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Dysregulated interferon-gamma responses during lethal cytomegalovirus brain infection of IL-10-deficient mice

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Abstract

Murine cytomegalovirus (MCMV) brain infection induces a transient increase in chemokine production, which precedes the infiltration of CD3⁺ lymphocytes. In this study, we hypothesized that an absence of anti-inflammatory cytokines would result in sustained proinflammatory neuroimmune responses. Direct intracerebroventricular injection of MCMV into IL-10 knockout (KO) mice produced an unexpected result: while wild-type animals controlled MCMV, the infection was lethal in IL-10 KO animals. Identical infection of IL-4 KO animals did not produce lethal disease. To further characterize the role of IL-10, infected brain tissue from both wild-type and IL-10 KO animals was assessed for cytokine and chemokine levels, as well as viral gene expression. These data show 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 KO animals. However, MCMV viral load, glycoprotein B mRNA levels, and titers of infectious virus were similar in both IL-10 KO and wild-type animals. Separation of cells isolated from murine brain tissue into distinct populations using FACS, along with subsequent quantitative RT real-time PCR, showed that brain-infiltrating CD45(hi)/CD11b(-) and CD45(hi)/CD11b(int) were the cellular source of IL-10 in the brain. Taken together, these data demonstrate that MCMV brain infection of IL-10-deficient mice causes lethal disease, which occurs in the presence of a dysregulated IFN- γ mediated neuroimmune response.

Keywords

MCMV; IFN- γ ; IL-10; encephalitis; chemokines

1. Introduction

Neuroimmune responses against viral infections must control invading pathogens while simultaneously preventing extensive brain damage (Patterson et al., 2002). We have previously reported that CD8⁺ T lymphocytes possess the ability to restrict intracerebral spread of murine cytomegalovirus (MCMV) brain infection through a perforin-dependent mechanism (Cheeran et al., 2004; Cheeran et al., 2005). In these studies, we detected the presence of a CD3⁺ lymphocyte infiltrate in the brains of MCMV-infected immunodeficient (C.B-17 SCID/Bg) mice 5 days following adoptive transfer of total splenocytes via tail-vein injection. It is likely that these peripheral immune T-cells infiltrated the brain in response to glial cell-produced chemotactic factors. We further demonstrated that viral infection of wild-type mice induces a

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transient increase in the IFN- γ -inducible chemokines CXCL9 and CXCL10, with kinetics that precede lymphocyte infiltration into the brain. Regulation of the production of chemokines, as well as other neuroinflammatory mediators, in the brain by anti-inflammatory cytokines may play a key role in maintaining the delicate balance between control of viral infection and immunopathogenesis.

Exaggerated proinflammatory neuroimmune responses must be downregulated in order to avoid irreparable brain damage and IL-10 and IL-4 are prototypical anti-inflammatory cytokines. One of the main functions of these cytokines is thought to be their role as a host mechanism to limit tissue damage and turn off proinflammatory responses (Mills, 2004). The well-established, neuroprotective effects of IL-10 are most likely due to its effect on macrophages and microglia, because these cells respond to inflammatory stimuli by producing proinflammatory mediators that are suppressed by this cytokine. Indeed, IL-10 treatment of glial cells has been reported to inhibit the production of a number of proinflammatory mediators including TNF- α , IL-12, NO, and superoxide (Chao et al., 1995; Hu et al., 1995; Ledebor et al., 2000; Lodge and Sriram, 1996; Molina-Holgado et al., 2001; Sawada et al., 1999). Like IL-10, it is well established that IL-4 also possesses a similar ability to downregulate production of proinflammatory mediators by microglia (Furlan et al., 2000; Ledebor et al., 2000). It has been reported that the neuroprotective mechanisms of IL-4 may be mediated through downregulation of brain inflammation, and subsequent increased neuronal survival, by directly inducing cell death of activated microglia (Park et al., 2005).

Lymphocytes communicate with glial cells through chemokine mediators. For this reason, the well-documented neuroprotective action of IL-10 could be related to its ability to inhibit chemokine-driven neuroinflammation. We have previously reported that MCMV brain infection of immunocompetent mice induces a transient increase in chemokine production (Cheeran et al., 2004), and further experiments went on to demonstrate that adoptive transfer of splenocytes into immunodeficient mice dampened chemokine production in the brain. Because lower levels of chemokines were found in the brains of immunodeficient animals receiving adoptive transfer of splenocytes than those without adoptive transfer, in the present study we hypothesized that in the absence of anti-inflammatory cytokines; in particular IL-4 and IL-10, sustained proinflammatory neuroimmune responses would be seen during MCMV brain infection. To our surprise, MCMV brain infection of IL-10 KO mice resulted in a lethal pathological condition.

