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Borna disease virus and schizophrenia

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Abstract

The development of a new serological assay method to detect antibodies in human sera recognizing Borna disease virus (BDV) proteins and a clinical pilot study are presented. Psychiatric patients from a schizophrenia research clinic in Baltimore, Maryland, were examined for antibodies to BDV antigen with traditional indirect immunofluorescence assays (IFA) that used both single and double labeling techniques and also with a Western blot assay capable of detecting antibodies to the three BDV proteins from a human neuroblastoma cell line. Thirteen of 90 (14.4%) patients and 0/20 control subjects had antibodies that recognized more than one BDV protein on the Western blot. Three patients had antibodies that recognized all three BDV proteins. Magnetic resonance imaging assessments of the volume of the putamen (with controls for total cranial volume) differentiated BDV+ from BDV− patients, and there were trend differences for bilateral amygdalae and the left amygdala-hippocampal process. We conclude that: (1) the Western blot assay is superior to IFA assays in BDV serology studies, (2) detection of antibodies to more than one BDV protein is a useful working criterion for seropositivity, (3) the 14.5 kDa BDV protein is 10 times more predictive of seropositivity than either the 38/40 kDa or the 24 kDa protein, (4) there is tentative evidence for a schizophrenia-control difference in the prevalence of anti-BDV antibodies, and (5) it is likely that there are neuroanatomical/behavioral features that differentiate seropositive from seronegative schizophrenic patients.

Keywords: Immunology; Antibodies; Magnetic resonance imaging; Putamen

1. Introduction

Reports of recent serological studies of patients with antibodies that recognize Borna disease virus (BDV) antigen contain proposals that the host range of BDV may include humans and that BDV may be a candidate causal agent in neuropsychiatric diseases (Amsterdam et al., 1985; Rott et al., 1985; Bode et al., 1988, 1992, 1993; Bechter et al., 1992; Fu et al., 1993). This report extends earlier work to a well-characterized cohort of schizophrenic patients and control subjects who were studied with a Western blot analysis of antibodies to all three BDV antigens and comparison with traditional BDV antibody detection by indirect immunofluorescence.

Borna disease, in its natural form, is a fatal meningoencephalitis in horses and sheep in central and eastern Europe. Three primary factors suggest that

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BDV could be implicated in human neuropsychiatric disease: (1) BDV has a very broad experimental host range including primates (Mayr, 1972; Mayr and Danner, 1974; Ludwig et al., 1988); (2) the manifestations of disease vary greatly and include asymptomatic infection (Kao et al., 1993) and abnormalities in social affiliation (Sprankel et al., 1978) seen in human neuropsychiatric diseases; and (3) serological screening studies have detected anti-BDV antibodies in humans in the United States (Amsterdam et al., 1985; Rott et al., 1985; Bode et al., 1988; Fu et al., 1993). In addition, the relative tropism for the limbic system (Narayan et al., 1983*a*) and excitatory fields in the hippocampus (Gosztonyi and Ludwig, 1984) make BDV a plausible etiological agent in schizophrenia, in particular, because of the possibility that the hippocampus may be involved in schizophrenic symptomatology (Gur and Pearlson, 1993). A recent serological screening study of neurologically asymptomatic horses from the U.S. middle Atlantic region, including Maryland, detected antibodies against the BDV 38/40 kDa antigen, suggesting a wider geographic distribution and an asymptomatic infection in horses (Kao et al., 1993).

The results of studies of human cohorts for an association between BDV and neuropsychiatric disease are mixed. The first studies (Amsterdam et al., 1985; Rott et al., 1985) revealed an association with psychiatric illness in patients with affective disorder compared with normal control subjects. Bechter et al. (1987, 1988) reported that antibodies occurred in 3% of surgical control subjects and 6.8% of psychiatric patients across several diagnostic groups, including schizophrenia, affective disorders, and personality disorders. Bechter et al. (1988) state that there were no large differences in rates across the different diagnostic groups, except that there were twice as many residual syndrome schizophrenic patients among the subjects who were seropositive for BDV. Neither diagnostic criteria nor Borna seropositive rates for schizophrenia were specified. Subsequent studies have demonstrated a rate of seropositivity of 2% in nonpsychiatric control subjects (Bode et al., 1988, 1990*a*), and one study (Bode et al., 1988) failed to demonstrate an association of seropositivity with psychiatric illness in a mixed diagnosis cohort. Associations of BDV seropositivity with multiple sclerosis and human immunodeficiency virus serological status have been reported as well (Bode et al., 1988, 1990*a*). Although some patients with schizophrenia are among psychiatric subjects reported in the aforementioned studies, BDV serological status in schizophrenia defined by *DSM-III-R* criteria (American Psychiatric Association, 1987) has never been specifically addressed.

There is only limited information available on the clinical characterization of seropositive patients. Bechter et al. (1987, 1988) have examined magnetic resonance imaging (MRI) of serologically characterized patients and reported an association between the presence of anti-BDV antibodies and T_2-weighted hyperluciencies that are like those seen in inflammatory conditions.

