Mesocestoides corti intracranial infection as a murine model for neurocysticercosis

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SUMMARY

Neurocysticercosis (NCC) is the most common parasitic disease of the central nervous system (CNS) caused by the larval form of the tapeworm *Taenia solium*. NCC has a long asymptomatic period with little or no inflammation, and the sequential progression to symptomatic NCC depends upon the intense inflammation associated with degeneration of larvae. The mechanisms involved in these progressive events are difficult to study in human patients. Thus it was necessary to develop an experimental model that replicated NCC. In this review, we describe studies of a murine model of NCC in terms of the release/secretion of parasite antigens, immune responses elicited within the CNS environment and subsequent pathogenesis. In particular, the kinetics of leukocyte subsets infiltrating into the brain are discussed in the context of disruption of the CNS barriers at distinct anatomical sites and the mechanisms contributing to these processes. In addition, production of various inflammatory mediators and the mechanisms involved in their induction by the Toll-like receptor signaling pathway are described. Overall, the knowledge gained from the mouse model of NCC has provided new insights for understanding the kinetics of events contributing to different stages of NCC and should aid in the formulation of more effective therapeutic approaches.

Key words: Neurocysticercosis, *Taenia solium*, *Mesocestoides corti*, Blood Brain Barrier, Toll-like receptors, neuroinflammation, immunomodulation.

INTRODUCTION

Neurocysticercosis (NCC) is an infection of the central nervous system (CNS) caused by the metacestode (larval form) of the tapeworm *Taenia solium* (White, 1997; White *et al*. 1997; Garcia and Del Brutto, 2005). NCC is common throughout Latin America, most of Asia, sub-Saharan Africa and parts of Oceania, and is the greatest cause of acquired epilepsy worldwide (Nash *et al*. 2006). It is now increasingly diagnosed in more developed countries due to immigration of tapeworm carriers from endemic zones (White, 2000). In the life cycle of *T. solium*, man develops the parasite's adult stage (taeniasis) in the intestine after ingesting undercooked pork infected with metacestodes. NCC results from ingestion of ova shed by a human tapeworm carrier. The oncosphere hatches in the intestine penetrating the intestinal mucosa and entering the bloodstream where they preferentially migrate to the CNS and the eye.

Although the metacestodes reach their mature size within a few weeks, evidence indicates that prior to clinical manifestations, there is a long asymptomatic period (months or even years) thought to be the result of mechanisms used by *T. solium* to modulate the host's immunity (White, 1997; White *et al*. 1997). Eventually, clinical symptoms appear as a result from the parasite's loss of the ability to suppress the host immune response or after the life-span of the tissue metacestode (cyst) is complete and begins dying (White, 2000). The clinical manifestations are varied, non-specific and depend on location and viability of the parasite. Epileptic seizures are the most common manifestation of NCC and generally represent the primary or sole manifestation of the disease. Seizures occur in 50–80% of patients with metacestodes or calcifications lodged in parenchyma but are less common in other forms of the disease (Del Brutto, 1992; Garcia *et al*. 2004; Nash *et al*. 2006). Intracranial hypertension, hydrocephalus, or both are seen in 20–30% of cases. This syndrome is associated with metacestodes located in the cerebral ventricles or basal cisterns, where they block circulation of the

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cerebrospinal fluid (Del Brutto, 1997; White, 2000). Motor deficits can also arise because of oedema secondary to cyst degeneration, or as a result of a stroke complicating NCC (Barrinagarrementeria, 2002). Compromise of the spine occurs in about 1% of cases of NCC, presenting with symptoms associated with compression (Parmar *et al*. 2001).

The severity of the symptoms is thought to be associated with the intensity and type of immune response (Escobar, 1983; Restrepo *et al*. 2001*a*, *b*). Parasites lodged in leptomeninges often induce a strong inflammation, perhaps due to their higher exposure to the immunosurveillance mechanisms operating in the CNS (Bandres *et al*. 1992; Restrepo *et al*. 1998). However, parasites residing in brain parenchyma also induce a strong inflammatory response in the chronic stage of the disease characterized by granuloma formation, angiogenesis and fibrosis (Restrepo *et al*. 2001*a*, *b*). Characterization of the first and even later stages of the immune response has been a very difficult task due to the lack of biological samples and to the long asymptomatic period observed with NCC patients. To study different aspects of the cestode-host interaction in the context of the CNS, our laboratory has used a closely related cestode (*Mesocestoides corti*) to infect mouse brain and thus, replicate the human disease in a murine model.

ESTABLISHMENT OF THE MODEL AND SIMILARITIES WITH HUMAN NCC

The study of the immunopathogenesis in NCC has been limited by the chronic progression of the disease, the influence of anti-helminth/steroid treatment, and the scarcity of patients undergoing surgical intervention. NCC pathology seems to be predominately related to the immune response against the metacestode (Restrepo *et al*. 1998, 2001*a*, *b*; Sciutto *et al*. 2007). Thus, a complete characterization of the host response elements involved is required to fully understand the disease process. In NCC, the immune response varies according to the incubation period and the number, location and stage of the cysts (Escobar, 1983; Flisser *et al*. 1986; Restrepo *et al*. 1998, 2001*a*, *b*; Alvarez *et al*. 2002*a*, *b*). Due to the high number of variables and to the lack of an animal model displaying the infection in the CNS, we developed a NCC murine model in which the multiple stages of the host immune response could be studied in the brain. As *T. solium* is not infectious in mice, a cestode with a known life cycle, easy to culture *in vitro*, conveniently maintained in laboratory animals and closely related to *T. solium* was required. Taeniids such as *T. crassiceps and T. solium* have been used to study NCC in laboratory animals, but these models lack the host-pathogen interaction in the tissue of interest as metacestodes are injected in the peritoneal cavity or subcutaneously (Hernandez-Mendoza *et al*. 2005; Nakaya *et al*. 2006). Intracranial injection of *T. crassiceps* was attempted in the laboratory, but as the metacestodes displaced most of the nervous tissue, this parasite could not be used for an animal model for studying a CNS infection. Another cestode, *Mesocestoides corti*, which meets the parameters previously mentioned, was then chosen (Cardona *et al*. 1999). The *M. corti* life cycle is somewhat similar to *T. solium* as it also has an intermediate host (mouse or lizard) harbouring metacestodes that, upon ingestion by a definitive host (dog, cat, or skunk), mature into intestinal tapeworms able to reproduce, release eggs and maintain the cycle, although no CNS infection has been reported in the intermediate or definitive hosts. The model has the limitation of injecting the parasite in the brain as *M. corti* does not infect the CNS through the faecal-oral route as occurs with *T. solium* in humans. But even with this constraint, to have metacestodes within the CNS environment provides an excellent tool to study different aspects of the host-parasite interaction.

In the laboratory, *M. corti* metacestodes are maintained by serial intraperitoneal inoculations of 8 to 12 wk old female BALB/c mice. To simulate the disease in humans, *M. corti* metacestodes are injected intracranially into 3–5 wk old female BALB/c mice using a 25-gauge needle inserted at the junction of the superior sagittal and the transverse sutures to avoid penetration of the brain tissue. The distribution of *M. corti* metacestodes parallels *T. solium* as the parasite locates in the subarachnoid space, ventricular areas and parenchyma. During the first 24 h of infection, most of the metacestodes are found in the leptomeningeal space where they bind and traverse the arachnoid-pia complex to move into the nervous tissue (Fig. 1a). After 48 h some of the metacestodes penetrate the parenchymal tissue and by one week, about 50% of the parasites are detected in leptomeninges-parenchyma interface (Fig. 1b). The parasite distribution within the CNS continues to resemble human NCC as metacestodes are also detected in the ventricular areas at early times (Fig. 1c) and throughout the infection. The number of parasites increases in the parenchymal tissue with about 50% of the metacestodes found there after 3 wks of infection (Fig. 1d). This percentage shows a minor increase in later times post-infection and the numbers of parasites plateau in the chronic part of the infection with about 60% located in parenchyma and 40% found in leptomeningeal areas and ventricles (Cardona *et al*. 1999). In addition, the number of immune cells infiltrating the CNS and surrounding the parasites in the first weeks of infection is low taking in consideration the size of these microorganisms (Fig. 1) (Cardona *et al*. 1999; Alvarez and Teale, 2006). Thus, it can be speculated that murine NCC can mimic the unknown mechanisms occurring in early and mid-human NCC, which is one of the

