Virology 409 (2011) 198–203

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Importance of macrophage inflammatory protein-1α and splenic macrophages in neurodegeneration induced by PVC-211 murine leukemia virus

Xiujie Li, Xiang Zhang, Joan L. Cmarik, Sandra Ruscetti *

Laboratory of Cancer Prevention, National Cancer Institute-Frederick, Frederick MD 21702, USA

ARTICLE INFO

Article history:
Received 13 July 2010
Returned to author for revision
4 October 2010
Accepted 7 October 2010
Available online 4 November 2010

Keywords:
Retrovirus
PVC-211 MuLV
Neuropathogenesis
Chemokine
MIP-1α
Microglia
Splenectomy

ABSTRACT

We recently reported that infection of rats with the neurodegenerative disease-causing retrovirus PVC-211 MuLV results in elevated levels of the chemokine MIP-1α followed by the accumulation of activated microglia in the brain. To investigate the importance of MIP-1α in recruitment of microglia to the brain, we treated rats with MIP-1α antibodies before and after PVC-211 MuLV infection. This caused a delay in the development of paralysis which was associated with a decrease in activated microglia without affecting virus expression. To determine the source of activated microglia, rats were splenectomized 4 days after virus infection. Splenectomized rats showed a delay in disease development that was associated with decreased numbers of activated microglia without affecting virus expression. Together, these results suggest that MIP-1α is directly involved in the neurodegeneration induced in rats by PVC-211 MuLV by recruiting macrophages/microglia from the periphery into regions of the brain that eventually become diseased.

Published by Elsevier Inc.

Introduction

PVC-211 murine leukemia virus (MuLV) induces a rapid, age-dependent spongiform neurodegenerative disease in rodents, resulting in paralysis (Kai and Furuta, 1984; Masuda et al., 1992). The primary target of PVC-211 MuLV infection within the central nervous system (CNS) is the brain capillary endothelial cell (BCEC) as shown by both *in vivo* (Hoffman et al., 1992) and *in vitro* (Jinno-Oue et al., 2001) experiments, suggesting that neurodegeneration caused by PVC-211 MuLV is an indirect result of virus infection of blood vessels within the CNS. Our recent data showed that brain vessels were altered and microglia were activated following virus infection, and that depletion of macrophages/microglia by clodronate-containing liposomes significantly blocked PVC-211 MuLV-induced neurodegeneration (Li et al., 2009). This suggests that microglia, which can secrete many cytokines/chemokines involved in neurodegeneration (Block et al., 2007; Langmann, 2007), are the major cells contributing to the neuropathogenesis induced by PVC-211 MuLV. We further showed that the chemokine MIP-1α is elevated in both serum and brain tissues of rats infected with PVC-211 MuLV (Li et al., 2009). MIP-1α can act as a leukocyte chemoattractant (Menten et al., 2002) and elevated levels of this chemokine play a role in a number of neurodegenerative diseases (Balashov et al., 1999; Man et al., 2007; Montanheiro et al., 2007; Reale et al., 2009; Tripathy et al., 2007; Wu and Proia, 2004). However, it is unclear whether MIP-1α, by virtue of its ability to attract macrophages/microglia, is directly involved in the neurodegeneration caused by PVC-211 MuLV. It is also not known whether the activated microglia found in the regions of the brain of virus-infected rats that eventually become diseased are recruited from resident microglia in the brain or from a peripheral organ such as the spleen, a major source of macrophages/microglia.

In this study, we determined the effects on the course of PVC-211 MuLV-induced disease of treatment with antibodies to MIP-1α, which reduced serum levels of the chemokine, as well as splenectomy, which removed a major source of peripheral macrophages/microglia available for recruitment by MIP-1α. Our data indicate that both treatments decreased the number of activated microglia in the brains of virus-infected rats without altering virus expression, leading to a significant delay in the development of neurodegeneration.

Results

Anti-MIP-1α treatment delays PVC-211 MuLV-induced paralysis

Our previous data showed that increased expression of the chemokine MIP-1α is associated with neurodegeneration induced by PVC-211 MuLV (Li et al., 2009). In order to further understand the importance of MIP-1α in the disease, we treated PVC-211 MuLV-

* Corresponding author. Laboratory of Cancer Prevention, Building 567, Room 152, National Cancer Institute-Frederick, Frederick, MD 21702-1201, USA. Fax: +1 301 846 6164.
E-mail address: ruscetts@mail.nih.gov (S. Ruscetti).

0042-6822/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.virol.2010.10.012

infected rats with anti-MIP-1α neutralizing antibodies via i.c. injection starting at birth (1 day prior to virus injection) and continuing for a total of 9 injections, ending at 17 dpi (see Materials and methods). The dose was adjusted for the age of the rat. As shown in Fig. 1, the paralysis phenotype was markedly delayed in the PVC-211 MuLV-infected rats that had been treated with anti-MIP-1α antibodies compared to PVC-211 MuLV-infected control rats. At 26 days post virus injection, only 16% (3/19) of the infected rats treated with anti-MIP-1α antibodies became paralyzed, and the brains of these rats showed markedly less spongiform neurodegeneration (data not shown) than control rats 44% (8/18) of which were paralyzed by 26 dpi. At 32 days post virus injection (and over 2 weeks after the last antibody injection), only 21% (4/19) of rats treated with anti-MIP-1α antibodies showed complete paralysis in contrast to 78% (14/18) of control rats. However, by day 40 post virus injection, 67% (10/15) of anti-MIP-1α-treated rats became paralyzed. These data suggest that MIP-1α plays a causal role in the neurodegeneration and paralysis induced by PVC-211 MuLV.

