Journal of NeuroVirology, 15: 334–342, 2009

ISSN 1355-0284 print / 1538-2443 online
DOI: 10.1080/13550280903062797

informa
healthcare

Reduced lymphocyte infiltration during cytomegalovirus brain infection of interleukin-10–deficient mice

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Interleukin (IL)-10 deficiency results in highly elevated levels of interferon (IFN)-γ, as well as the IFN-γ–inducible chemokines CXCL9 and CXCL10 within murine cytomegalovirus (MCMV)-infected brains. To test the hypothesis that these elevated chemokine levels would result in enhanced brain infiltration, we compared immune cell infiltration in response to MCMV brain infection between wild-type and IL-10 knockout (KO) mice. Longitudinal analysis following adoptive transfer of cells from β-actin–luciferase transgenic wild-type mice showed maximal brain infiltration by peripheral immune cells occurred at 5 days post infection. Although the overall percentage of CD45(hi) cells infiltrating the brain was not altered by IL-10 deficiency, paradoxically, despite elevated chemokine levels, reduced T lymphocyte (CD8+) and natural killer (NK) (CD49b+) cell infiltration into the brain was observed in IL-10–deficient animals. This decreased lymphocyte infiltration was associated with elevated levels of the lymph node homing receptor L-selectin/CD62L on CD8+ T cells. Lymph node cells obtained from MCMV-infected mice deficient in IL-10 also displayed reduced migration towards CXCL10 when compared to wild-type animals. Taken together, these data show that despite elevated chemokine levels, absence of IL-10 results in reduced lymphocyte infiltration into MCMV-infected brains. *Journal of NeuroVirology* (2009) **15,** 334–342.

Keywords: brain; IL-10; lymphocytes; MCMV

Introduction

We have recently shown that absence of the anti-inflammatory cytokine interleukin (IL)-10 results in dysregulated neuroimmune responses and turns a benign murine cytomegalovirus (MCMV) brain infection lethal (Cheeran *et al*, 2007). This lethal infection is associated with vastly elevated levels of interferon (IFN)-γ, and the IFN-γ–inducible chemokines CXCL9 and CXCL10, as well as IL-6, in brain homogenates obtained from IL-10 knockout (KO) mice, when compared to either wild-type or IL-4 KO animals (Cheeran *et al*, 2007). In this study, we tested the hypothesis that elevated levels of infection-induced chemokines observed in the brains of MCMV-infected IL-10–deficient mice would result in greater infiltration by cells of the peripheral immune system when compared to wild-type animals.

Anti-inflammatory cytokines, such as IL-10, IL-4, and transforming growth factor (TGF)-β, have been described as inhibitors of proinflammatory responses in the central nervous system (CNS), and specifically as suppressors of microglial cell function (Sawada *et al*, 1999; Suzumura *et al*, 1993, 1994). One of the main functions of anti-inflammatory cytokines is thought to be their role as a host mechanism to limit tissue damage and turn off proinflammatory responses (Mills, 2004). Because

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This study was funded in part by U.S. Public Health Service grant NS-038836.

Received 20 January 2009; revised 10 April 2009; accepted 9 May 2009.

resident glial cells and infiltrating lymphocytes communicate through cytokine and chemokine mediators, the well-documented neuroprotective action of anti-inflammatory cytokines is likely related to their ability to inhibit chemokine-driven neuroinflammation.

MCMV brain infection of both wild-type and immunodeficient mice induces chemokine production (Cheeran *et al*, 2004). However, chemokine levels are vastly elevated in the brains of infected animals, which lack lymphocytes when compared to wild-type mice (Cheeran *et al*, 2004). It appears that infiltrating IL-10–producing leukocytes possess the ability to suppress proinflammatory chemokine production by microglial cells in the brain. Adoptive transfer of primed splenocytes from wild-type animals into immunodeficient mice dampens this excessive chemokine production because lower levels of chemokines are found in the brains of immunodeficient SCID/Bg animals receiving adoptive transfer of splenocytes than those not receiving adoptive transfer (Cheeran *et al*, 2004). Also, previous studies have shown that induction of proinflammatory cytokines by endogenous brain cells alone is not sufficient to protect immunodeficient SCID/Bg mice against MCMV infection, but successful defense of the brain requires participation of T lymphocytes (Cheeran *et al*, 2005; Reuter *et al*, 2005).

It is clear IL-10 facilitates protection against MCMV brain infection because wild-type mice survive intracerebroventricular (i.c.v.) viral injection, but the same injection is lethal in IL-10–deficient animals (Cheeran *et al*, 2007). More severe MCMV-induced clinical manifestations in IL-10 KO mice (C57BL/6) have also recently been reported following intraperitoneal (i.p.) infection with the Smith strain (Oakley *et al*, 2008). Here, we assessed brain infiltration by peripheral immune cells using both wild-type and IL-10 knockout (KO) mice. Paradoxically, we found that despite the elevated levels of T cell–attracting chemokines in the brain and a more robust neuroimmune response to MCMV infection, absence of IL-10 resulted in reduced lymphocyte infiltration into MCMV-infected brains.