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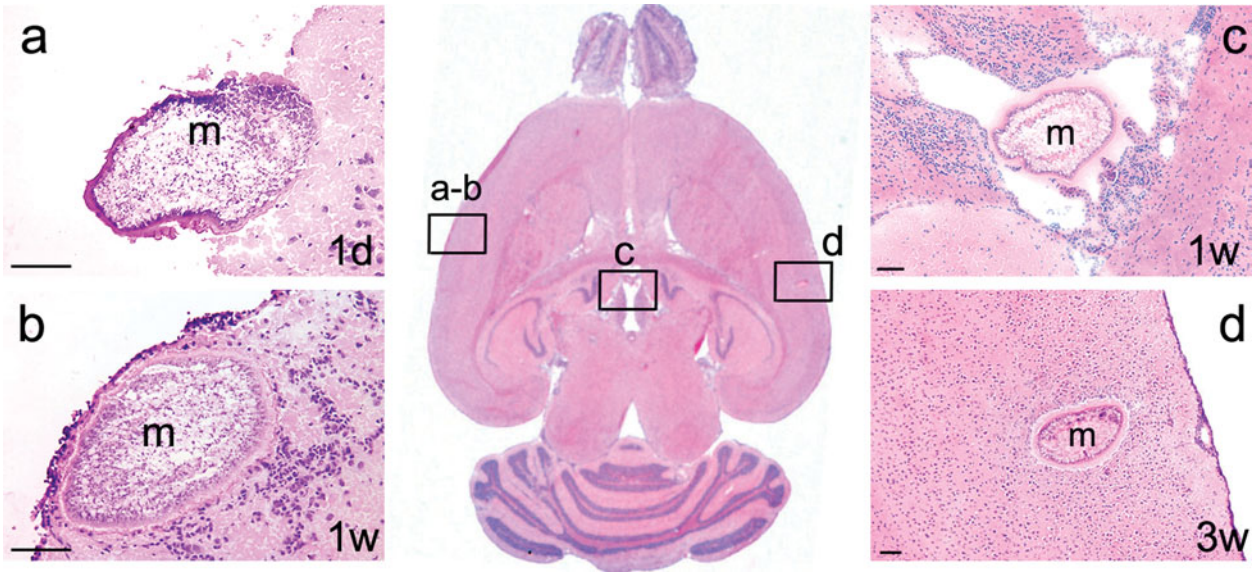

Fig. 1. *M. corti* distribution in the CNS during the course of murine NCC. In the murine model of NCC, mice are intracranially (IC) injected with *M. corti* metacestodes. Analysis of the dissemination of the parasite within the CNS was done by hematoxylin and eosin (H&E) histological analysis. The axial section of mouse brain in the centre indicates the areas of parasite distribution during the course of the infection and shown in a to d. 5X (a) *M. corti* metacestode (m) invading the parenchymal tissue from the leptomeninges after 1 day of infection. (b) Similar area presented in a, but after 1 wk of infection the m has completely traversed the leptomeninges. (c) Third ventricle displaying a m 1 wk post-infection. (d) *M. corti* m located in parenchyma after 3 wks of infection. Scale bars: 50 μm.

great advantages of this model and has been a main focus of our laboratory.

PARASITE GLYCOCONJUGATES, COMPARATIVE ANALYSIS IN MURINE, PORCINE AND HUMAN NCC AND THEIR POTENTIAL ROLE IN IMMUNOMODULATION

In the NCC field, major emphasis has been placed on determining the molecular nature of the parasite antigens associated with disease for their use in serological diagnosis and vaccine development (Tsang *et al*. 1989; Restrepo *et al*. 2000; Obregon-Henao *et al*. 2001, 2003). The glycosidic portion of glycoproteins and other glycoconjugates (GCs) expressed by *T. solium* metacestodes is highly antigenic, and it has been shown to be recognized by the serum of infected patients (Tsang *et al*. 1989; Restrepo *et al*. 2000; Obregon-Henao *et al*. 2001; Lopez-Marin *et al*. 2002). Although most studies focused on establishing their potential in serological diagnosis and vaccines, these GCs also appear to be involved in the complex interaction between the host and parasite in addition to modulation of the immune response (White, 1997; White *et al*. 1997). The parasite's external surface or tegument is the structure involved in this process, and it is dynamically responsive to changes in the host environment or immune attack. It can be rapidly released and thus protect the microorganism from antibodies, complement and activated immune cells (Maizels *et al*. 1993). Therefore, the release of tegument material by *T. solium* and *M. corti* has been a field of study in our laboratory. The GCs present in the tegument of *M. corti* metacestodes were studied at different times post-infection, and parallel studies with *T. solium* were performed by studying parasites in porcine skeletal and cardiac muscle in stages I to IV (Alvarez *et al*. 2002*a*, *b*) as well as metacestodes obtained from human brain (Alvarez *et al*. 2008).

M. corti metacestodes are localized in the subarachnoid space 1 day post-infection (Fig. 1a) and 24 h later can actively invade the nervous tissue exhibiting a progressive loss of their tegument in areas of close contact with the nervous tissue. In contrast, areas of the parasite located outside of the tissue are thicker and appear to remain intact, while metacestodes that have completely invaded the tissue exhibit a much thinner tegument on their surface. Thus, the parasite's degree of contact and penetration directly correlates with the amount of material released from the tegument (Alvarez *et al*. 2008). The tegument is made up of two zones; the distal cytoplasm, which is the external layer involved in the formation and replacement of the outer-surface membranes, and the proximal cytoplasm that contains multiple organelles required for the synthesis of molecules that replenish the distal cytoplasm (Hess, 1980; MacGregor *et al*. 1988). Fluorochrome-specific labeling of the GCs in these two structures demonstrated that *M. corti* sheds or secretes carbohydrate-rich material from the distal cytoplasm as early as 12 h post-infection, whereas GCs present in the proximal cytoplasm are slowly and continually released and appear to be a

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minority of the glycoantigens secreted by the parasite in the first week of infection (Alvarez *et al*. 2008). But, studying the phenomenon of material being released by *T. solium* during the infection in human or porcine tissue is difficult due to multiple technical limitations. Particularly problematic is analyzing the early events of the infection process highlighting the utility of the mouse model, particularly for understanding the role of released parasitic antigens during infection. Nonetheless, the differential release of GCs observed with *M. corti* tegument was also observed with *T. solium* by using fluorochrome-conjugated lectins and even showed similar GCs in the tegument of both species. Acetyl-D-galactosamine ends and α-D-galactosyl residues, which are carbohydrates bound by Isolectin B4 (IB4), are mainly found in the distal cytoplasm. The same pattern of staining is seen for N-acetylglucosaminyl residues bound by wheat germ agglutinin (WGA) in both parasite species (Fig. 2), whereas terminal β-galactose residues recognized by *Arachis hypogaea* lectin (PNA) and α-mannopyranosyl and α-glucopyranosyl residues bound by Concavalin A (ConA) were present in the whole tegument and in the parenchyma of the parasites (Alvarez *et al*. 2008). Interestingly, the material bound by IB4 is rapidly released and it is almost absent after 1 wk of infection. In contrast, WGA- and ConA-bound GCs are constantly released and are taken up by cells in the infiltrates around the metacestodes (Fig. 2). Active immune cells in the infiltrates could potentially up-regulate glycoproteins to levels detectable by the lectin concentrations used. Therefore, antibodies specific to *M. corti* (*M. corti* supernatant $\gamma 1 - \mathrm{MCS}\gamma 1$) and to a highly immunodominant antigen of *T. solium* (glycoprotein 12) were used to distinguish between these possibilities. In both parasites and in the 3 different hosts tested the GCs found in the immune infiltrates, particularly on macrophages, correspond to antigens released by the metacestodes (Fig. 2) (Alvarez *et al*. 2008).

In terms of their role in host-parasite interactions, the IB4 fluorochrome-labeled GCs, may well be important in the evasion of the early stages of the host response, as they are quickly released upon infection and correlate with a reduction in the rate of tegument build up and thus, less antigenic exposure in the initial steps of parasite invasion (Alvarez *et al*. 2008). But as NCC is a chronic and long-lasting infection, additional mechanisms to evade or modulate the host immune response are presumably associated with the constant release of GCs, like those bound by WGA. Thus, studies using the murine model of NCC along with porcine and human tissues infected with *T. solium* had determined that structural changes and selective release of GCs occurs during the course of infection (Fig. 2). These observations confirm that these organisms have the ability to modify their composition and metabolism as part of an adaptive response to the host microenvironment. The specific composition and potential role of these GCs as immunomodulators are currently being explored and will provide insights on how the parasite is able to remain silent in the CNS for extended periods of time.

LEUKOCYTE TRAFFICKING AND CHANGES IN THE CNS BARRIERS

Parasite-shed GCs accumulated in perivascular areas close to the metacestode as well as far from it, presumably through circulation of the cerebral spinal fluid (Alvarez *et al*. 2008). These antigens seem to be involved in promoting the infiltration of immune cells into the brain and altering the integrity of the CNS barriers, namely the Blood Brain Barrier (BBB) and Blood Cerebrospinal fluid Barrier (BCB) (Alvarez and Teale, 2006, 2007 *a*, *b*). Perivascular inflammation resulting from damage to the BBB is considered one of the pathological hallmarks of NCC (White, 1997; White *et al*. 1997). In the healthy brain, the movement of leukocytes and immune mediators from the bloodstream to the CNS is controlled by the BBB and the BCB (Kniesel and Wolburg, 2000). These barriers selectively restrict the molecular and cellular trafficking between the periphery and the CNS. Potential sites of leukocyte infiltration include parenchymal vessels, pial vessels, and ventricular blood vessels in the central stroma of the choroid plexuses (Ransohoff *et al*. 2003; Abbott, 2005). CNS vessels associated with the BBB (parenchymal and pial) and the choroid cells of the BCB are formed by highly specialized endothelial and epithelial cells that inhibit the transcellular and paracellular diffusion of macromolecules and cells through their unique structural and functional properties (Brightman and Kaya, 2000; Gloor *et al*. 2001). One of these properties is the paracellular cleft between adjacent lateral endothelial/epithelial membranes, which is almost completely sealed because of the continuous strands of junctional complex proteins that include tight junctions (TJs) (Kniesel and Wolburg, 2000; Wolburg and Lippoldt, 2002; Vorbrodt and Dobrogowska, 2003; Harhaj and Antonetti, 2004) and adherens junctions (AJs) (Schulze and Firth, 1993). TJs are located in the most apical section of the plasma membrane, and they are composed of a combination of transmembrane (occludin and claudins) and cytoplasmic molecules (zonula occludens proteins ZO1, ZO2, ZO3 and others) (Petty and Lo, 2002). The AJs are ubiquitous in the vasculature and ventricular epithelium, where they mediate the adhesion of cells to each other and partially the regulation of paracellular permeability (Brown and Davis, 2002; Vorbrodt and Dobrogowska, 2003; Bazzoni and Dejana, 2004). They consist of the transmembrane proteins cadherins and the cytoplasmic proteins catenins.

