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EXPERIMENTALLY INDUCED DISEASE

Expression Kinetics of RANTES and MCP-1 in the Brain of Deer Mice (*Peromyscus maniculatus*) Infected with Vesicular Stomatitis New Jersey Virus

L. P. Mesquita[*,†], F. R. P. Bruhn[‡], P. C. Maiorka[*] and E. W. Howerth[†]

[*] *Department of Pathology, School of Veterinary Medicine and Animal Science, University of Sao Paulo, Sao Paulo, Brazil,* [†] *Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, USA and* [‡] *College of Veterinary Medicine, Federal University of Pelotas, Pelotas, Brazil*

Summary

The vesicular stomatitis virus (VSV) causes encephalitis in mice when inoculated intranasally. The deer mouse (*Peromyscus maniculatus*), a native New World rodent, is also susceptible to VSV infection and develops similar central nervous system (CNS) lesions to those observed in other rodent species. Chemokines, such as regulated on activation, normal T-cell expressed and secreted (RANTES; CCL-5) and monocyte chemoattractant protein (MCP)-1 (CCL-2), which are important for chemotaxis and activation of inflammatory cells, are expressed during the course of VSV encephalitis. However, the role of CNS resident cells in chemokine expression is poorly characterized. Here, we show that during vesicular stomatitis New Jersey virus (VSNJV) encephalitis in deer mice, RANTES and MCP-1 are expressed only in the olfactory bulb (OB), where the virus was localized. This chemokine expression was followed by the influx of inflammatory cells to the OB later in the course of acute disease. Neurons, astrocytes and microglia expressed RANTES, while MCP-1 was expressed by neurons and astrocytes. Although astrocytes and microglia responded to VSNJV infection by expressing chemokines, neurons were the cell type that was predominantly infected. Therefore, infected neurons may have a critical role in initiating an immune response in the OB. The signalling between neurons and other CNS resident cells is most likely the mechanism by which astrocytes and microglia are activated during the course of VSV encephalitis.

Keywords: chemokine; deer mouse; encephalitis; vesicular stomatitis virus

Introduction

Vesicular stomatitis virus (VSV) is an enveloped, single-stranded negative-sense RNA virus of the genus *Vesiculovirus* of the family Rhabdoviridae. VSV causes acute encephalitis in mice when inoculated intranasally. Initially, VSV infects olfactory receptor neurons (ORNs) of the neuroepithelium and travels caudally to the olfactory bulb (OB), and then spreads to more caudal regions of the central nervous system (CNS) (Huneycutt *et al.*, 1994). The deer mouse (*Peromyscus maniculatus*), a native New World rodent, is susceptible to infection by vesicular stomatitis New Jersey virus (VSNJV) (Cornish *et al.*, 2001). When inoculated intranasally, VSNJV can spread throughout the CNS of the deer mouse through retrograde transneuronal transport and viral spread within the ventricular system (Cornish *et al.*, 2001). Since the pathogenesis of infection by VSNJV in deer mouse is similar to that described for other rodents (Cornish *et al.*, 2001), this rodent species can be used as a model for infection of the CNS.

In mice, VSV infects neurons with a rapid activation of microglia and astrocytes, which contributes

Correspondence to: E. W. Howerth (e-mail: howerth@uga.edu).

