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Astrocyte response to St. Louis encephalitis virus

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ABSTRACT

St. Louis encephalitis virus (SLEV), a flavivirus transmitted to humans by *Culex* mosquitoes, causes clinical symptoms ranging from acute febrile disorder to encephalitis. To reach the central nervous system (CNS) from circulating blood, the pathogen must cross the blood–brain barrier formed by endothelial cells and astrocytes. Because astrocytes play an essential role in CNS homeostasis, in this study these cells were infected with SLEV and investigated for astrogliosis, major histocompatibility complex (MHC)-I-dependent immune response, and apoptosis by caspase-3 activation. Cultures of Vero cells were used as a positive control for the viral infection. Cytopathic effects were observed in both types of cell cultures, and the cytotoxicity levels of the two were compared. Astrocytes infected with a dilution of 1E-01 (7.7E + 08 PFU/mL) had a reduced mortality rate of more than 50% compared to the Vero cells. In addition, the astrocytes responded to the flavivirus infection with increased MHC-I expression and astrogliosis, characterized by intense glial fibrillary acidic protein expression and an increase in the number and length of cytoplasmic processes. When the astrocytes were exposed to higher viral concentrations, a proportional increase in caspase-3 expression was observed, as well as nuclear membrane destruction. SLEV immunostaining revealed a perinuclear location of the virus during the replication process. Together, these results suggest that mechanisms other than SLEV infection in astrocytes must be associated with the development of the neuroinvasive form of the disease.

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1. Introduction

St. Louis encephalitis virus (SLEV) is an enveloped, positive, single-strand RNA virus that belongs to the family Flaviviridae, genus *Flavivirus* (Monath, 2015), and is serologically related to the Japanese encephalitis complex, which includes viruses such as Japanese encephalitis virus (JEV) and West Nile virus (WNV). SLEV is transmitted to humans mainly by *Culex* spp. bite (Monath and Tsai, 2002; Trent et al., 1980; Rodrigues et al., 2010). The virus is widely disseminated in the Americas (Spinsanti et al., 2008; Auguste et al., 2009; Rodrigues et al., 2010), and once inoculated, it may infect the central nervous system (CNS), causing encephalitis (Lim et al., 2008; Monath 2015).

During the process of invading the CNS, the virus must cross the blood–brain barrier (BBB), formed by endotheliocytes and astrocytes, to reach the brain and spinal cord. The BBB is a highly selective, permeable membrane that regulates the passage of several substances into the brain's extracellular fluid (Suen et al., 2014). If a virion succeeds in penetrating an endotheliocyte and crosses the basement membrane, it still has to infiltrate the astrocyte body to reach a neuron. Astrocytes, the key glial component of the CNS, are responsible for maintaining homeostasis for perfect neuronal function through the secretion of growth factors, glycogen storage, and detoxification of metals and xenobiotics (Chen et al., 2010; Furr and Marriot, 2012; Rao et al., 2012). In addition, these cells control BBB permeability, mainly due to their involvement in inflammatory processes through the expression of cytokines and nitric oxide synthase, inducing leucocyte migration and microglial activation (Bi et al., 1995). Astrocytes also express several molecules that are intimately involved in immune responses, such as major histocompatibility complex (MHC)-I and -II, antiviral interferon *via* pattern recognition receptors, interleukin (IL)-6, tumor necrosis factor α, and IL-1β (Kawai and Akira, 2008; Chauhan et al., 2010; Yoneyama and Fujita, 2010).

Once a virion encounters an astrocyte, both innate and adaptive immune responses are triggered, culminating in the release of cytokines and chemotactic factors that recruit defense cells from

