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Borna disease virus — does it infect humans and cause psychiatric disorders?

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Abstract

Antibodies recognizing Borna disease virus (BDV) antigens were first demonstrated in the blood of psychiatric patients ≈15 years ago. Since that time, a highly controversial debate arose whether BDV infects humans and whether it causes psychiatric disorders. In this review, we critically discuss the results of numerous studies that assessed this possibility by using virological and serological methods. We conclude that there is presently no strong experimental evidence supporting the notion that BDV is a human pathogen. The possibility remains, however, that an antigenically related agent is associated with human psychiatric disorders.

Keywords: Borna disease virus; Antibodies; RT-PCR; Psychiatric disorders

1. Introduction

Borna disease virus (BDV) is the causative agent of Borna disease, an often fatal meningoencephalitis originally described in horses in Germany (Ludwig et al., 1985). It is named after the town Borna in Saxony/Germany, where an epidemic outbreak of Borna disease occurred in cavalry horses ≈100 years ago. Sheep and other farm animals, e.g. donkeys and cattle, are also natural hosts of BDV (Rott and Becht, 1995; Staeheli et al., 2000). The molecular structure of BDV has been investigated in detail. It is an enveloped virus, with a negative-stranded non-segmented RNA genome of ≈8.9 kb. It replicates and transcribes its genome in the nucleus and uses the cellular RNA splicing machinery to regulate viral gene expression. Mainly because of these features, BDV has been classified as a prototype of a new virus family, Bornaviridae, within the non-segmented negative-stranded RNA viruses of the order Mononegavirales (Gonzalez-Dunia et al., 1997; Schwemmle et al., 1999a).

Several properties of BDV render it an attractive possible agent of human psychiatric disorders: (i) it is neurotropic and infects mainly neurons; (ii) it shows a broad host range; (iii) it has high affinity for the limbic circuitry which is

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involved in the regulation of behavior, memory and emotions and which seems to play a critical role in the etiopathology of several human psychiatric disorders; and (iv) experimental infection of animals with BDV may result in symptoms, such as aggression and hyperactivity, apathy or motor symptoms which resemble, at least in part, core features of human psychiatric disorders, such as depression or schizophrenia (for review see Briese et al., 1999). After first reports showed enhanced antibody titres to BDV or a related agent in sera of psychiatric patients (Amsterdam et al., 1985; Rott et al., 1985), a highly controversial debate arose whether BDV might infect humans and cause human neuropsychiatric disorders (Lieb et al., 1998). In this article, we will critically discuss the results of recent studies that investigated a possible association of BDV with human disease.

2. Serological evidence for infection of humans with BDV

Three different serological methods have been used to detect antibodies to BDV antigens in human sera: indirect immunofluorescence (IFA), Western blot and ELISA. The main advantage of serological methods for diagnostic work is that as a result of immunological memory, serum antibodies allow the detection of persisting as well as resolved viral infections. Unfortunately, for the diagnosis of BDV infections in animals or humans, serological methods are not optimal because BDV-specific antibodies are usually not abundant. For example, IFA titers are frequently below 1:40 in naturally infected animals with full-blown Borna disease (Herzog et al., 1994). Fig. 1(A) shows the typical punctate immunostaining in nuclei of BDV-infected cells of a reactive human serum when analyzed by IFA. This staining pattern is indistinguishable from that seen with sera of horses with autopsy-confirmed Borna disease (Fig. 1C).

In 1985, a first serological study by IFA on BDV-infected cells showed that sera from a significant proportion of psychiatric patients contained immunoglobulins with specificity for BDV antigens (Amsterdam et al., 1985; Rott et al., 1985). Since such antibodies were found much less frequently in sera of healthy controls, it was concluded that BDV infection might be associated with human psychiatric disorders. Using this and other serological methods, other researchers came to the same conclusion (Bode et al., 1988, 1992; Bechter et al., 1992; Fu et al., 1993; Waltrip et al., 1995; Auwanit et al., 1996; Sauder et al., 1996; Takahashi et al., 1997; Gonzalez-Dunia et al., 1997; Iwahashi et al., 1997; Chen et al., 1999b). Overall, BDV-reactive antibodies were detected in $\approx 10\%$ of psychiatric patients and 3% of normal controls. Although these findings suggested an association between psychiatric disorders and BDV infection, several methodological problems question this view. First, when identical samples of human sera were analyzed in blinded studies, the results frequently differed between laboratories (Nübling et al., 1999). A much more consistent picture emerged when the analysis was performed with sera of animals with confirmed

