Arch Virol (1993) [Suppl] 7: 101–109

Borna disease virus: nature of the etiologic agent and significance of infection in man

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Summary. This review presents data on the characterization of Borna disease virus (BDV) and its potential as a possible causative agent in humans. The isolation of (i) BDV-specific cDNA clones that encode various BDV-specific proteins and (ii) partially purified virus particles led to the conclusion that the viral genome consists of negative-sense, single-stranded RNA. The organization of the BDV-specific RNA species appears to be a nested set of overlapping subgenomic RNA transcripts. Furthermore, evidence is presented that BDV can infect humans and may cause certain psychiatric and neurological disorders. This concept is supported by (i) the finding of virus-specific antibodies in sera of patients with neuropsychiatric diseases and (ii) results obtained during attempts to isolate BDV or a BDV-related agent from the cerebrospinal fluid of seropositive patients.

Introduction

Borna disease (BD) is an infectious, immunopathological disease of the central nervous system (CNS), characterized by a disseminated meningoencephalomyelitis [9]. BD occurs as a natural infection in horses and sheep and probably other species and has been transmitted experimentally to birds, rodents, ruminants and non-human primates. BD occurs only sporadically and has not been recognized in countries other than Germany and Switzerland. In naturally infected animals the disease characteristically results in paralysis and death [9]; occasionally recovery occurs, but the infected animals may still exhibit motoric and behavioral alterations. Recent seroepidemiological surveys of BD in horses have shown that BDV-specific antibodies are present in many horses [13],

surprisingly also in sera of horses from Africa and the USA (Herzog, unpubl. results), without clinical signs of the disease.

Most laboratory studies on the pathogenesis of BDV have involved experimentally inoculated Lewis rats. Under these conditions, BDV infection occurs only after mandatory replication in nervous tissues, and disseminates via neural pathways from the central nervous system (CNS) to the peripheral nervous system (PNS) or vice versa [12, 17]. In immunocompetent rats no infection is found in the extraneural tissues, whereas in newborn or Cyclosporin A-treated rats the virus also spreads to nonneural tissues in the vicinity of nerve endings [10, 27]. The clinical outcome of a BDV-infection in rats can vary greatly and depends on the passage history of the virus used for inoculation. Thus, a variety of BDV-variants exist including strains which induce long-lasting behavioral changes, obesity syndrome with fertility disturbances, paralysis with a high percentage of mortality, or inapparent infections using a MDCK-cell-adapted virus variant (Herzog, unpubl. results). In all cases, intracerebral (i.c.) or intransal (i.n.) inoculation resulted in productive replication in the nervous system. Therefore, it can be assumed, that the virus can tolerate various mutations and the virus inoculum seems to be genetically heterogeneous and may be selected by various host factors.

Borna disease virus

Borna disease can be induced by an infectious agent, the Borna disease virus (BDV) [31], but until recently little was known about the nature of the agent. BDV is present in relatively high concentrations in the brain tissue of affected animals and it can be readily induced in susceptible animals by inoculation of infectious brain material. The infectivity titers in infected tissue and cells were reduced or eliminated by exposure to UV light, detergents [5, 7, 9] or incubation with cycloheximide or actimomycin D [5, 7]. Therefore, it was proposed that BDV contains nucleic acid as genetic material, is likely to be enveloped and to have physical and biological properties of a conventional virus [7, 16, 22]. BDV particles have never been visualized in infectious material, but BDV-infection is associated with the expression of at least three virus-specific proteins with molecular weights of 14, 24 and 38/39 kd, respectively [8, 25, 28].

BDV-specific cDNAs were recently isolated in three laboratories from infected rat brain and tissue culture cells using subtractive cloning methods [6, 14, 21, 29, 30]. The establishment of subtractive cDNA libraries from infected material resulted in the identification of cDNAs coding either for the 14 kd and 24 kd (p14, p24) [6, 14, 21, 29, 30] or

the 38/39 kd protein (p38) [19]. Database searches of nucleotide and deduced amino acid sequences for the p14, p24 and p38 proteins of BDV have shown only limited regions of homology. The p38 protein showed small regions of homology with some viral polymerases and matrix proteins; this may suggest that the p38 BDV-protein associates with RNA or ribonucleoprotein [19]. Analysis of the deduced amino acid sequence for the p24 and p38 proteins revealed nuclear targetting motifs in the p24 and p38 proteins [19, 29]. Southern blot hybridization experiments using digested genomic and episomal DNA from persistently infected tissues failed to show positive hybridization signals [6, 14, 21, 30]. Therefore, BDV is unlikely to be a DNA virus or retrovirus and the cDNA clones represent no host encoded genes.