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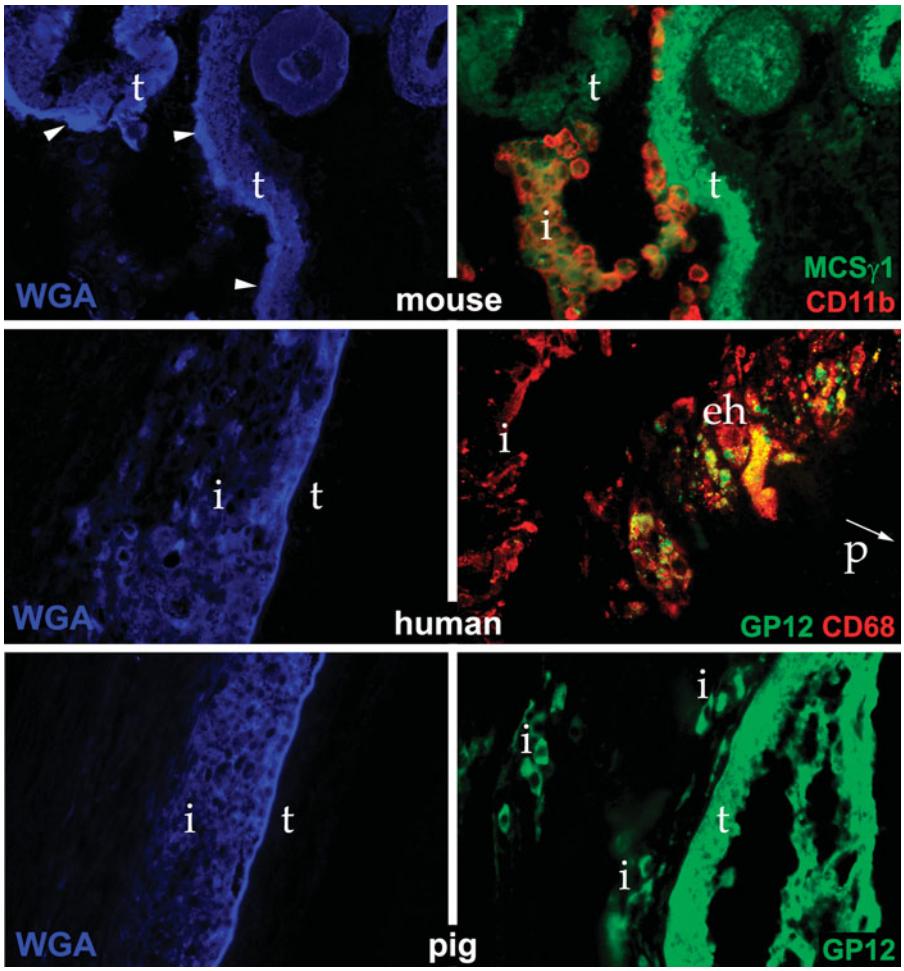

Fig. 2. *M. corti* and *T. solium* glycoconjugates are similarly released and taken up by host cells. The pattern of glycoconjugates (GCs) release was studied in sections of brain (murine and human NCC) and skeletal muscle (porcine NCC-naturally infected pigs) using lectins and specific antibodies against *M. corti* (anti-*M. corti* supernatant $\gamma 1$ – MCS$\gamma 1$) and *T. solium* (anti-glycoprotein 12 – gp12). In the upper panels *M. corti* metacestodes lodged in nervous tissue display WGA (blue) staining in tegument (t), preferentially in distal cytoplasm (arrowheads). The t and the CD11b+ infiltrates (i) are also labeled with MCS$\gamma 1$. Middle panels show WGA bound material in t and i of a human NCC section. Parasite (p) surrounded by granuloma with i shows some epithelioid histiocytes (eh) labeled with anti-GP12 (orange-yellow). Lower panels show parasite lodged in skeletal muscle displaying WGA and GP12 staining in t and i. All images were acquired at 40X.

The mechanisms of leukocyte infiltration in NCC and other parasitic diseases have been largely uncharacterized, and in other neuroinflammatory diseases this process has been mainly described in the context of parenchymal vessels or pial vessels (Brown *et al*. 1999; Minagar and Alexander, 2003; Lan *et al*. 2004), but rarely as a complex process involving distinct anatomical and vascular compartments of the CNS. Thus, the murine model of NCC has been used to study the kinetics of leukocyte infiltration and the mechanisms associated with it. Analysis of different anatomical areas of the infected brain indicated increased permeability of all the types of vessels/structural sites evidenced by fibronectin and leukocyte extravasation but with considerable differences in the timing and extent of these permeability changes (Table 1). Parenchymal vessels were the most resilient to changes whereas pial vessels were the least. Choroid plexus vessels of the ventricles also appeared less susceptible to increased permeability compared with pial vessels and ependyma. In addition, reactive astrocytes juxtaposed to blood vessels and ependyma showed increased expression of cytokines known to regulate brain barrier function and correlate with the increased permeability displayed by these structures during infection (Alvarez and Teale, 2006).

The differential permeability and barrier breakdown detected in the distinct anatomical areas/vasculature studied correlate with changes in the organizational structure of junctional complex proteins (TJs and AJs). The extent and timing of these changes differed between pial vessels of the meninges, ependyma, choroid plexus and vessels of the

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Table 1. Kinetics of CNS barriers changes during the course of murine NCC

Structure/ barrier	Time	Barrier disruption		
		Serum extravasation Leukocyte infiltration	Junctional proteins	MMP activation
Pial vessels BBB	1–3 d	+	altered	+
	1 w	++	altered	++
	3 w	+++	altered	+++
	5 w	+++	altered	+++
Parenchyma BBB	1–3 d	–	normal	–
	1 w	–	normal	–
	3 w	–	normal	–
	5 w	+	altered	+
Ependyma	1–3 d	–	normal	–
	1 w	+	altered	+
	3 w	++	altered	++
	5 w	++	altered	++
Ch plexus BCB	1–3 d	–	normal	–
	1 w	–	normal	–
	3 w	+	normal	+
	5 w	+	normal	+

The relative extent of extravasation, leukocyte infiltration and MMP activation at time post-infection were arbitrarily assigned as follows: – = absent to +++ = abundant. The modification in junctional proteins relate to positional changes in their structure, and were assigned as normal or altered. d = days post-infection, w = weeks post-infection.

parenchyma (Alvarez and Teale, 2007*a*, *b*). The junctional proteins of pial vessels were disrupted within days, whereas those in parenchymal vessels were not affected until 5 weeks post-infection (Table 1) (Alvarez and Teale, 2007*a*). Structural alterations of these proteins were also evident in ependyma, but the infection did not change the expression pattern of junctional complex proteins in choroid plexus. Thus, damaged subependymal pial vessels together with a disrupted ependyma explained the predominant source of leukocytes infiltrating ventricles rather than the CP and suggest an alternative route of leukocyte infiltration in the CNS (Alvarez and Teale, 2007*b*). For all the structures analyzed the kinetics and magnitude of changes in the junctional proteins directly correlated with the extent of barrier breakdown and leukocyte infiltration (Table 1). In some cases, particular TJ and AJ proteins were undetectable or appeared to be undergoing proteolysis (Alvarez and Teale, 2007*a*, *b*).

To examine mechanisms associated with changes in the pattern of junctional proteins, the expression and activity of matrix metalloproteinases (MMPs) were analyzed in distinct CNS barriers in infected mice. mRNA analysis of whole brain showed that the majority of MMPs tested (MMP-2, -3, -7, -8, -9, -12, -13 and -16) were up-regulated as a result of the infection (Alvarez and Teale, 2008). The expression and activity of multiple MMPs were mainly detected in leukocytes migrating into the brain. These cells displayed polarized active expression of several MMPs as early as 1 day post-infection. In contrast, leukocytes expressing active MMPs and extravasating parenchymal vessels were not detected until 5 weeks of infection. In ventricular areas, most of the MMP activity was detected in leukocytes traversing the ependyma from leptomeningeal infiltrates. In contrast to the multiple active MMPs in ependymal cells and leukocytes moving through this cellular layer, the choroid plexus and the cells extravasating it showed a more limited expression of MMPs (Table 1) (Alvarez and Teale, 2008). The pattern of MMP activity in different anatomical sites of the brain correlate with the differential kinetics in the disruption of the CNS barriers (Table 1), suggesting that various MMPs are required to support the complex nature of immune cell infiltration observed in this model. Studies in this area are clinically relevant for human NCC as particular sites of inflammation in the CNS play a significant role in the extent of morbidity and mortality associated with this infection.

