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Lack of CCR2 Results in Increased Mortality and Impaired Leukocyte Activation and Trafficking Following Infection of the Central Nervous System with a Neurotropic Coronavirus¹

Benjamin P. Chen,* William A. Kuziel,[†] and Thomas E. Lane^{2*‡}

In the present study, we evaluated the role of CCR2 in a model of viral-induced neurologic disease. An orchestrated expression of chemokines, including the CCR2 ligands monocyte chemoattractant protein-1/CCL2 and monocyte chemoattractant protein-3/CCL7, occurs within the CNS following infection with mouse hepatitis virus (MHV). Infection of mice lacking CCR2 (CCR2^{-/-}) with MHV resulted in increased mortality and enhanced viral recovery from the brain that correlated with reduced ($p \leq 0.04$) T cell and macrophage/microglial (determined by F4/80 Ag expression, $p \leq 0.004$) infiltration into the CNS. Moreover, MHV-infected CCR2^{-/-} mice displayed a significant decrease in Th1-associated factors IFN- γ ($p \leq 0.001$) and RANTES/CCL5 ($p \leq 0.002$) within the CNS as compared with CCR2^{+/+} mice. Further, peripheral CD4⁺ and CD8⁺ T cells from immunized CCR2^{-/-} mice displayed a marked reduction in IFN- γ production in response to viral Ag and did not migrate into the CNS of MHV-infected recombination-activating gene (RAG)1^{-/-} mice following adoptive transfer. In addition, macrophage/microglial infiltration into the CNS of RAG1^{-/-} mice receiving CCR2^{-/-} splenocytes was reduced ($p \leq 0.05$), which correlated with a reduction in the severity of demyelination ($p \leq 0.001$) as compared with RAG1^{-/-} mice receiving splenocytes from CCR2^{+/+} mice. Collectively, these results indicate an important role for CCR2 in host defense and disease by regulating leukocyte activation and trafficking. *The Journal of Immunology*, 2001, 167: 4585–4592.

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 infection of susceptible mice with neuroadapted strains of mouse hepatitis virus (MHV),³ a positive-strand RNA virus, results in an acute encephalomyelitis followed by a chronic demyelinating disease similar to the pathology of the human demyelinating disease multiple sclerosis (1, 2). The collective evidence indicates that the immune response to MHV infection is critical in host defense as well as the development of demyelination (3–8). Both CD4⁺ and CD8⁺ T lymphocytes are important in clearance of infectious virus from the brain during acute disease (4–7). In addition, recent studies point to a role for T lymphocytes in contributing to macrophage infiltration and activation that leads to myelin destruction (7, 8). One mechanism through which T lymphocytes may accomplish this is the secretion of both cytokines and chemokines. MHV in-

fection of the CNS results in an orchestrated expression of chemokine genes including IFN inducible protein of 10 kDa/CXCL10, monokine induced by IFN- γ /CXCL9, CCL2, macrophage inflammatory protein-1 β /CCL4, CCL5, and CCL7, suggesting an important role for these molecules in participating in leukocyte infiltration (9). Indeed, recent studies from our laboratory have demonstrated that administration of neutralizing antisera specific for either CXCL10 or CXCL9 to MHV-infected mice resulted in increased mortality accompanied by decreased T lymphocyte infiltration and IFN- γ expression as well as delayed viral clearance from the brain (10, 11). In addition, anti-CCL5 treatment of MHV-infected mice resulted in reduced macrophage infiltration into the CNS that correlated with a significant decrease in the severity of demyelination (7). Collectively, these studies indicate an important role for chemokines in host defense and demyelination by attracting T lymphocytes and macrophages into the CNS.

Chemokines activate leukocytes by binding to seven transmembrane receptors coupled to G-proteins (for review, see Refs. 12 and 13). Among the chemokines expressed within the CNS of MHV-infected mice are CCL2 and CCL7 (9). Previous studies have indicated that these chemokines are potent chemoattractants for T cells as well as macrophages, presumably through binding and signaling through CCR2 expressed on the surface of these cells (14–19). Based on experiments that have established that T cells and macrophages are important in both host defense and demyelination, it would be of great interest to determine the functional contributions of CCR2 in these separate processes. To this end, we have infected CCR2^{-/-} mice with MHV and assessed the severity of disease.

Materials and Methods

Virus and mice

MHV-4 (wild type) and MHV-V5A13.1 (containing a 142-aa deletion in the surface glycoprotein) were kindly provided by M. Buchmeier (The Scripps Research Institute, La Jolla, CA) (20). CCR2^{+/+} mice (B6129F2/J,

*Department of Molecular Biology and Biochemistry and [†]Reeve-Irvine Research Center, University of California, Irvine, CA 92612; and [‡]Department of Molecular Genetics and Microbiology and Institute for Cellular and Molecular Biology, University of Texas, Austin, TX 78712

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² Address correspondence and reprint requests to Dr. Thomas E. Lane, Department of Molecular Biology and Biochemistry, University of California, 3205 Biological Sciences II, Irvine, CA 92697-3900. E-mail address: tlane@uci.edu

³ Abbreviations used in this paper: MHV, mouse hepatitis virus; i.c., intracerebral; RAG, recombination-activating gene; RPA, RNase protection assay; LFB, luxol fast blue; EAE, experimental autoimmune encephalomyelitis; p.i., postinfection; MOG, myelin oligodendrocyte glycoprotein.

obtained from The Jackson Laboratory, Bar Harbor, ME), CCR2^{-/-} (129B6F2-Cmkbr2^{tm1Kuz}), and recombination-activating gene (RAG)1^{-/-} mice (B6129S-Rag1^{tm1Mom}, obtained from The Jackson Laboratory) were housed under specific pathogen-free conditions in enclosed filter-top cages (14). RAG1^{-/-} mice do not produce mature T or B lymphocytes (21). Age-matched (5–8 wk) mice were anesthetized by inhalation of methoxyflurane (Pitman-Moore, Washington Crossing, NJ), followed by intracerebral (i.c.) injection with 10 PFU of MHV-V5A13.1 suspended in 30 μ l of sterile saline. Control (sham) animals were injected with sterile saline alone. Mice were sacrificed at scheduled time points, when one-half of each brain was used for plaque assay on the DBT astrocytoma cell line to determine viral burden, and the remaining halves were either fixed for histologic analysis, stored at -80°C for RNA isolation, homogenized in sterile PBS and stored at -20°C for ELISA, or used for FACS analysis (7, 22).

RNase protection assay (RPA)

Total RNA was extracted from brains of MHV-infected and sham animals using TRIzol reagent (Life Technologies, Rockville, MD) (9). Chemokine mRNA transcripts were determined using the mCK-5 multitemplate probe set (BD PharMingen, San Diego, CA). RPAs were performed with 15 μ g total RNA using a previously described protocol (7, 9). For quantification of signal intensity, autoradiographs were scanned, and individual chemokine bands were normalized as the ratio of band intensity to the L32 control present in the probe set (7, 9). Analysis was performed using NIH Image 1.61 software (9, 23, 24).

Mononuclear cell isolation and flow cytometry

Mononuclear cells were obtained from brains of mice as previously described (7). FITC-conjugated rat anti-mouse CD4 and CD8 Abs were used to detect infiltrating CD4⁺ and CD8⁺ T cells (GK1.5 and Ly-2, respectively, obtained from BD PharMingen). FITC-conjugated rat anti-mouse F4/80 (C1:A3-1, Serotec, Oxford, U.K.) was used to detect activated macrophage/microglial cells (7). An isotype-matched FITC Ab was used as a control. Cells were incubated with Abs for 30 min at 4°C , washed, fixed in 1% paraformaldehyde, and analyzed on a FACStar (BD Biosciences, Mountain View, CA) (7). Data are presented as the percentage of positive cells within the gated population.

