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Production of IL-8, IL-17, IFN-gamma and IP-10 in human astrocytes correlates with alphavirus attenuation

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ABSTRACT

Venezuelan equine encephalitis virus (VEEV) is an important, naturally emerging zoonotic pathogen. Recent outbreaks in Venezuela and Colombia in 1995 indicate that VEEV still poses a serious public health threat. Astrocytes may be target cells in human and mouse infection and they play an important role in repair through gliosis. In this study, we report that virulent VEEV efficiently infects cultured normal human astrocytes, three different murine astrocyte cell lines and astrocytes in the mouse brain. The attenuation of virus replication positively correlates with the increased levels of production of IL-8, IL-17, IFN-gamma and IP-10. In addition, VEEV infection induces release of basic fibroblast growth factor and production of potent chemokines such as RANTES and MIP-1-beta from cultured human astrocytes. This growth factor and cytokine profile modeled by astrocytes *in vitro* may contribute to both neuroprotection and repair and may play a role in leukocyte recruitment *in vivo*.

1. Introduction

Venezuelan equine encephalitis virus (VEEV) is an enveloped virus with a non-segmented, positive-sense RNA genome of approximately 11.4 kb that belongs to the genus *Alphavirus* in the *Togaviridae* family. The 5′ two-thirds of the genome encodes four nonstructural proteins (nsP1–nsP4) that form an enzyme complex required for viral replication (Strauss and Strauss, 1986, 1994; Strauss et al., 1995). The approximately 4-kb-long, subgenomic RNA corresponds to the 3′ one-third of the viral genome and is translated into a structural polyprotein that is proteolytically cleaved into the capsid protein and the envelope glycoproteins E2 and E1 (Rice and Strauss, 1981).

VEEV is a zoonotic pathogen and a member of the VEE serocomplex, which is divided into six distinct antigenic subtypes (Walton and Grayson, 1988; Young, 1972; Young and Johnson, 1969). Subtypes IAB and IC were previously associated with major epidemics and equine epizootics. During the most recent major outbreak in Venezuela and Colombia in 1995 involving subtype IC VEEV, about 100,000 human cases occurred, with over 300 clinical encephalitis cases estimated (Rivas et al., 1997). Other recent epidemics indicate that VEEV still represents a serious public health problem (Weaver et al., 1996).

The murine model of Venezuelan equine encephalitis (VEE), in which C57BL/6 mice are infected with the virulent ZPC738 strain of VEEV, is a well-established model of the pathogenesis of neurological disease characterized by the development of encephalitis, paralysis and, subsequently, death (Paessler et al., 2003, 2006, 2007). To develop an

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attenuated, immunogenic vaccine, we have previously generated chimeric viruses that encode the replicative machinery from a relatively apathogenic member of the genus, Sindbis virus (SINV), and structural genes derived from VEEV (strain ZPC738, subtype ID) (Paessler et al., 2003). The chimeric vaccine candidate SIN/ZPC was selected and extensively tested for its safety and immunogenicity in hamsters and in a variety of mouse strains with selected immune deficiencies (Ni et al., 2007; Paessler et al., 2006). It has been shown that SIN/ZPC induces a high level of protection in animals with functional αβ T cells and that the passive transfer of $CD3^+$ T cells from vaccinated immunocompetent donors protects mice lacking αβ T cells (Paessler et al., 2007).

To establish an *in vitro* model of human infection that would allow us to study the characteristics of attenuation of different VEEV viruses, we have performed studies in cultured normal (derived from primary tissue culture) human astrocytes (NHA). Among the potential targets of VEEV infection in the CNS, *e.g.*, neurons, astrocytes and glial cells, it appears from examination of the brains of VEEV infected mice that neurons are the major target, but astrocytes may be also infected in lower numbers (16). In order to allow comparison of the results with the mouse model, we also performed experiments with 3 different astrocyte cell lines of murine origin (Alliot and Pessac, 1984). While the neuronal cell population is the major target for VEEV infection, it is unclear to what degree astrocytes may be involved in amplifying the virus and/or orchestrating the inflammatory response (Schoneboom et al., 2000, 1999). It is known, however, that reactive hyperplasia and hypertrophy of astrocytes (gliosis) may be detected in infected brains (Audouy et al., 1999; Caccuri et al., 2003).

