Demonstration of Borna Disease Virus RNA in Peripheral Blood Mononuclear Cells Derived from Domestic Cats in Japan

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Borna disease virus (BDV) naturally infects horses, sheep, and several other species, including humans, and it is believed to be related to neurological disorders. BDV infection in domestic cats has also been demonstrated by serological assays. We demonstrated for the first time BDV RNA in peripheral blood mononuclear cells from 11 of 83 (13.3%) randomly selected domestic cats in Japan by nested reverse transcriptase-PCR. The BDVs from cats were similar to but slightly different from those from horses and humans, as shown by sequencing the reverse transcriptase-PCR products. None of the cats was positive for both BDV RNA and anti-BDV antibodies.

Borna disease virus (BDV) is a neurotropic, yet unclassified, nonsegmented, negative, single-stranded RNA virus that naturally infects horses, sheep, cats, cattle, and ostriches (for a review, see reference 8). Serological data suggest the association of BDV, or a related agent, with specific psychiatric diseases in humans (2, 24), although healthy individuals also contain antibodies to BDV at a low prevalence (1, 3, 23). Experimental BDV infection in animals such as rats induces behavioral changes that resemble some types of affective neuropsychiatric disorders in humans (14, 20, 21). However, whether or not BDV is a natural pathogen of humans remains to be determined, since infectious BDV, or a related virus, has not yet been isolated from human tissue or body fluids, although efforts have been made to isolate BDV from the cerebrospinal fluid of seropositive patients.

Recently, we demonstrated the presence of BDV RNA in peripheral blood mononuclear cells (PBMCs) from 17 of 57 (29.9%) healthy horses (19) and from 22 of 60 (36.7%) humans with psychiatric disorders (12) by nested reverse transcriptase-PCR (RT-PCR) to amplify the region within the p24 gene. The phosphorylated p24 protein is encoded in the second open reading frame, and it may correspond to polymerase cofactor found in other nonsegmented, negative-stranded RNA viruses (8). Essentially similar results were also obtained by another group (4). They performed RT-PCR to amplify the BDV p40 (nucleoprotein) or p24 regions and found that all six of their psychiatric patients contained BDV p40 or p24 in their PBMCs, whereas none of 10 randomly selected healthy blood donors contained BDV RNA in their PBMCs. In contrast, even in the PBMCs of healthy humans, we found BDV RNA at a low rate (8 of 172 individuals; 4.7%) under the same conditions (11). Thus, the origin of BDV in humans should be clarified. One possibility is horizontal transmission from infected animals to humans. In fact, an epidemiological study of BDV infection of three groups in Israel indicated horizontal transmission from ostriches to humans (18, 26), although the similarity or dissimilarity of both BDVs has not been clarified.

Investigators in Sweden first reported a spontaneous neurological disease in cats which is characterized by behavioral and motor disturbances; this neurological disease is referred to as “staggering disease” (13). Serological and/or immunohistochemical screening yielded no specific correlation with feline infectious peritonitis virus, feline leukemia virus (FeLV), feline immunodeficiency virus (FIV), pseudorabies virus, tick-borne encephalitis virus, canine distemper virus, Borrelia burgdorferi, or Toxoplasma gondii (15). An extremely high seroprevalence of BDV (44%) has been identified in cats with neurological signs of staggering disease in Sweden (16, 17), although about 7% seroprevalence has been found among randomly selected cats in Germany (16). Despite the striking serological evidence for a BDV infection in cats with staggering disease, all attempts to demonstrate BDV, viral antigen, or viral RNA in the brains failed (22). Here, we determined for the first time the prevalence of BDV-specific RNA in PBMCs from randomly selected domestic cats in two cities in Japan.

We studied PBMCs from 51 and 32 domestic cats hospitalized in animal hospitals in Sapporo and Iwamizawa, respectively (Table 1). All cats examined had never had neurological disorders. The PBMCs were isolated from EDTA-treated blood by centrifugation on Ficoll-Paque (density, 1.077 g/mL). Total cellular RNA was prepared with an RNA extraction kit (RNAol B; Cinna/Biotex Laboratories International, Inc., Friendwood, Tex.) as described previously (6). To detect BDV-specific RNA, the extracted RNA was amplified by nested RT-PCR as described elsewhere (11, 12, 19) to obtain a fragment of the p24-coding region, which is relatively conserved within the BDV genome (25). The control experiment included MDCk cells uninfected or persistently infected with BDV (10). Briefly, 1 µg of cellular RNA was amplified by nested RT-PCR with two sets of primers, as follows: for the first PCR, 5'-TGACCCCAACCGTGAAGACCA-3' at nucleotides 1387 to 1405 and 5'-GTCCCATTCATCCGTGTC-3' at nucleotides 1865 to 1847 and for the second PCR 5'-TCA GACCCAGACCCGAACA-3' at nucleotides 1443 to 1461 and 5'-AGCTGGGATAATGCGCG-3' at nucleotides
1834 to 1816. RT-PCR consisted of reverse transcription and amplification of the viral cDNA and was performed by the protocol described for the Ex rtTh RNA PCR kit (Perkin-Elmer Corporation, Branchburg, N.J.). The final products were separated by 1.5% agarose gel electrophoresis, blotted onto a nylon membrane, and Southern hybridized with four 32P-labeled synthetic oligonucleotides: sense nucleotides 1462 to 1485, 1485 to 1507, and 1637 to 1658 and antisense nucleotides 1811 to 1791. The PCR products were cloned in a PCR II vector by using a TA cloning kit (Invitrogen Corporation, San Diego, Calif.), and several representative clones were sequenced by the protocol described for the CircumVent Thermal Cycle Dideoxy Sequencing Kit (New England Biolabs Ltd., Beverly, Mass.) by using the same 32P-labeled primers used for the first PCR. All of the numbers for BDV nucleotide sequences described here correspond to the previously reported numbering scheme (7).