Intracellular cytokine staining

Intracellular cytokine staining was performed using a previously described procedure (8). In brief, CCR2^{+/+} and CCR2^{-/-} mice were infected i.p. with 2×10^5 PFU of MHV-4. Splenocytes were isolated 8 days after infection, pooled together, and 1×10^6 total cells were stimulated with peptide corresponding to either the CD8 epitope in the surface glycoprotein spanning residues 510–518 (25, 26) or the CD4 epitope present in the membrane protein at residues 133–147 (27). After incubation for 6 h at 37°C in medium containing GolgiStop (Cytofix/CytoPerm kit, BD PharMingen), cells were washed and blocked with PBS containing 10% FBS and a 1/200 dilution of CD16/32 (BD PharMingen). Cells were then stained for surface Ags with either FITC-conjugated CD4, CD8, or rat IgG-2b (as control) for 45 min at 4°C . Cells were fixed and permeabilized using the Cytofix/CytoPerm kit and stained for intracellular IFN- γ using PE-conjugated anti-IFN- γ (1:50; XMG1.2, BD PharMingen) for 45 min at 4°C . Cells were analyzed on a FACStar (7). Data are presented as the percentage of positive cells within the gated population. The absolute numbers of Ag-specific CD4⁺ or CD8⁺ T lymphocytes was calculated by multiplying the fraction of dual-positive cells by the total number of cells obtained from the spleen.

ELISA

Brains were homogenized in 1 ml sterile PBS and centrifuged at $400 \times g$ for 5 min at 4°C . The supernatants were collected and stored at -20°C . Protein levels of cytokines and chemokines in brain samples of MHV-infected and sham mice at days 6 and 7 postinfection (p.i.) were assessed using specific Quantikine M murine immunoassay kits (R&D Systems, Minneapolis, MN) according to the manufacturer's specifications (7, 10).

Adoptive transfer

Splenocytes from CCR2^{+/+} or CCR2^{-/-} mice isolated 8 days p.i. (2×10^5 PFU MHV-4, i.p. injection) were adoptively transferred (5×10^6 cells suspended in 200 μ l sterile HBSS) via injection into the retro-orbital sinus to RAG1^{-/-} or CCR2^{-/-} mice 3 days following i.c. infection with 10 PFU of MHV-V5A13.1 (8, 28). Mice were sacrificed 7 days p.i., and brains and spinal cords were removed. One-half of the brains was used for flow analysis, and the remaining half was used to determine viral titers. Spinal cords were stained with luxol fast blue (LFB) to assess the severity of demyeli-

nation. Control animals included MHV-infected (i.c.), RAG1^{-/-}, and CCR2^{-/-} mice receiving sterile HBSS.

Histology

Spinal cords were fixed by immersion overnight in 10% normal buffered formalin, after which the tissues were embedded in paraffin. The severity of demyelination was scored on slides stained with LFB. Slides were coded and read blindly by three investigators. Scoring was 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 significant increase in myelin stripping; and 4, intense areas of inflammation accompanied by numerous phagocytic cells engulfing myelin debris (7, 24). Scores are presented as mean \pm SEM.

Statistical analysis

Statistically significant differences between groups of mice were determined by *t* test using Sigma-Stat 2.0 software, (Jandel, San Rafael, CA), and *p* values of ≤ 0.05 were considered significant.

Results

MHV-infection of CCR2^{-/-} mice

Intracranial infection of CCR2^{-/-} mice with 10 PFU of MHV-V5A13.1 resulted in a dramatic acceleration in mortality as compared with CCR2^{+/+} mice (Fig. 1). As early as day 5 p.i., CCR2^{-/-} mice began to succumb to infection, and by day 10 p.i., 100% of CCR2^{-/-} mice died. In marked contrast are the results of MHV infection of CCR2^{+/+} mice, of which 100% survived. The increased mortality in CCR2^{-/-} mice correlated with enhanced viral recovery from the brains at days 3 (5.5 ± 0.1 PFU/g, *n* = 4) and 6 p.i. (6.3 ± 0.2 PFU/g, *n* = 8 and *p* ≤ 0.02) when compared with titers present in the brains of infected CCR2^{+/+} mice (day 3, 5.3 ± 0.1 PFU/g, *n* = 3; day 6, 5.4 ± 0.4 PFU/g, *n* = 6) (Table I).

Reduced T cell and microglia/macrophage infiltration into the CNS of MHV-infected CCR2^{-/-} mice

Both CD4⁺ and CD8⁺ T cells are important in clearance of MHV from the CNS (4–7). Therefore, one potential explanation for the acceleration in mortality and increase in viral titers within the brains obtained from MHV-infected CCR2^{-/-} mice is a reduction in T lymphocyte infiltration into the CNS following viral infection.

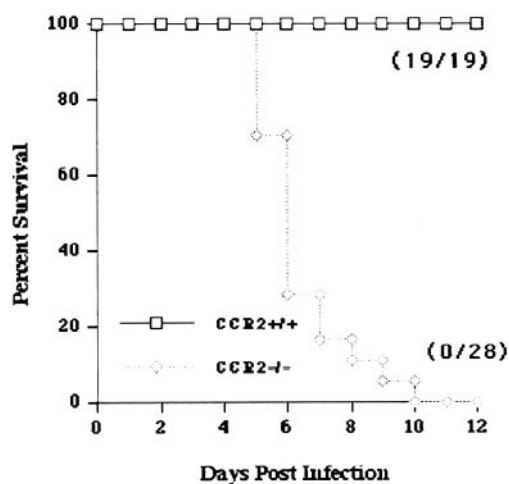


FIGURE 1. Increased mortality in MHV-infected CCR2^{-/-} mice. Mice were infected with 10 PFU of MHV-V5A13.1, and survival was recorded. Infected CCR2^{-/-} mice displayed a marked increase in mortality as compared with CCR2^{+/+} mice such that, by day 10 p.i., 100% of CCR2^{-/-} died, whereas all CCR2^{+/+} mice survived until day 12 p.i. Results presented are from six separate experiments with a total of 19 CCR2^{+/+} mice and 28 CCR2^{-/-} mice.

Table I. Viral burden after i.c. infection with MHV^a

Mouse	Days p.i.	Brain Titer Log (PFU/g)	n
CCR2 ^{+/+}	3	5.3 ± 0.1	3
	6	5.4 ± 0.4	6
	12	3.5 ± 0.2	7
CCR2 ^{-/-}	3	5.5 ± 0.1	4
	6	6.3 ± 0.2 ^b	8
	12	ND	

^a Results presented are from three separate experiments.^b $p \leq 0.02$ as compared to CCR2^{+/+} mice at day 6 p.i.

