Increased Accumulation of Regulatory Granulocytic Myeloid Cells in Mannose Receptor C Type 1-Deficient Mice Correlates with Protection in a Mouse Model of Neurocysticercosis

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Neurocysticercosis (NCC) is a central nervous system (CNS) infection caused by the metacestode stage of the parasite *Taenia solium*. During NCC, the parasites release immunodominant glycan antigens in the CNS environment, invoking immune responses. The majority of the associated pathogenesis is attributed to the immune response against the parasites. Glycans from a number of pathogens, including helminths, act as pathogen-associated molecular pattern molecules (PAMPs), which are recognized by pattern recognition receptors (PRRs) known as C-type lectin receptors (CLRs). Using a mouse model of NCC, we have investigated the role of mannose receptor C type 1 (MRC1), a CLR which recognizes high-mannose-containing glycan antigens. Here we show that MRC1−/− mice exhibit increased survival times after infection compared with their wild-type (WT) counterparts. The decreased disease severity correlates with reduced levels of expression of markers implicated in NCC pathology, such as interleukin-1β (IL-1β), IL-6, CCL5, and matrix metalloproteinase 9 (MMP9), in addition to induction of an important repair marker, fibroblast growth factor 2 (FGF2). Furthermore, the immune cell subsets that infiltrate the brain of MRC1−/− mice are dramatically altered and characterized by reduced numbers of T cells and the accumulation of granulocytic cells with an immune phenotype resembling granulocytic myeloid-dependent suppressor cells (gMDSCs). The results suggest that MRC1 plays a critical role in myeloid plasticity, which in turn affects the adaptive immune response and immunopathogenesis during murine NCC.

During NCC, the parasites release and display non-host-like immunodominant N-glycan antigens in the CNS environment. We have shown that glycan antigens released from the parasites are taken up by host cells in the CNS environment during both human and murine NCC (9). A growing body of evidence indicates that glycans from a number of pathogens, including helminths, act as pathogen-associated molecular pattern molecules (PAMPs), which are recognized by C-type lectin receptors (CLRs) through their carbohydrate recognition domains (CRDs) and function as important pattern recognition receptors (PRRs) (16, 17). Emerging evidence suggests a profound role of CLRs in a variety of biological functions, including first-line defense against pathogens, inflammation, phagocytosis, immune suppression, and tolerance (16, 18). Our data show that several CLRs, including mannose receptor C type 1 (MRC1), are induced during NCC in CNS cells as well as infiltrating cells (19, 20). MRC1 is an endocytic receptor which has been shown to play a crucial role in mediating inflammatory immune responses against pathogens such as *Mycobacterium tuberculosis*, *Cryptococcus neoformans*, and *Candida albicans* (21–24). It is a 175-kDa type I membrane protein which...
contains 8 C-type lectin domains (CTLDs), 1 fibronectin type II domain, and 1 cysteine-rich (CR) domain. The CTLDs recognize sugars containing D-mannose and N-acetylgalcosamine moieties in a Ca2+-dependent manner (25), which are abundantly released by the parasites during infection (9, 11, 26).

The aim of the current study was to evaluate the role of MRC1 in the immune response against M. corti. Infected wild-type (WT) and MRC1−/− mice were evaluated and compared for disease severity, immune mediators, and potential changes in infiltration of leukocyte subsets. Our data show that MRC1 deficiency leads to a dramatic accumulation of granulocytic myeloid cells that express markers associated with downregulating immune responses. The accumulation of these cells also correlates with decreased numbers of T cells. Taken together, our data indicate that MRC1 plays a critical role in mediating inflammation that directs subsequent immune responses and contributes to the immunopathology associated with NCC.

**MATERIALS AND METHODS**

**Animals.** MRC1−/− mice (27) were provided by Chiung-Yu Hung, University of Texas at San Antonio (originally from Michel C. Nussenzweig, Rockefeller University, New York, NY), MRC1−/− mice with a C57BL/6 background were back-crossed with C57BL/6 mice for 10 generations. Homozygous mice were bred at the University of Texas at San Antonio animal facility to obtain experimental mice. Control age- and sex-matched mice with a C57BL/6 background were obtained from Charles River Laboratories. Four- to five-week-old mice were used for experimental procedures. Experiments were conducted under the guidelines of the IACUC, University of Texas System, and the U.S. Department of Agriculture and National Institutes of Health.

