23
Borna Disease Virus: Impact on Mood and Cognition

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23.1
Introduction

The aim of integrating behavioral neurology and biological psychiatry into a better understanding of brain–behavior relationships has been accompanied by an overwhelming number of studies in recent years. The suggestion that central nervous system infections lead to severe neurological diseases and secondary psychosis as a result of structural brain damage has been readily accepted. Neurotropic viruses like herpes viruses, rabies virus, influenza virus, and HIV can cause encephalitis. The measles virus may lead to sub-acute sclerosing pan-encephalitis, and Creutzfeldt–Jakob disease is known to be an infectious spongiform encepalopathy. In addition to viruses, numerous bacteria and parasites are also able to induce fatal destructive brain diseases [1].

Unlike many infectious agents, there is only one known virus, namely the Borna disease virus (BDV) that under natural and experimental conditions primarily afflicts limbic structures of the brain and causes a non-cytopathogenic (non-destructive) persistent infection in animals [2]. The limbic system consists of several evolutionary "old" brain structures, such as hippocampus and amygdala, which are connected to each other and cortical areas by a complex network of neurons. A deeper understanding of information processing pathways, from cortex to limbic system and reciprocally, is one of the most fascinating topics of modern neurobiology, of which biological psychiatry is one important area. Meanwhile, it is textbook knowledge [3] that disturbances of these pathways are mainly due to dysfunction or modulation of important monoamine neurotransmitter networks, such as glutamate, serotonin, dopamine, and GABA. As severe pathological changes may result in clinical symptoms affecting mood, cognition, and behavior, it is feasible that this infection may present as major depression (MDD), bipolar disorder (BP I and II), obsessive–compulsive disorder (OCD), anxiety disorders, or as part of the chronic fatigue syndrome (CFS).

BDV is a unique enveloped RNA virus, which has adapted to target a vulnerable part of the brain in warm-blooded animals (mammals, including man, and birds)

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[2]. This process has most probably occurred during millions of years of co-evolution, given its extremely conserved non-segmented, negative-stranded RNA genome [4]. The majority of infected subjects have sub-clinical symptoms and their hosts are asymptomatic carriers; therefore, only a minority of infected subjects will have life-long relapsing behavioral syndromes [5]. Experimental animal infections (mainly studied in rats) have already emphasized the effectiveness of BDV in disturbing cognitive functions, such as learning and memory [6, 7]. These CNS disturbances are likely to affect the neurotransmitters, in particular glutamate [8], which is the most important excitatory neurotransmitter. The existence in humans of a similar BDV infection of possible clinical relevance has been the subject of heated debate [5] since the discovery of the presence of an antibody against BDV in psychiatric patients [9]. Recently however, the evidence linking BDV and depression has become more compelling due to significant improvements in methods of detection [10] and reports that an antiviral therapeutic approach produces beneficial results in some patients [5, 11–13].

The possibility that an infectious agent may contribute to the etiology of major psychiatric disorders such as MDD is very provocative, as this would certainly lead to a major paradigmatic shift in biological psychiatry. This chapter will therefore provide a summary of the data pertaining to this fascinating virus. The intriguing ability of BDV to influence mood and cognition may provide novel insights into biological psychiatry and may have an important impact on the provision of alternative therapeutic approaches for the treatment of MDD. We believe that this topic is of broad relevance to clinical medicine and neuroscience research.

23.2 Milestones of Discovery in Human Infection

23.2.1 Antibodies

The history of BDV infections in humans can be traced back a quarter of a century to 1976, when sera from psychiatric patients (including the patient ISOLA with an epilepsy-like syndrome), kindly provided by Jay Amsterdam of the University of Pennsylvania, Philadelphia, were found to be antibody-positive in serologic assays (Ludwig, Rott, and Koprowski, unpublished observations). Ten years later, samples from a collection of hospitalized psychiatric patients in Germany and the US reacted positively in immune fluorescence (IF) antibody assays against BDV [9]. These observations were confirmed by studies describing the presence of BDV infection in control individuals and in immune-compromised patients with HIV infection [14, 15]. An overview of serologic data from 17 771 subjects collected worldwide, namely in Germany, Japan, Africa, and the USA, and investigated mainly by our and Rott's group, convincingly demonstrated significant serologic reactivity (2–23%) to BDV [16]. These individuals suffered from a variety of psychiatric syndromes, including affective disorders and schizophrenia, chronic fatigue syndrome or

neurological diseases including multiple sclerosis (MS); they also suffered from infections with various other viruses (EBV, HIV) and blood parasites (malaria, schistosomiasis). A small percentage of healthy volunteers and blood donors (1–2%) were also found to be serologically reactive to BDV.

23.2.2 Nucleic Acid

The discovery of antigen- and BDV-specific RNA in peripheral white blood cells (PBMC) [17, 18] was a milestone for human BDV research. This discovery was followed by the recovery of the first BDV isolates [19] in humans. These findings initiated numerous investigations, including the report of the first Japanese isolate from the brain of a schizophrenic patient [20], and it supported a broader worldwide acceptance of human BDV infection [5]. However, BDV diagnosis in blood has revealed inconsistent results because different groups used heterogeneous methods and markers (antibodies or RNA or both; for reviews see [5, 21]). These inconsistencies have initiated controversial debates in the field. The clinical significance of BDV in humans is still questionable, despite the proven disease association in animals (for review see [7]).

Even after BDV-specific RNA was found in post-mortem brain tissue from psychiatric patients [20, 22–24], criticisms still persist, given the sensitivity problems related to the low replication rate of BDV, and the low number of infected PBMC in blood [25], in addition to the low IF antibody titres. The specificity of human BDV IF antibodies has recently been in doubt [26] but was confirmed later, by the very same group [27]. Prior to the availability of human-specific antibodies, human BDV isolates were thought to be laboratory contaminants [28], which seemed reasonable due to the known low genetic divergence of animal and human BDV [29, 30]. However, those assumptions were wrong [31] with respect to three strains originating from two bipolar and one OCD patients (Section 23.5.1) [19, 30, 31], but recently the isolate described by another German group [32, 33] was found to be a contaminant.

23.2.3 Pathological Antigens and Immune Complexes

In 2001, the discovery of circulating immune complexes (CIC), a product of antigenemia (release of viral proteins into the blood plasma) and host antibodies, resolved the main discrepancies regarding BDV infections [10]. BDV-CIC formation explained the transient disappearance of antibodies and also clarified the crucial aspect of this condition, which was the realization that persistent BDV infection alternates between activated and dormant phases. Activation is characterized by an over-expression of two major structural proteins (N, p40 and P, p24), while replication to form new infectious particles is low, which is in stark contrast to most other viruses [2, 5].

We have suggested that BDV proteins have a relevant role in the disease, because infected rats who show sub-clinical symptoms but have abundant amounts of BDV

protein present in structurally normal hippocampal neurons exhibit learning deficiencies [6]. Extensive investigations in the rat model have recently been summarized [8, 34]. They supported evidence that these proteins have the ability to interfere with the function of a non-NMDA glutamate receptor (activated by kainate1) [34]. The presence of BDV proteins in cerebrospinal fluid (CSF) of human patients with recurrent major depression (MDD) in a double-blind study [35] confirmed the presence of BDV replication in human brain. This last data suggest a likely association of BDV infection and symptoms because more than 90% of acutely depressed patients with MDD and bipolar disorder (BP I and II) were positive for BDV-CIC and the severity of depression correlated with high levels of plasma antigen (pAg) [10].

23.2.4 Infection and Disease

CIC have been confirmed to be the predominant markers of BDV infection (see Section 23.3.4). Their detection also disclosed an infection rate of around 30% for asymptomatic carriers; this incidence is about 10-fold higher than that estimated by IF antibody tests. Although the mode(s) of transmission is still elusive, the existence of healthy carriers that represent the majority of BDV infections in humans and animals is key to the basic understanding of both clinical presentations and BDV epidemiology. The prevalence of antigen markers (CIC and pAg) in healthy subjects is significantly different from that among sick patients; thus it is possible that differences in individual risks for virus activation can lead to antigenemia. In addition to a genetic predisposition for MDD and BP (refer to Chapters 29 to 31), psychosocial stress seems to be one of the major factors that could promote such events [36]. Differences in individual vulnerability may account for high or low morbidity risk for BDV infection and lead to approximately 5% of disease relapse among 30% of healthy carriers (Figure 23.1). These facts do not imply that BDV is the only cause of "limbic system disorders" such as MDD or BP, but they suggest

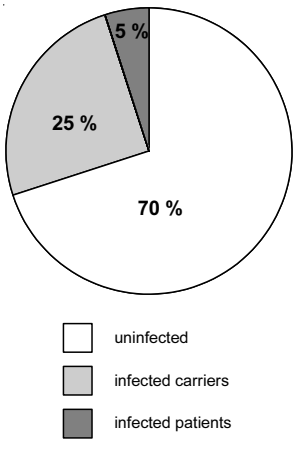

Figure 23.1 Assessment of the prevalence of silent and symptomatic human BDV infections. Data are based on the screening of randomized sets of blood samples for BDV-specific CICs (circulating immune complexes). Of infected subjects, the majority are healthy carriers (25%, gray segment) with low CIC levels, versus a vulnerable minority (5%, black segment), presenting with elevated CIC levels and clinical symptoms (e.g. depression).
(For details see Sections 23.2.4, 23.3.2, and 23.4.1.)