Immunophenotyping of mononuclear cell infiltrates into the CNS by flow cytometric analysis revealed no differences in leukocyte infiltration between the two populations of mice at day 2 p.i. (Table II). However, by day 4 p.i., CCR2^{-/-} mice exhibited a 45 and 55% reduction in CD4⁺ and CD8⁺ T lymphocyte infiltration, respectively, as compared with CD4⁺ and CD8⁺ T lymphocyte levels present within the CNS of CCR2^{+/+} mice. During acute disease (days 6 and 7 p.i.), there was a significant reduction ($p \leq 0.04$) in CD4⁺ T lymphocyte entry into the CNS of CCR2^{-/-} mice ($5.7\% \pm 1.6\%$, $n = 6$) when compared with levels present in CCR2^{+/+} mice ($14.6\% \pm 2.5\%$, $n = 6$). In addition, analysis of CD8⁺ T lymphocyte infiltration at this time revealed an ~45% decrease in CCR2^{-/-} mice ($8.3\% \pm 2.1\%$, $n = 6$) as compared with CCR2^{+/+} mice ($15.1\% \pm 2.2\%$, $n = 6$). Macrophage/microglial staining (determined by F4/80 Ag expression) was significantly decreased ($p \leq 0.004$) in CCR2^{-/-} mice ($9.5\% \pm 2.2\%$, $n = 3$) in comparison to CCR2^{+/+} mice ($26.6\% \pm 2.0\%$, $n = 3$) during acute disease (Table II). Although levels of F4/80⁺ cells were lower in sham-infected CCR2^{-/-} mice as compared with sham-infected CCR2^{+/+} mice, this difference was not significant (Table II). Therefore, it is unlikely that reduced numbers of resident microglia are present within the CNS of CCR2^{-/-} mice and that this accounts for the clinical disease phenotype.

Diminished Th1 response in the CNS of MHV-infected CCR2^{-/-} mice

Chemokine mRNA transcripts were analyzed in MHV-infected CCR2^{-/-} and CCR2^{+/+} mice at days 2, 4, and 6 p.i. by RPA, and the data are presented in Fig. 2. Both CCR2^{+/+} and CCR2^{-/-} mice exhibited a similar chemokine profile at all time points tested. Semiquantitative analysis of chemokine mRNA signal intensity was performed by densitometric scanning of the autoradiographs. These results indicated that both strains of mice expressed similar levels of chemokine transcripts following infection with virus (Fig. 2, A and B). The only differences detected were at day 6 p.i., in

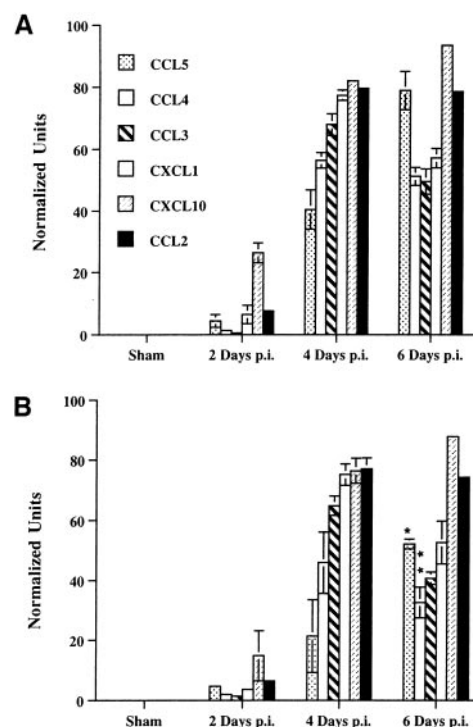


FIGURE 2. RPA analysis showing kinetics of chemokine expression within the brain following MHV infection. Total RNA was isolated from the brains of mice at the indicated time point. No signal for any chemokine tested was detected in sham mice. Shown are densitometric analysis of chemokine transcripts from CCR2^{+/+} mice (A) and CCR2^{-/-} mice (B) performed on the scanned autoradiographs using NIH Image 1.61 software. MHV-infected CCR2^{-/-} mice exhibited significantly decreased transcript levels for CCL5 (*, $p \leq 0.02$) and CCL4 (**, $p \leq 0.002$) at day 6 p.i. as compared with infected CCR2^{+/+} mice. MHV-infected mice: CCR2^{+/+}: day 2, $n = 3$; day 4, $n = 4$; day 6, $n = 3$; CCR2^{-/-} mice: day 2, $n = 2$; day 4, $n = 2$; day 6, $n = 4$. Sham for both strains, $n = 2$. Results are presented as mean \pm SEM and represent two separate experiments.

which CCR2^{-/-} mice displayed reduced levels of CCL5 ($p \leq 0.02$) and CCL4 ($p \leq 0.002$) transcripts in the brains of CCR2^{-/-} mice as compared with transcript levels present in CCR2^{+/+} mice. Analysis of chemokine and cytokine protein expression within the CNS of mice during acute disease (days 6 and 7 p.i.) indicated >80% decrease ($p \leq 0.002$) in CCL5 within CCR2^{-/-} mice (155 ± 36 pg/ml, $n = 5$) as compared with CCR2^{+/+} mice (836 ± 159 pg/ml, $n = 5$) (Table III). Furthermore, there was an ~10-fold reduction in IFN- γ ($p \leq 0.001$) within the CNS of CCR2^{-/-} mice (3.8 ± 0.8 pg/ml, $n = 10$) as compared with CCR2^{+/+} mice

Table II. Reduced leukocyte entry into the CNS of MHV-infected CCR2^{-/-} mice^a

Mouse	MHV	Days p.i.	n	CD4 ⁺	CD8 ⁺	F4/80 ⁺
CCR2 ^{+/+}	+	2	3	2.9 ± 1.6	1.7 ± 0.2	10.1 ± 1.2
CCR2 ^{-/-}	+	2	3	1.2 ± 0.1	1.0 ± 0.5	5.5 ± 1.6
CCR2 ^{+/+}	+	4	3	10.6 ± 2.5	6.0 ± 1.6	19.1 ± 3.3
CCR2 ^{-/-}	+	4	3	5.8 ± 1.6	2.7 ± 0.8	6.1 ± 1.1
CCR2 ^{+/+}	+	6 and 7 ^b	6	14.6 ± 2.5	15.1 ± 3.0	26.6 ± 2.0 ^c
CCR2 ^{-/-}	+	6 and 7	6	5.7 ± 1.6 [*]	8.3 ± 2.1	9.5 ± 2.2 ^{**}
CCR2 ^{+/+}	—	—	4	1.41 ± 0.71	0.24 ± 0.22	9.03 ± 1.58
CCR2 ^{-/-}	—	—	4	0.1 ± 0.2	0.37 ± 0.24	4.01 ± 1.64

^a Results represent three separate experiments. Data are presented as percentage of infiltration and represent mean \pm SEM.^b Analysis of T lymphocyte infiltration was performed at both 6 and 7 days p.i.^c $n = 3$ for F4/80 determination.*, $p \leq 0.04$.**, $p \leq 0.004$.

Table III. Chemokine and cytokine protein expression in the CNS during acute disease^a

Mice	MHV	IFN- γ	IL-4	IL-5	CCL5	CCL2
CCR2 ^{+/+}	+	39.4 \pm 7.2 (8 ^b)	<1 (5)	<1 (5)	836 \pm 159 (5)	5140 \pm 475 (5)
CCR2 ^{+/+}	—	<1 (3)	<1 (3)	<1 (2)	ND ^c	ND
CCR2 ^{-/-}	+	3.8 \pm 0.8 (10)*	3.8 \pm 3.8 (5)	<1 (5)	155 \pm 36 (5)**	5656 \pm 193 (5)
CCR2 ^{-/-}	—	<1 (2)	<1 (2)	<1 (2)	ND	ND

^a Results represent three separate experiments. All data are presented as mean \pm SEM (picograms per milliliter).^b Number of mice.^c Not done.*, $p < 0.001$ as compared to level present in CCR2^{+/+} mice.**, $p \leq 0.002$ as compared to level present in CCR2^{+/+} mice.