**Parasites and infection.** Parasite maintenance and intracranial (i.c.) infection were performed by using a protocol previously developed in our laboratory (10). M. corti metacestodes were maintained by serial intraperitoneal (i.p.) inoculation of 8- to 12-week-old female BALB/c mice. For intracranial inoculations, parasites were aseptically collected from the peritoneal cavity of mice that had been infected for about 4 to 6 months. Harvested parasites were extensively washed in Hanks balanced salt solution (HBSS). After that, the metacestodes (70 parasites) were suspended in 50 μl of HBSS and injected i.c. into 3- to 5-week-old mice by using a 1-ml syringe and a 25-gauge needle. The needle was inserted to a 2-mm depth at the junction of the superior sagittal and the transverse sutures. This allowed insertion of the needle into a protective cuff, avoiding penetration of the brain tissue. Control mice were injected with 50 μl sterile HBSS by using the same protocol. Before intracranial inoculation, mice were anesthetized intramuscularly with a 50-μl mixture of ketamine HCl and xylazine (30 mg/ml ketamine and 4 mg/ml xylazine).

**Determination of markers related to pathology.** Mock-infected and M. corti-infected WT and MRC1−/− brains were collected in 2 ml of sterile phosphate-buffered saline (PBS) containing a protease inhibitor cocktail (Roche, Indianapolis, IN). Brains were then immediately homogenized and frozen at −80°C. A minimum of 300 μl was sent to Rules Based Medicine (Austin, TX) to be analyzed by the Rodent MAP v 2.0 assay in order to assess the level of immunological mediators present in the various homogenized brains.

**Tissue processing and histological analysis.** Tissue processing and histological analysis were performed by using a previously described protocol (28). Briefly, mock-infected and M. corti-infected brains were immediately removed from perfused animals, embedded in optimal cutting temperature (OCT) resin (Sakura, Torrance, CA), and snap-frozen. Serial horizontal cryosections 10 μm in thickness were placed onto xylene prep slides (Sigma-Aldrich, St. Louis, MO). One in every five slides was fixed in formalin for 12 min at room temperature (RT) and stained with hematoxylin and eosin (H&E) to evaluate the infiltrates. H&E-stained slides were analyzed with a Leica DMR microscope (Leica Microsystems, Wetzlar, Germany). Images were acquired by using a cooled charge-coupled-device (CCD) Spot RT camera (Diagnostic Instruments, Inc., Sterling Heights, MI) and processed as well as analyzed by using Adobe Photoshop 7.0 (Adobe, Mountain View, CA). The remainder of the slides was air dried overnight and fixed in fresh acetone for 20 s at room temperature. Acetone-fixed sections were wrapped in aluminum foil and stored at −80°C or processed immediately for immunofluorescence (IF) analysis.

**Antibodies.** Biotinylated anti-mouse CD11b, biotinylated anti-mouse CD11c, and anti-mouse I-A/I-E (major histocompatibility complex class II [MHCII]) antibodies were purchased from BD Pharmingen (San Diego, CA). Biotinylated primary antibodies were detected by using Alexa Fluor 488 or rhodamine red X-labeled streptavidin (Molecular Probes). Antibodies against surface markers were purchased as follows: CD3 and F4/80 were purchased from AbD Serotec (Raleigh, NC), CD115 was purchased from eBioscience (San Diego, CA), GR-1 was purchased from BD Pharmingen (San Diego, CA), 7/4 was purchased from Cedarlane (Ontario, Canada), and arginase 1 (ARG1) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). For indirect immunofluorescence, appropriate fluorescence-conjugated secondary antibodies (Jackson Immunoresearch Laboratories, West Grove, PA) were used. Directly labeled isotype controls and antibodies against surface markers for flow cytometry were purchased as follows: CD45 (rat IgG2b, κ chain) was purchased from eBioscience (San Diego, CA), CD11b (rat IgG2b, κ chain) and Ly6C (rat IgM, κ chain) were purchased from BD Pharmingen (San Diego, CA), 7/4 (rat IgG2a) was purchased from Cedarlane, (Ontario, Canada), and Ly6G (rat IgG2a) was purchased from Biolegend (San Diego, CA).

**Immunofluorescence microscopy.** Brain sections from infected and mock-infected mice were thawed at RT for 30 min. Tissues were fixed in −20°C acetone for 10 min and then hydrated in PBS. To perform single staining and double-immunofluorescence labeling, the sections were blocked and incubated with either one set or two sets of primary and secondary antibodies. Non-specific immunoglobulin binding was blocked by 30 min of incubation at RT with serum from the same species from which the fluorochrome-conjugated antibodies to be used were derived. Sections were incubated for 40 min with previously optimized concentrations of primary antibodies diluted in species-specific serum. Sections were washed 7 times for 3 min each after incubation with specified antibodies. When secondary antibodies were necessary, they were incubated for 30 min at RT. The second set of primary antibodies were then incubated with their respective secondary antibodies. When primary antibodies were directly labeled with fluorochrome, the secondary antibody step was omitted. Sections were then mounted by using Fluorsave reagent (Calbiochem, La Jolla, CA) containing 0.3 μM 4,6-diamidino-2-phenylindole dilactate (DAPI) (Molecular Probes, Eugene, OR). Negative controls using secondary antibodies alone were included in each experiment and found to be negative for staining. Fluorescence was visualized with a Leica microscope (Leica Microsystems, Wetzlar, Germany). Images were acquired and processed by using IP lab software (Scanalytics, Inc.) and Adobe Photoshop CS2 (Adobe, Mountain View, CA) (28).

