Research News

Borna disease virus and neuropsychiatric disease – a reappraisal

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Despite progress in understanding the molecular biology and pathobiology of Borna disease virus, its epidemiology and role in human disease remain controversial. The challenges encountered in this field are a paradigm for the investigation of diseases potentially linked to complex host–microorganism interactions.

In 1985, Rott and Koprowski reported that serum from patients with bipolar disorder reacted with cells infected with Borna disease virus (BDV)[1], an unclassified infectious agent named after a town in Saxony (Eastern Germany) where an outbreak of equine encephalitis in the late 1800s crippled the Prussian cavalry[2]. Intrigued by the notion that an infectious agent might be implicated in human neuropsychiatric disease, we and others began to pursue the characterization of this elusive neurotropic virus. BDV nucleic acids were isolated by subtractive hybridization in the late 1980s, the first successful application of subtractive cloning in pathogen discovery[3,4]. Over the next five years, the genome was cloned, and the virus was visualized and classified as the prototype of a new genus of non-segmented negative-strand (NNS) RNA virus with unusual properties: nuclear replication and transcription, post-transcriptional modification of selected mRNA species by splicing, low level productivity, a broad host range, neurotropism and the capacity for persistence[5]. It was widely thought, and proposed in a review in this journal in 1995, that the introduction of specific reagents such as recombinant proteins and nucleic acid probes would allow rapid assessment of the role of BDV in human disease[6]. How have we fared? A recent publication by Fukuda and colleagues provides a context within which to review our knowledge of BDV epidemiology[7].

BDV infection and detection

Incredibly, the global distribution, natural reservoir(s) and mode(s) of BDV transmission remain unknown. Infection has been reported only sporadically in Europe, North America and parts of Asia (Japan, Israel and Iran); however, it is plausible that the virus is distributed more widely but is not recognized owing either to the lack of methods and reagents for diagnosis of infection or a failure to even consider the possibility of BDV infection. Recent reports of asymptomatic naturally infected animals indicate that the virus could be more widespread than we previously appreciated. Rodents have been proposed as reservoir candidates because experimental infection of neonatal rats results in virus persistence and is associated with the presence of virus in saliva, urine and feces; however, there is no evidence of natural infection of rodents. An olfactory route of transmission has been proposed because intranasal infection is efficient in experimental animals and the olfactory bulbs of naturally infected horses show inflammation and edema early in the course of disease. Reports of BDV nucleic acid and proteins being detected in peripheral blood also indicate the possibility of hematogenous transmission[8,9].

Natural infections have been described in birds, wild and domestic cats, dogs, horses, sheep and cattle. Most publications implicating BDV in human disease have focused on neuropsychiatric disorders including unipolar depression, bipolar disorder and schizophrenia; however, BDV has also been linked to chronic fatigue syndrome, AIDS encephalopathy, multiple sclerosis, motor neuron disease

Table 1. Serum immunoreactivity to BDV[a]

Disease	Prevalence		Assay	Ref.
	Disease	Control		
Psychiatric (various)	0.6% (4/694)	0% (0/200)	IFA	1
	2% (13/642)	2% (11/540)	IFA	19
	4 –7% (200–350/5000)	1% (10/1000)	WB/IFA	20
	12% (6/49)	–	IFA	21
	30% (18/60)	–	WB	22
	14% (18/132)	1.5% (3/203)	WB	23
	24% (13/55)	11% (4/36)	IFA	24
	0% (0/44)	0% (0/70)	IFA/WB	25
Affective disorders	4.5% (12/265)	0% (0/105)	IFA	26
	4% (12/285)	0% (0/200)	IFA	1
	38% or 12% (53 or 17/138)	16% or 4% (19 or 5/117)	WB (N or P)	27
	37% (10/27)	–	IFA	21
	12% (6/52)	1.5% (3/203)	WB	23
	0–0.8% (0–1/122)	0% (0/70)	IFA/WB	25
	2% (1/45)	0% (0/45)	WB	7
Schizophrenia	25% (1/4)	–	IFA	21
	9–28% (8 or 25/90)	0–20% (0 or 4/20)	WB (N or P)	28
	17% (15/90)	15% (3/20)	IFA	28
	14% (16/114)	1.5% (3/203)	WB	23
	20% (2/10)	–	WB	29
	0–1% (0–2/167)	0% (0/70)	IFA/WB	25
	9% (4/45)	0% (0/45)	WB	7
CFS	24% (6/25)	–	WB	30
	0% (0/69)	0% (0/62)	WB	17
MS	13% (15/114)	2.3% (11/483)	IP/IFA	31
	0% (0/50)	–	IFA	32

[a]Abbreviations: BDV, Borna disease virus; CFS, chronic fatigue syndrome; IFA, immunofluorescence assay; IP, immunoprecipitation; MS, multiple sclerosis; N, nucleoprotein; P, phosphoprotein; WB, western immunoblot.

