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Viral mouse models of multiple sclerosis and epilepsy: Marked differences in neuropathogenesis following infection with two naturally occurring variants of Theiler's virus BeAn strain

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ABSTRACT

Following intracerebral inoculation, the BeAn 8386 strain of Theiler's virus causes persistent infection and inflammatory demyelinating encephalomyelitis in the spinal cord of T-cell defective SJL/J mice, which is widely used as a model of multiple sclerosis. In contrast, C57BL/6 (B6) mice clear the virus and develop inflammation and lesions in the hippocampus, associated with acute and chronic seizures, representing a novel model of viral encephalitis-induced epilepsy. Here we characterize the geno- and phenotype of two naturally occurring variants of BeAn (BeAn-1 and BeAn-2) that can be used to further understand the viral and host factors involved in the neuropathogenesis in B6 and SJL/J mice. Next generation sequencing disclosed 15 single nucleotide differences between BeAn-1 and BeAn-2, of which 4 are coding changes and 3 are in the 5′-UTR (5′-untranslated region). The relatively minor variations in the nucleotide sequence of the two BeAn substrains led to marked differences in neurovirulence. In SJL/J mice, inflammatory demyelination in the spinal cord and its clinical consequences were significantly more marked following infection with BeAn-1 than with BeAn-2. Both BeAn substrains caused lymphocyte infiltration and increase of MAC3-positive cells in the hippocampus, but hippocampal damage and seizures were only observed in B6 mice. Seizures occurred in one third of BeAn-2 infected B6 mice, but not in BeAn-1 infected B6 mice. By comparing individual mice by receiver operating characteristic (ROC) curve analysis, the severity of hippocampal neurodegeneration and amount of MAC3-positive microglia/macrophages discriminated seizing from non-seizing B6 mice, whereas T-lymphocyte brain infiltration was not found to be a crucial factor. These data add novel evidence to the view that differential outcome of infection may be not invariably linked to a distinct viral burden but to a finely tuned balance between antiviral immune responses that although essential for host resistance can also contribute to immunopathology.

1. Introduction

Intracerebral infection of T-cell defective SJL/J mice with the neurotropic Theiler's murine encephalomyelitis virus (TMEV, a positive-sense single-stranded RNA virus that is a member of the Picornaviridae family) is one of the most widely used models of multiple sclerosis and has provided important insight into the immunopathogenesis of demyelination (Lipton et al., 2005; Owens, 2006; Drescher and Sosnowska, 2008; Tsunoda and Fujinami, 2010; Libbey et al., 2014). The strains of TMEV are divided into two groups based on their neurovirulence following intracerebral inoculation of mice (Libbey and Fujinami, 2011). The less neurovirulent group is the Theiler's original (TO) group and includes the TO, Daniels (DA), BeAn 8386 (BeAn) and WW strains of TMEV. The highly

Abbreviations: AUC, area under curve; B6, C57BL/6; CNS, central nervous system; DA, Daniels; dpi, days post-infection; IFN, interferon; IL, interleukin; ISG, interferon-stimulated gene; PFU, plaque-forming units; pi, post-infection; PKR, protein kinase R; ROC, receiver operating characteristic; SNP, single nucleotide polymorphism; TCS, total clinical score; TMEV, Theiler's murine encephalomyelitis virus; TNF, tumor necrosis factor; TO groups, Theiler's original group; TiHo, University of Veterinary Medicine; 5′-UTR, 5′-untranslated region; UU, University of Utah; VP, virus capsid protein.

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neurovirulent group of TMEV is the GDVII group, which includes the GDVII and FA strains, which produce a rapidly fatal encephalitis in adult mice. Strains from the two TMEV subgroups share about 90% sequence identity at the nucleotide level and about 95% identity at the amino acid level (Stein et al., 1992).

Following intracerebral inoculation, the DA and BeAn strains of TMEV induce a biphasic disease in susceptible mouse strains such as SJL/J. During the acute phase, 1 week after infection, TMEV causes polioencephalomyelitis characterized by infection and apoptosis of neurons in the gray matter of the brain. During the chronic phase, about 1 month after infection, the virus infects glial cells and macrophages, and induces inflammatory demyelination with oligodendrocyte apoptosis and axonal degeneration in the white matter of the spinal cord (Brahic et al., 2005; Tsunoda and Fujinami, 2010). In contrast to susceptible mouse strains, resistant mouse strains such as C57BL/6J (B6) have the ability to clear the virus during the first 2–4 weeks following infection and do not develop the demyelinating disease (Lipton and Dal Canto, 1979; Chamorro et al., 1986; Drescher et al., 1997; Brahic et al., 2005). However, more recently, intracerebral infection of B6 mice with the DA and BeAn strains of TMEV was shown to induce acute symptomatic seizures from which the animals recover and later develop epilepsy with spontaneous recurrent seizures, which was proposed to provide the first infection-driven animal model for epilepsy (Libbey and Fujinami, 2011; Vezzani et al., 2016). Thus, the same virus (BeAn or DA) induces two completely different diseases in different strains of mice.