Results

Kinetics of peripheral leukocyte migration into the brain in response to viral infection

Using bioluminescent imaging of live animals, we first examined the kinetics of peripheral leukocyte infiltration longitudinally in both MCMV-infected and sham-infected (i.e., saline injected) mice. Splenocytes and lymph node cells were obtained from primed luciferase transgenic mice (8 days post priming) and were delivered via tail-vein injection to MHC-matched FVB (H-2^q) mice 24 h prior to i.c.v. infection with MCMV (RM461). Within 24 h of adoptive transfer (i.e., −1 day post infection [d.p.i.]), luciferase positive cells were detected in the spleen of all animals. Movement of these peripheral leukocytes into the brain was observed by 24 h p.i., and peaked at 5 d.p.i. (Figure 1A). Additionally, the presence of labeled cells in the cervical lymph nodes and spleen was assessed by imaging the ventral side (Figure 1B). Luciferase signal intensity, which is directly proportional to the number of splenocytes present, was quantified in photons per second per cm^2 at the indicated time points in both brain (Figure 1C) and cervical lymph nodes (Figure 1D) over the 9-day time course of the experiment.

Lymphocyte infiltration is reduced in the absence of IL-10

To identify peripheral immune cell types involved in neuroimmune responses to MCMV brain infection, leukocytes were isolated from brains of infected animals at 5 d.p.i using a Percoll gradient. These isolated cells were first immunostained with markers characteristic of microglial cell and macrophage populations (i.e., CD45 and CD11b). In these experiments, two distinct populations of CD45(+) CD11b(+) cells were apparent in MCMV-infected brains: one expressing intermediate levels, CD45(int), which comprised the resident microglia; and another displaying high levels of CD45, which represented infiltrating macrophages. At 5 d.p.i., the highest proportion of CD45(hi) infiltrating leukocytes also expressed the macrophage marker CD11b, $55.2\% \pm 2.0\%$ and $68.9\% \pm 2.9\%$ for wild-type and IL-10 KO mice, respectively . When a similar analysis was performed using brain tissues obtained from sham-infected, control animals, infiltrating CD45(hi) cells were found to be absent (5.6%). Interestingly, although the overall percentage of CD45(hi) cells infiltrating the brain was not altered by IL-10 deficiency ($80.8\% \pm 1.8\%$ versus $82.8\% \pm 1.7\%$ for wild-type and IL-10 KO mice, respectively), decreased infiltration of the CD45(hi)CD11 b(-) cell population was observed in mice that were deficient in IL-10, $21.6\% \pm 0.5\%$ versus $11.2\% \pm 2.2\%$ for wild-type and IL-10 KO mice, respectively.

Having observed reduced infiltration of the CD45(hi)CD11b(−) cell population into the brains of IL-10 KO animals compared to wild-type mice, we went on to determine whether these cells were lymphocytes. Similar flow cytometric studies using allophycocyanin (APC)−labeled anti-CD45 and Cy7-phycoerythrin (PE)–labeled anti-CD3 antibodies (Abs) showed that IL-10 deficiency resulted in reduced T-lymphocyte ingress into the brain in response to MCMV infection (i.e., $5.2\% \pm 0.2\%$ versus $13.4\% \pm 0.5\%$, respectively, $P < .01$ Student's *t* test) (Figure 2A). We then assessed the involvement of natural killer (NK) cells. These lymphocytes were stained using APC-conjugated anti-CD45 and