Screening techniques for BDV antibodies have generally employed indirect immunofluorescence (IFA) on BDV-infected canine kidney cells with human sera as described by Rott et al. (1985). More recently, techniques such as double-labeling IFA with monoclonal mouse serum as a second primary antibody and sandwich enzyme immunoassay have been used (Bode et al., 1990*b*, 1992, 1993). Bode et al. (1992, 1993) used a double IFA technique with monoclonal anti-BDV p38/40 as a second primary antibody on the rationale that antibodies against the 38/40 kDa BDV protein are always observed. A Western blot was applied as a screening technique by Fu et al. (1993), who used immunoaffinity-purified 38/40 kDa and 24 kDa, but not 14.5 kDa antigens (Schädler et al., 1985) from a rabbit kidney cell line. Antibodies were detected that recognized the 38/40 kDa and 24 kDa BDV proteins in sera from psychiatric patients with affective disorders. A 38/40 kDa single BDV protein Western blot has also been used to screen the sera of horses in U.S. studies (Kao et al., 1993).

Because of our experience that IFA techniques identify a large number of seropositive normal subjects and our concern about issues of specificity, sensitivity, and reliability/reader subjectivity, we developed a Western blot assay that used all three BDV proteins obtained from infected human neural tissue culture cells. The Western blot results

were then compared with the IFA technique, and Western blot data were used in a pilot study to subgroup schizophrenic patients and were examined in relation to longitudinal course, neuroimaging, and neurobehavioral variables.

2. Methods

2.1. Subjects

Ninety outpatients, selected in order of appointment from research clinics at the Maryland Psychiatric Research Center, and 20 normal control subjects, derived from the community, were evaluated (see Table 1). The gender, racial, and age characteristics of the patient and control groups were as follows: patients: 65 men, 25 women, 53 white, 37 black, mean age = 35.4 years, SD = 7.4; control subjects: 14 men, 5 women, 18 white, 1 black, mean age = 34.8 years, SD = 7.4 (demographic data were missing for one control subject). Sixty-three of the patients' samples were analyzed in an open design in which samples were known to have been derived from schizophrenic patients, and 27 samples were analyzed in a blind design in which samples from patients were interspersed with those from control subjects. *DSM-III-R* (American Psychiatric Association, 1987) criteria for schizophrenia guided research psychiatrists in a best-estimate diagnostic conference that used all available data from clinical interviews, medical records, longitudinal observation, and family informants. The control subjects, who were all evaluated with the Structured Clinical Interview for *DSM-III-R* (Spitzer et al., 1989), were determined to have no Axis I diagnoses. Premorbid functioning, at age periods from childhood to adulthood, was assessed with the Premorbid Adjustment Scale (Cannon-Spoor et al., 1982). A subset of patients had undergone MRI examinations with volumetric analysis of brain structures as a research procedure. A subset of the subjects had also been evaluated with the Neurological Evaluation Scale (Buchanan and Heinrichs, 1989), an instrument which contains items that assess sensory integration, motor coordination, sequencing of complex motor performance, and a group of other tests of frontal release signs, eye movement abnormalities, and short-term memory. At the time of blood sampling, patients were either drug-free or were receiving neuroleptic medications of various types. Informed consent was obtained as approved by the University of Maryland at Baltimore Human Volunteers Research Committee. An aliquot of the serum was stored in sterile glass containers at 4 °C until IFA was performed, while the remainder was stored at −70 °C. Western blots were done on freshly thawed samples that had been stored at −70 °C. The Western blots were read without knowledge of the subject's identity.

2.2. Antibody assays

Indirect immunofluorescence screening was carried out according to the method described by Rott et al. (1985). Samples were diluted 1:10 in 2% normal goat serum (NGS) in phosphate buffered saline (PBS) and applied to both uninfected Madine Darby canine kidney (MDCK) cells and an MDCK cell line persistently infected with BDV (MBV cells) (Herzog and Rott, 1980). Fluorescein isothiocyanate (FITC) conjugated anti-human heavy and light chain immuno-γ-globulin (IgG, Cappel, Durham, NC) was used as secondary antibody. Sera with elevated background staining were adsorbed against MDCK cells or swine liver powder at a 1:10 dilution. Positive sera were titered using serial two-fold dilutions in 2% NGS. Antiserum from a rabbit with Borna disease (RbαBV) or unequivocally positive human serum (positive control subjects) and known negative human serum (negative control subjects) or normal rabbit serum (NRS) were run with each assay.

Double-antibody indirect immunofluorescence, with two primary and two secondary antibodies, was used for confirmation of samples defined as positive by single IFA screening. Incubation of the patient's serum on both MDCK and MBV cells was followed by incubation of a 1:50 dilution in 2% NGS of RbαBV (titer > 1:16 000) on one set of MDCK and MBV cells, and the same dilution of NRS on the other set. FITC-conjugated donkey anti-human heavy and light chain IgG and Lissamine rhodamine sulfonyl chloride-conjugated donkey anti-rabbit heavy and light chain IgG (Jackson, West Grove, Pa.) were applied and fluorescence microscopy performed (see Fig. 1). For