TOLL-LIKE RECEPTORS AS REGULATORS OF INFLAMMATORY RESPONSES IN MURINE NCC

The CNS has a remarkable set of mechanisms that work in concert to maintain its immune privileged status under normal circumstances, but can orchestrate inflammatory responses during infections and injury. During infection, several mechanisms act together leading to production of inflammatory

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mediators such as chemokines, adhesion molecules and cytokines (Chavarria and Alcocer-Varela, 2004) that culminate in infiltration of leukocytes and development of pathogen-specific adaptive immune responses (Ransohoff *et al*. 2003). One of the key mechanisms involved in this process is thought to be the direct recognition of microbial molecules by nervous tissue cells, and the subsequent generation of inflammatory conditions which is critical in initiating and mounting an overall immune response in the CNS.

Recent studies have demonstrated that the host innate immune system plays a critical role in recognition of pathogen-associated molecular patterns (PAMPs) which constitute a diverse family of microbial molecules (Kumar *et al*. 2009). PAMPs are recognized by families of pattern recognition receptors (PRRs) expressed by host cells such as Toll-like receptors (TLRs), NOD-like receptors (NLRs) and the RIG-like molecules (Akira *et al*. 2006). Among these PRRs, TLRs detect conserved motifs from a wide range of pathogens including bacteria, viruses, protozoa and fungi (Iwasaki and Medzhitov, 2004; Pasare and Medzhitov, 2005; von Bernuth *et al*. 2008; Martinon *et al*. 2009). NLRs detect primarily intracellular bacteria, while RIG-like molecules detect viral genomes (Takeuchi and Akira, 2009). Once engaged, signaling through PRRs leads to activation of the oxidative burst and nitric oxide (NO) release that can directly kill the microbial pathogen (Medzhitov, 2009; Mogensen, 2009). In addition, production of pro-inflammatory mediators leads to recruitment of immune cells and establishment of an appropriate anti-microbial adaptive immune response (Fritz *et al*. 2006). Among these PRR families, the TLR family of proteins has been demonstrated to play a critical role in host innate immunity across a wide range of pathogenic conditions (Hoebe *et al*. 2004). Thirteen mammalian TLR paralogues have now been identified so far (10 in humans and 12 in mice) (Beutler, 2004). Once engaged, TLRs signal through a common pathway involving myeloid differentiation primary-response gene 88 (MyD88) with the exception of TLR3 which acts through Toll-interleukin-1 receptor domain-containing adaptor inducing IFN-β instead (Rock *et al*. 1998). TLR signaling through MyD88 leads to downstream activation of the NF-κB and MAP kinase pathways and induction of a Th1 type pro-inflammatory cytokine response (McDermott and O'Neill, 2002). Emerging evidence indicates that TLRs can also elicit anti-inflammatory regulatory T cell (Treg) and T helper 2 (Th2)-associated responses (Didierlaurent *et al*. 2004; Dillon *et al*. 2004; Redecke *et al*. 2004; Piggott *et al*. 2005), indicating an important role for TLRs in multiple immune regulatory mechanisms.

In murine NCC, all of the TLRs (TLRs1-13), except TLR5 (which was not detected at the protein level) are up-regulated and differentially expressed among various CNS cell types and infiltrating leukocytes (Mishra *et al*. 2006, 2008). Among them, TLR2 almost exclusively localizes to nervous tissue cells, while TLR1 and TLR9 are essentially limited to infiltrating leukocytes. All other TLRs can be detected in both nervous tissue cells and infiltrating immune cells. Some of the TLRs are abundantly expressed in distinct nervous tissue cell types: neurons (TLRs 2, 11-13), ependymal cells (TLRs 2, 6, 7), neurofilaments (TLR8), and astrocytes (TLRs 2, 3, 13). Additionally, TLRs detected in infiltrating leukocytes during NCC are largely confined to $CD11b^{+}$ myeloid and $\gamma\delta$ T cells. The selective spatio-temporal expression of various TLRs underscores the likely importance of these receptors in both the immune response and pathogenesis of NCC and the likely cross-talk among infiltrating leukocytes, nervous tissue cells and parasite.

We next tested mice deficient in MyD88 ($MyD88^{-/-}$), an essential adaptor molecule for signaling through most of the TLRs. Infected $MyD88^{-/-}$ mice exhibited reduced numbers of multiple infiltrating leukocyte subsets including macrophages, B cells, $\alpha\beta$ T and $\gamma\delta$ T cells during the course of infection. This decrease in cellular infiltration correlated with a decrease in BBB permeability as measured by reduced fibrinogen leakage. Moreover, MyD88-deficient mice exhibited a reduction in brain levels of pro-inflammatory cytokines and chemokines such as TNF-α, IFN-γ, MCP-1, IL-6, MIP1-α, and MIP1-β. Importantly, this coincided with an enhanced ability to survive the infection along with reduced neuropathology as compared to wild-type (WT) mice (Mishra *et al*. 2009). Thus, MyD88-dependent mechanisms, particularly the production of pro-inflammatory mediators, likely contribute to the observed pathology and severity of NCC. This is in sharp contrast to other infections where the lack of MyD88 signaling results in increased susceptibility to the pathogen and a more rapid mean time to death (Ulevitch *et al*. 2004; Takeda, 2005; Schnare *et al*. 2006; West *et al*. 2006; Mitchell *et al*. 2007; Ropert *et al*. 2008; Rakoff-Nahoum and Medzhitov, 2009). However, in the case of NCC, MyD88 associated signaling is likely involved in the development of a persistent hyper-inflammatory response and associated with the clinical symptoms of NCC.

A critical question is whether the innate immune system and especially the TLR- associated responses have other functions besides regulating inflammation in NCC. TLRs are presumed to participate in innate immune response-mediated neuroprotection (Nguyen *et al*. 2002). In this light, constitutive expression and infection-induced up-regulation of particular TLR proteins is observed in distinct nervous tissue cell types, especially ependymal cells (TLRs 2, 7) and neurons (TLRs 2, 11-13) (Mishra

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et al. 2008). Some ependymal cells are in close contact with pial vessels and upon infection this area can support immune activation and constitutes an alternative route of leukocyte migration into the ventricular space (Alvarez and Teale, 2007*a*). On the other hand, expression of TLRs in neurons suggests that neurons might be able to recognize some forms of infection. Previous studies indicated that neurons express TLR2 (Kurt-Jones *et al*. 2004; Mishra *et al*. 2006) and TLR3 (McKimmie *et al*. 2005; Ma *et al*. 2006; Cameron *et al*. 2007). In the latter study, unlike glial cells, neuroblastoma cells could not synthesize IFN-α or pro-inflammatory cytokines upon stimulation. Alternatively, or in addition, perhaps activation of neurons could augment the neuroprotective functions via TLR-associated signaling (Ma *et al*. 2006; Cameron *et al*. 2007; Gosselin and Rivest, 2008; Hanisch *et al*. 2008). Indeed, other studies have correlated TLR signaling with adult neurogenesis (Ziv *et al*. 2006; Rolls *et al*. 2007). Thus, the innate immune system and particularly the TLR family of receptors, functions in initiation of immune reactions and contributes to the deleterious sequelae observed in chronic murine NCC. The neuroprotective role possibly played by the TLRs is yet to be determined.

IMMUNE RESPONSES IN THE MURINE MODEL OF NCC: CORRELATION WITH *TAENIA SOLIUM* INFECTIONS

We have shown that *M. corti* metacestodes permanently shed tegument molecules upon brain invasion. These molecules bind to the lectin IsoB4 and metacestodes that have fully invaded the brain tissue no longer express GCs able to bind this lectin. Moreover, these molecules are rapidly taken up by phagocytic cells (Alvarez *et al*. 2008). Thus, the initial immune response against the parasite is likely directed to molecules no longer attached to the organism providing an important early immune invasion mechanism. Unlike many other helminth infections (Wang *et al*. 2008), the initial immune response in murine NCC is of the Th1 type but does not resolve the infection, possibly due the above evasion strategy. However, metacestodes removed from intraperitoneally (i.p.) infected animals display the characteristic thicker tegument that continues to bind the IsoB4 lectin. In a study in which mice infected i.p. by the metacestode stage of the related parasite *T. crassiceps*, a Th1-type cytokine response is elicited with NO production resulting in clearance/inhibition of parasite growth and immunity to the infection (Alonso-Trujillo *et al*. 2007). This was further supported by studies in STAT6$^{-/-}$ mice, which produce high levels of IgG2a, IFN-γ, TNF-α and inducible nitric oxide synthase, resulting in a more efficient control of the infection (Rodriguez-Sosa *et al*. 2002). These studies provide evidence for the role of Th1 type responses in controlling parasite growth to this cestode in peritoneal infections where tissue invasion is not predominant. In murine NCC low doses of *M. corti* may be able promote an initial Th1 response with the potential to eliminate very few microorganisms.

The viable *M. corti* metacestode continues to release GCs such as those binding WGA throughout the infection. This likely contributes further to the inflammatory response although their importance in immunomodulatory/evasion mechanisms is also possible. Thus, isolating these molecules is a priority. Importantly, GCs with similar lectin specificities derived from *T. solium* were also identified (Fig. 2, Alvarez *et al*. 2008). Histopathological analyses clearly demonstrated that brains from asymptomatic individuals (from autopsies) contain viable encysted parasites with little or no evidence of surrounding inflammation (Fig. 3 acute bottom panel) (unpublished findings). Similar results were found in early cysts from *T. solium* infected pigs (Fig. 3 acute middle panel) (Alvarez *et al*. 2002*a*, *b*; Londono *et al*. 2002). Moreover, we have recently demonstrated that in human patient specimens, macrophages around the metacestode display a low expression of MHC-II molecules, although it appears to be higher in cells located further away from the parasite (Alvarez *et al*. 2008). These studies further demonstrate the intrinsic capacity of the viable parasite and its associated molecules to down-regulate immune cell activity in NCC.