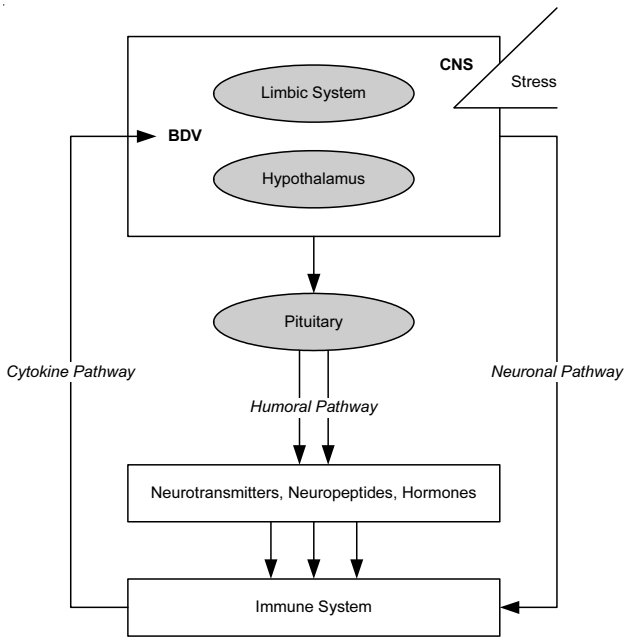

Figure 23.2 Model of viro-psycho-immunological interactions in symptomatic BDV infection. Illustration of how BDV may contribute to a subtype of affective disorder within interdependent reactions of host-determined factors. (For details see Section 23.4.3; figure modified from [37].)

that this viral infection represents a major factor that triggers the disease in vulnerable individuals within a multi-factorial scenario of disease-promoting events [37, 38] (Figure 23.2).

23.2.5 Therapy

Our discovery that antiviral treatment can result in amelioration of depressive symptoms has provided important information that strongly supports a link between BDV and depression [11]. During the course of this line of investigation, amantadine sulfate was shown to be very effective in one case of therapy-resistant depression in bipolar disorder and in the prevention of infection with human BDV *in vitro*, as well as in the inhibition of replication in infected cell cultures [11]. Again, controversies emerged because the *in vitro* efficacy of amantadine against BDV was difficult to replicate in laboratory strains of BDV [39–41]. In contrast to wild-type BDV of human or equine origin, laboratory strains were found to be resistant to amantadine [5, 7, 42].

The unexpected finding that amantadine is efficacious in the treatment of depressed patients infected by BDV was further substantiated by two open trials [12, 13]. The history of human BDV infections and their particular psychiatric

manifestations can be compared with previous data which confirmed the pathogenicity of this virus in animals. Extensive reviews have summarized the broad spectrum of BDV infections in animal species [7, 43–45] and have highlighted analogies with current knowledge regarding human infections [5, 16].

This enveloped virus has a non-segmented, single and negative-stranded RNA and replicates in the nucleus where it activates a complex splicing machism. With regard to the taxonomic classification of Bornavirus, it has recently been assigned to its own family, *Bornaviridae*, within the order *Mononegavirales*. This order includes several other neurotropic viruses, such as rabies, measles, and canine distemper virus [4]. The unique feature of the non-cytopathogenic Bornavirus is its ability to cause persistent CNS infection [2].

The study of natural BDV infections in animals (with and without the manifestation of disease) and their diagnosis provides valuable information which can be applied to humans. Extensive studies of experimental infections in animals as model systems will be discussed below (see Section 23.6) in relation to the human patient.

23.3 Properties of the Virus and Diagnosis

23.3.1 Virus Components

About a decade ago, electron microscopy studies of purified virus preparations from cell culture supernatants revealed BDV to exist as icosahedral particles of 90 and 50–60 nm in size [46]. The BDV virion is enveloped and therefore, its infectivity can be rapidly destroyed by disinfectants containing organic solvents, detergents, chlorine or formaldehyde. Viral RNA is also sensitive to ultraviolet irradiation and heat treatment (3 days at 56 °C) [2].

The viral genome consists of a linear non-segmented single-stranded RNA of 8915 kilobases (kb) with negative polarity [47, 48], and its replication is similar to that of other members of the order *Mononegavirales*. However, two outstanding differences in the replication process, namely nuclear (instead of cytoplasmic) replication and multiple splicing, have led to the establishment of a new family, the *Bornaviridae* family, of which Bornavirus is the sole member at the present time. Genomic RNA strands with positive and negative polarities are present in the cell nucleus, where transcription and replication occur [4]. The BDV genome encodes at least six open reading frames (ORF = genes) which start from the 3′ end: N (nucleoprotein), P (phosphoprotein), X (p10-protein), M (matrix protein), G (glycoprotein), and L (large polymerase). All of these genes are highly conserved (< 5% divergence at the nucleotide level) [47, 48], which suggests a long period of evolution.

In infected hosts, the proteins N, P, M, and G are the basis for the antigenicity of BDV, but N and P play the principal role. The N protein is expressed early during the infectious cycle, and exists in two isoforms (38/40 kDa) [49]. This protein covers

and protects the RNA, probably in conjunction with the P protein (24 kDa) and the L polymerase. Ribonucleoproteins (RNPs) have been isolated from cell nuclei and have been shown to be infectious [50]. Prior to the availability of genetic data, a protein complex known as the "s (soluble)-antigen", formed by the N and P protein, was found to be abundant in supernatants obtained from brain cells of infected animals or tissue culture preparations [2]. As previously mentioned, these two proteins are the major antigenic components which play a key role in the diagnosis of an infection; they also contribute to the unique pathogenicity of the virus [5].

23.3.2
Virus Properties

BDV preferentially targets limbic system neurons, but it can also infect other cells inside (glial cells, astrocytes) [8] and outside the brain (at least PBMC in blood) [17, 18]. Furthermore, BDV has an unusually broad spectrum of hosts, which covers a wide variety of mammalian and avian species (Figure 23.3) [2, 7]. Viral persistence that most probably lasts throughout the life-span of the host, is achieved with the help of several effective immune-escape strategies: (a) non-cytopathogenicity (no structural cell loss), (b) restricted reactivation most likely controlled by variations in host vulnerability, (c) low replication rates (producing low numbers of infectious particles) and over-expression of core proteins N and P, which elicit no protective

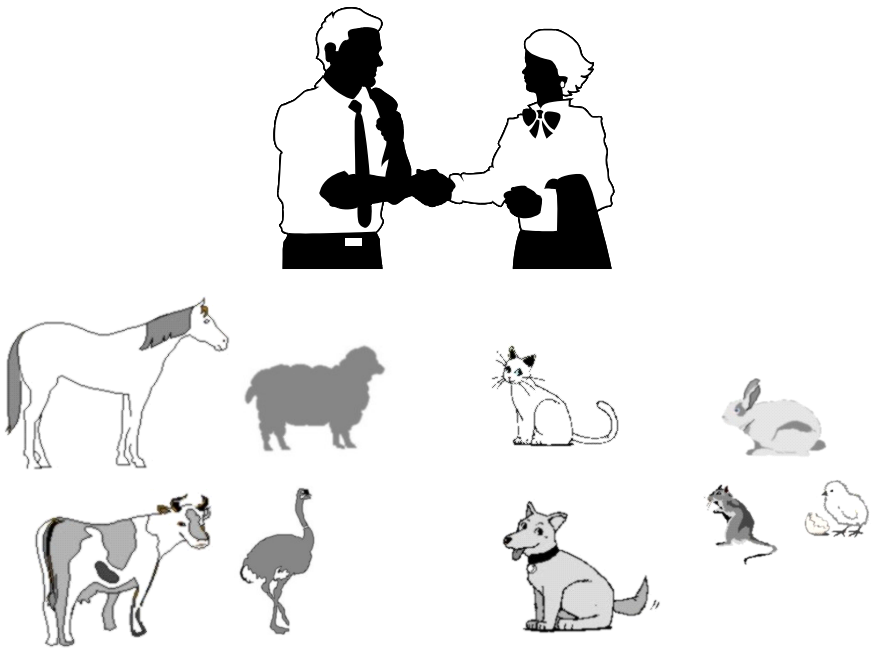

Figure 23.3 Host spectrum of BDV: relevant host species in natural and experimental infections. (For details see Sections 23.3, 23.4, 23.6.)

host immunity, and (d) production of very low amounts of functional glycoprotein G, thereby avoiding any relevant virus-neutralizing antibody capacity of the host [5, 51]. Together, these strategies result in relatively low pathogenicity, which would promote a fairly high infection prevalence of 30% (recently estimated based on positive CIC test) in healthy humans [10]. The prevalence rates are even higher among randomly selected horses from different geographic areas in Germany [7]. Based on elevated antigen levels (antigen/CIC) in plasma that reflect a higher frequency and/or longer duration of virus activity phases (Figure 23.1), approximately 5% of the human population appear to have an elevated morbidity risk. Intriguingly, the percentage of high-risk individuals in the population (5%), matches the worldwide prevalence of MDD [3, 52].

