Virology 527 (2019) 107–115

Contents lists available at ScienceDirect

Virology

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

# Neuronal transcriptomic responses to Japanese encephalitis virus infection with a special focus on chemokine CXCL11 and pattern recognition receptors RIG-1 and MDA5

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ARTICLE INFO

*Keywords:*
Japanese encephalitis virus
Transcriptome
RNA microarray
Proinflammatory mediators
Neuronal infection
CXCL11
RIG-1
MDA5

ABSTRACT

Japanese encephalitis virus (JEV) causes central nervous system neuronal injury and inflammation. A clear understanding of neuronal responses to JEV infection remains elusive. Using the Affymetrix array to investigate the transcriptome of infected SK-N-MC cells, 1316 and 2737 dysregulated genes ($\geq 2/-2$ fold change, $P < 0.05$) were found at 48 hours post-infection (hpi) and 60 hpi, respectively. The genes were mainly involved in anti-microbial responses, cell signalling, cellular function and maintenance, and cell death and survival. Among the most highly upregulated genes ($\geq 10$ folds, $P < 0.05$) were chemokines CCL5, CXCL11, IL8 and CXCL10. The upregulation and expression of CXCL11 were confirmed by qRT-PCR and immunofluorescence. Pathogen recognition receptors retinoic acid-inducible gene-1 (RIG-1) and melanoma differentiation-associated protein 5 (MDA5) were also upregulated. Our results strongly suggest that neuronal cells play a significant role in immunity against JEV. CXCL11, RIG-1 and MDA5 and other cytokines may be important in neuropathogenesis.

## 1. Introduction

Japanese encephalitis virus (JEV) is an approximately 50 nm, spherical, enveloped virion containing a single strand, positive-sense RNA genome of 11 kb. The genome comprises 3′ and 5′ end untranslated regions, and an open reading frame that encodes for three structural proteins (C, prM, E) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5). JEV belongs to the family *Flaviviridae* and genus *Flavivirus* (Misra and Kalita, 2010; Unni et al., 2011), which includes the West Nile virus (WNV), dengue virus (DENV) and tick-borne encephalitis virus (TBEV).

JEV is one of the leading causes of mosquito-borne encephalitides in the world. An annual estimated incidence of about 68,000 cases of Japanese encephalitis (JE), resulting in 13,600–20,400 deaths was reported in affected areas (WHO, 2015). The fatality rate ranges from 25% to 50%, and more than 50% of survivors develop permanent neurological complications (Libraty et al., 2002). Nevertheless, most JEV infections are subclinical, with only a chance of 1:25–1:1000 of developing into symptomatic JE (Solomon and Vaughn, 2002). The virus is maintained in an enzootic cycle between birds, swine and mosquitoes (*Culex*.sp). Human beings are incidental dead-end hosts, in which there is no significant post-infection viremia (Tiroumourougane et al., 2002).

JE is a meningoencephalitis characterized by perivascular cuffing and parenchymal infiltration by inflammatory cells with microglial nodule formation, edema, neuronophagia and necrosis in the central nervous system (CNS) (German et al., 2006). The neuron is the main viral target as evidenced by the neuronal localization of viral antigens and RNA in the cerebral grey matter, thalamus, brainstem, cerebellum, hippocampus, spinal cord and other parts of the CNS (German et al., 2006; Wong et al., 2012). A positive correlation between fatality rate and cytokine levels of IFN-α, IFN–γ, TNF-α, IL2, 4, 6 and 8 in the cerebrospinal fluid (CSF) and serum had been demonstrated (Babu et al., 2006; Ravi et al., 1997; Singh et al., 2000; Winter et al., 2004), suggesting that these inflammatory mediators have significant roles in neuropathogenesis. At present, there is little information with regards to direct immune responses from viral-infected neurons in general, and in particular, JEV-infected neurons.

In order to study early neuronal responses to JEV infection, the microarray technology was used to characterize the entire

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https://doi.org/10.1016/j.virol.2018.10.015
Received 6 July 2018; Received in revised form 1 October 2018; Accepted 16 October 2018