To exclude that the delay in the development of virus-induced neurodegeneration after treatment with anti-MIP-1α antibodies was caused by a decrease in the level of virus infection, we carried out western blotting analysis to determine the levels of envelope protein (gp70) of PVC-211 MuLV. As shown in Fig. 1B, similar amounts of viral gp70 were detected in the spleen, brainstem and serum of PVC-211 MuLV-infected control rats and those treated with anti-MIP-1α antibodies, indicating comparable viral load in treated and control rats.

PVC-211 MuLV-infected rats treated with anti-MIP-1α show decreased levels of MIP-1α in the serum and fewer activated microglia in the brain

In order to confirm that the anti-MIP-1α antibodies were neutralizing MIP-1α in the virus-infected rats, we carried out a specific immunoassay to detect the expression of MIP-1α in the serum. As shown in Fig. 2A, PVC-211 MuLV-infected control rats showed high levels of MIP-1α at 14 dpi, as previously reported (Li et al., 2009). In contrast, the virus-infected rats given anti-MIP-1α antibody showed significantly ($P<0.05$) less MIP-1α at 14 dpi, indicating that the antibody was effective in neutralizing MIP-1α.

To determine whether activation of microglia is influenced by administration of anti-MIP-1α antibody, immunostaining of brain tissue with ED1 monoclonal antibody, which detects activated microglia, was performed. At 21 dpi, less ED1 staining was found in both the brainstem (Fig. 2B) and the cerebellum (data not shown) of virus-infected rats treated with anti-MIP-1α antibody as compared with controls, suggesting that lower amounts of MIP-1α delayed the recruitment of activated microglia to the brain.

Splenectomy delays the paralysis phenotype in PVC-211 MuLV-infected rats without decreasing virus infection

The source of activated microglia in the region of the brain that becomes diseased can be from resident microglia that are recruited by MIP-1α to those regions and from macrophages/microglia in the periphery that are recruited by MIP-1α into the brain. Since the spleen is one of the major peripheral sources of macrophages/microglia available for recruitment by chemokines to the brain, we tested the effect of removing the spleen on the development of neurodegeneration induced by PVC-211 MuLV. Splenectomies were performed at 4 days of age (2 days after virus injection). As shown in Fig. 3A, splenectomized rats showed a marked delay in the development of neurodegeneration and paralysis compared with control rats. By 26 days post-infection, only 19% (4/21) of the splenectomized, virus-infected rats had become paralyzed, while 73% (8/11) of the non-splenectomized, virus-infected rats became paralyzed. By 40 days after infection, 67% (14/21) of the splenectomized, PVC-211 MuLV-infected rats had become paralyzed, perhaps reflecting the recruitment of macrophages/microglia from other peripheral organs such as the bone marrow.

Since the spleen is an organ where the virus has been shown to replicate and removing this organ might decrease viral load, we carried out western blot analysis to determine the level of PVC-211 MuLV envelope protein (gp70) in the brain. Splenectomy did not significantly decrease the level of MuLV gp70 in the brain at 24 dpi (Fig. 3B) or 14 dpi (data not shown) and actually led to an increase in expression in the bone marrow (Fig. 3B) compared with non-splenectomized rats, allowing us to exclude the possibility that the delay in disease after splenectomy was the result of lower viral titers in the rats. The increased expression of MuLV gp70 in the bone marrow of PVC-211 MuLV-infected splenectomized rats may result from a high rate of proliferation in the bone marrow to compensate for cells removed by splenectomy.

Increased MIP-1α levels and fewer activated microglia were found in PVC-211 MuLV-infected, splenectomized rats compared with controls

To determine if the delayed paralysis phenotype found in splenectomized rats was associated with a change in the levels of MIP-1α, which we showed plays a role in neurodegeneration, we carried out a specific immunoassay to detect MIP-1α in the serum of splenectomized and control rats at various times after injection of PVC-211 MuLV. As shown in Fig. 4A, MIP-1α levels in the serum of PVC-211 MuLV-infected control rats were increased at 14 dpi, as previously shown (Li et al., 2009). Interestingly, MIP-1α levels at

