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CCR1 Deficiency Increases Susceptibility to Fatal Coronavirus Infection of the Central Nervous System

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ABSTRACT

The role of CC chemokine receptor 1 (CCR1) in host defense and disease development was determined in a model of viral-induced neurologic disease. Intracerebral (IC) infection of mice with mouse hepatitis virus (MHV) results in an acute encephalitis followed by a chronic demyelinating disease similar in pathology to the disease multiple sclerosis (MS). No increase in mortality was observed during the acute phase of disease following MHV infection of mice lacking CCR1 ($CCR1^{-/-}$) as compared to wild-type ($CCR1^{+/+}$) mice. However, by 21 d post-infection, 74% of $CCR1^{-/-}$ mice had succumbed to death compared to only 32% mortality of $CCR1^{+/+}$ mice, indicating that chemokine signaling through CCR1 significantly ($p \leq 0.04$) enhanced survival following IC infection with MHV. Increased mortality in $CCR1^{-/-}$ mice was not associated with increased viral recovery from the CNS, although CCR1 deficiency correlated with reduced T-cell accumulation within the CNS during acute, but not chronic, disease. Despite the reduction in T-cell trafficking into the CNS of $CCR1^{-/-}$ mice during acute disease, components of host defense remained unaltered; T-cell effector functions including cytolytic activity and proliferation and the expression of IFN-γ within the CNS were not significantly different between $CCR1^{+/+}$ and $CCR1^{-/-}$ infected mice. In addition, macrophage infiltration into the CNS was unaffected in MHV-infected $CCR1^{-/-}$ mice when compared to $CCR1^{+/+}$ mice. Furthermore, assessment of neuropathology revealed no difference in the severity of demyelination between CCR1-deficient and wild-type mice. Together, these findings reveal that T-cell and macrophage trafficking are not dependent on CCR1 and highlight an important role for CCR1 signaling in promoting survival during chronic MHV infection.

INTRODUCTION

VIRAL INFECTION OF THE central nervous system (CNS) often results in a dramatic increase in expression of proinflammatory factors that regulate leukocyte migration, extravasation, and infiltration into the CNS parenchyma (28,31). Growing evidence suggests that chemokines are important molecules that participate in these events. In support of this are studies demonstrating robust chemokine gene expression following CNS infection with a wide variety of viruses (1,17,20). Therefore, chemokine expression may help initiate inflammatory events that result in elimination of foreign antigen. Paradoxically, chronic expression of chemokines may contribute to disease by maintaining an inflammatory state that ultimately results in tissue damage (23,27).

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We have used a model of viral-induced neurologic disease to better understand the contributions of chemokines and chemokine receptors in host defense and disease development within the context of the CNS. Intracerebral (IC) infection of susceptible mice with neuroadapted strains of mouse hepatitis virus (MHV), a positive-strand RNA virus, results in an acute encephalomyelitis followed by a chronic demyelinating disease (19,21). The collective evidence indicates that the immune response to MHV infection is critical in both host defense and the development of demyelination (10,29). Both $CD4^+$ and $CD8^+$ T lymphocytes are important in clearance of virus from the brain during acute disease (37). In addition, recent studies point to a role for T lymphocytes in enhancing macrophage activation and infiltration into the CNS, thereby amplifying the severity of myelin destruction (23,38). One mechanism by which T lymphocytes may accomplish this is via the secretion of both cytokines and chemokines (11,23,27). MHV infection of the CNS results in an orchestrated expression of both CXC and CC chemokine ligands including CXCL9, CXCL10, CCL2, CCL3, and CCL5 (20,25). Furthermore, there is a corresponding increase in chemokine receptors within the CNS that are capable of binding expressed chemokine ligands, suggesting these signaling pathways are important for leukocyte infiltration into the CNS (12–14). Indeed, recent studies from our laboratory using either chemokine-specific neutralizing antibodies or chemokine knockout mice have revealed both non-redundant and redundant roles for these molecules in participating in leukocyte migration into the CNS in response to MHV infection (6,11,14,15,25–27,36). Similarly, ablation of chemokine receptors capable of binding chemokine ligands expressed in response to MHV infection also results in increased susceptibility to viral-induced neurologic disease. For example, MHV infection of mice lacking CCR5 (a receptor for CCL3 and CCL5) demonstrates an important role for this receptor in influencing macrophage accumulation within the CNS and ultimately amplifying the severity of myelin destruction (14,34). Collectively, these studies indicate an important role for chemokines and chemokine receptors in both host defense and disease by attracting distinct leukocyte populations into the CNS.

CCR1 is a chemokine receptor that is expressed on a variety of cell types including lymphocytes and monocytes, and is capable of binding both CCL3 and CCL5 (5,8,32). Having previously demonstrated that these chemokines are expressed within the CNS of MHV-infected mice, it is of interest to determine the functional contributions of CCR1 with regard to regulating leukocyte activation and/or trafficking to the CNS following MHV infection (20). To this end, we have infected mice lacking CCR1 ($CCR1^{-/-}$) with MHV in order to determine the functional role of CCR1 in both host defense and disease.