Astrocytes play a central role in the CNS in several aspects. First, astrocytes improve neuronal survival in a variety of injury models by taking up toxic amino acids and/or by producing factors such as neurotrophic factors needed for neuronal survival (Rosenberg and Aizenman, 1989; Zhao et al., 2004). For example, molecules that are secreted by astrocytes protect neurons against a variety of insults such as hypoglycemic damage (Cheng and Mattson, 1991), excitotoxicity (Mattson and Rychlik, 1990) and anoxia (Vibulsreth et al., 1987). Second, astrocytes have a role in neuronal differentiation and maturation (Chamak et al., 1987). A variety of growth factors produced by astrocytes, such as epidermal growth factor (EGF), insulin-like growth factor-1 and basic fibroblast growth factor (bFGF) modulate neuron–glia interactions and have mitogenic effects on astrocytes (Stitt and Hatten, 1990). Based on these important functions within the CNS, it is clear that the infection of astrocytes by VEEV may have an impact on neuronal survival either by direct "killing" of astrocytes or by changing their physiological profiles and indirectly influencing their neuroprotective function and their ability to proliferate.

In this study, we demonstrate that normal human astrocytes (NHA) in culture are highly susceptible to infection with VEEV. Additionally, the infection of human and mouse astrocytes by virulent VEEV results in: (1) high infectious virus yield; (2) low-level proinflammatory/innate response; (3) chemokine induction; (4) increased release of bFGF and (5) accumulation of viral nucleocapsids in the nucleus of human astrocytes. Additionally, the infection of murine astrocytes in cell culture and in the brain is demonstrated. We have also performed a more detailed investigation of the immune response to attenuated chimeric virus, which replicates at lower levels but induces a robust proinflammatory/innate response *in vitro*. This model may be useful for determining the potential mechanisms responsible for attenuation of chimeric alphaviruses and to study the role of early proinflammatory response in controlling viral infection in human cells targeted by VEEV.

2. Results

2.1. Growth curves

2.1.1. Susceptibility of cultured human and murine astrocytes to VEEV

To evaluate the validity of *in vitro* studies of VEEV attenuation in selected CNS cell lines as a parallel model of human infection, we infected cultured normal human astrocytes with three strains of VEEV that exhibit differential virulence characteristics in the murine model.

Human astrocytes were susceptible to infection with all the VEEV strains tested (Fig. 1A). ZPC738, the virulent

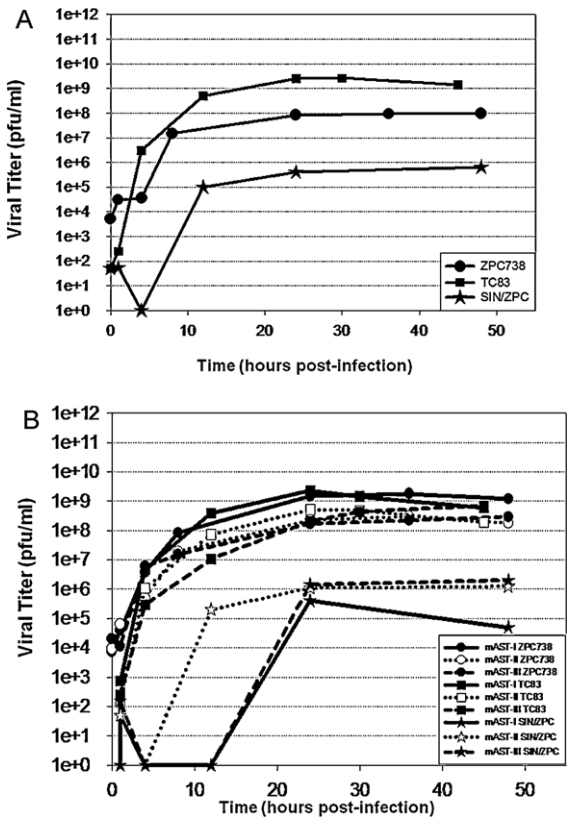

Fig. 1. VEEV can replicate in cultured astrocytes. Human (A) or murine (B) astrocytes infected with virulent ZPC738 (1 moi), vaccine strain TC83 (0.1 moi) or chimeric strain SIN/ZPC (0.1 moi) of VEEV. Viral replication was determined by plaque assay using supernatants collected 0–48 h post infection.