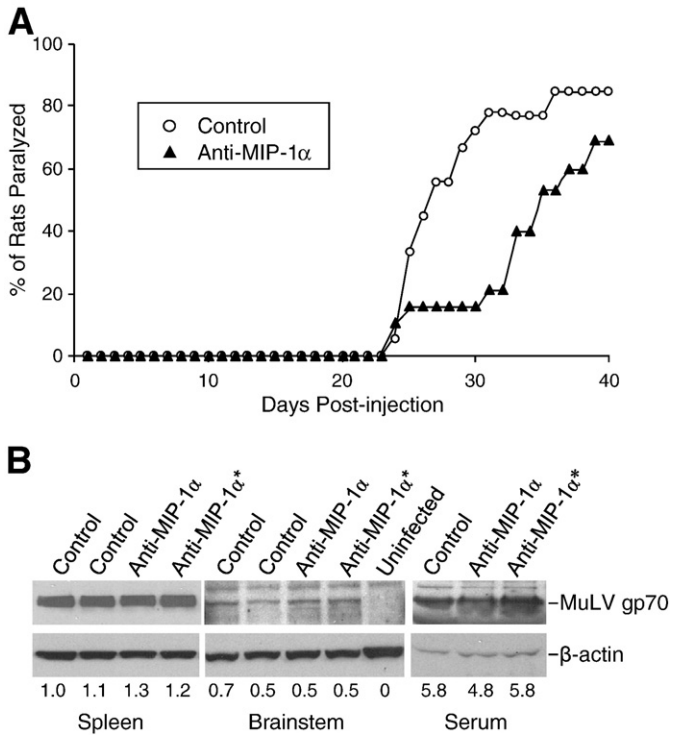

Fig. 1. Treatment of rats with neutralizing antibodies to MIP-1α delays spongiform neurodegeneration and paralysis induced by PVC-211 MuLV but does not alter viral replication. PVC-211 MuLV-infected rats were treated with anti-MIP-1α antibodies beginning on the day prior to virus injection and continuing for 8 more injections until 17 days post virus injection. Control PVC-211 MuLV-infected rats were treated with non-immune serum or water. A total of 19 anti-MIP-1α-treated rats and 18 control rats from 4 different experiments were followed. A. Percentage of PVC-211 MuLV-infected rats with complete paralysis at various time points after virus injection. ▲: PVC-211 MuLV-infected rats given anti-MIP-1α antibodies and ○: PVC-211 MuLV-infected control rats. B. PVC-211 MuLV gp70 envelope expression in the spleen, brainstem and serum from anti-MIP-1α-treated, control PVC-211 MuLV-infected or uninfected rats at 24 dpi or at the end of the observation period (40 dpi) (asterisk). β-actin was used as a loading control. The amount of MuLV gp70 was quantitated in relationship to the loading control and is represented as a gp70/actin ratio at the bottom of each lane.

