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Does astroglial protein S100B contribute to West Nile neuro-invasive syndrome?

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ABSTRACT

The clinical spectrum of West Nile Virus (WNV) infection ranges from a flu-like febrile condition to a more severe neuro-invasive disease that can cause death. The exact mechanism of neurodegeneration in neuro-invasive form of WNV infection has not been elucidated; however, a destructive role played by glial cells in promoting WNV mediated neurotoxicity has widely been speculated. The clinical studies revealed that the astroglial protein S100B is significantly elevated in the blood and CSF of patients with WNV infection, even in the absence of neuro-invasive disease. Therefore, the present study was designed to explore the potential role of S100B in the pathophysiology of WNV infection. The overarching hypothesis was that WNV primes astroglia to release S100B protein, which leads to a cascade of events that may have deleterious effects in both acute and chronic stages of WNV disease. To justify our hypothesis, we first ascertained increased levels of S100B in post-mortem tissue samples from WNV patients. Next, we looked at the effects of UV-inactivated WNV particles on astroglia using astroglial cell lines or primary cultures. Astroglial activation was measured as an increase in the expression of S100B and was analyzed by immunofluorescence and real-time PCR. Further, the in vitro effects of purified S100B protein on neutrophil migration and glutamate uptake were also determined in astroglial cell lines or primary cultures. We found that incubation of cultured astroglial cells with UV-inactivated WNV particles caused induction of S100B both at the mRNA and protein levels. Varying concentrations of S100B stimulated neutrophil migration in vitro. In addition, varying amounts of S100B caused inhibition of glutamate uptake in astroglia in a dose-dependent manner. Our data suggest that inactivated WNV particles are capable of inducing S100B synthesis in astroglia in vitro. We speculate that S100B release by activated astroglia may have multiple roles in the pathophysiology of WNV neuro-invasive disease, including induction of neutrophil migration to the sites where blood brain barrier is disrupted as well as glutamate neurotoxicity. To further elucidate the WNV-S100B neurotoxic pathway, in vivo studies using mouse models are warranted.

1. Introduction

West Nile virus (WNV) is an RNA arbovirus capable of infecting both animals and humans [1–3]. Humans suffering from acute WNV infection present with various symptoms, ranging from a mild, flu-like illness to neuro-invasive forms, such as encephalitis, meningitis, and/or acute flaccid paralysis due to the loss of spinal motor neurons [4]. The recovery from WNV infection may be prolonged and incomplete with many infected persons complaining of lingering or delayed-onset symptoms, from non-specific subjective fatigue to persistent neurologic impairments [5–7].

The precise mechanism of WNV neuro-invasive syndrome is uncertain. In vitro studies show that WNV infection in neurons is robust, rapid and destructive, lasting less than a week. In contrast, the astrocytes seem to be more resistant but remain infected for several weeks [8]. This raises a question as to the role of astrocytes in WNV infection given that they regulate neuronal excitability and function by controlling glutamate reuptake and release of neurotrophic factors. If astrocytes are damaged or activated by a pathologic process, they may destroy neurons via glutamate excitotoxicity or by releasing neurotoxic factors. Indeed, a selective loss of astrocytic glutamate transporter 1 within the lumbar spinal cord gray matter was found in advance of motor neuron injury in mice infected with Neuroadapted Sindbis virus

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(NSV) [9,10]. The reported similarities in spinal cord histopathology between NSV-infected mice and human autopsy tissue of cases presenting with acute flaccid paralysis make the astrocyte-mediated glutamate excitotoxicity hypothesis plausible [11].

Our recent studies provided evidence in support of astroglial activation in human WNV infection. The levels of glial fibrillary acidic protein (GFAP)-SM 126 and S100B were significantly elevated in the cerebrospinal fluid and serum of confirmed WNV cases with symptom onset between 2 and 18 days prior to sampling [12,13]. This raises a more specific question as to whether increased levels of S100B, a calcium-binding protein secreted mainly by astrocytes, interferes with glutamate reuptake and leads to excitotoxicity. This is plausible because S100B in nanomolar concentrations is neurotrophic, whereas in micromolar concentrations it is neurotoxic [14].

S100B also plays a role in neuroinflammation. Several members of S100 family of proteins (S100A8, S100A9, S100A8/A9) are potent stimulators of neutrophils and are involved in neutrophil migration to inflammatory sites [15]. This leaves open the possibility that S100B may facilitate cell migration across brain–blood barrier (BBB). S100B was found to be increased in CSF and serum of virtually all recently infected WNV patients irrespective of clinical presentation [16,17]. Thus, it is conceivable that high levels of S100 may be chemotactic and potentially increase transmigration of blood-borne inflammatory cells across brain capillaries, thereby causing a more severe and prolonged inflammatory response.