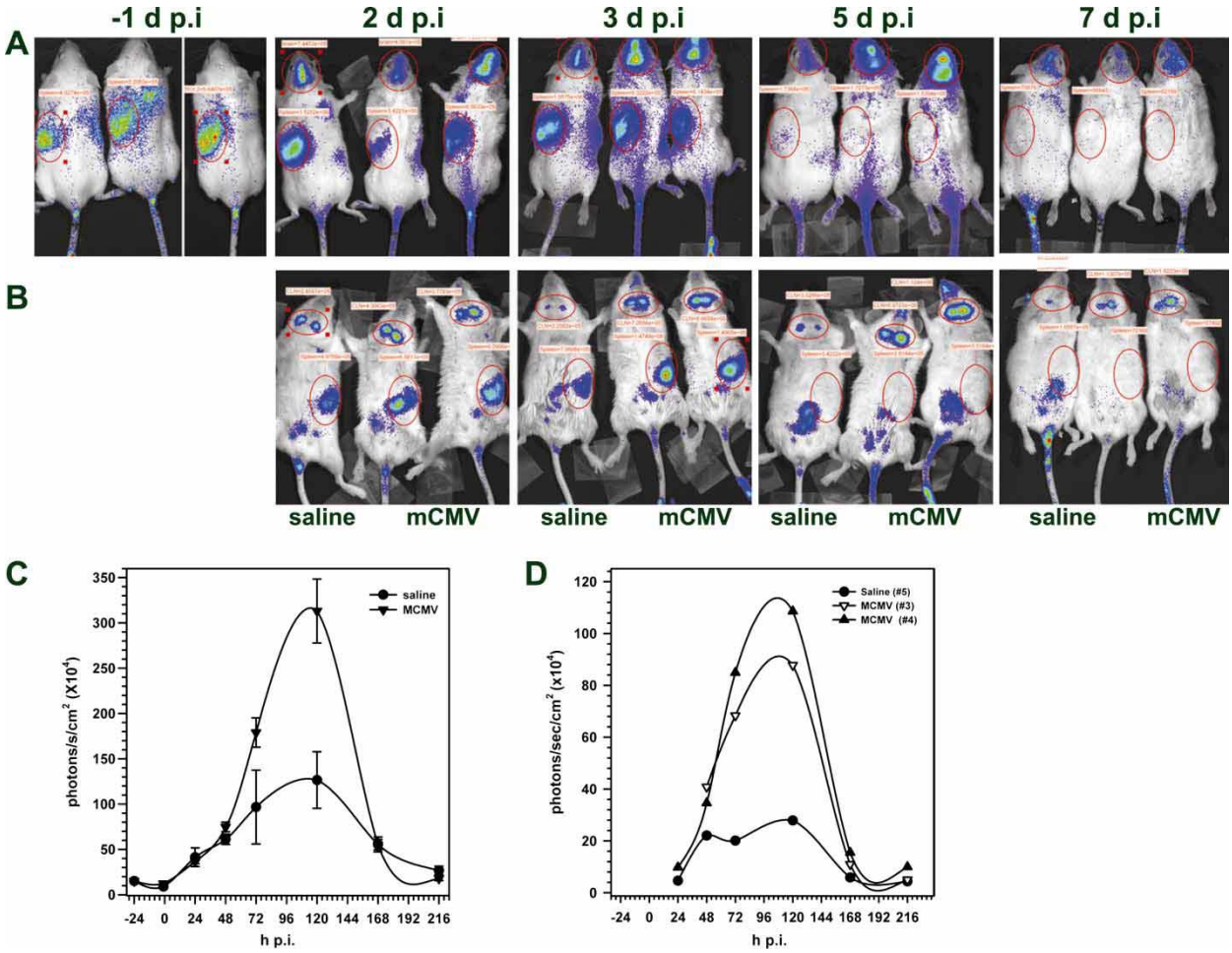

Figure 1 Bioluminescent imaging of immune cell trafficking into MCMV-infected brains. Splenocytes and lymph node cells from primed β-actin luciferase transgenic mice were harvested 7 days post priming and delivered, via tail-vein injection to MHC-matched FVB (H-2^q) animals 24 h prior to icv infection with MCMV. Images of recipients show peripheral immune cell infiltration into sham-injected (saline) and virus-injected (mCMV RM461) murine brains (**A**) and cervical lymph nodes and spleen (**B**) at the indicated times p.i. (dorsal and ventral views, respectively). The signal intensity of the luciferase-positive cells, which is directly related to their number, was quantified in photons per second per cm^2 in the brain (**C**) and cervical lymph nodes (**D**) at the indicated time points. Data are presented as mean intensities ($\pm$ SEM) from at least three animals for brain, and as individual animals for cervical lymph nodes, plotted over the 9-day time course.

PE-labeled anti-CD49b Abs and examined using flow cytometry. Results generated during these experiments demonstrated that NK cells infiltrated the brain of wild-type mice in response to MCMV infection and that infiltration of these cells was also decreased in animals with IL-10 deficiency, $10.2\% \pm 2.6\%$ versus $5.5\% \pm 0.2\%$, respectively, $P < .01$ Student's t test (Figure 2B). Significant reduction in the absolute numbers of infiltrating NK cells was also observed in the IL-10 KO mice ($5.7 \pm 0.8 \times 10^4$ versus $1.1 \pm 0.4 \times 10^5$, $P = .03$). Further, confirmation of reduced T-lymphocyte ingress into the brain was obtained through in situ immunohistochemical staining for the CD3 cell surface antigen in brain sections obtained from MCMV-infected mice. These immunostaining experiments clearly showed a decreased number of lymphocytes within brain sections from MCMV-infected, IL-10 KO mice when compared to wild-type animals (Figure 2C).

Profile of the T-cell infiltrate in wild-type versus IL-10 KO mice

Similar analysis to determine the absolute number of brain-infiltrating CD45(hi)CD3(+) T cells again demonstrated that there was significantly less infiltration of T lymphocytes into the brains of MCMV-infected IL-10 KO mice, when compared to wild-type animals, $P < .01$ (Figure 3A). Data obtained from these experiments also showed that the total number of infiltrating CD8(+) T cells was decreased to a greater extent than the CD4(+) subpopulation (Figure 3A). We then went on to determine the distribution of CD4(+) and CD8(+) lymphocytes within the infiltrating CD3(+) population. In these experiments, as well as being

