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Bornavirus is the only known member of the family Bornaiviridae. It is an enveloped virus with a diameter of about 100 nm. Its RNA genome is roughly 8.9 kb, has negative polarity, is non-segmented and codes for at least six proteins: a nucleoprotein (p40), a phosphoprotein (p24), a matrix protein, a glycoprotein, a polymerase and a protein of unknown function (p10) (for a review see Schneemann et al., 1995). BDV exhibits several interesting biological features. It replicates and transcribes its genome in the nucleus of infected cells. It uses the cellular splicing machinery to generate some of its messenger RNAs. It is non-cytolytic in cell culture and in the brain of infected animals. It is highly neurotropic and it can persist lifelong in the central nervous system (for a review see Gonzalez-Dunia et al., 1997).

Bornavirus affects mainly horses and sheep. In addition, some infections of cows, donkeys, dogs and other animals have been reported. Disease usually starts with behavioural changes followed by gait disturbances and hyperactivity. In the terminal stages of the disease, one can observe strong ataxia and paresis, and the animals can no longer drink and eat properly. Autopsy of animals with clinical signs of Bornavirus shows meningoencephalitis which can vary from mild to very severe. Perivascular infiltrates mainly consist of CD8-positive T-cells. Usually there is little or no detectable loss of brain cells in diseased animals (for a review see Gosztonyi and Ludwig, 1995; Stitz et al., 1995).

The non-cytolytic nature of BDV determines its pathology. One of the most remarkable features of BDV-induced disease is that clinical symptoms mainly result from the immune response of the infected host. BDV infection of the central nervous system by itself does not seem to cause disease. From experiments in rodent models it became clear that CD8-positive T-cells are the major players in the disease process (Hausmann et al., 1999; Noske et al., 1998). Recent work in my laboratory indicates that T-cells do not act by lysing infected neurons (Hausmann et al., 2000). Rather, the disease seems to result from neuron damage mediated by a cytokine storm which follows infiltration of lymphocytes into the virus-infected central nervous system.

Another interesting aspect of the biology of BDV is that animals, which for several reasons do not mount an efficient anti-viral immune response, remain healthy in spite of the fact that the brain of these animals is heavily infected. Sophis-
icated studies in the rat and mouse model systems have shown that tolerant persistently infected animals exhibit subtle defects in learning and that they present with behavioural disturbances (Dittrich et al., 1989; Sauder et al., in press). These findings led to the exciting suggestion that BDV could potentially be used to study human neurological disorders with similar features (Briese et al., 1999).

Here I shall discuss the question of whether or not BDV can induce human psychiatric disorder. During the last few years we have seen a large number of reports in highly respected scientific journals which all seemed to indicate that BDV is associated with some forms of depression, schizophrenia, chronic fatigue syndrome and possibly several other psychiatric disorders (reviewed in Gonzalez-Dunia et al., 1997; Lieb and Staeheli, 2001). During our own investigations of these proposed associations, I came to the conclusion that the existing experimental data cannot convincingly support this concept.

Starting with a report in 1985 by Rott and collaborators (Rott et al., 1985) followed by similar communications by several other investigators (reviewed in (Lieb and Staeheli, 2001), it became clear that human sera contain antibodies that recognise BDV antigens. It also got clear that such antibodies are mainly found in patients with psychiatric disorders. However, two major problems remained with these findings that I find disturbing. One problem is that depending on the method used for analysis (indirect immunofluorescence analysis (IFA), ELISA, or western blot analysis), one usually does not end up with comparable results (reviewed in Staeheli et al., 2000). These problems were initially attributed to different peoples' expertise in the various techniques. However, a more controlled multicenter study yielded a similar confusing picture (Nübling et al., 1999). A second problem is that a recent study of my laboratory has show that the avidity of reactive human sera to BDV antigens is extremely low (Allmang et al., in press). We performed straight-forward affinity tests by IFA. Basically, we first allowed the sera to react with virus antigen of infected cells and then subjected the antigen–antibody complexes to a quick wash with buffer containing three molar urea. We argued that if an antibody binds with low affinity to the antigen, it should remain bound to its target under these conditions. However, if the affinity of the antibody is low, it should be washed off. When we used serum from experimentally infected mice for these studies, we found that virus antigen remained unaffected by the 3 M urea treatment. This was also true for sera from infected rabbits and for sera from horses with Borna disease (Table 1). IFA staining of BDV antigen by serum from an experimentally infected monkey was also not influenced by 3 M urea. However, when we screened human sera under identical conditions, we observed that the fluorescent signals were strongly reduced, indicating that the human sera contained antibodies with low affinity for viral antigen. We then analysed the 20 highest scoring samples from a large collection of sera from human psychiatric patients (Bechter et al., 1992). When no urea was present in the wash buffer, we measured titres between 1:20 and 1:640. When 3 M urea was present, the titres dropped 16- to 32-fold and were often below the detection limit. One possible explanation was that the observed drops in titre occurred because these