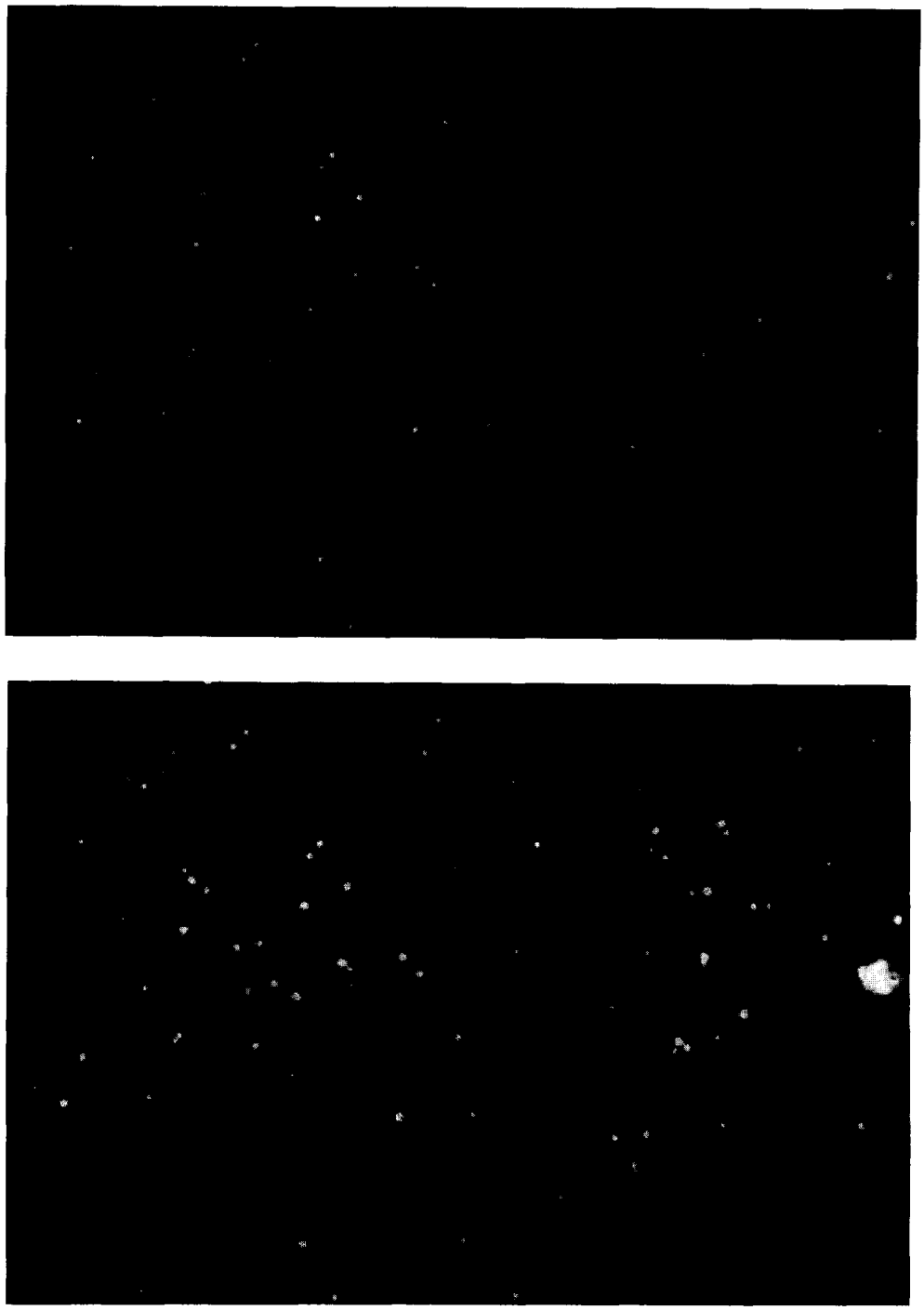

Fig. 1. Double primary antibody indirect immunofluorescence using rabbit anti-BDV polyclonal serum and serum from a patient with schizophrenia as primary antibodies. Secondary antibodies were fluorescein donkey anti-human IgG and rhodamine donkey anti-rabbit IgG. Photomicrographs of the same fields were taken with either a fluorescein (top) excitation filter detecting human antibodies or a rhodamine (bottom) excitation filter detecting rabbit antibodies. Note the similarity of the immunofluorescence pattern in the top and bottom panels.

each double IFA, a control negative human serum was also run as above.

A Western blot antibody assay was developed using lysate from a persistently BDV-infected human neuroblastoma SK-N-SY5Y cell line (Ross et al., 1983; Carbone et al., 1993) (SY5YBV). Western blot using SY5YBV as a source of antigen was chosen after screening lysates from a variety of persistently BDV-infected cell cultures against IFA-positive human sera using both Western blot analysis of the immunoprecipitation products of human sera and cell lysate and direct Western blot of cell lysates reacted with human sera. Lysate of SY5Y and SY5YBV was prepared in Laemmli sample buffer. Polyacrylamide gel electrophoresis was carried out using a 15% acrylamide gel in reducing conditions (Laemmli, 1970). The gels were electroblotted onto 0.2 μm nitrocellulose, and incubated in a blocking solution (BS, 10% (v/v) normal horse serum, 1% (w/v) powdered milk, and 0.5% (v/v) Tween 20 in PBS). Primary antibody solutions in BS, including patient and control samples, were applied and the strips rotated overnight at 4 °C. Human sera were run at a 1:20 dilution. After washes and reaction with secondary antibody, the primary antibody was visualized by Enhanced Chemiluminescence (ECL, Amersham, Arlington Heights, IL; see Fig. 2).

