Journal of NeuroVirology (1999) 5, 196–199

http://www.jneurovirology.com

Short Communication

Failure to demonstrate Borna disease virus genome in peripheral blood mononuclear cells from psychiatric patients in Korea

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RNA, extracted from peripheral blood mononuclear cells (PBMC) obtained from 81 Korean psychiatric patients (39 with schizophrenia, 33 with bipolar affective disorders and nine with major depression), was analyzed for a 391-nucleotide, highly conserved region of the p24 protein-encoding ORF II of Borna disease virus (BDV), using nested reverse transcription-polymerase chain reaction (RT–PCR). BDV genomic RNA was not detected in PBMC from any of the 81 Korean psychiatric patients. These data do not support an etiologic association between BDV infection and neuropsychiatric disorders in humans.

Keywords: Borna disease virus; RT–PCR; PBMC; psychiatric disorders

Aberrations in interleukin 2 (IL-2) regulation, including elevated soluble IL-2 receptor in sera of Caucasian and Korean schizophrenic patients (Rapaport *et al*, 1994) and decreased IL-2 production after mitogen stimulation (Kim *et al*, 1998), have been regarded as indirect evidence that schizophrenia may have an infectious or autoimmune basis. That viruses (or autoimmunity stimulated by viruses) may be involved in the pathogenesis of major psychiatric disorders, such as schizophrenia, bipolar disorder and major depression (Kirch and Alexander, 1992), is a concept which is gaining increasing support. Recent interest in the potential role of viruses in the pathogenesis of major psychiatric disorders has focused on specific candidate viruses. Originally isolated from horses with behavioral abnormalities and since detected in sheep, cats, ostriches and cattle, Borna disease virus (BDV), a member of the Bornaviridae family, possessing a non-segmented, negative-sense, single-stranded RNA genome with five open reading frames (ORF), is one such candidate.

Although BDV has not been definitively shown to cause any human disease, an etiologic association between BDV infection and major psychiatric disorders is tantalizing. Striking clinical similarities have been found between particular animal models of BDV infection and patients with affective disorders or schizophrenia. For example, behavioral disturbances in experimentally infected rats are reminiscent of affective disorders, such as bipolar and monopolar depression, in humans (Lipkin *et al*, 1995). In particular, the observations that BDV-infected rats have high levels of viral nucleic acid in prefrontal cortex, as well as abnormal mesocortical dopamine activity and abnormal nucleus accumbens dopamine system, suggest the potential for BDV being pathogenetically linked to psychiatric conditions having a dopaminergic substrate, such as schizophrenia and affective disorders (Solbrig *et al,* 1996a, b).

As determined by the indirect immunofluorescence test (Amsterdam *et al*, 1985; Rott and Becht, 1995; Bode *et al*, 1993) and Western blot analysis

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This work was presented in part at the 151st annual meeting of American Psychiatric Association in Toronto, Canada, May 30–June 4, 1998
Received 10 July 1998; revised 25 August 1998; accepted 26 August 1998

(Fu *et al*, 1993; Waltrip II *et al*, 1995), antibodies against BDV have been reported previously in patients with psychiatric disorders. By contrast, genomic analyses of BDV in psychiatric patients have yielded conflicting results. Whereas researchers in Germany and Japan have detected BDV nucleic acid in peripheral blood mononuclear cells (PBMC) of patients with schizophrenia and affective disorders (Bode *et al*, 1995; Kishi *et al*, 1995; Sauder *et al*, 1996; Igata-Yi *et al*, 1996), other investigators have been unsuccessful in detecting BDV genomic sequences in brain tissue, cerebrospinal fluid or PBMC (Sierra-Honigmann *et al*, 1995; Richt *et al*, 1997). Using nested reverse transcription-polymerase chain reaction (RT–PCR), we analyzed RNA extracted from PBMC of Korean patients with schizophrenia, bipolar affective disorders and major depression for a 391-nucleotide, highly conserved region of the p24 protein-encoding ORF II of BDV.

After obtaining informed consent, 81 inpatients (39 with schizophrenia, 33 with bipolar affective disorders and nine with major depression) (Table 1) from Korea University Hospital and Yong-In Mental Hospital, diagnosed according to DSM-IV criteria (American Psychiatric Association 1994), were studied during January to August 1997. Quantitative evaluations were based on the Brief Psychiatric Rating Scale (Overall and Gorham, 1962) for patients with schizophrenia, the Rating Scale for Mania (Young *et al*, 1978) for patients with bipolar affective disorders, and the Hamilton Rating Scale for Depression (Hamilton, 1960) for patients with major depression. All patients had psychotic or active symptoms at the time of study enrollment (Table 1), and at the time of blood collection, patients were drug-free or being maintained on various neuroleptic or other types of medications. All study participants lived in urban areas and none had contact with horses, sheep or cattle.

Table 1 Descriptive features of study subjects.

Diagnosis	*Numbers*	*Mean age (years)*	*Test score*[a]
Schizophrenia	39	38.3±9.2	45.4±12.3
Paranoid	18		
Undifferentiated	14		
Disorganized	5		
Catatonic	2		
Bipolar disorder	33	28.5±7.6	30.4±8.5
Manic/hypomanic	31		
Depressed	2		
Major depression	9	43.0±7.1	34.5±5.3

[a]Quantitative test scores (mean±s.d.) were based on the Brief Psychiatric Rating Scale for patients with schizophrenia, the Rating Scale for Mania for patients with bipolar affective disorders, and the Hamilton Rating Scale for Depression for patients with major depression.