**Brain leukocyte isolation and flow cytometry analysis.** After perfusion, WT and MRC1−/− mock-infected or M. corti-infected brains were collected in 3 ml of Ca2+/Mg2+-free HBSS supplemented with 0.1% fetal calf serum (FCS) plus 10 mM HEPES/buffer. Pooled brain tissues (4 mice/experimental group) were dispensed with a glass Dounce homogenizer (20 times with type A pestles) (Fisher), and the volume was brought up to 7 ml. Subsequently, 3 ml of 100% isotonic Percoll (Sigma-Aldrich) was added to make a suspension of 30% isotonic Percoll, which was layered on top of a 70% isotonic Percoll solution in a 15-ml polypropylene conical tube. After centrifugation (30 min at 500 × g), the 70%/30% interphase was collected, and cells were washed, counted, and used for flow cytometry for subset analysis and sorting or magnetic bead-mediated isolation of granulocytes. If staining of cell surface antigens for fluorescence-activated cell sorter (FACS) analysis was performed as described previously (29). After staining, cells were washed three times. Cell pellets were then suspended in 300 μl of staining buffer and analyzed on a BD LSRII flow cytometer.
cytometer (BD Biosciences) or processed with a FacsAria instrument (BD Biosciences) for sorting. FlowJo software (Three Star, Inc.) was used to analyze the FACS data. Alternatively, whole-leukocyte preparations were used for isolation of Ly6G<sup>+</sup> granulocytes by using magnetic beads coated with anti-Ly6G antibody and a magnetic microbead-activated cell sorting (MACS) system (Miltenyi Biotech, Auburn, CA) according to the manufacturer’s instructions (data from 3 to 5 mice/experimental group were pooled). A small number of cells either sorted or isolated through the MACS systems were cytocoentrifuged at 1,000 rpm for 7 min, followed by Diff-Quik staining (Dade Behring, Inc., Newark, DE) for phenotype analysis. Data using FACS-sorted cells are shown.

Generation of bone marrow chimeric mice. Recipient mice (5 to 6 weeks old) were irradiated with a dose of 9 Gy and allowed to recover overnight before bone marrow reconstitution. Bone marrow cells were isolated from femur and tibia as previously described (30, 31). Briefly, mice were sacrificed by CO<sub>2</sub> asphyxiation followed by cervical dislocation. Flushed bone marrow cells were resuspended in Iscove’s medium without fetal bovine serum (FBS) at 15 × 10<sup>6</sup> cells/ml. Recipient mice were anesthetized for 1 to 2 min (or until animals lost righting reflexes) via an induction chamber with an oxygen flow rate of 1 liter/min and isoflurane delivery to 3 to 4%, and 15 × 10<sup>6</sup> to 20 × 10<sup>6</sup> cells were injected via the retro-orbital sinus in a volume of 150 μL. Mice were placed into a clean cage and monitored until righting reflex was regained. Efficiency of engraftment was confirmed by flow cytometry 4 weeks after bone marrow reconstitution. Six weeks after reconstitution, mice were intracranially infected, and brains were analyzed at 2 weeks postinfection (p.i.).

RNA isolation and real-time PCR analysis. Granulocytes from WT and MRC1<sup>−/−</sup> M. corti-infected brains were sorted and used to determine the relative expression levels of immunoregulatory genes. Isolated cells were obtained from pooled brain cells from 3 to 5 mice/experimental group. Total RNA was extracted with a Pico Pure RNA isolation kit (Arcturus Bioscience, Mountain View, CA) according to the manufacturer’s protocol and quantified by using a NanoDrop ND-1000 instrument. cDNA was prepared from 1 μg total RNA by using the High Capacity cDNA Archive kit (Applied Biosystems). The cDNA derived from WT and MRC1<sup>−/−</sup> granulocytes was then loaded onto microfluidic cards preloaded with fluorogenic probes and primers in custom-designed TaqMan low-density arrays (Applied Biosystems, CA) for regulatory immune markers and the housekeeping genes β-actin, 18S rRNA, and GAPDH (glyceraldehyde-3-phosphate dehydrogenase). These cards were then loaded onto an ABI Prism 7900 HT sequence detection system (Applied Biosystems) for thermal cycling. Analysis of gene expression was determined by using ABI Prism 7900 sequence detection system software (Applied Biosystems). The target expression levels were normalized to levels of the 18S rRNA, β-actin, and GAPDH housekeeping genes in the same sample. Expression of each specific gene in infected samples was determined as the fold change over that in control samples, calculated by using the formula 2<sup>−(ΔΔCT)</sup> (20, 32). The experiment was repeated with granulocytes isolated by the MACS magnetic bead system (3 to 5 pooled brain cells/experimental group), with similar results.

