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Borna Disease Virus in Mice: Host-Specific Differences in Disease Expression

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We developed a mouse model of Borna disease to facilitate immunopathogenesis research by adaptation of Borna disease virus to mice through serial passage in mouse brain tissue. Borna disease virus replication, antibody production, inflammation, and Borna disease expression in several different strains of mice were examined.

Borna disease virus (BDV) is a neurotropic, negative-strand RNA virus which causes encephalomyelitis and neurobehavioral disease in a wide range of experimentally inoculated species (3, 5, 18). BDV infection causes severe encephalitis and death in horses, sheep, rabbits, and adult Lewis rats; encephalitis, abnormal social behavior, and hyperactivity in tree shrews (*Tupaia glis*); and asymptomatic encephalitis in Wistar rats, black-hooded rats, and hamsters (1, 10, 14, 23). Some BDV-infected mice have been reported to have slowed growth rates and minimal encephalitis, but no signs of neurobehavioral disease have been reported since the initial findings by Nicolau et al. in 1927 (11, 14, 16, 18, 19). Studies of the immunopathogenesis of BDV infection would be greatly facilitated by a mouse model of Borna disease. We developed a mouse model by adaptation of BDV to the mouse through serial passage in mouse brain cells and identified a Borna disease-susceptible strain.

Five 4-week-old SJL mice (Jackson Laboratory, Bar Harbor, Maine) were inoculated intracranially (i.c.) with 10^5 50% tissue culture infective doses ($TCID_{50}$) of second-passage BDV-infected Lewis rat brain homogenate (BDV-R-P_2), prepared as previously described (3) (BDV-M-P_1). Brain homogenate from the inoculated mice was serially passaged in mice four times: BDV-M-P_2 through BDV-M-P_5. No signs of Borna disease were noted.

Sera from BDV-infected mice were tested for anti-BDV antibody by indirect immunofluorescence assay (IFA) on acetone-fixed monolayers of a persistently BDV-infected tissue culture cell line, C6BV (2). All mice inoculated with BDV had anti-BDV antibody titers ranging from 1:1,000 to 1:64,000 (Table 1).

Tissue sections of paraformaldehyde-fixed, paraffin-embedded brains from uninfected and BDV-infected mice were stained with hematoxylin and eosin and examined under light microscopy for meningoencephalitis and graded 0 to 4+. A mononuclear cell encephalitis was present in the BDV-M-P_1 mice (2+) (Fig. 1A) and increased with subsequent passages (Fig. 1B).

By using an avidin-biotin indirect immunohistochemistry assay (ABC; Vector Laboratories, Burlingame, Calif.), paraffin-embedded sections of mouse brain were stained with polyclonal rabbit anti-BDV for detection of BDV proteins. Other brain sections were treated with OX6 (Accurate Chemical & Scientific Corp., Westbury, N.Y.), an antibody which recognizes major histocompatibility complex (MHC) class II antigens. BDV proteins were detected in neurons of SJL mice (P_2 through P_5; data not shown). MHC class II antigens were seen in perivascular and meningeal inflammatory cells in brain sections from SJL mice (Fig. 1C).

Mouse brain tissue was homogenized and sonicated in RPMI 1640 (GIBCO BRL, Gaithersburg, Md.) with 2% fetal bovine serum (GIBCO BRL) at 20% (wt/vol) and serially diluted in log or half-log increments on a primary fetal rabbit brain cell line (FRB) as described previously (3). Viral infectivity was detected by IFA, and viral content was reported as $TCID_{50}$ per gram, based on undiluted, prehomogenized mouse brain weight. Infectious virus was not recovered from the BDV-M-P_1 SJL mouse brain homogenate. Nonetheless, BDV-M-P_1 and subsequent passages initiated productive BDV infection in mice (Fig. 2), while BDV from early passages of rat brain tissue, BDV-R-P_2 (our data) or BDV-R-P_1 to -P_3 (11), was unable to initiate productive BDV infection in mice, as determined by recovery of infectious BDV.