(39.4 \pm 7.2 pg/ml, $n = 8$) (Table III). No differences in levels of CCL2 were detected between the two strains of mice, suggesting that the absence of CCR2 did not alter production of the CCL2 ligand (Table III). To determine whether decreased production of the Th1-associated factors IFN- γ and CCL5 correlated with increased levels of Th2 cytokines, CNS levels of IL-4 and IL-5 were determined. As shown in Table III, there were no differences in CNS levels of any of these cytokines between the two strains of mice, suggesting that a Th2 response does not predominate within the CNS in the absence of a robust Th1 response.

CCR2-deficient T cells and macrophage/microglia exhibit impaired trafficking into the CNS following adoptive transfer

The experimental results presented above suggest that leukocyte trafficking into the CNS is impaired in mice lacking CCR2. However, in an attempt to more thoroughly characterize the contributions of CCR2 in contributing to neuroinflammation following MHV infection of the CNS, a series of adoptive transfer experiments were performed. Immune splenocytes were obtained from CCR2^{+/+} mice and transferred i.v. to either RAG1^{-/-} or CCR2^{-/-} mice at 3 days after i.c. infection with MHV. Transfer of immune splenocytes from CCR2^{+/+} mice to MHV-infected RAG1^{-/-} mice resulted in T cell infiltration into the CNS that correlated with a reduction in brain viral titers as compared with MHV-infected RAG1^{-/-} mice receiving only HBSS (Table IV). Surprisingly, transfer of immune CCR2^{+/+} splenocytes did not protect CCR2^{-/-} mice from MHV-induced disease, as all recipient mice ($n = 6$) died by day 6 p.i. Furthermore, adoptive transfer of splenocytes obtained from immunized CCR2^{-/-} mice into RAG1^{-/-} mice resulted in ~2- and 1.3-fold reductions in numbers of CD4⁺ T cells ($p \leq 0.05$) and CD8⁺ T cells, respectively, within the CNS, which correlated with increased titers of virus ($p \leq 0.007$) as compared with RAG1^{-/-} mice receiving immune

CCR2^{+/+} splenocytes (Table IV). In addition, macrophage/microglia infiltration into the CNS was reduced ~2-fold ($p \leq 0.05$) in RAG1^{-/-} recipients of CCR2^{-/-} splenocytes (Table IV). Collectively, these data suggest that expression of CCR2 by both host and donor cells is important in allowing cells to migrate to the CNS and contribute to viral clearance.

CCR2 expression accelerates demyelination

Both T cells and macrophages have been shown to be important in contributing to demyelination in MHV-infected mice (7, 8, 28, 29). To determine whether reduced macrophage entry into the CNS of RAG1^{-/-} recipients of CCR2^{-/-} splenocytes correlated with a reduction in the severity of demyelination, spinal cords were stained with LFB, and myelin damage was evaluated. As shown in Fig. 3, there is a marked reduction in inflammatory foci accompanied by limited myelin stripping in RAG1^{-/-} mice receiving CCR2^{-/-} splenocytes as compared with spinal cords obtained from RAG1^{-/-} mice injected with CCR2^{+/+} splenocytes. Quantitative analysis revealed a reduction ($p \leq 0.001$) in the severity of demyelination in CCR2^{-/-} recipients (0.7 \pm 0.1, $n = 6$) as compared with CCR2^{+/+} recipients (1.9 \pm 0.1, $n = 7$) (Table IV). Control mice showed limited myelin stripping and inflammation (0.6 \pm 0.2, $n = 6$) (Table IV).

CCR2 expression and T cell response to virus

In addition to trafficking, CCR2 may also contribute to the development of an effective T cell response following MHV infection. Previous studies have determined that the immunodominant CD8⁺ T cell epitope is located in the surface glycoprotein at residues 510–518 (25, 26). Xue et al. (27) have mapped a CD4⁺ T cell epitope to the membrane protein located at residues 133–147. Splenocytes from immunized CCR2^{+/+} and CCR2^{-/-} mice were stimulated in vitro with either the CD4 or CD8 viral epitopes, and

Table IV. Analysis of leukocyte infiltration, viral titers, and demyelination in the CNS of MHV-infected-RAG1^{-/-} mice at day 4 following adoptive transfer of splenocytes^a

Mouse	MHV	Received	Infiltration into CNS			Viral Titer Log (PFU/g)	DM ^b	n
			No. of CD4 ⁺ cells	No. of CD8 ⁺ cells	No. of F4/80 ⁺ cells			
RAG1 ^{-/-}	+	CCR2 ^{+/+} splenocytes	2.9 $\times 10^5 \pm 5.4 \times 10^4$	1.2 $\times 10^5 \pm 6.7 \times 10^4$	1.2 $\times 10^6 \pm 2.4 \times 10^5$	5.4 ± 0.1	1.9 ± 0.1	7
RAG1 ^{-/-}	+	CCR2 ^{-/-} splenocytes	1.5 $\times 10^5 \pm 1.8 \times 10^4$ ^d	9.0 $\times 10^4 \pm 2.3 \times 10^4$	5.6 $\times 10^5 \pm 6.2 \times 10^4$ ^d	6.0 ± 0.1 ^e	0.7 ± 0.1 ^f	6
RAG1 ^{-/-}	+	HBSS	BD ^c	BD	1.9 $\times 10^5 \pm 5.3 \times 10^3$ ^d	5.8 ± 0.1 ^e	0.6 ± 0.2 ^f	6

^a Results are from two separate experiments. All data are presented as mean \pm SEM.^b Demyelination scores were assigned on a scale from 0 to 4 as described in *Materials and Methods*.^c Below level of detection.^d $p \leq 0.05$ as compared to recipients of wild-type splenocytes.^e $p \leq 0.007$ as compared to recipients of wild-type splenocytes.^f $p \leq 0.001$ as compared to recipients of wild-type splenocytes.

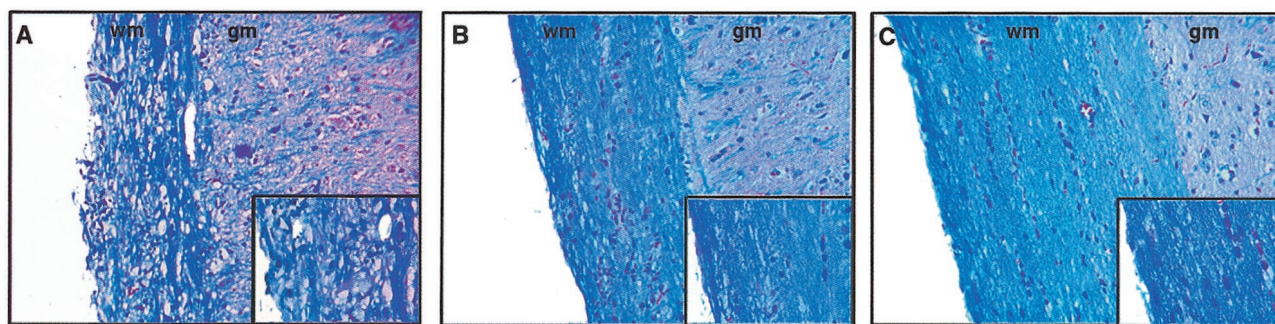


FIGURE 3. Demyelination is reduced in CCR2^{-/-} mice. LFB staining was performed on spinal cords of MHV-infected RAG1^{-/-} mice receiving splenocytes that were adoptively transferred from either immunized CCR2^{+/+} or CCR2^{-/-} mice. Increased inflammation and demyelination are observed in the white matter tract of RAG1^{-/-} mice receiving CCR2^{+/+} splenocytes as compared with recipients of CCR2^{-/-} splenocytes. Representative spinal cord sections from RAG1^{-/-} mice that have received CCR2^{+/+} splenocytes (A), RAG1^{-/-} mice that have received CCR2^{-/-} splenocytes (B), or RAG1^{-/-} mice that have received HBSS only (C). Wm, white matter; gm, gray matter. Magnification, $\times 100$; insert, $\times 400$.

intracellular staining for IFN- γ was performed. Such analysis revealed an ~ 6 -fold reduction ($p \leq 0.05$) in the number of CD8⁺ T cells responding to the surface 510–518 epitope in CCR2^{-/-} mice ($7.9 \times 10^4 \pm 5.1 \times 10^4$ cells) as compared with CCR2^{+/+} mice ($4.6 \times 10^5 \pm 7.5 \times 10^4$ cells) (Fig. 4, A and B). In addition, there was an ~ 2.5 -fold reduction ($p \leq 0.05$) in numbers of CD4⁺ T cells responding to the membrane 133–147 epitope in CCR2^{-/-} mice ($4.7 \times 10^4 \pm 2.0 \times 10^4$ cells) when compared with CCR2^{+/+} mice ($1.2 \times 10^5 \pm 2.0 \times 10^4$) (Fig. 4, C and D).