MATERIALS AND METHODS

Virus and mice

MHV strain J2.2V-1 was used for all intracranial infections (7). Age matched (5- to 7-week-old) and sex matched (male) $CCR1^{+/+}$ and $CCR1^{-/-}$ mice were used for our experiments (9). Both $CCR1^{+/+}$ (purchased from the National Cancer Institute, Frederick, MD) and $CCR1^{-/-}$ mice (bred at the University of California, Irvine vivarium) were on the C57BL/6 $H\text{-}2^b$ background. The animal protocols used for these studies were reviewed and approved by the institutional animal care review board. Following anesthetization by intraperitoneal (IP) injection with a ketamine/xylazine mixture, mice were injected IC with 500–1000 PFU of MHV suspended in 30 μL of sterile saline (20,23). Sham animals were injected IC with 30 μL sterile saline alone. Animals were sacrificed at defined time points and brains and spinal cords were removed for analysis in the studies described here. One-half of each brain at each time point was used for FACS analysis or ELISA, while the other half was used to determine viral burden on the DBT astrocytoma cell line (16,23). Spinal cords were isolated and used to determine viral burden as above, or fixed in 10% normal buffered formalin, and then embedded in paraffin and sectioned for histological study. Immune splenocytes were obtained from $CCR1^{+/+}$ and $CCR1^{-/-}$ mice injected IP with MHV at day 7 post-infection and used for flow cytometry as described below.

Mononuclear cell isolation and flow cytometry

Mononuclear cells were obtained from half-brains at days 7, 12, 15, and 18 post IC infection using a previously described method (23). Splenocytes were isolated on day 7 post IP infection (35). Immuno-phenotyping of cells isolated from the brain or spleen was performed using allophycoerythrin-conjugated rat anti-mouse CD4 (Pharmingen, San Diego, CA), allophycoerythrin- or PerCP-conjugated rat anti-mouse CD8 (Pharmingen), PE-conjugated anti-mouse MHC class I tetramer specific for the CD8 immunodominant epitope spanning amino acids 510–518 of the surface (S) glycoprotein (S510-518) (Beckman Coulter, San Diego, CA), FITC-conjugated rat anti-mouse F4/80 (Serotec, Oxford, UK), and allophycoerythrin- or PE-conjugated rat anti-mouse CD45 (Pharmingen) (15). Virus-specific $CD4^+$ and $CD8^+$ T cells recognizing their respective immunodominant epitope between amino acids 133 and 147 of the membrane (M) glycoprotein (M133–147) and surface glycoprotein

(S510-518) were determined by intracellular IFN-γ staining using previously described methods (10,12). In all cases, isotype-matched control antibodies were used. Cells were incubated with antibodies for 45 minutes at 4°C, washed, and fixed in 1% paraformaldehyde. Following fixation, flow cytometry was performed using a FACStar flow cytometer (Becton Dickinson, Mountain View, CA). Frequency data are presented as the percentage of positive cells within the gated population. Total cell numbers were calculated by multiplying these values by the total number of live cells isolated.

Histology

Spinal cords were fixed in normal balance formalin for 24 hours and then embedded in paraffin (23). The severity of demyelination was determined on sectioned spinal cords stained with Luxol fast blue. Demyelination was scored as follows: 0, no demyelination;1, mild inflammation accompanied by loss of myelin integrity; 2, moderate inflammation with increasing myelin damage; 3, numerous inflammatory lesions accompanied by a significant increase in myelin stripping; and 4, intense areas of inflammation accompanied by numerous areas of phagocytic cells engulfing myelin debris (18,22,23).

In vivo *T-cell proliferation assay*

$CCR1^{+/+}$ and $CCR1^{-/-}$ mice were IP infected with MHV and treated with 1.0 mg of BrdU (Sigma-Aldrich Co. Ltd., Gillingham, Dorset, UK) in sterile saline on days 3, 5, and 6 post-infection. Mice were sacrificed on day 7 post-infection, and splenocytes were isolated and proliferation of T cells as well as virus-specific T cells was determined by evaluating the frequency of BrdU-positive cells present within the gated populations.

Cytolytic T lymphocyte (CTL) assay

Effector-induced death of target cells was measured by ^{51}Cr release assay. Effector $CD8^+$ T cells were purified from immune splenocytes by positive selection using CD8 magnetic beads and MACS columns (Miltenyi Biotec, Auburn, CA). The number of S510-518-tetramer-positive $CD8^+$ double-positive T cells in each culture was determined using a flow cytometric analysis. RMA-S ($H\text{-}2^b$) cells were used as targets, and were preincubated with 5 μM S510-518 peptide or 5 μM ovalbumin (OVA) peptide as a control and labeled with 100 μCi $Na^{51}CrO_4$ (Amersham, Arlington Heights, IL) overnight at 26°C. Target cells were then transferred to 37°C for 2 hours before being plated at 5×10^4 cells/mL. S510-518-specific $CD8^+$ effector T cells and RMA-S cells were plated at a range of effector:target ratios in round-bottom 96-well plates. As a control, spontaneous release or maximal release target cells were incubated with media alone or 100 μL 1% Triton X-100, respectively. ^{51}Cr release was determined after a 5-h incubation of effector and target cells. Supernatants were collected using the Scatron SCS system (Skatron, Sterling, VA) and the released ^{51}Cr was counted in a Beckman gamma counter (Beckman Instruments, Fullerton, CA). Specific lysis was calculated from the mean of triplicate samples using the following formula (cpm = counts per minute): $100 \times$ (cpm experimental release – cpm spontaneous release)/(cpm maximal release – cpm spontaneous release).