Expression of disease induced by other neurotropic viruses, e.g., mouse hepatitis virus and Semliki Forest virus, can vary with the host (12, 24). Four 4-week-old mice of each of the MRL/*lpr*, MRL/+, BALB/c (Jackson Laboratory), and BALB/c (Charles River Laboratories, Stone Ridge, N.Y.) strains were inoculated i.c. with 10^4 $TCID_{50}$ from SJL BDV-M-P_4 in order to identify a strain of mouse susceptible to Borna disease (7, 8, 25). By day 55 postinfection (p.i.), all infected mice had mononuclear cell encephalitis (Fig. 3A), ranging from 1+ in infected BALB/c mouse brain tissue to 3+ in infected MRL/+ mouse brain tissue (Fig. 3B and C, respectively). MHC class II antigens were seen in inflammatory cells in brain sections of MRL/+ and MRL/*lpr* mice (data not shown). In contrast to BDV-infected rats, encephalitis in mice persisted through the last time points (4 to 6 months). All uninfected mice showed no inflammation on histological examination, with the exception of the MRL/*lpr* mice, which had a 1 to 2+ meningitis/choroiditis, a phenomenon that has been described for this strain of mice (25). BDV proteins were also detected in brain sections of MRL/+ and MRL/*lpr* mice (Fig. 3D). Infectious virus was recovered from all strains (Fig. 4).

The MRL/+ mice exhibited behavioral signs of Borna disease not exhibited by the uninfected mice: hyperactivity

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TABLE 1. Antibody titers and severities of encephalitis in SJL mice infected with serially passaged BDV-M

Passage no.	No. of mice	Days p.i.	Encephalitis	Antibody titer
1	5	50	2+	1:4,000
2	4	35	2+	1:1,000
3	4	56	2+	1:64,000
4	5	42	3+	1:32,000
4	2	76	3+	1:64,000
4	5	170	3+	1:64,000
5	2	31	4+	1:8,000
5	2	110	4+	1:64,000

(e.g., running wildly about the cage without provocation and jumping out of the cage when the lid was lifted) and aggression (e.g., wild fighting and loud squealing). No other signs of neurological disease (e.g., ataxia and limb or tail weakness) were noted.

At 2 months p.i., three BDV-infected MRL/*lpr* and MRL/+ mice, along with the same number of age- and gender-matched uninfected mice, were analyzed individually for hyperactivity in Omnitech Digiscan activity chambers (Omnitech Electronics, Columbus, Ohio). Horizontal activity was measured over a 26-h period, with data compiled every 2 h (13 samples). The first 2-h sample was discarded because of initial exploratory activity of the mice. BDV-infected MRL/+ mice showed hyperactivity more than threefold greater than that of uninfected MRL/+ mice (Fig. 5). In contrast, no difference in activity was noted between the BDV-infected and uninfected MRL/*lpr* mice.

The BDV-R stock is not molecularly or biologically cloned and probably represents a mixture of viral strains. Therefore, serially passaging BDV-R in mouse brain tissue rather than rat brain tissue may have supported rapid development of a small subset of the original inoculum capable of replicating in the mouse, selecting for the mouse-permissive subset, BDV-M. Our findings could be due to mouse strain differences or to infection with our mouse-adapted BDV-M rather than BDV-R.

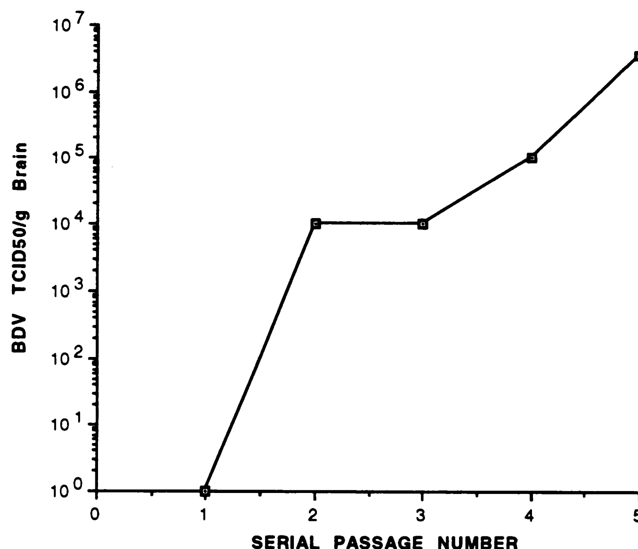


FIG. 2. BDV replication following serial passage in SJL mice.