Statistical analysis. Statistics were calculated by using the Student t test with GraphPad Prism 5.01 (GraphPad Software, Inc., La Jolla, CA). Survival analysis was generated by using the Kaplan-Meyer curve, and statistics were calculated by using a log-rank test.

RESULTS

MRC1<sup>−/−</sup> mice display reduced susceptibility. To determine if MRC1 affects disease outcome in murine NCC, MRC1<sup>−/−</sup> and WT mice were infected with M. corti and observed for 4 weeks. The progression of disease was found to be faster in WT mice. Infected WT mice displayed earlier signs of infection, such as mild piloerection, ruffled fur, weight loss, tilted head, and repetitive walking in circles, that progressively worsened until death. The onset of the disease signs in infected MRC1<sup>−/−</sup> mice was delayed, and a significant percentage of mice survived for the time period observed (P < 0.017) (Fig. 1).

Expression of mediators relevant to pathology. To determine if the reduced disease severity in MRC1<sup>−/−</sup> mice correlated with less inflammation, several markers related to pathology were assessed. Whole-brain homogenates from WT and MRC1<sup>−/−</sup> mice at 2 to 3 weeks p.i. were compared for the concentration of the inflammatory/pathology mediators interleukin-1β (IL-1β) (33, 34), IL-6 (35), CCL5 (29, 36), and matrix metalloproteinases 9 (MMP9) (35, 37) in addition to the repair mediator fibroblast growth factor 2 (FGF2) (38, 39) by using Luminex technology (Fig. 2). The concentrations of the inflammatory cytokines IL-1β, CCL5, and IL-6 as well as the protease MMP9 were found to be higher in WT brain homogenates than in MRC1<sup>−/−</sup> mice. In contrast, a comparatively higher level of FGF2, a growth factor that promotes angiogenesis (38, 39), was detected in infected MRC1<sup>−/−</sup> homogenates (Fig. 2). These data suggest less immunopathogenesis in MRC1<sup>−/−</sup> mice, correlating with disease outcome.

Infected MRC1<sup>−/−</sup> mice display an increased accumulation of granulocyte-type cells. As disease outcome was altered in MRC1<sup>−/−</sup> mice, it was important to determine if there were differences in the immune infiltrates in infected knockout (KO) animals. H&E-stained serial sections from brains of WT and MRC1<sup>−/−</sup> mock-infected and 3-week-infected mice were examined. The time point of 3 weeks p.i. was chosen because of the maximum differences in the disease phenotype between WT and MRC1<sup>−/−</sup> mice and because the time point of 3 weeks is typically when peak leukocyte infiltration occurs in WT animals (10, 28). H&E staining showed normal tissue morphology with a scarce presence of infiltrating immune cells in mock-infected control brains of WT (Fig. 3A1) and MRC1<sup>−/−</sup> (Fig. 3A2) mice. Infected WT brain sections showed leukocytes present mainly in the meninges/subarachnoid spaces, with fewer vessels showing signs of leukocyte infiltration in the neuropile/parenchymal area, consistent with our previous studies (28). In addition, WT brain sections showed a mixed population of monocytic and granulocyte-type cells but with a predominance of monocytic cells (Fig. 3A3 and A3'). Similar to WT mice, MRC1<sup>−/−</sup> mice showed most of the infiltrating leukocytes localized to meninges/subarachnoid spaces (Fig. 3A4). However, high-power images of nuclear morphology

![FIG 1 MRC1<sup>−/−</sup> mice display reduced susceptibility to M. corti infection.](http://iai.asm.org/)

FIG 1 MRC1<sup>−/−</sup> mice display reduced susceptibility to M. corti infection. WT and MRC1<sup>−/−</sup> mice (n = 15/group) were i.c. infected with M. corti, and disease severity and susceptibility were observed. Survival of MRC1<sup>−/−</sup> mice was significantly higher, as determined by a log-rank test (P = 0.017).
indicate that infiltrates in MRC1−/− mice are dominated by mostly granulocyte-type cells (Fig. 3A4, arrows). Interestingly, such differences between the infiltrates of infected WT and MRC1−/− brains were not apparent at 1 week p.i. (data not shown).