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Table 2. BDV RNA, virus or protein in subjects with various diseases[a]

Disease	Tissue	Prevalence		Divergence[b]	Refs
		Disease	Controls		
Psychiatric (various)	PBMC	67% (4/6)	0% (0/10)	0–3.6%	33
	PBMC	37% (22/60)	–		22
	PBMC	42% (5/12)	0% (0/23)	0–4.0%	23
	PBMC-co-culture	9% (3/33)	0% (0/5)	0.07–0.83%	10,34
	PBMC	2% (2/106)	0% (0/12)		25
	PBMC	0% (0/24)	0% (0/4)		29
	PBMC	37% (10/27)	15% (2/13)		35
Affective disorders	PBMC	33% (1/3)	0% (0/23)		23
	PBMC	17% (1/6)	0% (0/36)		24
	Brain	40% (2/5)	0% (0/10)		36
	PBMC	4% (2/49)	2% (2/84)	0–5.1%	37
	Brain (CSF)	5% (3/65)	0% (0/69)	(Protein)	38
	PBMC	2% (1/45)	0% (0/45)		7
Schizophrenia	Brain	0% (0/3)	0% (0/3)		39
	CSF	0% (0/8)	0% (0/8)		39
	PBMC	0% (0/7)	0% (0/7)		39
	PBMC	64% (7/11)	0% (0/23)		23
	PBMC	10% (5/49)	0% (0/36)		24
	PBMC	100% (3/3)	–	4.2–9.3%	40
	PBMC	0% (0/10)	0% (0/10)		29
	Brain	53% (9/17)	0% (0/10)		36
	PBMC	4% (3/77)	2% (2/84)	0–5.1%	37
	PBMC	14% (10/74)	1% (1/69)		41
	PBMC	0% (0/45)	0% (0/45)		7
	Brain	25% (1/4)	–	(RNA, virus, protein)	11
CFS	PBMC	12% (7/57)	–		42
	PBMC	12% (3/25)	–	6.0–14%	30
Hippocampal sclerosis	Brain	80% (4/5)	–		34
MS	Brain (CSF)	11% (2/19)	0% (0/69)	(Protein)	38
Normal controls	PBMC		5% (8/172)		43
	Brain		6.7% (2/30)		44

[a]Abbreviations: BDV, Borna disease virus; CFS, chronic fatigue syndrome; CSF, cerebrospinal fluid; MS, multiple sclerosis; PBMC, peripheral blood mononuclear cells.
[b]Divergence of P-gene nucleotide sequence from common BDV isolates (strain V and He/80).

and brain tumors (glioblastoma multiforme) (Tables 1,2). Infectious virus has only rarely been isolated from animals other than horses. Infection diagnosis is typically based on serology or PCR amplification of BDV genetic sequences in blood or tissues. Virus has been isolated from blood of three subjects with neuropsychiatric disease[10] and brain of one subject with schizophrenia[11]. There are two reports in which BDV nucleic acids were found in human brain (hippocampal sclerosis and schizophrenia) by *in situ* hybridization[11,12]. Most investigators whose results indicate infection of human blood or brain have used nested RT-PCR, a method that is prone to artifacts as a result of inadvertent introduction of template from laboratory isolates or cross-contamination of samples. Amplification products representing bona fide isolates and those caused by the amplification of low-level contaminants cannot be readily distinguished by sequence analysis because, unlike other NNS RNA viruses, BDV is characterized by high sequence conservation[13,14]. Thus, sequence similarities between putative new isolates and confirmed isolates cannot be used to exclude the former as artifacts.

BDV infection and PBMCs

Fukuda *et al.* recently published a detailed analysis of 45 subjects each with mood disorders, schizophrenia or no disease (blood-donor controls) for evidence of BDV infection in peripheral blood[7]. Plasma antibodies to BDV were assayed in western immunoblot and bead-based electrochemiluminescence assays using recombinant BDV nucleoprotein or phosphoprotein, and in immunofluorescence assays of infected cells. Peripheral blood mononuclear cells (PBMC) were tested using nested RT-PCR to detect the presence of viral mRNA encoding the phosphoprotein, and specific proliferative responses to recombinant BDV nucleoprotein or phosphoprotein. Although western immunoblot assays initially indicated a significantly higher prevalence of plasma antibodies to viral proteins in subjects with europsychiatric disease, adsorption with recombinant viral protein did not abrogate immunoreactivity in the majority of subjects. No significant differences were found between the groups; however, two subjects with bipolar disorder had both antibody responses to one viral protein and proliferative responses to two viral proteins, and a single subject with major depressive disorder was found to have BDV RNA in PBMCs in two separate specimens.