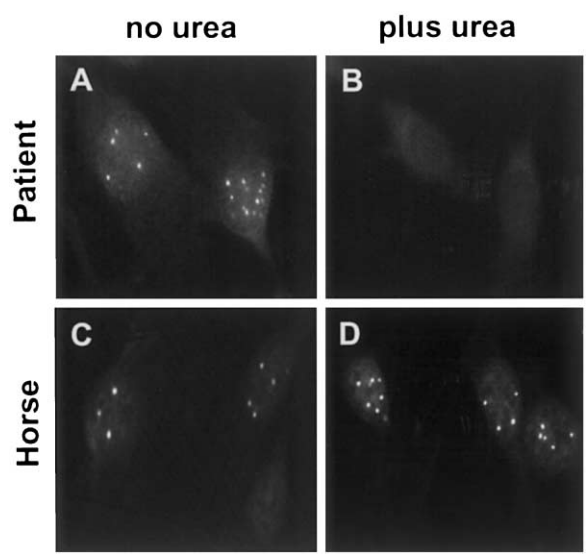

Fig. 1. Visualization by IFA of antibodies that react with BDV antigens. Note the strong staining of distinct punctate structures in the nucleus of BDV-infected C6 rat glioblastoma cells by reactive sera of a psychiatric patient (A) and by serum of a horse with Borna disease (C). The reactivity of the human serum (B) but not of the horse serum (D) was lost if the samples were subjected to washing buffer containing 3 M urea, indicating that target recognition of reactive human antibodies occurred with low avidity.

Borna disease. Second, Western blot analyses using recombinant BDV proteins demonstrated that reactive human sera usually recognized only one of the two major BDV antigens, e.g. p24 or p40 (Fu et al., 1993; Sauder et al., 1996; Iwahashi et al., 1997; Chen et al., 1999b). It is difficult to understand why BDV should frequently induce antibodies to both major viral antigens in animals, but only to one or the other in humans. Third, recent IFA studies from our laboratory indicated that the avidity of human IgG towards BDV antigens is surprisingly low (Allmang et al., 2001). Human antibodies could easily be removed from BDV antigens with washing buffer containing 3 M urea (Fig. 1B), whereas antibodies from horses with Borna disease could not (Fig. 1D). The reactive human serum antibodies persisted for many years without gaining high avidity for BDV antigens, indicating that they were probably not induced by BDV, but rather by infection with an antigenically related microorganism of unknown identity or by exposure to a related cellular immunogen which is up-regulated during psychiatric disorders. Thus, the diagnostic significance of human BDV serology remains questionable to date.

3. Is BDV present in samples of human peripheral blood?

The initial detection of BDV-derived nucleic acid (Bode et al., 1995) and infectious virus (Bode et al., 1996) in peripheral blood of psychiatric patients encouraged several other laboratories to investigate samples from additional patients (Table 1). The various studies analyzed samples of patients with schizophrenia, mood disorders or other psychiatric disorders, including chronic fatigue syndrome from Austria, Germany, Japan, Korea, Sweden, Taiwan and the US. The numbers of patient samples investigated in each study varied between six and 159, three publications were case reports (Planz et al., 1998, 1999; Nowotny and Kolodziejek, 2000). Most studies included normal controls for comparison of BDV status. In one report, a family study was performed (Nakaya et al., 1996). The various studies yielded very divergent results, with prevalence rates between 0 and 66% in patients and 0 and 57% in controls. Five studies found no evidence of BDV infection of human peripheral blood (Lieb et al., 1997a; Richt et al., 1997; Bachmann et al., 1999; Evengard et al., 1999; Kim et al., 1999). Divergent results were similarly obtained in recent multicenter studies performed in Germany (Nübling et al., 1999), where numbers of positive patient samples varied from 0 to 30% between participating laboratories that investigated the same blood samples in a blinded fashion.