Comparison of infiltrating leukocytes by cell surface markers using IF microscopy. The composition of infiltrates was further assessed by IF staining of brain sections from 3 weeks p.i. for various myeloid cell surface markers: F4/80 (monocytes), CD115 (monocytes), 7/4 (granulocytes and activated monocytes), CD11c (dendritic cells [DCs]), and MHCII (antigen-presenting cells). The results showed low to undetectable expression levels of the surface antigens F4/80, 7/4, CD11c, and MHCII and a basal level of CD11b expression in mock-infected WT and MRC1−/− brain sections (data not shown). However, in infected WT brain sections, abundant expression of the surface markers F4/80, CD11c, MHCII, CD11b, and CD115 (Fig. 4A1, A2, A3, A4, and B1, respectively) and relatively less expression of GR-1 and 7/4 were detected (Fig. 4B2 and C1). The data obtained from WT mice verified and further showed the presence of a mixed population of myeloid subsets consisting of monocytes, dendritic cells, and some granulocytes. In comparison to the WT, MRC1−/− brain sections showed lower expression levels of the surface antigens F4/80, CD11c, CD115, and MHCII, characteristic of antigen-presenting myeloid cells (Fig. 4A1′, A2′, A3′, and B1′, respectively); higher expression levels of Gr-1 and 7/4 (Fig. 4B2′ and C2′), character-
istic of granulocytic myeloid cells; and no apparent change in CD11b expression (Fig. 4A4). Furthermore, double-IF staining with MRC1−/− mouse brain sections showed that the majority of CD11b+ cells also expressed Gr-1 and 7/4 (Fig. 4B2 and C2), unlike the WT (Fig. 4B2 and C2). Altogether, the data indicate that there is a decrease in the numbers of DCs and monocytes/macrophages in MRC1−/− mice and an increased accumulation of granulocytic myeloid cells in infected MRC1−/− mice in comparison to WT mice.

FACS analyses of myeloid subsets in infected WT and MRC1−/− mice. To confirm differences in the frequency of granulocytic cells, leukocytes were isolated from 3-week-infected WT and MRC1−/− mice (and mock-infected mice) by Percoll gradients; stained with antibodies to CD45 (infiltrating leukocytes), CD11b, 7/4, and Ly6G (granulocyte marker); and analyzed by FACS (Fig. 5). Figure 5C shows the counts for CD45hi CD11bhi 7/4hi Ly6Ghi cells (granulocytic myeloid cells) and indicates a significant increase of this population in the MRC1−/− mice. This cell population was not detectable in brains from mock-infected animals (data not shown).

We also compared spleen tissues for differences in myeloid and lymphoid populations by FACS analysis to determine if there was an increased proportion of granulocytes in uninfected MRC1−/− mice. There was no apparent difference between WT and MRC1−/− mice in the frequency of CD45hi CD11bhi 7/4hi Ly6Ghi or CD11c cells in spleens, suggesting that the increased accumulation of CD45hi CD11bhi 7/4hi Ly6Ghi cells in MRC1−/− mice is not inherently skewed but infection induced (data not shown).

Skewing of myeloid subsets in infected MRC−/− brains is dependent upon peripheral MRC−/− cells. To determine if the increased frequency of granulocytic myeloid-dependent suppressor cells (gMDSCs) in the CNS is due to deficiency of MRC1 in peripheral myeloid cells, we generated WT→WT and MRC1−/−→WT chimeric mice. Flow cytometry analysis revealed around 95% engraftment (Fig. 6A and B). Upon infection, the MRC1−/−→WT chimeras showed an abundance of gMDSCs, as shown by an increased proportion of CD11b+ Ly6G/7/4-positive cells (Fig. 6C to E), which also expressed CD244 (Fig. 6F), a recently described marker which correlates with suppressive gMDSCs (40).

MRC1−/− granulocytes display a regulatory phenotype. As the accumulation of granulocytic cells correlated with decreased disease severity, it was important to assess the potential function of these cells. Leukocytes were isolated, as described above, from 3-week-infected WT and MRC1−/− mice and sorted for CD45hi CD11bhi 7/4hi and Ly6Ghi cells (Fig. 7). We first cytocentrifuged the sorted cells onto slides and stained them with Diff-Quik. The results highlight the different phenotypes of the sorted cells from WT versus MRC1−/− brains. The sorted WT cells displayed a nuclear morphology more typical of activated neutrophils (Fig. 7A3). In contrast, many of the sorted MRC1−/− cells had a more rounded nucleus or donut-shaped nucleus (Fig. 7A4) and resembled the unique phenotype associated with gMDSCs (41).