IFN-γ ELISA

To determine IFN-γ expression levels within the CNS, brains from IC infected or sham mice were collected and homogenized in 1 mL PBS plus protease inhibitor (Roche, Mannheim, Germany) and clarified by centrifugation at 4°C (25). Supernatants were collected and the aqueous volume recorded for total protein quantification using Bio-Rad Protein Assay (Bio Rad, Hercules, CA). Harvested supernatants were quantified for IFN-γ using the Mouse IFN-γ DuoSet (R&D Systems, Inc., Minneapolis, MN, USA).

Statistical analysis

For survival studies, the log-rank test was used to determine significance between groups of mice and p values ≤ 0.05 were considered significant. For all other analyses, statistically significant differences between groups of mice were determined by the Mann-Whitney ranked sum test and p values < 0.05 were considered significant.

RESULTS

Increased mortality in $CCR1^{-/-}$ mice following MHV infection

To determine if expression of CCR1 is important in host defense, $CCR1^{+/+}$ and $CCR1^{-/-}$ mice were IC infected with MHV and their ability to survive was monitored. Approximately 70% of $CCR1^{+/+}$ mice survived until day 21 post-infection, whereas mice lacking CCR1 began to die at a faster rate between days 15 and 20 post-infection, with only 26% surviving by day 21 post-infection (Fig. 1A). Statistical analysis of the Kaplan-Meier plot shows that survival was significantly impaired in MHV-infected $CCR1^{-/-}$ mice ($p = 0.04$).

To determine if the increased mortality observed in MHV-infected $CCR1^{-/-}$ mice was due to increased viral burden in the CNS, the amount of virus in the CNS of IC infected $CCR1^{+/+}$ and $CCR1^{-/-}$ mice was evaluated via plaque assay. There were no differences in viral

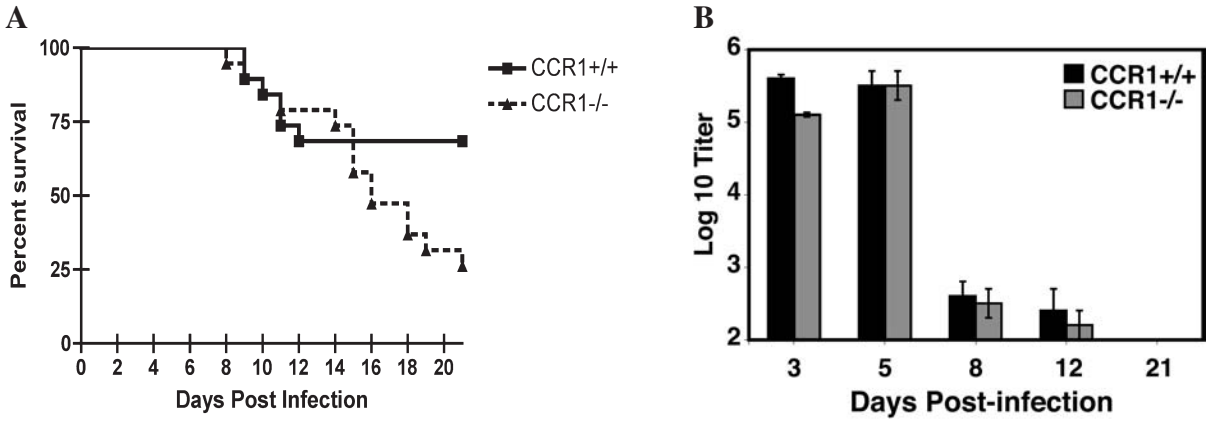

FIG. 1. Survival and viral titer in $CCR1^{+/+}$ and $CCR1^{-/-}$ mice following IC infection with MHV. (**A**) $CCR1^{+/+}$ and $CCR1^{-/-}$ mice were IC infected with MHV and their ability to survive infection and clear virus from the CNS was evaluated. $CCR1^{-/-}$ mice exhibited a reduced ability to survive beginning at day 15 post-infection in comparison to $CCR1^{+/+}$ mice ($p \leq 0.04$). Data shown were compiled from three separate experiments. $CCR1^{+/+}$ mice: n = 19; $CCR1^{-/-}$ mice: n = 19. (**B**) Virus is cleared below detectable levels (~2.0 log10 PFU/g tissue) from the brain in both $CCR1^{+/+}$ and $CCR1^{-/-}$ mice. Data presented are derived from two separate experiments with a minimum of two mice/time point and presented as mean ± SEM.

titers within the brains of $CCR1^{+/+}$ and $CCR1^{-/-}$ mice at any of the time points examined, indicating the increased susceptibility to death in $CCR1^{-/-}$ mice was not the result of an inability to clear virus from the CNS (Fig. 1B). Additionally, there were no differences in viral titers in spinal cords between wild-type and knockout mice at any time point examined (data not shown). Finally, there was no difference in cell populations infected by MHV in $CCR1^{+/+}$ mice compared to $CCR1^{-/-}$ mice, indicating that altered cellular tropism by the virus in CCR1-deficient animals was not accounting for the increase in mortality (data not shown).