Discussion

CCR2 is the primary receptor for CCL2 and is found on monocytes, activated T cells, B cells, and NK cells (30–33). Recent studies have indicated that CCR2^{-/-} mice display deficiencies in T lymphocyte and macrophage trafficking in response to stimuli or infection (14, 34–36). Previous studies have also demonstrated that CCL2 can activate T lymphocytes and macrophages, presumably through binding to CCR2 expressed on the surface of these cells (15–17, 37). Indeed, leukocyte populations from CCR2-deficient mice do not bind CCL2 and exhibit defects in activation and recruitment to sites of inflammation (14, 18, 19). Infection of CCR2-deficient mice with various microbial pathogens has increased our understanding of how this receptor contributes to immunological events involved with host protection. For example, CCR2-deficient mice infected in the lungs with *Cryptococcus neoformans* or in the skin with *Leishmania major* exhibit a diminished protective Th1 response accompanied by a switch to a strong Th2 response (35, 38). This switch in Th responses correlates with prolonged infection and greater dissemination of the infecting agent throughout the body. Thus, CCR2 is required for the development of a protective Th1 response in both the lungs and skin. Analysis of CCR2-deficient mice infected with *L. donovani* or immunized with Schistosomal Ag revealed an impaired cellular immune response characterized by reduced T lymphocyte and macrophage recruitment to sites of Ag localization as well as decreased expression of IFN- γ (36, 39). Collectively, these studies strongly suggest that CCR2 expression is required for the development of protective Th1 responses following microbial challenge.

In the present study, we provide evidence that supports and extends these previous observations. The major finding presented in this report is that CCR2 signaling is important in regulating events contributing to a protective immune response following MHV infection of the CNS. These data are consistent with a recent report by Dawson et al. (34) that demonstrated enhanced recovery of influenza A virus from the lungs of infected CCR2^{-/-} mice as compared with wild-type mice, which correlated with a delayed T

cell response to virus. Our results indicate that mice lacking CCR2 exhibited accelerated mortality accompanied by enhanced recovery of MHV from the brain. Although the reasons contributing to death in MHV-infected CCR2^{-/-} mice are unknown, underlying factors include the dramatic reduction in numbers of CD4⁺ and CD8⁺ T cells infiltrating into the CNS. Numerous studies have documented the importance of both populations of cells in controlling viral replication within the CNS and protecting mice from death (4–7). Moreover, the dramatic decrease in IFN- γ expression within the brains of infected CCR2^{-/-} mice may also contribute to the increased viral burden, as this cytokine has been shown to contribute to clearance of MHV from the brain (40). Collectively, these results indicate that CCR2^{-/-} mice are not able to mount a protective Th1 response characterized by T cell infiltration and IFN- γ production, and this may contribute to viral spread within the brain and enhanced mortality.

Interestingly, the brains of infected CCR2-deficient mice did not show increased levels of Th2 cytokines, and expression of the Th2-associated chemokine receptors CCR3 and CCR4 was undetectable (data not shown). The absence of a strong shift from a Th1 to a Th2 response in the MHV-infected CCR2-deficient mice stands in sharp contrast to results from the earlier infection studies with *C. neoformans*, *L. major*, and *L. donovani* (35, 38, 39). Together, these data are consistent with the hypothesis that there are organ-specific and/or pathogen-specific differences in immune responses and that the response in the CNS differs markedly with the response generated in the lungs and skin. It is likely that differential expression of soluble factors underlies the development of a Th2 response in the lungs and skin in the absence of CCR2. There is compelling evidence, including information from CCL2-deficient mice, that CCL2 itself is critical for the development of Th2 responses (41–43), an idea that appears at odds with the strong Th2 responses exhibited by mice lacking the CCR2 receptor for CCL2 (35, 38). The fact that high exogenous amounts of CCL2 show biological activity in CCR2-deficient mice suggests that there might be another receptor for CCL2 (14) and that the Th2-inducing effects are mediated by this receptor. Although others have also suggested the possibility of a second, functional CCL2 receptor (44, 45), such a CCL2 receptor has not been unambiguously identified thus far. An alternative hypothesis follows from the observation that significant accumulation of CCL2 occurs in CCR2-deficient mice after infection or immunization (35, 46). Unnaturally high levels of CCL2 that build up in the absence of CCR2 might allow promiscuous binding to one or more other chemokine receptors, a situation that would not occur in wild-type mice or in CCL2-deficient mice. The fact that we did not find a difference in