In Northern hybridization experiments, cDNA clones coding for the p24 and p38 protein detected four BDV-specific RNAs of 10.5 (supposably the viral genome), 3.6, 2.1 and 1.40–0.85 kb in extracts from infected rat brain and infected culture cells; the p24 cDNA clone hybridizes to the 0.85 kb RNA, whereas the p38 cDNA clone hybridizes to the 1.40 kb RNA [19]. All of these RNAs seem to be enriched by polyadenylate [poly (A)] selection [30]. In contrast, Lipkin et al. [14] reported that the largest RNA species is 8.5 kb and not polyadenylated. The BDV-specific RNAs were sensitive to digestion with pancreatic RNase [6]. These findings are in accordance with the view that BDV is a single-stranded RNA virus.

The cDNA clones isolated in our laboratories and coding for the p24 protein contained two open reading frames (ORF) with 217 and 77 amino acids (aa) [21, 30], the clone coding for the p38 protein contained a single ORF with 357 aa [19]. In vitro transcription and translation of the p24 cDNA clone produced two major proteins of ~14 and 24 kd (Fig. 1); in vitro transcription and translation of the p38 clone resulted in a 38 kd protein, recognized by both monoclonal and polyclonal antibodies to BDV. Recently we have amplified and cloned p24 and p38 BDV-specific sequences from horse brain RNA using the polymerase chain reaction (PCR) and oligonucleotides derived from the BDV-specific cDNA clones encoding for the p24 or p38 BDV-proteins (Richt et al., unpubl. results). The horse brain RNA was derived from a seropositive animal with clinical BD, which was found to be positive for BDV-infectivity on fetal rabbit brain cells (FRB) and BDV-antigens by immunohistological methods.

The genetic organization and polarity of the BDV-specific RNA-species was determined using strand-specific oligonucleotide probes. Oligonucleotides with negative polarity from coding and noncoding regions of the p24 cDNA clone all hybridized to the same four positive-stranded BDV-RNAs (Fig. 2; 10.5, 3.6, 2.1 and 0.85 kb) whereas com-