T-cell infiltration into the CNS is reduced in $CCR1^{-/-}$ mice

Previous studies have determined that CCR1 signaling contributes to the migration of activated T cells and macrophages to sites of inflammation following microbial infection (2). CCR1 transcripts are detected in virus-specific T cells in response to MHV infection, suggesting a potential role in promoting T-cell trafficking (12,13). Immunophenotyping of the cellular infiltrate within the brains of $CCR1^{+/+}$ and $CCR1^{-/-}$ mice was performed to determine if CCR1 influences leukocyte migration into the CNS following MHV infection. The frequency of $CD4^+$ T cells within the brains of both $CCR1^{+/+}$ and $CCR1^{-/-}$ mice was similar at all of the time points examined (Fig. 2A). Fewer $CD4^+$ T cells infiltrate the CNS of $CCR1^{-/-}$ mice compared to $CCR1^{+/+}$ mice during acute disease (Fig. 2B), with significant ($p = 0.05$) reduction in total numbers on day 12 post-infection. Assessment of the presence of $CD4^+$ T cells recognizing the immunodominant viral epitope M133-147 in the brains of infected $CCR1^{+/+}$ and $CCR1^{-/-}$ mice revealed a similar frequency and total number at days 7, 15, and 18 post-infection, but reduced levels at day 12 post-infection ($p = 0.05$; Fig. 2C and D). The frequency of $CD8^+$ T cells was significantly reduced ($p = 0.05$) in the CNS of $CCR1^{-/-}$ mice on day 12 post-infection compared to $CCR1^{+/+}$ mice (Fig. 2E). Lack of CCR1 resulted in reduced $CD8^+$ T cell infiltration into the brain on days 7 and 12 post-infection ($p = 0.05$) when compared to $CCR1^{+/+}$ mice (Fig. 2F). However, there were no differences in the overall numbers of $CD8^+$ T cells on days 15 and 18 post-infection between $CCR1^{+/+}$ and $CCR1^{-/-}$ mice (Fig. 2F). The number and frequency of virus-specific S510-518 $CD8^+$ T cells was evaluated and the comparison of $CCR1^{+/+}$ and $CCR1^{-/-}$ mice showed comparable populations in the CNS at all time points examined, indicating that the generation of virus-specific $CD8^+$ T cells is not dependent on chemokine signaling through CCR1 (Fig. 2G and H). These data demonstrate that CCR1 signaling enhances T-cell infiltration into the CNS during acute disease (e.g., at days 7 and 12), but does not significantly affect accumulation and/or retention of T cells at later time points, such as at days 15 and 18 post-infection, which are representative of chronic disease (7,29).

T-cell proliferation is not affected in the absence of CCR1

One potential mechanism contributing to the overall diminished numbers of T cells present within the brains