BDV RNA was amplified by RT–PCR from PBMC of the psychiatric patients. PBMC separated by Ficoll-hypaque gradient centrifugation from blood samples collected from 81 psychiatric patients. Total RNA was extracted from PBMC using RNA zol (GIBCO/BRL, Gaithersburg, MD, USA). cDNA was initially synthesized for 1 h at 42°C using Superscript II RNase H-Reverse Transcriptase (GIBCO/BRL). RNA extracted from BDV-infected rat brain was used as a positive control. BDV sequences were amplified by nested PCR using the following previously described oligonucleotide primers which afforded amplification of a 391-nucleotide region of the p24-encoding ORF II of BDV (Kishi *et al*, 1995) : 5′-TGACCCAACCAGTAGACCA-3′+1387; 5′-GTCCCATTCATCCGTTGTC-3′−1865; 5′-TCAGACCCAGACCAGCGAA-3′+1443; 5′-AGCTGGGGAAATGCGCG-3′−1834. Primers for β-actin were 5′-TGGAATCCTGTGGCATCCATGAAA-3′ and 5′-TAAAACGCAGCTCAGTAACAGTCCG-3′ (product size, 348 bp) (Tokunaga *et al*, 1986). Primers were used at a final concentration of 0.1 μM in a 50 μL reaction mixture (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM each dNTP) containing 2.5 μL of cDNA and 2.5 units of AmpliTaq polymerase (Perkin Elmer Co., Norwalk, CT, USA).

Reaction mixtures were cycled 40 times: denaturation for 1 min at 94°C, annealing for 1 min at 50–55°C, and extension for 2 min at 72°C in a DNA thermal cycler (Perkin-Elmer model 480). Amplicons of first round PCR were subjected to 40 cycles of nested PCR using the same cycling conditions. Amplified products were size fractionated by electrophoresis on 1.5% agarose gels containing ethidium bromide at 0.5 μg/ml. PCR products of positive control and β-actin were purified by Wizard PCR Preps DNA Purification System (Promega, Madison, WI, USA). DNA sequencing was performed in both directions of each PCR product, using the dye termination cycle sequencing ready reaction kit (Applied Biosystems Inc., Foster City, CA, USA) on an automated sequencer (Model 377, Perkin Elmer Co.).

Using nested RT–PCR, successful amplification of BDV-specific RNA and β-actin mRNA was achieved with BDV-infected rat brain (Figure 1). However, specific viral nucleic acid sequences were not detected in any of the PBMC samples from the 81 psychiatric patients tested.

Previously, Bode *et al* (1995) reported BDV nucleic acid in four of six (66.7%) psychiatric patients, and Sauder *et al* (1996) found such sequences in 13 (seven patients with schizophrenia, one with affective disorder and five with other psychiatric disorders) of 26 (50%) neuropsychiatric patients. Moreover, in Japan, BDV-specific RNA was detected in 22 of 60 (37%) neuropsychiatric patients (Kishi *et al*, 1995) and in six (five patients with schizophrenia, one with depression) of 55

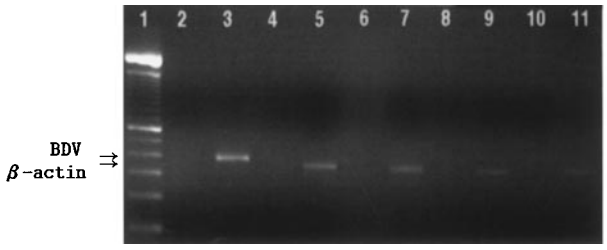

Figure 1 RT–PCR amplification of Borna disease virus (BDV) RNA in PBMC from Korean psychiatric patients. Lane 1: molecular weight marker (100 bp DNA Ladder, GIBCO BRL); lane 2: negative control; lane 3: BDV-infected rat brain cDNA control, showing BDV-specific 391 bp product; PBMC cDNA from patients with schizophrenia (lanes 4 and 5), bipolar mania (lanes 6 and 7), bipolar depression (lanes 8 and 9), and major depression (lanes 10 and 11). As shown, the 348 bp β-actin gene product was successfully amplified in all samples (lanes 5, 7, 9 and 11).

(10.9%) psychiatric patients, compared to none of 36 blood donor controls (Igata-Yi *et al*, 1996). Finally, in examining the limbic structures of postmortem brain samples from 75 North American and European individuals with various brain disorders for BDV P gene mRNA by RT–PCR, Salvatore *et al* (1997) reported the presence of BDV nucleic acids in the brains from nine of 17 patients with schizophrenia and from two of five patients with bipolar disorder. However, BDV p24 genome has also been found in normal human brain tissues (Haga *et al*, 1997), suggesting that BDV may infect the human brain without leading to neuropsychiatric disease.

Although we used the same oligonucleotide primers as Kishi *et al* (1995) for amplification of BDV p24, we were unable to demonstrate BDV-specific RNA in PBMC samples from 81 Korean psychiatric patients. Recently, Kubo *et al* (1997), using the identical primers, also demonstrated no association between BDV infection and psychiatric disorders among Japanese patients. In addition, three other groups have similarly failed to detect BDV-specific RNA in PBMC and/or brain biopsy samples from patients with schizophrenia and affective disorders (Sierra-Honigmann *et al*, 1995; Lieb *et al*, 1997; Richt *et al*, 1997). Neither infectious virus nor BDV-specific RNA was detectable in PBMC of psychiatric patients who exhibited serum antibodies reactive to the p24 and/or p38 antigens of BDV (Sierra-Honigmann *et al*, 1995; Richt *et al*, 1997). This raises concerns about the actual source of the antigenic stimulus in humans, and whether antigenic mimicry is involved.

The precise reasons for these conflicting data are not readily apparent. The potential for laboratory contamination with BDV RNA or cDNA and the exquisite sensitivity of nested RT–PCR may account for the above-mentioned discrepancies. Thus, definitive support for an etiologic association between BDV infection and neuropsychiatric disorders in humans is still lacking.

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