Borna disease virus infection in psychiatric patients: are we on the right track?

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Animals infected with Borna disease virus (BDV) typically present with neurological dysfunction including behavioural abnormalities. Sero-epidemiological surveys suggested that BDV infection can occur in human beings and is associated with mental disorders. Partly contradictory results from studies employing RT-PCR and serological screening led to debate over whether BDV can infect people at all. Critical evaluation of available data led to doubts about the diagnostic value of RT-PCR-based test results. A more consistent picture has emerged from serological studies because seropositive cases were found more frequently among psychiatric patients than among normal controls, supporting the notion that BDV might indeed be responsible for some psychiatric disorders. This view is now challenged by the observation that human BDV-reactive antibodies are of low avidity and might therefore represent cross-reacting antibodies. It remains to be shown whether these antibodies are indeed induced by BDV or by related antigens of unknown identity.

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Epidemiological evidence suggests that infectious agents may have a role in the etiology of neuropsychiatric disorders such as schizophrenia.[1] Among the long-suspected candidates are viruses such as influenza virus, herpes simplex virus, retroviruses, cytomegalovirus, and Borna disease virus (BDV).[2] This virus is the causative agent of Borna disease, an infectious and often fatal neurological disease that occurs sporadically in horses and sheep in central Europe,[3] and has been known since the 18th century.[4] Mode of transmission is unknown, but is assumed to be through salival, nasal, or conjunctival secretions.[5] Rodents are suspected carriers of the virus but the true host reservoir of BDV has not been identified.[3,5]

The complete nucleotide sequence of the virus was determined 7 years ago.[6,7] BDV is an enveloped virus with a nonsegmented negative-strand RNA viral genome (figure 1). Natural or experimental infection of a broad variety of warm-blooded animals leads, in most cases, to a lifelong persistence of this neurotropic virus in the central nervous system (CNS).[2] Within the CNS, BDV preferentially infects

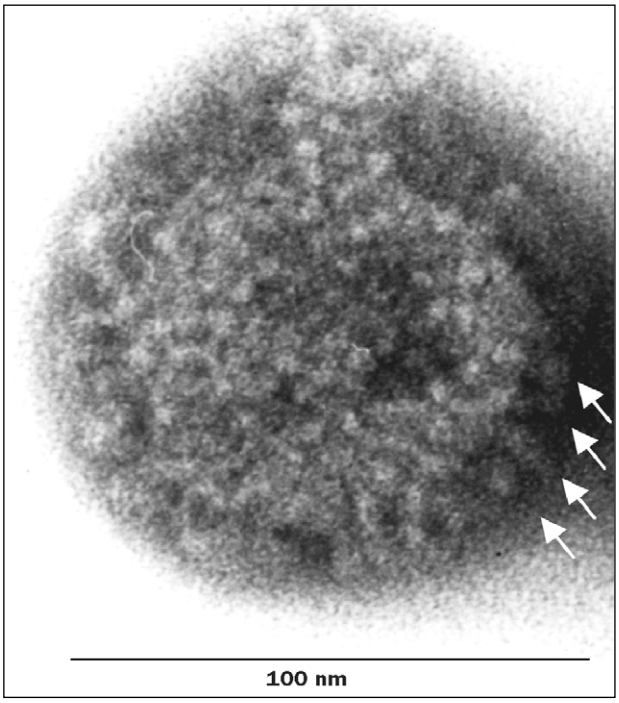

Figure 1. Electron micrograph of purified BDV particle visualised by negative-contrast staining. Arrows indicate glycoprotein spikes (courtesy of M Eickmann, University of Marburg, Germany).

neurons of the limbic system. This system is involved in behaviour, memory, and emotions and seems to have a critical role in the aetiopathology of several human psychiatric disorders.[2,8] In fact, a range of abnormalities is observed in some of the animal models after BDV infection.[2,8] Adult infected rats show an immune-mediated biphasic behavioural disease progression with an initial acute phase of aggression and hyperactivity followed by a chronic phase of apathy.[9,10] In tree shrews (*Tupaia glis*) BDV infection is often associated with a disruption in social interactions.[11]

Based on the similarity between BDV-induced behavioural abnormalities in animal models and mental

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disorders of human beings, large numbers of psychiatric patients have been screened by serological methods for the presence of BDV over the past 16 year[9–32] since this was first done by Rott and colleagues.[31,32] In these studies, BDV-reactive antibodies were found exclusively in a small percentage of psychiatric patients, suggesting a possible involvement of this virus in human psychiatric disturbances. Subsequent studies used both serological and non-serological methods to confirm or refute these observations. Despite enormous efforts from many laboratories it remains unclear whether BDV infection is associated with psychiatric diseases.