2. Methods

2.1 Viruses and animals

RM461, a recombinant MCMV expressing *E. coli* β -galactosidase under the control of the human ie1/ie2 promoter/enhancer (Stoddart et al., 1994), was provided by Edward Mocarski. Viral stocks were passaged in murine salivary glands to retain their virulence. Virus isolated from the salivary glands was then passaged once on NIH3T3 fibroblasts, followed by purification of the stock by centrifugation over a sucrose gradient. Sucrose gradient-purified RM461 was used for all intracerebroventricular (*icv*) infections. Stocks of MCMV Smith Strain (ATCC, Rockville, MD), used to prime donor animals for adoptive transfer, were grown and titered using 50% tissue culture infective dose (TCID₅₀) assay on NIH 3T3 fibroblasts. BALB/c mice were obtained from Charles River Laboratories (Wilmington, MA), while IL-4 KO and IL-10 KO mice were purchased from The Jackson Laboratory (Bar Harbor, ME).

2.2 Intracerebroventricular infection

Icv infection of mice was performed as previously described (Cheeran et al., 2004). Briefly, female wild-type BALB/c, IL-4 KO, and IL-10 KO mice (8–10 weeks) 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 Co., 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. Salivary gland passaged MCMV RM461 (1.5×10^5 TCID₅₀), was injected slowly 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 fitted to a 25 G cannula. The injection was delivered over a period of 3–5 min. The opening in the skull was sealed with bone wax and the skin closed using 9 mm wound clips (Stoelting Co., Wood Dale, IL).

2.3 Real-time PCR

Total RNA and DNA were extracted from brain tissue homogenates using the Trizol Reagent (Invitrogen, Carlsbad, CA). cDNA was synthesized using 1 µg of total RNA, SuperScript II reverse transcriptase (Invitrogen Life Technologies, Carlsbad, CA) and oligo dT_{6–12} primers (Sigma-Genosys, The Woodlands, TX). Quantitative real-time PCR was performed using the FullVelocity SYBR Green QPCR master mix (Stratagene, La Jolla, CA) following the manufacturer's specifications. The 25 µl final reaction volume consisted of pre-made reaction mix (SYBR Green I dye, reaction buffer, Taq DNA polymerase, and dNTPs), 0.3 mM of each primer, and 0.5 ng cDNA in water. Reaction conditions for PCR for the Mx3000P QPCR System (Stratagene) were as follows: polymerase activation at 95°C for 5 min, 40 denaturation cycles of 95°C for 10 s, annealing at 60°C for 10 s and elongation at 72°C for 10 s. Primer sequences used in the amplification of cytokines and chemokines will be provided on request. For real-time viral DNA PCR, the DNA was eluted in water and stored at –80°C until quantification using real-time PCR. Primers for MCMV were designed from the gene encoding glycoprotein B (GenBank accession no. M86302, 5'-CGCTGGTCGTCTTTCAGTTC-3' and 5'-CTGTTCGTGTCGCAGTTCTC-3', 112 bp product). Primers recognizing the housekeeping gene β-actin were designed from the mouse β-actin DNA sequence (GenBank accession no. NM_007393, 5'-GGGCTATGCTCTCCCTCAC-3' and 5'-GATGTCACGCACGATTTCC-3', 100 bp product). A melting curve analysis was performed to assess primer specificity and product quality by denaturation at 95 °C, annealing at 65 °C and melting at a rate of 0.1 °C/sec to 95° C. The relative levels of viral DNA were quantified using the 2(-Delta Delta C_T) method (Livak and Schmittgen, 2001).

2.4 Cytokine and chemokine ELISA

A previously described sandwich ELISA-based system (Peterson et al., 1997) was used to quantify cytokine and chemokine levels from murine whole brain tissue extract (homogenized in TPER; Pierce, Rockford, IL.). ELISA plates were coated with rat-anti-mouse chemokine capture antibodies (R&D Systems, Minneapolis, MN). Detection antibodies (biotinylated goat anti-mouse chemokine) were also obtained from R&D Systems. Absorbance values at 450 nm were used to quantify chemokines levels based on the standard concentration curve generated from serial dilutions of cytokines and chemokines.