In a manner similar to human NCC, our unpublished data using the murine model showed down-regulation of MHC-II expression in many CD11b^{+} infiltrating myeloid cells *in vivo*. In addition, expression of co-stimulatory molecules was diminished and detected on fewer cells. Moreover, exposure to *T. solium* factors (TSF) and *M. corti* soluble factors (MCSF) resulted in down-regulation of multiple APC maturation markers. Additionally, prior exposure of monocytes to TSF or MCSF inhibited LPS-induced inflammatory cytokine production. These findings clearly suggest that neurocysticercal antigens can directly down-regulate APC maturation and activation, which, in turn, can diminish anti-parasitic immune responses and facilitate the establishment of a chronic infection. The biochemical nature of complex carbohydrates, as those present in *M. corti* and *T. solium*, contributes to their inability to be digested effectively by the lysosomal enzymes of the phagolysosome (Leyva-Cobian *et al*. 1997). Moreover, intracellular accumulation of indigestible glycoantigens could interfere with the intracellular pathways involved with presentation of antigens by histocompatibility molecules thus inhibiting T cell responses (Fruth *et al*. 1993; Leyva-Cobian *et al*. 1997). Thus, studying the potential role of specific parasite GCs in APC inhibition will be critical.

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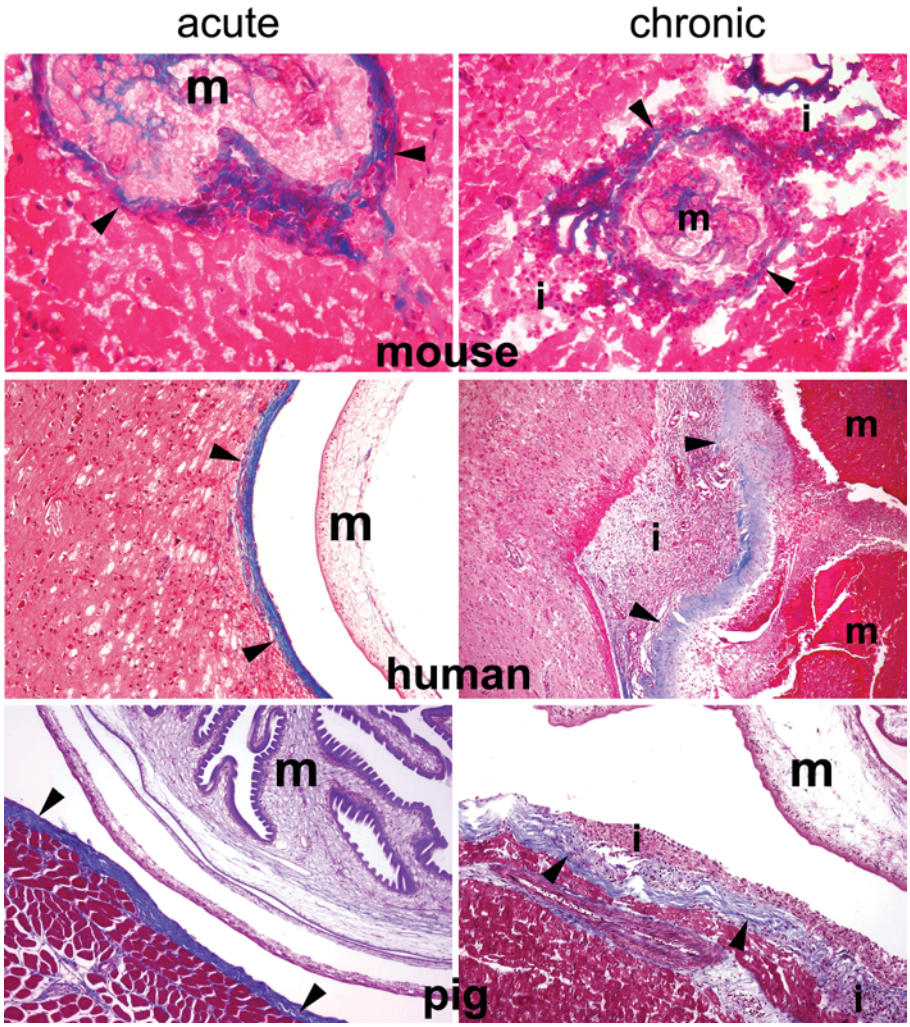

Fig. 3. Granulomatous response in murine, human and porcine NCC. Trichromic stain (labels collagen type IV in blue, background is red) was done in mice, human and pigs (naturally infected) displaying acute and chronic granulomas against *M. corti* and *T. solium* metacestodes. In the murine model of NCC, the granulomatous response is not evident in the first 2 months of infection when 50 metacestodes are injected in the CNS. The sections shown correspond to mice injected with low doses of metacestodes (5 to 10) and infected for 6 to 12 months. The left panels show collagen deposition (arrowheads) in the periphery of metacestodes (m) in the 3 hosts and they are termed acute because of the minimal infiltration of leukocytes between the m and the fibrotic capsule (arrowhead) and between the later and the nervous tissue. For murine NCC the infection period was 7 months, the human specimen is an autopsy finding from a patient dying from NCC unrelated conditions and the porcine specimen is a stage I parasite. The right panels display chronic specimens characterized by an increase in fibrosis (arrowheads), and immune cell infiltrates (i) located between the parasite, the fibrotic capsule (arrowhead) and the nervous tissue. The infection period for murine NCC was 12 months, the human specimen was surgically removed as treatment and the porcine sample is a stage 3 parasite.

In addition, other parasitic molecules from *T. solium* have been characterized for their ability to modulate the host's immune and inflammatory responses directly (Sciutto *et al*. 2007). For example, a highly antigenic parasitic molecule Antigen B (Plancarte *et al*. 1983) was demonstrated to bind complement such as factor C1q and to inhibit the classical pathway of complement activation (Laclette *et al*. 1991). Some other important molecules include secreted proteases of *T. solium* can degrade cytokines, immunoglobulins and even T cells (Molinari *et al*. 2000; Baig *et al*. 2005), while parasitic proteinase inhibitors like taeniaestatin hinder activation of the complement pathway, lymphocytes as well as cytokine production (White *et al*. 1997).

Virtually all cases of symptomatic NCC are characterized by prominent immunological responses in host nervous tissue (Correa *et al*. 1985; Grewal *et al*. 2000), with an immune response in the CNS consisting of an overt Th1 phenotype (Restrepo *et al*. 1998), or a mixed Th1, Th2, and Th3 phenotype depending upon the absence or presence of granuloma formation and the stage of the granulomatous response (Restrepo *et al*. 2001*b*). Similar results have been found with *M. corti* granulomas

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(unpublished findings), liver granulomas from *T. crassiceps* (White *et al.* 1997) and *T. crassiceps* granulomas induced subcutaneously and then injected in mouse brain (Stringer *et al.* 2003; Patil *et al.* 2006). It is likely that early granulomas with little evidence of inflammatory infiltrates (Fig. 3, acute) are somewhat protective depending upon their number and location. As the organism dies, and the granuloma dissipates (Fig. 3, chronic), a Th1 inflammatory response prevails and causes the symptomatic phase associated with severe neuropathology and even mortality (White, 1997; White *et al.* 1997). Importantly, these distinct stages were found in humans and pigs infected with *T. solium* as well as *M. corti* infected mice that survived for 6–12 months (Fig. 3) and exhibited very few infectious organisms compared to mice that survive for shorter periods of time.

In the murine model of NCC, infection of the CNS by *M. corti* is characterized by an initial accumulation of parasites and inflammatory cells in extraparenchymal areas of the brain including meninges and subarachnoid spaces starting at 1–3 days after infection (Cardona *et al.* 1999). Interestingly, in the first week or two the animals show no overt signs of illness suggesting some degree of inflammatory cells and cytokine production in the CNS can be tolerated. As the infection progresses, parasites and immune cells penetrate/sequester into the brain parenchyma and the degree of inflammation and leukocyte infiltration in the CNS increases and correlates with clinical symptoms (Cardona *et al.* 1999). The cytokines produced by infiltrating cells in the CNS during *M. corti* infection (IL-2, IL-12, IL-15, IFN-γ, and TNF-α) are indicative of a cell mediated, Th1 type of response (Cardona *et al.* 1999). The production of anti-inflammatory cytokines such as IL-4 (Th2 type) and IL-10 (regulatory type) were detected at low levels (Cardona *et al.* 1999). Interestingly, this Th1 type of response in the mouse model is largely mediated by $\gamma\delta$ T cells that infiltrated early during the infection and persist throughout the course of murine NCC (Cardona *et al.* 1999). Moreover, mice that lack $\gamma\delta$ T cells show reduced CNS inflammation by way of decreased expression of the Th1 cytokine responses, lower leukocyte trafficking and increased survival time (Cardona and Teale, 2002). Collectively these findings indicate that the inflammatory responses elicited by $\gamma\delta$ T cells play a prominent role in the development of neuropathology and disease severity. Interestingly, similar studies in pigs infected with *T. solium* also exhibited infiltration of T cells that lacked CD4 and CD8 and were presumed to be $\gamma\delta$ T cells (Restrepo *et al.* 2001*b*). As glycolipids make up part of the GCs identified in *M. corti*, it is likely that these released/secreted molecules are involved in the recruitment of this cell type (unpublished findings).