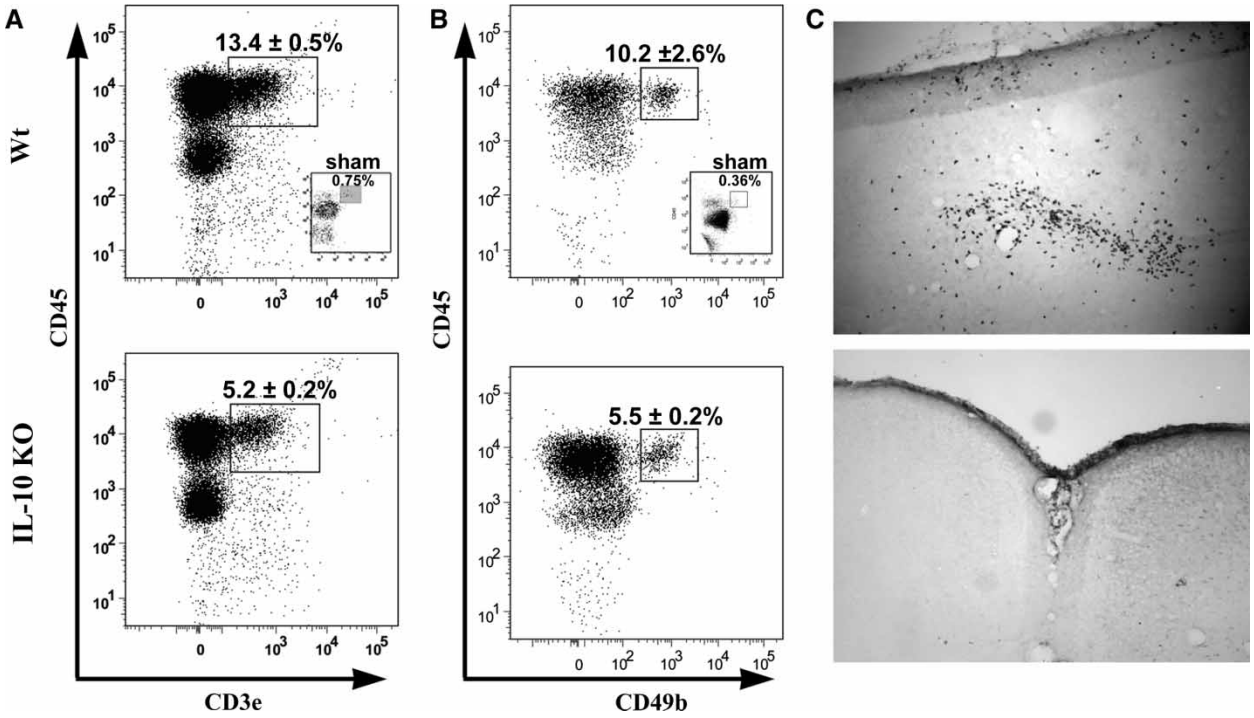

Figure 2 Reduced lymphocyte infiltration into the brains of MCMV-infected IL-10 KO mice. (**A**) Brain-infiltrating leukocyte subpopulations obtained from wild-type (WT) and IL-10–deficient (IL-10 KO) mice were further examined using anti-CD45 Abs along with Cy7-PE-labeled anti-CD3ε Abs and analyzed using flow cytometry. Ratios of the cell population expressing a high level of each marker are presented as mean ± SEM from 3 independent experiments ($n = 3$–5 animals each). (**B**) Brain leukocytes isolated as described above from wild-type (WT) and IL-10–deficient (IL-10 KO) mice at 5 d.p.i. were also stained using APC-conjugated anti-CD45 and PE-labeled anti-CD49b Abs to determine natural killer (NK) cell infiltration. Data are presented as mean ± SEM percentage of cells displaying CD45(hi)CD49b(+) expression from 3 to 4 independent experiments ($n = 6$–8 animals each). (**C**) Immunohistochemical staining of the CD3 cell surface antigen in brain sections obtained from MCMV-infected wild-type (*top panel*) versus IL-10 KO (*bottom panel*) mice.

reduced in total number, CD8(+) lymphocytes were also found to make up proportionally less of the cellular infiltrate in the brains of IL-10–deficient animals (Figure 3B).

Retention of CD8(+) T cells in the lymph nodes of IL-10–deficient animals

To determine whether deficiency in IL-10 disrupts the migration of immune cells out of the lymph nodes, cervical lymph node cells were isolated from MCMV-infected wild-type versus IL-10–deficient animals. The isolated cells were then double-stained for expression of CD8 or CD4, as well as the lymph node homing receptor L-selectin/CD62L and assessed using flow cytometry. Representative data show that proportionally more CD8(+) cells were retained in the lymph nodes of MCMV-infected IL-10 KO mice when compared to infected wild-type animals (Figure 4A). Analysis of pooled data obtained from 4 experiments (using 3–4 animals/experiment) at 5 d.p.i., demonstrated significantly increased expression of L-selectin/CD62L on CD8(+) T lymphocytes obtained from the cervical lymph nodes of the IL-10 deficient animals when compared to wild-type controls (Figure 4B).

Reduced chemotaxis by lymph node cells obtained from IL-10–deficient animals

To determine whether deficiency in IL-10 also inhibits cellular migration towards a chemotaxic stimulus, the ability of cervical lymph node cells isolated from MCMV-infected IL-10 KO mice to migrate towards CXCL10 was compared to that of wild-type control animals. In these studies, analysis of pooled data obtained from 3 experiments ($n = 3$ animals/experiment) demonstrated that cells from IL-10–deficient mice displayed a significant inhibition of chemotaxis towards CXCL10, a well-known lymphocyte chemoattractant (Figure 5).

