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Rabies virus glycoprotein is an important determinant for the induction of innate immune responses and the pathogenic mechanisms

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ABSTRACT

Our previous studies have suggested that street and fixed rabies viruses (RABVs) induce diseases in the mouse model via different mechanisms. In the present study, attempts were made to determine if it is the glycoprotein (G) that is responsible for the observed differences in the pathogenic mechanisms. To this end, an infectious clone from fixed virus B2c was established and used as a backbone for exchange of the G from street viruses. The rate of viral replication, expression of viral proteins, and the induction of innate immune responses were compared in cells or in mice infected with each of the viruses. Furthermore, the infiltration of inflammatory cells into the CNS and the enhancement of blood–brain barrier (BBB) permeability were also compared. It was found that fixed viruses induced stronger innate immune responses (expression of chemokines, infiltration of inflammatory cells, and enhancement of BBB permeability) than street RABV or recombinant viruses expressing the G from street RABVs. Fixed viruses induce disease via an immune-mediated pathogenic mechanism while street viruses or recombinant viruses expressing the G from street RABVs induce diseases via a mechanism other than immune-mediated pathogenesis. Therefore, RABV G is an important determinant for the induction of innate immune responses and consequently the pathogenic mechanisms.

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1. Introduction

Rabies is an ancient zoonotic disease and today still causes more than 55,000 human deaths around the globe each year (WHO, 2005). The causative agent, rabies virus (RABV), belongs to the genus *Lyssavirus* in the family *Rhabdoviridae*. Its genome is a single-strand, negative-sense RNA of approximately 12 kb in length and encodes for five proteins in the order of nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and RNA-dependent RNA polymerase (RdRp) or large protein (L) (Tordo et al., 1986; Wunner, 2007). RABV N plays important roles in regulating viral transcription and replication (Patton et al., 1984; Wunner, 2007) via encapsdation of the genomic RNA (Sokol et al., 1969). RABV P binds to the nucleoprotein-RNA template and is also part of the RdRp (Mebatsion, 2001; Raux et al., 1997; Takamatsu et al., 1998; Vidy et al., 2007). The P has also been reported to interact with cellular factors such as dynein, contributing to RABV spread (Raux et al., 2000) and promoting RABV transcription (Tan et al., 2007). The viral envelope is composed of the M and the transmembrane G proteins. The G is the only surface protein of the rabies virion and thus capable of inducing virus neutralizing antibodies (VNA) (Benmansour et al., 1991; Wiktor et al., 1973). The G plays important roles in rabies pathogenesis

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(Ito et al., 2001; Morimoto et al., 1999; Wunner, 2007) by binding to neural receptors such as acetylcholine receptor (Lentz et al., 1982, 1984), neural cell adhesion molecules (NCAM) (Thoulouze et al., 1998), or low affinity neurotrophic receptor (Tuffereau et al., 1998), contributing to the exclusive neurotropism and neuroinvasiveness of RABV (Morimoto et al., 2000).

Despite the lethality of rabies, only mild inflammation and little neuronal destruction were observed in the central nervous system (CNS) of rabies patients (Miyamoto and Matsumoto, 1967; Murphy, 1977). On the other hand, fixed (laboratory-adapted) RABV induces extensive inflammation and neuronal degeneration in experimentally infected animals (Miyamoto and Matsumoto, 1967; Murphy, 1977). It was further shown that fixed RABV induces inflammation by activating the innate immune responses (type 1 interferon, chemokines and complements) in the CNS (Johnson et al., 2006; Nakamichi et al., 2004; Prehaud et al., 2005). The induction of innate immune responses and inflammation in the CNS is also associated with the enhancement of blood–brain barrier (BBB) permeability (Fabis et al., 2008; Phares et al., 2007; Roy and Hooper, 2007; Roy et al., 2007). The BBB was more permeable in mice infected with fixed RABV than in those infected with street RABV. It is hypothesized that the induction of inflammatory responses, on one hand, can lead in the clearance of the RABV from the CNS (virus attenuation) when the virus dose is low. However, extensive inflammation in the CNS can result in diseases and death when large doses of fixed virus are used to infect mice (Sarmento et al., 2005; Wang et al., 2005). These studies led to the hypothesis that street and fixed RABV induce disease and death by different mechanisms (Kuang et al., 2009; Wang et al., 2005). Infection with fixed RABV induces diseases via immune-mediated pathogenesis while the pathogenic mechanism by which street RABV induces rabies remains to be determined.