Non-serological evidence for human infection with BDV

The promising observation that the otherwise neurotropic BDV can be detected in the blood of experimentally infected newborn rats[12] stimulated various groups to screen peripheral blood samples of psychiatric patients. Bode et al (1995) were the first to identify viral antigen and nucleic acid in the blood of four psychiatric patients by highly sensitive RT-PCR.[13] Encouraged by this finding, several laboratories investigated blood samples of patients from Europe, USA, Japan, and other countries with psychiatric disorders including schizophrenia and mood disorders (reviewed in references 14 and 15). The results obtained in more than 20 studies are controversial: the observed prevalence rate of viral nucleic acid detected in the blood of patients and normal controls varied between 0–66% and 0–57%, respectively. Five independent studies found no evidence for BDV in blood at all.[16–20] Reasons for the discrepancies between individual studies are unclear.[3,14] Although differences in protocols are reported, the sensitivity of the individual RT-PCR and the initial amount of blood investigated are comparable between at least some of these studies.[14] Several studies that failed to detect BDV RNA included in their screenings blood samples from patients from known BDV endemic regions. This finding suggests that the observed discrepancies are not simply due to differences in patients' geographical origins.[14]

It was also argued that the blood levels of BDV may fluctuate and that threshold levels of BDV RNA are reached only at undefined time points.[21] These levels may be below the detection limit of RT-PCR. However, even in naturally infected horses it remains to be shown whether there is a period of viraemia during the course of infection in which BDV can be isolated from blood. If a BDV-related viraemia is linked to acute psychiatric disorders, the virus should readily be detected in acutely ill patients. This seems not to be the case in general as one study detected no viral RNA in blood samples of such patients.[16]

An alternative explanation for the high frequency of BDV in some positive cases could involve accidental contamination of blood samples in the laboratory. Comparison of the RT-PCR-derived sequences from psychiatric patients with commonly used laboratory strains revealed complete identity in some cases, arguing for a contamination of the human blood samples.[3,22] However, such comparisons can be misleading due to the high degree of sequence conservation between laboratory strains and BDV isolates of naturally infected animals. Furthermore, contamination problems can only partly explain the controversial findings between the various laboratories, since RT-PCR-positive samples were found more frequently in patients than in control groups. A BDV subtype named No/98 has been identified that exhibits a significant degree of genetic divergence with respect to all other known BDV strains.[33] Intriguingly, viral RNA of this new horse-derived isolate could not be amplified with primers commonly used in many laboratories for the screening of human samples. Therefore, it seems possible that human BDV-like viruses escaped detection by this technique.

Since BDV replicates preferentially in the limbic system some studies were initiated to identify viral nucleic acid mainly by RT-PCR in brain tissues from psychiatric patients.[23–28] Similar to the conflicting results mentioned above, the analysis of the brain samples from individuals with a history of mental disorders including schizophrenia, bipolar depression, and atypical hippocampal degeneration revealed a variation in prevalence rate of 0–53%. Interestingly, the rate of BDV-positive cases in healthy controls varied only between 0 and 6·5%. Reasons for the differences in frequency of BDV-positive cases between the various studies are not understood. They cannot simply reflect differences of RT-PCR sensitivity or the numbers of individual brain tissues investigated, since one study found no viral nucleic acid among 86 cases with various forms of psychiatric disorders by a highly sensitive RT-PCR analysis.[24] However, long postmortem intervals, which eventually led to the degradation of BDV RNA in human brain tissues, may have contributed to the negative findings. Some intrinsic problems may further complicate the detection of BDV nucleic acid in brain tissues. The appropriate brain region would have to be sampled to detect a focal infection. In addition, detection of BDV nucleic acid would be difficult to achieve if the infection occurred very early in life, but the brain samples were taken many years later after the virus was eliminated.

Regardless of all the conflicting results and potential problems, two laboratories independently confirmed the presence of BDV RNA in brain tissues of three psychiatric patients with atypical hippocampus sclerosis and clinical histories of neuropsychiatric symptoms.[24,25] Thus, the suspicion that BDV can infect people was apparently proven to be true. However, it is still puzzling that the BDV-positive patients who lived in the USA harboured the same BDV strain that killed a German horse 20 years ago.[14]

A few groups have succeeded in isolating BDV from blood or brain tissue by either a complicated cell culture procedure or infection of newborn gerbils.[28–30] The significance of these findings is however, clouded by the observation that the genome sequences of the human isolates were almost identical with the laboratory strains used for experiments in the laboratories where isolation took place.[3,22] Thus, accidental contamination cannot formally be excluded.