Two 'wrinkles' have been introduced that impact on the interpretation of published reports. The work of Planz and colleagues demonstrated that cells harboring BDV nucleic acid were found in granulocytes rather than PBMCs (Ref. 15). If this holds true as a general principle, the majority of reports in the literature, which are based on PBMC analyses, could be misleading. Similarly, Nowotny and colleagues sequenced an equine strain of BDV in Austria that was sufficiently different to preclude primer binding in industry-standard RT-PCR assays for BDV detection based on strains V and He/80 (Ref. 16). Using primers based on the Austrian horse sequence, Fukuda *et al.* found no evidence of BDV RNA in blood from 16 schizophrenic subjects[7]; however, the template for these experiments was extracted from PBMCs rather than other blood fractions.

Perspective

What can we conclude? The findings of Fukuda and co-workers[7] suggest that many subjects previously described as being seroreactive to BDV proteins could in fact have shown non-specific binding. A similar conclusion was reached by Evengård and co-workers following a study of 169 Swedish subjects with chronic fatigue syndrome – in 41% of subjects with severe disease, immunoreactivity to recombinant proteins in an ELISA was not confirmed by western blot[17], and by Allmang and

colleagues, who recently reported that positive sera from psychiatric patients have lower avidity for BDV proteins than sera from naturally or experimentally infected non-human hosts[18]. Although such data do not indicate an involvement of BDV, they do have a serendipitous aspect: investigating the mechanisms of cross-reactivity to BDV could provide clues to the pathogenesis of these disorders. Do these latest findings allow us to exclude a role for BDV in human neuropsychiatric disease? Certainly not. Future work must incorporate the latest sequence information and, when assaying blood, employ techniques that do not exclude cell populations that might harbor viral transcripts.

At the time of writing, a multicenter study (Microbiology and Immunology of Neuropsychiatric Disorders; MIND) comprising investigators in Australia, Germany, Poland and the USA has begun. The MIND study will use standardized methods for clinical diagnosis and blinded laboratory assessment of BDV infection in subjects with affective disorders, schizophrenia and controls matched for geography, age, gender and socioeconomic status. To minimize the potential for experimental error, multiple aliquots of sera and white blood cells (rather than PBMC) will be randomly distributed for analysis using serological methods designed to detect both conformational and linear epitopes, and real-time PCR assays based on all known BDV strains. A similar effort could be undertaken using brain tissues; however, given evidence from neonatal rodent models of BDV infection, in which specific patterns of damage and behavioral dysfunction can occur during gestation or infancy, it might be necessary to examine human materials from earlier time points.

Retrospective studies might be pursued using samples stored for other purposes, such as blood spots collected from neonates to rule out metabolic disorders. Indeed, recent work with blood spots has provided insights into disturbances in the levels of cytokines and growth factors at birth in children with cerebral palsy, mental retardation and autism (Karin Nelson, pers. commun.). More powerful yet would be programs designed to assess the role of microbial, immune and toxic factors in the pathogenesis of a wide range of chronic diseases in which risk is dependent upon exposure during gestation or at later time points. There is increasing support for prospective studies of maternal and child health, microbial ecology and toxicology. Such studies, in concert with host genetic profiling, will undoubtedly uncover new associations between infectious agents and diseases, and contribute to strategies for enhancing human health and welfare.

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Response from Takahashi and Fukuda

In a recent publication, we presented a detailed analysis of the T-cell proliferative and antibody responses to Borna disease virus (BDV) proteins and RNA of peripheral blood mononuclear cells (PBMCs) in 90 psychiatric patients and 45 controls to evaluate more precisely the role, if any, of BDV infection in neuropsychiatric disease[1]. In this study, three different methods [western blot analysis, electrochemiluminescence immunoassay (ECLIA) and immunofluorescence assay] were used to detect anti-BDV antibodies. Our western blot analysis included an inhibition test by adsorption of specific antibodies with purified BDV proteins to ensure the specificity of detection of BDV antibodies in human sera. We found that the majority of subjects gave false-positive readings in the first screening assay and similar results were also obtained using ECLIA. The prevalence of antibodies against BDV proteins in psychiatric patients was therefore not significantly different from that in blood donors. These data are convincing as the inhibition test ensures the specificity of the anti-BDV antibodies detected. What is the reason for the false-positive results? We speculate that they might be caused by SDS denaturation of BDV proteins forming new epitopes to which unrelated antibodies can bind. As intact BDV proteins do not have these new epitopes, they cannot inhibit the reaction.

Concerning the detection of BDV RNA in granulocytes rather than PBMCs of schizophrenic patients by Planz *et al*.[2], we have also tried to detect BDV RNA in granulocytes (1×10^7 cells) from 20 schizophrenic patients. However, no BDV RNA was detected in granulocytes by our RT-PCR system, which can detect 100 copies of BDV RNA. We therefore think it is unlikely that granulocytes preferably harbor BDV.

We agree with the comments of Lipkin *et al*. on BDV research and also support the ongoing multicenter study. By using standardized methods, hopefully including an inhibition test, in multicenter laboratories worldwide, we expect that sufficient virological and immunological data on BDV infection will be generated to reveal the associations between BDV infection and psychiatric disorders. A similar analysis, including precise molecular histopathological studies, of BDV infection in brain tissues should also be carried out.

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