It has been reported that virulent or street RABV expresses low level, while fixed RABV expresses high level, of the G (Morimoto et al., 1998; Yan et al., 2001). The high level of G expression by fixed RABV has been correlated with the induction of apoptosis, enhancement of the BBB permeability, and increases of innate immune responses in the CNS (Faber et al., 2002). On the other hand, street RABV expresses low level of the G, thus evading the host innate immune responses (Wang et al., 2005). In the present study attempts were made to determine if RABV G is a determinant for the induction of innate immune responses and the pathogenic mechanisms by exchanging the G between street and fixed RABVs. It was found that fixed viruses induced stronger innate immune responses and inflammation than street RABV or recombinant viruses expressing the G from street RABVs. These results confirm that fixed viruses induce disease via an immune-mediated pathogenic mechanism while street viruses or recombinant viruses expressing the G from street RABVs induce diseases via mechanism other than immune-mediated pathogenesis. Therefore, RABV G is an important determinant for the induction of innate immune responses and consequently the pathogenic mechanisms.

2. Materials and methods

2.1. Viruses, cells and antibodies

Three parent RABVs were used in this study and they are silver-haired bat rabies virus (SHBRV), dog rabies virus (DRV), and BHK-adapted CVS virus (B2c). SHBRV is a wt RABV isolated from a human patient (Morimoto et al., 1996). DRV is a wt virus originated from a Mexican dog (Dietzschold et al., 2000). B2c is a fixed virus isolated from challenge virus standard (CVS-24) by serial passaging in BHK cells (Morimoto et al., 1998). Virus stocks were prepared as described previously (Sarmento et al., 2005; Wang et al., 2005). Briefly, one-day-old suckling mice were inoculated with 10 μl of viral preparation by the intracerebral (IC) route. When moribund, mice were euthanized and brains removed. A 10% (w/v) suspension was prepared by homogenizing the brain in Dulbecco's modified Eagle's medium (DMEM). The homogenate was centrifuged to remove debris and the supernatant collected and stored at −80 °C. Fluorescein isothiocyanate (FITC)-conjugated antibody against the RABV N was purchased from FujiRebio (FujiRebio Diagnostic Inc., PA). Anti-RABV N monoclonal antibody (N42) was prepared as described (Jiang et al., 2010). Anti-RABV G polyclonal antibody was prepared in rabbits as described (Fu et al., 1993) and has been shown to have similar affinity to the G from street and fixed RABVs (Yan et al., 2001). Anti-CD3 polyclonal antibody was purchased from Dako (Dako North America, CA). Mouse neuroblastoma (NA) cells were maintained in RPMI 1640 medium (Mediatech, Herndon, VA) supplemented with 10% fetal bovine serum (FBS, Gibco, Grand Island, NY). BSR cells, a cloned cell line derived from BHK-21 cells, were maintained in Dulbecco's modified Eagle's medium (Mediatech, VA) containing 10% FBS. Four to six weeks old female ICR mice and 6–8 weeks old female Balb/c mice were purchased from Harlan (Harlan, IN) and housed in temperature- and light-controlled quarters in the Animal Facility, College of Veterinary Medicine, University of Georgia. All animal experiments were carried out under Institutional Animal Care and Use Committee-approved protocols (animal welfare assurance no. A3085-01).

2.2. Sequencing the complete B2c virus genome

Total RNA was extracted from B2c-infected mouse brain tissue using TRIZOL® LS Reagent (Invitrogen Life Technologies, Carlsbad, CA) according to the manufacturer's recommendation. Briefly, 250 μl of the brain suspension was mixed with 3 volumes of TRIzol LS reagent and extracted with 240 μl of chloroform. The RNA containing aqueous phase was precipitated with 600 μl of isopropanol. The RNA was used to amplify the B2c sequences using reverse transcription polymerase chain reaction (RT-PCR) with 10 pairs of oligonucleotide primers (Supplemental Table 1) based on the genomic sequences of the HEP-Flury and CVS-AVO1 strains (Inoue et al., 2003; Wang et al., 2010). PCR amplification was performed for a total of 35 cycles (denaturation at 95 °C for 15 s, annealing at 55–58 °C for 30 s, and extension at 68 °C for 1 min per kb pair) in the presence of 10 μM each of forward and reverse primers, 0.3 mM of dNTPs, 20 mM KCl, 30 mM Tris–HCl (pH

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