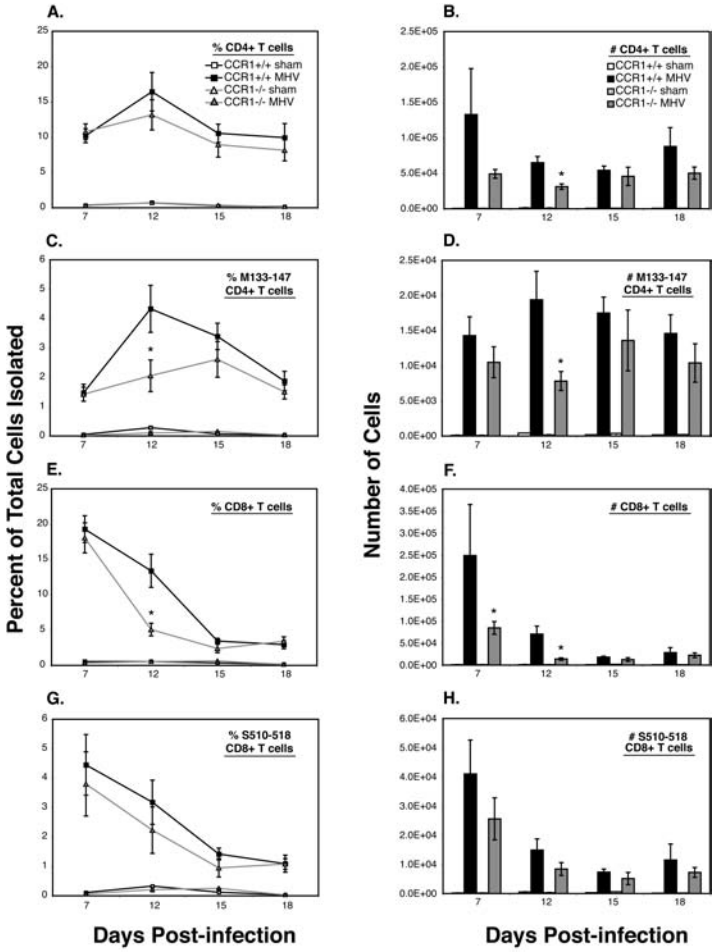

FIG. 2. $CD4^+$ and $CD8^+$ T-cell infiltration into the CNS during acute and chronic disease following IC infection with MHV. $CCR1^{+/+}$ and $CCR1^{-/-}$ mice were IC infected with MHV and the frequency and numbers of T-cell populations in the CNS was determined. $CD4^+$ T cells (**A** and **B**) and $CD8^+$ T cells (**E** and **F**), as well as virus-specific M133-147 $CD4^+$ T cells (**C** and **D**) and virus-specific S510-518 $CD8^+$ T cells (**G** and **H**) in the CNS were evaluated using flow cytometry at defined time points post-infection. The frequency of positive cells represents the percentage of positive cells present within the gated population of live cells. Data presented are derived from three separate experiments with a minimum of three mice/time point and presented as mean ± SEM. $*p \leq 0.05$ using the Mann-Whitney ranked sum test.

of $CCR1^{-/-}$ mice during acute disease is a reduction in the ability of T cells to proliferate following antigenic challenge. To evaluate the functional role for CCR1 in T-cell proliferation, mice were IP infected with MHV and subsequently injected with BrdU. Proliferation of $CD4^+$ and $CD8^+$ T cells isolated from the spleen was determined by evaluating the frequency of cells that incorporated BrdU following challenge with virus. There were no differences in the ability of total $CD4^+$ or $CD8^+$ T cells to proliferate in MHV-infected $CCR1^{+/+}$ or $CCR1^{-/-}$ mice. Both the frequency and number of BrdU-positive $CD4^+$ and $CD8^+$ T cells was similar between infected $CCR1^{+/+}$ and $CCR1^{-/-}$ mice (Fig. 3A and B). Moreover, there were comparable frequencies of proliferating virus-specific $CD4^+$ and $CD8^+$ T cells, indicating that lack of CCR1 did not alter the generation of an adaptive immune response (Fig. 3C). These data illustrate that chemokine signaling through CCR1 does not affect T-cell proliferation, and suggests that the reduction in T-cell accumulation in the CNS of $CCR1^{-/-}$ mice during acute disease is the result of a deficiency in T-cell trafficking.

Cytolytic activity and IFN-γ secretion

A potential mechanism contributing to the increase in mortality observed at later stages of disease in $CCR1^{-/-}$ mice could relate to enhanced pathogenic activity by T

