Journal of NeuroVirology (2000) 6, 89–93

www.jneurovirology.com

Short Communication

Analysis of the Fv1 alleles involved in the susceptibility of mice to lactate dehydrogenase-elevating virus-induced polioencephalomyelitis

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Development of polioencephalomyelitis in mice infected with lactate dehydrogenase-elevating virus (LDV) requires expression of N-tropic ecotropic MuLV retroviruses. 129/Sv mice are resistant to N-tropic MuLV expression and therefore do not develop LDV-induced polioencephalomyelitis. The *Fv1* gene determines the susceptibility to retrovirus replication. We sequenced the open reading frame of the *Fv1*^{nr} allele of 129/Sv mice. It differs by only one nucleotide, modifying one amino acid in the encoded protein, from the *Fv1*^{n} allele of susceptible AKR and C58 animals. We excluded that the resistance of 129/Sv mice to LDV-induced polioencephalomyelitis resulted from the absence of endogenous N-tropic retrovirus, by infecting (129/Sv × C58/J) F1 animals. Therefore it is possible that the amino acid that defines the *Fv1*^{nr} allele is responsible for resistance of 129/Sv mice to N-tropic MuLV expression and to LDV-induced polioencephalomyelitis. *Journal of NeuroVirology* (2000) **6,** 89–93.

Keywords: lactate dehydrogenase-elevating virus; gene; polioencephalomyelitis; retrovirus

After infection with a neurovirulent quasispecies of lactate dehydrogenase-elevating virus (LDV), polioencephalomyelitis may develop in some mouse strains such as C58 or AKR, but not in others such as C57Bl/6, DBA/2 or C3H mice (Pease and Murphy, 1980; Martinez *et al*, 1980; Nawrocki *et al*, 1980; Murphy *et al*, 1983; Godeny *et al*, 1993; Stroop and Brinton, 1983; Faaberg *et al*, 1995; Chen *et al*, 1997). In the susceptible animals, the LDV-triggered neuron destruction requires some degree of natural or induced immunosuppression (Duffey *et al*, 1976; Bentley and Morris, 1982; Bentley *et al*, 1983; Murphy *et al*, 1983; Anderson *et al*, 1995a; Monteyne *et al*, 1997), and expression of N-tropic ecotropic MuLV retrovirus during the LDV infection (Pease and Murphy, 1980; Contag and Plagemann, 1988, 1989; Inada *et al*, 1993; Anderson *et al*, 1995b). The interactions between MuLV and LDV are not well understood. Although anti-LDV immune responses control the replication of the neurotropic quasispecies of LDV and prevent polioencephalomyelitis (Chen *et al*, 1997; 1999), immunosuppression may also induce an increase of retrovirus expression in the glial cells of the spinal cord (Contag and Plagemann, 1989; Anderson *et al*, 1995b). This MuLV infection of glial cells then renders, through unknown indirect mechanism, motor neurons susceptible to infection by LDV, leading to destruction of these neurons and to paralysis (Contag and Plagemann, 1989; Anderson *et al*, 1995b).

Susceptibility to LDV-induced polioencephalomyelitis depends on gene *Fv1*, which controls the tropism of different subgroups of MuLV retroviruses (Mayer *et al*, 1978; Kozak and Chakraborti, 1996). The products of the *Fv1*^{n} and *Fv1*^{b} alleles, the most frequent *Fv1* alleles in inbred mouse strains, exclusively allow the replication of N-tropic and B-tropic retroviruses, respectively (Odaka, 1969; Pincus *et al*, 1971; Lilly and Pincus, 1973; Steeves and Lilly, 1977; Jolicoeur, 1979). Gene *Fv1* contains a single large exon (Best *et al*, 1996; EMBL accession numbers X97719 and X97720). The *Fv1*^{n} and *Fv1*^{b} alleles differ by a 1.3 kb deletion, present in the 3′

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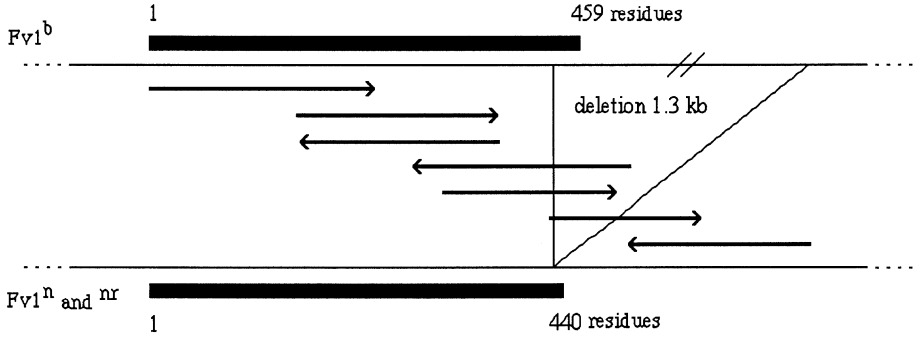
Received 14 June 1999; revised 2 August 1999; accepted 8 September 1999