Sorted CD45hi CD11bhi 7/4hi Ly6Ghi cells from infected WT and MRC1−/− brains were then used to isolate RNA for RT-PCR so that transcripts specific for immune mediators could be assessed, particularly those known to be regulatory. The mediators
cells from MRC1\(^{-/-}\) mice compared to WT mice (Fig. 8A). Furthermore, ARG1, YM1, and LGALS3 were confirmed at the protein level by IF staining of brain cryosections from MRC1\(^{-/-}\) mice using antibodies specific for these mediators. Costaining for ARG1 with anti-7/4 antibody indicated extensive colocalization of the mediators with these granulocytic myeloid cells. The results for ARG1 are shown in Fig. 8B.

Infected MRC1\(^{-/-}\) mice exhibit diminished numbers of T cells. As the data show increased expression levels of several regulatory mediators in the granulocytic myeloid cells that accumulated in infected MRC1\(^{-/-}\) mice, we questioned whether there were alterations in the T cell responses. Infected WT and MRC1\(^{-/-}\) brains were assessed for the relative frequency of infiltrating T cells by in situ IF microscopy and FACS analysis using antibodies against CD3 and \(\alpha\beta\)-T cells, respectively (Fig. 9). The results indicate that at 3 weeks p.i., there is a substantial reduction in the number of T cells in MRC1\(^{-/-}\) brains compared with WT brains, indicating an inhibition of T cell responses.

DISCUSSION

We have shown that during murine NCC, parasites release both host-like and immunodominant non-host-like glycan antigens in the CNS environment, similar to human NCC. Among the non-host-like glycan antigens, highly mannosylated N-glycans are a characteristic feature of pathogenic helminths. Previously, we have shown that \(M.\) corti and \(T.\) solium parasites produce N-glycans, as they are preferentially recognized by the lectins IB4 (recognizes acetyl-D-galactosaminyl ends and \(\alpha\)-D-galactosyl residues), wheat germ agglutinin (WGA) (recognizes \(N\)-acyethylgalactosamine), and concanavalin A (ConA) (\(\alpha\)-mannopyranosyl and \(\alpha\)-glucopyranosyl ends) (9). In vivo studies using murine NCC further provide evidence that glycan antigens are released/secreted in a time-dependent fashion and that such N-glycans (WGA binding glycans) are taken up by infiltrating immune cells, some of which appear immunodominant by antibody production (9). Similarly, \textit{ex vivo} studies have shown the presence of multiple immunodominant molecules present in N-glycan fractions of \(T.\) solium fluid, such as GP-12, -16, and -18 as well as others (11, 26, 45). However, there is little understanding about how these glycans are recognized and the subsequent immunological consequences. Our data show that the mannose binding CLR MRC1 appears to be an important mediator of injurious inflammation, and its absence correlates with increased regulatory mediators and a slower disease process.

Inflammatory signals have been shown to induce MMP expression in both CNS residential cells and infiltrating leukocytes, which in turn facilitate several biological events, such as leukocyte extravasation, cytokine/chemokine activation, and tissue remodeling (46). By performing \textit{in situ} zymography, we have found that among various MMPs, MMP9 is active in murine NCC as well as other CNS diseases (35, 37). \(M.\) corti infection of MMP9\(^{-/-}\) mice diminished leukocyte infiltration in extraparenchymal sites during the acute phase of infection as well as in the parenchyma during the chronic phase (37). In addition, multiple MMPs were found to be induced and likely contribute to pathology and disease progression, as treatment with the broad MMP inhibitor doxycycline led to reduced morbidity and mortality of the animals (35). Also relevant are our data shown here of an...
increased expression level of FGF2, a repair molecule involved in angiogenesis during cerebral ischemia and neuroprotection (38, 39). Increased FGF2 levels could also contribute to the increased survival of MRC1−/− mice.