ADDITIONAL INSIGHTS FROM IMMUNE RESPONSES IN MURINE NCC

We have recently found that i.c. infection of mice with *M. corti* also results in infiltration of macrophages bearing markers associated with alternatively activated macrophages (aaM), namely Fizz1 'found in inflammatory zone-1' antigen and Arginase-1. This is interesting in that aaMs are generally thought to be induced by the IL-4/IL-13 Th2 axis (Munder *et al.* 1998; Pauleau *et al.* 2004) cytokines that are detected in low amounts in our model. *T. crassiceps* has also been shown to induce aaMs (Reyes and Terrazas, 2007). However, it is now known that glycans are among the several apparent stimuli required for the development of aaMs (Atochina *et al.* 2008; Martinez *et al.* 2009). Moreover, it has recently been shown that aaMs expressing Fizz1 and Arg1 actually suppress Th2 responses (Pesce *et al.* 2009*a*, *b*). The abundant release/secretion of GCs observed with cestodes like *Mesocestoides* and *Taenia* may well be responsible for the induction of aaMs and thus, suppress Th2 responses in these infections. The exact role of aaMs in murine NCC remains to be determined but presumably involves regulation of the Th1/Th2 balance to control the parasite but minimize damage to the CNS.

CONCLUDING REMARKS

A complex regulation of both the innate and adaptive immune responses during parasite infection results in either asymptomatic or symptomatic NCC. These polarized stages during NCC correlate with either an absence or presence of inflammatory response, respectively. The immune evasion mechanisms associated with the asymptomatic stage of the infection in NCC are not well understood. Murine NCC provides an important model for understanding the underlying mechanisms involved in different stages of NCC. We have determined that the initial response to *M. corti* in the brain is a Th1 type of response with infiltration of macrophages, neutrophils and $\gamma\delta$ T cells expressing proinflammatory cytokines followed by the infiltration of $\alpha\beta$ T cells and B cells. Using this model, we determined the kinetics of BBB disruption at distinct anatomical sites and mechanisms involved in this process. Moreover, the mechanisms regulating the inflammatory responses which ultimately underlie these changes are now being characterized which include alterations in the activity of a variety of innate immune cell receptors involving TLRs and their downstream signaling pathways. Importantly, we have discovered the release of distinct GCs in a stage-specific manner during NCC and are in the process of isolating these molecules. They will be used to define the pro-inflammatory responses

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contributing to symptomatic NCC and also anti-inflammatory/suppressor pathways responsible for immune evasion strategies as well as immune responses that minimize neuropathology. Such studies should aid in the development to new therapeutic strategies.

REFERENCES

Abbott, N. J. (2005). Dynamics of CNS barriers: evolution, differentiation, and modulation. *Cellular and Molecular Neurobiology* **25**, 5–23.

Akira, S., Uematsu, S. and Takeuchi, O. (2006). Pathogen recognition and innate immunity. *Cell* **124**, 783–801.

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

Alvarez, J. I., Colegial, C. H., Castano, C. A., Trujillo, J., Teale, J. M. and Restrepo, B. I. (2002*a*). The human nervous tissue in proximity to granulomatous lesions induced by *Taenia solium* metacestodes displays an active response. *Journal of Neuroimmunology* **127**, 139–144.

Alvarez, J. I., Londono, D. P., Alvarez, A. L., Trujillo, J., Jaramillo, M. M. and Restrepo, B. I. (2002*b*). Granuloma formation and parasite disintegration in porcine cysticercosis: comparison with human neurocysticercosis. *Journal of Comparative Pathology* **127**, 186–193.

Alvarez, J. I., Rivera, J. and Teale, J. M. (2008). Differential release and phagocytosis of tegument glycoconjugates in neurocysticercosis: implications for immune evasion strategies. *PLoS Neglected Tropical Diseases* **2**, e218.

Alvarez, J. I. and Teale, J. M. (2006). Breakdown of the blood brain barrier and blood-cerebrospinal fluid barrier is associated with differential leukocyte migration in distinct compartments of the CNS during the course of murine NCC. *Journal of Neuroimmunology* **173**, 45–55.

Alvarez, J. I. and Teale, J. M. (2007*a*). Differential changes in junctional complex proteins suggest the ependymal lining as the main source of leukocyte infiltration into ventricles in murine neurocysticercosis. *Journal of Neuroimmunology* **187**, 102–113.

Alvarez, J. I. and Teale, J. M. (2007*b*). Evidence for differential changes of junctional complex proteins in murine neurocysticercosis dependent upon CNS vasculature. *Brain Research* **1169**, 98–111.

Alvarez, J. I. and Teale, J. M. (2008). Multiple expression of matrix metalloproteinases in murine neurocysticercosis: Implications for leukocyte migration through multiple central nervous system barriers. *Brain Research* **1214**, 145–158.

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

Baig, S., Damian, R. T., Molinari, J. L., Tato, P., Morales-Montor, J., Welch, M., Talhouk, J., Hashmeys, R. and White, A. C., Jr. (2005). Purification and characterization of a metacestode cysteine proteinase from *Taenia solium* involved in the breakdown of human IgG. *Parasitology* **131**, 411–416.

Bandres, J. C., White, A. C., Jr., Samo, T., Murphy, E. C. and Harris, R. L. (1992). Extraparenchymal neurocysticercosis: report of five cases and review of management. *Clinical Infectious Diseases* **15**, 799–811.

Barrinagarrementeria, F. C. C. (2002). *Cerebrovascular Manifestations of Neurocysticercosis*, CABI Publishing, Oxfordshire.

Bazzoni, G. and Dejana, E. (2004). Endothelial cell-to-cell junctions: molecular organization and role in vascular homeostasis. *Physiological Reviews* **84**, 869–901.

Beutler, B. (2004). Innate immunity: an overview. *Molecular Immunology* **40**, 845–859.

Brightman, M. W. and Kaya, M. (2000). Permeable endothelium and the interstitial space of brain. *Cellular and Molecular Neurobiology* **20**, 111–130.

Brown, H., Hien, T. T., Day, N., Mai, N. T., Chuong, L. V., Chau, T. T., Loc, P. P., Phu, N. H., Bethell, D., Farrar, J., Gatter, K., White, N. and Turner, G. (1999). Evidence of blood-brain barrier dysfunction in human cerebral malaria. *Neuropathology and Applied Neurobiology* **25**, 331–340.

Brown, R. C. and Davis, T. P. (2002). Calcium modulation of adherens and tight junction function: a potential mechanism for blood-brain barrier disruption after stroke. *Stroke* **33**, 1706–1711.

Cameron, J. S., Alexopoulou, L., Sloane, J. A., Dibernardo, A. B., Ma, Y., Kosaras, B., Flavell, R., Strittmatter, S. M., Volpe, J., Sidman, R. and Vartanian, T. (2007). Toll-like receptor 3 is a potent negative regulator of axonal growth in mammals. *Journal of Neuroscience* **27**, 13033–13041.

Cardona, A. E., Restrepo, B. I., Jaramillo, J. M. and Teale, J. M. (1999). Development of an animal model for neurocysticercosis: immune response in the central nervous system is characterized by a predominance of gamma delta T cells. *Journal of Immunology* **162**, 995–1002.

Cardona, A. E. and Teale, J. M. (2002). Gamma/delta T cell-deficient mice exhibit reduced disease severity and decreased inflammatory response in the brain in murine neurocysticercosis. *Journal of Immunology* **169**, 3163–3171.

Chavarria, A. and Alcocer-Varela, J. (2004). Is damage in central nervous system due to inflammation? *Autoimmunity Reviews* **3**, 251–260.

Correa, D., Dalma, D., Espinoza, B., Plancarte, A., Rabiela, M. T., Madrazo, I., Gorodezky, C. and Flisser, A. (1985). Heterogeneity of humoral immune components in human cysticercosis. *Journal of Parasitology* **71**, 535–541.

Del Brutto, O. H. (1992). Cysticercosis and cerebrovascular disease: a review. *Journal of Neurology, Neurosurgery and Psychiatry* **55**, 252–254.

Del Brutto, O. H. (1997). Neurocysticercosis. *Current Opinion in Neurobiology* **10**, 268–272.

Didierlaurent, A., Ferrero, I., Otten, L. A., Dubois, B., Reinhardt, M., Carlsen, H., Blomhoff, R., Akira, S., Kraehenbuhl, J. P. and Sirard, J. C. (2004). Flagellin promotes myeloid differentiation factor 88-dependent

development of Th2-type response. *Journal of Immunology* **172**, 6922–6930.

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

Escobar, A. (1983). *The Pathology of Neurocysticercosis*. Charles C Thomas, Springfield, Illinois, USA.

Flisser, A., Espinoza, B., Tovar, A., Plancarte, A. and Correa, D. (1986). Host–parasite relationship in cysticercosis: immunologic study in different compartments of the host. *Veterinary Parasitology* **20**, 95–102.

Fritz, J. H., Ferrero, R. L., Philpott, D. J. and Girardin, S. E. (2006). Nod-like proteins in immunity, inflammation and disease. *Nature Immunology* **7**, 1250–1257.

Fruth, U., Solioz, N. and Louis, J. A. (1993). *Leishmania major* interferes with antigen presentation by infected macrophages. *Journal of Immunology* **150**, 1857–1864.