23.3.3 Pathogenic Proteins

The presence of antibodies against the N and P protein complex (anti-s-antigen) in the blood of human psychiatric patients marked the first indication of human infection 20 years ago (for a review see [16]). Detection of these antibodies was first achieved using immune fluorescence (IF) and the technique is still widely used today. However, we have recently developed reliable sensitive assays that can detect disease-relevant infection markers such as BDV antigen (N/P proteins), in human (and animal) blood (for review see [5]). These assays have been successfully used in PBMCs (cAg) [17, 18], plasma (pAg) and immune complexes (CIC) [10].

The pathogenic significance of one of these antigens was independently and directly demonstrated in non-infected transgenic mice in 2003. These mice showed distinct behavioral and neurotransmitter alterations based solely on the expression of BDV P protein in glial cells in the absence of cell damage [53]. This experimental finding supported the concept that the primary pathological mechanism used by the virus is located in the brain, which is substantiated by the non-cytopathogenic properties of the agent, periodicity, and type of symptoms (at least in mood disorders). Our concept considers functional (instead of structural) disturbances of brain neurotransmitter circuits either by direct or indirect interference of N/P proteins (or their components) with neurotransmitter receptor sites, thereby changing and/or modulating their sensitive balance [5, 8, 7, 19, 34, 37]. Experimental data obtained using persistently infected rats that displayed cognitive deficits, have suggested the involvement of a non-NMDA glutamate receptor (activated by kainate-1) [34] (see Section 23.4.3).

23.3.4 Virus Markers and Assays for Diagnosis

Detection of viral proteins is the main priority for the conclusive diagnosis of BDV infection. They are also useful in the determination of antigen load in symptomatic patients, and they can also be used to monitor the therapeutic efficacy of treatments. We became pioneers in applying this approach and developing easy-to-use versatile

assays in standardized format (enzyme immune assays (EIA)). These assays monitor the relevant proteins that appear during a period of virus activity. The assays target N and P proteins, which are abundantly produced by PBMCs (cAg) and other cells/organs in the body; these proteins are finally released into the bloodstream (pAg), and circulate as N/P complexes supposedly bound to host transport proteins. Plasma antigens will induce antibodies (Ab) and form circulating immune complexes (CIC); the latter predominate as a result of this dynamic process [10]. Our "triple-EIA" system mainly detects CIC, but also free pAg and antibodies, and thus provides a reliable method with which to establish the presence of infection even if antigenemia is absent or present only at a very low (Figure 23.4).

In 2002, competitive groups in Germany evaluated this versatile and novel BDV triple-EIA. In a workshop held at the Robert Koch Institute in Berlin, this test was found to be reproducible, robust, and easy to handle. Meanwhile, investigators in other countries have successfully applied this system to the determination of infection prevalence and the study of patients (Australia, Italy, UK, Czech Republic) in collaborative projects. Our own laboratories have evaluated the sensitivity and specificity of these assays over the past 2 years (20,000 samples; 2/3 human, 1/3 horses and other animals) [5, 7]. The specificity of such tests is of course, crucial and should be carefully controlled. Two monoclonal antibodies (W1 against N; Kfu2 against P) [54] have shown their extraordinarily high quality in terms of both

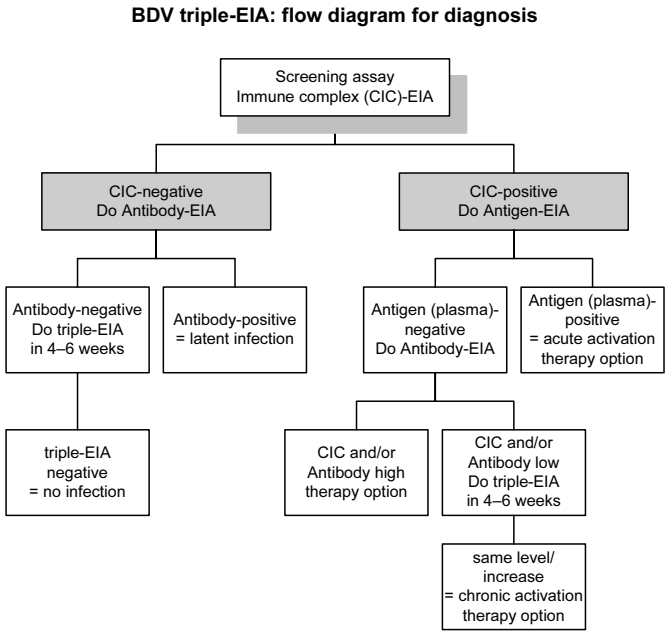

Figure 23.4 Diagnosis of BDV infection in blood with an enzyme immune assay (EIA) system. Flow diagram illustrating how monitoring of antibodies, immune complexes (CIC) and antigens facilitates the identification of positive individuals and disease- and therapy-relevant antigen/CIC load.

specificity and sensitivity, because they have high binding capacities to the native conformation of N and P protein [10, 42]. These assays are described in full detail elsewhere [10]. In addition to what has already been published, the mapping of epitopes on the N and P proteins of natural antibodies has confirmed the specificity of the EIA and its superiority to the widely used IF technique [5].

Nevertheless, BDV infection studies in human subjects as well as in animals, still rely on several methods and infection markers which are too insensitive and/or do not relate to the disease. We were the first to amplify BDV nucleic acid from PBMC of patients [18] spearheading worldwide studies (for reviews see [7, 21]). However, we do not recommend RNA detection (by RT-PCR) in PBMC as the best diagnostic tool. RNA detection is less reliable than the detection of antigens due to the low replication of this virus [25]. However, as BDV genome sequences have been published and are easily and widely accessible, RNA detection assays can be easily implemented. Several studies showed a lack of correlation between IF-antibodies and RNA-positive cells (for a review see [5]). This issue can now be explained because CIC formation accounts for the decline of antibodies in plasma. But this discrepancy adds to the ongoing controversies in this field, thus investigators who have consistently doubted the existence of BDV infections in humans continue to avoid considering their potential clinical impact [26, 28, 55].

Current systems used for the detection of BDV infection have recently been summarized [7]; a comparative ranking of the value of these techniques was undertaken to provide a guide for psychiatrists, patients and those who are engaged in the study of this viral infection [5]. To date our assays offer a clear answer to the following questions: (1) is the patient infected? (2) Is the infection active? (3) How intense is the antigen/CIC load? As detailed below (Section 23.5), the possibility of an infection contributing to MDD pathophysiology could provide a new antiviral therapeutic option for patients who suffer from a relapsing mood disorder.

23.4
Relationship of the Virus to Major Mood Disorders

The etiologic relationship between BDV and human disease remains the key issue to be clarified in BDV research. An important, if not inevitable prerequisite, for any clinical approach is to consider that the majority of infected individuals are healthy carriers. However, the evidence which supports the discovery and validation of human infections has focused much more on patients than normal subjects. Initial reports focused on the detection of antibodies in large patient cohorts that included those with psychiatric disorders (for a review see [16]); subsequent reports have focused on the detection of antigen [17] and nucleic acid in PBMCs of patients with mood disorders [18] and schizophrenia (for reviews see [5, 21]). Viruses have been isolated from PBMCs [19] and post-mortem brain tissue [20]. BDV RNA has been detected in brain bank samples of psychiatric patients [22, 23] and BDV antigens have been found in the CSF of depressed patients [35]. Recent studies report the presence of CIC and antigenemia in the plasma of MDD and BP patients [10].