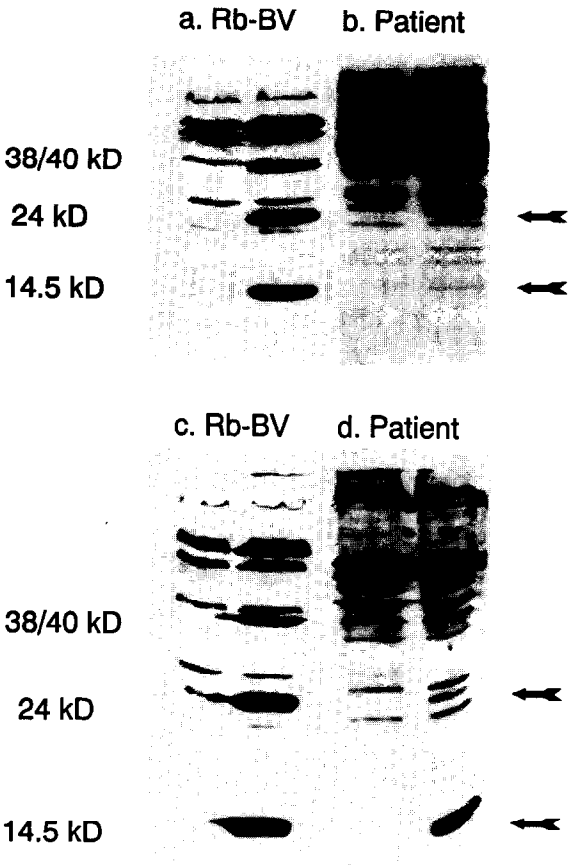

Fig. 2. Immunoblots of BDV-infected and -uninfected cell lysates stained with either rabbit anti-BDV or patient sera. Blots stained with rabbit anti-BDV (a, c) or sera from patients (b, d). In each blot, lanes contain SK-N-SY5Y (left) or SK-N-SY5YBV (right). Note that all three BDV proteins are recognized by rabbit anti-BDV. The two patient sera each recognize the 24 kDa and 14.5 kDa BDV proteins (see arrows).

2.3. MRI examination

Imaging data were collected on a Siemens 2-Tesla Magnetom system operating at 1.5 Tesla. The high-resolution spin-echo technique was used to evaluate the whole brain in the coronal plane in 3-mm contiguous slices, with a repetition time of 600 ms, an echo time of 17 s, and a matrix size of 256 × 256 pixels, with two excitations. The data were transported on magnetic tape for morphometric analysis. The morphometric analyses were conducted on the Loats image analysis system (Loats and Holcomb, 1989) using previously described methods of structure delineation (Bryant et al., 1993; Buchanan et al., 1993; Elkashef et al., 1994). The interrater reliabilities, by intraclass correlation (Bartko and Carpenter, 1976), for all structures were above 0.90.

3. Results

Table 1 contains the seropositivity rates by demographic characteristics and Table 2 by single IFA alone, single IFA combined with double antibody IFA confirmation, and Western blot. Table 2 displays two seropositivity criteria for Western blot: (1) A liberal criterion of any pattern of BDV-specific protein recognition (patient rate/control rate = 32.2%/20%, $\chi^2 = 0.03$, $df = 1$, $P = 0.85$) and (2) a restrictive criterion of specific recognition of more than one BDV protein (patient rate/control rate = 14.4%/0, Fisher's exact $df = 1$, $P = 0.06$). The rates, using restrictive Western blot

Table 1
Descriptive information

Subjects	Age		Sex		Race		Duration of illness		
	Mean	SD	M/F	%Male	W/B	%White	Mean	SD	*n*
Seropositive patients ($n = 13$)	37.3	8.1	10/3	76	5/8	38	12.2	4.3	12
Seronegative patients ($n = 77$)	35.2	7.3	55/22	71	48/29	62	14.1	6.8	60
All patients ($n = 90$)	35.4	7.4	65/25	72	53/37	59	13.8	6.5	72
Control subjects ($n = 20$)	34.8	7.4	14/5	74	18/1	95			

Duration-of-illness data were available for a subset of cases.

criteria, for patients whose samples were analyzed in open vs. blind designs were 17.5% vs. 7.4% ($\chi^2 = 0.83$, $df = 1$, $P = 0.36$). In addition, Table 2 contains the results of calculations of relative risk, sensitivity, and specificity for the IFA and Western blot methods. The sensitivity and specificity calculations were based on the restrictive criterion Western blot as a "gold standard." On the assumption that it minimizes false-positive findings, the restrictive criterion Western blot will be used as the measure of BDV seropositivity for the remainder of the results.

Table 3 presents the difference in rates for individual bands between patients and control subjects. To compare the contribution of antibody recognition of each BDV protein to the likelihood of a seropositive finding, Table 3 also reports the results of odds-ratio calculations, which were performed to place antibodies to the three BDV proteins on a single metric of importance in their contribution to seropositivity for comparison purposes. For example, samples that have antibodies recognizing the 38/40 kDa BDV protein have 40.71 times the risk of being seropositive than samples that do not have anti-38/40 kDa antibodies, and samples that have antibodies to the 14.5 kDa protein have 528 times the risk of being seropositive.

3.1. Demographics, premorbid adjustment, and duration of illness

Table 1 presents demographic and duration-of-illness information (see Table 4 for diagnostic

Table 2
BDV serology methods

	Rate in subjects				Relative risk	Sensitivity	Specificity
	Patients		Control				
	n	%	*n*	%			
Single IFA	22/90	24.4	4/20	20	1.29	0.54	0.80
Single IFA + double IFA confirmation	15/90	16.7	3/20	15	1.13	0.53	0.89
Recognition of 1 or more BDV bands on Western blot (liberal criterion)	29/90	32.2	4/20	20	1.90	0.39	0.93
Recognition of > 1 BDV band on Western blot (restrictive criterion)	13/90	14.4	0		—	—	—

Relative risk was not assessed for the restrictive Western blot criterion due to 0 rate in control subjects. The restrictive Western blot criterion was used as the "gold standard" for sensitivity and specificity.