Nested RT-PCR revealed clear, positive signals for 7 of 51 cats (13.7%) from Sapporo and 4 of 32 cats (12.5%) from Iwamizawa (I series). Thus, the prevalence of BDV RNA averaged 13.3%. The results for and clinical records of BDV RNA-positive cats are summarized in Table 1. Representative RT-PCRs are shown in Fig. 1. Most of the PCR products showed a discrete band of the predicted size (374 bp), which corresponded to that of the fragment from persistently BDV-infected MDCK cells. However, the amplified levels and the intensities of Southern blots in these samples were lower compared with those of samples from healthy horses (19) and humans with psychiatric disorders (12). Direct sequencing of a random sampling (cat S-10) of the PCR products revealed some differences from the reported sequence of horse-derived BDV (He/80-1) (25) (Fig. 2A). In addition, the sequences of two DNA clones (clones pFBDV1-7a and pFBDV1-7b) derived from the products of cat I-7 were also slightly different from those of horse-derived BDV (25) (Fig. 2A). Nucleotide divergence at nucleotides 1596 to 1811 was about 6% between cat- and horse-derived BDVs. There was only a slight difference (7% divergence) in the deduced amino acids according to the sequences (Fig. 2B). In addition, the sequence of cat-derived BDV was also slightly different from that of human-derived BDV at nucleotides 1671 to 1781, which we showed previously by sequencing the RT-PCR product from a sample from psychiatric patient (12). The sequence divergence at this region was 13.6 to 14.5% between cat- and human-derived BDVs, while it was 5.5 to 7.3% between cat- and horse-derived BDVs and 21.8% between human- and horse-derived BDVs. Thus, at this time the BDVp24 sequence is different in the individual host.

The plasma from the same samples used for the detection of BDV RNA in PBMC preparations was also examined for the presence of anti-BDV p24 antibodies. The full-length p24 protein prepared as a fusion protein with glutathione S-transferase (GST) in Escherichia coli (27) was used as the antigen for immunoblotting. GST was used as a negative control protein. Both GST-p24 and GST proteins were used after purification by glutathione Sepharose 4B (Pharmacia Biotech AB, Uppsala, Sweden) column chromatography. The proteins were separated by sodium dodecyl sulfate–12% polyacrylamide gel electrophoresis and were then blotted onto a nitrocellulose membrane. Representative results for positive and negative samples are provided in Fig. 3. The clinical records of all cats positive for anti-BDV antibody are summarized in Table 1. None of the cats was positive for both BDV RNA and antibodies to BDV (Table 1).

Our results suggest that BDV or a similar, related agent is also a hematopoietic cell-related virus in domestic cats, as well as in horses (19) and humans (4, 11, 12). None of the cats examined in the present study had ever had a neurological disorder. In addition, the prevalence of BDV infection in FeLV- and/or FIV-seropositive cats was similar to that in the virus-free cats. In contrast, the clinical manifestations of staggering disease in Swedish cats are reported to be strikingly similar to those of borna disease in horses, as determined by

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**TABLE 1. Profiles and summarized results of BDV-infected domestic cats**