2.5 Isolation of brain leukocytes and sorting using FACS

Brain leukocytes were isolated from MCMV-infected or sham-infected murine brains using a previously described procedure with minor modifications (Ford et al., 1995; Marten et al., 2000). Briefly, brain tissues harvested from 3–5 animals were minced finely in RPMI (2 g/L D-glucose and 10mM HEPES) and digested in 0.25% trypsin (Ca/Mg free HBSS) at room temperature for 20 min. The single cell suspension was resuspended in 30% Percoll and banded on a 70% Percoll cushion at 900 xg at 15°C. Brain leukocytes obtained from the 30–70% Percoll interface were stained with anti-mouse CD45-Allophycocyanin (APC) (eBioscience,

San Diego, CA) and anti-mouse CD11b-FITC (BD Biosciences, San Jose, CA) for 45 min at 4°C. For FACS, live leukocytes were gated using forward scatter and side scatter parameters. Non-overlapping populations were separated using a fluorescence-activated cell sorter (FACS, BD FACS Aria). Total RNA, isolated from the sorted cell populations, was analyzed by quantitative PCR for IL-10 expression.

3. Results

3.1 Lethal MCMV brain infection in animals with IL-10 deficiency

We first investigated the kinetics of MCMV brain infection in both IL-4 and IL-10 deficient animals. In these experiments, wild-type (BALB/c, n=12), IL-4 KO (n=11), and IL-10 KO (n=15) mice (also BALB/c, H-2^d) were infected by injecting salivary gland-passaged, virulent MCMV stereotactically into the right lateral ventricle and followed over an 18-day time-course. A plot of surviving animals expressed as percent of the total mice examined in each group over the time-course is shown in Fig. 1A. These data demonstrate that while wild-type and IL-4 KO animals controlled MCMV brain infection, the identical infection was lethal in IL-10 deficient animals. In real-time PCR experiments, we detected highly elevated levels of IL-10 transcription in the brains wild-type mice at early time points during the course of infection (Fig. 1B). Additionally, IL-10 protein levels in brain homogenates of wild-type mice during MCMV infection were measured at 5 days post-infection (p.i.) using ELISA (Fig. 1C).

3.2 Dysregulated IFN- γ and IL-6 gene expression in the brains of IL-10 KO mice relative to wild-type mice

To further characterize the role of IL-10 in regulating cytokine production during this viral brain infection, the levels of selected proinflammatory cytokines in whole brain homogenates, obtained from infected wild-type BALB/c versus IL-10 KO mice, were measured using quantitative real-time RT-PCR. The cytokine mRNA levels which appeared different between the two groups of animals by RT-PCR were then further confirmed at the protein level using ELISA. Cytokine levels were assessed using primers specific for IFN- γ (Fig. 2A), followed by confirmation using ELISA (Fig. 2B); and IL-6 (Fig. 2C), followed by confirmation with ELISA (Fig. 2D). mRNA levels for TNF- α (Fig. 2E) and IL-1 β (Fig. 2F) were not found to be different between wild-type and IL-10 KO animals. All cytokine mRNA expression was normalized to HPRT (hypoxanthine guanine phospho-ribosyl transferase) and is presented as fold increase in relative mRNA induction levels over sham-infected controls. Brains from MCMV-infected wild-type and IL-10 KO mice were harvested at 5 days p.i. for ELISA. These data demonstrate that IFN- γ and IL-6 levels, but not TNF- α or IL-1 β are dysregulated in the brains of IL-10 KO mice compared to wild-type mice.

