclusion we have provided experimental evidence for an immunosuppressive role of AAMϕs during experimental cysticercosis by using in vivo macrophage depletion. Importantly, blockade of recruitment of macrophages at the site of infection resulted in prevention of *T. crassiceps* growth whilst the Th2 response and recruitment of eosinophils remained intact.

The studies described here suggest that the presence of AAMϕs favor parasite survival, while early or full depletion of AAMϕs help to eliminate the parasite. These studies represent, to our knowledge, the first demonstration that AAMϕ populations are critically involved in favoring susceptibility during a helminth infection.

Acknowledgments

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References

Anthony, R.M., Urban Jr., J.F., Alem, F., Hamed, H.A., Rozo, C.T., Boucher, J.L., Van Rooijen, N., Gause, W.C., 2006. Memory T(H)2 cells induce alternatively activated macrophages to mediate protection against nematode parasites. Nat. Med. 12, 955–960.

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 metalloaminopeptidases from *Taenia crassiceps* metacestodes. J. Parasitol. 91, 983–987.

Beiting, D.P., Gagliardo, L.F., Hesse, M., Bliss, S.K., Meskill, D., Appleton, J.A., 2007. Coordinated control of immunity to muscle stage *Trichinella spiralis* by IL-10, regulatory T cells, and TGF-beta. J. Immunol. 178, 1039–1047.

Brys, L., Beschin, A., Raes, G., Ghassabeh, G.H., Noel, W., Brandt, J., Brombacher, F., De Baetselier, P., 2005. Reactive oxygen species and 12/15-lipoxygenase contribute to the antiproliferative capacity of alternatively activated myeloid cells elicited during helminth infection. J. Immunol. 174, 6095–6104.

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.

D'Elia, R., Behnke, J.M., Bradley, J.E., Else, K.J., 2009. Regulatory T cells: a role in the control of helminth-driven intestinal pathology and worm survival. J. Immunol. 182, 2340–2348.

Donnelly, S., O'Neill, S.M., Sekiya, M., Mulcahy, G., Dalton, J.P., 2005. Thioredoxin peroxidase secreted by *Fasciola hepatica* induces the alternative activation of macrophages. Infect. Immun. 73, 166–173.

Donnelly, S., Stack, C.M., O'Neill, S.M., Sayed, A.A., Williams, D.L., Dalton, J.P., 2008. Helminth 2-Cys peroxiredoxin drives Th2 responses through a mechanism involving alternatively activated macrophages. FASEB J. 22, 4022–4032.

Fabre, V., Beiting, D.P., Bliss, S.K., Gebreselassie, N.G., Gagliardo, L.F., Lee, N.A., Lee, J.J., Appleton, J.A., 2009. Eosinophil deficiency compromises parasite survival in chronic nematode infection. J. Immunol. 182, 1577–1583.

Gordon, S., 2003. Alternative activation of macrophages. Nat. Rev. Immunol. 3, 23–35.

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.

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.

Jenkins, S., Allen J.E., 2010. Similarity & diversity in macrophage activation by nematodes, trematodes & cestodes. J Biomedicine & Biotechnology, in press.

Khan, W.I., Blennerhasset, P., Ma, C., Matthaei, K.I., Collins, S.M., 2001. Stat6 dependent goblet cell hyperplasia during intestinal nematode infection. Parasite Immunol. 23, 39–42.

Kreider, T., Anthony, R.M., Urban Jr., J.F., Gause, W.C., 2007. Alternatively activated macrophages in helminth infections. Curr. Opin. Immunol. 19, 448–453.

Leendertse, M., Willems, R.J., Giebelen, I.A., Roelofs, J.J., van Rooijen, N., Bonten, M.J., van der Poll, T., 2009. Peritoneal macrophages are important for the early containment of *Enterococcus faecium* peritonitis in mice. Innate Immun. 15, 3–12.

Loke, P., MacDonald, A.S., Robb, A., Maizels, R.M., Allen, J.E., 2000. Alternatively activated macrophages induced by nematode infection inhibit proliferation via cell-to-cell contact. Eur. J. Immunol. 30, 2669–2678.

Marillier, R.G., Michels, C., Smith, E.M., Fick, L.C., Leeto, M., Dewals, B., Horsnell, W.G., Brombacher, F., 2008. IL-4/IL-13 independent goblet cell hyperplasia in experimental helminth infections. BMC Immunol. 9, 11.

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.

McSorley, H.J., Harcus, Y.M., Murray, J., Taylor, M.D., Maizels, R.M., 2008. Expansion of Foxp3+ regulatory T cells in mice infected with the filarial parasite *Brugia malayi*. J. Immunol. 181, 6456–6466.

Nair, M.G., Cochrane, D.W., Allen, J.E., 2003. Macrophages in chronic type 2 inflammation have a novel phenotype characterized by abundant expression of Ym1 and Fizz 1 that can be partly replicated in vitro. Immunol lett 85, 173–180.

Nair, M.G., Gallagher, I.J., Taylor, M.D., Loke, P., Coulson, P.S., Wilson, R.A., Maizels, R.M., Allen, J.E., 2005. Chitinase and Fizz family members are a generalized feature of nematode infection with selective upregulation of Ym1 and Fizz1 by antigen-presenting cells. Infect Immun 73, 385–394.

Nair, M.G., Du, Y., Perrigoue, J.G., Zaph, C., Taylor, J.J., Goldschmidt, M., Swain, G.P., Yancopoulos, G.D., Valenzuela, D.M., Murphy, A., Karow, M., Stevens, S., Pearce, E.J., Artis, D., 2009. Alternatively activated macrophage-derived RELM-{alpha} is a negative regulator of type 2 inflammation in the lung. J. Exp. Med. 206, 937–952.

Noel, W., Raes, G., Hassanzadeh Ghassabeh, G., De Baetselier, P., Beschin, A., 2004. Alternatively activated macrophages during parasite infections. Trends Parasitol. 20, 126–133.

Ostrand-Rosenberg, S., Sinha, P., 2009. Myeloid-derived suppressor cells: linking inflammation and cancer. J. Immunol. 182, 4499–4506.

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.

Pesce, J.T., Ramalingam, T.R., Wilson, M.S., Mentink-Kane, M.M., Thompson, R.W., Cheever, A.W., Urban Jr., J.F., Wynn, T.A., 2009. Retnla (relmalpha/fizz1) suppresses helminth-induced Th2-type immunity. PLoS Pathog. 5, e1000393.

Raes, G., Beschin, A., Ghassabeh, G.H., De Baetselier, P., 2007. Alternatively activated macrophages in protozoan infections. Curr. Opin. Immunol. 19, 454–459.

Rausch, S., Huehn, J., Kirchhoff, D., Rzepecka, J., Schnoeller, C., Pillai, S., Loddenkemper, C., Scheffold, A., Hamann, A., Lucius, R., Hartmann, S., 2008. Functional analysis of effector and regulatory T cells in a parasitic nematode infection. Infect Immun 76, 1908–1919.

Reece, J.J., Siracusa, M.C., Scott, A.L., 2006. Innate immune responses to lung-stage helminth infection induce alternatively activated alveolar macrophages. Infect Immun. 74, 4970–4981.

Renshaw, M., Rockwell, J., Engleman, C., Gewirtz, A., Katz, J., Sambhara, S., 2002. Cutting edge: imapaired 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.

Reyes, J.L., Terrazas, C.A., Vera-Arias, L., 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.

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., 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., Rosas, L.E., David, J.R., Bojalil, R., Satoskar, A.R., Terrazas, L.I., 2003. Macrophage migration inhibitory factor plays a critical role in mediating protection against the helminth parasite *Taenia crassiceps*. Infect Immun. 71, 1247–1254.

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.

Schmidt-Weber, C.B., Rittig, M., Buchner, E., Hauser, I., Schmidt, I., Palombo-Kinne, E., Emmrich, F., Kinne, R.W., 1996. Apoptotic cell death in activated monocytes following incorporation of clodronate-liposomes. J. Leukoc. Biol. 60, 230–244.

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.

Taylor, M.D., van der Werf, N., Harris, A., Graham, A.L., Bain, O., Allen, J.E., Maizels, R.M., 2009. Early recruitment of natural CD4+ Foxp3+ Treg cells by infective larvae determines the outcome of filarial infection. Eur. J. Immunol. 39, 192–206.

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., 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.

van Rooijen, N., Sanders, A., van den Berg, T.K., 1996. Apoptosis of macrophages induced by liposome-mediated intracellular delivery of clodronate and propamidine. J. Immunol. Methods 193, 93–99.

Voehringer, D., van Rooijen, N., Locksley, R.M., 2007. Eosinophils develop in distinct stages and are recruited to peripheral sites by alternatively activated macrophages. J. Leukoc. Biol. 81, 1434–1444.

Weigert, A., Brune, B., 2008. Nitric oxide, apoptosis and macrophage polarization during tumor progression. Nitric oxide 19, 95–102.

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.

Zhao, A., Urban Jr., J.F., Anthony, R.M., Sun, R., Stiltz, J., van Rooijen, N., Wynn, T.A., Gause, W.C., Shea-Donohue, T., 2008. Th2 cytokine-induced alterations in intestinal smooth muscle function depend on alternatively activated macrophages. Gastroenterology 135, 217–225.

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Taenia crassiceps infection abrogates experimental autoimmune encephalomyelitis

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ABSTRACT

Helminth infections induce strong immunoregulation that can modulate subsequent pathogenic challenges. *Taenia crassiceps* causes a chronic infection that induces a Th2-biased response and modulates the host cellular immune response, including reduced lymphoproliferation in response to mitogens, impaired antigen presentation and the recruitment of suppressive alternatively activated macrophages (AAMΦ). In this study, we aimed to evaluate the ability of *T. crassiceps* to reduce the severity of experimental autoimmune encephalomyelitis (EAE). Only 50% of *T. crassiceps*-infected mice displayed EAE symptoms, which were significantly less severe than uninfected mice. This effect was associated with both decreased MOG-specific splenocyte proliferation and IL-17 production and limited leukocyte infiltration into the spinal cord. Infection with *T. crassiceps* induced an anti-inflammatory cytokine microenvironment, including decreased TNF-α production and high MOG-specific production of IL-4 and IL-10. While the mRNA expression of TNF-α and iNOS was lower in the brain of *T. crassiceps*-infected mice with EAE, markers for AAMΦ were highly expressed. Furthermore, in these mice, there was reduced entry of $CD3^{+}Foxp3^{-}$ cells into the brain. The *T. crassiceps*-induced immune regulation decreased EAE severity by dampening T cell activation, proliferation and migration to the CNS.