Various explanations for these controversial results are possible. First, it might be argued that the divergent results are due to the fact that patients in the reports with negative results came from areas where Borna disease is not endemic in animals. This, however, seems unlikely because in two of these reports (Lieb et al., 1997a; Richt et al., 1997), a high proportion of patients came from endemic areas. Second, BDV in peripheral blood might be missed if brief transient viremic phases rather than viral persistence in blood are the rule. It was argued that if blood is collected at an inappropriate time after infection, the true infection status of the patient might not be revealed (Nowotny and Kolodziejek, 2000). However, if BDV viremia is related to acute psychiatric disorders, the virus should be found in acutely ill patients, which clearly was not the case (Lieb et al., 1997a; Richt et al., 1997). Third, differences in assay sensitivities might explain negative findings in some studies (Sauder and De la Torre, 1998). However, in one report with negative results (Lieb et al., 1997a), the sensitivity of the assay system was demonstrated to be very high. Fourth, it was argued that negative results might be due to insufficient volumes of blood collected (Sauder and De la Torre, 1998). Although this argument may apply for our own study (Lieb et al., 1997a), at least two other studies with negative results used 10–35 ml of blood for RNA extraction (see Table 1). A fifth possibility to explain the divergent results is accidental contamination of the samples in the laboratories. In fact, a comparison of BDV sequences from human peripheral blood supports the contamination hypothesis: viruses found in human

Table 1
Studies aimed at detecting BDV by RT-PCR in samples of human peripheral blood[a]

Viral gene analyzed	Cell type (ml blood analyzed)	Origin of blood donors	Health status	Positives	Reference
p24, p40	PBMCs, (9 ml)	Germany	Various diagnoses; healthy controls	4/6 (66.7%); 0/10 (0%)	(Bode et al., 1995)
p24	PBMCs (unknown)	Japan	Various diagnoses; controls	22/60 (36.7%); 8/172 (4.6%)	(Kishi et al., 1995a,b)
p24	PBMCs (unknown)	Japan	Schizophrenia; depression; healthy controls	5/49 (10.2%); 1/6 (16.7%); 0/36 (0%)	(Igata-Yi et al., 1996)
p24	PBMCs (unknown)	Japan	CFS	3/25 (12%)	(Nakaya et al., 1996)
p24, p40	PBMCs (15–20 ml)	Germany	Various diagnoses; healthy controls	13/26 (50%); 0/23 (0%)	(Sauder et al., 1996)
p24	Whole blood (unknown)	Japan	Schizophrenia; medical staff	6/61 (9.8%); 0/26 (0%)	(Iwahashi et al., 1997)
p24	PBMCs (7–10 ml)	Japan	Various diagnoses; healthy controls	2/106 (1.9%); 0/12 (0%)	(Kubo et al., 1997)
p24	PBMCs (unknown)	Japan	Blood donors	4/7 (57.1%)	(Takahashi et al., 1997)
p40	Whole blood (5 ml)	Germany	Various diagnoses	0/159 (0%)	(Lieb et al., 1997a)
p24, p40	PBMCs (10–20 ml)	USA, Germany	Various diagnoses; healthy controls	0/52 (0%); 0/14 (0%)	(Richt et al., 1997)
p24	PBMCs (15 ml)	Japan	Schizophrenia; mood disorder; healthy controls	2/49 (4.1%); 3/77 (3.9%); 2/84 (2.4%)	(Iwata et al., 1998)
p24, p40	Whole blood (unknown)	Germany	Schizophrenia for 8 months	1/1	(Planz et al., 1998)
p40	Granulocytes (5 ml)	Germany	Schizophrenia; mood disorder; healthy controls	1/1; 2/2; 0/3 (0%)	(Planz et al., 1999)
p24, p40	PBMCs (10 ml)	Taiwan	Schizophrenia; healthy controls; mental health workers	10/74 (13.5%); 1/69 (1.4%); 7/45 (15.5%)	(Chen et al., 1999a)
p24	PBMCs (unknown)	Korea	Various diagnoses	0/81 (0%)	(Kim et al., 1999)
p40	PBMCs (5×10^6 cells)	Switzerland	Various neurological disorders	0/27 (0%)	(Bachmann et al., 1999)
p24	PBMCs (unknown)	Japan	Two families with CFS	6/10 (60%)	(Nakaya et al., 1999)
p24, p40	PBMCs (35 ml)	Sweden	CFS	0/18 (0%)	(Evengard et al., 1999)
p24, p40, gp18	PBMCs (unknown)	Austria	CFS	1/1	(Nowotny and Kolodziejek, 2000)