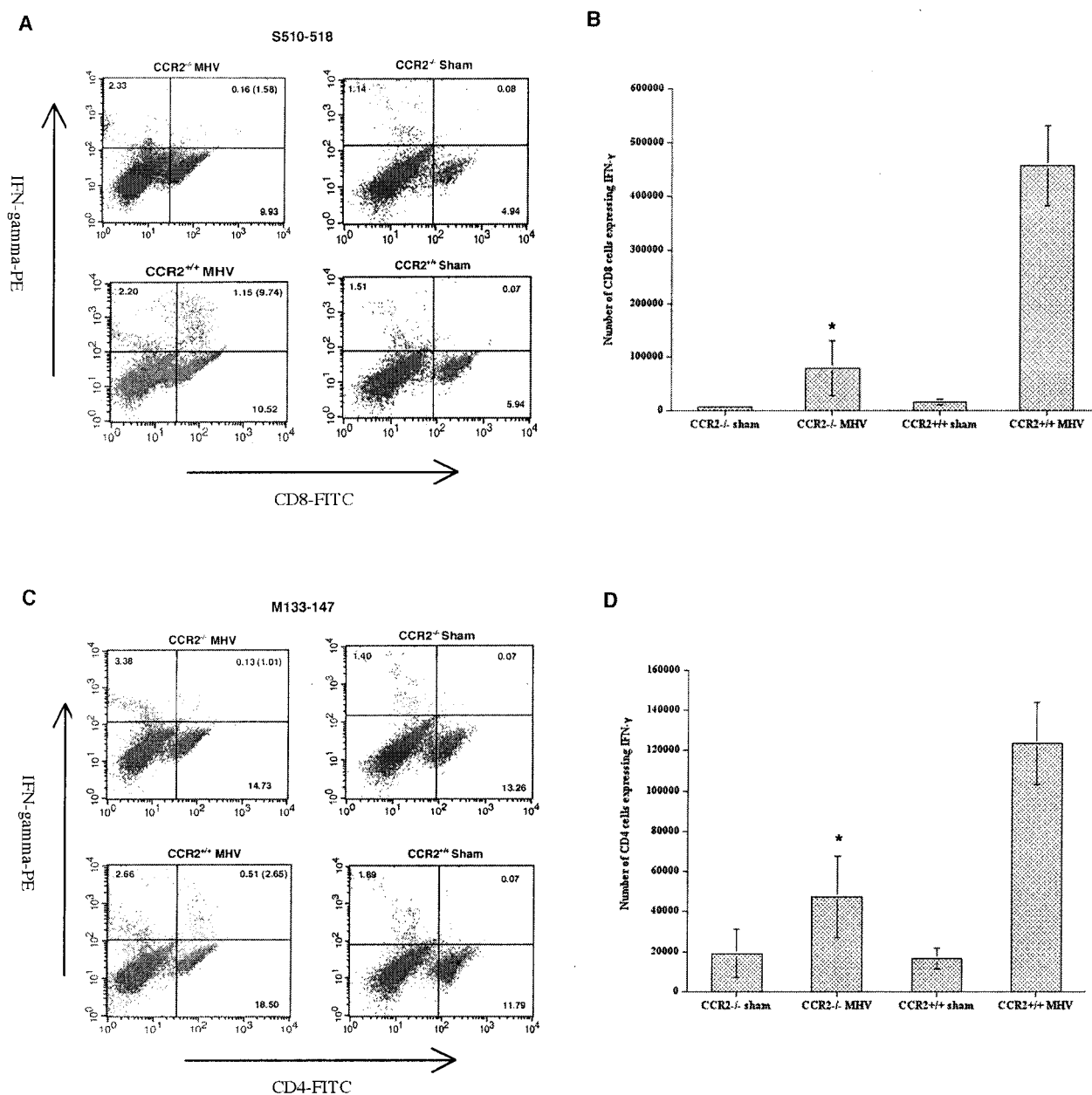


FIGURE 4. Analysis of MHV-specific T cells. Lymphocytes were harvested from spleens at 8 days after immunization with MHV, and the CD4 and CD8 response to virus was determined. Cells were stained for either CD8 or CD4, and IFN- γ expression was evaluated following stimulation with the CD8 epitope surface 510–518 (S510–518) (A) or the CD4 epitope membrane 133–147 (M133–147) (C). The percentage of dual-positive cells in infected mice is indicated in the parentheses in the upper-right quadrants of A and C. Total numbers of dual-positive CD8⁺ T cells responding to the surface 510–518 epitope membrane are shown in B. Similarly, total numbers of dual-positive CD4⁺ T cells responding to the membrane 133–147 epitope are shown in D. Total numbers of cells were determined as described in *Materials and Methods* and represent the results of pooled splenocytes from two separate experiments with a minimum of three mice per experiment (*, $p \leq 0.05$).

CCL2 levels in the MHV-infected CNS of wild-type mice or CCR2-deficient mice may account for the lack of a switch to a Th2 response in the knockout animals (Table III).

The defining role of chemokines and their receptors is leukocyte activation and trafficking to areas of infection and injury. In support of this is the demonstration of the dramatic reduction in T cell and macrophage/microglia infiltration into the CNS of CCR2^{-/-} mice following i.c. infection with MHV, indicating that CCR2 is important in allowing migration of leukocytes into the CNS following infection. Adoptive transfer experiments confirmed these observations. Transfer of splenocytes obtained from MHV-infected CCR2^{-/-} mice into MHV-infected RAG1^{-/-} animals re-

vealed a diminished ability of T cells to enter the brain and eliminate virus (Table IV). Surprisingly, similar results were obtained following adoptive transfer of immune splenocytes from CCR2^{+/+} mice into MHV-infected CCR2^{-/-} mice, as all recipient mice died by day 6 p.i., presumably due to an inability of T cells to enter the brain and participate in viral clearance because these mice exhibited similar mortality as CCR2^{-/-} mice that did not receive donor splenocytes. Although these data indicate that CCR2 expression on cells present in both donor and recipient mice is important in T cell trafficking, these results also suggest that CCR2 may be important in the development of an effective T cell response following MHV infection. In support of this possibility is the demonstration of a

pronounced decrease in the numbers of CD4⁺ and CD8⁺ T cells responding to defined viral epitopes, as determined by intracellular staining for IFN- γ (Fig. 4). These results support and extend earlier studies that implied that CCR2-dependent production of IFN- γ by T cells is important in host defense following infection with *C. neoformans* (35) or *L. donovani* (39). Moreover, Izikson et al. (47) reported that T cells from CCR2^{-/-} immunized mice showed decreased Ag-induced proliferation and IFN- γ production compared with wild-type mice in the experimental autoimmune encephalomyelitis (EAE) model following challenge with myelin oligodendrocyte glycoprotein (MOG). Whether the reduction in Ag-specific T cells present within the spleens of MHV-infected CCR2^{-/-} mice is the result of impaired CCR2 signaling by T cells or the result of impaired trafficking of APCs, as has recently been reported, is unknown at this time (39, 48).

In addition to CCR2, two other CC chemokine receptors, CCR1 and CCR5, have been shown to regulate the gateway for inflammatory cell entry into the CNS. CCR1-deficient mice have reduced CNS inflammatory responses in the MOG-induced EAE model (49), and CCR5-deficient mice have negligible inflammation after disseminated *C. neoformans* infection of the CNS (50). Although it may be assumed that the absence of CCR1, CCR2, or CCR5 from leukocytes is responsible for these phenotypic changes, a recent report by Andjelkovic and Pachter (51) suggests that the expression of CCR1, CCR2, and CCR5 on brain microvessels might also have a role in allowing leukocytes to traverse the blood-brain barrier. This idea is supported by the fact that adoptive transfer of immune splenocytes obtained from CCR2^{+/+} mice failed to protect MHV-infected CCR2^{-/-} mice from death, suggesting that leukocytes were not able to enter the brain. It will be interesting to determine the relative roles of these chemokine receptors on leukocytes and on the brain microvascular endothelium in mediating inflammatory responses in the CNS.

Our present results with intracranial injection of MHV into CCR2-deficient mice are consistent with the recent demonstration that CCR2 is critical for CNS inflammatory responses induced by s.c. immunization with MOG in a model of EAE (47, 52). CCR2-deficient mice were strongly protected against disease induction, and the animals demonstrated little evidence of CNS inflammation. Moreover, Fife et al. (52) have correlated the reduction in disease severity with reduced macrophage entry into the CNS. These data are consistent with the data presented in this study showing that CCR2 is important in macrophage trafficking into the CNS of MHV-infected mice and contributing to demyelination (Fig. 3 and Table IV) (47, 52). The data from these two very different models demonstrate that CCR2 is important in regulating CNS inflammation. It also appears that CCR2 is important for inflammatory cell invasion of the peripheral nervous system in a mouse model of Wallerian degeneration (53). In addition to CCR2, recent studies from our laboratory have documented the importance of CCR5 in contributing to macrophage infiltration into the CNS and demyelination in MHV-infected mice (54). Therefore, macrophage/microglia expression of both CCR2 and CCR5 is important in allowing macrophage/microglia to migrate to white matter tracts and participate in myelin destruction. We have detected reduced expression of chemokine receptor transcripts including CCR5 within the brains of CCR2^{-/-} mice following infection with MHV (data not shown), suggesting that receptor cross-talk signaling may be necessary for optimal expression of chemokine receptors.