end of *Fv1*^{n}, and by point mutations in codons corresponding to residues 358 and 399 of the putative 50 kD protein (Best *et al*, 1996). Mice that are susceptible to LDV-induced polioencephalomyelitis, such as C58 or AKR animals (Stroop and Brinton, 1983), have several MuLV copies in their genome and carry the *Fv1*^{n} allele (Pease *et al*, 1982) of which product renders them permissive to N-tropic retrovirus replication. In contrast, in C57BL/10J or (C58/M X C57BL/10J) F_1 mice carrying the *Fv1*^{b} allele, N-tropic retrovirus replication is absent and no polioencephalomyelitis occurs after LDV infection (Anderson *et al*, 1995b). These results suggest that the deletion and the two amino acid changes characteristic of the *Fv1*^{n} allele determine the genetic susceptibility to N-tropic virus infection and therefore to LDV-induced polioencephalomyelitis.

In addition to *Fv1*^{n} and *Fv1*^{b}, three other alleles have been described. The *Fv1*^{o} allele controls a nonrestrictive phenotype allowing replication of both N- and B-tropic leukemia viruses (Kozak, 1985). Because this allele was described mostly in wild mice, its role in LDV-induced polioencephalomyelitis is not known. The *Fv1*^{d} allele, present in DBA/2 mice, restricts B-tropic virus replication, although not as severely as *Fv1*^{n} (Kozak and Chakraborti, 1996). Analysis of recombinant inbred strains between DBA/2 (*Fv1*^{d}) and AKR (*Fv1*^{n}) animals indicated that the resistance of DBA/2 mice is caused by the absence of replication-competent ecotropic MuLV, and not by a genetic defect linked to the *Fv1* gene (Anderson *et al*, 1995b). The *Fv1*^{d} allele codes for the same predicted protein as *Fv1*^{n}, but there is a difference in the non-coding sequence of the gene (Best *et al*, 1996). Finally, 129, RF, NZB, NZW animals, and some wild mice carry the *Fv1*^{nr} allele of which product restricts the expression of B-tropic and of a subgroup of N-tropic viruses such as AKV-1 (Emv-11), which is present in mice susceptible to LDV-induced polioencephalomyelitis (Herr and Gilbert, 1982). However, *Fv1*^{nr} product allows the replication of other N-tropic MuLV, such as AKR-L1 (Mayer *et al*, 1978; Kozak, 1985). *Fv1*^{nr} mice do not develop polioencephalomyelitis when infected with LDV (Stroop and Brinton, 1983). This might be due either to a genetic background determining resistance or to the absence of the appropriate endogenous retrovirus.

A preliminary analysis of the *Fv1*^{nr} allele from 129/Sv animals unexpectedly suggested the presence of a deletion in the 3′ end of the gene, similar to that reported for *Fv1*^{n} (Best *et al*, 1996). We sequenced the *Fv1* alleles of C58 (*Fv1*^{n}) and 129/Sv (*Fv1*^{nr}) mice. DNA was prepared from the tail of mice by phenol/chloroform extraction after tissue digestion with proteinase K. Overlapping fragments of DNA were amplified by polymerase chain reaction (PCR), as indicated in Figure 1A. The PCR products were purified with a QIAquick PCR Purification Kit (Qiagen, Hilden, Germany), and sequenced with the BigDye Terminator Cycle Sequencing kit (PE Applied Biosystems, Warrington, UK).

A



B

Fv1	352	358	399	438 ...
b	S	E	R	GLTSVGSVGVLSLSPWKHQSNS*
n	S	K	V	TKL*
nr	F	K	V	TKL*

Figure 1 (**A**) Analysis of *Fv1* alleles. *Fv1*^{n} and *Fv1*^{nr} alleles were sequenced in DNA from C58/J and 129/Sv mice, respectively, after amplification of overlapping DNA fragments shown by arrows. Black boxes represent the *Fv1* open reading frames. (**B**) Predicted amino acid differences of the *Fv1* proteins. *Fv1*^{n} and *Fv1*^{nr} sequences were compared to the published *Fv1*^{b} sequence (Best *et al*, 1996). *Indicates a stop codon.

As shown in Figure 1B, the *Fv1*^{n} allele of C58/J mice is identical to that of AKR animals (Best *et al*, 1996). This was expected, considering the similar susceptibility of these strains to LDV-induced polioencephalomyelitis. In 129/Sv mice, the *FV1*^{nr} allele appeared very similar to the *FV1*^{n} allele: we found both the 1.3 kb deletion at the 3′ end of the ORF and the amino acids at positions 358 and 399 that distinguish this allele from *FV1*^{b} (Best *et al*, 1996). The only difference was a nucleotide change in codon 352 (TTT in *FV1*^{nr} instead of TCT in *FV1*^{n} mice), modifying the S of the *FV1*^{n} allele into F in *FV1*^{nr}.

