Experimental Neurology 279 (2016) 57–74

Contents lists available at ScienceDirect

Experimental Neurology

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

Brain inflammation, neurodegeneration and seizure development following picornavirus infection markedly differ among virus and mouse strains and substrains

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ARTICLE INFO

Article history:
Received 29 October 2015
Received in revised form 25 January 2016
Accepted 13 February 2016
Available online 15 February 2016

Keywords:
Seizures
Epileptogenesis
Hippocampal damage
Encephalitis
Infection
Interferon
ISG15
Macrophages
Microglia

ABSTRACT

Infections, particularly those caused by viruses, are among the main causes of acquired epilepsy, but the mechanisms causing epileptogenesis are only poorly understood. As a consequence, no treatment exists for preventing epilepsy in patients at risk. Animal models are useful to study epileptogenesis after virus-induced encephalitis and how to interfere with this process, but most viruses that cause encephalitis in rodents are associated with high mortality, so that the processes leading to epilepsy cannot be investigated. Recently, intracerebral infection with Theiler's murine encephalomyelitis virus (TMEV) in C57BL/6 (B6) mice was reported to induce early seizures and epilepsy and it was proposed that the TMEV mouse model represents the first virus infection-driven animal model of epilepsy. In the present study, we characterized this model in two B6 substrains and seizure-resistant SJL/J mice by using three TMEV (sub)strains (BeAn-1, BeAn-2, DA). The idea behind this approach was to study what is and what is not necessary for development of acute and late seizures after brain infection in mice. Receiver operating characteristic (ROC) curve analysis was used to determine which virus-induced brain alterations are associated with seizure development. In B6 mice infected with different TMEV virus (sub)strains, the severity of hippocampal neurodegeneration, amount of MAC3-positive microglia/macrophages, and expression of the interferon-inducible antiviral effector ISG15 were almost perfect at discriminating seizing from non-seizing B6 mice, whereas T-lymphocyte brain infiltration was not found to be a crucial factor. However, intense microglia/macrophage activation and some hippocampal damage were also observed in SJL/J mice. Overall, the TMEV model provides a unique platform to study virus and host factors in ictogenesis and epileptogenesis.

1. Introduction

Following viral encephalitis, the risk of seizures, particularly acute symptomatic ("early") seizures, is increased more than 20% over the risk of seizures in the general population (Getts et al., 2008; Libbey and Fujinami, 2011; Vezzani et al., 2016). In addition, viral encephalitis increases the risk of developing epilepsy with late unprovoked seizures; 4–20% of viral encephalitis survivors go on to develop epilepsy (Getts et al., 2008; Misra et al., 2008). Over 100 different neurotropic viruses cause encephalitis in humans, and of these, several different viruses have been suggested to play a role in the development of seizures and epilepsy (Misra et al., 2008; Singhi, 2011; Vezzani et al., 2016). Viruses from the herpes virus group are prominent among these. In animals, viral encephalitis also leads to seizures; the best known example is canine distemper (Spitzbarth et al., 2012). However, the processes leading from viral encephalitis to early and late seizures are poorly understood. Animal models are useful to study these processes, but most viruses that cause encephalitis in rodents are associated with high mortality, so that the processes leading to epilepsy cannot be investigated (Libbey and Fujinami, 2011).

An exception is Theiler's murine encephalomyelitis virus (TMEV), a non-enveloped, positive-sense, single-stranded RNA virus of the *Picornaviridae* family and *Cardiovirus* genus, which is a naturally occurring enteric pathogen of the mouse (Libbey and Fujinami, 2011). Intracerebral infection of SJL/J mice with TMEV results in an acute encephalitis

Abbreviations: B6, C57BL/6; B6H, C57BL/6JOlaHsd; B6J, C57BL/6J; CNS, central nervous system; DA, Daniel's; DG, dentate gyrus; dpi, days post-infection; EEG, electroencephalogram; IFN, interferon; IL, interleukin; ISG, interferon-stimulated gene; NK, natural killer; OAS1, 2′5′-oligoadenylate synthetase 1; PFU, plaque-forming units; pi, post-infection; PKR, protein kinase R; PMN, polymorphonuclear leukocyte; ROC, receiver operating characteristic; TCS, total clinical score; TMEV, Theiler's murine encephalomyelitis virus; TNF, tumor necrosis factor.

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http://dx.doi.org/10.1016/j.expneurol.2016.02.011
0014-4886/

which, due to virus persistence and virus spread, is followed by a chronic inflammatory demyelinating disease predominantly in the spinal cord, so that TMEV-infected SJL/J mice are widely used as an animal model of multiple sclerosis (Drescher and Sosnowska, 2008; Libbey and Fujinami, 2011). In contrast, infection of C57BL/6 (B6) mice with TMEV also causes acute encephalitis but, unlike SJL/J mice, B6 mice can clear the virus during the first weeks following infection, so that this mouse strain is considered resistant to the TMEV-induced demyelinating disease (Libbey and Fujinami, 2011). More recently, the groups of Robert Fujinami and Steve White at the University of Utah have recognized the importance of B6 mice as a model of viral encephalitis-induced early and late seizures. They described that approximately 50–75% of B6 mice (male and female) infected with the Daniel's (DA) strain of TMEV developed acute behavioral (early) seizures (Libbey et al., 2008). Typically, seizures were first observed on day 3 post-infection (pi), the highest seizure activity was on day 6 pi and no seizures were observed after day 10 pi. The development of seizures appeared to be specific for B6 mice as no SJL/J (male and female), FVB/N (male) or BALB/c (male) mice developed seizures after intracerebral infection with the DA strain of TMEV (Libbey et al., 2008). The development of seizures was not specific to the DA strain of TMEV, but other TMEV strains and mutants were also able to induce seizures to various degrees; e.g. 40% of B6 mice exhibited acute seizures after infection with the BeAn 8386 (BeAn) strain (Libbey et al., 2011b).

