J. Comp. Path. 2009, Vol. 140, 113–126

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Comparative Pathological Study of the Murine Brain after Experimental Infection with Classical Rabies Virus and European Bat Lyssaviruses

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Summary

European bat lyssaviruses (EBLVs) types 1 (EBLV-1) and 2 (EBLV-2) cause rabies in terrestrial species, but the pathological changes associated with neuroinvasion have yet to be fully elucidated. Swiss OF-1 mice were inoculated peripherally with strain RV61 (classical rabies virus), RV1423 (EBLV-1) or RV1332 (EBLV-2) to compare the nature and extent of histopathological changes produced. Inoculated animals developed varying degrees of non-suppurative encephalitis, and lyssavirus infection was confirmed by the detection of viral antigen. The lesions produced, which included perivascular cuffs and gliosis, were more severe after RV1423 or RV1332 infection than after RV61 infection. Perivascular cuffs were mainly localized to caudal brain regions, irrespective of the infecting strain; after RV1332 infection, however, they were particularly abundant, being composed of large numbers of inflammatory cells. T cells were the predominant lymphocytic component of the inflammatory infiltrate in both the Virchow–Robin space and the brain parenchyma. Viral antigen, which was widespread throughout the brain, was apparently unrelated to the degree of cuffing. The study suggested that there was increased immune activation after inoculation with strain RV1423 or RV1332, particularly the latter, but that this did not affect the final outcome.

Keywords: encephalitis; European bat lyssavirus; mouse; rabies; viral infection

Introduction

Rabies is an acute and invariably fatal viral encephalitis caused by highly neurotropic RNA viruses belonging to the *Lyssavirus* genus of the family Rhabdoviridae. The disease is characterized by a progressive infection of the central nervous system (CNS) that causes encephalitis and a range of clinical features, including ataxia, hydrophobia and photosensitivity (Lafon, 2004). European bat lyssavirus (EBLV) types 1 and 2 (genotypes 5 and 6) can infect terrestrial species, including sheep and man (Fooks *et al*., 2003; Tjornehoj *et al*., 2006), causing clinical disease indistinguishable from that produced by classical rabies virus (RABV). In the UK, serological evidence of a low prevalence of EBLV-2 in Daubenton's bats (*Myotis daubentonii*), together with the occurrence of fatal human infection, indicates that EBLVs represent a potential threat to animal and human health (Johnson *et al*., 2003; Harris *et al*., 2006). The neurotropism and clinical effects of EBLV and classical RABV are known to be comparable, but the host immune response to natural or experimental EBLV infection has not been fully characterized and compared with that of classical RABV.

CNS pathology, following natural or experimental infection with RABV, is typified by a non-suppurative meningoencephalomyelitis with neuronal necrosis, which may be accompanied by neuronophagia, focal gliosis, lymphocytic perivascular infiltrates and Negri bodies (Charlton, 1984; Charlton *et al*., 1987). However, these features are neither uniformly reproduced in all RABV infections nor convincingly associated with viral antigen distribution,

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0021-9975/$ - see front matter
doi:10.1016/j.jcpa.2008.09.001

particularly in human cases (Tirawatnpong *et al.*, 1989). Severity and distribution of lesions appear to vary, depending upon host and viral strain. In mouse inoculation models, encephalitic changes have been reported throughout the thalamus, hypothalamus, midbrain and pons (Sugamata *et al.*, 1992). In other such studies, however, similar encephalitic features (perivascular cuffing, apoptosis and necrosis) were absent after infection with the highly neuro-invasive silver-haired bat rabies virus (Morimoto *et al.*, 1996; Yan *et al.*, 2001; Roy *et al.*, 2007). Moreover, infection models in which Duvenhage (genotype 4) and Danish bat variants (EBLV-1) were compared with wild-type genotype 1 RABV strains revealed distinct differences in the type of encephalitis produced (Fekadu, 1988). Human beings naturally infected with RABV rarely exhibit CNS inflammation, although minimal inflammatory changes, including increased microglial activation, may be observed in patients with prolonged periods of clinical illness (Murphy, 1977; Hemachudha *et al.*, 2006). In contrast, human beings infected with non-RABV lyssaviruses have pronounced CNS lesions (Familusi *et al.*, 1972; Hanna *et al.*, 2000).