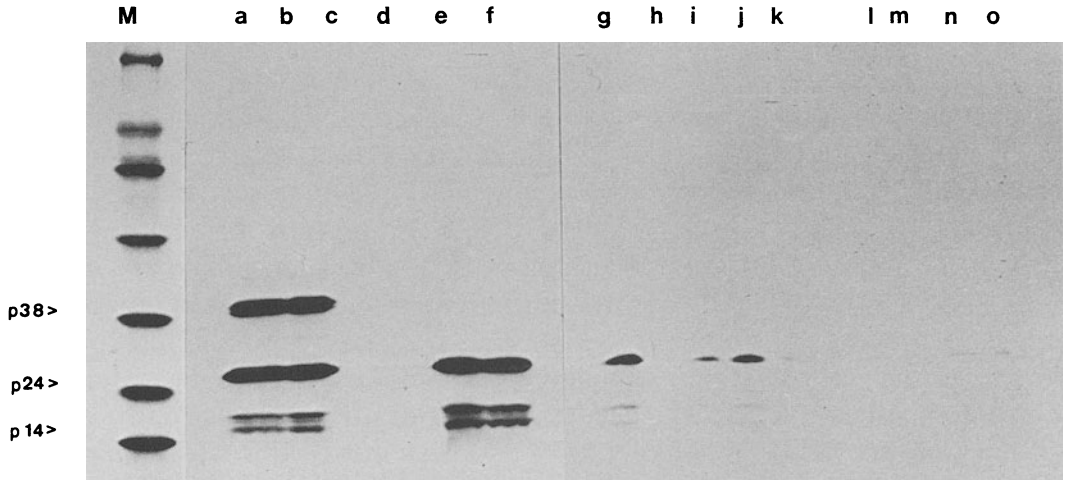

Fig. 1. Immunoprecipitations of BDV-specific proteins translated from the cDNA clone B8. Poly(A)-selected RNA from BDV-infected rat brain was translated in vitro and immunoprecipitated with polyclonal anti-BDV sera from rat (*a*) and rabbit (*b*). (−)-strand RNA (*c*, *d*) from cDNA clone B8 and (+)-strand RNA (*e*–*o*) from cDNA clone P4 (B8 subcloned in reverse orientation) were synthesized and translated in vitro and immunoprecipitated with polyclonal anti-BDV sera from rat (*c*, *e*) and rabbit (*d*, *f*) or human sera (*g*–*o*). Normal human sera (*l*–*o*) and various human sera, all positive for BDV-antigens in indirect immunofluorescence assays were analysed: human anti-p24 (*g*); human anti-p38 (*h*); patient 112 (*i*); patient 114 (*j*); patient 115 (*k*). Lane M contains the molecular weight markers from top to bottom: 200, 92.5, 69, 46, 30, 21.5, 14.3 kd proteins

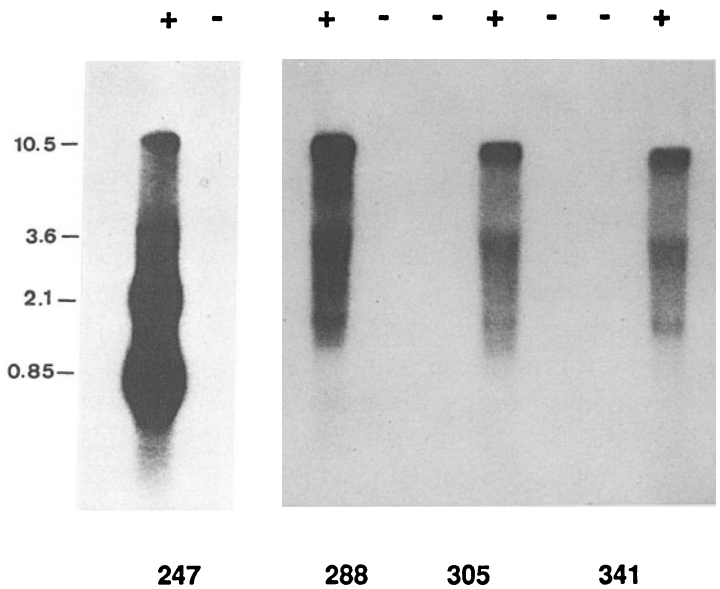

Fig. 2. Nothern blot analysis of RNA from rat brain. 10 μg total RNA from uninfected rat brain (−) and BDV-infected rat brain (+) were hybridized with a negative sense ^{32}P-labelled oligonucleotide (#247), or with positive sense ^{32}P-labelled oligonucleotides (#288, #305, #341) from different regions of cDNA clone B8 as previously described [21]

plementary positive-stranded oligonucleotides all hybridized to three BDV-specific RNAs of 10.0, 3.5 and 1.7 kb (Fig. 2) in size [21]. This suggests that the organization of the BDV-specific RNAs is a nested set of overlapping negative- and positive-stranded subgenomic RNA-species [21], similar to members of the coronavirus superfamily [11].

The question of genome polarity has been ambiguous, because purification procedures for BDV particles were not available. The relative abundance of the negative-stranded genomic RNA over the complementary positive-stranded RNA species provided a basis for the suggestion that BDV is likely to be a negative-stranded RNA virus [14], whereas the presence of a nested set of cross-hybridizing mRNAs indicates a striking similarity of BDV to the positive-stranded coronaviruses [21]. To identify the polarity of genomic BDV-RNA, the isolation of intact viral particles is required. We have recently partially purified BDV from infected tissues with the lipid solvent Freon-113 [20]. This allowed us to examine BDV-specific RNA species and proteins after extraction in a two-phase Freon-gradient. This treatment resulted in infectious particles with a bouyant density of 1.16–1.22 g/ml. All three virus-specific proteins regularly detected in BDV-infected tissue homogenates could be demonstrated after Freon treatment. Both positive- and negative-stranded RNA species found in BDV-infected rat brain and cells were present in the Freon-extracted preparations. When these preparations were treated with RNase A prior to RNA-extraction, only negative-stranded, genomic RNA was detected in Northern blot hybridizations using sense and antisense RNA probes [20]. No loss of infectivity was observed after RNase A digestion of Freon-extracted material. Therefore, all data available suggest that BDV appears to be a negative, single-stranded RNA virus.