During murine NCC, large proportions of inflammatory cytokines and MMPs are produced by infiltrating leukocytes (47). The infiltrates in infected WT mice have been shown to be heterogeneous and comprised of macrophages, γδ-T cells, some neutrophils, αβ-T cells, and B cells (10). Our data show that MRC1−/− mice display an altered composition of infiltrates dominated by granulocytic myeloid cells. Moreover, our use of chimeric mice confirms that the increased number of granulocytic myeloid cells is dependent upon a lack of MRC1 expression by peripheral myeloid cells. Multiple lines of evidence suggest that MRC1−/− granulocytes are of a regulatory nature rather than the classical inflammatory phenotype described in the literature. Granulocytes (neutrophils) are known to be a major source of MMP9 in CNS disease (48). Importantly, despite the increased proportion of granulocytic cells in MRC1−/− brains, there was a reduction in MMP9 expression levels compared with those in WT brains. Furthermore, granulocytic cells from MRC1−/− mice have rounded/donut-shaped nuclei that are less fragmented, which is a characteristic of the noninflammatory phenotype of granulocytes (41, 49). Furthermore, MRC1−/− granulocytes express molecules

**FIG 6** gMDSCs are abundant in MRC1−/−→WT chimeric mice. (A) Bone marrow radiation chimeric mice revealed >95% engraftment of donor bone marrow cells in peripheral blood. PBMC, peripheral blood mononuclear cells. (B to D) At 2 weeks postinfection, brain mononuclear cells were isolated (B) and characterized by expression of the myeloid marker CD11b (C) and the granulocytic markers Ly6G and 7/4 (D) by flow cytometry. (E and F) Chimeric MRC1−/−→WT mice showed an increased proportion of CD11b−/7/4− cells (E) that exhibited a CD244− phenotype characteristic of gMDSCs (F, arrows).
known to contribute to tissue growth, wound healing, and fibrosis, such as ARG1, YM1, and LGALS3 (50–53). Thus, it is likely that MRC1−/− granulocytes participate in repair and immune regulation.

Furthermore, levels of DCs, monocytes/macrophages, and the antigen presentation marker MHCII are highly reduced in infected MRC1−/− mice, creating a microenvironment with compromised antigen presentation to T cells, which is required for T

FIG 7 Cell sorting using surface markers for immunophenotype analysis. WT and MRC1−/− brain leukocytes were isolated at 3 weeks p.i. by using Percoll gradients, stained, and sorted with a FACS Aria instrument. (A1) Example of the gates based on side scatter (SSC) (area/width), forward scatter (FSC) (area/width), CD45, CD11b, 7/4, Ly6C, and Ly6G for the WT. (A2) Similar gate displaying increased numbers of CD45+CD11b+Ly6G+ granulocytic cells in MRC1−/− mice. (A3 and A4) Sorted cells from WT (A3) and MRC1−/− (A4) mice were placed onto slides and stained by using Diff-Quik reagent, showing a striking difference in nuclear morphology.
cell retention in the CNS microenvironment (54). Consistent with this, we saw a substantial reduction in the number of T cells in MRC1$^{-/-}$ mice at 3 weeks p.i., which is typically the time point for peak inflammation (10, 28). One report suggested a role of MRC1 in $\alpha$-selectin-mediated adhesion of lymphocytes to lymphatic endothelium (55). Deficiency of MRC1 could influence the trafficking of T cells into the CNS. However, examination of infection of MRC1$^{-/-}$ mice at 1 week p.i. did not show a significant change in the accumulation of T cells. This evidence suggests an antigen presentation- or suppression-related mechanism for a reduction of the T cell response. Furthermore, ARG1 induces T cell dysfunction by depleting extracellular availability of L-arginine, which leads to an arrest in proliferation of antigen-specific T cells and reexpression of the CD3$\zeta$ chain (43, 56, 57). Furthermore, ARG1 and IL-10 have been shown to induce proliferation/expansion of Tregs, which further downregulate immune responses (58). Thus, it will be of interest to know the phenotype of the remaining T cells in the infected MRC1$^{-/-}$ brain. The mechanisms by which MRC1$^{-/-}$ granulocytes lead to a compromised T cell response or influence the functional phenotype are currently being investigated.

Interestingly, our data show that other CLRs, such as MGL1 and LGALS3, are upregulated in isolated MRC1$^{-/-}$ granulocytic myeloid cells. It is possible that these CLRs are involved in skewing myeloid cells to the granulocytic phenotype in infected MRC1$^{-/-}$ mice, as LGALS3 and MGL1 recognize host-like sugar moieties (59–61). Thus, parasites are known to harbor host-like glycans, and the tumor-associated antigens T and Tn are found in the tegument of M. corti (62). An additional host-like glycan, the tumor-associated antigen “Tk,” has also been reported in Taenia hydatigena, M. corti, and Taenia crassiceps, (63). Our group has

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**FIG 8** Expression of regulatory markers by MRC1$^{-/-}$ granulocytes. (A) WT and MRC1$^{-/-}$ granulocytes sorted by using a FacsAria instrument. Sorted granulocytes from 3 mice were pooled for each group. Pooled samples ($n = 3$) were used for mRNA isolation and transcript analysis by real-time RT-PCR. Results were analyzed by using the $\Delta\DeltaCT$ method and are represented as fold changes over the WT. MGL1, LGALS3, S100A9, YM1, IL-10, and ARG1 were found to be upregulated in MRC1$^{-/-}$ granulocytes in comparison to WT granulocytes. (B) MRC1$^{-/-}$ brain cryosections were used to verify the expression of Arg1 by using the granulocyte-specific marker 7/4 and ARG1.