The typical changes of viral encephalitis described after RABV infection were also observed in a stone marten (*Martes foina*) naturally infected with EBLV-1, although the inflammatory changes were restricted in distribution and associated with vascular activation (Muller *et al.*, 2004). Experimental infection of foxes with EBLV-1 resulted in encephalitis, multifocal in distribution and variable in severity (Vos *et al.*, 2004). Similarly, intracranial challenge of sheep and peripheral (footpad) inoculation of mice with EBLV-2 produced encephalitis typical of lyssavirus infection (Brookes *et al.*, 2005, 2007); experimental infection of ferrets, however, failed to produce clinical disease (Vos *et al.*, 2004).

Perivascular cuffing and parenchymal infiltration within the brain, which are the result of inflammatory cell recruitment to sites of infection, are changes that may be associated with RABV or EBLV infection. However, due to variability in response, the influence of lymphocyte recruitment on the resolution of rabies infection remains unclear. A number of investigations with nude mice (B cell-deficient, T cell-deficient and CD4 T cell-depleted) have highlighted the protective nature of the host immune response and isolated CD4 T cells as the key mediator of protection in abortive virus infections (Iwasaki *et al.*, 1977; Hooper *et al.*, 1998). Depletion of CD4 T cells transforms an abortive infection into an acute fatal encephalitis (Weiland *et al.*, 1992; Galelli *et al.*, 2000). Street rabies virus-resistant mouse strains (SJL/J and BALB/cByJ) effectively lose their resistance after the targeting of CD4 cells by neutralizing antibodies. A similar depletion of CD8 T cells produced no measurable effect on resistance (Perry and Lodmell, 1991).

Comparison of abortive with fatal RABV infection models demonstrated that, initially, inflammatory cell recruitment, migration and parenchymal infiltration were similar, but divergence occurred 7 days after infection (Irwin *et al.*, 1999; Camelo *et al.*, 2000; Lafon, 2005). This divergence was marked by widespread T-cell depletion, coinciding with an ''upregulation'' of the Fas/FasL apoptotic pathway, in fatal infection models (Baloul *et al.*, 2004). T-cell apoptosis effectively attenuates the immune response, preventing the clearance of infection (Galelli *et al.*, 2000; Baloul and Lafon, 2003; Lafon, 2005).

The variable pathological manifestations of RABV infection and the questionable nature of the underlying immune response, combined with the inherent failure of the innate and adaptive immune response to curtail disease, indicate the need for further investigation. The purpose of the present study was to compare the histopathological changes produced in mice by inoculation with RABV, EBLV-1 or EBLV-2.

Materials and Methods

Viruses, Mice and Experimental Procedure

Three viral strains representative of genotypes 1, 5 and 6 were used. Strain RV61 (wild-type RABV, genotype 1) was isolated from a human being, bitten by a dog in India in 1987. Strain RV1423 was isolated from a naturally infected serotine bat (*Eptesicus serotinus*) in Germany in 1997 (EBLV-1 genotype 5; kindly provided by Dr T. Muller, Friedrich-Loeffler-Institut, Wusterhausen, Germany). Strain RV1332 (EBLV-2, genotype 6) was isolated from a naturally infected Daubenton's bat in the UK in 2002 (Johnson *et al.*, 2003). Each strain was initially grown to 100% infection in tissue culture before a mouse lethal dose 50% (MLD_{50}) assay was undertaken.

Groups of genetically homogeneous female Swiss OF-1 mice (Charles River, L'Arbresis, France) aged 5 weeks were anaesthetized; each group was then inoculated peripherally (hind left foot pad) with one of the three virus strains (dose, 5MLD_{50} in a volume of 30 µl). Negative control mice ($n = 4$) were mock-infected with 30 µl of N2A tissue culture medium (Sigma–Aldrich, Poole, Dorset). The mice were killed when showing clinical signs of rabies (RV61-infected mice, $n = 9$; RV1423-infected mice, $n = 13$; RV1332-infected mice, $n = 16$). They were all used in the perivascular cuffing studies (see below); and those exhibiting the more advanced clinical sign of bipedal paralysis (RV61, $n = 4$; RV1423, $n = 4$; RV1332, $n = 7$) were