Discussion

The anti-inflammatory cytokines IL-10, IL-4, and TGF-β play key roles in maintaining the delicate balance between control of viral infection and immunopathogenesis. In the central nervous system (CNS), IL-10 has been shown to be secreted by both resident microglial cells and infiltrating Tr1 regulatory T cells (i.e., Tregs) (Burkhart *et al*, 1999; Groux *et al*, 1997; OGarra *et al*, 2004; Sheng *et al*, 1995; Williams *et al*, 1996). Previous *in vitro* studies from

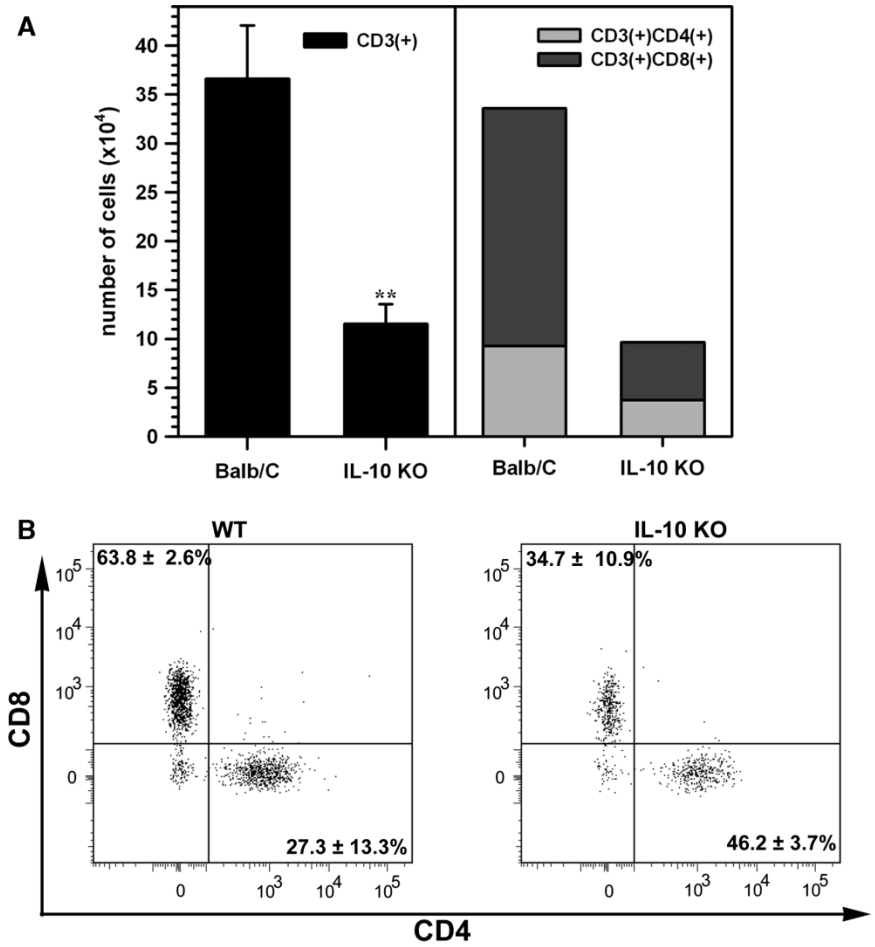

Figure 3 Phenotypic profile of the T-cell infiltrate in wild-type versus IL-10 KO mice. (**A**) Total numbers of infiltrating CD45(hi)CD3(+) cells in the brains of MCMV-infected wild-type (Balb/C) versus IL-10 KO mice were determined at 5 d.p.i. Data showing the mean (± SEM) absolute number of cells infiltrating the brain were pooled from 3 to 5 experiments. $^{**}P < .01$ versus wild-type Balb/C. (**B**) PE-labeled anti-CD8 and FITC-labeled anti-CD4 Abs were used to determine the total number of CD4(+) and CD8(+) lymphocytes within the infiltrating CD3(+) population. Data presented show the mean absolute number of cells in each population. (**C**) A representative dot plot shows the distribution of CD4(+) and CD8(+) lymphocytes within the infiltrating CD3(+) population. The average percentage of positive T cells (mean ± SEM) from 3 independent experiments is shown.

our laboratory have demonstrated that infection of primary human microglia with human cytomegalovirus (CMV) results in CXCL10 production (Cheeran *et al*, 2003). This virus-induced chemokine production was found to be suppressed following treatment with IL-10 and IL-4, but not transforming growth factor (TGF)-β (Cheeran *et al*, 2003). Importantly, follow-up *in vivo* studies using MCMV infection of mice have shown that the IL-10 that is necessary to control dysregulated virus-induced, IFN-γ–mediated neuroimmune responses during viral brain infection is provided by an infiltrating peripheral CD45 (hi)CD11b(-) or CD45(hi)CD11b(int) immune cell type, not resident microglial cells (Cheeran *et al*, 2007). Interestingly, human CMV carries a homolog of IL-10 (i.e., cmvIL-10), which also inhibits virus-induced chemokine production from microglial cells (Cheeran *et al*, 2003), presumably to subvert host defenses. The presence of an IL-10 homolog carried by MCMV has not been reported.