In addition to CCL2, other chemokines contribute to host defense following MHV infection of the CNS. Neutralization of the T cell chemoattractant CXCL9 or CXCL10 by administration of specific antisera to MHV-infected mice resulted in reduced T lymphocyte infiltration of the CNS, a dramatic reduction in IFN- γ

expression, and consequently, an increased viral burden within the CNS accompanied by increased mortality (10, 11). The data presented here extend this work and demonstrate unambiguously that CCR2 is crucial for the development of an effective host response against MHV infection of the CNS. These data further illustrate the complexity of chemokine and chemokine receptor interactions and expression patterns as they relate to host defense mechanisms following viral challenge of the CNS. More importantly, it is clear that these proinflammatory molecules exert nonredundant roles in generating a protective Th1 response against MHV infection of the CNS.

References

- Houtman, J. J., and J. O. Fleming. 1996. Pathogenesis of mouse hepatitis virus-induced demyelination. *J. Neurovirol.* 2:361.
- Lane, T. E., and M. J. Buchmeier. 1997. Murine coronavirus infection: a paradigm for virus-induced demyelinating disease. *Trends Microbiol.* 5:9.
- Houtman, J. J., and J. O. Flemming. 1996. Dissociation of demyelination and viral clearance in congenitally immunodeficient mice infected with murine coronavirus JHM. *J. Neurovirol.* 2:101.
- Pearce, B. D., M. V. Hobbs, T. S. McGraw, and M. J. Buchmeier. 1994. Cytokine induction during T-cell-mediated clearance of mouse hepatitis virus from neurons in vivo. *J. Virol.* 68:5483.
- Williamson, J. S. P., and S. A. Stohman. 1990. Effective clearance of mouse hepatitis virus from the central nervous system requires both CD4⁺ and CD8⁺ T cells. *J. Virol.* 64:4589.
- Yamaguchi, K., N. Goto, S. Kyuwa, M. Hayami, and Y. Toyoda. 1991. Protection of mice from a lethal coronavirus infection in the central nervous system by adoptive transfer of virus-specific T cell clones. *J. Neuroimmunol.* 32:1.
- Lane, T. E., M. T. Liu, B. P. Chen, V. C. Asensio, R. M. Samawi, A. D. Paoletti, I. L. Campbell, S. L. Kunkel, H. S. Fox, and M. J. Buchmeier. 2000. A central role for CD4⁺ T cells and RANTES in virus-induced central nervous system inflammation and demyelination. *J. Virol.* 74:1420.
- Wu, G. F., A. A. Dandekar, L. Pewe, and S. Perlman. 2000. CD4 and CD8 T cells have redundant but not identical roles in virus-induced demyelination. *J. Immunol.* 165:2278.
- Lane, T. E., V. C. Asensio, N. Yu, A. D. Paoletti, I. L. Campbell, and M. J. Buchmeier. 1998. Dynamic regulation of α - and β -chemokine expression in the central nervous system during mouse hepatitis virus-induced demyelination disease. *J. Immunol.* 160:970.
- Liu, M. T., B. P. Chen, P. Oertel, M. J. Buchmeier, D. Armstrong, T. A. Hamilton, and T. E. Lane. 2000. The T cell chemoattractant IFN-inducible protein 10 is essential in host defense against viral-induced neurologic disease. *J. Immunol.* 165:2327.
- Liu, M. T., D. Armstrong, T. A. Hamilton, and T. E. Lane. 2001. Expression of Mig (monokine induced by interferon- γ) is important in T lymphocyte recruitment and host defense following viral infection of the central nervous system. *J. Immunol.* 166:1790.
- Luster, A. D. 1998. Chemokines—chemotactic cytokines that mediate inflammation. *N. Engl. J. Med.* 338:436.
- Murphy, P. M., M. Baggiolini, I. F. Charo, C. A. Hebert, R. Horuk, K. Matsushima, L. H. Miller, J. J. Oppenheim, and C. A. Power. 2000. International union of pharmacology. XXII. Nomenclature for chemokine receptors. *Pharmacol. Rev.* 52:145.
- Kuziel, W. A., S. J. Morgan, T. C. Dawson, S. Griffin, O. Smithies, K. Ley, and N. Maeda. 1997. Severe reduction in leukocyte adhesion and monocyte extravasation in mice deficient in CC chemokine receptor 2. *Proc. Natl. Acad. Sci. USA* 94:12053.
- Carr, M. W., S. J. Roth, E. Luther, S. S. Rose, and T. A. Springer. 1994. Monocyte chemoattractant protein 1 acts as a T-lymphocyte chemoattractant. *Proc. Natl. Acad. Sci. USA* 91:3652.
- Gu, L., B. Rutledge, J. Fiorillo, C. Ernst, I. Grewal, R. Flavell, R. Gladue, and B. J. Rollins. 1997. In vivo properties of monocyte chemoattractant protein-1. *J. Leukocyte Biol.* 62:577.
- Rollins, B. J. 1996. Monocyte chemoattractant protein 1: a potential regulator of monocyte recruitment in inflammatory disease. *Mol. Med. Today* 2:198.
- Boring, L., J. Gosling, S. W. Chensue, S. L. Kunkel, R. V. Farese, Jr., H. E. Broxmeyer, and I. F. Charo. 1997. Impaired monocyte migration and reduced type 1 (Th1) cytokine responses in C-C chemokine receptor 2 knockout mice. *J. Clin. Invest.* 100:2552.
- Kurihara, T., G. Warr, J. Loy, and R. Bravo. 1997. Defects in macrophage recruitment and host defense in mice lacking the CCR2 chemokine receptor. *J. Exp. Med.* 186:1757.
- Dalziel, R. G., P. W. Lampert, P. J. Talbot, and M. J. Buchmeier. 1986. Site-specific alteration of murine hepatitis virus type 4 peplomer glycoprotein E2 results in reduced neurovirulence. *J. Virol.* 59:463.
- Mombaerts, P., J. Iacomini, R. S. Johnson, K. Herrup, S. Tonegawa, and V. E. Papaioannou. 1992. RAG-1-deficient mice have no mature B and T lymphocytes. *Cell* 68:869.
- Hirano, N., T. Murakami, K. Fujiwara, and M. Matsumoto. 1978. Utility of mouse cell line DBT for propagation and assay of mouse hepatitis virus. *Jpn. J. Exp. Med.* 48:71.