23.4.1 Healthy Carriers

Prior to the realization that CIC are the predominant markers of BDV infection in the blood, it was unfeasible to obtain an accurate prevalence rate of this infection in healthy people. A prevalence rate of 0 to 2–3% was reported in studies that used detection of IF antibodies as an indication of infection (for a review see [16]), and studies which used RNA detection in PBMCs, reported prevalence rates between 0 and 4–5%. However this data could not be substantiated in corresponding samples, using these two markers [7, 21]. Although in the majority of studies, the frequency of antibody- and/or RNA-positive samples obtained from psychiatric patients was higher than that from healthy subjects [16, 21, 55, 56], the lack of concordance in prevalence data promoted controversies about the clinical role of this virus. Thus, studies focusing solely on BDV antibodies and RNA have "muddied the waters", because the absence of serum antibodies or of RNA in PBMCs cannot exclude infection, as antibodies may bind to plasma antigen forming CICs [10], and RNA may be present in only one per 10^5 PBMCs [25]. Unlike viral antigens and CIC, free antibodies and (PBMC) RNA have no meaning in terms of identifying a currently active infection state. Antibodies, if found alone, may only indicate a dormant state or previous infection [5]. As mentioned earlier, the introduction of CICs revealed a substantially higher prevalence of infection (20–30%) in people with no clinical signs.

23.4.2 Patients with Affective Disorders

A high prevalence of healthy carriers would imply that the agent has relatively low pathogenicity, and this would suggest little or no morbidity risk for the majority of infections. This is in line with the finding of a prevalence rate of about 100% in patients with MDD or BP who presented with an acute episode of depression (Figure 23.5), thus supporting a link between infection and disease symptoms. According to WHO data [52], MDD has a worldwide lifetime prevalence of at least 5%. In summary, prevalence data based on the assessment of CIC levels indicated significant differences between healthy carriers and sick patients. The levels of antigenemia, as measured by pAg and CIC levels, during an acute depression episode correlate with the severity of symptoms (Figure 23.5).

In such patients, persistent BDV infection is characterized by a significant level of markers of viral activity (pAg and CIC), while in the majority of healthy infected subjects, only low levels of CIC, with or without free antibodies, have been documented. A small proportion (about 4–5%) of silent carriers however, exhibited elevated CIC and/or pAg levels in independent cross-sectional studies [5]. Due to data protection (in the case of anonymized samples from blood donors) access to clinical records and follow-up investigations was not possible in some studies. Nevertheless, this 5% prevalence intriguingly mirrors the prevalence of MDD; therefore, these patients could be considered to have an elevated risk of morbidity.

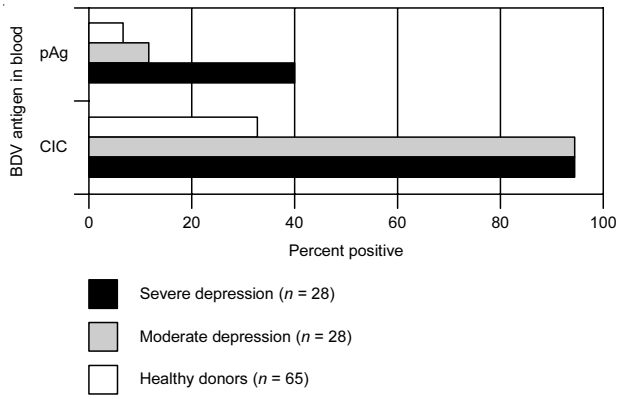

Figure 23.5 Prevalence of BDV antigenemia in acutely depressed patients vs. healthy carriers. Patients with Major Depressive Disorder (MDD) or Bipolar disorder (BP I and II) differed significantly from healthy infected subjects in terms of both infection prevalence (> 90 vs. 30%) and virus antigen load (CIC, immune complexes; pAg, plasma antigen), when their blood was tested during an acute episode. (For details see Section 23.4; figure modified from [5].)

The main significant difference between infected individuals who stay healthy, and infected individuals who become symptomatic, seems to be related to the level of antigen production by the virus, which results in a variable prevalence of CIC. Evidence which supports an etiologic role for BDV in mood disorders is presently based on the following findings (for a review see [5]):

1. Temporal relationship of BDV infection markers and symptomatic episode [10, 18]
2. Frequent presence of antigenemia (CIC and/or pAg) in acute depression [5, 10]
3. Strength/duration of antigenemia correlating with severity of acute symptoms [10]
4. Isolation of virus from PBMCs of severely sick patients with high antigenemia [19]
5. Correlation of BDV activation and elevated stress hormone levels in acute depression [36]
6. Long-term benefit of antiviral treatment in infected depressed patients [11–13] (details in Section 23.5)
7. Analogies in clinical and virological parameters between infected animals and humans [7] (details in Section 23.6).

23.4.3
Concept of a Link between BDV and Affective Disorders

The possibility of a significant contribution of an infectious agent to the pathogenesis of affective and other neuropsychiatric disorders implies a change of paradigm,

and requires the consideration of other infection-associated factors, especially immune defense mechanisms because they are the first line of defense. Empirical evidence suggests that there are alterations in immune functions in subgroups of mentally-ill patients; these alterations may lead to activation or suppression of the immune system. The following interdependent possibilities should be considered in the etiology of mental disorders:

- Immune system alterations may occur as a result of stress
- Neuroendocrine and humoral functions may be altered in neuropsychiatric (e.g. depressive) patients due to genetic factors
- Different viruses, particularly BDV, may be activated by alterations in the immune system (as a result of stress)
- Viral infections may directly interfere with psychiatric diseases

In line with previous concepts [5, 10, 19, 37, 38] we hypothesize that BDV, due to its unique properties, is supposedly the only virus that satisfies the above profile, provided that the individual host is vulnerable to frequent viral attacks by reason of their inherent genetic make-up and levels of stress; this in turn, may directly contribute to the frequency, severity, and duration of affective episodes, or other neuropsychiatric disorders. This concept integrates host and virus factors as interdependent contributors in a spiral-event cascade which eventually, after reaching an individual threshold, leads to overt clinical symptoms. Once the cycle has started the source of the initial signal, either the host or virus, becomes less relevant given their close interdependence.

A scheme of possible events for a subtype of affective disorder is detailed below [37] (Figure 23.2). We propose that the initial step in our model is the presence of acute and chronic stressors [57] which causes alterations in the immune system via neuronal and humoral pathways which then induce a new clinical episode (and involve neurotransmitters, neuropeptides, and hormones). Second, altered immune functions may be responsible for the reactivation of persistent BDV in limbic structures. Activation of a persistent virus, particularly as a consequence of immune deficiency is known to occur with several other neurotropic viruses, e.g. herpes viruses [58, 59], but unlike BDV infection, these viruses cause cell damage and encephalitis. Third, BDV activation in limbic structures which involves over-expression of structural core proteins N and P may disrupt neurotransmitter system circuits. They may be influenced directly, due to the affinity of BDV for glutamate and aspartate receptors [8, 34] or indirectly, via immune mediators, namely cytokines [60, 61]. A secondary reaction of the immune system following virus activation may occur and lead to immunological signs of an inflammatory reaction [62]. Furthermore, changes in limbic neurotransmitter systems may also influence information processing by serotonergic and dopaminergic pathways. Direct interference of BDV with these systems may also be possible. This cascade of events would finally culminate in a major imbalance of neurotransmitter systems, which may be responsible for the various neurobehavioral changes observed in psychiatric disorders. Once such a disorder has been established, it may indirectly influence

the immune system via neuronal and humoral pathways and could lead to further activation of BDV, thereby promoting relapsing episodes. Moreover, these mechanisms may initiate a cycle of disruption of psychosocial, immunological, virological, and neuroendocrine factors that would synergistically influence the complex disease process.