Considering that the *FV1*^{nr} sequence is much closer to *FV1*^{n} than to *FV1*^{b}, we tested whether the *FV1*^{nr} phenotype determined susceptibility to LDV-induced polioencephalomyelitis. We examined the development of the disease in F1(129/Sv × C58/J) animals. These mice are homozygous for the deletion and the two amino acid changes at positions 358 and 399, and carry from their C58/J genitor the N-tropic ecotropic MuLV required for polioencephalomyelitis development. This approach had showed that F1 hybrids between *FV1*^{n} C58/M mice, carrying the N-tropic ecotropic MuLV retrovirus, and *FV1*^{n} CE/J mice, whose genome is devoid of MuLV copies, express the retrovirus in their central nervous system and are susceptible to LDV-induced polioencephalomyelitis (Anderson *et al*, 1995b). C58/J mice obtained from the Jackson Laboratory (Bar Harbor, Maine) were maintained in microisolators with sterile food and water. Preliminary experiments with 129/Sv mice bred by G Warnier at the Brussels branch of the Ludwig Institute for Cancer Research confirmed that these animals do not develop polioencephalomyelitis when infected with LDV. F1(129/Sv × C58/J) animals were bred in our animal facility,. After immunosuppression with 5 mg cyclophosphamide (Cycloblastine®, Farmitalia Carlo Erba), polioencephalomyelitis was induced by intraperitoneal injection of LDVc (kindly provided by EK Godeny, Martinez *et al*, 1980). The virus was amplified once in C58/J mice, and the equivalent of 2 μl of plasma from 1 day-infected mice was diluted in 500 μl saline and used for infection. As expected, immunosuppressed C58/J mice developed polioencephalomyelitis after LDV infection, leading often to death (shown in Figure 2 for a typical experiment). In contrast, none of the F1(129/Sv × C58/J) mice showed any symptom of a neurological disease. This result indicates that the 1.3 kb deletion and the two amino acid changes that characterize the protein coded by *Fv1*^{n} allele are not sufficient to confer susceptibility to LDV-induced polioencephalomyelitis, and thus probably to N-tropic MuLV.

Retrovirus restriction by *Fv1* product occurs at an infection step between reverse transcription of viral RNA and integration of DNA into the host genome (Jolicoeur and Baltimore, 1976). Sequence homology between *Fv1* and *Gag* of human and mouse endogenous retroviruses (HERV-L and MuERV-L; Best *et al*, 1996; Bénit *et al*, 1997), lead to the hypothesis that the *Fv1* gene product interfered with Gag proteins of the infecting virus (Best *et al*, 1996; Coffin, 1996; Goff, 1996). As the only difference beween the *Fv1*^{n} and *Fv1*^{nr} products is a S > F substitution at position 352, it is possible that this modification changes the conformation of the protein, inducing resistance to N-tropic viruses. However, it cannot be excluded at this stage that the resistant phenotype of *Fv1*^{nr} mice results from other genetic differences, for example in the non-coding sequence of *Fv1*.

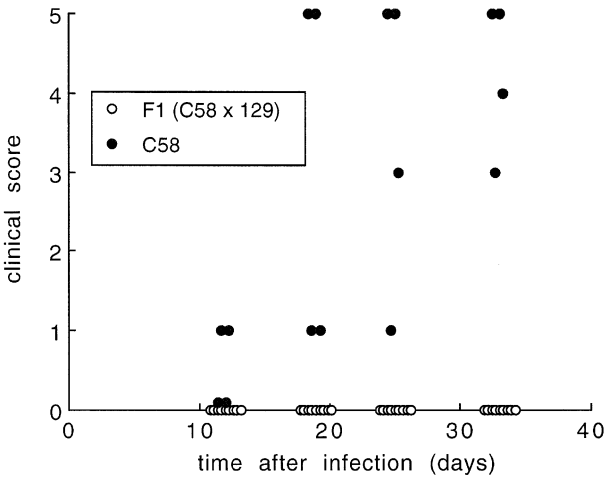


Figure 2 Development of polioencephalomyelitis in C58/J and F1(129Sv × C58/J) mice. Two to three month-old female mice were infected with LDVc. Five mg cyclophosphamide were injected at days −2, +5, and +12. At different times after infection, the intensity of the disease was estimated with an arbitrary score as follows: 1=paresis of one or two legs; 2=paresis of more than two legs; 3=flaccid paralysis of more than two legs; 4=total flaccid paralysis; 5=death.

Acknowledgements

The authors are indebted to Drs PL Masson and J Van Snick for critical reading of this manuscript, and to C Muller, M-D Gonzales, and T Briet for expert technical assistance. Drs EK Godeny is gratefully acknowledged for gift of reagents. This work was supported by the Fonds National de la Recherche Scientifique (FNRS), Fonds de la Recherche Scientifique Médicale (FRSM), Loterie Nationale and the State-Prime Minister's Office - S.S.T.C. (Interuniversity attraction poles, grant no 44) and the French Community (concerted actions, grant no 88/93-122), Belgium. J-P Coutelier is a research director with the FNRS.

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