The relationship between viral infection with acute symptomatic seizures and the subsequent development of epilepsy was examined in this model by studying B6 mice infected with the DA strain of TMEV (Stewart et al., 2010a). Monitoring by using long-term video–EEG at 2, 4 and 7 months pi of the TMEV-infected B6 mice that had experienced acute seizures demonstrated that a significant proportion (65%) of the mice developed spontaneous recurrent epileptic seizures following a latent period in which no behavioral seizures were observed (Stewart et al., 2010a). In addition, those animals with epilepsy had hippocampal damage characterized by neuronal cell loss in the CA1/CA2 pyramidal cell layers and gliosis, reminiscent of hippocampal sclerosis in patients with infection-induced temporal lobe epilepsy (Stewart et al., 2010a). Based on these findings, the TMEV-induced seizure model was proposed to represent the first infection-driven animal model for epilepsy (Libbey and Fujinami, 2011).

The present study had various aims. First, because all previous experiments on the novel TMEV model of infection-induced epilepsy have been performed by the same group of researchers, we wanted to replicate their findings. This was initiated by the fact that we had used the BeAn strain of TMEV in thousands of SJL/J and B6 mice over the last ~10 years in studies on mechanisms involved in virus-induced demyelination but never observed any seizures in B6 mice (Ulrich et al., 2006; Ulrich et al., 2008; Jafari et al., 2012; Prajeeth et al., 2014). Thus, we hypothesized that either the substrain of B6 mice or the BeAn substrain used in our experiments may have been responsible for the lack of seizures. This hypothesis was addressed by comparing two B6 and two BeAn substrains, including the mouse and virus substrains used in the original studies of Fujinami and White. In addition, we compared the potency of the BeAn and DA TMEV strains to induce seizures and epilepsy in mice. In all experiments, the severity of inflammation and hippocampal damage was examined and correlated with the development of seizures. Finally, because interleukin (IL)-6 and interferon (IFN)-β production of macrophages is thought to play a crucial role in the development of seizures following TMEV infection (Cusick et al., 2013; Moore et al., 2013), the expression of IFN-inducible antiviral effectors was examined in the hippocampus.

2. Materials and methods

2.1. Animals

Overall, 198 mice were used in this study. 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.

We compared two B6 substrains from the different breeders: C57BL/6JOlaHsd (B6H, obtained from Harlan) and C57BL/6J (B6J, obtained from Charles River); the latter substrain was identical to the substrain used in the experiments of Fujinami and White (cf., Libbey and Fujinami, 2011). The B6H and B6J substrains originally descended from the same C57BL/6 breeding stock and were maintained at the Jackson Laboratory (JAX; Bar Harbor, Maine, USA), but subsequent history differed, leading to two distinct substrains (Festing, 1996; Wotjak, 2003) which, because of genetic drift, differ in many characteristics, including seizure susceptibility (Bankstahl et al., 2012). In the present study, for all investigated parameters for which the two substrains did not differ significantly, all B6 mice were pooled. Additionally, we had to change the SJL/J mouse substrain from Harlan twice in the initial phase of the experiments, as the originally used SJL/JHan™Hsd and SJL/JOlaHsd animals were not available anymore, so that we had to change to SJL/JCrHsd mice from Harlan. Seizures were not observed in any of these SJL/J substrains from Harlan, so that we pooled the data of the SJL/J substrains for final analysis and comparison with B6 mice; however, most data from SJL/J mice shown in this paper were obtained from SJL/JCrHsd 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 33.9-42502-04-09/1770 and 33.9-42502-04-11/0516).

2.2. Viruses

We evaluated three different virus (sub)strains of TMEV: two BeAn-derived strains and one DA strain. The first BeAn substrain, (termed "BeAn-1" here), is routinely used in one of our laboratories in Hannover for studies on demyelinating infections and was originally obtained from Dr. Howard L. Lipton (University of Illinois, Chicago, Illinois, USA). Another BeAn substrain, "BeAn-2", which has been reported to cause acute seizures after intracerebral infection in C57/BL6 mice (Libbey et al., 2008), 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). Robert Fujinami's group also kindly provided the third virus strain, the Daniel's (DA) strain, which was isolated in 1948 from a mouse in the Harvard colony (Daniels et al., 1952). DA is more virulent than BeAn, so that the DA strain was more effective than the BeAn-2 strain in inducing seizures (Libbey et al., 2011b), depending on the viral dose that correlated with the percentage of mice having seizures, ranging from 30% at 3×10^3 to 80% at 3×10^6 plaque-forming units (PFU). This group routinely used doses between 2×10^4 and 3×10^5 PFU to induce seizures (Libbey et al., 2008; Kirkman et al., 2010; Stewart et al., 2010a; Smeal et al., 2012; Cusick et al., 2013; Umpierre et al., 2014) and based on their reports, we started our first experiments with the BeAn-1 strain with a similar titer (1.26×10^5 PFU). However, the lack of early seizures prompted us to test higher doses (see the Results section), so that we injected also 4.6×10^7 PFU of BeAn-1 and stayed with a higher titer for our following experiments with the other virus substrains (8.1×10^6 for BeAn-2, and 2.44×10^7 for DA).

2.3. Experimental procedures

Fig. 1 gives an overview of the experimental procedures. Mice were randomized into four groups: mock, BeAn-1, BeAn-2, and DA (cf., Table 1).