3.3 CXCL9 and CXCL10 gene expression in the brain was unrestrained in IL-10 KO relative to wild-type mice

We next examined the levels of selected proinflammatory chemokines in the central nervous system (CNS) using quantitative real-time RT-PCR and ELISA. Total RNA was extracted, DNase-treated and reverse transcribed. For confirmation by ELISA, brains from MCMV-infected wild-type and IL-10 KO mice were harvested at 5 days p.i. cDNA obtained was analyzed by real-time PCR using primers specific to CXCL9 (Fig. 3A), followed by confirmation using ELISA (Fig. 3B); and CXCL10 (Fig. 3C), followed by confirmation using ELISA (Fig. 3D). RT-real-time PCR was also performed for CCL5 (Fig. 3E), followed by ELISA (Fig. 3F). RNA levels were normalized to HPRT and are presented as mean of normalized expression from averaged data obtained using three animals per group at each time point. Data obtained during these experiments show that, similar to the situation with IFN- γ , infected mice which were deficient in IL-10 were unable to downregulate the IFN- γ -inducible chemokines CXCL9 and CXCL10 relative to wild-type animals. We also compared the amount

of infection-induced CCL2, CXCL9, and CXCL10 mRNA in the brains of IL-4 KO mice to those of wild-type animals and found similar levels in both groups.

3.4 Similar amounts of virus were present in the brains of IL-10 KO and wild-type animals

We went on to quantify the level of viral DNA replication in the brains of both groups of animals using real-time DNA PCR to assess number of viral genomes (Fig. 4A). We further examined viral load by directly measuring the amount of infectious virus present in brains of infected animals from both groups. Serial dilutions of brain homogenates obtained from animals at 5 days p.i. were plated onto indicator cells and viral titers were determined using TCID₅₀ assay (Fig. 4B). In addition to measuring viral load, we also evaluated viral gene expression in the brains of MCMV-infected IL-10 KO and wild-type animals by RT-real-time PCR. Levels of MCMV glycoprotein B mRNA in whole brain homogenates were measured using total RNA extracted from animals in each group at 5 days p.i. (Fig. 4C). Since similar levels of viral infection were observed in wild-type and IL-10 KO mice, these data suggest that the increased mortality seen in the IL-10 KO animals is not due to failure to control viral replication. Our previous studies using this MCMV brain infection model examined immune-mediated viral clearance from the brains of wild-type versus SCID/Bg mice and found vast differences in viral load between these animals. For comparison of viral levels in the brains of animals with or without immune control, data derived from quantitative real-time PCR experiments show vastly elevated levels of MCMV mRNA expression in the brains of SCID/Bg mice when compared to wild-type mice (Fig. 4D).

3.5 The inflammatory cell infiltrate was the cellular source of IL-10 in the infected brain

Finally, we determined the source of IL-10 in MCMV-infected brain tissue. In these experiments, brain leukocytes isolated from sham-infected and MCMV-infected (5 days p.i.) murine brain tissues were sorted into four distinct populations (A through D) by FACS using anti-CD45 and anti-CD11b Abs (Fig. 5A, B). These sorted populations were then assayed for IL-10 mRNA expression using quantitative RT real-time PCR. While little if any IL-10 mRNA was detected in the brains of animals injected with saline, vastly elevated levels of IL-10 mRNA were seen in the CD45(hi)/CD11b(-) and CD45(hi)/CD11b(int) populations isolated from infected MCMV-mice (Fig. 5C). Additionally, no significant amounts of IL-10 mRNA were detected in the CD45(hi)/CD11b(hi) or CD45(int)/CD11b(+) cell populations (Fig. 5C).

4. Discussion

Results reported in this paper clearly demonstrate that *icv* injection of MCMV triggers a lethal infection in IL-10-deficient mice, which occurs in the presence of a dysregulated and unrestrained IFN- γ -mediated neuroimmune response. While there is no doubt that the immune system serves to protect and defend the host from invading pathogens, an uncontrolled proinflammatory response could also create a potentially deleterious situation within the enclosed CNS microenvironment. Anti-inflammatory cytokines, such as IL-4, IL-10, and TGF- β , have been described as inhibitors of proinflammatory responses in the CNS, and specifically as suppressors of microglial cell function (Suzumura et al., 1994; Suzumura et al., 1993). In addition, previous *in vitro* studies from our laboratory have demonstrated that infection of primary human microglia with human cytomegalovirus (CMV) resulted in CXCL10 production from these cells (Cheeran et al., 2003). This CXCL10 production, in response to CMV, was found to be suppressed following treatment of the microglia with IL-10 and IL-4, but not transforming growth factor (TGF)- β (Cheeran et al., 2003). It is interesting to note that human cytomegalovirus carries a homolog of IL-10 (i.e. cmvIL-10) which also inhibits CXCL10 production (Cheeran et al., 2003), presumably to subvert host defences. The presence of a similar IL-10 homolog in the MCMV genome has not been identified. Further *in vitro* studies have shown that treatment of human microglial cells with IL-10 prior to infection with

herpes simplex virus (HSV)-1 suppresses virus-induced TNF- α , IL-1 β , and CCL5 production, through a mechanism involving inhibition of infection-induced NF- κ B activation (Marques et al., 2004).