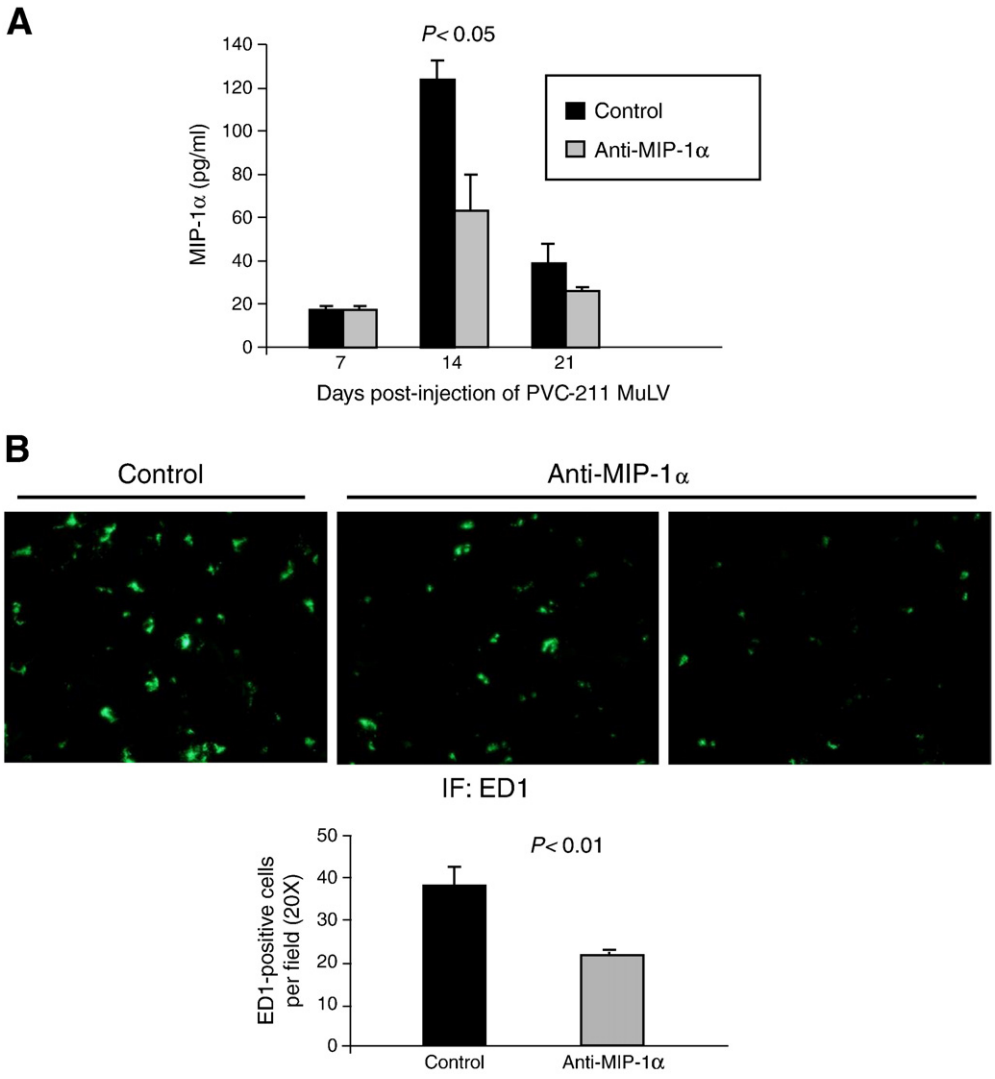

Fig. 2. PVC-211 MuLV-infected rats treated with anti-MIP-1α antibodies show decreased levels of MIP-1α in the serum and fewer activated microglia in the brain. A: Protein levels of MIP-1α were detected by a specific immunoassay using serum from PVC-211 MuLV-infected rats collected at different time points after virus injection. Gray bars: rats given anti-MIP-1α and black bars: control rats. Data represent means ± SEM. $P<0.05$ at 14 dpi and $P>0.05$ at 7 and 21 dpi. B: Activated microglia (upper panels) were detected at 21 dpi in frozen sections of brainstem from PVC-211 MuLV-infected rats by immunofluorescent (IF) staining with ED1 antibody (indicated by green color). Left: Control PVC-211 MuLV-infected rats and middle and right: PVC-211 MuLV-infected rats treated with anti-MIP-1α antibodies. 20×. Comparison of the average number of activated microglia (as determined by counting the number of ED1 antibody-positive cells per 20× field) in the brainstem of 3 control rats and 3 anti-MIP-1α antibody-treated rats is shown at the bottom. Equivalent numbers of cells (as determined by DAPI staining; not shown) were examined in each case.

14 dpi in the virus-infected splenectomized rats were even higher than those in the control rats ($P<0.05$), most likely representing a compensatory response to the loss of macrophages/monocytes resulting from the spleen removal. However, analysis of the blood of splenectomized and control rats at 4, 11 and 18 days after splenectomy revealed no significant differences in their leukocyte or red blood cell counts (data not shown).

To determine whether splenectomy delayed microglia activation in the brain, we performed immunostaining with ED1 antibody. As shown in Fig. 4B, considerably fewer activated microglia were detected at 21 dpi in the PVC-211 MuLV-infected rats after splenectomy.

Discussion

Previous studies have indicated that the neuropathogenesis induced in rats by PVC-211 MuLV is the indirect result of virus replication in brain capillary endothelial cells (Hoffman et al., 1992; Masuda et al., 1992). We recently demonstrated that PVC-211 MuLV infection of BCEC results in the production of the vascular permeability factor VEGF and speculated that this is responsible for the vascular changes seen in the brains of the virus-infected rats, leading to activation of microglia in response to the vascular damage (Li et al., 2009). The importance of microglia activation was shown by depleting macrophages/microglia from rats before virus infection, which caused a significant reduction in the development of neurodegeneration (Li et al., 2009). We also demonstrated that the chemokine MIP-1α is specifically elevated in the brains and serum of rats infected with PVC-211 MuLV and that this precedes the accumulation of activated microglia in the diseased areas of the brain (Li et al., 2009). In this study, we carried out experiments to determine the importance of MIP-1α in activation of microglia and development of neurological disease as well as experiments to determine the source of the microglia recruited into diseased areas of the brain by MIP-1α. Our studies indicate that blocking MIP-1α markedly reduces microglia activation and delays the development of paralysis in PVC-211 MuLV-infected rats, supporting a causal role for MIP-1α in our virus-induced neurodegenerative disease. Our studies further demonstrate that removing the spleen 2 days after infection of rats with PVC-211 MuLV also reduces microglia activation and clearly delays the development of paralysis, indicating that the spleen is an