1. Introduction

Helminth parasites possess sophisticated and efficient mechanisms to regulate the immune response to survive inside their hosts, including the skewing of the immune response toward a Th2 response and the generation of regulatory cells. In addition to Th2 polarization, the recruitment of immunomodulatory cell populations, such as T regulatory cells (Tregs) and alternatively activated macrophages (AAMΦs), has also been described in different helminthic infections. However, the role of these cells is also dependent on the particular helminth [1,2]. For example, in nematode infection, AAMΦs actively participate in parasite clearance [3], while in *Taenia crassiceps* infection, AAMΦs favor parasite persistence [4]. Interestingly, experimental infection with *Brugia malayi, Schistosoma mansoni* or *Litomosoides sigmodontis* induces an increased and rapid recruitment of $CD4^{+}$ $CD25^{+}$ $Foxp3^{+}$ Tregs cells, which can be beneficial for both the parasite and the host [5–7]. It is clear that the strong regulatory mechanisms developed by helminthes are necessary either to successfully colonize their hosts or to complete their life cycle while minimizing damage to the host.

Given the impact of helminth infections on subsequent challenge with pathogens, including *Mycobacterium*, *Leishmania*, *Trypanosoma* and *Plasmodium* [8–13], helminth-induced immunomodulation has been heavily studied. Recent data have shown that helminth-induced immunomodulation can also ameliorate autoimmune and allergic diseases, even though these diseases are Th1/Th17 or Th2 dependent, respectively [14,15]. For example, *S. mansoni* and its antigens, *Trichinella spiralis* or *Heligmosomoides polygyrus* are able to modulate Type 1 diabetes (T1D) in NOD mice, mainly due to the Th2 skewing [16–19]. *S. mansoni* infection has also been shown to decrease the severity of experimental autoimmune encephalomyelitis (EAE) [20]. Furthermore, allergic disorders, which are typically Th2 dependent, are improved in *S. mansoni*- or *H. polygyrus*-infected mice despite high circulating IgE levels [21,22].

T. crassiceps naturally infects rodents where it can reproduce by budding, and the final hosts are canines. Nevertheless, there are reports demonstrating that immuno-compromised humans can develop *T. crassiceps*-cysticercosis [23]. Additionally, antigenic similarities have been very well established between *Taenia solium* and *T. crassiceps* metacestodes [24]. Therefore, sera from human patients with neurocysticercosis can recognize *T. crassiceps* antigens. Similar to other helminthes, *T. crassiceps* and its antigens can skew the host immune response toward a Th2 cytokine profile [25]. In addition to the increased Th2 response, a striking growth of *T. crassiceps* metacestodes and the recruitment of suppressive AAMΦs have been observed [25]. *T. crassiceps* infection also

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appears to enhance *Trypanosoma cruzi* and *Leishmania* spp. replication in co-infected hosts [9,10]. Recently, *T. crassiceps* infection has been shown to attenuate chemically induced T1D [26]; however, the effect of *T. crassiceps* on the development of other autoimmune diseases has not been determined.

EAE is a T cell-driven autoimmune disease of the central nervous system (CNS) that shares many clinical and pathological features with multiple sclerosis (MS) [27]. Treatments that interfere with costimulation or co-inhibition have been shown to alter the progression of EAE [28–31].

In this study, we evaluated the impact of *T. crassiceps* infection on the onset and develop of EAE. We show that concurrent infection with *T. crassiceps* induces T cell hyporesponsiveness that impedes MOG-specific T cell proliferation and is associated with decreased leukocyte infiltration into the CNS and significantly less severe disease. Furthermore, we demonstrate that an anti-inflammatory microenvironment and the over-expression of AAMΦ markers, such as Arginase-1 (Arg-1), Relm-α and YM-1, are features of *T. crassiceps*-infected mice that did not develop EAE.

2. Materials and methods

2.1. Mice, parasites and infection

Six- to eight-week-old female C57BL/6 mice were purchased from Harlan Laboratories (México) and were maintained in a pathogen-free environment at the FES-Iztacala, UNAM animal facility in accordance with Institutional and National guidelines.

T. crassiceps (ORF strain) metacestodes 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 phosphate-buffered saline (PBS; 0.15 M NaCl, 0.01 M sodium phosphate, pH 7.2) and used for infection. Because C57BL/6 mice are considered resistant to *T. crassiceps* infection, we performed parasite burden kinetics to determine the dose required to make these mice susceptible to infection. We found that infection with 40 metacestodes was enough to break resistance. Therefore, i.p. infection with 40 metacestodes was carried out for each experiment.

2.2. EAE induction and assessment

To induce EAE, *T. crassiceps*-infected mice (at 8 weeks post-infection), as well as uninfected mice, were immunized subcutaneously with 300 μg myelin oligodendrocyte glycoprotein peptide 35–55 (MOG_{35-55}) (Life tein, Piscataway, NJ) emulsified in complete Freund's adjuvant containing 400 μg/ml *Mycobacterium tuberculosis* (Sigma Aldrich). The mice were given 200 ng of Pertussis toxin (Fluka,) intraperitoneally on the day of immunization and 48 h later.

All animals were observed daily for clinical signs and scored (blinded) as follows: grade 0, no abnormality; grade 1, limp tail; grade 2, limp tail and hind limb weakness; grade 3, partial hind limb paralysis; grade 4, complete hind limb paralysis; and grade 5, death.

2.3. Histology

For histological evaluation of EAE, animals were euthanized at the peak of disease. The spinal cord was removed and fixed in 4% formalin. Tissue samples were embedded in paraffin, and 5-μm sections were cut on a microtome and stained with hematoxylin and eosin for histological examination.

2.4. Splenocyte cultures

At the peak of EAE (21 d.p.i.), spleens were aseptically removed, and single-cell suspensions were prepared by gently teasing apart the spleen in RPMI-1640 media supplemented with 10% FBS, 100 U of penicillin/streptomycin, 2 mM glutamine, 25 mM HEPES and 1% non-essential amino acids (all from GIBCO, Grand Island, NY). The cells were centrifuged at 1000*g*, and erythrocytes were lysed by resuspending the cells in Boyle's solution (0.17 M Tris and 0.16 M ammonium chloride). Following two washes with PBS, the viable cells were counted using Trypan blue exclusion, and the splenocytes were adjusted to 3×10^6 cells/ml in the same medium. The cells (100 μl per well) were seeded in 96-well plates (Costar, Cambridge, MA) and stimulated with 30 μg MOG_{35-55}. Additionally, adherent cell-depleted splenocytes were stimulated in an anti-CD3-coated plate. The proliferation was quantified after 72 h of incubation at 37 °C and 5% CO_2 by pulsing the cells for 18 h with 0.5 μCi [^{3}H] thymidine (Amersham Biosciences). The cells were harvested on a 96-well harvester (Tomtec, Finland) then counted using a 1450 micro β-plate counter (Trilux, Finland). The values are presented as counts per min (CPM) from triplicate wells.

Supernatants from similar cultures were frozen and stored at −80 °C until used for detection of cytokines.

2.5. Cytokine measurements

The IL-4, IL-10, IL-17, TNF-α and IFN-γ levels were quantified in mouse serum and splenocyte culture supernatants at the indicated point times. Antibody pairs were used according to the manufacturer's instruction (Peprotech México, México DF, and Biolegend, San Diego, CAL, USA, for IL-17).

2.6. Macrophage suppression ability

To determine whether the macrophages from infected animals retained their suppressive ability, the co-culture of macrophages with $CD90^+$ cells was performed. Macrophages were obtained at the peak of EAE and adjusted to 5×10^5 cells/ml. Splenocytes were prepared from naïve mice and enriched for $CD90^+$ cells (95% by flow cytometry) using CD90 magnetic beads (MACS, Miltenyi Biotec, CA). $CD90^+$ cells were plated in 96-well flat bottom plates (Costar, Cambridge, MA) that were pre-coated with 1 μg/ml anti-CD3 and anti-CD28 antibodies (Biolegend). After 3 h, macrophages were added to the $CD90^+$ T cells at ratios of 1:4, 1:8, 1:16 or 1:32 (Macrophages:$CD90^+$), and the co-cultures were maintained at 37 °C and 5% CO_2 for 72 h. Following this incubation, 0.5 μCi/well [^{3}H] thymidine (185 GBb/mmol activity, Amersham, England) was added, and the cultures were incubated for an additional 18 h. The cells were then harvested, and the incorporation of radioactivity was assessed as described above.

2.7. Flow cytometry

It has been previously shown that macrophages recruited by *T. crassiceps* to the site of infection express alternatively activated and suppressive markers, such as mannose receptor (MR), PD1 ligand 1 (PD-L1) and PD-L2 only in susceptible hosts. To determine whether C57BL/6 mice infected with 40 metacestodes mimic the infection observed in susceptible BALB/c mice, flow cytometry was performed on peritoneal exudates cells (PECs) from EAE or *T. crassiceps*-infected EAE mice. Briefly, 8 weeks after infection, PECs were aseptically obtained, and 1×10^6 cells were incubated with anti-CD16 and anti-CD32 (Biolegend, San Diego, CA, USA) to block non-specific antibody binding. The cells were then stained with APC-conjugated anti-F4/80, FITC-conjugated anti-MR, PE-conjugated anti-PDL1 and PE-conjugated anti-PDL2 (all from Biolegend)

and incubated for 30 min at 4 °C in FACS staining buffer (1% FBS, 0.5% sodium azide in PBS). The cells were analyzed using a FACSCalibur and Cell Quest software (Becton Dickinson).

2.8. RT-PCR

RNA was extracted from PECs isolated after 8 weeks of infection using the TRIzol Reagent (Invitrogen, Carlsbad, CA) and the isopropanol–chloroform technique. The RNA was quantified, and 5 μg of RNA was reverse-transcribed using the Superscript II First Strand Synthesis Kit (Invitrogen). PCR reactions containing 5× PCR Buffer blue, 10 mM dNTP, 40 nM each forward and reverse primers, 1 U Taq DNA polymerase (Sacace Biotechnologies, Italy) and 2 μl of the cDNA were prepared in a 25 μl final volume. The PCR conditions consisted of an initial denaturation step 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 at 72 °C for 4 min in a thermal cycler (Corbett Research, Australia). The amplified products were mixed with loading buffer containing SYBR green and observed in a 1.5% agarose gel with the Fujifilm FLA 5000 scanner (Fuji, Japan) using the image reader V2.1 software to capture the images.

2.9. Real Time PCR in brain

At peak disease total brain RNA was extracted from different experimental groups and reverse transcription to obtain cDNA was achieved. By using the SYBR green jumpstart Taq Ready mix (Sigma Aldrich St. Louis MO, USA) in a final volume of 25 μl each sample and following manufacturer's conditions samples were submitted to 40 cycles in a real time thermal cycler (Rotor gene RG 3000, Corbett Research). Sequences for our target genes were glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Forward 5′ CTC ATg ACC ACA gTC CAT gC 3′ Reverse 5′ CAC ATT ggg ggT Agg AAC AC3′), tumor necrosis factor (TNF)-α (Forward 5′ ggC Agg TCT ACT TTg gAg TCA TTg C 3′ Reverse 5′ ACA TTC gAg gCT CCA gTg AAT TCg 3′). Gene expression was normalized to the expression of the constitutively expressed gene GAPDH.