As a first step toward examining the putative role of S100B in pathophysiology of WNV infection, this study was designed with two main objectives in mind. The first objective was to determine if more S100B is present in the nervous system of West Nile patients who presented with acute flaccid paralysis and died several weeks after the onset of infection. Our rationale was that such evidence would provide support and justify further in vitro and in vivo studies of S100B. After confirming increased S100B in human autopsy tissue, we proceeded with our second objective to examine possible role of S100B in pathophysiology of WNV infection. We designed three experiments. Our first experiment was to determine if UV inactivated WNV particles may induce S100B in the cultured astrocytic cells both at the mRNA and protein level. The second experiment was to determine if WNV-induced high S100B concentrations may impair glutamate uptake into astrocytes, as an upstream indicator of interference with glutamate transport. The third experiment was to determine if WNV-induced S100B could stimulate neutrophil migration in vitro.

2. Materials and methods

2.1. Materials

Rabbit polyclonal anti-S100B and mouse monoclonal beta-III tubulin antibodies were obtained from Abcam Inc., Cambridge, MA, USA. Dulbecco's Modification of Eagle's Medium (DMEM), Dulbecco's Phosphate-Buffered Saline, 1X, and Trypsin 10X were provided by Mediatech, Inc. A Corning Subsidiary, Manassas, VA, USA. Boyden chamber was purchased from Cell Biolabs, Inc., San Diego, CA, USA. The S100B protein purified from bovine brain was purchased from Sigma-Aldrich (St. Louis, MO, USA). Fetal bovine serum was purchased either

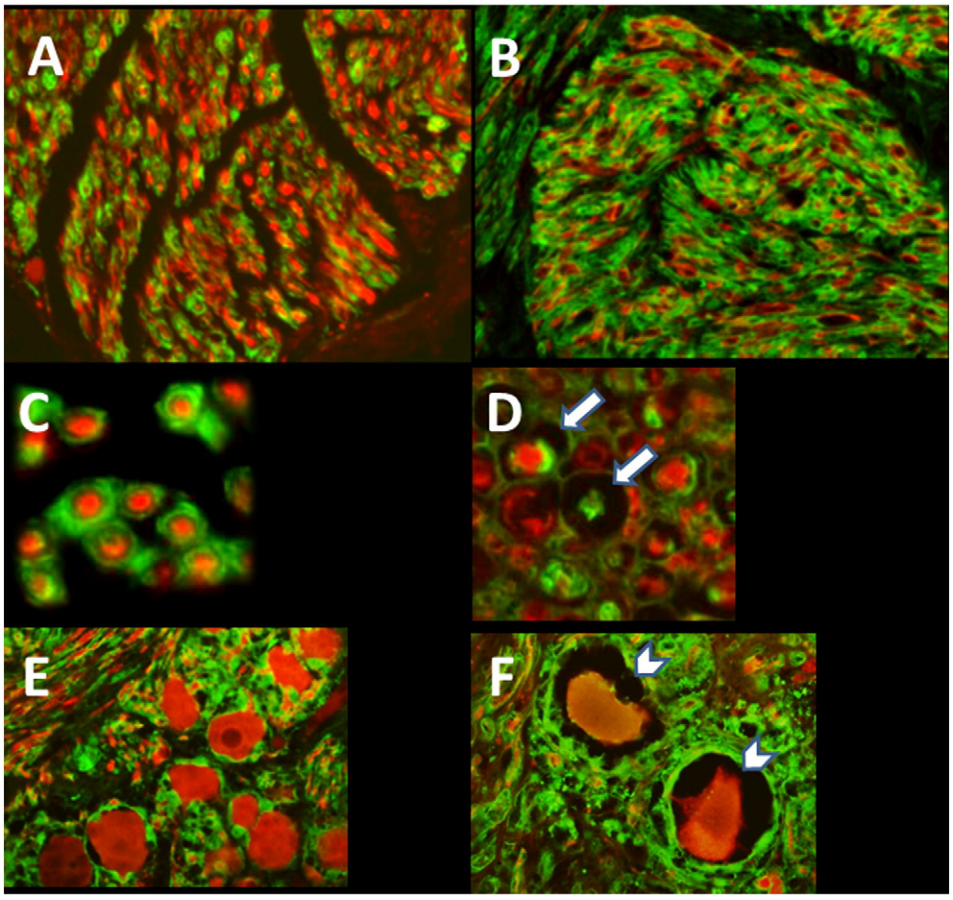

Fig. 1. (A–D) Double-immunostained sections of a nerve originating from lumbosacral spinal cord showing S100B (green) and B-tubulin III (red) in a control (A, C, E) and a WNV-infected patient (B, D, F). S100B is localized to myelinated axons and astrocytes, whereas beta III tubulin labeled individual axons. Axonal swelling and loss of tubulin was visible in WNV-infected tissue section (arrows). WNV-infected tissue (B) also showed increased S100B immunoreactivity, where many axons were degenerating (loss of myelin sheath), in contrast to the control section (A). Neurons in the dorsal root ganglion were markedly enlarged in WNV (arrowheads in F) compared to the control (E).