The Freon-treated preparations have also been used in ultrastructural studies. The only virus-like structures which could be identified by transmission electron microscopy were particles of 60–82 nm diameter. The presence of a rim-like structure surrounding these particles suggests the presence of an envelope. Similar particles were described previously by Ludwig and Becht [15]. It cannot be assumed with certainty that these virion-like structures correspond to the original native virus particle, because few of these structures were found and the virus preparations had been treated with the lipid solvent Freon-113.

In conclusion, BDV-specific cDNA clones encoding the p24 and p38 BDV-proteins were isolated from persistently BDV-infected tissues. Four poly(A) mRNAs of 10.5, 3.6, 2.1, and 1.40–0.85 kb were recognized by negative-stranded RNA probes, whereas RNA transcripts of 10.0, 3.5 and 1.7 kb were recognized by positive-stranded RNA probes. No hybridization was detected in infected or uninfected MDCK or rat

brain DNA, indicating that the p24 and p38 cDNAs were not host encoded and that BDV is unlikely to be a DNA virus or a retrovirus. The nature and polarity of the BDV genome was determined after isolation of BDV with Freon-113 from infected tissues. Treatment of Freon 113-treated virus preparations with RNase A prior to RNA-extraction revealed, that only negative-stranded, genomic RNA could be detected in Northern blots using sense and antisense RNA probes. Therefore, BDV appears to be a negative, single-stranded RNA virus.

Borna virus infection in man

The nature of the behavioral disturbances observed in rats [18] and tree shrews [26] appear related to certain mental disorders in human beings. Therefore, sera and cerebrospinal fluid (CSF) of patients with neuropsychiatric diseases were examined for BDV-specific antibodies. Antibodies were present in 4–7% of sera derived from more than 5,000 psychiatric and neurological patients from Germany, USA and Japan [23, 24; Ikeda, pers. comm.]. In some of the seropositive patients, specific antibodies were concomitantly detected in the CSF, but always lower than those found in the corresponding sera [23]. The highest percentage of seropositive patients came from a region in Southern Germany, where BD is known to be endemic among horses and sheep. The antibodies from human patients reacted either with the p24 or p38 BDV-proteins or with both BDV-antigens in immunoblots. The BDV-specificity of the human antibodies was recently reinforced by the finding that antibodies from seropositive patients recognized the p24 protein (Fig. 1) expressed by a BDV-specific cDNA clone [30]. There is no evidence for a major clinical manifestation of infection with BDV in man. Seropositive patients were found with a broad range of mental disorders, with a predominance of schizophrenia, affective psychoses and certain personality disorders [1]. About 1% of 1,000 randomly collected sera from hospital patients also showed antibodies specific for BDV-proteins [1, 23]. This seems to indicate that inapparent infection with BDV or a BDV-related agent might take place in human beings, but clinical manifestation is only observed in yet unknown circumstances similar to naturally infected horses [13]. Serological examination of patients infected with human immunodeficiency virus (HIV) by Bode et al. (1990) has shown an incidence of BDV-specific antibodies of ~8%. The same investigators have reported a high incidence of BDV-specific antibodies among patients with chronic inflammatory neurological disorders such as multiple sclerosis.