2.10. Analysis of the brain microenvironment

To determine the brain microenvironment at the peak of disease, total RNA was extracted from brains, and RT-PCR was performed as described above. For flow cytometry, the brains were dissected from mice transcardially perfused with 10 mL of ice-cold PBS and dissociated by passage through a 70 μm cell strainer (Beckton Dickinson). Additionally, brain debris was incubated with collagenase for 60 min at 37 °C and 5% CO_2. The obtained cells were stained with APC-conjugated anti-CD3 and PE-conjugated anti-Foxp3 in FACS buffer, as previously described, and analyzed on a FACSCalibur.

Table 1
T. crassiceps-infected mice have reduced incidence and severity of EAE.

	Incidence (%)	Onset day	Higher score	CDI[a]	Peak score[b]
EAE	17/20 (85)	10 ± 2	4	65	3.25 ± 0.61
Taenia/EAE	10/20 (50)*	11 ± 2	1*	15*	0.51 ± 0.31*

* $p < 0.05$ compared against *Taenia* infected and immunized mice.
[a] Cumulative disease index (CDI) was calculated by adding the based on the sum of clinical scores over the entire observation period for each animal and averaged within each group ± SD.
[b] Peak score was also measured over the duration of disease per animal and averaged ± SD.

2.11. Statistical analysis

The significance of the differences between the experimental groups was measured using a Student's *t*-test with the Graph Pad Prism 4.0 software.

3. Results

3.1. C57BL/6 mice challenged with 40 T. crassiceps metacestodes are susceptible to infection and develop a Th2-type response

Because the MOG_{35-55} EAE mouse model has been established and studied largely on a C57BL/6 genetic background, our first goal was to characterize *T. crassiceps* infection in this mouse strain. It has been reported that *T. crassiceps* metacestodes could not grow in the C57BL/6 mouse strain [32]. However, in these studies, a dose as low as 10 metacestodes was used for infection. Here we determined the number of parasites that must be inoculated to establish an infection. Infections with 20, 30 or 40 metacestodes were performed, and we found that C57BL/6 mice infected with 40 metacestodes were susceptible to *T. crassiceps* infection (Fig. 1A). Importantly, mice infected with 40 metacestodes developed parasite loads as heavy as 800 parasites in the peritoneal cavity after 8 weeks of infection, which is very close to the loads reported for susceptible BALB/c mice (Fig. 1A). Furthermore, following infection with 40 metacestodes, the serum IL-4, *T. crassiceps*-specific IgG_1 and total IgE levels were higher than the serum IFN-γ and *T. crassiceps*-specific IgG_{2a} levels, suggesting that the C57BL/6 mice predominantly generated a Th2 immune response (Fig. 1B–E). Therefore, whereas a low dose of metacestodes (10–20) cannot subvert the immune response in C57BL/6 mice, a higher dose (40 metacestodes) allows for *T. crassiceps* growth in a strain that was previously considered resistant.

3.2. T. crassiceps infection recruits AAMΦ but suppresses the Foxp3$^+$ CD4$^+$ T regulatory cell population

We have previously found that following a dose of 10 metacestodes, only susceptible (BALB/c) mice recruited AAMΦs to the infection site [32]. As seen in Fig. 2A, infection with 40 *T. crassiceps* metacestodes induced recruitment of AAMΦs, and the overexpression of genes associated with AAMΦs, such as Arg-1, Relm-α, YM-1 and TREM-2, was detected. Together, these data demonstrate a clear recruitment of AAMΦs as the infection progressed. Furthermore, as detected using flow cytometry, the increased expression of PD-L1 and PD-L2, as well as mannose receptor (MR), could be associated with the suppressive activity of the AAMΦs in *T. crassiceps* infection (Fig. 2B).

Similar to AAMΦs, another regulatory cell population, Foxp3$^+$ CD4$^+$ CD25$^+$ Tregs, has been described during both experimental and natural infection with helminthes [6,33]. In fact, Tregs, rather than AAMΦs, have been shown to play an important role in parasite persistence; however, in some cases, both populations of cells are generated [5,34,35]. Unlike other models, the dynamics of Foxp3$^+$ Tregs in experimental *T. crassiceps* infection have not been examined. Therefore, we tested for the presence of Foxp3$^+$ Tregs in the spleen and mesenteric lymph nodes (MLN). Interestingly, 8 weeks after *T. crassiceps* infection, we observed a significant reduction in the fraction of Tregs in spleen (Fig. 2C; 2% uninfected versus 0.3% 8 weeks infected); no changes were observed in the fraction of Tregs in the MLN (Fig. 2D). Therefore, unlike other helminth models, *T. crassiceps* infection induces AAMΦs but not Foxp3$^+$ Tregs.

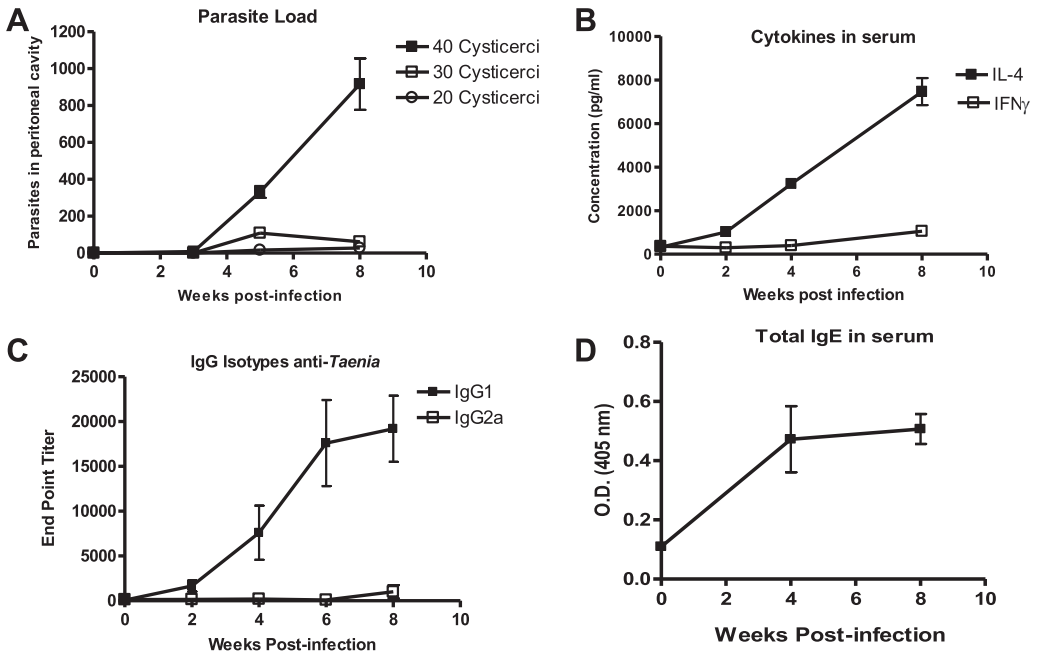

Fig. 1. Th2-biased response in C57BL/6 mice inoculated with a large dose of *T. crassiceps* metacestodes. (A) The kinetics of the parasite load after infection with 20–40 metacestodes. A clear Th2 response, based on increased production of IL-4 (B) and IgG_1 (C) compared to IFN-γ and IgG_{2a}, was detected in mice infected with 40 metacestodes. (D) The total IgE levels increased as the infection progressed. The data shown are representative of two independent experiments, with four mice per group. The bars indicate the standard deviation.

3.3. Concurrent infection with *T. crassiceps* significantly reduces EAE severity

After detection of a strong Th2 response, AAMΦ recruitment and a reduced $Foxp3^+$ Treg population in C57BL/6 mice 8 weeks after infection with *T. crassiceps*, we immunized mice with $MOG_{35–55}$ peptide to analyze the impact of the presence of *T. crassiceps* prior to EAE onset. Following induction of EAE, we found that the C57BL/6 mice infected with *T. crassiceps* had significantly less severe clinical signs of disease during the acute phase of EAE (Fig. 3A). Although we observed no difference in the day of onset (Fig. 3A and Table 1), *T. crassiceps*-infected animals had a reduced incidence of EAE (50% versus 85% in uninfected mice), cumulative disease index and peak clinical score (Table 1). Therefore, the course of EAE in *T. crassiceps*-infected mice was significantly modified, as the clinical symptoms in these mice were clearly diminished.

3.4. *T. crassiceps* infection results in fewer spinal cord-infiltrating cells and a systemic mixed cytokine profile

Next, we analyzed spinal cord sections to determine the extent of the inflammatory cell infiltration. The presence of *T. crassiceps* resulted in less leukocyte infiltration (Fig. 3B). Therefore, the decreased severity of the EAE symptoms in mice infected with *T. crassiceps* was the result of fewer spinal cord-infiltrating cells. Moreover, *T. crassiceps*-infected EAE mice displayed a significant increase in the serum levels of IL-4 and IL-17 during the progression of EAE compared to uninfected mice with EAE; the serum level of IFN-γ was unchanged between the groups. We also observed significantly decreased and delayed production of TNF-α in the serum of *T. crassiceps*-infected EAE mice (Fig. 4A–D).

3.5. *T. crassiceps* infection modifies the MOG-specific immune response

To understand the immune mechanisms underlying the observed effect of *T. crassiceps* on EAE, we investigated the MOG-specific antibody and splenic T cell response at the disease peak. In line with the high levels of circulating IL-4 and IFN-γ in *T. crassiceps*-infected EAE mice, the MOG-specific IgG_{2a} levels were similar in uninfected EAE mice, but the MOG-specific IgG_1 level was increased (data not shown). Additionally, the total IgE level was greater in *T. crassiceps*-infected EAE mice (Data not shown). While the proliferative response of splenocytes in response to MOG or anti-CD3 in uninfected EAE mice was strong, the response of *T. crassiceps*-infected mice was weak against either stimulus, suggesting that the presence of *T. crassiceps* somehow modulated T cell proliferation (Fig. 5A and B).

$Foxp3^+$ Tregs have been shown to modulate the course of EAE because, following MOG immunization, this population is induced most prominently during the recovery phase of disease [36]. However, in our system, *T. crassiceps* alone was unable to increase the fraction of $Foxp3^+$ Tregs. We examined whether $Foxp3^+$ Tregs were collaborating to ameliorate EAE in infected mice. We first analyzed the fraction of $Foxp3^+$ Tregs in the spleen at the peak of disease. As seen in Fig. 5C, 21 days after MOG immunization, the fraction of Tregs had increased almost threefold compared to naïve mice; however, the fraction of Tregs was similar in *T. crassiceps*-infected mice with less severe or no EAE symptoms. Next, we developed an assay to determine if AAMΦs from *T. crassiceps*-infected or uninfected EAE mice were able to suppress T cell proliferation. Naïve T cells were stimulated with anti-CD3 and co-cultured with different ratios of macrophages isolated from the peritoneal cavities. Only macrophages obtained from *T. crassiceps*-infected EAE mice were able to suppress T cell proliferation in a dose-dependent manner (Fig. 5D). Furthermore, the expression of AAMΦ markers was analyzed in adherent splenocytes from uninfected or *T. crassiceps*-infected EAE mice using RT-PCR. As seen in Fig. 5E, only adherent splenocytes from *T. crassiceps*-infected EAE mice expressed AAMΦ markers.