Initiation of encephalitis has been temporally linked to the onset of Borna disease in rats (3, 17, 22). Our data, as well as

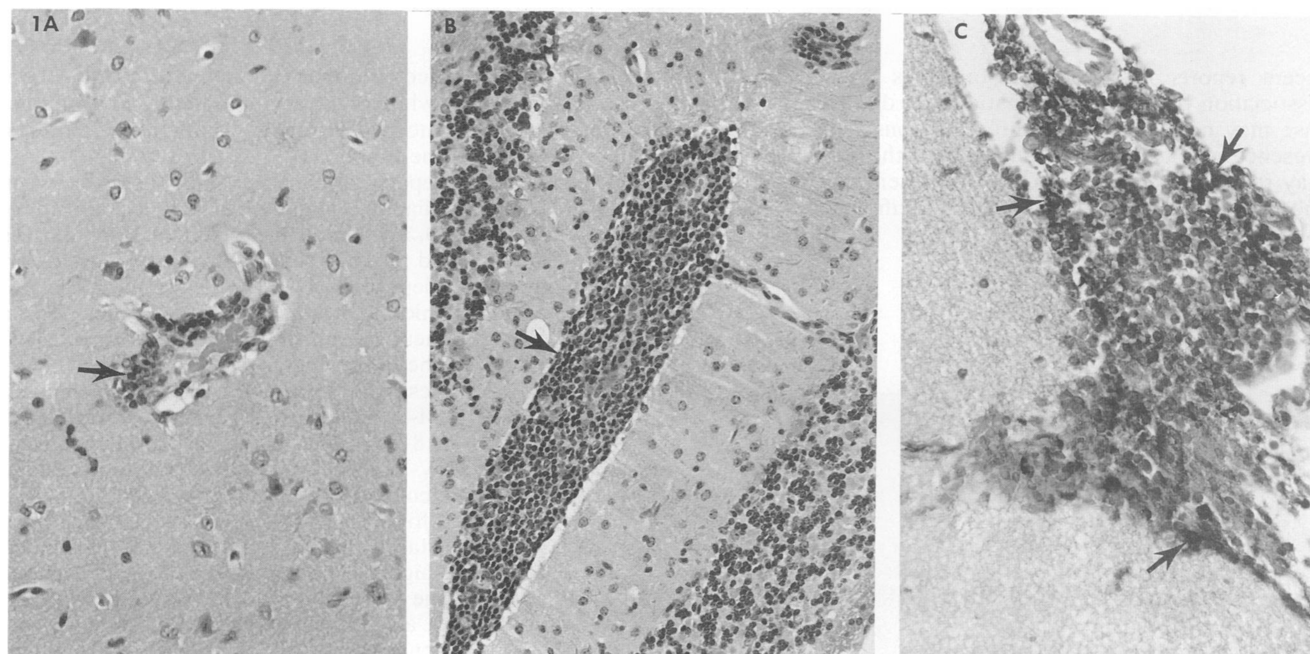


FIG. 1. Mononuclear inflammatory responses to BDV infection in SJL mouse brain sections. (A) BDV-M-P_1 mouse brain section with 2+ encephalitis (arrow); (B) BDV-M-P_5 mouse brain section with 4+ encephalitis (arrow) (A and B, hematoxylin and eosin stain; magnification, ×304); (C) BDV-M-P_5 mouse brain section with MHC class II expression on perivascular and meningeal inflammatory cells (arrows) (immunohistochemical stain with hematoxylin counterstain; magnification, ×304).