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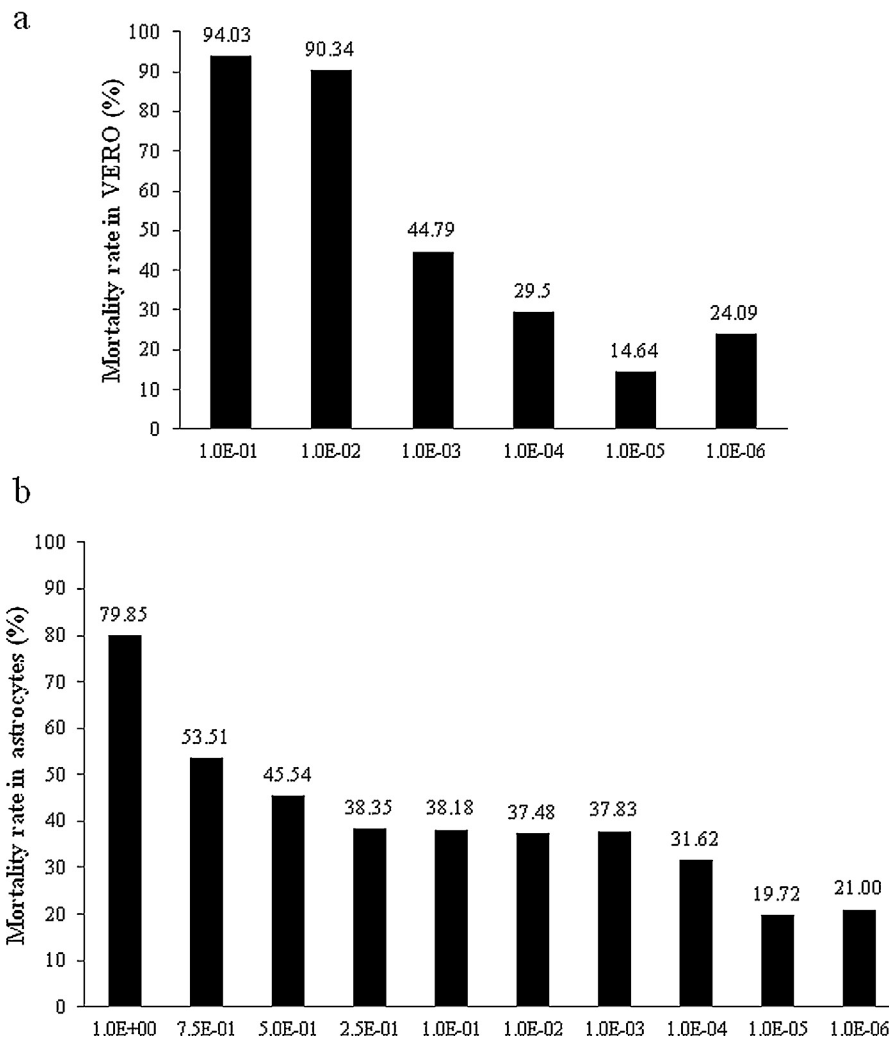

Fig. 1. Mortality rate in (a) Vero cells and (b) astrocytes at six days post-infection with different SLEV dilutions. Note that the viral concentration responsible for 94.03% of mortality in Vero cells caused only 38.18% of mortality in astrocytes.

the bloodstream. Thus, activated astrocytes (or astrogliosis) produce IL-6, IL-8, IL-1β, IL-18, RANTES, interferon γ-inducible protein 10, glial fibrillary acidic protein (GFAP), glutamate aspartate transporter, glutamate transporter 1, nerve growth factor (NGF), ciliary neurotrophic factor (CNTF), and brain-derived neurotrophic factor (BDNF) (Chen et al., 2010). The production of hepatocyte growth factor, ceruloplasmin, glutamine synthetase, vimentin, and reactive oxygen species by astrocytes has also been reported (Mishra et al., 2007). Astrogliosis increases the permeability of the BBB, allowing leukocytes in the bloodstream to cross into the nervous extracellular matrix. Once the BBB is disrupted, more virions can penetrate the nervous tissue coming from the blood, especially in patients with high viremia or immunologic deficiency, resulting in a massive infection of the brain and spinal cord (Dong and Benveniste, 2001; Fischer and Reichmann, 2001; Furr and Marriott, 2012).

Considering that astrocytes are the maestros of BBB homeostasis, the investigation of this cell response to SLEV infection may contribute to a better comprehension of the mechanisms of viral infection of the CNS and encephalitis. To address this issue, this study aimed to investigate the astrocyte–SLEV interaction using primary astrocyte cultures compared to viral infection in the Vero cell lineage. Parameters such as astrogliosis, expression of MHC-I molecules, and apoptosis were evaluated in both infected cell cultures.

2. Material and methods

2.1. Production of virus stock

SLEV (BeH 355964 strain) was cultured by continuous passage in *Aedes albopictus* C6/36 cell linage and maintained in L15 culture medium (Sigma-Aldrich Co., St. Louis, MO, USA) supplemented with 5% fetal bovine serum (FBS; Nutricell, Campinas, Brazil), 100 U/mL penicillin, 100 μL/mL streptomycin (Sigma-Aldrich) at 28 °C. Cultures were harvested after 7 days or when cytopathic effect was observed. For harvesting, cells were frozen at −70 °C (ultrafreezer Nuare, Plymouph, MN, USA) and immediately thawed. The suspension was transferred to a 15 mL Falcon tube and centrifuged at 1000 × *g* for debris removal. Supernatant was removed and the aliquots were stored at −70 °C, constituting the virus stock.

2.2. Astrocyte primary culture

Astrocyte primary cultures were made by dissociation of cerebral cortex of neonate Swiss mice (1–2 days postnatal). Animals were immersed in ice until reaching unconsciousness and decapitated for brain removal. All procedures in animals were submitted and approved by the Ethics Committee on animal use of the Federal University of Uberlandia (protocol number CEUA148/13).

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