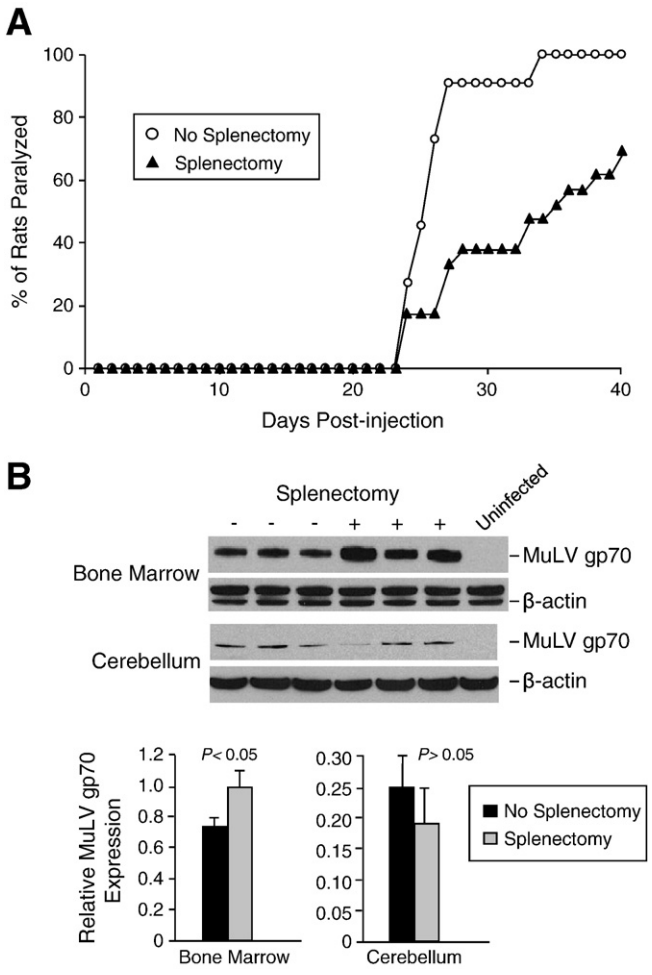

Fig. 3. Splenectomy causes a delay in the paralysis phenotype of PVC-211 MuLV-infected rats without decreasing virus replication. PVC-211 MuLV-infected rats were splenectomized at 4 days of age. A total of 21 splenectomized rats and 11 PVC-211 MuLV-infected control rats from 2 different experiments were followed. A. Percentage of PVC-211 MuLV-infected rats with complete paralysis at various time points after virus injection. ▲: splenectomized PVC-211 MuLV-infected rats and ○: non-splenectomized PVC-211 MuLV-infected rats. B. PVC-211 MuLV gp70 expression at 24 dpi in bone marrow and cerebellum from PVC-211 MuLV-infected rats with or without splenectomy. The amount of MuLV gp70 was determined by Western blot using anti-MuLV gp70 antiserum. β-actin was used as a loading control. The amount of MuLV gp70 was quantitated in relationship to the loading control and is represented as relative MuLV gp70 expression (gp70/actin) in the bar graph. Black bars: control rats and gray bars: splenectomized rats. Data represent means ± SEM.

important source of microglia that become activated in the diseased areas of the brains of the virus-infected rats. Taken together, our data further supports the crucial role of activated microglia in the neurodegenerative disease induced by PVC-211 MuLV.

MIP-1α is a CC-type chemokine that is a strong chemoattractant for monocytes (Davatelis et al., 1988). MIP-1α plays a beneficial role in wound repair (DiPietro et al., 1998), but its overproduction has been associated with neurodegenerative diseases in mice and humans (Balashov et al., 1999; Man et al., 2007; Montanheiro et al., 2007; Reale et al., 2009; Tripathy et al., 2007; Wu and Proia, 2004). In our model, we propose that MIP-1α recruits large numbers of macrophages/microglia to the brain and that the activated microglia not only release MIP-1α to recruit more microglia but also release other factors [e.g. nitric oxide, reactive oxygen species and glutamate (Langmann, 2007)] that can cause neuronal damage, leading to paralysis. Neutralization of MIP-1α stops recruitment of macrophages/microglia to the brain, essentially preventing the accumulation of sufficient activated microglia to cause neurodegeneration. Although treatment of rats with anti-MIP-1α antibodies significantly delayed the development of neurological disease induced by PVC-211 MuLV, it did not prevent it. Up to day 32 after virus injection and over two weeks after stopping treatment with MIP-1α neutralizing antibody, only 21% of the treated rats became paralyzed, in contrast to 78% of the control rats. The fact that a few treated rats became paralyzed during this time period may be due to variations from rat to rat in the level of reduction of MIP-1α. After day 32, more of the MIP-1α antibody-treated rats gradually became paralyzed, reaching 67% by day 40. This most likely reflects the decreasing effects of the MIP-1α antibody treatment, which was stopped on day 17 post virus injection.

Our data also shed some light on the source of the activated microglia in the diseased areas of the brains of PVC-211 MuLV-infected rats. Activated microglia can come from two sources: resident brain microglia or macrophages/microglia from peripheral tissues such as the spleen or bone marrow. The spleen, which is a major resource of macrophages/microglia, can respond to injury of the brain by producing elevated levels of circulating cytokines (Dimagl et al., 1999; Offner et al., 2006). Subsequently, macrophages/microglia stored in the spleen are released into the bloodstream, from which they infiltrate the brain and promote a secondary response that enhances neurodegeneration (Ajmo et al., 2008). This major source of macrophages/microglia is removed upon splenectomy. Different neurological disease models have implicated either resident brain microglia or peripheral macrophages/microglia recruited to the brain in disease. In the rat middle cerebral artery occlusion (MCAO) model of stroke, several groups have shown that the majority of the macrophages in the neurodegenerating regions of the brain are resident microglia (Denker et al., 2007; Schilling et al., 2003). However, in another study using a rat MCAO model of neurodegeneration, the spleen was shown to play a significant role in neurodegeneration, indicating that infiltrating monocytes/macrophages from spleen were important (Ajmo et al., 2008). Also, in the murine model for Sandhoff disease, a lysosomal storage disease, researchers demonstrated that macrophages were recruited via MIP-1α from the periphery into the CNS and implicated this in the pathogenesis of the disease (Wu and Proia, 2004). In our model of neurodegeneration, the results of splenectomy on PVC-211 MuLV-induced neurodegenerative disease have provided evidence that macrophages/microglia from the periphery, such as the spleen, but not local resident microglia, may be the main source of the activated microglia in the brain. Although splenectomy delayed the development of paralysis after PVC-211 MuLV infection, it did not prevent it. This is most likely due to a compensatory response of the rat to the splenectomy, which would be to produce more macrophages to replace the loss. In the splenectomized PVC-211 MuLV-infected rats, we observed an increase in the level of MIP-1α after splenectomy, and this may result in the production of more macrophages in other organs, eventually creating another large peripheral pool of cells which can be recruited by MIP-1α to the brain to cause paralysis.