Table 3
BDV-specific protein recognition

BDV-specific protein	Antibody rate in subjects				Odds ratio of seropositivity (95% confidence)	
	Patients		Controls		Mean	Range
	n	%	*n*	%		
38/40 kDa	8	8.9	0		40.71	6.90–240.20
24 kDa	25	27.8	4	20	56.47	6.87–463.96
14.5 kDa	12	13.3	0		528	44.21–6306.29

information). Most notable is the differing racial composition of the the control group vs. the patient group ($\chi^2 = 7.37$, $df = 1$, $P = 0.007$). While there is not a statistically significant association between race and seropositivity ($\chi^2 = 2.62$, $df = 1$, $P = 0.1$), the finding of a 9.4% rate among white subjects and a 21.6% rate among black subjects in this small group raises the possibility of such an association.

Premorbid Adjustment Scale (Cannon-Spoor et al., 1982) and duration-of-illness data were available for a subset of the cases. In 12 of the seropositive subjects, the mean duration of illness was 12.2 (SD = 4.3) years, and in 60 of the seronegative subjects, the mean duration of illness was 14.1 (SD = 6.8) ($F = 0.88$; $df = 1, 70$; $P = 0.35$). The age of first psychotic symptom was 21 (SD = 6.0) for seronegative subjects vs. 24.1 (SD = 7.0) for seropositive subjects ($F = 2.8$; $df = 1, 85$; $P = 0.10$) and the age of first hospitalization was 22.9 (SD = 6.4) for seropositive subjects and 27 (SD = 7.4) for seronegative subjects ($F = 3.37$; $df = 1, 74$; $P = 0.07$). The mean number of hospitalizations was 4.7 (SD = 6.3) in the seronegative subjects and 2.2 (SD = 1.7) in the seropositive subjects ($F = 1.89$; $df = 1, 83$; $P = 0.17$) while the total weeks of hospitalization was 62.5 (SD = 107) for the seronegative subjects and 28.5 (SD = 56.7) for the seropositive subjects ($F = 1.15$; $df = 1, 81$; $P = 0.29$). There were neither premorbid adjustment differences in any of the age epochs nor differences in the Premorbid Adjustment Scale total scores between seropositive subjects and seronegative subjects.

Table 4
Diagnostic information

Diagnosis	*DSM-III-R*	Seropositive rate	
		n	%
Schizophrenia, disorganized	295.1	2/8	25
Schizophrenia, paranoid	295.3	5/25	20
Schizophrenia, undifferentiated	295.9	6/48	12.5
Schizophrenia, residual type	295.6	0/2	—
Schizoaffective disorder	295.70	0/2	—
Schizophreniform disorder	295.40	0/2	—
Delusional disorder	297.10	0/1	—
Psychotic disorder, not otherwise specified	298.90	0/2	—
Total		13/90	14.4

3.2. Neurological Evaluation Scale

Findings from the Neurological Evaluation Scale (Buchanan and Heinrichs, 1989) were available for a subset of cases. There were no significant differences on the Neurological Evaluation Scale between seropositive subjects and seronegative subjects; however, the mean scores deserve mention because the pattern of scores may indicate increasing neurological abnormality with serological status (control subjects < seronegative subjects < seropositive subjects) and because all scores except the sequencing of complex motor tasks differentiated seropositive subjects from control subjects. Table 5 contains the mean scores and

Table 5
Neurological Evaluation Scale

	Anti-BDV serological status				Buchanan et al. (1989)	
	Control subjects ($n = 20$)	Seronegative ($n = 77$)	Seropositive ($n = 13$)	ANOVA ($df = 2, 83$)	Control subjects	Patients
Motor coordination	0.90	1.50	2.75	$F = 3.86$ $P = 0.025$	0.78	1.94
Sensory integration*	1.90 $n = 10$	3.52 $n = 62$	4.50 $n = 12$	$F = 3.18$ $P < 0.05$	2.68	4.11
Sequencing of complex motor tasks	0.60	1.18	1.58	$F = 2.20$ $P = 0.12$	1.32	2.78
Total score	7.90	16.58	21.08	$F = 8.54$ $P = 0.0004$	9.66	17.42

*Sensory integration data exist on a subset of the cases.

results from analysis of variance for the groups and, for comparison, published scores from control subjects and schizophrenic patients from Buchanan and Heinrichs (1989).

3.3. Medication

Medication data were available for 77 of the patients who had been treated in the Outpatient Program. The patients were taking 10 different neuroleptics, including clozapine. Three patients (3.9%) were drug-free; 8.2% of the patients were receiving treatment with more than one neuroleptic, and 58.9% were taking an anticholinergic. There was no association of restrictive criterion Western blot seropositivity with any particular neuroleptic, conventional neuroleptic vs. clozapine, one neuroleptic vs. two, type of anticholinergic, anticholinergic vs. no anticholinergic, or neuroleptic dose.