Peripheral immune cells infiltrate the brain in response to glial cell-produced chemotactic factors. Here, we first used bioluminescence imaging to quantify the overall kinetics of immune cell trafficking into MCMV-infected brains. The results of these studies clearly demonstrate that these cells move into the brain in response to viral infection. Infiltration of the brain by cells of the peripheral immune system was also evident through histopathological examination of brain sections obtained from MCMV-infected animals. When these sections were examined, inflammatory cells were easily detected in the ventricles, periventricular regions, and meninges. However, no histopathological differences were apparent between wild-type and IL-10 KO animals at 5 d.p.i. (data not shown).

The early neuroimmune responses to MCMV infection were dominated by the influx of macrophages in both the wild-type and IL-10 KO mice. These infiltrating CD45(hi)CD11b(+) cells

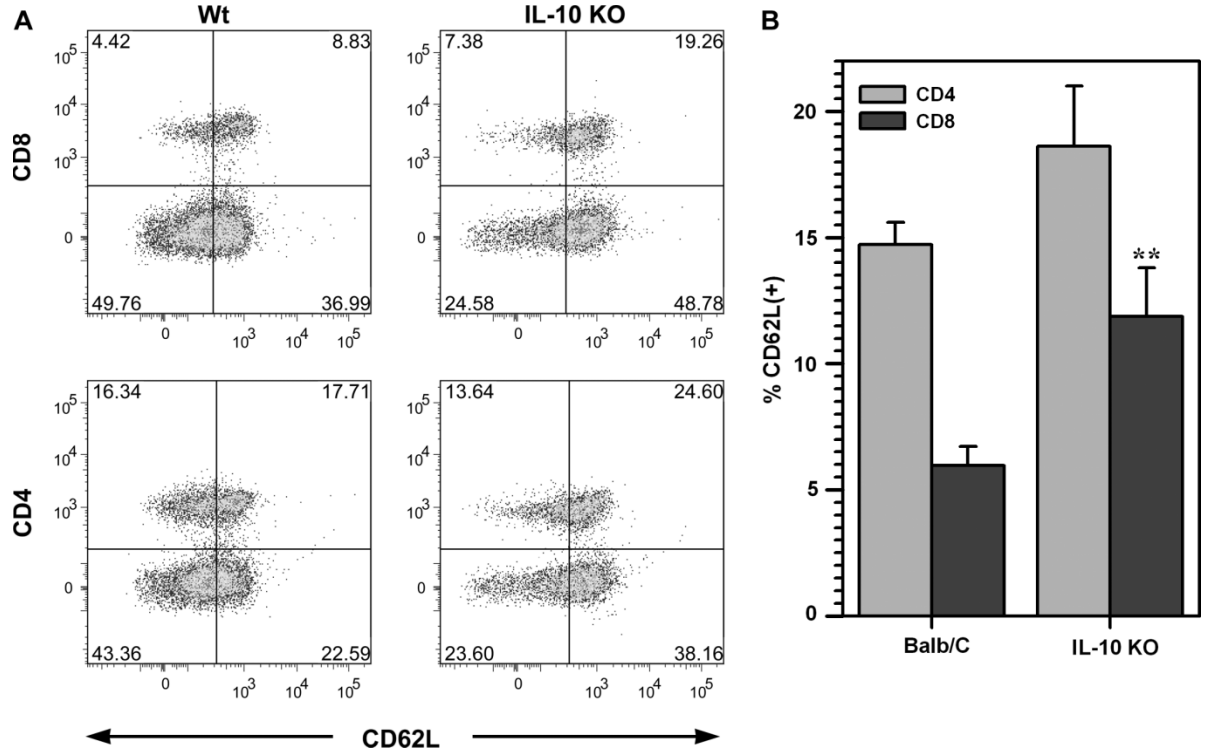

Figure 4 Retention of CD8(+) T cells in the lymph nodes of IL-10–deficient animals. (**A**) Cervical lymph node cells were isolated from MCMV-infected wild-type Balb/C (Wt) and IL-10 KO animals and examined for expression of CD8 and CD4 as well as the lymph node homing receptor L-selectin/CD62L. Representative dot plots from 4 experiments ($n = 3$–4 animals/experiment) at 5 d p.i. are presented. Relative percentages of each cell population in the representative experiment are shown. (**B**) Pooled data indicating ratios of the various cell populations present in the lymph nodes are expressed as mean ± SEM. $^{**}P < .01$ versus Balb/C.

were in an activated state, as assessed by up-regulation of major histocompatibility complex (MHC) class II expression (data not shown). Resident microglia (i.e., CD45(int)CD11b(+) cells), which normally do not express MHC class II, become activated either in response to the virus itself or in response to IFN-γ, which has been shown to induce MHC class II expression (Hamo *et al*, 2007). Interestingly, when macrophage infiltration in response to MCMV brain infection was compared between wild-type and IL-10 KO animals, it was not found to be affected by the IL-10 deficiency.