In summary, our studies of PVC-211 MuLV-induced neurodegeneration demonstrate that the chemokine MIP-1α and splenic macrophages are important for microglia activation in the brain and the development of paralysis in virus-infected rats. These studies suggest that human neurodegenerative diseases associated with elevated cytokines/chemokines and microglial activation, such as Alzheimer's disease, Parkinson's disease and multiple sclerosis (Block et al., 2007), may benefit by targeting cytokines/chemokines, and perhaps their receptors, as well as depleting peripheral sources of microglia.

Materials and methods

Virus and animals

PVC-211 MuLV was grown in Rat-1 or NIH 3T3 fibroblasts in Dulbecco's minimal essential medium plus 10% fetal calf serum, as

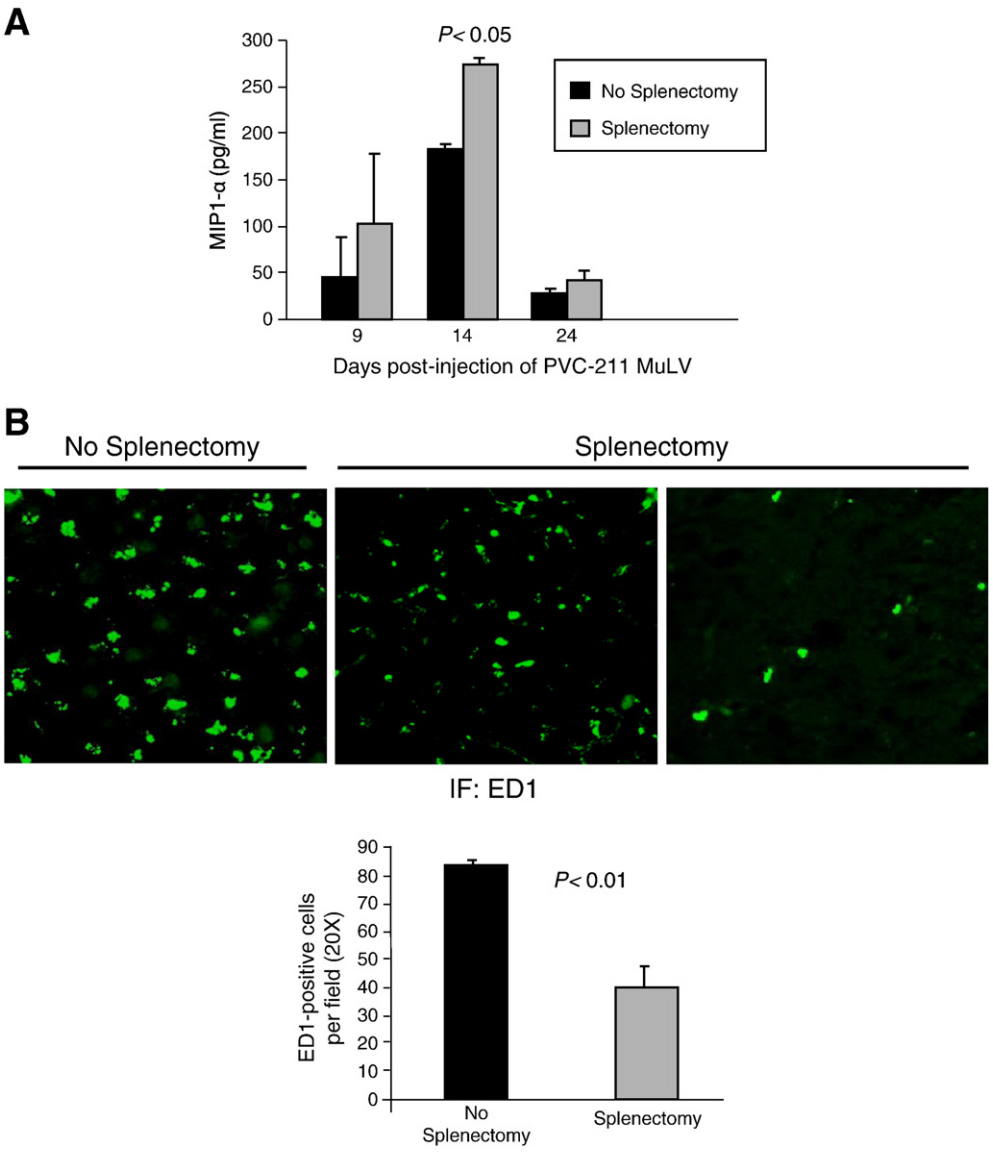

Fig. 4. Splenectomized PVC-211 MuLV-infected rats show increased levels of MIP-1α in the serum and fewer activated microglia in the brain. A: The protein levels of MIP-1α were detected by a specific immunoassay using serum from PVC-211 MuLV-infected rats collected at different time points after virus injection. Gray bars: splenectomized rats and black bars: control rats. Data represent means ± SEM. $P<0.05$ at 14 dpi and $P>0.05$ at 9 and 24 dpi. B: Activated microglia (upper panels) were detected at 21 dpi in frozen sections of the brainstems of non-splenectomized (left panel) or splenectomized (right and middle panel) PVC-211 MuLV-infected rats by immunofluorescent (IF) staining with ED1 antibody (indicated by green color). 20×. Comparison of the average number of activated microglia (as determined by counting the number of ED1 antibody-positive cells per 20× field) in the brainstem of 3 control rats and 3 splenectomized rats is shown at the bottom. Equivalent numbers of cells (as determined by DAPI staining; not shown) were examined in each case.