3.4. MRI correlates

Brain structure volumes obtained by MRI were available for 46 patients and 10 control subjects for the following structures: amygdala, hippocampus, prefrontal cortex gray matter, prefrontal cortex white matter, head of the caudate, body of the caudate, putamen, globus pallidus, temporal lobe, amygdala-hippocampus complex, prefrontal cortex, and caudate. Volumes of the structures were compared between seropositive patients (restrictive Western blot criterion), seronegative patients, and control subjects by analysis of covariance (with total cranial volume as the covariate). Several features differentiated the groups. The left putamen was significantly larger in seropositive patients (6.21 cm^3, $n = 5$) than in seronegative patients (5.1 cm^3, $n = 18$) or in control subjects (5.22 cm^3, $n = 5$) ($F = 7.97$; $df = 2, 28$; $P = 0.002$). The left amygdala-hippocampus complex was significantly smaller in seropositive subjects (5.8 cm^3, $n = 9$) and seronegative subjects (6.12 cm^3, $n = 32$) when compared with control subjects (7.04 cm^3, $n = 10$) ($F = 5.6$; $df = 2, 47$; $P = 0.007$). There was a trend toward decreasing amygdala size bilaterally from control (right = 1.69, left = 1.81, $n = 10$) > seronegative subjects (right = 1.46, left = 1.50, $n = 33$) > seropositive subjects (right = 1.29, left = 1.32, $n = 9$) (analysis of covariance for right: $F = 3.18$; $df = 2, 48$; $P = 0.05$; left: $F = 3.04$; $df = 2, 48$; $P = 0.057$). In contrast, the left body of the caudate was larger in seropositive subjects (1.41 cm^3, $n = 9$) than control subjects (1.05 cm^3, $n = 10$) (seronegative subjects = 1.20 cm^3, $n = 34$; $F = 3.31$; $df = 2, 49$; $P = 0.04$), and the left caudate was larger in the seropositive subjects (4.37 cm^3, $n = 9$) and seronegative subjects (4.36 cm^3, $n = 34$) than in control subjects (3.8 cm^3, $n = 10$; $F = 4.2$; $df = 2, 49$; $P = 0.02$).

4. Discussion

The study of a possible BDV-like agent in humans is in its infancy. There is at present no consensus on a serological method; however, the majority of studies have used indirect immunofluorescence assays (IFA). The use of IFA has the advantages of being relatively rapid and inexpensive compared with Western blot or immunoprecipitation techniques. The reading of IFA slides requires training and is inherently subjective. In the case of BDV serology in humans, IFA has poor reliability in low-titer samples (Bechter et al., 1987). The combination of single and double IFA with the initial single IFA optimized for sensitivity and the confirmatory double antibody IFA optimized for specificity should, in theory, improve the sensitivity while maintaining specificity. Even with combined IFA methods, however, our control rates seemed excessively high. This provided the impetus to develop the Western blot assay reported here.

The specific Western blot method described in this study adds to the serological armamentarium by providing a method of detecting human antibodies to all three BDV proteins. In contrast to the method described by Fu et al. (1993), the method used in this study allows detection of the 14.5 kDa BDV protein. The odds-ratio results in Table 3 indicate approximately a 10-fold greater likelihood of restrictive criterion Western blot seropositivity in the presence of antibodies to the 14.5 kDa BDV protein than with antibodies to either the 38/40 kDa or 24 kDa proteins. This emphasizes the importance of detecting antibodies to the 14.5 kDa protein.

The Western blot technique has the advantage of providing data on the pattern of recognition of specific BDV proteins by human sera. The observation that defining seropositivity by recognition of more than one BDV-specific protein (restrictive criterion) in Western blot excluded the control subjects and included 14.4% of patients with schizophrenia suggests that refinement of the serology may be clinically meaningful. Fu et al. (1993) reported an association between one of the four possible anti-BDV antibody patterns detected in the present study and psychiatric patient status. They found the 38/40 kDa–24 kDa Western blot banding pattern in a higher rate in affective disorder patients (9/138 or 6.5%) than in control subjects (1/117 or 0.85%). Both Fu et al. (1993) and the present study found high rates of antibodies recognizing a single BDV protein in both patients and control subjects. This emphasizes that a method such as IFA that does not discriminate between recognition of one, two, or three BDV proteins may lead to false-positive results. IFA, in our experience, can vary considerably in signal intensity, and there is considerable variation across samples in level and type of nonspecific background reactions. This often necessitates multiple assays on certain problematic samples for categorization and may make Western blot, with its lower sample-to-sample variability in background, a more economical and reliable serological method than IFA.

The rate and relative risk information in Table 2 indicates that there is very little increased risk of being categorized as a patient if seropositive by IFA; however, for subjects defined as seropositive by liberal criterion Western blot, the relative risk rises to 1.9. Use of the restrictive criterion of recognition of more than one BDV-specific protein leads to a seropositivity rate of 14.4% in the patients and 0 in control subjects. We are reporting higher rates of seropositivity by IFA than have been reported by some groups (Amsterdam et al., 1985; Rott et al., 1985). This may be due to nonspecificity introduced into the assay by our attempt to initially screen with a bias toward sensitivity so as not to exclude low-titer sera that might read falsely negative (Bechter et al., 1987).

Fu et al. (1993) reported rates of 38% and 16% for the 38/40 kDa protein and 12% and 4% for the 24 kDa protein for patients and control subjects, respectively. The differences in band pattern recognition between the Fu et al. (1993) affective cohort and our schizophrenia cohort (see Table 3) may relate to antigen source or preparation. Fu et al. (1993) derived antigen from BDV-infected *rabbit* kidney cells while we used a persistently BDV-infected *human* neuroblastoma cell line. The studies used different diagnostic groups of patients as well.