Based on our previous in vitro results, for the present study we hypothesized that in the absence of anti-inflammatory cytokine-mediated downregulation of the neuroimmune response, a more robust and sustained proinflammatory response to MCMV brain infection would be observed. To our surprise, while wild-type BALB/c animals controlled viral brain infection, direct icv injection of MCMV into IL-10 knockout (KO) mice resulted in a lethal infection. In fact, these IL-10 KO animals succumbed to MCMV brain infection as rapidly as highly immunosuppressed SCID/beige mice (Cheeran et al., 2003). An identical infection of IL-4 KO animals, however, did not induce lethal disease. At the present time, we do not know why MCMV brain infection is lethal in these IL-10 KO animals, but proper regulation of neuroimmune responses may be crucial in controlling immunopathological brain damage associated with clearing viral infections.

We then set out to further characterize the functional role of IL-10 in regulating the host neuroimmune response to MCMV brain infection by assessing the levels of several prototypical proinflammatory cytokines and chemokines in infected brain tissue obtained from both wild-type and IL-10 KO animals. The data obtained from these experiments show 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 KO animals versus those observed in wild-type mice. CXCL9 and CXCL10 are chemokines which are best known for their ability to attract activated T lymphocytes to sites of viral infection (Farber, 1997). Interestingly, the levels of several other proinflammatory mediators (i.e., TNF- α , IL-1 β , and CCL5) were not altered in the IL-10 KO mice. Taken together, a failure to restrain IFN- γ , as well as IL-6, production and to downregulate the IFN- γ -inducible chemokines CXCL9 and CXCL10 was seen in IL-10 deficient mice relative to wild-type animals.

We next examined whether IL-10-deficient mice failed to control the amount of virus present during MCMV brain infection. For these studies we used real-time PCR experiments designed to quantify both the number of viral genome copies (i.e., viral load) and assess viral gene expression in the brains of MCMV-infected IL-10 KO versus wild-type animals. Similar amounts of viral nucleic acid were found in the brains of animals from both groups using either DNA (for genome copies) or RNA (for transcripts) PCR. The PCR results obtained correlated well with the actual levels of infectious virus as assessed by TCID₅₀ assay. Using all three assays, similar amounts of virus were seen in the brains of wild-type and IL-10 KO mice. Interestingly, both wild-type and IL-10 KO mice were able to restrict MCMV replication and spread when compared to immunodeficient SCID/bg animals (Cheeran et al., 2003). Taken together, these data suggest that the increased mortality seen in IL-10 KO animals is not simply due to failure to control viral replication.

Taken together with our previous studies, results presented here indicate that control of MCMV brain infection by the immune response and control of excess brain inflammation by IL-10 may be independent actions mediated through different mechanisms. IL-10 has been shown to be secreted by both microglial cells and Tr1 regulatory T cells (Tregs) (Burkhart et al., 1999; Groux et al., 1997; OGarra et al., 2004; Sheng et al., 1995; Williams et al., 1996). Results generated during these experiments suggest that the IL-10 necessary to control dysregulated virus-induced, IFN- γ -mediated neuroimmune response in wild-type mice is provided by a peripheral CD45⁺ immune cell type. Thus, experiments are currently underway to characterize the phenotypic profile of the inflammatory brain infiltrate and determine which cell type is responsible for IL-10 production.

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Fig. 1.

Lethal MCMV brain infection in IL-10 KO mice. (A) MCMV RM461 was injected slowly into the right lateral ventricle of IL-10 KO (closed circles, n=15), IL-4 KO (open squares, n=11), and wild-type (Balb/C) (open triangles, n=12) adult mice. Data are expressed as percent of mice in each group surviving at the indicated time point, followed over the 18 d time-course of the experiment. (B) Kinetics of IL-10 expression in the brains of wild-type BALB/c mice (closed circles) in response to MCMV infection. IL-10 mRNA levels were examined over the 15 day time-course of infection using RT real-time PCR. Transcript levels were normalized to HPRT and are presented as mean ($\pm$ SD) fold increase over animals injected with saline from averaged data obtained using three to ten animals per time point. (C) Levels of IL-10 (pg/mg protein) in brain homogenates obtained from sham-injected (saline) and MCMV-injected wild-type (Balb/C) mice during MCMV infection were measured at 5 days p.i. using ELISA.

