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Short communication

Changes in Borna disease virus genome with adaptation to host

Satoshi Okayama [a], Nanako Miura [a], Masaru Murakami [b], Masayuki Funaba [c], Yoshii Nishino [a,*]

[a] *Laboratory of Veterinary Immunology, Azabu University School of Veterinary Medicine, 1-17-71 Fuchinobe, Sagamihara 229-8501, Japan*
[b] *Laboratory of Molecular Biology, Azabu University School of Veterinary Medicine, Sagamihara 229-8501, Japan*
[c] *Laboratory of Nutritional Science, Kyoto University Graduate School of Agriculture, Kyoto 606-8502, Japan*

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Abstract

The CRNP5 variant of Borna disease virus (BDV) has stronger pathogenesis than the CRP3 variant in which only 4 nucleotides in the whole genome are different. The CRP3 is produced by 3 passages in rat brains of BDV, whereas the CRNP5 is produced by 5 passages in mouse brains after 2 passages in rat brains of the BDV. Thymidylic acids at nt 3608 and 3673 were replaced by cytidylic acids during 3 passages in mice. Three passages in mice caused replacement of adenylic acid at nt 7936 by guanylic acid. No replacement at nt 8742 occurred during passages in mice.

Keywords: Borna disease virus; Genome; Pathogenesis

1. Introduction

Borna disease virus (BDV) is a nonsegmented negative-strand RNA virus [1] belonging to the family *Bornaviridae* [1]. BDV naturally infects a wide range of warm-blooded hosts [2]. In horses and sheep, BDV causes classical Borna disease (BD), a fatal mononuclear inflammatory encephalomyelitis with severe signs of neurological disease [2].

Classical BD is in large part due to immunopathogenic damage to the nervous system by blood-borne inflammatory cells [3]. Responses to BDV infection vary according to differences in species, animal strain or the age of the host at the time of infection [4], indicating the onset of BD by host-dependent factors.

In a previous study, however, the importance of the virus-specific factor in addition to the host-specific factor for the expression of BD has been shown; inoculation of a BDV variant CRNP5 to newborn rats severely and rapidly induced neurological disorders, as compared with the inoculation of another variant CRP3 [5]. CRNP5 was produced after two serial passages in rat brain (CRP2) followed by five serial passages in mouse brain (a total of seven passages in the brain), while CRP3 had a single brain passage in rats beyond CRP2 (a total of three passages in the brain). Comparison of the whole genome sequence of CRP3 with CRNP5 indicated differences of only four nucleotides with encoding amino acid differences [5]. Thus, single or multiple nucleotide substitutions of CRP3 are a key event eliciting a stronger pathogenesis in rats infected with CRNP5.

Currently, however, it is unclear whether the number of passages in the brain or host species for BDV passage or both is involved in the nucleotide variations. The present study examined the nucleotides differing in CRP3 and CRNP5 variants during viral passages in rats and mice.

2. Methods

2.1. Virus and passage

BDV-infected MDCK cells (He/80 strain) [6] were kindly provided by Dr. R. Rott (University of Giessen, Germany). The virus passage is outlined in Fig. 1. The cells were disrupted by ultrasonication, and clarified by centrifugation (2880 × *g* for

* Corresponding author. Tel.: +81 42 850 2473; fax: +81 42 754 7661.
E-mail address: nishino@azabu-u.ac.jp (Y. Nishino).

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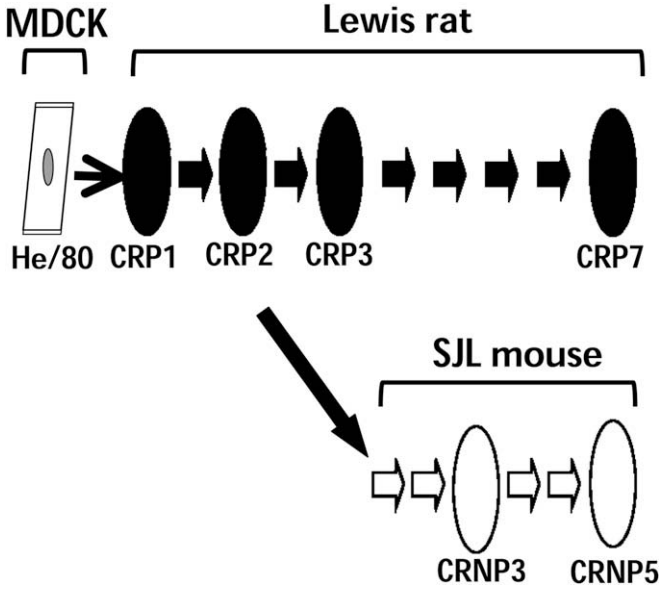