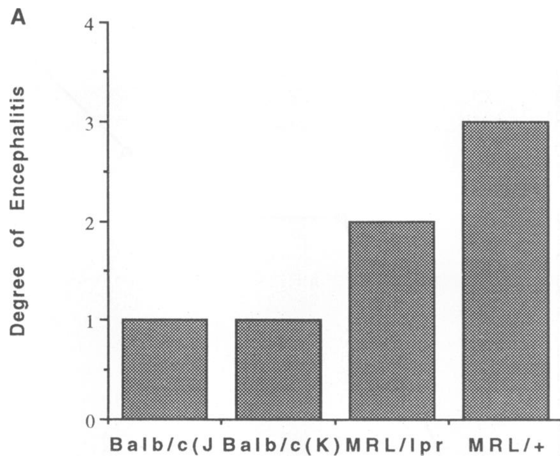
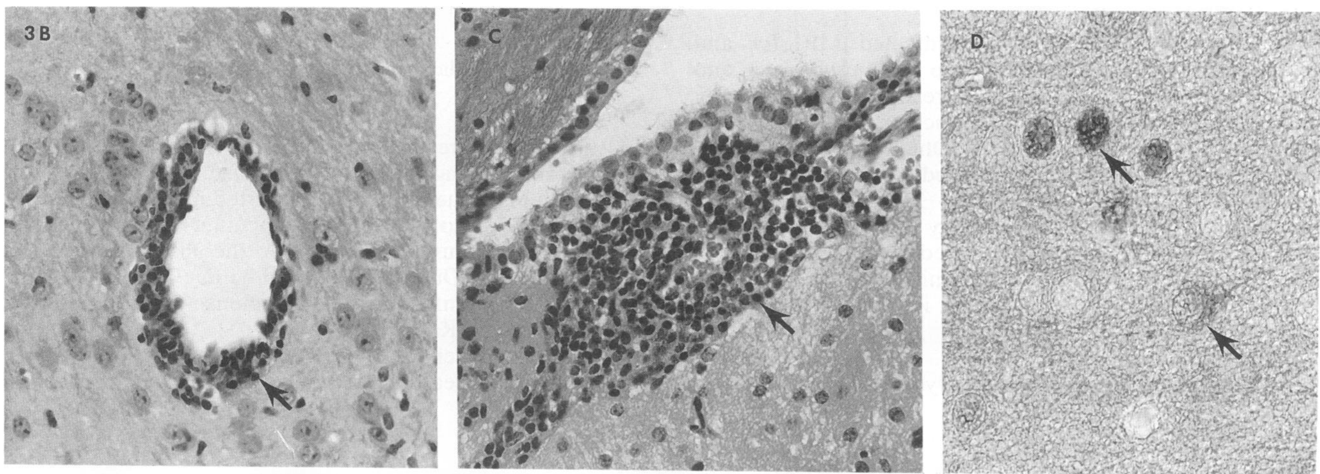


FIG. 3. BDV infection in various strains of mice. (A) Encephalitic response to BDV; (B) BDV-M-P_5 BALB/c mouse brain tissue with minimal encephalitis (1+) (arrow); (C) BDV-M-P_5 MRL/+ mouse brain tissue with 3+ encephalitis (arrow) (B and C, hematoxylin and eosin stain; magnification, ×288); (D) BDV-M-P_5 MRL/+ mouse brain tissue with BDV protein-expressing cells (arrows) (immunohistochemical stain, no counterstain; magnification, ×360).



recent reports from other laboratories (4, 9), suggest a dissociation between inflammation and disease. Borna disease may not simply be a direct response to the physical presence of mononuclear infiltrates in the brain but rather may result from the strain-specific presence of a particular subset of inflammatory cells and/or specific factors, perhaps released by the infected animal's immune cells (4). It remains to be shown whether the asymptomatic and symptomatic rodents with encephalitis have a different spectrum of cells or factors in the brain.

Despite earlier reports of a causal relationship between MHC class II-mediated inflammation and expression of Borna disease (6, 20, 21), Borna disease expression in Lewis versus black-hooded rats was found to be genetically determined but was independent of MHC class II genes (9). Indeed, MRL/*lpr* mice showed no signs of clinical Borna disease, although these mice have increased levels of MHC class II mRNA in the brain (15).

Brain homogenates from SJL BDV-M-P_5 (asymptomatic) and MRL/+ BDV-M-P_5 (diseased) mice were inoculated i.c. into three and four 8-week-old Lewis rats, respectively, to assess whether BDV-M caused Borna disease in rats. Seven Lewis rats were inoculated i.c. with BDV-R as controls. The rats were observed for signs of Borna disease and sacrificed on day 28 p.i. Mortality in both groups of rats inoculated with BDV-M was higher (three of seven rats) than in the BDV-R group (none of seven rats) by day 28. BDV-M-inoculated rats had an anti-BDV antibody titer of greater than 1:12,000, 4+ encephalitis, BDV antigen in the brain, and equivalent titers of infectious BDV (10^5 $TCID_{50}$/g) (data not shown). Although BDV-R and BDV-M were unable to produce Borna disease in most of the mouse strains tested, BDV-M caused Borna disease in rats.

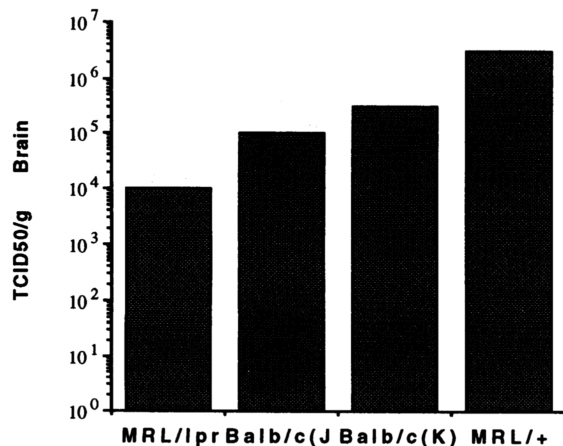


FIG. 4. BDV replication in different mouse strains.