We also examined the levels of different pro-and anti-inflammatory cytokines that have been shown to be involved in EAE regulation. Whereas the production of IFN-γ and TNF-α from MOG-specific splenocytes was no different between the groups, we observed increased production of IL-4 from splenocytes isolated from infected mice compared to uninfected mice. This result illustrates the induction of a MOG-specific Th2 response, which is known to be non-encephalitogenic. In contrast, whereas

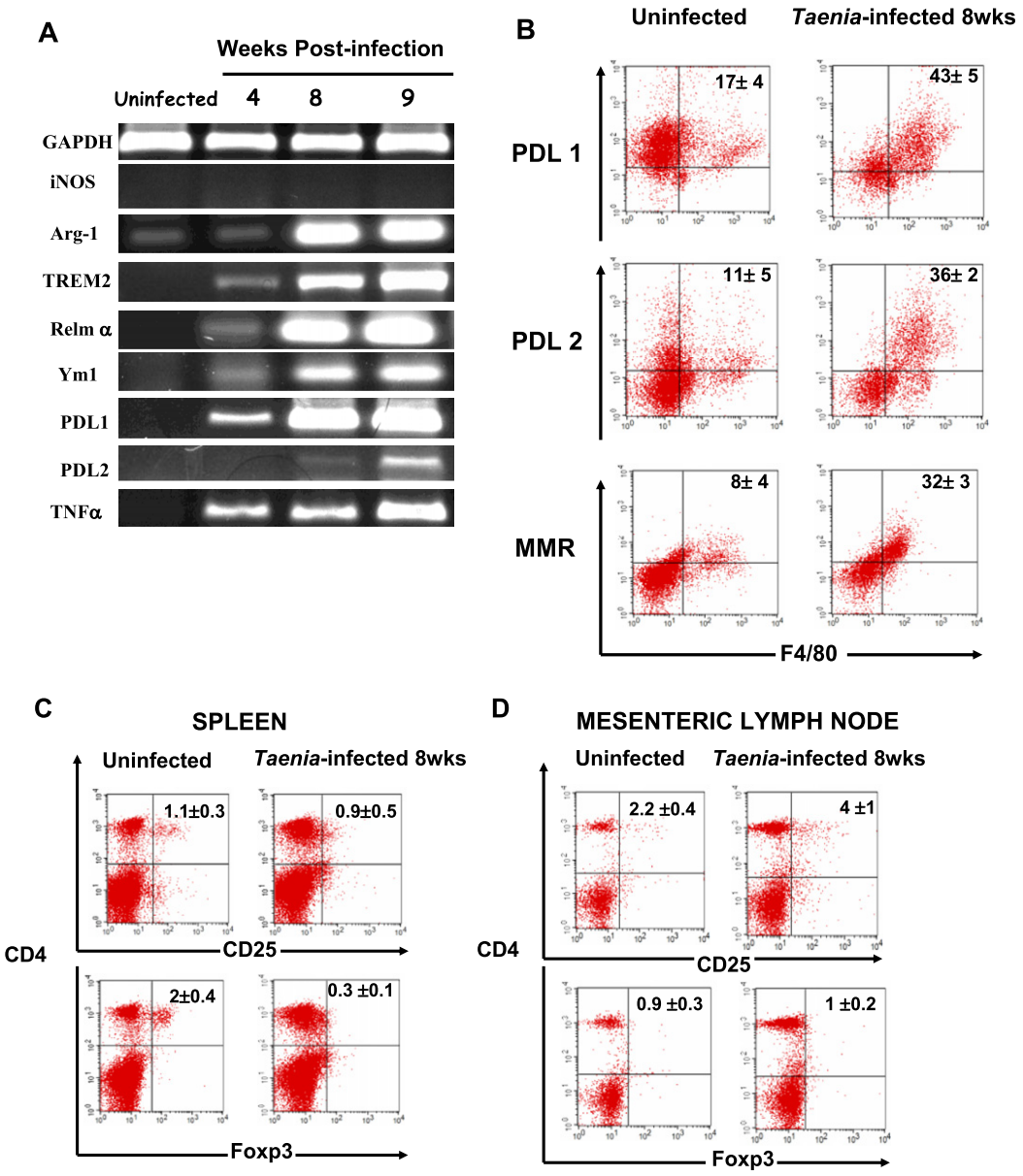

Fig. 2. A high-dose *T. crassiceps* infection induces peritoneal recruitment of AAMΦs after 8 weeks of infection. (A) RT-PCR analysis of adherent peritoneal macrophages recovered from animals infected with 40 metacestodes 8 weeks p.i. The increased expression of genes associated with AAMΦs, such as Arginase1, TREM-2, RELM-α, Ym1, PD-L1 and PD-L2, was detected. (B) Total PECs were assayed for the surface expression of F4/80, PD-L1, PD-L2 and MR using flow cytometry. (C and D) Flow cytometry of $CD4^+$ $CD25^+$ $Foxp3^+$ T regulatory cells in secondary lymphoid organs (spleen, (C); mesenteric lymph node, (D)) 8 weeks p.i. with *T. crassiceps*. Note that the while $CD4^+$ $CD25^+$ cells were increased in the infected group compared to the uninfected mice, the level of $CD4^+Foxp3^+$ cells was the same in either the absence or presence of *T. crassiceps*. The dot plots represent four mice per group, and the data are from two independent experiments.

uninfected EAE mice demonstrated high MOG-specific production of IL-17, the MOG-specific production of IL-17 was abrogated in *T. crassiceps*-infected mice (Fig. 6A–D). This finding demonstrates the poor generation of MOG-specific IL-17-producing T cells in *T. crassiceps*-infected mice. Interestingly, in the same cell cultures, we detected high MOG-specific IL-10 production only from splenocytes isolated from *T. crassiceps*-infected EAE mice (Fig. 6E).

3.6. Alternatively activated macrophage markers but not inflammatory genes or Foxp3 are over-expressed in the CNS of *T. crassiceps*-infected mice with EAE

In addition to the spinal cord, the brain is another target organ where demyelination occurs during EAE [37]. Therefore, we analyzed brain tissue using RT-PCR and flow cytometry to examine how *T. crassiceps* infection impacts an anatomically distinct organ. At the peak of EAE symptoms, the mice were perfused, and total RNA was extracted from the brain for gene expression analysis. Because we were interested in suppressor AAMΦs, we analyzed the expression of AAMΦ markers 21 days after MOG immunization. As shown in Fig. 7A, genes such as Relm-α and YM-1 were over-expressed in mice infected with *T. crassiceps* compared to uninfected EAE mice. In contrast, the expression of TREM-2 remained unchanged, suggesting the presence of AAMΦs only in the *T. crassiceps*-infected mice, at least at this time point. Additionally, while the expression of Arginase-1 was increased in infected animals, iNOS was predominantly detected in the uninfected EAE mice which displayed a higher clinical score. The expression of cytokines, such as TNF-α and IL-23 (p19 subunit), which are required to maintain EAE symptoms, were also analyzed by RT-PCR. Surprisingly, the expression of IL-23 (p19 subunit) was comparable between the experimental groups regardless of

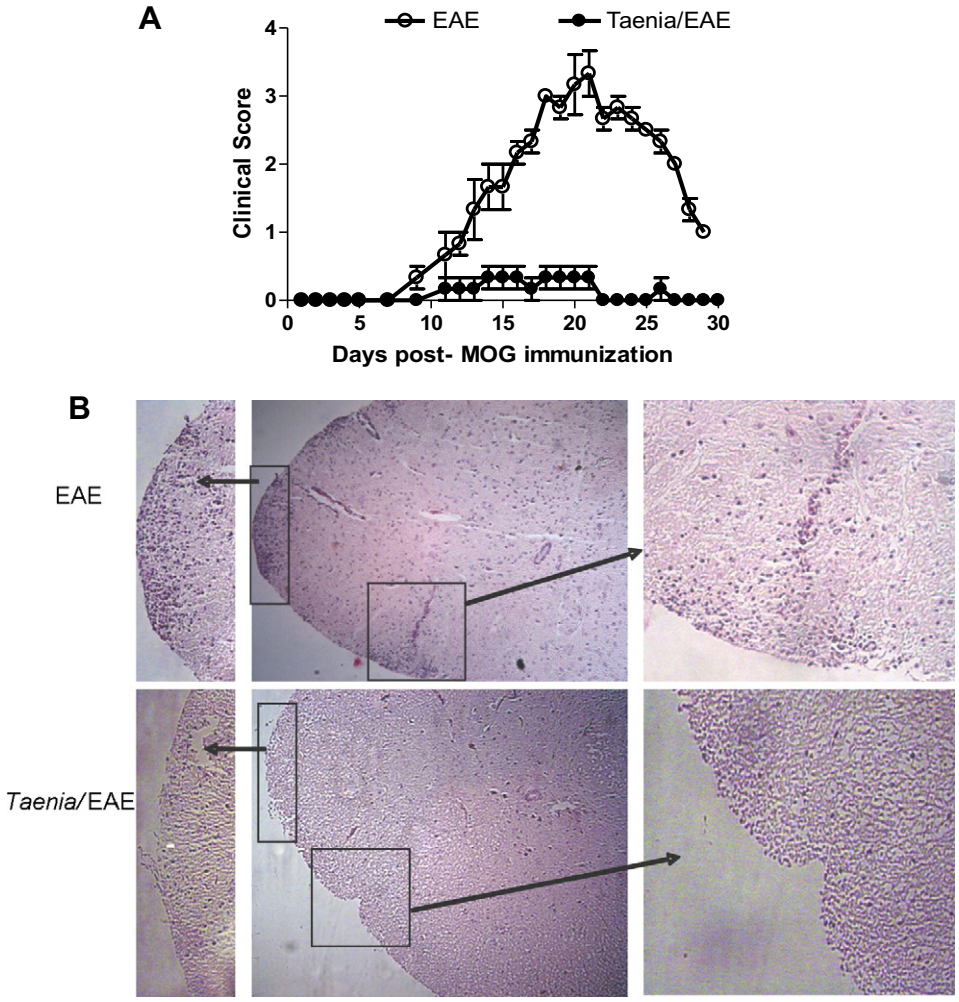

Fig. 3. *T. crassiceps* infection abrogates the development of EAE. After 8 weeks of infection, mice were immunized with MOG peptide and compared with uninfected but similarly immunized mice. (A) The open circles represent the normal course of EAE, with onset at day 11 post-immunization, peak disease at day 21 and an average maximum disease score of 3.5. The EAE course in *T. crassiceps*-infected mice displayed a slight delay in onset (13 days) and significantly less severe symptoms, with a maximum score of 1 (closed circles). (B) The spinal cord from mice in both groups was extracted at the peak of disease, and H&E-stained sections are shown. The uninfected EAE mice demonstrated infiltrating cells, whereas the infected EAE mice had fewer cells infiltrating the spinal cord. The inset is a magnification of the indicated area. The clinical evaluation shown is representative of 20 animals. $^{*}p < 0.01$ by unpaired Student's *t*-test.

whether the clinical signs were significantly different (Fig. 7B). In contrast, we observed a decrease in the expression of TNF-α in the brain of *T. crassiceps*-infected EAE mice by using both RT-PCR and Real time RT-PCR analysis (Fig. 7B and C), which displayed significantly less severe EAE symptoms. Finally, it is well known that Th1 cells, in contrast to Th2 cells, are necessary to cause EAE, and in turn, transcription factors such as T-bet and GATA-3 are required for Th1 and Th2 lineage commitment, respectively. Therefore, we analyzed the expression of these transcription factors to determine which Th profile dominated at disease peak. As seen in Fig. 7D, T-bet transcripts were over-expressed in uninfected EAE mice, whereas a clear difference could not be seen in the expression of GATA-3 in any group. The expression of Foxp3 was also examined in brain tissue, and while only minimal expression was observed in the EAE group, no expression was detected in the *T. crassiceps*-infected EAE group. This result once again demonstrates that this regulatory subpopulation is not increased at the peak of EAE in mice infected with *T. crassiceps*.