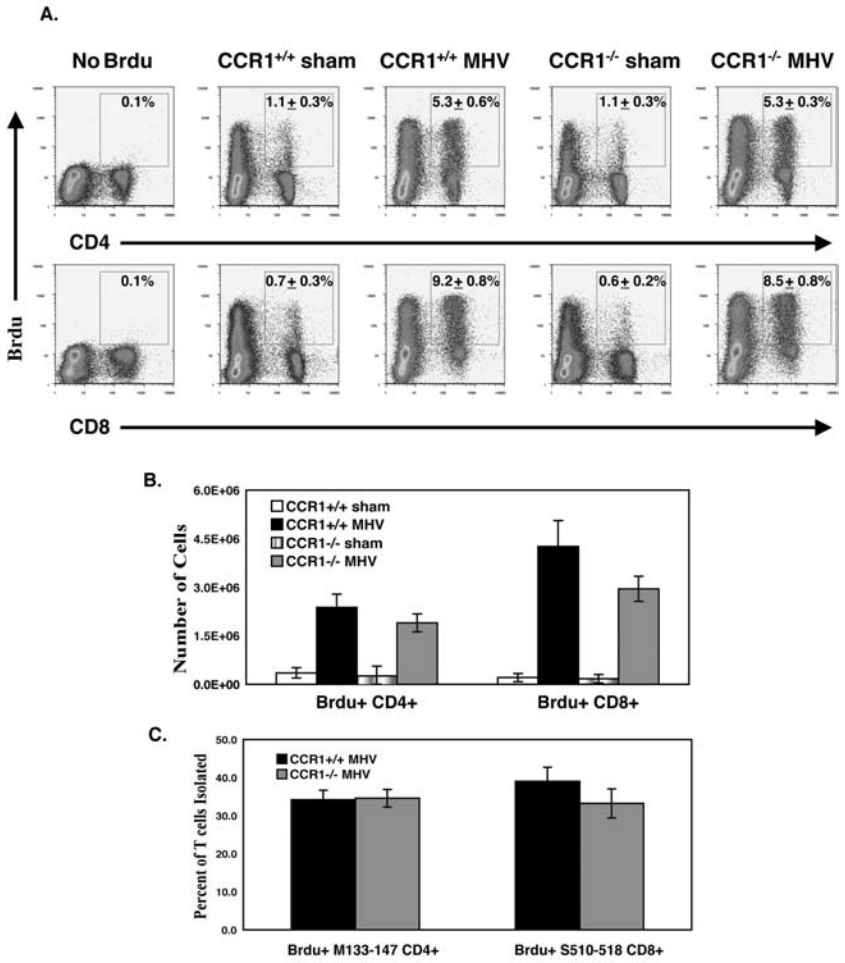

FIG. 3. T-cell proliferation is not affected following MHV infection of CCR1$^{-/-}$ mice. CCR1$^{+/+}$ and CCR1$^{-/-}$ mice were infected IP with MHV and treated with BrdU on days 3, 5, and 6 post-infection. Splenocytes were isolated at day 7 post-infection and BrdU incorporation into either total T cells or virus-specific T cells was determined by flow cytometry. Shown are representative dot blots (**A**) demonstrating BrdU incorporation within T-cell populations with numbers representing the frequency of dual-positive cells, mean ± SEM. The number of total proliferating CD4$^+$ and CD8$^+$ T cells is presented (**B**), as well as the frequency of proliferating virus-specific T cells (**C**). The data presented are representative of three separate experiments with n = 8 for each experimental group. Data are presented as mean ± SEM.

cells resulting in death over an extended period of time. To address this possibility, the cytolytic activity of virus-specific S510-518 CD8$^+$ T cells was evaluated in CCR1$^{-/-}$ mice infected with MHV. Both CCR1$^{+/+}$ and CCR1$^{-/-}$ mice were challenged IP with MHV and the ability of virus-specific CD8$^+$ T cells to lyse target cells was determined. As shown in Fig. 4A, there were no differences in CTL responses between S510-518–specific CD8$^+$ T cells isolated from either CCR1$^{+/+}$ or CCR1$^{-/-}$ mice. A previous study by Miller *et al.* (30) indicated that the absence of CCR1 results in increased IFN-γ secretion following respiratory syncytial virus (RSV) infection. To determine if lack of CCR1 modulated IFN-γ expression, total IFN-γ levels within the brains of infected CCR1$^{+/+}$ and CCR1$^{-/-}$ mice were evaluated by ELISA. However, there were no differences in the amount of IFN-γ in brain homogenates isolated from IC infected CCR1$^{+/+}$ and CCR1$^{-/-}$ mice at any time points examined (Fig. 4B).

Macrophage trafficking and demyelination are not altered in the absence of CCR1

Macrophages are critical in amplifying the severity of myelin damage in mice persistently infected with MHV (14,27,39). The ability of macrophages to traffic into the CNS is dependent on specific chemotactic signals, including ligands for CCR1, derived within the CNS of MHV-infected mice (11,23,36). However, macrophage trafficking into the CNS was not affected in the absence