Subsequent immunostaining followed by flow cytometry demonstrated that T lymphocytes made up a high proportion of the CD45(hi)CD11b(-) population. Interestingly, unlike the situation with macrophages and contrary to our hypothesis of greater leukocyte infiltration into the brains of IL-10 KO mice, decreased CD8(+) T lymphocyte infiltration into infected brains was observed in animals which were deficient in IL-10. Likewise, NK cells were also found to infiltrate the brain of wild-type mice in response to MCMV infection and, as with T lymphocytes, this cellular infiltration was also significantly reduced in IL-10 KO animals.

Based on our previous studies, we anticipated that IL-10 deficiency would lead to enhanced neuroimmune responses against MCMV brain infection. Paradoxically, these data show that despite elevated chemokine levels, absence of IL-10 actually resulted in reduced lymphocyte infiltration into MCMV-infected brains. In the present paper, we report that this decreased lymphocyte brain infiltration is associated with increased expression of the lymph node homing receptor L-selectin/CD62L on CD8(+) T lymphocytes obtained from the cervical lymph nodes of MCMV-infected, IL-10–deficient animals. The increased expression of CD62L was significant only on CD8+ lymphocytes, which also demonstrated decreased brain infiltration. The decreased ability of IL-10–deficient animals to down-regulate L-selectin/CD62L during cellular activation in the cervical lymph nodes may lead to reduced or delayed recruitment of effector T cells and NK cells into the brain in response to viral infection. Using a murine model for mucosal herpes simplex virus (HSV)-2 infection, it has recently been reported that ablation of regulatory T cells disrupts the migration of immune cells out of the lymph nodes and profoundly reduces or delays recruitment of effector T cells and NK cells to sites of viral infection, thereby accelerating fatal infection (Lund *et al*, 2008). Although Tregs utilize multiple means to limit immune responses, IL-10 production by these cells has been found to be essential for keeping immune responses in check at particular environmental

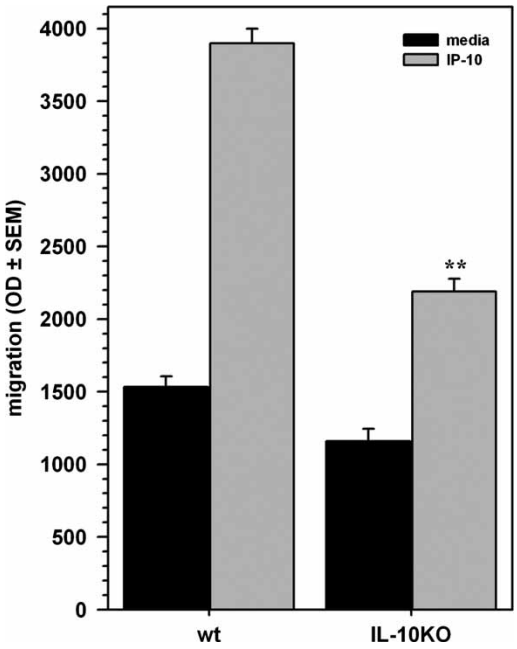

Figure 5 Lymph node cells from IL-10 KO animals display reduced chemotaxis towards CXCL10. Cells were isolated from the cervical lymph nodes of MCMV-infected wild-type (wt) and IL-10 knockout (IL-10 KO) mice (5 d.p.i.), and loaded into the upper chamber of a chemotaxis plate (3×10^5 cells/well). Cellular migration towards CXCL10 (100 ng/ml, 3 h) was quantified using alamarBlue uptake assay (excitation at 530 nm and emission at 570). Pooled data from triplicate samples/animal (n = 3 mice) are presented as mean ± SD. $^{**}P < .01$ versus wt.

interfaces such as the colon and lungs (Rubtsov *et al*, 2008). Distinct suppressor mechanism most likely play a prominent role in particular tissues and it remains to be seen if an analogous immune response-promoting role for IL-10 occurs in the brain during MCMV infection.

Although decreased CD8+ lymphocyte infiltration into the MCMV-infected brain that was observed in IL-10 knockout animals was associated with higher levels of CD62L on CD8+ T cells in the cervical lymph nodes, it is clear that this is not the sole responsible mechanism. The fact that NK cells from IL-10 KO animals did not display enhanced CD62L expression demonstrates the involvement of multiple mechanisms that likely work in concert to produce the observed phenotype. Further experiments also demonsrated that lymph node cells obtained from IL-10 knockout animals were deficient in their chemotaxic migration towards CXCL10 when compared to those obtained from wild-type mice.

We do not yet know why MCMV brain infection is lethal in IL-10 KO animals, but it does not appear to be due simply to higher overall viral load in the brain (Cheeran *et al*, 2007). In salivary glands, blockade of the IL-10 receptor with an antagonist Ab has been reported to increase INF-γ–secreting cells and subsequently decrease viral loads (Humphreys *et al*, 2007). In similar studies, Oakley *et al* reported that following i.p. injection of MCMV into IL-10 KO mice, the KO animals had more severe disease which was associated with elevated levels of IFN-γ, monocyte chemoattractant protein (MCP)-1, and IL-6, but was not attributable to viral replication (Oakley *et al*, 2008). Instead, proper regulation of the neuroimmune response appears to be crucial in controlling immunopathological brain damage associated with clearing viral infection. The neuroprotective effects of anti-inflammatory cytokines are believed to be mediated through down-regulation of brain inflammation. It is thought that the neuroprotective action of IL-10 is related to its ability to inhibit chemokine-driven neuroinflammation, turn off proinflammatory responses, and limit damage to brain tissue. Surprisingly, in this study IL-10 deficiency resulted in reduced infiltration of the brain by T lymphocytes and NK cells in response to MCMV infection.