3.7. A lower percentage of CD3^{+} Foxp3^{-} cells are detected in the CNS of T. crassiceps-infected mice with EAE

Finally, we evaluated whether *T. crassiceps* infection could prevent the infiltration of T cells into the CNS. The brains were digested with collagenase, and total cells were isolated and analyzed using flow cytometry. As seen in Fig. 7E, a twofold increase in the percentage of CD3^{+} Foxp3^{-} cells was detected in uninfected mice compared to both the healthy control mice and the *T. crassiceps*-infected EAE mice. However, a similar percentage of CD3^{+} Foxp3^{+} cells were found in the experimental and naïve (control) groups. This result suggests that the CD3^{+} cells in uninfected EAE mice might be effectors rather than regulatory cells. Therefore, while the Treg population increases in response to MOG immunization; it is not due to the presence of *T. crassiceps*, suggesting that the immunomodulatory effect of *T. crassiceps* is not mediated by the induction of Foxp3^{+} Tregs.

4. Discussion

There has been recent and considerable interest in defining the ways in which helminth infections perturb the immune system and induce a predominantly Th2 response that is associated with increased Treg and AAMΦ populations. Many experimental studies have shown that the immune perturbations induced by helminths are protective in animal models of autoimmunity (e.g., colitis, arthritis and diabetes) and allergy (reviewed in [38].

T. crassiceps cysticercosis has been shown to modulate the course of noninfectious (e.g., diabetes) and infectious diseases

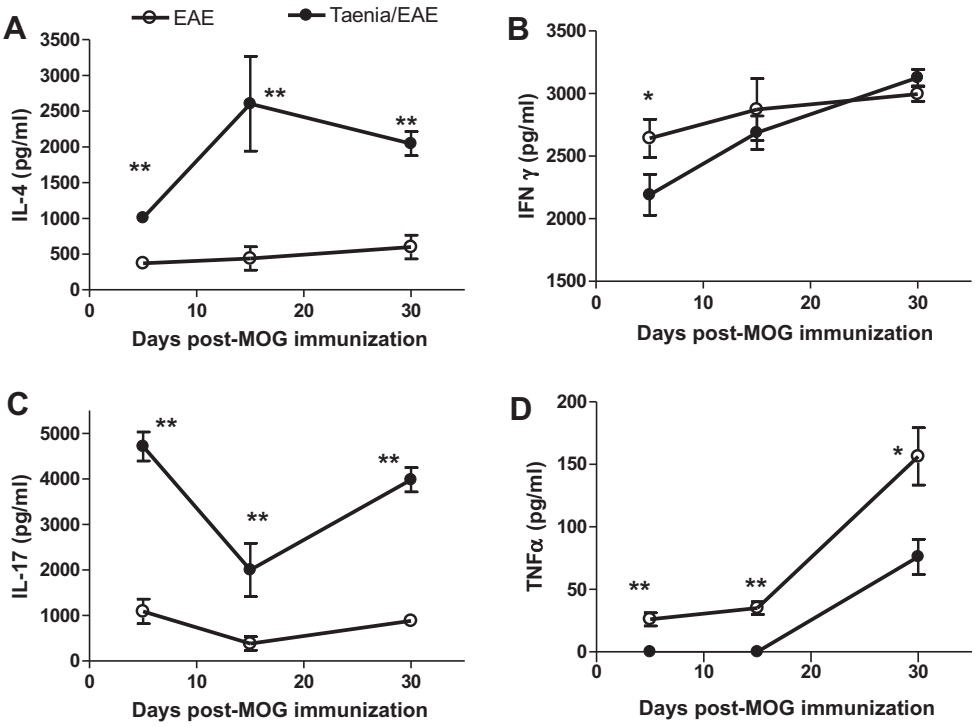

Fig. 4. *T. crassiceps*-infected EAE mice produce an anti-inflammatory and Th2-type cytokine microenvironment. (A) MOG immunization did not induce IL-4 production in uninfected mice (open circles); however, infected mice (closed circles) produced significantly more IL-4. (B) The production of IFN-γ was similar between the experimental groups. (C) Uninfected mice had significantly lower circulating IL-17 levels than infected mice. (D) The production of TNF-α was decreased and delayed in infected EAE mice compared with uninfected EAE mice. The data are presented as the mean ± standard error from eight animals per group. $^{*}p < 0.05$ unpaired Student's *t*-test. All cytokines in serum were detected using an ELISA.

(e.g., Chaga's disease, virus infection and leishmaniasis) in mice [9,10,26]. Infection of streptozotocin-induced diabetic mice with *T. crassiceps* significantly inhibited the development of diabetes, demonstrating that cysticercosis can inhibit the induction of an autoimmune disease, albeit by an undetermined mechanism [26]. In this study, we determined the impact of *T. crassiceps* infection on the development of EAE, and our data demonstrate that concurrent infection with *T. crassiceps* could delay the progression of, and in some cases abrogate the development of, EAE. The early improvement in the clinical scores suggests that *T. crassiceps* infection also reduces ongoing inflammation.

While the mechanism by which *T. crassiceps* infection (as well as other helminth infections) can alter the induction and development of EAE is still unclear, it most likely involves multiple factors, including the suppression of antigen-specific T cell responses, skewing of the immune response from a Th1/Th17 profile to Th2/Th1 profile and a regulatory role for alternatively activated macrophages and potentially B cells, as a source of IL-10, which can reduce CNS inflammation.

One of the main cytokines that has recently been associated with the development of autoimmune disease is IL-17, a new cytokine family containing five different isoforms. This cytokine is mainly secreted by a subpopulation of Th cells now called Th17 cells which have been shown to be increased in inflammatory/autoimmune conditions, including collagen-induced arthritis, T1D and EAE [39–43]. Nevertheless, more recent investigations have found several innate sources of IL-17 such as γδ T cells, iNKT and myeloid cells [44]. One of the possible mechanisms involved in the *T. crassiceps*-induced protection of EAE may be the very high levels of both circulating and MOG-specific IL-4 that was produced by splenocytes from *T. crassiceps*-infected EAE mice. As IL-4 is known to suppress Th17 development [45], the Th17 response, as well as the Th1 response, could also be suppressed in helminth-infected animals [46]. In fact, STAT6-dependent IL-4/IL-13 signaling has been shown to be essential in the suppression of colitis [47] and EAE [20] by *S. mansoni*; however, in these studies, the authors did not measure IL-17. In addition to these investigations, it has been recently found that schistosome-infected mice became resistant to experimental arthritis following the down-regulation of both the splenic Th1 and Th17 responses [38]. Similarly, Ruyssers et al. reported the suppression of chemically-induced colitis by schistosome antigens, which was accompanied by the down-regulation of IL-17 gene expression in the colon and MLN [48]. *H. polygyrus* infection has also been reported to suppress IL-17 production in MLN cells and lamina propria mononuclear cells [49], and the blocking of both IL-4 and IL-10 restored IL-17 production in vitro. Another study demonstrated that the *Fasciola hepatica*-induced down-regulation of the autoantigen-specific Th1 and Th17 responses (and protection from EAE) was dependent on TGF-β but not on IL-10 [50]. Surprisingly, in our study, we found higher levels of circulating IL-17 in infected mice with less severe EAE symptoms, such high levels of circulating IL-17 may come from the innate sources described above. On the other hand, these mice also had an increased IL-4 level; therefore, the presence of IL-4 may be affecting the pathogenic activity of IL-17. Interestingly, the MOG-specific production of IL-17 by splenocytes was greater in uninfected EAE mice but absent in *T. crassiceps*-infected mice. This finding suggests that fewer MOG-specific Th17 clones were generated in peripheral organs in the presence of *T. crassiceps*, thereby altering the normal EAE course which is in line with a considerable amount of evidence pointing out a critical role for Th17 specific MOG-cells in EAE development, mainly because these MOG-specific cells are able to cross to the CNS and initiate local inflammation and in turn the known EAE symptoms [51–53].

Although the mechanism underlying the downregulation of Th17 cells by helminths has not been established, some of the mechanisms