Seroprevalence of BDV in human beings

Test assay*	Origin of donor	Donor description	Number of patients	Positives (%)	References
IF	Germany	Various psychiatric disturbances	694	0·6	32
		Healthy volunteers	95	0	
IF	USA	Major depressive disorders	265		31
		Healthy volunteers	105		
				4·5	
				0	
IF	Germany	Various psychiatric disturbances	1003	6·8	34
		Surgery patients	133	3·5	
IF	Germany	HIV infected	460	7·8	36
		HIV antibody negative	125	1·6	
		HIV negative drug abusers	106	3·8	
IF	USA/ Germany/ Japan	Psychiatric/neurological patients	5000	4–7	35
		Control hospital patients	1000	1	
IF	East Africa	Schistosomiasis and malaria	193	9·8	37
IF	Germany	Psychiatric patients	2377	5·9	38
		Surgery patients	569	3·5	
IF/IP	Europe	Symptomless HIV infection	1024	7·1	37
		HIV infected	244	13·99	
		HIV-negative blood donors	118	2·5	
IF/IP	USA	Major depression	550	2·2	37
		Surgery patients	365	2·2	
IF	Germany	Acute psychiatric patients (follow up study)	71	19·7	39
WB	USA	Major depression	138	6·5	40
		Healthy controls/non-psychiatric patients	117	0·85	
ELISA/WB	Japan	Blood donors	100	1	41
WB	USA	Schizophrenic outpatients	90	14·4	42
		Normal controls	20	0	
WB	Japan	Psychiatric patients	60	30	43
WB	Germany	Various psychiatric disorders	416	9·6	44
		Healthy volunteers	203	1·4	
ELISA	Thailand	Symptomless HIV infection	60	15	45
		Patient with AIDS	67	17·9	
		HIV-negative blood donor	103	2·5	
IF	Germany	Multiple sclerosis	50	0	46

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In summary, RT-PCR-based detection of BDV nucleic acid in blood samples is highly controversial and not suitable as a screening method for human BDV infection. Similarly, virus isolation seems to be very inefficient and error-prone due to technical difficulties and the risk of contamination.

Serological evidence for human infection with BDV

Presence of BDV-reactive antibodies in the sera of psychiatric patients was first documented by a conventional indirect immunofluorescence assay (IFA), where cells infected with virus are used in the screen.[31,32] This test seems to be most suitable because viral replication occurs only in the nucleus of infected cells and nonspecific reactions of human sera with components of the cytoplasm can be ignored. The presence of BDV-reactive antibodies is simple to diagnose due to a typical punctate immunostaining of what are most likely aggregated viral ribonucleoprotein complexes in the nucleoplasm (figure 2). In initial studies,[31,32,34,35] BDV-reactive antibodies were found in a small but significant fraction of psychiatric patients with a variation in prevalence rate of 0·6% to 7%, and 0 to 3·5% in normal controls (table). Similar to the situation in naturally infected horses, antibody titres were frequently below 1/40 and thus, depending on the BDV-infected cell line used, difficult to detect by IFA.[32,59]

Subsequent studies[19,20,34–58] that used serological techniques such as western blot, ELISA, and ECLIA confirmed only to a certain extent the IFA-based findings (table). Interestingly, ECLIA and western blot analysis with recombinant forms of the two major viral proteins—the nucleoprotein (N) and phosphoprotein (P)—revealed that the reactivity of human antibodies is often directed against only one of these proteins.[42,53,58] It is presently unclear why BDV should induce, in the most cases, antibodies to only one or the other protein in humans, whereas in naturally infected horses reactivity to both proteins is normally observed. It is tempting to speculate that this could be related to preferential recognition of conformational epitopes on the N or P protein. Due to the high frequency with which sera from normal controls reacts with only one BDV protein, Waltrip et al[42] introduced a restrictive criterion by defining seropositivity as recognition of more than one BDV protein by western