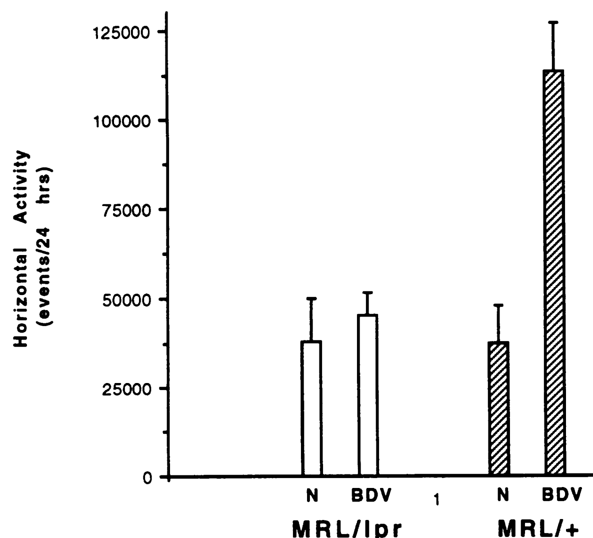


FIG. 5. Horizontal activity of normal and BDV-infected MRL/+ and MRL/*lpr* mice observed at 2 months p.i.

To compare anti-BDV antibody production in mice and rats, a Western immunoblot was performed with proteins isolated from rat brain homogenates by detergent extraction (Fig. 6). After the protein content was determined by using a bicinchoninic acid protein assay reagent kit (Pierce, Rockford, Ill.), 13 μg of protein was loaded per lane, and the proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and blotted onto nitrocellulose paper. Native immunoglobulins were removed with glycine-HCl treatment (13). The blots were stained via indirect immunohistochemical assay with anti-BDV antibodies from Lewis rats infected with BDV-R (Fig. 6A) or BDV-M (Fig. 6B) and with anti-BDV antibodies from MRL/+ mice infected with BDV-M (Fig. 6C) adjusted to equivalent titers by IFA. The proteins were visualized by using either the tetramethylbeinzidine membrane peroxidase substrate system (Kirkegaard & Perry Laboratories, Gaithersburg, Md.) or the enhanced chemiluminescence Western blotting detection system (Amersham, Arlington, Ill.).

In both Western blot systems, sera from BDV-infected mice and rats recognized BDV p38/40 and p24. Unlike

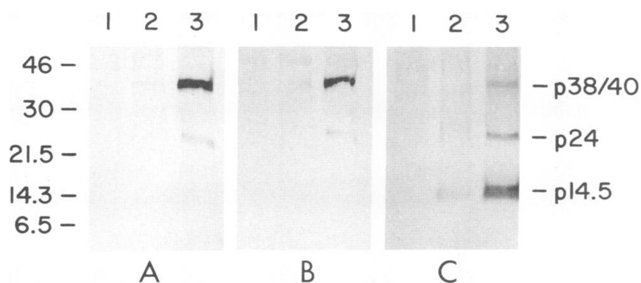


FIG. 6. Western blot analysis of proteins extracted from uninfected (lane 1), BDV-M-infected (lane 2), and BDV-R-infected (lane 3) rats. The blots were stained via indirect immunohistochemical assay with anti-BDV antibodies from Lewis rats infected with BDV-R (A) or BDV-M (B) and with anti-BDV antibodies from MRL/+ mice infected with BDV-M (C). Sizes on the left are expressed in kilodaltons.

BDV-infected mouse sera, rat sera did not recognize the p14.5 viral protein. With prolonged exposure, however, a faint band at 14.5 kDa was visualized with the BDV-infected rat sera with use of the ECL system. Laser densitometry analysis revealed a relative intensity of antibody signal for mouse sera compared with rat sera of 1.5 for p38/40, 1.1 for p24, and 7.8 for p14.5. Consistent with this observation, a 10-fold concentration of rat sera produced a p14.5 band equivalent to that seen with mouse sera (data not shown). This phenomenon could be attributed to antibody-antigen avidity or to a lower level anti-p14.5 antibody in BDV-infected rat sera than in BDV-infected mouse sera.

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