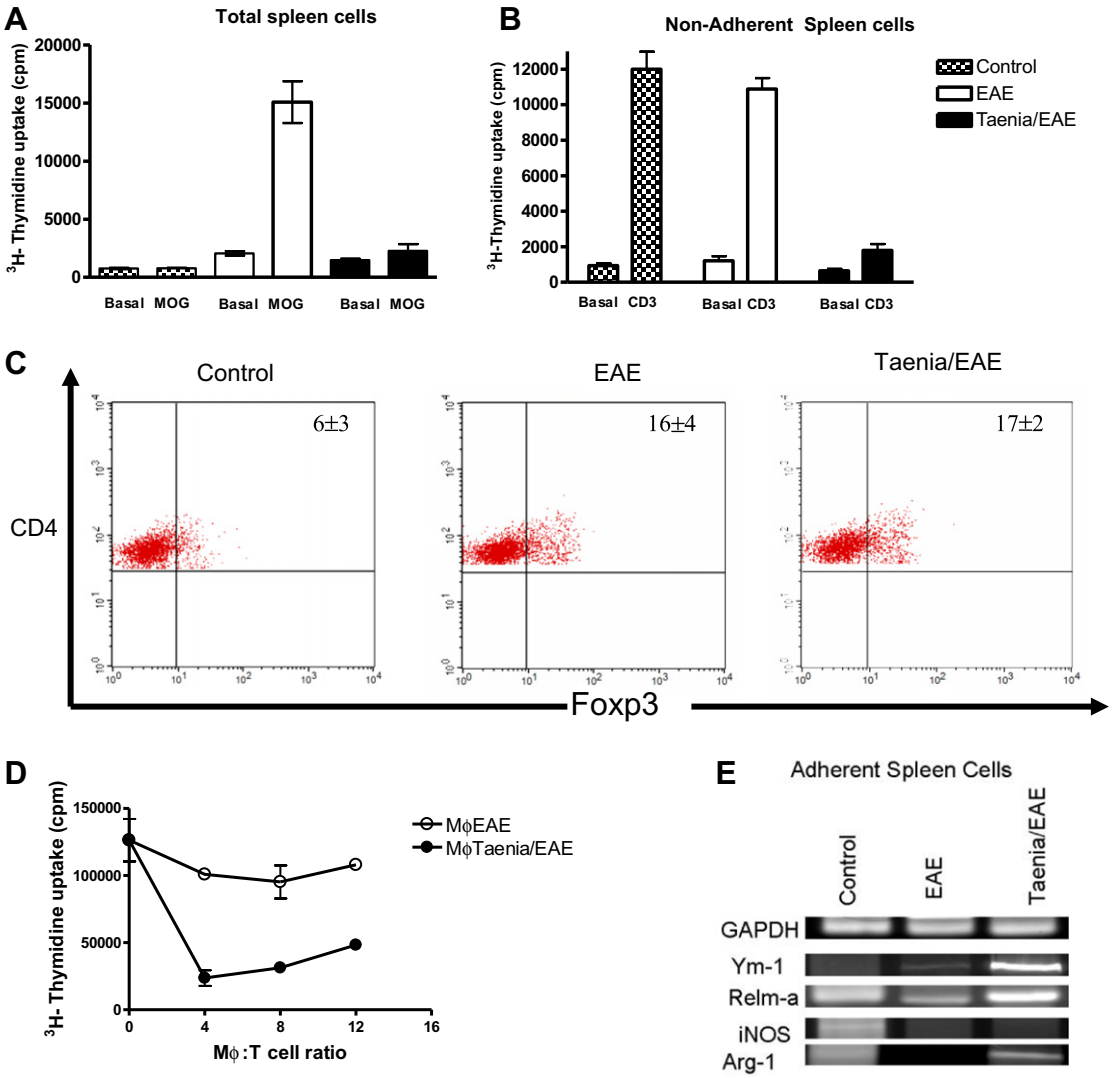

Fig. 5. Anergy in *T. crassiceps*-infected EAE mice dampens MOG-specific cell proliferation. (A) At the peak of disease, spleens were isolated from both experimental groups and processed. The cells obtained from control mice (untreated, uninfected) did not respond to MOG peptide. In sharp contrast, the cells from uninfected EAE mice (white bars) had a sevenfold increase in MOG-specific cell proliferation. However, the *T. crassiceps*-infected EAE mice (black bars) did not proliferate in response to MOG. (B) Because macrophages appear to play a suppressive role, we depleted the macrophages through adherence and re-stimulated the non-adherent cells with 1 μg of anti-CD3. The non-adherent cells from control and immunized mice proliferated robustly, whereas the cells isolated from *T. crassiceps*-infected EAE mice did not proliferate. (C) As detected using flow cytometry, control mice presented a basal fraction of $CD4^+$ $Foxp3^+$ cells in the spleen, whereas uninfected and *T. crassiceps*-infected EAE mice had an increased fraction of $CD4^+$ $Foxp3^+$ cells in the spleen. No difference was detected in the immunized groups. (D) Peritoneal macrophages obtained from uninfected mice (open circles) at the peak of EAE did not inhibit T cell proliferation. In contrast, macrophages from *T. crassiceps*-infected EAE mice (closed circles) suppressed T cell proliferation. (E) Representative RT-PCR of adherent splenocytes for specific markers of AAMΦs. The increased expression of these markers is clear only in the *T. crassiceps*-infected mice. The data shown are from four animals from two independent experiments.

may be common, such as the induction of IL-4 and IL-10 and down-regulation of TNF-α, while others may be distinct, such as Treg induction [54]. In our study on EAE, we did not observe increase in the expression of Treg-related genes (e.g., Foxp3) in the spleen, MLN or brain of *T. crassiceps*-infected mice with EAE compared to the uninfected EAE mice. Therefore, $Foxp3^+$ cells may have only a limited role in the modulating effect of *T. crassiceps* infection on EAE.

Additionally, following infection with *T. crassiceps*, we observed several phenotypic changes on APCs that had the potential to modify the encephalitogenic Th1/Th17 response. *T. crassiceps* infection increased both PD-L1 and PD-L2 expression on macrophages. The negative co-stimulatory molecules PD-L1 and PD-L2 have been shown to be up-regulated on AAMΦs induced by different helminths [25,55,56] and play a role in the regulation of EAE in mice [57–59]. It is well known that macrophages are flexible and can adapt to changes in the cytokine environment [60]. AAMΦs are the counterpart to inflammatory macrophages and can be found during any helminth infection. Due to their anti-inflammatory, suppressive and tissue-repairing abilities, AAMΦs appear to be important in disease outcome [1,2]. We examined if infection of C57BL/6 mice with 40 metacestodes also induced AAMΦs. Not only did we find AAMΦs in the peritoneal cavity and spleen, but we also found that these *T. crassiceps*-induced AAMΦs were able to suppress the proliferative response of T cells. Importantly, this suppression has been shown to be predominantly PD-L dependent [25,55]. Additionally, we also detected a decreased MOG-specific proliferative response in *T. crassiceps*-infected EAE mice, suggesting decreased induction of potentially pathogenic MOG-specific T cells. This finding is in line with our observation that fewer T cells ($CD3^+$ cells) were recovered from the brain of *T. crassiceps*-infected EAE mice, suggesting that *T. crassiceps* infection could prevent the migration of inflammatory cells into the CNS after induction of EAE. Therefore, AAMΦs may also play a critical role in the *T. crassiceps*-mediated protection from EAE.

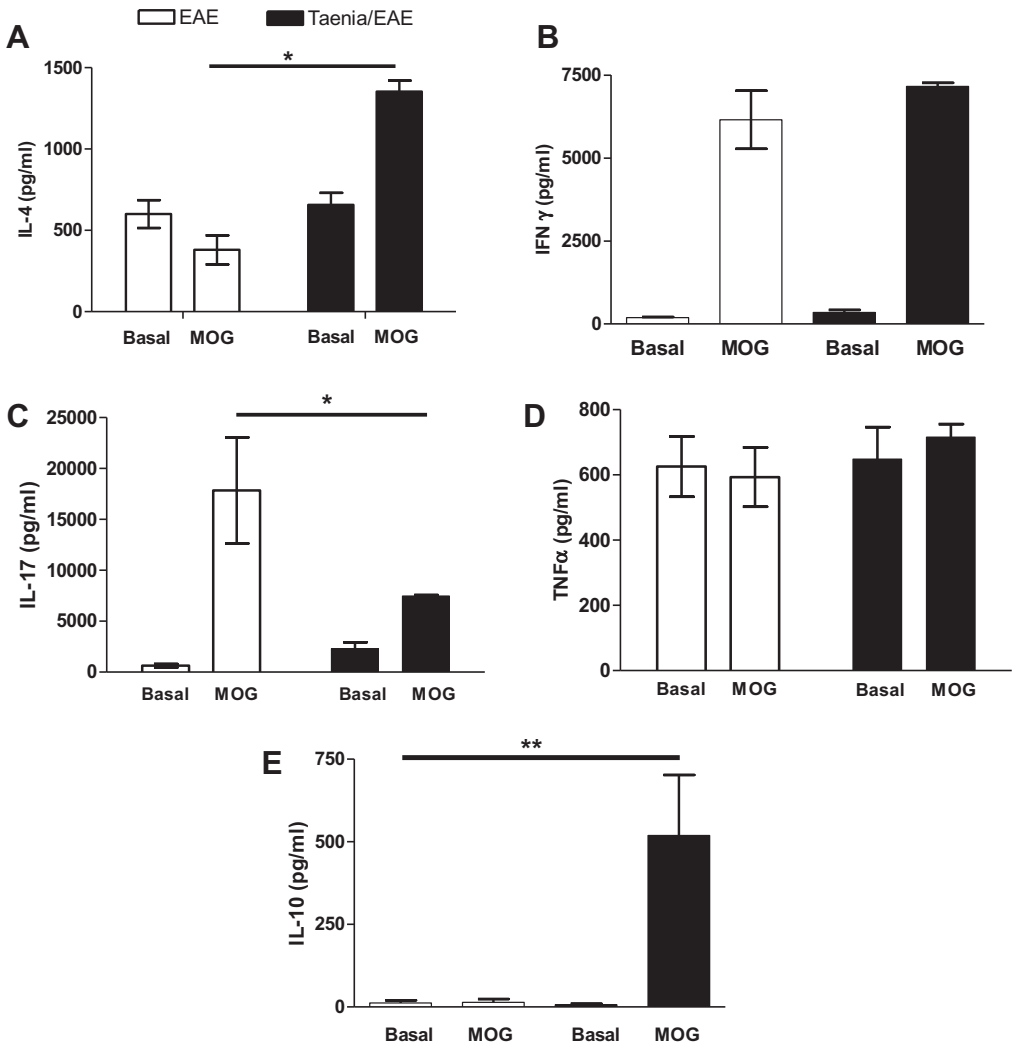

Fig. 6. *T. crassiceps* infection modifies a mixed Th1/Th17 MOG-specific response towards Th2/Th1 response. (A) Anti-MOG-specific IL-4 production in supernatants of total spleen cell cultures. (B) IFN-γ production was detected in similar levels in both experimental groups. (C) MOG-specific IL-17 levels were 2-fold higher in EAE/uninfected group than in *T. crassiceps*-infected EAE group. (D) TNF-α production in the same supernatants. (E) *Taenia*/EAE mice displayed significantly higher amounts of IL-10 than EAE/uninfected mice. Data shown are representative of two independent experiments (four mice per group) and averages from each sample by triplicate. Significances are $^{*}P < 0.05$ and $^{**}P < 0.01$ by unpaired Student's *t* test.

Another interesting finding was the high MOG-specific production of IL-10 in splenocytes from *T. crassiceps*-infected EAE mice. However, the source of this cytokine is unknown as IL-10 is not secreted by *T. crassiceps*-induced AAMΦs [25]. Furthermore, while another possible source is Tregs, as we have shown here, Tregs are not up-regulated during *T. crassiceps* infection. A possible source for the increased production of IL-10 may be B cells, which have been shown to negatively regulate EAE severity through the production of this cytokine [61]. Moreover, previous studies have shown that B cells from patients with MS produce less IL-10 than B cells from healthy controls and that B cells that emerge after treatment with rituximab secrete higher levels of IL-10 than B cells before treatment [62]. Recently, it has been demonstrated that regulatory B cells (also known as B10 cells) may participate in the inhibition of EAE induction through their production of IL-10 [63]. Although our study supports a role for AAMΦs in the regulation of EAE during cysticercosis, a protective role for B cells cannot be ruled out.

Our results show that chronic/concurrent *T. crassiceps* infection inhibits CNS inflammation and demyelinization, which correlates with the clinical severity of EAE. This finding could be partly explained because once the parasite is installed in the peritoneal cavity is able to recruit AAMΦs with potent suppressor activity given the high expression of the down-regulatory molecules PD-L1 and PD-L2, thus when the immune system of these hosts is challenged with MOG plus pertussis toxin the auto-reactive T cells can be anergized by these macrophages. Despite *T. crassiceps* is confined to the peritoneal cavity its immune-modulatory effects seem to be systemic. One possibility is that excretory/secretory molecules, which we have already reported with immune-modulatory activity [64,65], could reach different tissues and modulate the immune response in situ through targeting different cells such as dendritic cells [25] and macrophages, thus such modulated cells may also significantly influence the response to heterologous antigens, as MOG, and avoid the expansion of auto-reactive cells. Moreover as we detected AAMΦs markers on the spleen of *Taenia*/EAE mice it suggests that AAMΦs may also migrate to places where new challenges are going on.