Test assay*	Origin of donor	Donor description	Number of patients	Positives (%)	References
ELISA	Japan	Blood donors near horse farms	428	2·6–14·8	47
		Blood donors	100	1	
RS-ELISA	Japan	Chronic schizophrenia	70	0	48
		Healthy volunteers	40	0	
IF	Japan	Psychiatric disorders	346	0·9	49
		Healthy controls	70	0	
WB	Japan	Schizophrenic patients	67	45	50
		Medical staff	26	0	
IF	Germany	Major depression	65	3·1	51
		Bipolar disorders	8	0	
		Schizophrenic patients	27	0	
		Other psychiatric patients	28	0	
		Multiple sclerosis patients	19	0	
		Patient with cerebrospinal diseases	14	7·14	
		Other neurological diseases	69	0	
WB	Taiwan	Schizophrenic patients	314	12·1	52
		Symptom-free family members of schizophrenic patients	132	12·1	
		Blood donors, hospital patients	274	2·9	
		Mental health worker	82	9·8	
ECLIA	Japan	Schizophrenic patients	845	3·08	53
		Mood disorders	251	3·59	
		Various psychiatric patients	366	0·55	
		Various ocular diseases	1393	1·36	
		Multitransfused patients	66	4·55	
		Blood donors	917	1·09	
ELISA/WB	Sweden	Chronic fatigue syndrome	169	0	20
		Healthy controls	62	0	
IF	Switzerland	Patient with HIV	16	12·5	19
		Patient with AIDS dementia complex	25	8	
IF	Germany	Psychiatric patients	27	14·8	54
		Healthy controls	13	0	
IF	Surinam	Schizophrenic patients	29	3	55
		Healthy volunteers	26	6	
WB	Japan	Psychiatric patients	89	0	56
		Healthy volunteers	210	0	
ECLIA	Poland	Various psychiatric diseases	816	2	57
IF/ECLIA/WB	Japan	Patient with mood disorder	45	4	58
		Schizophrenic patients	45	9	

*Assays used to determine BDV prevalence: ECLIA=electrochemiluminescence immunoassay; IF=indirect immunofluorescence assay; IP=immunoprecipitation; RS-ELISA=reverse-type sandwich ELISA; RT-PCR=reverse transcriptase-PCR, WB=western blot.

blot analysis. Based on this restriction, 14·4% of patients with schizophrenia were BDV seropositive compared with 0% of normal controls.

Several studies employing western blot or ELISA as detection methods have completely failed to demonstrate BDV-reactive antibodies in sera of psychiatric patients with various disorders.[20,48] This finding could be related to low sensitivity of the serological assays or, alternatively, to stringent procedures that reduces the frequency of false positives. Comparison of serological methods in a German multicentre study indeed revealed great differences between assays in their capacity to identify BDV-reactive human sera.[60] This finding suggests that validation of a suitable screening test is urgently needed.

Although there is no "gold standard", BDV-reactive antibodies have been detected more frequently in psychiatric patients (0·6–45%) than in normal controls (0–4%), suggesting an association between psychiatric disorders and BDV-infection. Epidemiological studies with IFA or ELISA as screening methods further imply that production of antibodies to BDV also occurs in individuals with an impaired immune system such as HIV patients (table).

Is BDV serology misleading?

By contrast with the controversial debate on whether BDV RNA can be detected in blood or brain tissues, it seemed possible that improvements and validation of serologically based assays might result in a reliable diagnostic tool. However, the recent observation[59] that BDV antibodies bind their antigens with low avidity challenges this view and questions the diagnostic value of serological tests.

Low-avidity IgG antibodies appear very early during the course of many primary viral infections.[61] The maturation of low-avidity IgG towards high-affinity antibodies occurs subsequently during the selection process of B cells. The differences in strength with which low and high affinity antibodies recognise their antigens are often used as a diagnostic tool to differentiate between primary infection, re-infection, or reactivation.[61] Evaluation of antibody avidity is simple and can be carried out with most serological tests including IFA by a brief incubation with denaturing agents such as urea after antigen-binding. Low-avidity antibodies dissociate from their antigen after this procedure, whereas high-avidity antibodies remain bound to the antigen.