Garcia, H. H. and Del Brutto, O. H. (2005). Neurocysticercosis: updated concepts about an old disease. *Lancet Neurology* **4**, 653–661.

Garcia, H. H., Pretell, E. J., Gilman, R. H., Martinez, S. M., Moulton, L. H., Del Brutto, O. H., Herrera, G., Evans, C. A. and Gonzalez, A. E. (2004). A trial of antiparasitic treatment to reduce the rate of seizures due to cerebral cysticercosis. *New England Journal of Medicine* **350**, 249–258.

Gloor, S. M., Wachtel, M., Bolliger, M. F., Ishihara, H., Landmann, R. and Frei, K. (2001). Molecular and cellular permeability control at the blood-brain barrier. *Brain Research Reviews* **36**, 258–264.

Gosselin, D. and Rivest, S. (2008). MyD88 signaling in brain endothelial cells is essential for the neuronal activity and glucocorticoid release during systemic inflammation. *Molecular Psychiatry* **13**, 480–497.

Grewal, J. S., Kaur, S., Bhatti, G., Sawhney, I. M., Ganguly, N. K., Mahajan, R. C. and Malla, N. (2000). Cellular immune responses in human neurocysticercosis. *Parasitology Research* **86**, 500–503.

Hanisch, U. K., Johnson, T. V. and Kipnis, J. (2008). Toll-like receptors: roles in neuroprotection? *Trends in Neurosciences* **31**, 176–182.

Harhaj, N. S. and Antonetti, D. A. (2004). Regulation of tight junctions and loss of barrier function in pathophysiology. *International Journal of Biochemistry and Cell Biology* **36**, 1206–1237.

Hernandez-Mendoza, L., Molinari, J. L., Garrido, E., Cortes, I., Solano, S., Miranda, E. and Tato, P. (2005). The implantation of *Taenia solium* metacestodes in mice induces down-modulation of T-cell proliferation and cytokine production. *Parasitology Research* **95**, 256–265.

Hess, E. (1980). Ultrastructural study of the tetrathyridium of *Mesocestoides corti* Hoeppli, 1925: tegument and parenchyma. *Zeitschrift für Parasitenkunde* **61**, 135–159.

Hoebe, K., Janssen, E. and Beutler, B. (2004). The interface between innate and adaptive immunity. *Nature Immunology* **5**, 971–974.

Iwasaki, A. and Medzhitov, R. (2004). Toll-like receptor control of the adaptive immune responses. *Nature Immunology* **5**, 987–995.

Kniesel, U. and Wolburg, H. (2000). Tight junctions of the blood-brain barrier. *Cellular and Molecular Neurobiology* **20**, 57–76.

Kumar, H., Kawai, T. and Akira, S. (2009). Pathogen recognition in the innate immune response. *Biochemical Journal* **420**, 1–16.

Kurt-Jones, E. A., Chan, M., Zhou, S., Wang, J., Reed, G., Bronson, R., Arnold, M. M., Knipe, D. M. and Finberg, R. W. (2004). Herpes simplex virus 1 interaction with Toll-like receptor 2 contributes to lethal encephalitis. *Proceedings of the National Academy of Sciences, USA* **101**, 1315–1320.

Laclette, J. P., Landa, A., Arcos, L., Willms, K., Davis, A. E. and Shoemaker, C. B. (1991). Paramyosin is the *Schistosoma mansoni* (Trematoda) homologue of antigen B from *Taenia solium* (Cestoda). *Molecular and Biochemical Parasitology* **44**, 287–295.

Lan, K. P., Wang, C. J., Hsu, J. D., Chen, K. M., Lai, S. C. and Lee, H. H. (2004). Induced eosinophilia and proliferation in *Angiostrongylus cantonensis*-infected mouse brain are associated with the induction of JAK/STAT1, IAP/NF-kappaB and MEKK1/JNK signals. *Journal of Helminthology* **78**, 311–317.

Leyva-Cobian, F., Outschoorn, I. M., Carrasco-Marin, E. and Alvarez-Dominguez, C. (1997). The consequences of the intracellular retention of pathogen-derived T-cell-independent antigens on protein presentation to T cells. *Clinical Immunology and Immunopathology* **85**, 1–15.

Londono, D. P., Alvarez, J. I., Trujillo, J., Jaramillo, M. M. and Restrepo, B. I. (2002). The inflammatory cell infiltrates in porcine cysticercosis: immunohistochemical analysis during various stages of infection. *Veterinary Parasitology* **109**, 249–259.

Lopez-Marin, L. M., Montrozier, H., Lemassu, A., Garcia, E., Segura, E. and Daffe, M. (2002). Structure and antigenicity of the major glycolipid from *Taenia solium* cysticerci. *Molecular and Biochemical Parasitology* **119**, 33–42.

Ma, Y., Li, J., Chiu, I., Wang, Y., Sloane, J. A., Lu, J., Kosaras, B., Sidman, R. L., Volpe, J. J. and Vartanian, T. (2006). Toll-like receptor 8 functions as a negative regulator of neurite outgrowth and inducer of neuronal apoptosis. *Journal of Cell Biology* **175**, 209–215.

MacGregor, A. N., Kusel, J. R. and Wilson, R. A. (1988). Isolation and characterisation of discoid granules from the tegument of adult *Schistosoma mansoni*. *Parasitology Research* **74**, 250–254.

Maizels, R. M., Bundy, D. A., Selkirk, M. E., Smith, D. F. and Anderson, R. M. (1993). Immunological modulation and evasion by helminth parasites in human populations. *Nature* **365**, 797–805.

Martinez, F. O., Helming, L. and Gordon, S. (2009). Alternative activation of macrophages: an immunologic functional perspective. *Annual Reviews of Immunology* **27**, 451–483.

Martinon, F., Mayor, A. and Tschopp, J. (2009). The inflammasomes: guardians of the body. *Annual Reviews of Immunology* **27**, 229–265.

McDermott, E. P. and O'Neill, L. A. (2002). Ras participates in the activation of p38 MAPK by interleukin-1 by associating with IRAK, IRAK2, TRAF6, and TAK-1. *Journal of Biological Chemistry* **277**, 7808–7815.

McKimmie, C. S., Johnson, N., Fooks, A. R. and Fazakerley, J. K. (2005). Viruses selectively upregulate Toll-like receptors in the central nervous system. *Biochemical and Biophysical Research Communications* **336**, 925–933.

Medzhitov, R. (2009). Approaching the asymptote: 20 years later. *Immunity* **30**, 766–775.

Minagar, A. and Alexander, J. S. (2003). Blood-brain barrier disruption in multiple sclerosis. *Multiple Sclerosis* **9**, 540–549.

Mishra, B. B., Gundra, U. M. and Teale, J. M. (2008). Expression and distribution of Toll-like receptors 11–13 in the brain during murine neurocysticercosis. *Journal of Neuroinflammation* **5**, 53.

Mishra, B. B., Gundra, U. M., Wong, K. and Teale, J. M. (2009). MyD88-deficient mice exhibit decreased parasite induced immune responses but reduced disease severity in a murine model of neurocysticercosis. *Infection and Immunity* **77**, 5369–5379.

Mishra, B. B., Mishra, P. K. and Teale, J. M. (2006). Expression and distribution of Toll-like receptors in the brain during murine neurocysticercosis. *Journal of Neuroimmunology* **181**, 46–56.

Mitchell, J. A., Paul-Clark, M. J., Clarke, G. W., McMaster, S. K. and Cartwright, N. (2007). Critical role of toll-like receptors and nucleotide oligomerisation domain in the regulation of health and disease. *Journal of Endocrinology* **193**, 323–330.

Mogensen, T. H. (2009). Pathogen recognition and inflammatory signaling in innate immune defenses. *Clinical Microbiology Reviews* **22**, 240–273.

Molinari, J. L., Mejia, H., White, A. C., Jr., Garrido, E., Borgonio, V. M., Baig, S. and Tato, P. (2000). *Taenia solium*: a cysteine protease secreted by metacestodes depletes human CD4 lymphocytes *in vitro*. *Experimental Parasitology* **94**, 133–142.

Munder, M., Eichmann, K. and Modolell, M. (1998). 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. *Journal of Immunology* **160**, 5347–5354.

Nakaya, K., Mamuti, W., Xiao, N., Sato, M. O., Wandra, T., Nakao, M., Sako, Y., Yamasaki, H., Ishikawa, Y., Craig, P. S., Schantz, P. M. and Ito, A. (2006). Usefulness of severe combined immunodeficiency (scid) and inbred mice for studies of cysticercosis and echinococcosis. *Parasitology International* **55** (Suppl.), S91–S97.

Nash, T. E., Singh, G., White, A. C., Rajshekhar, V., Loeb, J. A., Proano, J. V., Takayanagui, O. M., Gonzalez, A. E., Butman, J. A., Degiorgio, C., Del Brutto, O. H., Delgado-Escueta, A., Evans, C. A., Gilman, R. H., Martinez, S. M., Medina, M. T., Pretell, E. J., Teale, J. and Garcia, H. H. (2006). Treatment of neurocysticercosis: current status and future research needs. *Neurology* **67**, 1120–1127.

Nguyen, M. D., Julien, J. P. and Rivest, S. (2002). Innate immunity: the missing link in neuroprotection and neurodegeneration? *Nature Reviews Neuroscience* **3**, 216–227.