Taken together, our data and those from previous studies suggest that there may be various mechanisms involved in the anti-autoimmune effects of helminth infections; however, further studies are required to determine the relative importance of these potential mechanisms.

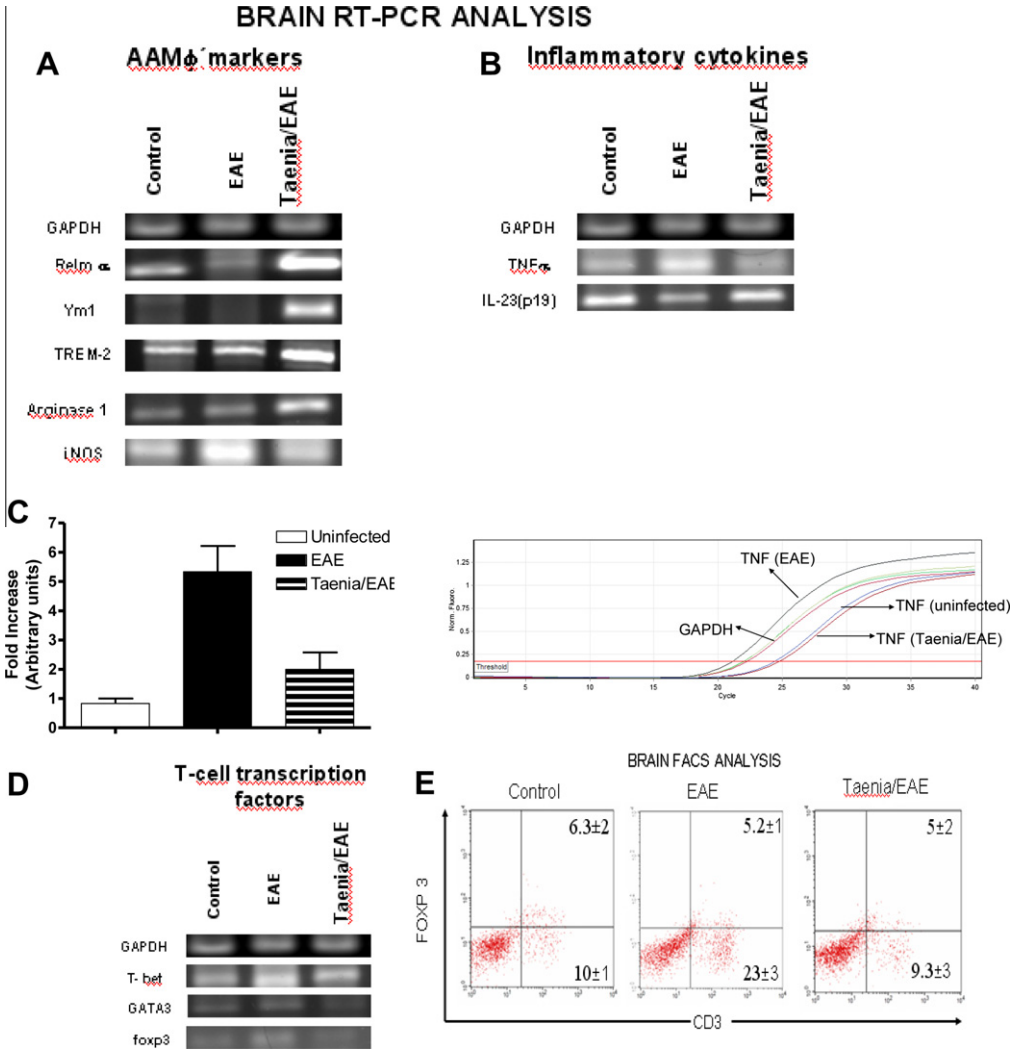

Fig. 7. *Taenia crassiceps* infection induces over-expression of anti-inflammatory/tissue repair-associated molecules in brain and restrains infiltration of $CD3^{+}Foxp3^{-}$ cells. Mice were perfused and total RNA from brains was extracted for gene expression analysis. (A) Markers such as Relm-α, Ym1, TREM-2 and Arginase-1 associated with AAMΦ which have tissue-repairing abilities were over-expressed only in mice harboring cestodes. Noteworthy is the fact that iNOS enzyme transcripts were higher in EAE/uninfected group. (B) RT-PCR analysis for inflammatory cytokines in brains. (C) Real time RT-PCR for TNF-α transcripts is shown together with the curves displayed by the equipment. (D) RT-PCR representative assay for T cell transcription factors. (E) Brain samples were digested with collagenase and total cells were stained for FACS analysis. Naïve mice had a basal percent of $CD3^{+}Foxp3^{-}$ cells. Uninfected/EAE group presented a significant increase of $CD3^{+}Foxp3^{-}$ effectors cells rather than regulatory T cells. *T. crassiceps* infected/EAE mice returned to basal level this infiltrating population. Data shown are from three animals per group from two independent experiments. Numbers in the quadrants indicate percent average ± standard deviation of the corresponding cell markers.

Acknowledgments

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References

[1] S.J. Jenkins, J.E. Allen, Similarity and diversity in macrophage activation by nematodes, trematodes, and cestodes, J. Biomed. Biotechnol. (2010) 262609.
[2] J.L. Reyes, L.I. Terrazas, The divergent roles of alternatively activated macrophages in helminthic infections, Parasite Immunol. 29 (2007) 609–619.
[3] R.M. Anthony, J.F. Urban Jr., F. Alem, H.A. Hamed, C.T. Rozo, J.L. Boucher, N. Van Rooijen, W.C. Gause, Memory T(H)2 cells induce alternatively activated macrophages to mediate protection against nematode parasites, Nat. Med. 12 (2006) 955–960.
[4] J.L. Reyes, C.A. Terrazas, J. Alonso-Trujillo, N. van Rooijen, A.R. Satoskar, L.I. Terrazas, Early removal of alternatively activated macrophages leads to *Taenia crassiceps* cysticercosis clearance in vivo, Int. J. Parasitol. 40 (2010) 731–742.
[5] M.D. Taylor, N. van der Werf, A. Harris, A.L. Graham, O. Bain, J.E. Allen, R.M. Maizels, Early recruitment of natural CD4+ Foxp3+ Treg cells by infective larvae determines the outcome of filarial infection, Eur. J. Immunol. 39 (2009) 192–206.
[6] H.J. McSorley, Y.M. Harcus, J. Murray, M.D. Taylor, R.M. Maizels, Expansion of Foxp3+ regulatory T cells in mice infected with the filarial parasite *Brugia malayi*, J. Immunol. 181 (2008) 6456–6466.
[7] M. Baumgart, F. Tompkins, J. Leng, M. Hesse, Naturally occurring CD4+Foxp3+ regulatory T cells are an essential, IL-10-independent part of the immunoregulatory network in Schistosoma mansoni egg-induced inflammation, J. Immunol. 176 (2006) 5374–5387.
[8] G.S. Noland, D.R. Chowdhury, J.F. Urban Jr., F. Zavala, N. Kumar, Helminth infection impairs the immunogenicity of a *Plasmodium falciparum* DNA vaccine, but not irradiated sporozoites, in mice, Vaccine 28 (2010) 2917–2923.
[9] M. Rodriguez-Sosa, I. Rivera-Montoya, A. Espinoza, M. Romero-Grijalva, R. Lopez-Flores, J. Gonzalez, L.I. Terrazas, 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 (2006) 61–71.
[10] M. Rodriguez, L.I. Terrazas, R. Marquez, R. Bojalil, Susceptibility to *Trypanosoma cruzi* is modified by a previous non-related infection, Parasite Immunol. 21 (1999) 177–185.