<table>
<thead>
<tr>
<th>Cat no.</th>
<th>Sex</th>
<th>Age</th>
<th>BDV RNA</th>
<th>Antibody to BDV</th>
<th>Clinical record</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-1</td>
<td>M</td>
<td>10 mo</td>
<td>+</td>
<td>+</td>
<td>Healthy</td>
</tr>
<tr>
<td>S-2</td>
<td>M</td>
<td>3 yr</td>
<td>+</td>
<td>Healthy</td>
<td>Healthy</td>
</tr>
<tr>
<td>S-3</td>
<td>M</td>
<td>8 yr</td>
<td>+</td>
<td>Healthy</td>
<td>Healthy</td>
</tr>
<tr>
<td>S-4</td>
<td>F</td>
<td>6 yr</td>
<td>+</td>
<td>FeLV infection</td>
<td></td>
</tr>
<tr>
<td>S-5</td>
<td>F</td>
<td>7 yr</td>
<td>+</td>
<td>FeLV infection</td>
<td></td>
</tr>
<tr>
<td>S-6</td>
<td>F</td>
<td>1 yr</td>
<td>+</td>
<td>Healthy</td>
<td></td>
</tr>
<tr>
<td>S-7</td>
<td>F</td>
<td>4 yr</td>
<td>+</td>
<td>Empyema</td>
<td></td>
</tr>
<tr>
<td>S-8</td>
<td>F</td>
<td>6 yr</td>
<td>+</td>
<td>Healthy</td>
<td></td>
</tr>
<tr>
<td>S-9</td>
<td>F</td>
<td>6 yr</td>
<td>+</td>
<td>Healthy</td>
<td></td>
</tr>
<tr>
<td>S-10</td>
<td>F</td>
<td>8 yr</td>
<td>+</td>
<td>Lymphosarcoma</td>
<td></td>
</tr>
<tr>
<td>S-11</td>
<td>F</td>
<td>8 yr</td>
<td>+</td>
<td>Healthy</td>
<td></td>
</tr>
<tr>
<td>I-1</td>
<td>M</td>
<td>1 yr</td>
<td>+</td>
<td>Healthy</td>
<td></td>
</tr>
<tr>
<td>I-2</td>
<td>M</td>
<td>1 yr</td>
<td>+</td>
<td>FeLV and FIV infections</td>
<td></td>
</tr>
<tr>
<td>I-3</td>
<td>M</td>
<td>Unknown</td>
<td>-</td>
<td>FeLV infection</td>
<td></td>
</tr>
<tr>
<td>I-4</td>
<td>M</td>
<td>8 mo</td>
<td>+</td>
<td>Healthy</td>
<td></td>
</tr>
<tr>
<td>I-5</td>
<td>M</td>
<td>6 yr</td>
<td>+</td>
<td>FIV infection</td>
<td></td>
</tr>
<tr>
<td>I-6</td>
<td>F</td>
<td>8 mo</td>
<td>+</td>
<td>Healthy</td>
<td></td>
</tr>
<tr>
<td>I-7</td>
<td>F</td>
<td>4 yr</td>
<td>+</td>
<td>Healthy</td>
<td></td>
</tr>
</tbody>
</table>

* Cats showing positive signals of BDV RNA in their PBMCs and/or anti-BDV plasma among 51 domestic cats from Sapporo (S series) and 32 domestic cats from Iwamizawa (I series).

* M, male; F, female.

* Results by nested RT-PCR of the p24 region.

* Results by immunoblotting with GST-p24.
Although there are no reports of such neurological disorders in Japanese cats, the prevalence of BDV RNA and seroprevalence were 13.3 and 8.4%, respectively. This seroprevalence was slightly higher than the 7% in German cats with unknown records, but it was slightly lower than the 13% in German cats with undefined neurological disorders (16). Similarly, we also demonstrated the presence of BDV RNA in healthy horses (19) and humans (11). Therefore, it is suggested that only part of the BDV RNA- or anti-BDV antibody-positive population, irrespective of animal species, will show neurological signs after long-term persistence. In addition to BDV infection, disease progression might be affected by a host factor(s), such as the age and immunostatus of the host, as shown by the induction of the disease in adult rats, whereas the infection in newborn rats is persistent and tolerated (5, 9). It is notable that there were at least two BDV-positive groups among domestic cats, namely, individuals positive only for BDV RNA and individuals positive only for anti-BDV antibodies. Similar results were also obtained with healthy blood donors (11). One explanation for the group that is positive only for BDV RNA signals is that BDV infection is passed onto newborns or they are born to infected mothers, both of which would induce tolerance to viral antigens. Therefore, the group that is positive only for anti-BDV antibodies might have been cats with mature immune systems that were infected as adults. The cats with staggering disease might be the latter group. The similarity and dissimilarity between feline and human BDVs should be clarified by a further comparison of their nucleotide sequences to understand the routes of natural transmission of this virus, such as horizontal transmission from infected cats to humans or vertical transmission from infected to uninfected individuals.

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**FIG. 2.** Comparison of the p24 nucleotide sequence of BDV derived from cat PBMCs with that of horse-derived BDV. The sequence of p24 at nucleotides 1596 to 1811 in two cDNA clones (pFBDVI-7a and pFBDVI-7b) corresponding to cat I-7 in Table 1 are shown (A). In addition, the result of direct sequencing from nucleotides 1680 to 1811 for the PCR product from cat S-10 is included (A). The sequence (He/80-1) of BDV from a horse (25) is also shown as a control (A). The deduced amino acids according to these sequences are shown (B).

**FIG. 3.** Immunoblots for anti-BDV antibodies in domestic cats. The purified GST-BDV p24 and the GST used as a control were similarly resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and were then blotted onto a nitrocellulose membrane. Fiftyfold dilutions of the plasma were reacted with the membrane. The positive sample (+) was plasma derived from a psychiatric patient (12). The samples with positive signals (samples S-1 and S-2) and the samples with no signals (samples S-6 and I-4) are shown in lanes 2, 3, 1, and 4, respectively. The molecular weights of the proteins were calculated by comparing their mobilities with those of marker proteins in a calibration kit (Bio-Rad).
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