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EXPERIMENTALLY INDUCED DISEASE

Differential Chemokine Responses in the Murine Brain Following Lyssavirus Infection

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Summary

The hallmark of lyssavirus infection is lethal encephalomyelitis. Previous studies have reported distinct lyssavirus isolate-related differences in severity of cellular recruitment into the encephalon in a murine model of infection following peripheral inoculation with rabies virus (RABV) and European bat lyssavirus (EBLV)-1 and −2. In order to understand the role of chemokines in this process, comparative studies of the chemokine pattern, distribution and production in response to infection with these lyssaviruses were undertaken. Expression of CCL2, CCL5 and CXCL10 was observed throughout the murine brain with a distinct caudal bias in distribution, similar to both inflammatory changes and virus antigen distribution. CCL2 immunolabelling was localized to neuronal and astroglial populations. CCL5 immunolabelling was only detected in the astroglia, while CXCL10 labelling, although present in the astroglia, was more prominent in neurons. Isolate-dependent differences in the amount of chemokine immunolabelling in specific brain regions and chemokine production by neurons *in vitro* were observed, with a greater expression of CCL5 *in vivo* and CXCL10 production *in vitro* after EBLV infection. Additionally, strong positive associations between chemokine immunolabelling and perivascular cuffing and, to a lesser extent, virus antigen score were also observed. These differences in chemokine expression may explain the variation in severity of encephalitic changes observed in animals infected with different lyssavirus isolates.

Keywords: chemokine; encephalitis; European bat lyssavirus; rabies

Introduction

The lyssavirus genus is comprised of neurotropic viruses including rabies virus (RABV) and 13 other viruses (Kuzmin *et al.*, 2010; Freuling *et al.*, 2011, 2012). Lyssavirus infection of the central nervous system (CNS) results in non-suppurative meningoencephalomyelitis, with variable degrees of neuronal necrosis, neuronophagia, focal gliosis and lymphocytic perivascular infiltration in the presence or absence of Negri bodies (Charlton, 1984; Charlton *et al.*, 1987; Fekadu, 1988; Hooper *et al.*, 1999). The severity of inflammatory changes after lyssavirus infection can vary depending on the incubation period (Murphy, 1977; Hemachudha *et al.*, 2006), host (Fekadu *et al.*, 1982, Fekadu, 1988) and virus strain (Sugamata *et al.*, 1992; Yan *et al.*, 2001; Roy *et al.*, 2007; Hicks *et al.*, 2009). These inflammatory changes are a non-specific response and are observed commonly in other virus infections of the CNS including West Nile virus (Kelley *et al.*, 2003), Japanese encephalitis virus (Johnson *et al.*, 1985), tick-borne encephalitis virus (Gelpi *et al.*, 2005),

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herpes simplex virus (Yen *et al*., 2009) and measles virus (Garg, 2002).

In a previous study, a murine model has been used to demonstrate lyssavirus isolate-related differences in the level of cellular recruitment, largely comprised of T cells, to the perivascular spaces surrounding blood vessels in the brain and cellular infiltration of the brain parenchyma itself (Hicks *et al*., 2009). The importance of T cells to the host immune response to RABV infection had been established previously using a number of T-cell deficient (Weiland *et al*., 1992; Galelli *et al*., 2000; Lafon, 2005) and B-cell deficient (Hooper *et al*., 2009) mouse models to investigate abortive lyssavirus infection. Despite the clear role for T cells in the clearance of abortive lyssavirus infection (Weiland *et al*., 1992; Galelli *et al*., 2000; Lafon, 2005) and in conferring resistance to lyssavirus infection to SJL/J and BALB/cByJ mice (Perry and Lodmell, 1991), the contribution of infiltrating T cells to the pathogenesis observed in acute lyssavirus infection is uncertain. People who are naturally infected with pathogenic RABV exhibit limited CNS inflammation (Murphy, 1977; Hemachudha *et al*., 2006), while the severity of encephalitis after infection with RABV in mice appears dependent on the virus isolate that is inoculated (Sugamata *et al*., 1992; Yan *et al*., 2001; Roy *et al*., 2007). The pathology of European bat lyssavirus (EBLV) infection has not been studied as intensively as that of RABV infection and preliminary data suggest that EBLV infection is associated with more severe encephalitic manifestations in experimental models (Hicks *et al*., 2009).