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to the development of encephalitis (Bi *et al.*, 1995). The recruitment of inflammatory cells to the CNS can be triggered by chemokines that are expressed by CNS resident cells including neurons, microglia and astrocytes (Asensio and Campbell, 1999). Chemokines are a family of small secretory proteins divided into two major subfamilies based on the arrangement of the two amino-terminal cysteine residues, depending on whether the first two residues are separated by an amino acid (CXC) or not (CC) (Zlotnik and Yoshie, 2000). During the course of VSV encephalitis in mice, many chemokines are expressed at the peak of inflammation, including monocyte chemoattractant protein (MCP)-1, also known as C–C motif ligand 2 (CCL2), and regulated on activation, normal T-cell expressed and secreted (RANTES), also known as CCL5 (Ireland and Reiss, 2006; Steel *et al.*, 2014). These chemokines are both expressed in inflammatory and degenerative disorders of the CNS. RANTES and MCP-1 are mainly involved in the infiltration of monocytes and T lymphocytes into the neuroparenchyma (Reiss *et al.*, 2002). In addition, MCP-1 is involved in the recruitment of natural killer cells (Deshmane *et al.*, 2009). In VSV encephalitis in mice, the expression of these chemokines is important for the activation of inflammatory cells such as $CD4^+$ and $CD8^+$ T lymphocytes within the CNS, and this correlates with the clearance of VSV infection (Ireland and Reiss, 2006). The expression of RANTES in the brain of mice infected with herpes simplex virus type 1 (HSV-1) is responsible for leucocyte rolling and adhesion to microvasculature, contributing to the development of meningoencephalitis (Vilela *et al.*, 2009). In neurodegenerative conditions such as experimental autoimmune encephalomyelitis (EAE), a model for multiple sclerosis, MCP-1 and RANTES expression are correlated with leucocyte adhesion to the cerebral microvasculature (dos Santos *et al.*, 2005). Although the expression of these chemokines is often correlated with inflammation and damage to the neuroparenchyma, in some conditions both RANTES and MCP-1 may exert a neuroprotective effect (Madrigal *et al.*, 2009; Tripathy *et al.*, 2010).

VSV infection in laboratory mice has been studied extensively; however, the immune response of deer mice to VSV infection has not yet been investigated. Although it is known that chemokines are crucial for the development of VSV encephalitis, few studies have attempted to assess the role of each resident cell of the CNS in chemokine production during VSV infection. Therefore, the aim of this study was to evaluate the expression and cellular source of RANTES and MCP-1 in the CNS during the course of VSNJV infection in adult deer mice.

Materials and Methods

Deer Mice and Virus

Tissues from 6-week-old female deer mice from a previous study were used (Cornish *et al.*, 2001). All experimental procedures were performed under a protocol approved by the University of Georgia's Institutional Animal Care and Use Committee. Before the experimental procedures, all animals were tested for serum neutralizing antibodies to VSNJV as previously described (Cornish *et al.*, 2001).

The VSNJV Colorado strain was provided by the National Veterinary Services Laboratories (NVSL), US Department of Agriculture (NVSL accession No. 95-44625). This virus was isolated in 1995 from a horse in Colorado, USA. The isolate was passaged and titrated by end point in confluent Vero cell culture monolayers (Cornish *et al.*, 2001). The infective dose used was 1×10^6 median tissue culture infective doses ($TCID_{50}$) in 50 µl of minimum essential medium (MEM).

Intranasal Inoculation

Thirty-five deer mice were anaesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) and inoculated intranasally with 1×10^6 $TCID_{50}$ of VSNJV-Colorado in 50 µl of minimum MEM (Cornish *et al.*, 2001). These mice were divided into groups of five and animals in each group were killed by CO_2 overdose on days 1–7 post inoculation (pi). Additionally, five mice were inoculated intranasally with MEM without virus and served as controls. Control mice were killed on day 7 pi. Necropsy examination was performed in all mice, including controls. The CNS and the nasal cavity of each animal were collected.

Histology and Immunohistochemistry

Tissues were fixed in formalin for 24–48 h followed by decalcification of the nasal cavity in 5% formic acid. Then, the tissues were processed routinely and embedded in paraffin wax. Sections (3 µm) were stained with haematoxylin and eosin (HE). Coronal sections of different CNS regions were evaluated, including the OB, septostriatal, caudal diencephalon (hippocampus and thalamus), rostral mesencephalon (caudal hippocampus) and rostral cerebellum with cerebellar peduncles.

Immunohistochemistry (IHC) for RANTES and MCP-1 was performed on all areas of the CNS collected for histology. MCP-1 and RANTES were detected as described previously (Hicks *et al.*, 2013), with some modifications. Sections (3 µm) were

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