Fig. 1. Scheme of the protocol used to generate viral passages in rats and mice. Twenty microliters of the supernatant of ultrasonicated and centrifuged BDV-infected MDCK cell lysate were inoculated intracranially (i.c.) into newborn Lewis rats (CRP1), and then passaged into a second litter (CRP2), a third litter (CRP3) and seventh litter (CRP7). CRP2 was serially passaged by i.c. inoculation into adult SJL mice an additional three (CRNP3) or five times (CRNP5).

20 min at 4 °C). Twenty microliters of the supernatant enriched with BDV (7.2×10^3 focus-forming units (FFU)/mL) were inoculated intracranially (i.c.) into newborn Lewis rats. Four weeks after inoculation, the rat brains were recovered in 4 volumes of minimum essential medium (MEM) containing 2% (v/v) fetal bovine serum (FBS), homogenized and sonicated. The supernatant after centrifugation ($1200 \times g$ for 25 min at 4 °C) was designed as CRP1 [5]. Twenty microliters of CRP1 were inoculated i.c. into newborn Lewis rats, and CRP2 was obtained as CRP1 [5]. CRP2 was then inoculated into newborn Lewis rats to obtain CRP3 and CRP7 or adult SJL mice to obtain CRNP3 and CRNP5 [5].

2.2. Virus titer determination

The virus titer was determined by indirect immunofluorescent assays using C6 rat glioma cells as described previously [5].

2.3. Determination of nucleotide differing in CRP3 and CRNP5 variants

RNA isolation from BDV-infected MDCK cells and brain samples and cDNA synthesis were conducted as described previously [5]. To specify the nucleotides at nt 3608 and nt 3673 encoding membrane glycoprotein gp94 (G protein) and at nt 7936 and nt 8742 encoding RNA-dependent RNA polymerase p190 (L-polymerase), the following oligonucleotides were used for PCR; 5′-CTACGGAACGCAGGCTGA-3′ and 5′-TGGTCGGTACGGTTTATTC-3′ for nt 3608 and nt 3673, 5′-AGACCTCGTCGCCAAGCT-3′ and 5′-TGCGTGACTTA-GACCAAGAA-3′ for nt 7936, and 5′-TTGTTGGGAAGCGTC CTGTG-3′ and 5′-CCAAGCACTGCACCACTGA-3′ for nt 8742. PrimeSTAR HS DNA polymerase (TaKaRa) was used as the DNA polymerase for PCR. The PCR products were purified from agarose gels (QIAquick gel extraction kit; Qiagen), and ligated into the pCR2.1 vector and transformed INVαF' *Escherichia coli* strain (TA cloning kit; Invitrogen) according to manufacturer's protocol. The nucleotide sequence of insertion into the pCR2.1 vector recovered from 20 to 45 independent colonies was determined using a genetic analyzer (PRISM 3100 genetic analyzer; Applied Biosystems).

2.4. BDV infection

To examine changes in pathogenesis during passages of BDV, each BDV strain was inoculated into the brain of newborn Lewis rats as described above. The pathogenesis of CRNP3 in newborn Lewis rats was not examined, since the virus titer of CRNP3 was below the detection limit (<10 FFU/mL). In addition, the virus titer of CRNP5 for inoculation was adjusted to equal that of CRP3, i.e., 2×10^3 FFU. The number of dead rats was counted within 3 weeks after the virus inoculation. All rat experimentation were approved by the Azabu University Animal Experiment Committee (No. 53).