[a] CFS, chronic fatigue syndrome; PBMCs, peripheral blood mononuclear cells.

samples were usually most strongly related to the BDV strains used for experiments by the reporting laboratories (Schwemmle et al., 1999b), suggesting contamination of the human specimens (for discussion see: Lancet 355, (2000) pp. 656–657/1462–1463). However, contamination problems cannot explain why all groups who reported positive results (Table 1) found that BDV-specific

RNA was present at a higher frequency in blood of patients than in controls.

4. Evidence for BDV infection of human brain tissue

As shown in Table 2, BDV antigen and/or BDV RNA was also detected in human autopsy brain samples from individuals with a history of various mental disorders (De la Torre et al., 1996; Haga et al., 1997a; Salvatore et al., 1997) and apparently normal controls (Haga et al., 1997b). These data contrasted an earlier study (Sierra-Honigmann et al., 1995) and a recent report from our laboratory (Czygan et al., 1999) in which, despite high sensitivity of the assay, no BDV RNA was detected in autopsy brain samples from different brain regions of patients with various psychiatric disorders and normal controls. Since the amount of RNA decreases with longer post mortem intervals, divergent prevalence findings of BDV RNA in autopsy brain samples might be explained by different post mortem intervals. Although nothing is known about the stability of BDV RNA in human post mortem brain tissue, it might be argued that the fairly short post mortem intervals of some BDV-positive brains (4–12 h) compared to the mostly longer post mortem intervals in the other brains (3–168 h) could account for negative findings.

Using non-nested RT-PCR, we were able to confirm (Czygan et al., 1999) the presence of both BDV p40 and p24 transcripts in the brains of three psychiatric patients with neuropathologically verified atypical hippocampus sclerosis and clinical histories of neuropsychiatric symptoms, including depression, memory loss and/or hallucinations, but no evidence of Alzheimer's disease (for a discussion see Collier, 2000). We further detected viral RNA corresponding to the first intergenic region of the BDV genome (Czygan et al., 1999). In these brains, BDV had previously been shown to be present by means of nested RT-PCR, in situ hybridization and immunohistochemistry (De la Torre et al., 1996). To our surprise, the p24 and p40 genes, as well as the first intergenic region of these viruses, were almost identical to the corresponding regions of laboratory strain He/80 (Czygan et al., 1999). Since large amounts of viral nucleic acid were present in the patient's brains that could only be detected if the samples were subjected to reverse transcription before PCR amplification, we argued that accidental contamination of the tissue samples had probably not occurred. It remains an unan-

Table 2
Studies investigating BDV antigen and/or BDV RNA in human autopsy brain samples of psychiatric patients and controls

Viral gene analyzed	Origin of subjects	Health status	Positives	References
p40	USA	Schizophrenia; healthy controls	0/3 (0%); 0/3 (0%)	(Sierra-Honigmann et al., 1995)
p40	USA	Atypical hippocampal degeneration	4/5 (80%)	(De la Torre et al., 1996)
p24	USA, Europe	Schizophrenia; bipolar disorder; depression; various diagnoses; healthy controls	9/17 (52.9%); 2/5 (40%); 0/6 (0%); 0/37 (0%); 0/10 (0%)	(Salvatore et al., 1997)
p24	Japan	Schizophrenia; Parkinson's disease; healthy controls	3/9 (33.3%); 1/6 (16.7%); 2/31 (6.5%)	(Haga et al., 1997a,b)
p24, p40	Germany	Atypical hippocampal degeneration[a]; various diagnoses; healthy controls	3/4 (75%); 0/86 (0%); 0/52 (0%)	(Czygan et al., 1999)
p24, p40	Japan	Schizophrenia; healthy controls	1/4 (25%); 0/2 (0%)	(Nakamura et al., 2000)

[a] Samples were from brains that were analyzed previously (De la Torre et al., 1996).

swered question why these patients, who lived in the US, contained virtually the same strain of BDV that killed a horse in Germany more than 20 years ago.