23. Asensio, V. C., and I. L. Campbell. 1997. Chemokine gene expression in the brains of mice with lymphocytic choriomeningitis. *J. Virol.* 71:7832.
24. Lane, T. E., H. S. Fox, and M. J. Buchmeier. 1999. Inhibition of nitric oxide synthase-2 reduces the severity of mouse hepatitis virus-induced demyelination: implications for NOS2/NO regulation of chemokine expression and inflammation. *J. Neurovirol.* 5:48.
25. Castro, R. F., and S. Perlman. 1995. CD8⁺ T cell epitopes within the surface glycoprotein of neurotropic coronavirus and correlation with pathogenicity. *J. Virol.* 69:8127.
26. Bergmann, C. C., Q. Yao, M. Lin, and S. A. Stohlman. 1996. The JHM strain of mouse hepatitis virus induces a spike protein-specific D^b-restricted CTL response. *J. Gen. Virol.* 77:315.
27. Xue, S., A. Jaszewski, and S. Perlman. 1995. Identification of a CD4⁺ T cell epitope within the M protein of a neurotropic coronavirus. *Virology* 208:173.
28. Wu, G. F., and S. Perlman. 1999. Macrophage infiltration, but not apoptosis, is correlated with immune-mediated demyelination following murine infection with a neurotropic coronavirus. *J. Virol.* 73:8771.
29. Wang, F., S. Stohlman, and J. Fleming. 1990. Demyelination induced by murine hepatitis virus JHM (MHV-4) is immunologically mediated. *J. Neuroimmunol.* 30:31.
30. Frade, J. M., M. Mellado, G. del Real, J. C. Gutierrez-Ramos, P. Lind, and A. C. Martinez. 1997. Characterization of the CCR2 chemokine receptor: functional CCR2 receptor expression in B cells. *J. Immunol.* 159:5576.
31. Rabin, R. L., M. K. Park, F. Liao, R. Swofford, D. Stephany, and J. M. Farber. 1999. Chemokine receptor responses on T cells are achieved through regulation of both receptor expression and signaling. *J. Immunol.* 162:3840.
32. Boring, L., J. Gosling, F. S. Monteclaro, A. J. Lusis, C. L. Tsou, and I. F. Charo. 1996. Molecular cloning and functional expression of murine JE (monocyte chemoattractant protein 1) and murine macrophage inflammatory protein 1 α receptors: evidence for two closely linked C-C chemokine receptors on chromosome 9. *J. Biol. Chem.* 271:7551.
33. Polentarutti, N., P. Allavena, G. Bianchi, G. Giardina, A. Basile, S. Sozzani, A. Mantovani, and M. Introna. 1997. IL-2-regulated expression of the monocyte chemoattractant protein-1 receptor (CCR2) in human NK cells: characterization of a predominant 3.4-kilobase transcript containing CCR2B and CCR2A sequences. *J. Immunol.* 158:2689.
34. Dawson, T. C., M. A. Beck, W. A. Kuziel, F. Henderson, and N. Maeda. 2000. Contrasting effects of CCR5 and CCR2 deficiency in the pulmonary inflammatory response to influenza A virus. *Am. J. Pathol.* 156:1951.
35. Traynor, T. R., W. A. Kuziel, G. B. Toews, and G. B. Huffnagle. 2000. CCR2 expression determines T1 versus T2 polarization during pulmonary *Cryptococcus neoformans* infection. *J. Immunol.* 164:2021.
36. Warmington, K. S., L. Boring, J. H. Ruth, J. Sonstein, C. M. Hogaboam, J. L. Curtis, S. L. Kunkel, I. R. Charo, and S. W. Chensue. 1999. Effect of C-C chemokine receptor 2 (CCR2) knockout on type-2 (Schistosoma antigen-elicited) pulmonary granuloma formation. *Am. J. Pathol.* 154:1407.
37. Furutani, Y., H. Nomura, M. Notake, Y. Oyamada, T. Fukui, M. Yamada, C. G. Larsen, J. J. Oppenheim, and K. Matsushima. 1989. Cloning and sequencing of the cDNA for human monocyte chemoattractant and activating factor (MCAF). *Biochem. Biophys. Res. Commun.* 159:249.
38. Sato, N., S. K. Ahuja, M. Quinones, V. Kostechki, R. L. Reddick, P. C. Melby, W. A. Kuziel, and S. S. Ahuja. 2000. CC chemokine receptor (CCR)2 is required for langerhans cell migration and localization of T helper cell type 1 (Th1)-inducing dendritic cells: absence of CCR2 shifts the *Leishmania major*-resistant phenotype to a susceptible state dominated by Th2 cytokines, b cell outgrowth, and sustained neutrophilic inflammation. *J. Exp. Med.* 192:205.
39. Sato, N., W. A. Kuziel, P. C. Melby, R. L. Reddick, V. Kostechki, W. Zhao, N. Maeda, S. K. Ahuja, and S. S. Ahuja. 1999. Defects in the generation of IFN- γ are overcome to control infection with *Leishmania donovani* in CC chemokine receptor (CCR)5-, macrophage inflammatory protein-1 α -, or CCR2-deficient mice. *J. Immunol.* 163:5519.
40. Parra, B., D. R. Hinton, N. W. Marten, C. C. Bergmann, M. T. Lin, C. S. Yang, and S. A. Stohlman. 1999. IFN- γ is required for viral clearance from central nervous system oligodendroglia. *J. Immunol.* 162:1641.
41. Karpus, W. J., N. W. Kukacs, K. J. Kennedy, W. S. Smith, S. D. Hurst, and T. A. Barrett. 1997. Differential CC chemokine-induced enhancement of T helper cell cytokine production. *J. Immunol.* 158:4129.
42. Karpus, W. J., K. J. Kennedy, S. L. Kunkel, and N. W. Lukacs. 1998. Monocyte chemoattractant protein 1 regulates oral tolerance induction by inhibition of T helper cell 1-related cytokines. *J. Exp. Med.* 187:733.
43. Gu, L., S. Tseng, R. M. Horner, C. Tam, M. Loda, and B. J. Rollins. 2000. Control of TH2 polarization by the chemokine monocyte chemoattractant protein-1. *Nature* 404:407.
44. Heesen, M., S. Tanabe, M. A. Berman, I. Yoshizawa, Y. Luo, R. J. Kim, T. W. Post, C. Gerard, and M. E. Dorf. 1996. Mouse astrocytes respond to the chemokines MCP-1 and KC but reverse transcriptase polymerase chain reaction does not detect mRNA for the KC or new MCP-1 receptor. *J. Neurosci. Res.* 45:382.
45. Schweickart, V. L., A. Epp, C. J. Raport, and P. W. Gray. 2000. CCR11 is a functional receptor for the monocyte chemoattractant protein family of chemokines. *J. Biol. Chem.* 275:9550.
46. Kim, Y., S. Sung, W. A. Kuziel, S. Feldman, S. M. Fu, and C. E. Rose, Jr. 2001. Enhanced airway Th2 response after allergen challenge in mice deficient in CC chemokine receptor-2 (CCR2). *J. Immunol.* 166:5183.
47. Izikson, L., R. S. Klein, I. F. Charo, H. L. Weiner, and A. D. Luster. 2000. Resistance to experimental autoimmune encephalomyelitis in mice lacking the CC chemokine receptor (CCR)2. *J. Exp. Med.* 192:1075.
48. Peters, W., M. Dupuis, and I. F. Charo. 2000. A mechanism for the impaired IFN- γ production in C-C chemokine receptor (CCR2) knockout mice: role of CCR2 in linking the innate and adaptive immune responses. *J. Immunol.* 165:7072.
49. Rottman, J. B., A. J. Slavin, R. Silva, H. L. Weiner, C. G. Gerald, and W. W. Hancock. 2000. Leukocyte recruitment during onset of experimental allergic encephalomyelitis is CCR1 dependent. *Eur. J. Immunol.* 30:2372.
50. Huffnagle, G. B., L. K. McNeil, R. A. McDonald, J. W. Murphy, G. B. Toews, N. Maeda, and W. A. Kuziel. 1999. Role of C-C chemokine receptor 5 in organ-specific and innate immunity to *Cryptococcus neoformans*. *J. Immunol.* 163:4642.
51. Andjelkovic, A. V., and J. S. Pachter. 2000. Characterization of binding sites for chemokines MCP-1 and MIP-1 α on human brain microvessels. *J. Neurochem.* 75:1898.
52. Fife, B. T., G. B. Huffnagle, W. A. Kuziel, and W. J. Karpus. 2000. CC chemokine receptor 2 is critical for induction of experimental autoimmune encephalomyelitis. *J. Exp. Med.* 192:899.
53. Siebert, H., A. Sachse, W. A. Kuziel, N. Maeda, and W. Bruck. 2000. The chemokine receptor CCR2 is involved in macrophage recruitment to the injured peripheral nervous system. *J. Neuroimmunol.* 110:177.
54. Glass, W. G., M. T. Liu, W. A. Kuziel, and T. E. Lane. 2001. Reduced macrophage infiltration and demyelination in mice lacking the chemokine receptor CCR5 following infection with a neurotropic coronavirus. *Virology* 288:8.