Fig. 2.

Dysregulated cytokine expression during MCMV brain infection in IL-10 knock out mice. The kinetics of cytokine mRNA expression were measured by quantitative real-time RT-PCR using total RNA extracted from whole brain homogenates obtained from infected wild-type (Balb/C, closed circles) versus IL-10 KO (open circles) mice. Total RNA was extracted, DNase-treated and reverse transcribed. cDNA obtained was analyzed by qPCR using primers specific to IFN- γ (A), along with ELISA for IFN- γ (B); and IL-6 (C) followed by IL-6 ELISA (D); TNF- α (E) and IL-1 β (F). Cytokine mRNA levels were normalized to HPRT and averaged data are expressed as mean $\pm$ SD fold increase in relative mRNA induction levels over sham-infected controls from 3–6 animals per time point. Brains from MCMV-infected wild-type and IL-10

KO mice were harvested at 5 days p.i. for ELISA and data are expressed as mean ($\pm$ SEM) of three to five animals per group.

Fig. 3.

Dysregulated chemokine expression during MCMV brain infection in IL-10 knock out mice. Brains from MCMV-infected wild-type (Balb/C) and IL-10 KO mice were harvested at 5 days p.i. Total RNA was extracted, DNase treated and reverse transcribed. The cDNA obtained was analyzed by quantitative real-time PCR using primers specific to CXCL9 (A) and CXCL9 ELISA (B); CXCL10 (C) and CXCL10 ELISA (D); CCL5 (E) and CCL5 ELISA (F). Chemokine mRNA expression was normalized to HPRT. Averaged data from 3–6 animals per time point are expressed as fold increase in relative mRNA induction over sham-infected controls. Brains from MCMV-infected wild-type and IL-10 KO mice were harvested at 5 days p.i. for ELISA and data are expressed as mean ($\pm$ SEM) of three to five animals per group.

Fig. 4.

Similar viral loads in the brains of IL-10 KO and wild-type animals. (A) Viral load in brain tissue homogenates from both groups of animals was quantified using real-time DNA PCR (5 days p.i.). The amount of MCMV DNA was normalized to β -actin and is presented as mean ($\pm$ SD) normalized copy number from averaged data using three to five animals per group. (B) Viral titers in brain homogenates of MCMV-infected mice obtained at 5 days p.i. Titers were obtained using TCID₅₀ assay. Data are presented as mean ($\pm$ SEM) titers from three animals per group. (C) MCMV gB mRNA expression was measured using quantitative real-time RT-PCR. Total RNA was extracted from whole brain homogenates of wild-type (Balb/C) or IL-10 KO animals at 5 days p.i. gB mRNA levels were normalized to HPRT (hypoxanthine guanine phospho-ribosyl transferase) and are presented as mean of normalized expression from averaged data obtained using four to eight animals per group. (D) Viral gB mRNA levels in the brains of infected, immunodeficient (SCID) versus wild-type (Balb/C) mice. Data are presented as mean ($\pm$ SEM) mRNA expression (normalized to HPRT) obtained using 5 SCID and 3 wild-type animals per group.

Fig. 5.

Expression of IL-10 mRNA by brain-infiltrating CD45(hi)/CD11b(-) and CD45(hi)/CD11b(int) inflammatory cells during MCMV infection. (A) Single cell suspensions of brain tissue obtained from saline-injected (sham) and (B) virus infected (MCMV infected) mice (5 animals/group at 5 days p.i) were banded on a 70% Percoll cushion. Brain leukocytes at the 30-70% Percoll interface were collected, labeled with APC-conjugated Abs specific for CD45 and FITC-labeled anti-CD11b Abs, and sorted into four populations (A through D) using FACS. Data presented are representative of three separate experiments using cells from 5 animals each. (B) Total RNA extracted from each of the separated populations (A through D) from virus-infected (infected) or saline-treated (sham) brains (5 days p.i) was analyzed by qRT-PCR for IL-10 expression. Transcript levels were normalized to HPRT expression and are presented as mean ($\pm$ SEM) using cells isolated from the brain tissues of five animals.