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Detection of anti-Borna Disease Virus (BDV) antibodies from patients with schizophrenia and mood disorders in Japan

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Abstract

The relationship between infection with the Borna Disease Virus (BDV) and the clinical symptoms of schizophrenia and mood disorders (DMS-IV) was investigated. Western blotting techniques were used to examine anti-p10-BDV antibodies in serum from 32 patients with schizophrenia and 33 patients with mood disorders in Japan. The results showed that 1 out of 25 controls (4.0%), 7 out of 32 patients with schizophrenia (21.9%) and 9 out of 33 patients with mood disorders (27.3%) were positive for anti-BDV-p10 antibodies. Compared with levels of anti- BDV-p10 antibodies in controls, the production of anti-BDV-p10 antibodies failed to show a statistically significant relationship with schizophrenia but did show a significant relationship with mood disorder. The subgroup of schizophrenia patients with positive syndromes had a non-significantly higher frequency of anti-BDV-p10 antibodies than the subgroup of patients with negative syndromes. Similarly, the production of anti-BDV-p10 antibodies was non-significantly higher among patients with the unipolar subtype of mood disorder than in those with the bipolar subtype.

Keywords: Anti-BDV-p10 antibody; Schizophrenia; Positive syndromes; Mood disorders; Unipolar types

1. Introduction

Borna Disease Virus (BDV) causes central nervous system (CNS) disease in several vertebrate species that manifest behavioral abnormalities and diverse forms of pathology (Wimmer et al., 1993). Horses, sheep, cats and rabbits have been regarded as the natural hosts of BDV (Richt et al., 1993; de la Torre, 1994; Rott and Becht, 1995). In these species, BDV can cause Borna disease (BD), an

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often fatal neurological disease mediated by the immune system.

BDV has been molecularly characterized as a non-segmented, negative-stranded RNA virus. According to its unique genetic and biological features, BDV is the prototypic member of a new family, *Bornaviridae*, within the order *Mononegavirales* (Cubitt et al., 1994; Schneeman et al., 1995; Malik et al., 1999). BDV is an envelope with at least six open reading frames, encoding nucleoprotein (p40), polymerase cofactor (p24), matrix protein (gp16), envelope protein (gp56), RNA polymerase (gp190) and X protein (p10) (de la Torre, 1994; Cubitt et al., 1994; Wehner et al., 1997; Malik et al., 1999).

There is no apparent connection between BDV infection and the following characteristics: age, age of onset, period of hospitalization, accompanying somatic disease, past history of tuberculosis, history of transfusions, family history, or doses of psychotropic drugs. There is also no significant relationship between contact with animals, such as horses, cattle, and cats, and patients found to be positive and negative for BDV antibodies and/or RNA. Therefore, the route of BDV infection in humans remains unknown (Hussin and Woldehiwet, 1994; Day et al., 1996; Gow et al., 1997; Iwahashi et al., 1997, in preparation; Isoshima et al., 1998; Fukuda et al., 2001). It is possible that BDV infection in patients with schizophrenia and with mood disorders may not be a nosocomial (hospital-acquired) infection (Iwahashi et al., 1997). But, in fact, significantly higher proportions of anti-BDV antibody and BDV RNA have been detected in patients with schizophrenia and mood disorders than in controls (Bode, 1995; Bode et al., 1988, 1992, 1993, 1996; Kishi et al., 1995; Nakaya et al., 1996; Iwahashi et al., 1997; Isoshima et al., 1998; Ferszt et al., 1999).

In this study, the relationship between BDV infection and schizophrenia and mood disorders was examined with respect to the clinical time course and symptom-based subtypes.

2. Methods

After the procedures had been fully explained and written informed consent obtained, anti-BDV antibodies were examined in serum from patients with schizophrenia and mood disorders from the Shikoku area in western Japan. The BDV antigens used for Western blotting were the horse BDV-derived recombinant full-length p10 (nucleoprotein) fusion proteins with GST (glutathione *S*-transferase), and the negative control antigen was GST alone (Ludwig et al., 1993; Kitani et al., 1996; Nakaya et al., 1996; Thorsten et al., 2000).

The schizophrenia group comprised 32 chronically hospitalized patients (17 men, 15 women; mean age = 50.2 years, S.D. = 10.0; mean duration of illness = 24.8 years, S.D. = 8.8, mean period of hospitalization = 16.6 years, S.D. = 8.2 years; mean age of onset = 28.8 years, S.D. = 7.4). The mood disorders group consisted of 33 patients (17 men, 16 women; mean age = 48.2 years, S.D. = 8.4; mean duration of illness = 6.2 years, S.D. = 2.8 years; mean period of hospitalization = 3.0 months, S.D. = 1.2; mean age of onset = 39.6 years, S.D. = 4.2). Diagnoses were made by psychiatrists according to DSM-IV criteria (American Psychiatric Association, 1994). A psychiatrist and a psychologist conducted the clinical interviews, which lasted approximately 1 h, followed by independent ratings with the 1–7-point, 30-item Positive and Negative Syndrome Scale (PANSS; Kay et al., 1987).