previously described (Masuda et al., 1992). Virus samples were collected 24 h after incubation of cells with fresh medium and stored at −80 °C until use. Pregnant Fisher 344 (F344) rats were obtained from the Small Animal Facility in NCI-Frederick. Two-day-old F344 rats were inoculated intracranially (i.c.) with 0.05–0.075 ml of supernatant from virus-producing cells or with medium.

Antibody administration

The administration of rabbit anti-rat MIP-1α antibody (PeproTech Inc., Rocky Hill, NJ; Cat. # 500-P77) was started at birth [1 day before virus injection (−1 dpi)] and continued on days 0, 3, 5, 7, 10, 12, 14 and 17 dpi. Three different doses were administered i.c. depending on the age of the rats: 0–7 days of age (40 ng), 7–14 days of age (80 ng) and 14–19 days of age (100 ng). The amount of anti-MIP-1α administered to rats was based on the concentration of MIP-1α found in serum of PVC-211 MuLV-infected rats (Li et al., 2009) and the neutralization concentration provided by the manufacturer. Control animals were injected with an equal amount of either normal rabbit serum or water. Since neither control treatment had an effect on the course of disease, the data was pooled.

Immunofluorescence

For immunostaining to detect ED1-positive cells, frozen sections were fixed in 50% acetone and 50% methanol at −20 °C for 30 min and then permeabilized in 0.3% Triton X-100/PBS for 10 min. After incubation in 5% milk in PBS for 1 h at RT, the slides were incubated

overnight at 4 °C with ED1 monoclonal antibody (1:50, MCA341GA; Serotec, Raleigh, NC). The next morning, the slides were washed with PBS and then incubated with goat anti-mouse IgG coupled to Alexa Fluor 488 (Invitrogen, Carlsbad, CA) for ED1 detection. Coverslips were applied to slides with Pro-Long Gold antifade reagent and DAPI (P36931; Invitrogen).

Measurement of MIP-1α in serum

The level of MIP-1α in serum was determined as previously described (Li et al., 2009) using a CCL3 immunoassay kit (Cat. # MMA00; R&D Systems, Minneapolis, MN) according to the manufacturer's directions.

Western blotting

Tissues were homogenized in lysis buffer (1% Triton X-100, 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate in PBS) and then resuspended in SDS-PAGE sample buffer (BioRad Laboratories, Hercules, CA) containing a reducing agent. Proteins were separated by electrophoresis on an 8% Tris–glycine gel (Invitrogen) and then transferred to a nitrocellulose membrane (Invitrogen). The membrane was then incubated with anti-RLV gp70 serum as previously described (Jinno-Oue et al., 2003). Bound antibody was detected using peroxidase-labeled secondary antibody and the enhanced chemiluminescence system (GE Healthcare, Buckinghamshire, UK). Quantitation of signals after autoradiography was performed by densitometry utilizing NIH-Image software.

Splenectomies

On day 4 after birth, spleens were removed as previously described (Gray et al., 1985). Briefly, rats were anaesthetized using isoflurane, and a small incision was made just below the thoracic area. The spleen was raised, the vessels between the spleen and pancreas were cut using cauterization, and the spleen was removed. The peritoneal wall was closed with 6–0 dexon and the skin was glued together with tissue adhesive. Sham operated control animals were treated the same way except that the spleen was not removed. Sham splenectomy did not delay the onset of paralysis in infected rats (data not shown). Therefore, non-splenectomized PVC-211 MuLV-infected rats were used as controls for the splenectomy studies.

Statistical analysis

Student's *t*-test was employed and *P* values less than 0.05 were considered statistically significant.

Acknowledgments

This work was supported by the Intramural Research Program of the National Institutes of Health, National Cancer Institute, Center for Cancer Research.

We thank Christina Robinson for her excellent technical assistance with our animal studies.

References

Ajmo, C.T., Vernon, D.O., Collier, L., Hall, A.A., Garbuzova-Davis, S., Willing, A., Pennypacker, K.R., 2008. The spleen contributes to stroke-induced neurodegeneration. J. Neurosci. Res. 86, 2227–2234.

Balashov, K.E., Rottman, J.B., Weiner, H.L., Hancock, W.W., 1999. CCR5+ and CXCR3+ T cells are increased in multiple sclerosis and their ligands MIP-1α and IP-10 are expressed in demyelinating brain lesions. Proc. Natl Acad. Sci. USA 96, 6873–6878.

Block, M.L., Zecca, L., Hong, J.S., 2007. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. Nat. Rev. Neurosci. 8, 57–69.