Obregon-Henao, A., Gil, D. L., Gomez, D. I., Sanzon, F., Teale, J. M. and Restrepo, B. I. (2001). The role of N-linked carbohydrates in the antigenicity of *Taenia solium* metacestode glycoproteins of 12, 16 and 18 kD. *Molecular and Biochemical Parasitology* **114**, 209–215.

Obregon-Henao, A., Londono, D. P., Gomez, D. I., Trujillo, J., Teale, J. M. and Restrepo, B. I. (2003). *In situ* detection of antigenic glycoproteins in *Taenia solium* metacestodes. *Journal of Parasitology* **89**, 726–732.

Parmar, H., Shah, J., Patwardhan, V., Patankar, T., Patkar, D., Muzumdar, D., Prasad, S. and Castillo, M. (2001). MR imaging in intramedullary cysticercosis. *Neuroradiology* **43**, 961–967.

Pasare, C. and Medzhitov, R. (2005). Toll-like receptors: linking innate and adaptive immunity. *Advances in Experimental Medicine and Biology* **560**, 11–18.

Patil, S., Robinson, P., Actor, J. K., Baig, S. and White, A. C., Jr. (2006). Proinflammatory cytokines in granulomas associated with murine cysticercosis are not the cause of seizures. *Journal of Parasitology* **92**, 738–741.

Pauleau, A. L., Rutschman, R., Lang, R., Pernis, A., Watowich, S. S. and Murray, P. J. (2004). Enhancer-mediated control of macrophage-specific arginase I expression. *Journal of Immunology* **172**, 7565–7573.

Pesce, J. T., Ramalingam, T. R., Mentink-Kane, M. M., Wilson, M. S., El Kasmi, K. C., Smith, A. M., Thompson, R. W., Cheever, A. W., Murray, P. J. and Wynn, T. A. (2009*a*). Arginase-1-expressing macrophages suppress Th2 cytokine-driven inflammation and fibrosis. *PLoS Pathogens* **5**, e1000371.

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

Petty, M. A. and Lo, E. H. (2002). Junctional complexes of the blood-brain barrier: permeability changes in neuroinflammation. *Progress in Neurobiology* **68**, 311–323.

Piggott, D. A., Eisenbarth, S. C., Xu, L., Constant, S. L., Huleatt, J. W., Herrick, C. A. and Bottomly, K. (2005). MyD88-dependent induction of allergic Th2 responses to intranasal antigen. *Journal of Clinical Investigation* **115**, 459–467.

Plancarte, A., Flisser, A. and Larralde, C. (1983). Fibronectin-like properties in antigen B from the cysticercus of *Taenia solium*. *Cytobios* **36**, 83–93.

Rakoff-Nahoum, S. and Medzhitov, R. (2009). Toll-like receptors and cancer. *Nature Reviews Cancer* **9**, 57–63.

Ransohoff, R. M., Kivisakk, P. and Kidd, G. (2003). Three or more routes for leukocyte migration into the central nervous system. *Nature Reviews Immunology* **3**, 569–581.

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Redecke, V., Hacker, H., Datta, S. K., Fermin, A., Pitha, P. M., Broide, D. H. and Raz, E. (2004). Cutting edge: activation of Toll-like receptor 2 induces a Th2 immune response and promotes experimental asthma. *Journal of Immunology* **172**, 2739–2743.

Restrepo, B. I., Aguilar, M. I., Melby, P. C. and Teale, J. M. (2001*a*). Analysis of the peripheral immune response in patients with neurocysticercosis: evidence for T cell reactivity to parasite glycoprotein and vesicular fluid antigens. *American Journal of Tropical Medicine and Hygiene* **65**, 366–370.

Restrepo, B. I., Alvarez, J. I., Castano, J. A., Arias, L. F., Restrepo, M., Trujillo, J., Colegial, C. H. and Teale, J. M. (2001*b*). Brain granulomas in neurocysticercosis patients are associated with a Th1 and Th2 profile. *Infection and Immunity* **69**, 4554–4560.

Restrepo, B. I., Llaguno, P., Sandoval, M. A., Enciso, J. A. and Teale, J. M. (1998). Analysis of immune lesions in neurocysticercosis patients: central nervous system response to helminth appears Th1-like instead of Th2. *Journal of Neuroimmunology* **89**, 64–72.

Restrepo, B. I., Obregon-Henao, A., Mesa, M., Gil, D. L., Ortiz, B. L., Mejia, J. S., Villota, G. E., Sanzon, F. and Teale, J. M. (2000). Characterisation of the carbohydrate components of *Taenia solium* metacestode glycoprotein antigens. *International Journal of Parasitology* **30**, 689–696.

Reyes, J. L. and Terrazas, L. I. (2007). The divergent roles of alternatively activated macrophages in helminthic infections. *Parasite Immunology* **29**, 609–619.

Rock, F. L., Hardiman, G., Timans, J. C., Kastelein, R. A. and Bazan, J. F. (1998). A family of human receptors structurally related to *Drosophila* Toll. *Proceedings of the National Academy of Sciences, USA* **95**, 588–593.

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

Rolls, A., Shechter, R., London, A., Ziv, Y., Ronen, A., Levy, R. and Schwartz, M. (2007). Toll-like receptors modulate adult hippocampal neurogenesis. *Nature Cell Biology* **9**, 1081–1088.

Ropert, C., Franklin, B. S. and Gazzinelli, R. T. (2008). Role of TLRs/MyD88 in host resistance and pathogenesis during protozoan infection: lessons from malaria. *Seminars in Immunopathology* **30**, 41–51.

Schnare, M., Rollinghoff, M. and Qureshi, S. (2006). Toll-like receptors: sentinels of host defence against bacterial infection. *International Archives of Allergy and Immunology* **139**, 75–85.

Schulze, C. and Firth, J. A. (1993). Immunohistochemical localization of adherens junction components in blood-brain barrier microvessels of the rat. *Journal of Cell Science* **104**, 773–782.

Sciutto, E., Chavarria, A., Fragoso, G., Fleury, A. and Larralde, C. (2007). The immune response in *Taenia solium* cysticercosis: protection and injury. *Parasite Immunology* **29**, 621–636.

Stringer, J. L., Marks, L. M., White, A. C., Jr. and Robinson, P. (2003). Epileptogenic activity of granulomas associated with murine cysticercosis. *Experimental Neurology* **183**, 532–536.

Takeda, K. (2005). Evolution and integration of innate immune recognition systems: the Toll-like receptors. *Journal of Endotoxin Research* **11**, 51–55.

Takeuchi, O. and Akira, S. (2009). Innate immunity to virus infection. *Immunological Reviews* **227**, 75–86.

Tsang, V. C., Brand, J. A. and Boyer, A. E. (1989). An enzyme-linked immunoelectrotransfer blot assay and glycoprotein antigens for diagnosing human cysticercosis (*Taenia solium*). *Journal of Infectious Diseases* **159**, 50–59.

Ulevitch, R. J., Mathison, J. C. and Da Silva Correia, J. (2004). Innate immune responses during infection. *Vaccine* **22** (Suppl. 1), S25–S30.

Von Bernuth, H., Picard, C., Jin, Z., Pankla, R., Xiao, H., Ku, C. L., Chrabieh, M., Mustapha, I. B., Ghandil, P., Camcioglu, Y., Vasconcelos, J., Sirvent, N., Guedes, M., Vitor, A. B., Herrero-Mata, M. J., Arostegui, J. I., Rodrigo, C., Alsina, L., Ruiz-Ortiz, E., Juan, M., Fortuny, C., Yague, J., Anton, J., Pascal, M., Chang, H. H., Janniere, L., Rose, Y., Garty, B. Z., Chapel, H., Issekutz, A., Marodi, L., Rodriguez-Gallego, C., Banchereau, J., Abel, L., Li, X., Chaussabel, D., Puel, A. and Casanova, J. L. (2008). Pyogenic bacterial infections in humans with MyD88 deficiency. *Science* **321**, 691–696.

Vorbrodt, A. W. and Dobrogowska, D. H. (2003). Molecular anatomy of intercellular junctions in brain endothelial and epithelial barriers: electron microscopist's view. *Brain Research Reviews* **42**, 221–242.

Wang, L. J., Cao, Y. and Shi, H. N. (2008). Helminth infections and intestinal inflammation. *World Journal of Gastroenterology* **14**, 5125–5132.

West, A. P., Koblansky, A. A. and Ghosh, S. (2006). Recognition and signaling by toll-like receptors. *Annual Review of Cell and Development Biology* **22**, 409–437.

White, A. C. (1997). Neurocysticercosis: a major cause of neurological disease worldwide. *Clinical Infectious Diseases* **24**, 101–113.

White, A. C., Jr. (2000). Neurocysticercosis: updates on epidemiology, pathogenesis, diagnosis, and management. *Annual Review of Medicine* **51**, 187–206.

White, A. C., Jr., Robinson, P. and Kuhn, R. (1997). *Taenia solium* cysticercosis: host-parasite interactions and the immune response. *Chemical Immunology* **66**, 209–230.

Wolburg, H. and Lippoldt, A. (2002). Tight junctions of the blood-brain barrier: development, composition and regulation. *Vascular Pharmacology* **38**, 323–337.

Ziv, Y., Ron, N., Butovsky, O., Landa, G., Sudai, E., Greenberg, N., Cohen, H., Kipnis, J. and Schwartz, M. (2006). Immune cells contribute to the maintenance of neurogenesis and spatial learning abilities in adulthood. *Nature Neuroscience* **9**, 268–275.