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individuals had acquired the BDV infections only recently. Fortunately, we had access to archival samples of sera from two of these patients. Analysis showed that antibodies detecting BDV antigen were already present in both early sera and had persisted for 3 and 7 years, respectively (Table I). Importantly, the sensitivity toward 3 M urea of the reactive antibodies in both sera did not change during the whole period, indicating that affinity maturation for BDY antigen had not occurred. These findings argued against the notion that we were dealing with patients who had acquired the BDY infection shortly before the blood samples were taken. They rather suggested that they contained cross-reactive antibodies. It should be noted that the nature of the proposed immunogen which induces BDY-reactive antibodies in humans remains unknown.

To further learn about the specificity of reactive human sera, we expressed the p40 and p24 antigens of BDV in *Escherichia coli*, immobilized them on agarose beads and used them to perform serum depletion experiments. We found that human and animal sera could both be depleted very efficiently of their reactivity toward BDY antigens when a mixture of both major BDV antigens was used, demonstrating that they indeed recognised epitopes of BDV antigens. To verify this, we expressed p24-encoding cDNA constructs in transfected Vero cells and used these cells to perform IFA. As expected, sera from animals with Borna disease clearly detected the transfected cells. Human sera with high reactivity titres also did, yielding direct proof that these sera indeed recognised this BDV antigen. Now it was of interest to determine what would happen if a washing step with buffer containing 3 M urea was included. We found that the reactive antibodies in animal sera remained bound to p24, whereas the reactive antibodies present in human sera were washed off (Allmang et al., in press). The most reasonable conclusion from these findings again was that the reactive antibodies in human sera exhibited cross-reactivity.

A second independent piece of evidence in favour of the notion that humans can be infected with BDV are the results of RT-PCR and virus isolation experiments. Many laboratories reported the successful detection of BDV RNA in human blood samples and in autopsy brain material. In the vast majority of studies, viral RNA was found at enhanced frequency in material of psychiatric patients (reviewed in Lieb and Staeheli, 2001). In my view there is one major problem with these data that makes it difficult to come up with a definitive conclusion. The problem is that viruses identified in human samples and common laboratory strains of BDV have virtually identical sequence (Schwemmle et al., 1999). This is of great concern because new data showed that there is surprisingly high genetic diversity among wild strains of BDV from distinct geographic areas. In Germany and Switzerland where Borna disease is endemic in farm animals, wild viruses are closely related. They show 5% or less genetic difference (reviewed in Staeheli et al., 2000). By contrast, a virus isolated from a diseased horse in Eastern Austria, designated No/98, is about 15% different from the reference strains (Nowotny et al., 2000). These findings indicate that the major central European type of BDY should not be expected to occur world-wide. This expectation fits with the observation that Borna disease in animals is restricted to few well-defined parts of Europe. Surprisingly, the data of molecular epidemiological studies of BDV in humans do not fit with this rational. Rather, the viral sequences detected in human samples tend to resemble strongly the various reference strains of BDV that are used by the reporting laboratories for experimental work (Fig. 1). The simplest explanation for these findings is that laboratory contamination may have clouded the picture (Schwemmle et al., 1999; Staeheli et al., 2000). It should be mentioned, however, that a few exceptional viral sequences were amplified by RT-PCR from human material (reviewed in Lieb and Staeheli, 2001). It remains unclear whether they indeed indicate the existence of genetically distinct human strains of BDV.

So, what is the role of BDV in human psychiatric disorders? I think it is fair to say that the presently available data do not convincingly support the notion that this virus is involved in human psychiatric disorders: the evidence from serological studies is difficult to evaluate due to the cross-reactivity problems and the evidence...
from RT-PCR and virus isolation studies is difficult to evaluate due to contamination problems.

Nevertheless, I find it most interesting that virtually all published epidemiological studies have reported an increased frequency of sera with reactive antibodies in psychiatric patients. In my view this strongly suggests a role of an related micro-organism. At present, we have no good clue to what this agent might be.

Acknowledgements

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References


Fig. 1. Relationship between BDV laboratory strains and selected viruses (in grey circles) reported to originate from human blood or brain tissue. A rooted phylogenetic consensus tree was generated by the neighbor-joining method using nucleotide sequences derived from regions coding for BDV protein p24 (nucleotides 1482–1814). The exception was isolate BDVHuP2br for which sequence information was restricted to a fragment comprising nucleotides 1573–1772 (Nakamura et al., 2000). Individual sequences are identified by their accession numbers. Sequences reported by Iwata et al. (Iwata et al., 1998) are marked by asterisks. Note the surprising clustering of putative human BDV sequences to laboratory strains. Also note the fact that viruses of clusters I, II, III and IV were reported by laboratories that are using strains He80, rat BDV, V and BDV-MDCK, respectively, for experimental work.