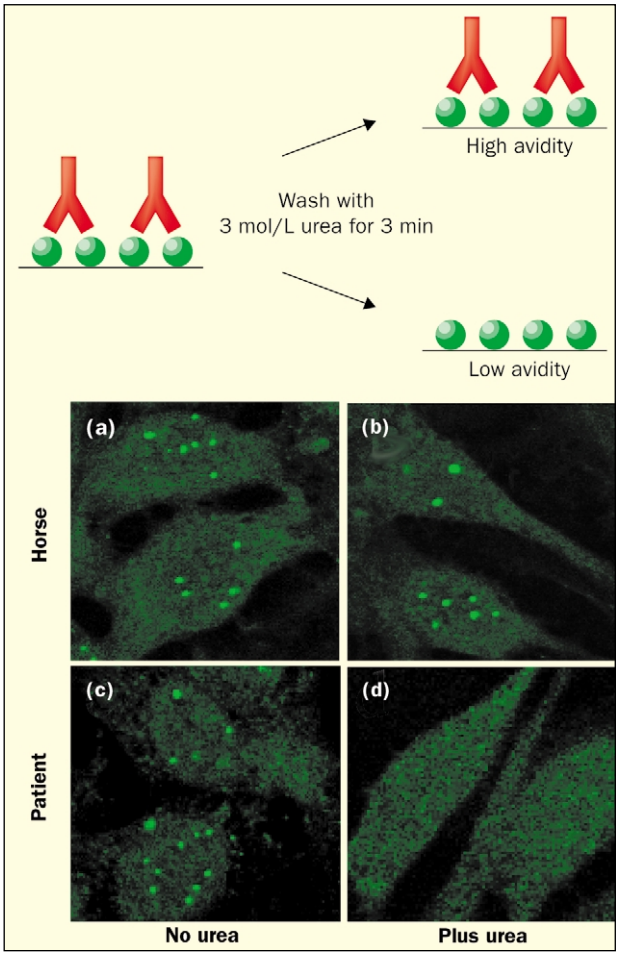

Figure 2. Determination of the avidity of BDV-reactive antibodies by indirect IFA. Top: to test avidity of BDV-reactive antibodies, a short washing step with 3 mol/L urea is introduced in the IFA procedure after antibodies have bound to antigens. High-avidity antibodies remain bound to BDV antigen, whereas low-avidity antibodies are washed away and are no longer visible in the IFA. Bottom: in IFA, antibodies from horses with documented Borna disease give a typical punctuate staining in the nucleus of BDV-infected C6 cells (a). These antibodies show high avidity since treatment with urea has no impact on the binding-efficacy (b). BDV reactive antibodies from patients with mental disorders frequently recognise BDV antigens in the nuclei of infected cells (c). However, the avidity seems to be very low since treatment with urea completely abolishes the specific signals in the IFA (d).

Measuring the avidity of 25 serum samples from psychiatric patients by the IFA, Allmang et al[59] observed in all cases a more than fivefold reduction in immunofluorescence signal intensities after treatment with urea (figure 2), indicating that the BDV-reactive antibodies are of the low-avidity phenotype. On the other hand, binding of BDV-specific antibodies from naturally infected horses (figure 2) or from experimentally infected monkeys was unaffected. One interpretation of these results is that all human samples investigated in this study were, by chance, obtained from patients with primary BDV infections. However, longitudinal studies with serum samples from two patients obtained at intervals from 1 to 4 years revealed, at all time points, a similarly high sensitivity to urea despite raised titres of BDV-reactive antibodies, suggesting that there is no maturation towards high-affinity antibodies. The simplest interpretation of these results is that reactive antibodies were not induced by BDV itself and are of a cross-reactive nature, especially since such antibodies are known to exhibit a low-avidity phenotype. The existence of cross-reacting antibodies may also explain in part the difficulty in developing a reliable serological test for human BDV-specific antibodies. Alternatively, BDV infection may generally result in a persistence of low-avidity IgGs only, by an unknown mechanism. It is tempting to speculate that threshold levels of viral antigen might be the cause for the impaired maturation towards high-affinity antibodies.

Due to the small number of sera investigated by Allmang et al,[59] it is too early to conclude that all previously BDV-positive sera will react with similar sensitivity to urea. In this respect, it will be interesting to analyse the avidity of those sera that recognise two or three viral proteins on western blots. Because there seems to be confirmed cases of human BDV infections by non-serological methods,[24] it is likely that we may also find BDV-specific antibodies with high avidity. However, the frequency of such serologically "true" cases will certainly be lower and such cases may no longer be linked to psychiatric diseases.

In conclusion, the low avidity of BDV-reactive antibodies questions the diagnostic value of serological assays. Incorporation of the avidity test into serological surveys is likely to reveal lower prevalence rates of BDV infection in humans. Independent of the avidity issue, there remains the seroepidemiological evidence that BDV-reactive antibodies are found more often in psychiatric patients than in normal controls. Therefore, identification of the agent responsible for the induction of these antibodies may finally lead to the discovery of a new pathogen or immunogen important in the development of psychiatric diseases. Since all BDV strains show extremely high conservation in their primary protein sequences,[3] it is tempting to speculate that this agent represents a new human BDV variant with only remote similarity to known animal-derived strains.

Search strategy and selection criteria

I have followed publications on BDV for more than a decade by computer-assisted search strategies based on keywords of Borna disease virus, psychiatric disorders, and antibody avidity, as well as the names of scientists who are colleagues in the study of this virus. Scrutiny of publications on BDV and psychiatric disorders includes papers in English and German. Data for this review were identified by searches of Medline.

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