Davatelis, G., Tekamp-Olson, P., Wolpe, S.D., Hermsen, K., Luedke, C., Gallegos, C., Coit, D., Merryweather, J., Cerami, A., 1988. Cloning and characterization of a cDNA for murine macrophage inflammatory protein (MIP), a novel monokine with inflammatory and chemokinetic properties. J. Exp. Med. 167, 1939–1944.

Denker, S.P., Ji, S., Dingman, A., Lee, S.Y., Derugin, N., Wendland, M.F., Vexler, Z.S., 2007. Macrophages are comprised of resident brain microglia not infiltrating peripheral monocytes acutely after neonatal stroke. J. Neurochem. 100, 893–904.

Dimagl, U., Iadecola, C., Moskowitz, M.A., 1999. Pathobiology of ischaemic stroke: an integrated view. Trends Neurosci. 22, 391–397.

DiPietro, L.A., Burdick, M., Low, Q.E., Kunkel, S.L., Strieter, R.M., 1998. MIP-1alpha as a critical macrophage chemoattractant in murine wound repair. J. Clin. Invest. 101, 1693–1718.

Gray, D., Chassoux, D., MacLennan, I.C., Bazin, H., 1985. Selective depression of thymus-independent anti-DNP antibody responses induced by adult but not neonatal splenectomy. Clin. Exp. Immunol. 60, 78–86.

Hoffman, P.M., Cimino, E.F., Robbins, D.S., Broadwell, R.D., Powers, J.M., Ruscetti, S.K., 1992. Cellular tropism and localization in the rodent nervous system of a neuropathogenic variant of Friend murine leukemia virus. Lab. Invest. 67, 314–321.

Jinno-Oue, A., Oue, M., Ruscetti, S.K., 2001. A unique heparin-binding domain in the envelope protein of the neuropathogenic PVC-211 murine leukemia virus may contribute to its brain capillary endothelial cell tropism. J. Virol. 7, 12439–12445.

Jinno-Oue, A., Wilt, S.G., Hanson, C., Dugger, N.V., Hoffman, P.M., Masuda, M., Ruscetti, S.K., 2003. Expression of inducible nitric oxide synthase and elevation of tyrosine nitration of a 32-kilodalton cellular protein in brain capillary endothelial cells from rats infected with a neuropathogenic murine leukemia virus. J. Virol. 308, 1314–1318.

Kai, K., Furuta, T., 1984. Isolation of paralysis-inducing murine leukemia viruses from Friend virus passaged in rats. J. Virol. 50, 970–973.

Langmann, T., 2007. Microglia activation in retinal degeneration. J. Leukoc. Biol. 81, 1345–1351.

Li, X., Hanson, C., Cmarik, J.L., Ruscetti, S., 2009. Neurodegeneration induced by PVC-211 murine leukemia virus is associated with increased levels of vascular endothelial growth factor and macrophage inflammatory protein 1 alpha and is inhibited by blocking activation of microglia. J. Virol. 83, 4912–4922.

Man, S.-M., Ma, Y.-R., Shang, D.-S., Zhao, W.-D., Li, B., Guo, D.-W., Fang, W.-G., Zhu, L., Chen, Y.-H., 2007. Peripheral T cells overexpress MIP-1α to enhance its transendothelial migration in Alzheimer's disease. Neurobiol. Aging 28, 485–496.

Masuda, M., Remington, M.P., Hoffman, P.M., Ruscetti, S.K., 1992. Molecular characterization of a neuropathogenic and nonerythroleukemogenic variant of Friend murine leukemia virus PVC-211. J. Virol. 66, 2798–2806.

Menten, P., Wuyts, A., Damme, J.V., 2002. Macrophage inflammatory protein-1. Cytokine Growth Factor Rev. 13, 455–481.

Montanheiro, P., Vergara, M.P., Smid, J., Duarte, A.J.S., de Oliveira, A.C., Casseb, J., 2007. High production of RANTES and MIP-1α in the tropical spastic paraparesis/HTLV-1-associated myelopathy (TSP/HAM). J. Neuroimmunol. 188, 138–142.

Offner, H., Subramanian, S., Parker, S.M., Afentoulis, M.E., Vandenbark, A.A., Hurn, P.D., 2006. Experimental stroke induces massive, rapid activation of the peripheral immune system. J. Cereb. Blood Flow Metab. 26, 654–665.

Reale, M., Iarlori, C., Thomas, A., Gambi, D., Perfetti, B., DiNicola, M., Onofrj, M., 2009. Peripheral cytokines profile in Parkinson's disease. Brain Behav. Immun. 23, 55–63.

Schilling, M., Besselmann, M., Leonhard, C., Mueller, M., Ringelstein, E.B., Kiefer, R., 2003. Microglial activation precedes and predominates over macrophage infiltration in transient focal cerebral ischemia: a study in green fluorescent protein transgenic bone marrow chimeric mice. Exp. Neurol. 183, 25–33.

Tripathy, D., Thirumangalakudi, L., Grammas, P., 2007. Expression of macrophage inflammatory protein 1-α is elevated in Alzheimer's vessels and is regulated by oxidative stress. J. Alzheimer's Dis. 11, 447–455.

Wu, Y.P., Proia, R.L., 2004. Deletion of macrophage-inflammatory protein 1 alpha retards neurodegeneration in Sandhoff disease mice. Proc. Natl Acad. Sci. USA 101, 8425–8430.