The data available on the subjects also allowed a preliminary examination of the relationship

between seropositivity and clinical variables, including demographics, schizophrenia subtype diagnosis, premorbid adjustment, course of disorder, and neurological and neuroanatomical abnormalities. While not statistically significant in our sample, the pattern of seropositive subjects by race suggests that there may be an association of seropositivity with black race which is confounded with lower socioeconomic status in our sample. This emphasizes the need for carefully matched control subjects in future studies. Examination of the age-of-onset and duration-of-illness data indicates that there is unlikely to be a duration-of-illness difference between seropositive subjects and seronegative subjects, while there may be a slightly later age of onset and age of first hospitalization in seropositive subjects. Seropositive subjects appear to have a lower number of hospitalizations and also to have spent less time in the hospital than seronegative subjects. This would argue against seropositivity being secondary to some institutional exposure. There were no age-epoch differences on the Premorbid Adjustment Scale to suggest a possible age of exposure to BDV, assuming that BDV is related to psychosis. Table 4 presents the *DSM-III-R* diagnoses by serological status. There is no clustering of seropositive cases in any of the standard schizophrenia subtype diagnoses.

Future studies may clarify a neurological/neuroanatomical pattern of findings related to BDV seropositivity. The consistency of the pattern of findings on the Neurological Evaluation Scale (Table 5) suggests that BDV seropositivity may be associated with increased neurological impairment. Further studies with larger cohorts will be needed to address this question directly. The MRI data suggest a regional specificity of structural pathology and an association with seropositivity in the patients. There is a clear difference in putamen volume, with seropositive patients having larger volumes than seronegative subjects, and there is also the possibility of smaller amygdalae and amygdala-hippocampal complex in the seropositive subjects. These findings have been reported in schizophrenia in general. Hippocampal damage is likely to be intrinsic to BDV infection in animals (Carbone et al., 1991). Pathology in the basal ganglia may explain the general effect on neurological function seen with the Neurological Evaluation Scale. Future studies will need to test for an effect of BDV serology on volumetric measures while controlling for possible confounding factors such as drug treatment and duration of illness.

It has been hypothesized by Rott et al. (1985) that BDV infection may be associated with the onset of an affective disorder. Our findings of 14.4% seropositivity in schizophrenia and earlier reports (Bechter et al., 1987, 1988, 1992; Bode et al., 1988, 1993) of seropositive schizophrenic patients among broader diagnostic cohorts provide limited support for a link between schizophrenia and BDV. The range of disease manifestations of experimental Borna infection in animals suggests that there could be a range of neuropsychiatric manifestations if a similar agent is causing disease in humans. The possibility must also be considered that these are antibodies directed at other proteins and that their presence does not indicate infection with a BDV-like human agent. Several of the serological studies in humans report high anti-BDV antibody rates in patients affected by other known processes that result in organic brain pathology, such as AIDS or multiple sclerosis. Finding anti-BDV antibodies in these patients leaves open the possibility of an association between the antibodies and an autoimmune process that may or may not require a precipitating injurious process. These possibilities need to be explored in further research to determine if anti-BDV antibodies are useful markers of a viral infection or underlying processes involving brain injury and, if so, what possible role they may play in the pathogenesis of clinical syndromes.

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References

American Psychiatric Association (1987) *DSM-III-R: Diagnostic and Statistical Manual of Mental Disorders*. 3rd rev. edn. APA, Washington, D.C.

Amsterdam, J.D., Winokur, A., Dyson, W., Herzog, S., Gonzalez, F., Rott, R. and Koprowski, H. (1985) Borna disease virus: a possible etiologic factor in human affective disorders? *Arch Gen Psychiatry* 42, 1093–1096.

Bartko, J.J. and Carpenter, W.T., Jr. (1976) On the methods and theory of reliability. *J Nerv Ment Dis* 163, 307–317.

Bechter, K., Herzog, S., Fleischer, B., Schüttler, R. and Rott, R. (1987) Kernspintomographische Befunde bei psychiatrischen Patienten mit und ohne Serum-Antikörper gegen das Virus der Bornaschen Krankheit. *Nervenarzt* 58, 617–624.

Bechter, K., Herzog, S., Fleischer, B., Schüttler, R. and Rott, R. (1988) Infection mit dem Virus der bornaschen Krankheit als Urasache von psychiatrisch relevanten Erkrankungen des Menschen? In: Beckmann, H. and Laux, G. (Eds.), *Biologische Psychiatrie*. Springer-Verlag, Berlin, pp. 196–198.

Bechter, K., Herzog, S. and Schüttler, R. (1992) Possible significance of Borna disease for humans. *Neurol Psychiatry Brain Res* 1, 23–29.

Bode, L., Ferszt, R. and Czech, G. (1993) Borna disease virus infection and affective disorders in man. *Arch Virol* Suppl. 7, 159–167.

Bode, L., Riegel, S., Czech, G., Lange, W. and Ludwig, H. (1990*a*) Increased incidence of Borna disease virus infection in chronically diseased patients. (W93-006) *Abstracts, VIIIth International Congress of Virology*, Berlin, Germany, p. 138.

Bode, L., Riegel, S., Lange, W. and Ludwig, H. (1992) Human infections with Borna disease virus: Seroprevalence in patients with chronic diseases and healthy individuals. *J Med Virol* 36, 309–315.

Bode, L., Riegel, S., Ludwig, H., Amsterdam, J.D., Lange, W. and Koprowski, H. (1988) Borna disease virus-specific antibodies in patients with HIV infection and with mental disorders. *Lancet* II, 689.