Materials and methods

Virus and animals

RM461, a recombinant virus expressing *Escherichia coli* β-galactosidase under the control of the human ie1/ie2 promoter/enhancer (Stoddart *et al*, 1994), was kindly provided by Edward S. Mocarski. Virulent, salivary gland–passaged, sucrose gradient–purified virus was used for all i.c.v. infections. Stocks of MCMV Smith Strain (ATCC, Rockville, MD), used to prime donor animals, were grown and titered by $TCID_{50}$ assay on NIH 3T3 fibroblasts. BALB/c and FVB/N mice were obtained from Charles River Laboratories (Wilmington, MA), whereas IL-10 KO animals were purchased from The Jackson Laboratory (Bar Harbor, ME). FVB/N β-actin promoter–luciferase transgenic mice were obtained from Xenogen (Alameda, CA).

Intracerebroventricular infection of mice

Intracerebroventricular infection of mice was performed as previously described (Cheeran *et al*, 2004). Briefly, female mice (8 to 10 weeks old) were anesthetized using a combination of ketamine and xylazine (100 mg and 10 mg/kg body weight, respectively) and immobilized on a small animal stereotactic instrument equipped with a Cunningham mouse adapter (Stoelting, Wood Dale, IL). The skin and underlying connective tissue were reflected to expose reference sutures (sagittal and coronal) on the skull. The sagittal plane was adjusted such that the bregma and lambda were positioned at the same coordinates on the vertical plane. Virulent, salivary gland–passaged MCMV RM461 (1.5×10^5 $TCID_{50}$ units in 10 μl), was injected into the right lateral ventricle at 0.9 mm lateral, 0.5 mm caudal to the bregma and 3.0 mm ventral to the skull surface using a Hamilton syringe

(10 μl) fitted to a 25-G cannula. The injection was delivered over a period of 3 to 5 min. The opening in the skull was sealed with bone wax and the skin was closed using 9 mm wound clips (Stoelting).

Adoptive transfer
Spleen cells were aseptically collected from MHC-matched FVB/N β-actin promoter–luciferase transgenic mice 8 days following i.p. injection of tissue culture-passaged MCMV Smith strain. Splenocytes were depleted of red blood cells by treatment with 0.87% ammonium chloride for 2 min followed by the addition of an equal volume of phosphate-buffered saline (PBS) containing 2% fetal bovine serum. Spleen cells (1×10^7) obtained from MCMV-primed mice (8 d.p.i.) were then transferred via tail vein injection into wild-type FVB/N mice 24 h prior to i.c.v. infection with RM461 (3×10^5 $TCID_{50}$).

Bioluminescence imaging
Imaging of firefly luciferase expression in live animals was performed using an IVIS50 (Xenogen, Alameda, CA) equipped with a charge-coupled camera device, as previously described with minor modifications (Luker *et al*, 2003). Briefly, 150 μg of D-luciferin (Gold Biotechnology, St. Louis, MO) was administered to anesthetized mice by i.p. injection. Animals were imaged 5 min after D-luciferin administration and data were acquired using a 5-min exposure window. Bioluminescence imaging studies were carried out with age-matched 8- to 10-week old female FVB/N mice as recipients of adoptive transfer. Signal intensity of luciferase expression, as measured by the total amount of transmitted light, was quantified as photons/sec/cm^2 using Living-Image and Igor (Wavemetrics, Lake Oswego, OR.) image analysis software.

Isolation of brain leukocytes and flow cytometry
Leukocytes were isolated from MCMV-infected murine brains using a previously described procedure with minor modifications (Cheeran *et al*, 2007; Ford *et al*, 1995; Marten *et al*, 2000). Briefly, brain tissues harvested from 4 to 6 animals were minced finely in RPMI (2 g/L D-glucose and 10 mM HEPES) and mechanically disrupted (in Ca/Mg-free Hanks' balanced salt solution [HBSS]) at room temperature for 20 min. Single-cell preparations from infected brains were resuspended in 30% Percoll and banded on a 70% Percoll cushion at $900 \times g$ at 15°C. Brain leukocytes obtained from the 30% to 70% Percoll interface were stained with anti-mouse immune cell surface markers for 45 min at 4°C (CD45-allophycocyanin [APC]) (eBioscience, San Diego, CA), CD11b-FITC (fluorescein isothiocyanate) or CD11b-APC-CY7, CD4-FITC, Ly6G-FITC, Ly6C-FITC, MHC class II–phycoerythrin (PE), CD8-PE, and CD3-PE-Cy7 (BD Biosciences, San Jose, CA) and analyzed by flow cytometry using a BD FACSCanto. Live leukocytes were gated using forward scatter and side scatter parameters and analyzed using FlowJo software (TreeStar).

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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This paper was first published online on iFirst on 20 July 2009.