A most recent report described the presence of BDV in the brain of a schizophrenic patient from Japan (Nakamura et al., 2000). Viral RNA was detected by RT-PCR and in-situ hybridization in scattered neurons in three of 12 brain regions that were investigated (hippocampus, pons and cerebellum). Microscopic analysis of the hippocampus revealed subtle neuropathological changes including mild lymphocyte infiltration that was not detected in BDV-negative brains. Several neurons in the hippocampus formation were stained by a polyclonal serum from a BDV-infected mouse. BDV was recovered by intracerebral injection of extracts of this human brain into newborn gerbils. The nucleotide sequences of p24 and p40 gene fragments of this virus isolate (designated BD-VHuP2br) differed by $\approx 2\%$ from standard BDV laboratory strains (Nakamura et al., 2000). However, they show complete identity to the corresponding regions in laboratory strain BDV-MDCK (Iwata et al., 1998 and Pleschka S, personal communication). Since this strain is frequently used for cell culture experiments in the reporting laboratory, contamination problems can also not be excluded in this case.

5. Treatment with amantadine

Despite the controversial debate regarding the frequency at which humans might be infected with BDV, oral application of amantadine was proposed for the treatment of BDV-related psychiatric disorders (Bode et al., 1997). Amantadine is a non-competitive *N*-methyl-D-aspartate (NMDA)-type glutamate receptor antagonist that also binds to the σ-receptor and the nicotinic acetylcholine-receptor (Kornhuber et al., 1994). The rationale for treating patients with amantadine came from cell culture experiments that indicated that amantadine would exhibit antiviral activity against BDV (Bode et al., 1997). Interestingly, treatment of a depressive patient with this drug showed antidepressant effects which coincided with clearance of BDV markers from the blood of this patient (Bode et al., 1997). In the meantime, several groups questioned the rationale for amantadine treatment by presenting evidence that this drug cannot inhibit the replication of BDV in cell cultures and experimentally infected animals (Cubitt and De la Torre, 1997; Hallensleben et al., 1997; Stitz et al., 1998). It was argued that human BDV isolates might differ from laboratory strains in amantadine sensitivity. However, since the human isolates were not made available to the research community, independent verification of these differences has, to date, not been possible. Further problems with amantadine as a drug for the treatment of BDV-related psychiatric disorders came from a recently published open trial of amantadine sulfate in chronically depressed patients expressing BDV markers in blood (Ferszt et al., 1999). In this study, the antidepressant activity of amantadine sulfate did not correlate with clearance of viral markers. Since antidepressant effects were shown for a range of NMDA-receptor antagonists in an animal model of depression (Moryl et al., 1993), the antidepressant activity of amantadine might be explained more easily by an interaction of this drug with neurotransmitter systems than by antiviral activity (Lieb et al., 1997b).

6. Conclusions

Our critical evaluation of available reports indicates that, at present, there is no conclusive evidence that BDV is infecting humans nor that it is causing human psychiatric disorders. Nevertheless, the possibility remains that BDV is linked to human psychiatric diseases, mainly because the various assays used to detect BDV infections are not performing optimally. The various serological assays have not yet been validated and the significance of the low avidity of BDV-reactive human antibodies is not understood. It remains to be determined whether these antibodies are indeed induced by infection with BDV. Alternatively, they might have been generated in response to infection with a related microorganism or exposure to a related cellular immunogen that is up-regulated in psychiatric patients.

Several studies reporting the detection of BDV RNA in human peripheral blood and brain samples may have yielded false results due to laboratory contamination of the human specimens. To clarify this point, independent confirmation of positive test results is of special importance. If BDV is infecting human brains only transiently rather than persistently, by definition, documentation of such cases will only be possible by serological tests which, as discussed in this review, are presently not very reliable.

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