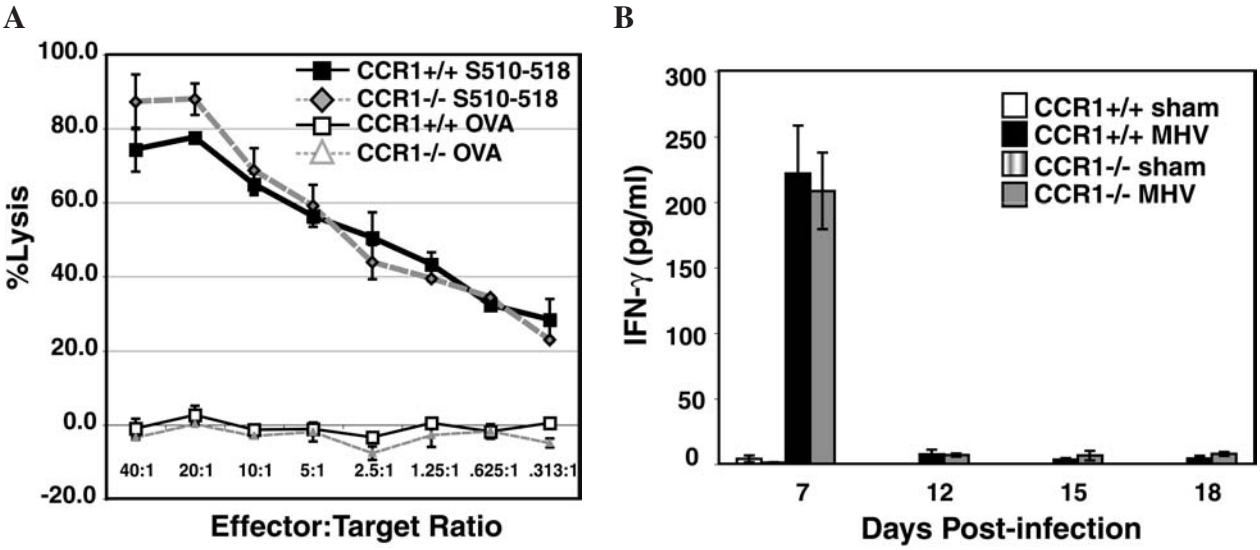

FIG. 4. Cytolytic activity and IFN-γ production. CCR1$^{+/+}$ and CCR1$^{-/-}$ mice were infected IP with MHV and the cytolytic activity of CD8$^+$ T cells was evaluated. (**A**) Splenocytes were obtained at day 7 post-infection, and the ability to lyse targets was determined using ^{51}Cr-loaded target RMA-S cells expressing either S510-518 or ovalbumin (OVA) peptide (control) at varying effector:target ratios. No difference in lytic activity was determined using CD8$^+$ T cells isolated from infected CCR1$^{+/+}$ or CCR1$^{-/-}$ mice. Data are representative from three mice/group and presented as average ± SEM. (**B**) CCR1$^{+/+}$ and CCR1$^{-/-}$ mice were IC infected with MHV and the expression of IFN-γ within the CNS was evaluated at defined time points post-infection. The level of IFN-γ expressed within the CNS was determined by ELISA using clarified supernatant from brain homogenates, and data represent concentrations from tissue supernatants with comparable total protein levels. Data are presented as average ± SEM and represent a minimum of two separate experiments with a minimum of three mice/time point.

of CCR1 at any of the time points examined, indicating that CCR1 signaling is not important for promoting the directional migration or accumulation of these cells within the parenchyma of the CNS in response to MHV infection (Fig. 5A). As a result, there was no difference in the severity of myelin damage between infected CCR1$^{+/+}$ and CCR1$^{-/-}$ mice at any of the time points examined (Fig. 5B and C).

DISCUSSION

CCR1 binds both CCL3 and CCL5, which are expressed in the CNS following infection with MHV (5,8,32). Previous work from our laboratory highlights important and non-redundant roles for these chemokines in regulating both host defense as well as disease progression following MHV infection. For example, antibody-targeting of CCL5 during acute disease results in delayed viral clearance from the CNS along with reduced T-cell entry into the brain (23). Treatment of mice persistently infected with MHV with an anti-CCL5 antibody results in an improvement in clinical disease and a reduction in the severity of myelin destruction correlating with reduced T-cell and macrophage entry into the CNS (11). Additionally, we have demonstrated that MHV infection of CCL3$^{-/-}$ mice affects T-cell egress from secondary lymphoid tissues correlating with impaired expression of chemokine receptors such as CXCR3 and CCR5, that aid in T-cell homing to the CNS (12,13,36).

The chemokine receptor CCR5 also binds both CCL3 and CCL5 (34). MHV infection of mice lacking CCR5 does not dramatically affect survival or viral clearance from the CNS, indicating that CCR5 is not essential in host defense during acute disease (14). However, macrophage infiltration was muted, indicating that signaling through CCR5 enhanced accumulation of these cells into the CNS following viral infection (14). Adoptive transfer of virus-specific T cells lacking CCR5 provided additional information regarding the role of this receptor in T-cell subset trafficking. Although S510-518–specific CCR5$^{-/-}$ CD8$^+$ T cells were able to gain entrance into the CNS of MHV-infected mice, migration of M133-147–specific CD4$^+$ T cells lacking CCR5 was notably reduced (12,13). These findings highlight the non-redundancy in chemokine receptor signaling on different subsets of immune cells with respect to lymphocyte trafficking in this particular model.

Our previous findings that T-cell migration was incompletely impaired in the absence of CCR5 combined with studies demonstrating an importance for both CCL3 and CCL5 for the generation of an effective immune re-