23.4.4 Impact on the Chronic Courses of Depression

After several acute episodes, many patients run into a chronic course of the disease that either does not respond or responds only partially to antidepressants. This phenomenon represents one of the most serious complications of recurrent MDD and BP and cannot be explained by popular theories. The introduction of BDV infection as a major contributor to these types of disorder provides two quite plausible reasons for the chronic course of the illness. One reason is that every relapse will be accompanied by an antigen production cycle and it may well be that despite recovery from the first episodes, antigen residues still remain in the central nervous system, and may therefore accumulate from one cycle to another. Indeed, biochemical analysis has revealed that BDV N and P proteins are quite resistant to cleavage. In addition, it is not known how these proteins are marked for apoptosis in surviving cells such as neurons. The other reason for the establishment of a chronic course of illness may relate to the parallel host adaptation processes. It is likely that the total absence of BDV proteins is not crucial for staying healthy, but the threshold level of antigen required for disease manifestation may vary between individuals. This threshold may change according to the number of relapses. Chronically depressed patients generally have low CIC and/or pAg levels (Figure 23.6) in contrast to those patients who have an episodic course of disease (compare with Figure 23.5). An adaptation process occurring during multiple activation cycles

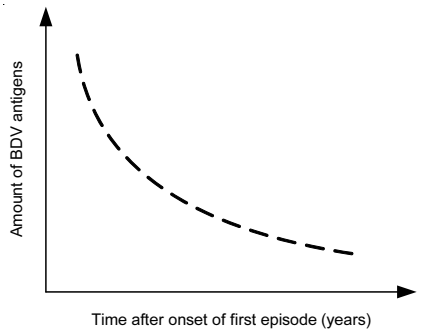

Figure 23.6 Decrease in BDV antigen load with number of episodes. In patients with chronic depression, it has frequently been observed that there is a reverse correlation of pathological proteins with previous episodes of depression and severity of symptoms, suggesting an increasing sensitivity to virus proteins with time. (For details see Section 23.4.4.)

would explain this surprising finding, which may eventually lead to the disease becoming evident at a lower threshold level of antigen residue i.e. increased sensitivity to the antigen. As a plausible consequence, each clinical episode may be initiated by lower antigen levels than the previous episode, eventually resulting in a chronically low level of both maintaining CICs and symptoms.

23.4.5
Other "Limbic Disorders"

As mentioned earlier, clinical studies have focused mainly on major affective disorders and schizophrenia. In contrast with the body of evidence for recurrent depression, the lack of clinical sub-typing and methodological limitations has prevented the establishment of a relationship between schizophrenia and BDV [21, 56].

The above viral disease model (Figure 23.2), which suggests that the central process of the disease focuses on interference/modulation of the monoamine neurotransmitter by BDV proteins, supports the hypothesis that several functional limbic system disorders may be associated with this infection. Thus, depending upon the respective structure that is affected by BDV (e.g. hippocampus and/or amygdala) and their multiple projections to associative cortical areas, a range of behavioral changes may be manifested, which include alterations in mood and interest, obsessive thoughts and compulsions, as well as attention deficit and anxiety. Although these emotional and cognitive changes are categorized into different clinical disorders, they seem to have in common, disturbed inhibitory or excitatory neurotransmitter circuits between the limbic system and cortical areas (particularly

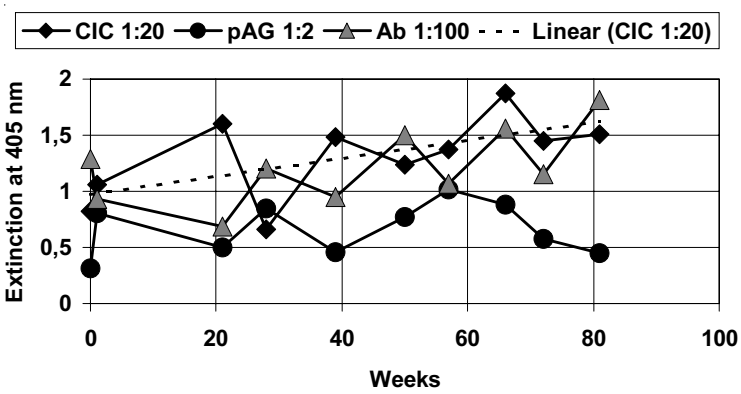

Figure 23.7 Chronic BDV antigenemia in a patient with severe obsessive-compulsive disorder (OCD). At 20-months follow-up blood monitoring in a chronically symptomatic OCD patient (male, age 26 years), indicated chronically activated infection and the maintenance of high values of immune complexes (CIC), plasma antigen (pAg), and antibodies (extinction at the indicated dilutions). (For details see Section 23.4.5.)

the ventro-medial part of the pre-frontal cortex). In this respect, it may not be so speculative to consider BDV as the unifying link between conditions that affect mainly cognitive functions that are emotionally controlled.

We have recently studied patients with obsessive-compulsive disorder (OCD), a proportion of whom presented with secondary depression [63]. We not only found a considerable prevalence of infection, but longitudinal investigations revealed enduring antigenemia (CIC and pAg) which paralleled the severe chronic course of OCD, indicating a chronically productive state of infection (Figure 23.7).

Interestingly, recent findings from event-related potentials (ERP) in BDV-infected OCD patients demonstrated a significant correlation between pathological changes in information processing and BDV antigen load (CICs) in the blood. This represents the first clinical finding suggesting that BDV infection can cause cognitive changes in human patients.

23.5 Antiviral Treatment

23.5.1 History and Controversies

The concept of an infectious etiology or an infectious contribution to mood disorders may provide new therapeutic approaches, namely the introduction of antiviral therapy. In the 1920s researchers in France used hexamethylenetetramine (hexamine) with some success for the treatment of horses with Borna disease [64]. Our group was the first to report an *in vitro* and *in vivo* antiviral effect of amantadine sulfate (a chemical relative of hexamine) against a human BDV isolate. A severely depressed bipolar patient infected with BDV improved dramatically under low-dose amantadine therapy, the course of improvement for that patient paralleled the disappearance of viral markers in the blood [11]. Amantadine is a well-known drug that has been used for 30 years; it was initially licensed to treat influenza-A virus infections [65], and it is now mainly prescribed for Parkinson's disease.

The novel finding that an antiviral treatment against BDV also had an antidepressant effect supports the hypothesis of a link between the virus and affective disorders, and has initiated debates both in psychiatry and virology. Psychiatrists have questioned whether the antidepressant efficacy of amantadine is associated with its antiviral properties, since this versatile compound is also known to have certain amphetamine-like, NMDA-receptor antagonistic, and other psycho-pharmacological effects (for a review see [66]). However, even if these properties do contribute to the improvement in symptoms, the decrease in BDV activity markers in patients who show a clinical response, as documented in several trials, cannot be ignored (see Section 23.5.3). Virologists in the field have hastily questioned our discovery, as there are *in vitro* studies that report a lack of antiviral efficacy with amantadine treatment [39–41]. However, it should be pointed out that these studies are not comparable to our study since they used BDV laboratory strains while we

used only wild-type human isolates [11] and laboratory strains are known to become highly adapted after multiple *in vivo* and *in vitro* cross-species passages [2, 51, 54].

23.5.2 Amantadine Studies In Vitro and Human Isolates

No genetically-defined wild-type virus existed until the mid-1990s; laboratory strains of BDV [2] were sequenced in 1994 [47, 48], and a few horse isolates had been previously identified [67]. A concerted effort was necessary to obtain the first four human isolates. The sources for these isolates were PBMCs from two bipolar (Hu-H1, Hu-H3), one obsessive-compulsive disorder (Hu-H2), and one (American) chronic fatigue syndrome (Hu-HUSA1) patient [19]. Virus could only be recovered from individuals during severe and lasting phases of major mood disorders, who exhibited a significant antigen load in the blood cells. Moreover, at least 10 blind passages in human oligodendria (OL) cells used for co-cultivation were required to allow adaptation to the tissue culture system. This may explain, why only six human isolates have been described so far, the sixth being recovered from the brain of a Japanese schizophrenic patient [20].

Unfortunately, the fifth isolate, which was obtained from granulocytes of a schizophrenic patient [32] has recently been withdrawn by the authors due to contamination problems [33]. Nevertheless, the contentious issue of contamination due to the divergence of laboratory strains [28] has been found to have little foundation in practice [31] (except for [32]). Human BDV strains are closely related to animal strains [29], and differ genetically from each other and animal strains only by unique point mutations in several genes [19, 30]. Biologically, they induce a different syndrome in rabbits [19], and also show a different sensitivity to amantadine [5, 42]. Their authenticity as human strains has undisputedly been proven by sequence identity of the original PBMC source and corresponding isolate [30].

There are numerous examples in other systems showing that one or a few mutations may cause significant phenotype variations that alter the characteristics of the virus [68]. This may also explain the remarkable difference in response to amantadine of wild-type and laboratory strains, not only with regard to the described inhibition-of-replication effects, but also to the prevention-of-infection [11, 42]. The ID_{50} (50% infection-inhibitory dose) of the most sensitive human strain differs by 6 $\log_{10}$ units from that of a resistant laboratory strain [42].