Bode, L., Riegel, S., Reckwald, P. and Ludwig, H. (1990*b*) Improved and rapid serodiagnosis of Borna disease virus infections in animals and man. (P31-064) In: *Abstracts, VIIIth International Congress of Virology*, Berlin, Germany, p. 302.

Bryant, N.L., Buchanan, R.W. and Breier, A. (1993) Superior temporal gyrus-limbic neurocircuits and positive symptoms in schizophrenia. Presented at the Annual Meeting of the American College of Neuropsychopharmacology, Honolulu, HI.

Buchanan, R.W., Breier, A., Kirkpatrick, B., Elkashef, A., Munson, R.C., Gellad, F. and Carpenter, W.T., Jr. (1993) Structural abnormalities in deficit and nondeficit schizophrenia. *Am J Psychiatry* 150, 59–65.

Buchanan, R.W. and Heinrichs, D.W. (1989) The Neurological Evaluation Scale (NES): a structured instrument for the assessment of neurological signs in schizophrenia. *Psychiatr Res* 27, 335–350.

Cannon-Spoor, H.E., Potkin, S.G. and Wyatt, R.J. (1982) Measurement of premorbid adjustment in chronic schizophrenia. *Schizophr Bull* 8, 470–484.

Carbone, K.M., Park, S.W., Rubin, S.A., Waltrip, R.W. and Vogelsang, G.B. (1991) Borna disease: association with a maturation defect in the cellular immune response. *J Virol* 65, 6154–6164.

Carbone, K.M., Rubin, S.A., Sierra-Honigmann, A.M. and Lederman, H.M. (1993) Characterization of a glial cell line persistently infected with Borna disease virus (BDV): influence of neurotrophic factors on BDV protein and RNA expression. *J Virol* 67, 1453–60.

Elkashef, A.M., Buchanan, R.W., Gellad, F., Munson, R.C. and Breier, A. (1994) Basal ganglia pathology in schizophrenia and tardive dyskinesia: an MRI quantitative study. *Am J Psychiatry* 151, 752–755.

Fu, F.F., Amsterdam, J.D., Kao, M., Shankar, V., Koprowski, H. and Dietzschold, B. (1993) Detection of Borna disease virus-reactive antibodies from patients with affective disorders by Western immunoblot technique. *J Affect Disord* 27, 61–68.

Gosztonyi, G. and Ludwig, H. (1984) Neurotransmitter receptors and viral neurotropism. *Neuropsychiatr Clin* 3, 107–114.

Gur, R.E. and Pearlson, G.D. (1993) Neuroimaging in schizophrenia research. *Schizophr Bull* 19, 337–353.

Herzog, S. and Rott, R. (1980) Replication of Borna disease virus in cell cultures. *Med Microbiol Immunol* 168, 153–158.

Kao, M., Hamir, A.N., Rupprecht, C.E., Fu, Z.F., Shankar, V., Koprowski, H. and Kietzschold, B. (1993) Detection of antibodies against Borna disease virus in sera and cerebrospinal fluid of horses in the USA. *Vet Rec* 132, 241–244.

Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 224, 680–685.

Loats, H.L. and Holcomb, H.H. (1989) Image analysis. In: Anderson, J.H. (Ed.), *Innovations in Diagnostic Radiology: Medical Radiology Series*. Springer-Verlag, Berlin.

Ludwig, H., Bode, L. and Gosztonyi, G. (1988) Borna disease: A persistent virus infection of the central nervous system. *Prog Med Virol* 35, 107–151.

Mayr, A. (1972) Borna virus: a new model for slow virus research. *Ann Inst Pasteur* 123, 545–552.

Mayr, A. and Danner, K. (1974) Persistent infections caused by Borna virus. *Infection* 2, 64–69.

Narayan, O., Herzog, S., Frese, K., Scheefers, H. and Rott, R.

(1983*a*) Behavioral disease in rats caused by immunopathological responses to persistent Borna virus in the brain. *Science* 220, 1401–1403.

Narayan, O., Herzog, S., Frese, K., Scheefers, H. and Rott, R. (1983*b*) Pathogenesis of Borna disease in rats: immune-mediated ophthalmoencephalopathy causing blindness and behavioral abnormalities. *J Infect Dis* 148, 305–315.

Ross, R.A.; Spengler, B.A.; and Biedler, J.I. (1983) Coordinate morphological and biochemical interconversion of human neuroblastoma cells. *J Natl Cancer Inst* 71, 740–747.

Rott, R., Herzog, S., Fleischer, B., Winokur, A., Amsterdam, J., Dyson, W. and Koprowski, H. (1985) Detection of serum antibodies to Borna disease virus in patients with psychiatric disorders. *Science* 228, 755–756.

Schädler, R., Diringer, H. and Ludwig, H. (1985) Isolation and characterization of a 14 500 molecular weight protein from brain and tissue cultures persistently infected with Borna disease virus. *J Gen Virol* 66, 2479–2484.

Spitzer, R.L., Williams, J.B.W., Gibbon, M. and First, M.D. (1989) *Structured Clinical Interview for* DSM-III-R *Patient Version (SCID-P)*. New York State Psychiatric Institute, New York.

Sprankel, H., Richarz, K., Ludwig, H. and Rott, R. (1978) Behavior alterations in tree shrews (Tupaia glis, Diard 1820) induced by Borna disease virus. *Med Microbiol Immunol* 165, 1–18.