However, over the about 10 years that we have used the BeAn strain of TMEV in studies on mechanisms involved in virus-induced demyelination, we never observed any seizures in B6 mice (Ulrich et al., 2006; Ulrich et al., 2008; Jafari et al., 2012; Prajeeth et al., 2014; Bröer et al., 2016), whereas Libbey et al. (2011) reported that in their experiments about 40% of B6 mice exhibited acute seizures after infection with the BeAn and about 60% after infection with the DA strain of TMEV, correlating with the viral dose. Both, the BeAn strain used in our studies and the strain used in the studies of Libbey et al. (2011) at the University of Utah (UU) originally descended from the same BeAn 8386 virus strain (Rozhon et al., 1983), but may differ in their phenotypic and genotypic characteristics, most likely as a result of spontaneous mutations during in vitro passaging since the virus was obtained from H.L. Lipton (see Materials and methods section). Such naturally occurring variants may provide important insights into the relationship between viral pathogenesis and immunological parameters (Kim et al., 1998). This prompted us to directly compare the two BeAn substrains used by our group ($BeAn^{TiHo}$ or "BeAn-1") and at the University of Utah ($BeAn^{UU}$ or "BeAn-2"), resulting in interesting phenotypic differences in B6 mice (Bröer et al., 2016). In the present study, we further characterized the two BeAn substrains and evaluated whether they also differ in their neurovirulence in SJL/J mice. In B6 mice, inflammation and neuronal damage were compared between mice that did and did not exhibit early seizures. Furthermore, in view of recent evidence that high expression of the interferon (IFN)-stimulated genes ISG15 (IFN stimulated gene 15) and PKR (protein kinase R) contributes to virus elimination in B6 mice (Li et al., 2015), that glial production of IFN-β modulates hippocampal network excitability (Costello and Lynch, 2013), and that ISG15 expression almost perfectly discriminates between seizing and non-seizing TMEV-infected B6 mice (Bröer et al., 2016), expression of IFN-related genes following infection with BeAn-1 and BeAn-2 were compared. Finally, the two BeAn substrains were sequenced to substantiate that they present naturally occurring variants of BeAn.

2. Materials and methods

2.1. Animals

Three-week-old female SJL/J and B6 mice were purchased from Harlan Laboratories (Eystrup, Germany) and from Charles River (Sulzfeld, Germany) and kept in groups of five to eight animals in isolated ventilated cages (Tecniplast, Hohenpeißenberg, Germany) under controlled environmental conditions (22–24 °C; 50–60% humidity; 12/12 h light/dark cycle) with free access to standard rodent diet (R/M-H; Ssniff Spezialdiäten GmbH, Soest, Germany) and tap water. Female mice were used to allow group housing (to enable normal social behavior and interactions between the animals) over several months without the problems owing to the naturally aggressive behavior of male mice. Libbey et al. (2008) have shown previously that the consequences of TMEV infection do not differ between male and female B6 mice. All animal experiments were conducted in accordance with the German Animal Welfare Law and were authorized by the local government (LAVES Oldenburg, Germany, permission numbers 509.6-42502-04/860, 33.9-42502-04-09/1770, 33.9-42502-04-11/0516, and 33.12-42502-04-15/1892).

2.2. Viruses

Two BeAn substrains were compared. The first BeAn substrain, termed "$BeAn^{TiHo}$" or "BeAn-1" here, is routinely used in one of our laboratories at the University of Veterinary Medicine (TiHo) in Hannover for studies on demyelinating infections and was originally obtained from Dr. Howard L. Lipton (University of Illinois, Chicago, Illinois, USA) about 15 years ago. The second BeAn substrain, termed "$BeAn^{UU}$" or "BeAn-2" here, which has been reported to cause acute seizures after intracerebral infection in B6 mice (Libbey et al., 2011), was kindly provided by Prof. Dr. Robert S. Fujinami (University of Utah, Salt Lake City, Utah, USA), who originally got it from the American Type Culture Collection (Manassas, VA, USA). Both substrains originally descended from the same BeAn 8386 virus strain (Rozhon et al., 1983). In the majority of the experiments shown in this study, virus dose used for intracerebral infection in B6 and SJL/J mice ranged between 8.1×10^6 and 4.6×10^7 plaque-forming units (PFU). Only some preliminary experiments with BeAn-1 were also performed with lower virus doses (1.26×10^5 and 1.63×10^6 PFU), resulting in a similar extent of hippocampal damage as seen at the higher doses (see Results section). Intracerebral virus inoculation in one hemisphere was performed as described previously (Bröer et al., 2016). Mock-infected mice were used as controls.

2.3. Sequencing of the two BeAn substrains and bioinformatic analysis

Total nucleic acid was extracted from cell culture supernatant using the Macherey-Nagel NucleoSpin (Macherey-Nagel, Düren, Germany) as per manufacturer's instructions. An on-column DNAse digestion step was introduced in order to get rid of the DNA. RNA sequencing libraries were generated using the ScriptSeq v2 RNA-Seq kit (Illumina, San Diego, CA) from approximately 15 ng total RNA as input, following manufacturer's instructions. Briefly, RNA was fragmented and reverse transcribed using random hexamers containing an adaptor overhang. Adaptor-ligated fragments were then amplified for 15 cycles and purified using Ampure XP beads (Beckman Coulter, Brea, CA). Quality control was performed using the Qubit HS dsDNA kit (Invitrogen, Carlsbad, CA) and the Agilent Bioanalyzer (Santa Clara, CA). Libraries were then sequenced on an Illumina MiSeq using the v2 chemistry generating 2×250 bp paired-end reads. Approximately 2 million reads were generated per sample.

Data quality was assessed using FastQC (http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc), and trimmed using prinseq (Schmieder and Edwards, 2011) and trim galore (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore). TMEV sequences were assembled using two different approaches in parallel: de novo assembly and reference-dependent assembly (mapping) as implemented in CLC Genomics Workbench v7 (CLC Bio, Aarhus, Denmark). All subsequent data analysis and visualization was performed in CLC. Variants which