[11] M. Segura, C. Matte, N. Thawani, Z. Su, M.M. Stevenson, Modulation of malaria-induced immunopathology by concurrent gastrointestinal nematode infection in mice, Int. J. Parasitol. 39 (2009) 1525–1532.
[12] D.L. Sewell, E.K. Reinke, L.H. Hogan, M. Sandor, Z. Fabry, Immunoregulation of CNS autoimmunity by helminth and mycobacterial infections, Immunol. Lett. 82 (2002) 101–110.
[13] H. Helmby, Gastrointestinal nematode infection exacerbates malaria-induced liver pathology, J. Immunol. 182 (2009) 5663–5671.
[14] A. Cooke, Review series on helminths, immune modulation and the hygiene hypothesis: how might infection modulate the onset of type 1 diabetes? Immunology 126 (2009) 12–17.
[15] A. Cooke, Infection and autoimmunity, Blood Cells Mol. Dis. 42 (2009) 105–107.
[16] P. Zaccone, O. Burton, N. Miller, F.M. Jones, D.W. Dunne, A. Cooke, Schistosoma mansoni egg antigens induce Treg that participate in diabetes prevention in NOD mice, Eur. J. Immunol. 39 (2009) 1098–1107.
[17] P. Zaccone, O.T. Burton, S. Gibbs, N. Miller, F.M. Jones, D.W. Dunne, A. Cooke, Immune modulation by *Schistosoma mansoni* antigens in NOD mice: effects on both innate and adaptive immune systems, J. Biomed. Biotechnol. (2010) 795210.
[18] K.A. Saunders, T. Raine, A. Cooke, C.E. Lawrence, Inhibition of autoimmune type 1 diabetes by gastrointestinal helminth infection, Infect. Immun. 75 (2007) 397–407.
[19] Q. Liu, K. Sundar, P.K. Mishra, G. Mousavi, Z. Liu, A. Gaydo, F. Alem, D. Lagunoff, D. Bleich, W.C. Gause, Helminth infection can reduce insulitis and type 1 diabetes through CD25- and IL-10-independent mechanisms, Infect. Immun. 77 (2009) 5347–5358.
[20] A.C. La Flamme, K. Ruddenklau, B.T. Backstrom, Schistosomiasis decreases central nervous system inflammation and alters the progression of experimental autoimmune encephalomyelitis, Infect. Immun. 71 (2003) 4996–5004.
[21] M.S. Wilson, M.D. Taylor, M.T. O'Gorman, A. Balic, T.A. Barr, K. Filbey, S.M. Anderton, R.M. Maizels, Helminth-induced CD19+CD23hi B cells modulate experimental allergic and autoimmune inflammation, Eur. J. Immunol. 40 (2010) 1682–1696.
[22] M.I. Araujo, B.S. Hoppe, M. Medeiros Jr., E.M. Carvalho, *Schistosoma mansoni* infection modulates the immune response against allergic and auto-immune diseases, Mem. Inst. Oswaldo Cruz 99 (2004) 27–32.
[23] K. Heldwein, H.G. Biedermann, W.D. Hamperl, G. Bretzel, T. Loscher, D. Laregina, M. Frosch, D.W. Buttner, D. Tappe, Subcutaneous *Taenia crassiceps* infection in a patient with non-Hodgkin's lymphoma, Am. J. Trop. Med. Hyg. 75 (2006) 108–111.
[24] L.A. Suzuki, G.C. Arruda, E.M. Quagliato, Q.L. Rossi, 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 (2007) 152–155.
[25] C.A. Terrazas, L. Gomez-Garcia, L.I. Terrazas, 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 (2010) 1051–1062.
[26] A. Espinoza-Jimenez, I. Rivera-Montoya, R. Cardenas-Arreola, L. Moran, L.I. Terrazas, *Taenia crassiceps* infection attenuates multiple low-dose streptozotocin-induced diabetes, J. Biomed. Biotechnol. (2010) 850541.
[27] M. Sospedra, R. Martin, Immunology of multiple sclerosis, Annu. Rev. Immunol. 23 (2005) 683–747.
[28] L. Adorini, Immunotherapeutic approaches in multiple sclerosis, J. Neurol. Sci. 223 (2004) 13–24.
[29] T. Chitnis, S.J. Khoury, Role of costimulatory pathways in the pathogenesis of multiple sclerosis and experimental autoimmune encephalomyelitis, J. Allergy Clin. Immunol. 112 (2003) 837–849.
[30] R.E. Gonsette, New immunosuppressants with potential implication in multiple sclerosis, J. Neurol. Sci. 223 (2004) 87–93.
[31] S.J. Khoury, M.H. Sayegh, The roles of the new negative T cell costimulatory pathways in regulating autoimmunity, Immunity 20 (2004) 529–538.
[32] J.L. Reyes, C.A. Terrazas, L. Vera-Arias, L.I. Terrazas, Differential response of antigen presenting cells from susceptible and resistant strains of mice to *Taenia crassiceps* infection, Infect. Genet. Evol. 9 (2009) 1115–1127.
[33] L.J. Wammes, F. Hamid, A.E. Wiria, B. de Gier, E. Sartono, R.M. Maizels, A.J. Luty, Y. Fillie, G.T. Brice, T. Supali, H.H. Smits, M. Yazdanbakhsh, Regulatory T cells in human geohelminth infection suppress immune responses to BCG and *Plasmodium falciparum*, Eur J Immunol 40 (2010) 437–442.
[34] M.D. Taylor, L. LeGoff, A. Harris, E. Malone, J.E. Allen, R.M. Maizels, Removal of regulatory T cell activity reverses hyporesponsiveness and leads to filarial parasite clearance in vivo, J. Immunol. 174 (2005) 4924–4933.
[35] M.D. Taylor, A. Harris, S.A. Babayan, O. Bain, A. Culshaw, J.E. Allen, R.M. Maizels, CTLA-4 and CD4+ CD25+ regulatory T cells inhibit protective immunity to filarial parasites in vivo, J. Immunol. 179 (2007) 4626–4634.
[36] R.A. O'Connor, S.M. Anderton, Foxp3+ regulatory T cells in the control of experimental CNS autoimmune disease, J. Neuroimmunol. 193 (2008) 1–11.
[37] M. Chopp, Y. Li, J. Zhang, Plasticity and remodeling of brain, J. Neurol. Sci. 265 (2008) 97–101.
[38] Y. Osada, T. Kanazawa, Parasitic helminths: new weapons against immunological disorders, J. Biomed. Biotechnol. (2010) 743758.
[39] E. Lubberts, Th17 cytokines and artritis, Semin. Immunopathol. 32 (2010) 43–53.
[40] E.M. Bradshaw, K. Raddassi, W. Elyaman, T. Orban, P.A. Gottlieb, S.C. Kent, D.A. Hafler, Monocytes from patients with type 1 diabetes spontaneously secrete proinflammatory cytokines inducing Th17 cells, J. Immunol. 183 (2009) 4432–4439.
[41] J.A. Emamaullee, J. Davis, S. Merani, C. Toso, J.F. Elliott, A. Thiesen, A.M. Shapiro, Inhibition of Th17 cells regulates autoimmune diabetes in NOD mice, Diabetes 58 (2009) 1302–1311.
[42] B.M. Segal, Th17 cells in autoimmune demyelinating disease, Semin. Immunopathol. 32 (2010) 71–77.
[43] H.H. Hofstetter, S.M. Ibrahim, D. Koczan, N. Kruse, A. Weishaupt, K.V. Toyka, R. Gold, Therapeutic efficacy of IL-17 neutralization in murine experimental autoimmune encephalomyelitis, Cell. Immunol. 237 (2005) 123–130.
[44] D.J. Cua, C.M. Tato, Innate IL-17-producing cells: the sentinels of the immune system, Nat. Rev. Immunol. 10, 479–489.
[45] J.P. van Hamburg, M.J. de Bruijn, C. Ribeiro de Almeida, M. van Zwam, M. van Meurs, E. de Haas, L. Boon, J.N. Samsom, R.W. Hendriks, Enforced expression of GATA3 allows differentiation of IL-17-producing cells, but constrains Th17-mediated pathology, Eur. J. Immunol. 38 (2008) 2573–2586.
[46] M. Rangachari, N. Mauermann, R.R. Marty, S. Dirnhofer, M.O. Kurrer, V. Komnenovic, J.M. Penninger, U. Eriksson, T-bet negatively regulates autoimmune myocarditis by suppressing local production of interleukin 17, J. Exp. Med. 203 (2006) 2009–2019.
[47] P. Smith, N.E. Mangan, C.M. Walsh, R.E. Fallon, A.N. McKenzie, N. Van Rooijen, P.G. Fallon, Infection with a helminth parasite prevents experimental colitis via a macrophage-mediated mechanism, J. Immunol. 178 (2007) 4557–4566.
[48] N.E. Ruyssers, B.Y. De Winter, J.G. De Man, A. Loukas, M.S. Pearson, J.V. Weinstock, R.M. Van den Bossche, W. Martinet, P.A. Pelckmans, T.G. Moreels, Therapeutic potential of helminth soluble proteins in TNBS-induced colitis in mice, Inflamm. Bowel Dis. 15 (2009) 491–500.
[49] D.E. Elliott, A. Metwali, J. Leung, T. Setiawan, A.M. Blum, M.N. Ince, L.E. Bazzone, M.J. Stadecker, J.F. Urban Jr., J.V. Weinstock, Colonization with Heligmosomoides polygyrus suppresses mucosal IL-17 production, J. Immunol. 181 (2008) 2414–2419.
[50] K.P. Walsh, M.T. Brady, C.M. Finlay, L. Boon, K.H. Mills, Infection with a helminth parasite attenuates autoimmunity through TGF-beta-mediated suppression of Th17 and Th1 responses, J. Immunol. 183 (2009) 1577–1586.
[51] S. Serada, M. Fujimoto, M. Mihara, N. Koike, Y. Ohsugi, S. Nomura, H. Yoshida, T. Nishikawa, F. Terabe, T. Ohkawara, T. Takahashi, B. Ripley, A. Kimura, T. Kishimoto, T. Naka, IL-6 blockade inhibits the induction of myelin antigen-specific Th17 cells and Th1 cells in experimental autoimmune encephalomyelitis, Proc. Natl. Acad. Sci. USA 105 (2008) 9041–9046.
[52] A.C. Murphy, S.J. Lalor, M.A. Lynch, K.H. Mills, Infiltration of Th1 and Th17 cells and activation of microglia in the CNS during the course of experimental autoimmune encephalomyelitis, Brain Behav. Immun. 24 (2010) 641–651.
[53] D.C. Fitzgerald, B. Ciric, T. Touil, H. Harle, J. Grammatikopolou, J. Das Sarma, B. Gran, G.X. Zhang, A. Rostami, Suppressive effect of IL-27 on encephalitogenic Th17 cells and the effector phase of experimental autoimmune encephalomyelitis, J. Immunol. 179 (2007) 3268–3275.
[54] J.P. van Hamburg, A.M. Mus, M.J. de Bruijn, L. de Vogel, L. Boon, F. Cornelissen, P. Asmawidjaja, R.W. Hendriks, E. Lubberts, GATA-3 protects against severe joint inflammation and bone erosion and reduces differentiation of Th17 cells during experimental arthritis, Arthritis Rheum. 60 (2009) 750–759.
[55] P. Smith, C.M. Walsh, N.E. Mangan, R.E. Fallon, J.R. Sayers, A.N. McKenzie, P.G. Fallon, *Schistosoma mansoni* worms induce anergy of T cells via selective up-regulation of programmed death ligand 1 on macrophages, J. Immunol. 173 (2004) 1240–1248.
[56] S. Huber, R. Hoffmann, F. Muskens, D. Voehringer, Alternatively activated macrophages inhibit T-cell proliferation by Stat6-dependent expression of PD-L2, Blood 116 (2010) 3311–3320.
[57] S. Hirata, S. Senju, H. Matsuyoshi, D. Fukuma, Y. Uemura, Y. Nishimura, Prevention of experimental autoimmune encephalomyelitis by transfer of embryonic stem cell-derived dendritic cells expressing myelin oligodendrocyte glycoprotein peptide along with TRAIL or programmed death-1 ligand, J. Immunol. 174 (2005) 1888–1897.
[58] X. Cheng, Z. Zhao, E. Ventura, B. Gran, K.S. Shindler, A. Rostami, The PD-1/PD-L pathway is up-regulated during IL-12-induced suppression of EAE mediated by IFN-gamma, J. Neuroimmunol. 185 (2007) 75–86.
[59] B. Schreiner, S.L. Bailey, T. Shin, L. Chen, S.D. Miller, PD-1 ligands expressed on myeloid-derived APC in the CNS regulate T-cell responses in EAE, Eur. J. Immunol. 38 (2008) 2706–2717.
[60] F.O. Martinez, L. Helming, S. Gordon, Alternative activation of macrophages: an immunologic functional perspective, Annu. Rev. Immunol. 27 (2009) 451–483.
[61] S. Fillatreau, C.H. Sweenie, M.J. McGeachy, D. Gray, S.M. Anderton, B cells regulate autoimmunity by provision of IL-10, Nat. Immunol. 3 (2002) 944–950.
[62] M. Duddy, M. Niino, F. Adatia, S. Hebert, M. Freedman, H. Atkins, H.J. Kim, A. Bar-Or, Distinct effector cytokine profiles of memory and naive human B cell subsets and implication in multiple sclerosis, J. Immunol. 178 (2007) 6092–6099.
[63] T. Matsushita, M. Horikawa, Y. Iwata, T.F. Tedder, Regulatory B cells (B10 cells) and regulatory T cells have independent roles in controlling experimental autoimmune encephalomyelitis initiation and late-phase immunopathogenesis, J. Immunol. 185 (2010) 2240–2252.
[64] R.J. Spolski, J. Corson, P.G. Thomas, R.E. Kuhn, Parasite-secreted products regulate the host response to larval *Taenia crassiceps*, Parasite Immunol. 22 (2000) 297–305.
[65] R.J. Spolski, P.G. Thomas, E.J. See, K.A. Mooney, R.E. Kuhn, Larval *Taenia crassiceps* secretes a protein with characteristics of murine interferon-gamma, Parasitol. Res. 88 (2002) 431–438.