23.5.3 Amantadine in Clinical Trials

Although the molecular mechanisms of viral inhibition and its putative mutations are still unknown, the *in vitro* efficacy of the amantadine against human BDV has encouraged further clinical trials. The earliest study addressing the effects of amantadine on depression [69] was carried out before it was hypothesized that infection with BDV may be involved; 40 outpatients with a "chronic depressive

syndrome" (32 women; mean age 34 years) were treated for 4 weeks. The antidepressant efficacy of amantadine was reported to be superior to placebo, but inferior to amitriptyline. This observation was ignored for more than 25 years, until we reported that amantadine had both antidepressant and antiviral effects in a case report [11].

23.5.3.1 **Open Studies**

Consequently, the therapeutic benefit of this well-known drug in the treatment of BDV-infected depressive (MDD and BP) patients was further evaluated in two independently-conducted open trials (OT). Patients in the OTs were treated daily with a mean oral dose of 200 mg amantadine sulfate (AS) twice a day for a mean of 12 weeks, according to a dosing regimen of 2–4 mg amantadine per kg body weight. An oral dose of 200 mg AS will result in a blood level of the drug of 0.4 µg/ml, which corresponds to the concentration found to give *in vitro* efficacy.

The majority of patients showed a significant and rapid clinical response after an average of 3 weeks of treatment (Figure 23.8) [12, 13]. Of the 68% (17/25) responders in the Hanover study [13], 70.6% showed no depression at all, and 29.4% had a > 50% decrease in symptoms, according to the 21-item Hamilton rating scale for depression (HAM-D). Bipolar I (BP I) patients showed a more rapid improvement and did not show any subsequent hypomania. In addition, BP II and MDD patients suffering from a melancholic subtype of depression responded significantly better than patients with evidence of a neurotic or "reactive" type of depression [70].

In the Berlin study [12], 63.3% (19/30) of the patients showed a significant decrease in depressive symptoms, measured by at least a 40% reduction in points on the Montgomery–Asberg Depression Rating Scale (MADRS). Remarkably, this considerable favorable effect of amantadine therapy, comparable to that of the Hanover study, was achieved in patients, who were recruited due to poor or even complete lack of response (17/30 = 56.7%) to any antidepressant for more than 1 year [12].

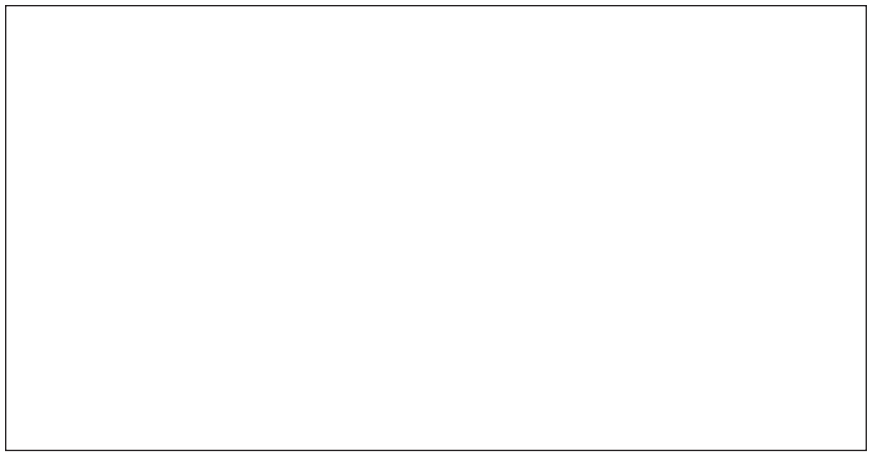

Figure 23.8

In both open trials improvement of depressive symptoms tended to parallel the decrease in viral activity, indicating a virus-static effect. No significant adverse effects were observed. In conclusion, the antidepressant efficacy in both OTs was considered to be comparable to standard antidepressants, and likely to be the result of antiviral efficacy against BDV rather than of short-term effects attributable to the psychopharmacological properties of the drug as mentioned above [66].

Further evidence of the beneficial use of amantadine may also be taken from studies and case reports of OCD patients infected with BDV [63]. In summary, the presently available data clearly support firstly, the view that infected patients significantly benefit from this drug, and secondly that the clinical response parallels the virus-static efficacy as indicated by a drop in antigen/CIC load in the blood.

At present there is no alternative antiviral compound against BDV which can compete with amantadine. We had earlier shown that a mannose-derivative targeting BDV matrix (M) protein (gp17) inhibited infection *in vitro* [71]. An almost universal antiviral compound, Ribavirin, was reported to interfere with BDV *in vitro*-replication [72, 73], and more recently, the inhibiting effects of Ara-C (1-β-D-arabinofuranosyl-cytosine) on both replication and virus spread were described [74]. However, even if *in vitro*-effective doses are analogously effective *in vivo*, clinical trials among psychiatric patients with either compound would not be advisable, given the known severe adverse effects of these drugs, in contrast to the well-tolerated drug amantadine.

23.6
Relevance and Role of Animal Models

In general, animal models are important in viral infections in the following cases:

- studies in which human virus had been transmitted to and was studied in animals (e.g. HSV or polio virus)
- etiologic studies in animals, using the human or a similar animal virus, allow insights into the disease processes in human patients by analogy (such as rotaviruses, corona viruses, or HIV), and
- studies in which the virus itself is used as a tool to manipulate and elucidate agent–cell, agent–organ, or agent–organism interactions without relevance to the natural host

BDV has been studied in relation to all three cases, although BDV isolates from human PBMCs have only been transmitted to rabbits by our group [19], and the Japanese brain isolate was only studied in gerbils [20]. Many studies in BDV research fall into the third category, such as those studying eye disease in rabbits [75, 76], brain alterations in monkeys [7], or neurodevelopmental and pharmacological effects in the rat [77–79]. To understand the impact of BDV infection on mood and cognition one must clearly differentiate between natural and experimental infection. In the former case small amounts of virus enter the animal, and in the latter case an

aliquot of brain- or cell culture-adapted virus is inoculated into the animal brain, which would be equivalent to the size and weight of a walnut as compared to the dimensions of the human brain. The significant difference between natural and artificial infection processes has often led to misinterpreted or over-interpreted conclusions.

The spectrum of BDV in nature is unusually broad (Figure 23.3). Host animals include the horse and ungulates, particularly sheep. Recently cats and dogs have been found to be virus carriers. Any data pertaining to possible viral transmission across the species barrier is based on pure speculation, and these possibilities are, at least in our experience, irrelevant. In addition to rabbits small rodents are also BDV susceptible, although infection can only occur after adaptation of the virus. Rats have served as the animal of choice [2, 7].

23.6.1 Experimental Infections

Many of the conclusions drawn from BDV rat experiments by major American and Japanese research groups, in addition to our own, especially those using variably adapted BDV strains, can be traced back to the pioneering studies of Nitzschke [80]. Basic information using the rat model was independently presented by Narayan's group [82] and our group [81] in 1983, followed by the first fundamental studies on behavioral changes and interference of BDV with learning and cognition [6].

However, it is becoming clear that these subtle emotional changes and learning deficiencies which appear in apparently healthy rats infected with BDV, are not associated with inflammatory reactions or obvious neural damage [6, 81], whereas in Narayan et al.'s study, immune-mediated processes were linked with behavioral abnormalities [82]. These discoveries paved the way for numerous studies in rats, the results of which changed mainstream thinking to include the concept that BDV may influence alterations in normal behavior.

Certainly the neonatal rat has evolved to present an optimal model for studying slight and subtle changes in normal instinctive behavior [2, 6, 78, 79, 83–86]. Similar data were obtained once BDV had been adapted to the mouse [87] with some heterogeneity among different mouse strains [88].

The main features of the vast literature on persistent, tolerant infections in rats, which can be compared to a certain degree with the impact of BDV on mood and cognition in man, are described below, together with the neuropharmacological alterations in the brain.

23.6.1.1 Neonatal Rat

Neonatal infection of rats resulted in locomotive hyperactivity, reduced anxiety, and significant deficits in spatial learning and memory [6, 89]. A review of the data reporting reduced resting behavior [6] and restlessness with hyperactivity which was also observed in infected adult animals such as the tree shrew [90], rhesus monkey [7] and rat [82], revealed a pattern of disturbance to normal movement

activity which appeared to be a prominent feature of infection. Quantification of this behavior together with exploration behavior in a novel environment by these rats amounted to a 5–10-fold increase in movement activity compared to normal animals [85]. Although the complex BDV antigen pattern which is visible at different times after infection and is characterized by localization of antigen residue in the limbic structures and accumulation of antigen in the Purkinje cells in the cerebellum, cannot completely explain these behavior changes, a reproducible periodical appearance of BDV antigens, first observed by our group, which seems to clear after 3 weeks along with the rostro-caudal progression of the infectious process [2, 8] may provide part of the explanation. These findings which have been replicated by many other groups [82, 85, 91] represent the neuropathological basis which helps to explain the complex modulation of functions controlled by the amygdala and cerebellar structures which result in altered behavior in rats. Vulnerability of the cerebellum in neonatal animals to viral infections has already been described as a characteristic feature [92].