Furthermore, efforts have been made recently to isolate BDV from the CSF of three seropositive patients. The CSFs were either applied to fetal rabbit brain cells or inoculated i.c. into rabbits, which are highly susceptible to BDV isolated from naturally or experimentally infected animals. In the cell cultures, small numbers of immunoreactive foci were found with BDV-specific antibodies 10–12 days after inoculation [23]. The cells, however, lost their antigen during subsequent passages. On the other hand, the i.c. inoculated rabbits developed no clinical signs of BD, no histological lesions in the CNS and no BDV-specific antigens could be found in the nervous system (NS) after an observation period of 5 months [23]. Nevertheless, these animals developed BDV-specific antibodies in their sera with titers ranging from 1:20 to 1:640. The brain homogenate from one rabbit was infectious for fetal rabbit brain cells as demonstrated by positive cell foci in immunofluorescence assay. However, again, the antigen disappeared during attempts to propagate the agent by subcultivation of the cells. These findings can be interpreted as typical of an abortive infection, and might indicate that infection in humans is caused by a BDV-related agent for which no suitable isolation method is available so far.

In conclusion, the presence of virus-specific antibodies and the results obtained during attempts to isolate the agent from CSF of seropositive patients support the concept, that humans can be infected with BDV or a related virus. From clinical studies it appears that this virus could induce acute and chronic meningoencephalitis with neurological symptomatology and might contribute to or initiate certain psychiatric disorders [1, 3]. There is also evidence that BDV might be horizontally transmitted from domestic animals to man [2]. Whether this agent induces disease might be dependent on genetic preposition of the host or other endogenous or exogenous factors. Evidence that such factors play a role in the outcome of BD has been described in naturally as well as experimentally infected animals. The advent of BDV-specific cDNA clones encoding the p24 and p38 BDV-proteins allows further studies on characterization, epidemiology and pathogenesis of BDV infection in animals and man.

Acknowledgement

The work done by the authors S.H., J.A.R. and R.R. were supported by the Deutsche Forschungsgemeinschaft (Ro 202/7-1; Ro 202/7-3; Ri 518/1-2), by the authors J.E.C., O.N. and J.P. by institutional grants from the Johns Hopkins University. We would like to thank Dr. Boschek for critically reading the manuscript and K. Haberzettl and E. Gottfried for excellent technical assistance.

References

1. Bechter K, Herzog S (1990) Über Beziehungen der Borna'schen Krankheit zu endogenen Psychosen. In: Kaschka WP, Aschauer HN (eds) Psychoimmunologie. Thieme, Stuttgart, pp 133–141
2. Bechter K, Schüttler R, Herzog S (1992a) Case of neurological and behavioral abnormalities: due to Borna disease virus encephalitis? (Letter). Psychiatr Res 42: 193–196
3. Bechter K, Schüttler R, Herzog S (1992b) Borna disease virus: Possible causal agent in psychiatric and neurological disorders in two families (Letter). Psychiatr Res 42: 291–294
4. Bode L, Riegel S, Ludwig H, Amsterdam JD, Lange W, Koprowski H (1988) Borna disease virus specific antibodies in patients with HIV infection and with mental disorders (Letter). Lancet ii: 689
5. Danner K, Mayr A (1979) *In vitro* studies on Borna virus. II. Properties of the virus. Arch Virol 61: 261–271
6. de la Torre JC, Carbone KM, Lipkin WI (1990) Molecular characterization of the Borna disease agent. Virology 179: 853–856
7. Duchala CS, Carbone KM, Narayan O (1989) Preliminary studies on the biology of Borna disease virus. J Gen Virol 70: 3507–3511
8. Haas B, Becht H, Rott R (1986) Purification and properties of an intranuclear virus-specific antigen from tissues infected with Borna disease virus. J Gen Virol 67: 235–241
9. Heinig A (1969) Die Bornasche Krankheit der Pferde und Schafe. In: Röhrer, H (ed) Handbuch der Virusinfektionen bei Tieren. VEB Fischer Jena 4: 83–148
10. Herzog S, Kompter C, Frese K, Rott R (1984) Replication of Borna disease virus in rats: age-dependent differences in tissue distribution. Med Microbiol Immunol 173: 171–177
11. Holmes KV (1990) Coronaviruses and their replication. In: Fields BN, Knipe DM (eds) Virology 2nd edn. Raven Press, New York, pp 841–856
12. Krey HF, Stitz L, Ludwig H (1982) Virus-induced pigment epithelitis in rhesus monkeys. Clinical and histological findings. Ophthalmologica 185: 205–213
13. Lange H, Herzog S, Herbst W, Schliesser T (1987) Seroepidemiologische Untersuchungen zur Bornaschen Krankheit (Ansteckende Gehirn-Rückenmarkentzündung) der Pferde. Tierärztl Umschau 12: 938–946
14. Lipkin WI, Travis GH, Carbone KM, Wilson MC (1990) Isolation and characterisation of Borna disease agent cDNA clones. Proc Natl Acad Sci USA 87: 4184–4188
15. Ludwig H, Becht B (1977) Borna disease: a summary of our present knowledge. In: ter Meulen V, Katz H (eds) Slow virus infections of the central nervous system. Springer, Berlin Heidelberg New York, pp 75–83
16. Ludwig H, Bode L, Gostonyi G (1988) Borna disease: a persistent virus infection of the central nervous system. Prog Med Virol 35: 107–151
17. Morales JA, Herzog S, Kompter C, Frese K, Rott R (1988) Axonal transport of Borna disease virus along olfactory pathways in spontaneously and experimentally rats. Med Microbiol Immunol 177: 51–68
18. Narayan O, Herzog S, Frese K, Scheefers H, Rott R (1983) Behavioral disease in rats caused by immunopathological responses to persistent Borna virus in the brain. Science 220: 1401–1403