Chemokines are low molecular weight pro-inflammatory soluble mediators vital to homeostasis, response to injury, synaptic transmission and development and the cellular recruitment process observed in disease-associated neuroinflammation (De Haas *et al*., 2007). Functional criteria and N-terminal structural motifs are used to categorie chemokines into four different groups: CXC, CC, C and CX_3C. The CXC subfamily, including CXCL10, is defined by an amino acid separating two conserved cysteine residues and is primarily involved in the recruitment of neutrophils. CXCL10 is a chemoattractant associated with monocyte/macrophage, T cell, natural killer (NK) cell and dendritic cell recruitment. It also aids T-cell adhesion to endothelial cells (Dufour *et al*., 2002) and has been associated with T-cell recruitment in response to CNS infection by measles virus (Patterson *et al*., 2003) and West Nile virus (Klein *et al*., 2005).

Chemokines, including CCL2 and CCL5, belonging to the CC subfamily are defined by the presence of conserved adjacent cysteine residues, and are principally involved in the recruitment of monocytes, T cells and macrophages (Banisadr *et al*., 2005). CCL2 is chemotactic for monocytes, memory T cells and dendritic cells and is produced in response to injury or infection (Carr *et al*., 1994). CCL2 can be produced by resident CNS cell populations including neurons, astrocytes and microglia (Banisadr *et al*., 2005; Hickman and El Khoury, 2010) and, consequently, CCL2 is thought to mediate neuroinflammatory processes including T-cell extravasation into the brain (Carrillo-de Sauvage *et al*., 2012). CCL5, a chemoattractant for T cells and monocytes (Amaral et al., 2011), has an established role in neuroinflammatory responses after infection with viruses including Dengue fever virus (Amaral *et al*., 2011), herpes virus (Savarin *et al*., 2010) and West Nile virus (Klein *et al*., 2005).

Experimental infection of mice with an abortive RABV demonstrated that cellular recruitment and virus clearance was preceded by the up-regulation of a variety of inflammatory mediators including chemokines such as CCL2, CCL5 and CXCL10 (Phares *et al*., 2006). Similarly, chemokine mRNA transcripts were up-regulated in mice after infection with acute RABV isolates, including the highly pathogenic silver-haired bat virus (Roy *et al*., 2007; Johnson *et al*., 2008), and EBLV-2 (Mansfield *et al*., 2008). The combination of mRNA transcript up-regulation and the presence of a predominately T cell inflammatory infiltrate observed in mice after infection with EBLV isolates (Mansfield *et al*., 2008; Hicks *et al*., 2009) indicates that CCL2, CCL5 and CXCL10 appear to be important candidates for involvement in cellular recruitment during encephalitis. Furthermore, because of the pivotal role of chemokines in cellular recruitment and the up-regulation of chemokine mRNA transcripts after lyssavirus infection, the variable encephalitis occurring after lyssavirus infection may be attributable to virus-dependent differences in chemokine expression. Comparison of the host innate immune response, including chemokine up-regulation, after the infection of mice with either the abortive RABV strain CVS-F3 or the acute silver-haired bat virus, have highlighted that significant difference in chemokine expression levels do not exist between these two species of lyssaviruses, despite the extensive cellular recruitment observed after infection with CVS-F3 (Roy *et al*., 2007). However, differences in chemokine expression levels may account for the variation in the severity of encephalitis observed between virus isolates belonging to different lyssavirus species.

The aims of this study were to establish whether the greater cellular recruitment into the brain observed in mice following infection with EBLVs when compared with RABV (Hicks *et al*., 2009) resulted from