The control group consisted of 25 unrelated medical staff members (nurses, doctors, social workers, medical technicians; 11 men, 17 women; mean age = 46.8 years; S.D. = 10.2).

In this study, the BDV antibody assay (Western blot) was performed at least twice, and frequencies of anti-BDV-p10 antibodies were also assessed using a χ^2 test with Yates' correction if indicated.

3. Results

Anti-BDV-p10 antibody carriers were found among 7 of the 32 patients with schizophrenia (21.9%) and 9 of the 33 patients with mood disorders (27.3%), while there was only one carrier of BDV antibody (4.0%) among the 25 controls (medical staff) (Tables 1–3; Fig. 1). There was no significant difference in age and period of hospitalization (patients)/engagement (medical staff) between the 65 patients and 25 controls.

Table 1
BDV antibodies and the Positive and Negative Syndrome Scale (PANSS) in 32 schizophrenic patients

No.	Sex	P10	Point	PANSS
1	M	−	−7	N
2	M	−	−5	N
3	M	−	−5	N
4	M	−	−4	N
5	F	−	−4	N
6	F	−	−3	N
7	F	−	−3	N
8	M	−	−2	N
9	M	−	−1	N
10	M	−	0	
11	M	−	0	
12	M	+	1	P
13	M	−	1	P
14	M	−	1	P
15	F	−	2	P
16	F	+	3	P
17	F	−	3	P
18	F	−	3	P
19	M	−	3	P
20	M	+	4	P
21	F	−	4	P
22	F	−	5	P
23	M	−	5	P
24	F	+	5	P
25	F	+	6	P
26	F	−	7	P
27	F	+	8	P
28	M	−	8	P
29	F	+	9	P
30	M	−	9	P
31	M	−	9	P
32	F	−	14	P

N = Negative syndrome; P = Positive syndrome.

Table 2
BDV antibodies and clinical symptoms in 33 mood disorder patients

No.	Sex	P10	Symptom
1	M	+	MDP
2	F	+	MDP
3	F	+	MDP
4	F	+	MDP
5	M	+	MDP
6	M	+	MDP
7	F	+	MDP
8	M	+	MDP
9	F	−	MDP
10	M	−	MDP
11	M	−	MDP
12	M	−	MDP
13	F	−	MDP
14	M	−	MDP
15	F	−	MDP
16	M	−	MDP
17	M	−	MDP
18	M	−	MDP
19	F	−	MDP
20	M	−	MDP
21	F	+	D
22	F	−	D
23	M	−	D
24	F	−	D
25	M	−	D
26	M	−	D
27	F	−	D
28	M	−	D
29	F	−	D
30	F	−	D
31	M	−	D
32	F	−	D
33	F	−	D

MDP = Unipolar type. D = Bipolar type

There were anti-BDV-p10 antibodies in 7 of 21 patient with positive syndromes (33%) and 0 of 9 patients with negative syndromes (0%) of schizophrenia (Table 1), and 8 of 20 patients of the unipolar type (40%) and 1 of 13 of patients of the bipolar type (7.7%) in the mood disorders group (Tables 2 and 3).

Table 3
Appearance frequency of anti-BDV–P10 antibodies in controls and patients

Anti-BDV-p10 antibodies frequency	−	+
Schizophrenic patients	25	7
Positive syndrome	14	7
Negative syndrome	9	0
Score 0	2	0
Mood disorder patients	24	9
Unipolar type	12	8
Bipolar type	12	1
Controls	24	1

4. Discussion

The production of anti BDV-p10 antibodies was not significantly related to a diagnosis of schizophrenia ($\chi^2 = 2.3829$, d.f. = 1, $P = 0.1127$). A significant difference did emerge, however, between