19. Pyper JM, Richt JA, Brown L, Rott R, Narayan O, Clements JE (1993) Genomic organization of the structural proteins of Borna disease virus revealed by a cDNA clone encoding the 38 kd protein. Virology (in press)
20. Richt JA, Clements JE, Herzog S, Pyper J, Becht H, Wahn K, Narayan O, Rott R (1993) Analysis of virus-specific RNA species and proteins in Freon-113 preparations of the Borna disease virus (submitted)
21. Richt JA, VandeWoude S, Zink MC, Narayan O, Clements JE (1991) Analysis of Borna disease virus-specific RNAs in infected cells and tissues. J Gen Virol 72: 2251–2255
22. Richt JA, VandeWoude S, Zink MC, Clements JE, Herzog S, Stitz L, Rott R, Narayan O (1992a) Infection with Borna disease virus: Molecular and immunobiological characerization of the agent. Clin Infect Dis 14: 1240–1250
23. Rott R, Herzog S, Bechter K, Frese K (1991) Borna disease, a possible hazard for man? Arch Virol 118: 143–149
24. Rott R, Herzog S, Fleischer B, Winokur A, Amsterdam J, Dyson W (1985) Detection of serum antibodies to Borna disease virus in patients with psychiatric disorders. Science 228: 755–756
25. Schädler R, Diringer H, Ludwig H (1985) Isolation and characterization of a 14,500 molecular weight protein from brains and tissue cultures persistently infected with Borna disease virus. J Gen Virol 66: 2479–2484
26. Sprankel H, Richarz K, Ludwig H, Rott R (1978) Behavior abnormalities in tree shrews (tupaia glis, Diard, 1920) induced by Borna disease virus. Med Microbiol Immunol 165: 1–18
27. Stitz L, Schilken D, Frese K (1991) Atypical dissemination of the highly neurotropic Borna disease virus during persistent infection in cyclosporin A-treated, immunosuppressed rats. J Virol 65: 457–460
28. Thiedemann N, Presek P, Rott R, Stitz L (1992) Antigenic relationship and further characterization of two major Borna disease virus proteins. J Gen Virol 73: 1057–1064
29. Thierer J, Riehle H, Grebenstein O, Binz T, Herzog S, Thiedemann N, Stitz L, Rott R, Lottspeich F, Niemann H (1991) The 24 kd protein of Borna disease virus. J Gen Virol 73: 413–416
30. VandeWoude S, Richt JA, Zink MC, Rott R, Narayan O, Clements JE (1990) A Borna virus cDNA encoding a protein recognized by antibodies in humans with behavioral diseases. Science 250: 1278–1281
31. Zwick W, Seifried O, Witte J (1927) Experimentelle Untersuchungen über die seuchenhafte Gehirn- und Rückenmarksentzündung der Pferde (Bornasche Krankheit). Z Infektionskr Haustiere 30: 42–136

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