**FIG 9** Reduced T cell accumulation in the CNS of MRC1$^{-/-}$ mice during murine NCC. IF staining using CD3 was performed in mock-infected and infected WT and MRC1$^{-/-}$ brain tissues. (A) During infection, fewer CD3$^{+}$ T cells were detected in infected MRC1$^{-/-}$ mice (A$'$) than in infected WT mice (A). (B) Infiltrating leukocytes were isolated by using a Ficoll gradient, and flow cytometric analysis was done by using antibody against the $\alpha$-$\beta$-T cell receptor, showing that MRC1$^{-/-}$ mice have reduced T cell accumulation in the CNS during infection.
also shown that during murine, porcine, and human NCC, the surfaces of *M. corti* and *T. solium* larva strongly label with peanut agglutinin (PNA), a lectin with affinity for O-glycans expressing the nonsubstituted T antigen (galactose–B1→3-N-acetylgalactosamine–α1→serine/threonine) (9). Furthermore, an antigen fraction from the cyst fluid of *T. solium* is recognized by Jaclin and VvB4 lectins specific to T antigens and Tn antigens, respectively (64). Thus, it is possible that in the absence of MRC1, preferential engagement of CLRs on myeloid cells, such as MGL1 and LGALS3, that recognize host-like glycans shifts the phenotype to regulatory granulocytic myeloid cells similar to the gMDSCs described in cancer biology (39–61). The idea that myeloid plasticity may be regulated by host-like versus non-host-like glycans is also supported by our preliminary data showing that infection of MGL1−/− mice results in an accumulation of an activated phenotype of granulocytes in the CNS that appear to be activated neutrophils and leads to more severe disease.

Similar to lymphoid populations, increasing evidence shows that myeloid cells are a highly plastic cell type whose activation can be skewed to participate in fighting infection by driving inflammation (65, 66) as well as suppressing inflammation and regulating repair of resulting tissue damage (41, 52, 66, 67). Myeloid-derived suppressor cells (MDSCs) are a good example of myeloid plasticity. MDSCs (CD11b+ Gr1+ [LY6C+/LY6G+] ) are believed to be a heterogeneous population of immature myeloid cells or myeloid progenitor cells (43), as myeloid cell differentiation markers such as F4/80, CD11c, MHCII, and CD80/86 have been described to have various degrees of expression (41, 43). Under steady-state conditions, this population quickly differentiates into mature granulocytes, macrophages, and dendritic cells. Under pathogenic conditions, especially those characterized by immunosuppression, such as cancer, MDSCs are expanded and consist of two major subsets of MDSCs, monocytic MDSCs (mMDSCs) (CD11b+ Ly6C+Ly6C− cells) and granulocytic MDSCs (gMDSCs) (CD11b+ Ly6C−Ly6C+ cells) (68, 69). Previous studies suggested that mMDSCs are highly plastic and can further differentiate into functional mature macrophages *in vivo* or *ex vivo* with a classical phenotype (M1), an alternative activation phenotype (AAM or M2), or dendritic cells, depending on the microenvironment (58, 68, 69). Myeloid cells, especially the monocytic population, have been shown to be modulated during several parasite infections (41). Our group as well as others have shown that during parasitic infections, including infections by *Brugia malayi*, *Schistosoma mansoni*, *Taenia crassiceps*, and *M. corti*, both the M1 and the M2 phenotypes of monocytic myeloid cells are induced, but increasingly, there is a shift to M2 or alternatively activated macrophages (20, 70, 71). Our data show that during parasitic infection, granulocytic myeloid cells can also be modulated to different functional phenotypes similar to that of monocytic myeloid cells (19, 20). Since there was no significant difference in myeloid subsets in the periphery of mock-infected WT and MRC1−/− mice, it is likely that the phenotypic changes are due to an antigen-specific effect.

Thus, our data show that CLRs play a critical role in the disease process of murine NCC. Due to the similarity between *T. solium* and *M. corti* glycan antigens, CLRs are likely to play an important role in human NCC. MRC1 in particular is involved in facilitating the inflammatory immune response against the parasite. Perhaps more importantly, MRC1 deficiency leads to granulocytes with a regulatory phenotype and less severe disease. A thorough understanding of the mechanisms leading to this phenotype may well lead to new therapeutic interventions in NCC (72, 73).

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