3. Results

Table 1 summarizes the effects of BDV passages on nucleotides involved in the virus-specific factor, the pathogenesis in newborn Lewis rats and the virus titer. MDCK He/80 cells are persistently infected with BDV isolated from a horse [6]. Inoculation of horse-originated BDV into newborn rats did not affect nt 3608 and nt 3673 encoding G protein, even if the virus was passaged in rats seven times. By contrast, three passages of the virus in mice (CRNP3) caused complete substitution of both nucleotides (thymidylic acids to cytidylic acids) with amino acid mutations (nt 3608: F–S; nt 3673: Y–H), and the change continued during two additional passages.

As for nt 7936 encoding L-polymerase, single inoculation of the BDV into a rat caused emergence of the changed nucleotide, guanylic acid, at a smaller percentage. The proportion of guanylic acid to total examined clones was maintained during an additional six passages in rats (<15%). Analyses of BDV passaged in mice, however, indicated complete substitution with guanylic acid within three passages in mice. Adenylic acid at nt 8742 was conserved during two passages in rats; however, clones at a smaller percentage showed guanylic acid instead of adenylic acid in CRP3, and the proportion increased in CRP7. By contrast, nucleotide 8742 was unaffected during the passages in mice.

We also found another nucleotide substitution at nt 7872, with amino acid replacement, i.e., guanylic acid (G) or adenylic acid (R). The proportion of guanylic acid gradually decreased with passage in rat brains, whereas it was completely substituted to adenylic acid in mouse brains (data not shown).

Table 1
Nucleotide of BDV gene involved in pathogenesis in newborn Lewis rats and the virus titer in whole brain.

	G protein				L-polymerase				Lethality	Titer (FFU/mL)
	nt 3608		nt 3673		nt 7936		nt 8742			
	T (Phe)	C (Ser)	T (Tyr)	C (His)	A (Lys)	G (Arg)	A (Arg)	G (Gly)		
MDCK He/80	100% (43/43)	0% (0/43)	100% (43/43)	0% (0/43)	100% (44/44)	0% (0/44)	100% (39/39)	0% (0/39)	0/9	7.2×10^3
CRP1	100% (32/32)	0% (0/32)	100% (32/32)	0% (0/32)	91.1% (41/45)	8.9% (4/45)	100% (24/24)	0% (0/24)	0/9	6.4×10^2
CRP2	100% (32/32)	0% (0/32)	100% (32/32)	0% (0/32)	85.3% (29/34)	14.7% (5/34)	100% (28/28)	0% (0/28)	0/9	7.0×10^3
CRP3	100% (30/30)	0% (0/30)	100% (30/30)	0% (0/30)	88.9% (24/27)	11.1% (3/27)	95.7% (22/23)	4.3% (1/23)	0/9	9.5×10^4
CRP7	100% (20/20)	0% (0/20)	100% (20/20)	0% (0/20)	91.7% (33/36)	8.3% (3/36)	28.9% (11/38)	71.1% (27/38)	0/15	5.2×10^5
CRNP3	0% (0/34)	100% (34/34)	0% (0/34)	100% (34/34)	0% (0/35)	100% (35/35)	100% (27/27)	0% (0/27)	NT[a]	<10
CRNP5	0% (0/30)	100% (30/30)	0% (0/30)	100% (30/30)	0% (0/37)	100% (37/37)	100% (36/36)	0% (0/36)	9/9	4.0×10^5

[a] NT: not tested.

BDV passaged even seven times in rat brains did not exhibit stronger pathogenesis in newborn Lewis rats. Consistent with the previous study [5], CRNP5 infection induced severe neurological disorder, leading to the death of all examined rats two to three weeks after infection. Histological analyses also indicated no evidence of inflammatory cell infiltration, an intensive gliosis, and neuronal degeneration in pyramidal cells in the hippocampus and cortex (data not shown), which was also consistent with the previous study [5].

The virus titer tended to increase during passage in rats, indicating efficient viral multiplication. By contrast, after CRP2 inoculation into SJL mice, the virus titer was below the detection limit, indicating rapid inhibition of viral multiplication; however, this inhibition was transient, and the virus titer was increased by additional passages in mouse brains.