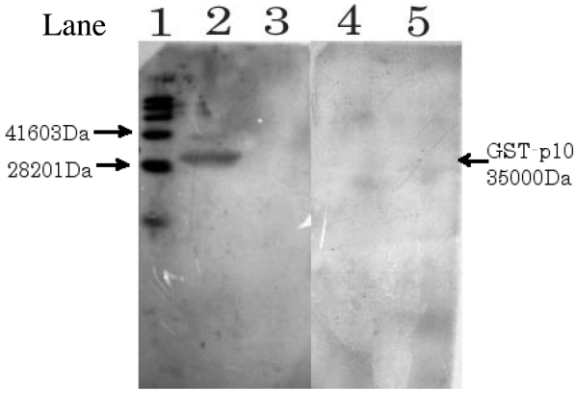

Fig. 1. Detection of anti BDV-p10 antibodies in the human plasma by Western blotting. Purified GST-fused BDV-p10 protein (Lane 2, 4) and GST alone (Lane 3, 5) were electrophoresed simultaneously by SDS-polyacrylamide gel and transferred to PVDF membranes. The human plasma absorbed with purified GST and diluted 200 times was incubated with the PVDF membrane, and the protein reactions were detected using peroxidase-conjugated anti human-IgG antibody and the ECL reagent (Amersham Pharmacia Biotech). Lane 1 is Dr Western (Oriental Yeast) for molecular quantity marker. (a) Anti BDV-p10 antibody positive (Lane 2, 3) (b) Anti BDV-p10 antibody negative (Lane 4, 5)

levels of anti-BDV-p10 antibodies in patients with mood disorder vs. controls ($\chi^2=3.8914$, d.f.$=1$, $P=0.0485$). The frequency of anti-BDV-p10 antibodies was non-significantly higher among patients with schizophrenia with positive syndromes than among those with negative syndromes ($\chi^2=2.2715$, d.f.$=1$, $P=0.1318$), and among mood disorder patients with the unipolar type than those with the bipolar type ($\chi^2=2.6772$, d.f.$=1$, $P=0.1018$).

The etiology of BDV-related psychosis is not yet understood, but BDV infection seems to be related to psychosis. It has been reported that both the BDV antigen and RNA were detected in brain samples from neuropsychiatric patients with clinical histories of mental disorders, and this demonstrated that BDV might infect human brain tissue, possibly contributing to the pathophysiologies of human neuropsychatric disorders (de la Torre et al., 1996). It is known that viruses persisting in the CNS can cause severe acute and chronic inflammation and that immunopathologic reactions may play a central role in the pathogenesis of a variety of neurologic diseases (Biler and Stitz, 1994). BDV infection may be associated with a maturation defect in the cellular immune response (Carbone et al., 1991) and BD may be a virus-induced immunopathologic disease of the CNS that results in changes in the levels of cytokines (growth factor of nervous cells), mRNAs in the brain, inflammation-induced tissue destruction, and finally cortical brain atrophy (de la Torre et al., 1996; Shanker et al., 1998). The virus may not be directly involved with the destruction of brain tissue, but it may cause damage indirectly by triggering cell-mediated immune responses (Shanker et al., 1998). It is also possible that the age at onset of the BDV infection, the brain area infected, the susceptibility (immune sensitivity or status) to BDV of the host, and BDV genetic variations influence the pathogenesis of BDV and the occurrence of psychotic features.

Crow (1980) in England and Andreasen (1982) in the United States have revised the heuristic concept of schizophrenia to encompass two syndromes, one characterized by positive (productive) features and the other by negative (deficit) features. They have proposed that the phenomenological distinction reflects differences in pathogenesis, neurobiological status, and prognosis. A positive syndrome is considered an aspect of hyperdopaminergia, hence portending neuroleptic sensitivity and a good outcome, whereas a negative syndrome is thought to be associated with a structural brain deficit, signaling neuroleptic resistance and poor prognosis (Crow, 1980; Andreasen, 1982; Kay et al., 1987).

In association with the detection of a virus or a gene product to BDV infection, there is a possible role of contamination in the laboratory. On the other hand, the antibody value of a BDV-positive sample is very low. And the positive sample of BDV-RNA did not always show positive antibodies of three kinds (p40, p24, p10). In fact, there may be some problems in detection sensitivity (Nishino et al., 1999; Thorsten et al., 2000). Methodological research into the detection of BDV is progressing. Recently, new methods for the detection of BDV using fusion proteins with GST were developed. The specificity and sensitivity of

methods of detection are now advancing (Hussin and Woldehiwet, 1994; Gow et al., 1997; Fukuda et al., 2001).

We reported (Iwahashi et al., 1998) that anti-BDV-p24 antibodies appeared significantly more frequently in negative syndromes of schizophrenia. Waltrip et al. (1997) similarly found that anti-BDV antibodies appeared significantly more often in the deficit syndrome of schizophrenia. Although analogous relationships of anti-BDV-p10 antibodies to subtypes of schizophrenia and mood disorders in the present study were not statistically significant, additional studies on the relationship between BDV infection and clinical subtypes should be performed in larger samples to more definitively examine the influence of BDV infection on the onset of and/or susceptibility to schizophrenia and mood disorders.

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