A series of experiments by our group have offered explanations for some of these unusual behaviors in the rat, for example a significant accumulation of BDV antigen in neurons which belong to structures of the limbic system. This has also been observed in natural infections of the horse [67]. Increased amounts of antigen (N and P protein) with characteristic stratified patterns in their basal and apical dendrites were reported to predominate in the hippocampal pyramidal neurons. The antigen appears to have an affinity for excitatory neurotransmitters leaving the inhibitory synapses devoid of antigen [8, 93]. Glutamate and aspartate are the neurotransmitters associated with the synapses of the strata oriens and radiatum which carry the antigen; the other two strata however, have no such affinity. Furthermore, CA3 neurons (carrying antigen) and those of the CA1 region (free of antigen), are both glutamatergic, the major difference being that only the CA3 neurons harbor the non-NMDA (kainate-1; KA-1) receptor. Thus the hypothesis was put forward that KA-1 represents the BDV receptor in the brain [34]. This assumption is supported by the fact that the retina, a tissue that has a high expression of glutamate (KA-1) receptors, is severely affected in several experimentally-infected animal species [7, 75, 82].

23.6.1.2 **Dentate Gyrus**

The historically early observation in BDV neuropathology [2, 8, 34] of the destruction of granular cells in the dentate gyrus (DG) during the course of infection, may explain some of the characteristic features of learning and memory processes [94]. Several groups have replicated this phenomenon. The disappearance of neurons in this hippocampal structure relates to selective vulnerability and apoptosis [84, 85]. Our group, however, offers a more stringent explanation, which is based on the treatment of infected rats with N-acetyl-cysteine, a known anti-oxidant, which was able to inhibit DG degeneration. The vulnerability of these sensitive neurons to toxic mechanisms which result from the induction of oxidative stress (free oxygen radicals) followed by destructive processes, was proposed as a plausible explanation for the involvement of DG and is still accepted today [34].

DG damage seems to be of general relevance in BDV infections, because transfer of infected cat brain material to rats induced the same pathological morphology, but lacked virus antigen expression [95].

23.6.1.3 **Neurodevelopmental Disorders**

Neonatal infection is also considered to be an appropriate model for studying neurodevelopmental disorders. Significant weight (growth) reduction was a prominent feature in sub-clinically but persistently infected mice; the lack of growth became apparent in the first week post infection and these animals were 30% smaller than their control littermates at 15 weeks [87]. Abnormal growth and physiology was also seen in the neonatal rat model, the reasons for which are as yet still unknown [77].

Other outstanding changes that accompany infection include disturbed play behavior [78] and impaired social communication between litters and mother [77], as described in the tree shrew [90]. It has been suggested that the neuropathological basis involves antigen accumulation in neurons or interactions of other infected brain cells located in the amygdala and hippocampus [94]. Neuropathological findings have been presented which led to the suggested explanation of an imbalance in the neurotransmitter equilibrium [8, 34, 93]. Although adult animals were used in the tree shrew model of BDV infection, the alterations in social behavior might fall into the same category as those in neonatal animals and may also be linked to dysfunction in limbic areas [90].

However, in general, animal infection models that display global inflammatory responses like those observed in the adult rat, rabbit, tree shrew, and rhesus monkey are regarded as experimental artifacts with no relevance to the understanding of human infections and their relationship to mood disorders [5, 7, 42]. This view on etiology is essentially shared by Hornig et al. [85].

23.6.1.4 **Cytokines**

Cytokine research is an important area to consider in the investigation of human infections. It is important to address the following questions: Are there similar changes in cytokine patterns after BDV infection? How can neuroimmunological profiles influence alterations in behavior?

Cytokine gene expression during CNS infection can be predicted by taking into account dysfunctions that occur during inflammatory processes in the body [62]. In BDV-infected neonatal rats the picture is still rather heterogeneous. Altered levels of IL-1alpha, IL-1beta, IL-6, TNF-alpha, and an increase in TGF-1beta with higher levels of Tissue Factor have been reported. Since cytokine mRNAs co-localize with a pathological morphology that indicates a disturbance in the metabolism of astrocytes and glial cells, activation of these cell groups may well correlate with cytokine production [77, 88, 96, 97].

The neuroimmunological profile of BDV-infected neonates still remains obscure. In common with other virus models, changes in Th1- and Th2-type reactivity from the acute to chronic stages of disease have been postulated [77]. Data concerning the immune reactions of neonates who are also receiving immune-suppressive

treatment are questionable, as results relating to the immune reactions in different animal strains have been contradictory [98]. From our basic studies using Wistar rats [81] or different mouse strains [87], it became clear that no cellular immune reaction was observed in the CNS, but a definite humoral response producing antibodies against the major antigenic components (N- and P-protein) together with the considerable production of neutralizing antibodies, was demonstrated during the course of such silent infections. The presence of virus outside the brain, which has also recently been shown to occur in infected humans, might have triggered these immune reactions [5, 8, 81].

23.6.1.5 **Neuropharmacology**

The original hypothesis that some of the changes in behavior can be linked to the interaction of structural elements of BDV with neurotransmitter receptors [93] has been strengthened by further neuropharmacological investigations [8, 34]. The affinity of BDV for glutamate-neurotransmitter neurons was evidenced by specific antigen co-localization in the limbic structures and in the retina. Furthermore, recent experiments have focused our interest on the importance of the KA-1 (non-NMDA) receptor as a specific protein formation in certain CNS areas, where BDV proteins may contribute to or protect against a selective vulnerability [34].

Additionally, Lipkin's and Solbrig's groups have amassed considerable evidence showing that the dopaminergic and cholinergic system might also be involved in BDV brain pathology, although these systems were mainly studied in the more mature rat brain (see also Table 1 in [79]), and have also discussed in detail the similarities with human psychiatric disorders [79].

A recent review by Solbrig and Koob [86] elegantly summarizes the complexity of neuropharmacological changes and the wealth of new information gained in the field of neuroscience from the study of experimentally-induced infection in the rat. However, any conclusions extrapolated from this data to explain the expression of psychiatric disorders in humans should be regarded with caution [5]. Interpretations of neuropathological and *in situ* hybridization (based on mRNA detection) patterns support the basic view that BDV infection disturbs a neurotransmitter balance in the rat brain [8, 93, 99]. By analogy this may serve as a blueprint for what may be occurring in natural infections of animals and man, but should be used with caution.

23.6.2
Natural Infections

In relation to the topic covered in this chapter of a book which has endeavoured to assemble the biological factors contributing to depression, only BDV-infected horses and cats are of any relevance to the comparison of behavioral changes with the symptoms attributable to BDV infections in human patients (see Section 23.4).

The transmission and infection mechanisms in mammals are most probably similar. The virus can be transmitted horizontally or vertically. Both methods of transmission can be demonstrated in the horse with a rostro-caudal progression of

CNS manifestation. Once the agent has reached its preferred sites in the older areas of the brain and structures of the limbic system, a spectrum of symptoms is expressed, depending on the instinctive behavioral repertoire of the particular animal species (ungulate or carnivore), namely, unusual ear position, trembling of skin areas, lowered head with anxious look in the eyes, sudden drop of performance, lack of drive, apathy and somnolence, head shaking, loss of appetite, colic, and stumbling. Along with the progression of neurological symptoms, mydriasis, paralysis mainly of the hind limbs, circling movements, ataxia, and falling down is the known sequence of events in the course of Borna disease in the horse [2, 7, 43, 83]. In cats a similar repertoire of symptoms has been reported which is characterized by more frequent mewing than usual, anxious mydriatic eyes expressing a staring gaze, and depressive symptoms. Infected animals avoid familiar persons, become shy, stop eating, and often show hyperaesthesia to light and noise. Occasionally the inability to withdraw their claws accompanies the increase in neurological symptoms [7, 95]. It is of interest that experimentally-infected cats only showed mild symptoms and recovered with no significant overt residual symptoms [100].