4. Discussion

BD development resulting from BDV infection is generally dependent on the host species [4]. Since the genomes of most BDV isolates differ by less than 5% [7,8], a host-specific factor has been thought to induce host-dependent expression of BD [4]; however, in the previous study [5], only four nucleotide differences with amino acid replacement of BDV induced clear differences in the pathogenesis in newborn rats as well as adult rats, indicating the importance of the virus-specific factor. The present study evaluated the effects of the passage number and animal species for BDV passage on the nucleotide variation and pathogenesis in newborn Lewis rats. As a result, the variations of four nucleotides were categorized into 3 patterns: 1) no changes during passages in rats, but complete replacement during passages in mice, as for nt 3608 and nt 3673, 2) emergence of changed nucleotides during passages in rats at a low and constant rate, and complete substitution during passages in mice, as for nt 7936, and 3) increased replacement during passages in rats, but no substitution during passages in mice, as for nt 8742.

Consistent with the previous study [5], five passages of BDV in mouse brains induced severe pathogenesis in newborn Lewis rats. By contrast, passages in rat brains did not affect the pathogenesis, even if BDV was passaged 7 times in rat brains and a higher amount of virus was infected. Changes in nucleotides at nt 3608 and nt 3673 to cytidylic acids are likely to be critical for the expression of BD in newborn Lewis rats infected with CRNP5, because the nucleotides were limited to the strains passaged in mice. In addition, guanylic acid at nt 7936 may also be involved in the severe pathogenesis of CRNP5. Although the corresponding nucleotide in a small population (<15%) of BDV strains passaged in the rat brain was the same as that of CRNP5, rat-adapted strains did not exhibit stronger pathogenesis in neonatal Lewis rats. Sanz-Ramos et al. [9] revealed that the population of pathogenic variants is an important determinant of the expression of virus-induced disease in foot-and-mouse disease virus.

The present results suggested that the nucleotide at nt 8742 is not sufficient for the stronger pathogenesis of CRNP5.

The nucleotide of CRNP5 at nt 8742 was the same as that of parental BDV and major variants of CRP3.

There are two possibilities for the differences in the BDV genome between animal species for BDV passage, i.e., spontaneous mutations of limited nucleotides during passages and predominant selection of a specific virus during passages. Currently, the reason for this difference is unclear. Because of the high mutation rate, RNA virus strains generally consist of complex populations differing in their genomic structure [10]; however, within a constant environment, these populations remain stable [11,12]. A rabies virus passaged in BHK cells (CVS-B2c) indicated differences in 10 amino acids in the glycoprotein as well as the pathogenesis from that passaged in neuroblastoma cells (CVS-N2c) [13]. The pattern of restriction enzyme digestion of nucleotides spanning the glycoprotein was reproducible during passages in BHK cells in 3 parallel experiments, suggesting that CVS-B2c was selected from a minor population of CVS-24 during passages in BHK cells. It is possible that a minor population of BDV with stronger pathogenesis is dominantly selected during passages in mice. A previous study revealed that virus replication efficiency was higher when molecularly cloned BDV with L-polymerase of mouse-adapted strains, i.e., guanylic acid at nt 7936 and adenylic acid at nt 8742, was inoculated into mouse brains [14]. In addition, enhanced activity of polymerase complexes containing the mouse-type L-polymerase was evident in rodent cells but not in human cells [14]. These results partly explain the reason of selection of mouse-adapted BDV strains.

The previous study indicated that a main nucleotide at nt 8742 of CRP3 was guanylic acid, and that adenylic acid was minor population [5], which is not consistent with the present result. At present, the reason for these inconsistent results is unknown. The number of samples may have been insufficient to determine nt 8742 of CRP3 in the previous study, although the nucleotide sequence was determined in more than 10 independent clones [5]. In addition, the proportion of guanylic acid at nt 8742 in rat brains increased with passage; the reason is also unclear. It is possible that the nucleotide is preferable to adaptation to rat brains but not to mouse brains.

In conclusion, the present study suggests that critical nucleotides of BDV elicit a severe pathogenesis. Large differences in virulence resulting from small number of virus genome changes have been shown in several viruses, including rabies virus [15], Sindbis virus [16], lymphocytic choriomeningitis virus [17], avian influenza A virus [18] and Semliki Forest virus [19]. Considering BDV infection in a wide range of animals, the reason for alteration of the pathogenesis with 3-nucleotide changes should be clarified from a molecular basis in future studies to prevent the onset of BD.

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