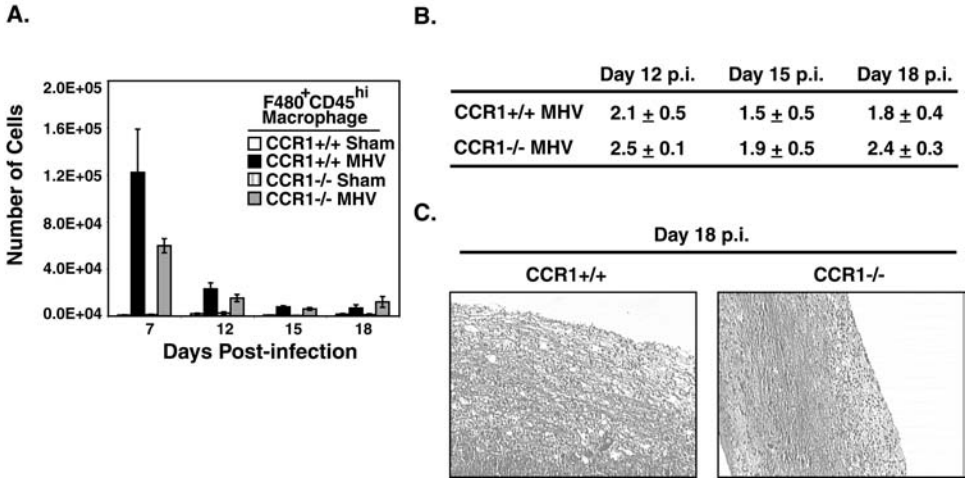

FIG. 5. CCR1 signaling does not regulate macrophage trafficking or the severity of myelin damage. $CCR1^{+/+}$ and $CCR1^{-/-}$ mice were IC infected with MHV and (**A**) numbers of infiltrating macrophages ($F480^{+}CD45^{hi}$) determined by flow cytometry at the indicated time points. Data presented represent a minimum of two separate experiments with a minimum of three mice/experimental group for each time point. Data are presented as average ± SEM. (**B**) Scores of demyelination (average ± SEM) are comparable between MHV-infected $CCR1^{+/+}$ and $CCR1^{-/-}$ mice at all time points examined. (**C**) These are representative spinal cords from infected $CCR1^{+/+}$ and $CCR1^{-/-}$ mice at day 18 post-infection.

sponse to MHV infection suggested that CCR1 signaling also aids in host defense. To address this possibility, the response to IC infection with MHV was evaluated in $CCR1^{-/-}$ mice. Analysis of T-cell infiltration revealed only a reduction in $CD4^{+}$ T-cell accumulation in the brain on day 12 post-infection, during the acute stage of disease. Although the total numbers of virus-specific $CD4^{+}$ T cells recognizing the M133-147 peptide within the CNS was also reduced at day 12 post-infection when compared to wild-type mice, there were no appreciable differences in the chronic stage of disease, at days 15 or 18 post-infection. Similarly, the number of $CD8^{+}$ T cells in the CNS was reduced during the acute stage of disease in $CCR1^{-/-}$ mice as compared to $CCR1^{+/+}$ mice. However, the frequency of infiltrating virus-specific S510-518-$CD8^{+}$ T cells was comparable between $CCR1^{-/-}$ and $CCR1^{+/+}$ mice at all time points examined. These data indicate that CCR1 enhances $CD4^{+}$ and $CD8^{+}$ T-cell infiltration into the CNS, but does not substantially contribute to trafficking during chronic disease. Importantly, these data indicate that the increase in mortality in MHV-infected mice lacking CCR1 was not a result of altered trafficking patterns of T cells.

Consistent with the demonstration that lack of CCR1 did not result in increased mortality during acute disease, viral clearance from the CNS was also not impaired. Although CCR1 contributes to the trafficking of T cells into the CNS during acute infection, the data presented clearly demonstrate that a CCR1 deficiency does not alter T-cell antiviral effector functions. The proliferation of virus-specific T cells and CD8 cytolytic activity were comparable between $CCR1^{+/+}$ and $CCR1^{-/-}$ mice, indicating that CCR1 signaling is not essential in tailoring an effective T-cell response following viral infection. These data are similar to those found in studies examining the functional role of CCR1 following infection with RSV, in which CCR1-deficient mice were able to clear virus yet displayed altered pathophysiologic responses (30). However, unlike RSV, MHV infection of $CCR1^{-/-}$ mice did not modulate the level of IFN-γ expression. MHV infection of $CCR1^{+/+}$ and $CCR1^{-/-}$ mice resulted in similar expression levels of IFN-γ within the CNS. Notably, mice displayed peak expression of IFN-γ on day 7 post-infection, correlating with the height of MHV infection (3). Altogether, these data indicate that CCR1 signaling does not modulate antiviral host defense following infection of the CNS.

CONCLUSION

Although CCR1 signaling does not influence the outcome from acute viral encephalitis, it is clearly important in contributing to survival, as there was a marked increase in mortality in $CCR1^{-/-}$ mice during chronic disease compared to $CCR1^{+/+}$ mice. Importantly, this was not the result of viral recrudescence, as there were no differences in viral titers within the brains or spinal cords between wild-type and knockout mice during the chronic stage of disease. Therefore, it is unlikely that CCR1 signaling on plasma cells is critical in controlling viral replication during chronic disease (24,33). In addi-

tion, there were comparable numbers of macrophages and T cells present within the CNS during chronic disease, and this correlated with similar levels of myelin damage. Therefore these data indicate that the increase in mortality in $CCR1^{-/-}$ mice is not the result of modulated inflammation or demyelination during viral persistence**.** Importantly, the difference in mortality is not a reflection of altered cellular tropism by MHV following infection of $CCR1^{-/-}$ mice compared to $CCR1^{+/+}$ mice. The diminished ability to survive during chronic disease in mice lacking CCR1 may reflect a more subtle role for CCR1 in affecting the biology of glia and/or neurons. Numerous studies have demonstrated that CCR1 is expressed on activated populations of both cells, and chemokine receptor signaling has been implicated as an important factor in promoting neuron survival in the face of inflammatory challenge (4,35). Therefore it is tempting to speculate that within the CNS of mice undergoing chronic inflammation, CCR1 expression is required for protection of resident cells of the CNS from death or irreversible damage.

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