Importantly, in both these mammals (horse, cat), Borna disease presents mainly with mild to moderate behavioral changes which may become more severe, and in rare cases, lead to severe neurological disease and death. This undoubtedly represents an etiopathogenetic sequence caused by the persistence of the virus in selected limbic areas. Moreover, recent studies in horses have considerably changed the previous opinion that the outcome of Borna disease is always fatal, because many clinical cases presented as transient episodes with spontaneous remission. As in human patients with affective disorders, symptomatic phases were paralleled by antigenemia (pAg, CIC) in the blood [5, 7, 10]. Interestingly, amantadine treatment using the same dose range as that for human patients (2–4 mg per kg body weight), was similarly effective and well tolerated. Silent carriers are also prevalent among the horse population, and their percentage in the population may even be twice that estimated for the prevalence of silent carriers in the human population, which can be as high as 60%; this novel finding has been facilitated by the determination of CICs in blood, and is in contrast to previous textbook information [43–45]. Thus, naturally-infected animals are supposedly a better guide for the comparative study of BDV infection and disease in humans than are experimental studies conducted on small rodents, as there is already a large literature base relating to natural infections in animals.

23.6.3 Critical Review of Animal Models

As a result of investigations spanning several decades [2, 5, 10, 18, 19, 83, 90, 101], we have now learned that the vast majority of efforts to study BDV infection in experimental animals have failed with respect to the elucidation of the mechanism of infection in the human patient. Unfortunately, they have misled our group and most other groups in this field. In our opinion, some of the data has been very

misleading, particularly the immune pathology data which endeavored to clarify the causal link between BDV and psychiatric disease.

These facts are summarized below:

- Age, virus strain passages, and immunological competence of the experimental animals clearly determine disease mechanisms and outcome [7]. For example, in Wistar rats two to three more passages of the adapted virus in rat brain tissues changed the neonatal symptoms from a persistent, tolerant infection into a dramatic sub-acute disease (see Figure 12 in [83]). Neuropathological analyses of these animals showed no immune pathological signs, but distinct cytopathological alterations in the brain (see Figure 14 in [8]). Similar observations were recently reported in gerbils infected during the neonatal period [102]. The response of different strains of rat (Wistar, Lewis, Black Hooded, Fischer etc.) to varying numbers of passages of Borna virus has yet to be defined.
- Animal models showing inflammatory reactions are seemingly irrelevant in relation to an explanation of BDV-specific pathogenesis in humans. We have over-interpreted the tree shrew model for the human patient, because although several tree shrews lived for a number of years without overt symptoms they were found to have severe neuropathological inflammatory reactions [7, 90]. The same is true for the experimental cat model. In this case persistently infected animals recovered from the early slight behavioral alterations, but were found to have considerable infiltrations of lymphocytes in the brain [100]. Furthermore, it is known that horses dying from Borna disease may or may not have inflammatory reactions. We assume that the virus infection, for unknown reasons, may run an unrestricted course, thereby inducing an overflow of antigen production in the brain, which could finally lead to fatal brain dysfunction. This leaves immune pathology as a secondary event that is unlikely to be a driving force behind the behavioral changes, and which may play no part in the fatal outcome resulting from a severe course of natural Borna disease; these views however, are contradictory to the general assessment of immune pathological events published by other scientists [98].
- The use of the wrong BDV strain (laboratory instead of wild-type) in in vitro inhibition assays [39, 40, 41] was sufficient to lead to an unfortunate setback and confusion in the use of amantadine against BDV infection, despite encouraging clinical studies with antidepressive and virus-static effectiveness, the details of which are discussed in Section 23.5.

In conclusion, experimental animal models might be useful in addressing neuroscience problems, but have not helped clarify the complex events associated with BDV infection in humans. The use of intracerebrally-infected animals has given some insight into the etiologic mechanisms of animal infections, but unfortunately these studies are of limited value for elucidating the different infection states in humans, and they cannot they explain the different morbidity risks. The theory of mild encephalitis in human patients due to BDV infection [103] has added even more confusion to understanding the pathological mechanisms [7].

23.7 Concepts for future studies and perspectives

Our survey on the broad body of data obtained from persistently sub-clinically infected animals has emphasized the manifold impact of BDV on behavior, mood regulation, and cognition, but has also referred to the often underestimated limitations of animal experiments. In natural animal infections, however, an impressive similarity of infection markers (antigenemia), symptom spectrum, and periodicity could be demonstrated, if horses with (transient) Borna disease and patients with mood disorders are compared [7, 10].

The study of natural infections in animals and humans provided evidence to support, first the concept of BDV as an unique virus that influences mood and cognition in vulnerable individuals of all host species; second, that periodical BDV antigenemia is the likely primary pathogenic factor, and third, that immune complexes and plasma antigen in the blood are suitable markers for monitoring the course of the disease and the efficacy of therapy.

Future studies should address:

- The implementation of a "gold standard" for the diagnosis of infection
- The identification of the spectrum of the pathogenicity of BDV in humans
- The benefit of antiviral treatment in different "limbic system" disorders
- The worldwide prevalence of human infection and morbidity risks of healthy carriers
- The identification of relevant modes of transmission and risk assessment in infected animals for application to humans

23.7.1 Future Diagnosis

The varying suitability of host and virus components for the determination of BDV infection in blood has been outlined in Sections 23.2 and 23.3. To overcome the present lack of comparability between studies from different research groups, a unifying "gold" standard should be introduced. According to both the pathological significance and predominant prevalence of immune complexes, we consider these markers to be appropriate standards for improving clinical and epidemiological approaches. BDV-specific tools for use in respective triple-EIAs (for detection of CICs) [10] should be made available for research on a collaborative basis as has already been implemented in some cases. In this context, we support multicenter and bilateral studies to further evaluate the assays, as well as participating in regular quality-control monitoring of collaborating laboratories.

Until an internationally accepted "gold" standard can be implemented, we recommend regular screening (at least) of BDV-CICs in clinical samples [5], supplemented by in-house tests of the respective laboratory, namely for antibodies and/or RNA.

23.7.2
Future Clinical Studies

Priority should be given to further investigations into the effectiveness of amantadine among depressed patients, who are either BDV-positive or BDV-negative. A multicenter approach using a double-blind randomized design seems appropriate for enrolling a larger number of patients as compared to previous studies. Furthermore, the use of multivariate analyses for repeated measures of BDV markers and standard depression inventories would allow further evaluation of the influence of infection on the efficacy of amantadine.

In addition to major depression, obsessive-compulsive disorder (OCD) should be a priority for future investigations and therapeutic trials (see Section 23.4.4), as should anxiety disorders, and chronic fatigue syndrome (CFS). Since only a subgroup of OCD patients were found to be infected [63], screening for BDV-CIC facilitates the identification of potential clinical and/or immunological differences between the groups. Added risk factors for BDV infection should be identified and treated.

23.7.3
Epidemiology and Risk Assessment in Carriers

The introduction of CICs for screening healthy individuals revised previous assumptions and revealed that 20–30% of the normal population were carriers (see Sections 23.4.1 and 23.4.2). Future longitudinal studies should investigate randomized cohorts in different countries and continents to obtain representative worldwide prevalence data with regard to carriers. In addition, these studies should address the morbidity risk in different age groups and for those with elevated CIC levels, investigate the potential transmission modes between family members, and finally should assess the potential risk of animal infections; all of these studies should be complemented by standardized questionnaires.

23.7.4
Conclusions and Perspectives

This chapter introduces a unique RNA virus, Borna disease virus (BDV), into biological psychiatry. The presence of persistent BDV infection in the brain and blood of humans and animals is the only evidence which supports the possibility of an infectious etiology in mood disorders and of BDV being the only candidate agent. This view at first seems provocative, but is supported by an intriguing body of correlative evidence which has been amassed so far.

Clinical data obtained from humans and animals fits the concept that BDV contributes to periodicity and symptoms by transiently disrupting the limbic system–cortical neurotransmitter circuits via its proteins (antigens), as part of a multifactorial cascade of events, which involves both host and virus in an interdependent manner. Virus activation, indicated by the presence of immune complexes and antigens,

can be monitored in blood samples with easy-to-use assays, and similarly, antiviral treatment with the low-risk drug amantadine is apparently effective. However, in contrast to this are controversies that have led to setbacks in human BDV research. This chapter aims to encourage biological psychiatrists who are interested in the fascinating impact of our concept, not to be deterred by these debates which detract attention from the scientific information associated with this complex research issue.

This chapter also aims to provide a guide for medical professionals who are interested in novel therapeutic options, and for patients who are suffering from often therapy-resistant mood disorders and are seeking help. A novel field of research will always provide more questions than answers, and will always have more opponents than advocates. At this point it is important to consider the aspects that cannot be disproved. Regarding the spectrum of properties of BDV and its preferential target cells in a crucial and sensitive area of the brain, both unusual efforts and unconventional approaches will be required to further confirm the impact of this agent on mood and cognition in mankind.

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