Mini-review

Borna disease virus: molecular analysis of a neurotropic infectious agent

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Introduction

Borna disease (BD) was originally described in the 1800s as an encephalitis in horses. Though it has been confirmed to occur as a natural infection only in horses and sheep, BD has been induced experimentally in birds, rodents and primates by inoculation with brain homogenates from infected animals.1 BD is characterized by behavioral abnormalities, meningeal and parenchymal inflammatory cell infiltrates and the early accumulation of BD-specific proteins in limbic system neurons.1 The most extensively studied animal model for BD is the infected rat. Rats infected as neonates have a persistent infection and show subtle disturbances in behavior.2 Rats infected as adults also have a persistent infection but show immune-mediated neurologic disease, progressive disturbances in behavior and motor function and abnormalities in brain levels of neurotransmitter mRNAs.3 5 The wide range of species susceptible to BD and the nature of the behavioral disturbances observed in adult-infected rats suggested that the BD-inducing agent, Borna disease virus (BDV), might cause neuropsychiatric disease. Though infectious material has not been isolated from human subjects, several groups have identified antibodies reactive with BD-specific proteins in patients with bipolar depression, schizophrenia and AIDS-encephalopathy.6 9 Thus, there is indirect evidence to suggest that BDV, or a related agent, might be a clinically significant human pathogen.

Subtractive cloning of BDV RNAs

BDV was considered to be an unconventional agent until recently. Because the potency of extracts from brains of animals with BD to induce disease in other animals is reduced by exposure to UV and detergents, it was proposed that the agent contained nucleic acids and was likely to be an enveloped virus.1 The inability of several laboratories to purify virus prompted the search for BD-specific sequences through subtractive cDNA cloning. By using this approach, two classes of cDNAs encoding either a 24 kDa protein (p24)9 11 or a 38/40 kDa protein (p40)10 were isolated from infected rat brain and tissue culture cells. Southern hybridization experiments performed with digested genomic and episomal DNA from persistently infected cells indicated that the agent was unlikely to be either a DNA virus or an integrated retrovirus.12
Characterization of BDV RNAs

In Northern hybridization experiments, cDNAs encoding the p24 and p40 detect RNAs of 8.5, 3.6, 2.1 and 1.2–0.8 kb in extracts from infected rat brain or infected cultured cells. Analysis by oligo d(T) column chromatography indicates that only the 3.6, 2.1 and 1.2–0.8 kb RNAs are poly(A)+; the 8.5 kb RNA is not. Recent experiments have allowed finer resolution of the 1.2–0.8 kb hybridization band: the p24 cDNA hybridizes to a 0.8 kb RNA; and the p40 cDNA hybridizes to a 1.2 kb RNA (Briese and Lipkin, unpublished data).

Most investigators believe that the largest RNA (8.5 or 10.5 kb, see below) represents the BVD genome. Treatment of extracts with RNase under conditions that degrade only single-stranded RNAs prevents hybridization. Northern hybridizations done with total RNA extracted from infected tissues and cultured cells using sense and antisense p24 and p40 sequences as probes show only sense polarity 3.6, 2.1, 1.2–0.8 kb RNAs. In contrast, the largest RNA (8.5 kb) is detected by both sense and anti-sense transcripts though the more abundant species is anti-sense (opposite in polarity to the smaller RNAs). These results are most consistent with a virus having a negative polarity genome.

Similar Northern hybridization results have been obtained by other investigators using p24 cDNA probes. However, VandeWoude, Richt and co-workers reported that the size of the largest RNA is different in extracts from rat brain (10.5 kb) or tissue culture (8.7 kb), all RNA species are poly(A)+ and the amounts of positive and negative polarity genomic RNA are equivalent in BD rat brain. Discrepancies in polyadenylation status of the largest RNA may be due to differences in methods for oligo d(T) selection. The majority of the 8.5 kb RNA is opposite in polarity to the smaller RNAs. RNAs must be diluted to concentrations below 1 mg/ml and boiled prior to chromatography to avoid annealing and copurification.

BDV genome polarity

The question of genome polarity has been resolved with the advent of a method for isolation of intact viral particles. Infectious virus is released from cells following exposure to hypertonic salt solutions. Released virus preparations are treated with nucleases to eliminate sequences external to viral particles, then RNA within the particles is extracted for Northern hybridization analysis with probes encoding the p24 and p40. Consistent with data suggesting that BDV was likely to be a negative-strand RNA virus, such experiments reveal only a negative polarity 8.5 kb RNA.

BDV proteins

Four proteins have been identified in BDV infected cells. Three proteins, termed s-antigens (soluble antigens), with molecular weight of 60, 38/40 and 24 kDa (p60, p40, p24, respectively), are released from infected brain or tissue culture preparations by sonication. Preliminary data indicate that p60 may be a multimer of p24: in Western blots, antibodies to p24 also detect p60. Moreover, incubation of s-antigen preparations or purified protein with dithiothreitol results in loss of p60 and an apparent increase in p24 signal (Briese and Ludwig, unpublished data). A 14.5 kDa (p15) proteinase K resistant protein has been extracted from infected brain and tissue culture preparations with detergents. Whether p15 is encoded by the host or BDV is not known.

The p40 and p24 are distributed in both the cytoplasm and the nucleus of infected cells. Analyses of deduced amino acid sequence for p24 and p40 have revealed nuclear
targeting motifs in both proteins.\textsuperscript{11,17} Database searches of deduced protein sequence for p24 have shown no significant similarities to other reported sequences.\textsuperscript{11,17} However, searches performed with deduced p40 sequence have shown similarities to L-polymerase proteins of monopartite negative-strand RNA viruses, paramyxoviridae and rhabdoviridae.\textsuperscript{17} These similarities were important not only for taxonomy but also because they identified a previously unidentified duplication in paramyxo- and rhabdoviridae L-polymerases. Furthermore, p40 does not contain two aspartate residues described as active sites of RNA polymerase activity.\textsuperscript{18-20} If p40 is a polymerase, it may be necessary to consider new models for RNA polymerase activity. Motifs conserved between p40 and L-polymerase proteins of paramyxoviridae and rhabdoviridae would be candidates for active sites of enzymatic activity.

**Site of BDV transcription**

The observation that BDV proteins accumulate in the nucleus of infected cells suggested the hypothesis that BDV, like influenza virus, might have a nuclear phase for transcription. Support for this hypothesis came from Northern hybridization experiments performed using RNA extracts from either the nucleus or the cytoplasm of infected cells. Such studies showed that whereas the 8.5 kb RNA was restricted to the nuclear fraction, the smaller RNAs were present in both the nuclear and the cytoplasmic fractions.\textsuperscript{12} These results were confirmed by \textit{in situ} hybridization experiments using strand-specific RNA probes that were designed to hybridize either to the smaller RNAs or the genome. Signal from the probe that hybridized to the 8.5 kb RNA was localized over the nucleus; signal from the probe that hybridized to the smaller RNAs was distributed diffusely over the entire cell.\textsuperscript{21} Nuclear transport studies provided conclusive evidence that the 3.6, 2.1, 1.2 and 0.8 kb RNAs are transcribed in the nucleus and that transport to the cytoplasmic compartment is ATP-dependent.\textsuperscript{15}

In the influenza virus system, nuclear localization is presumably due to a requirement for capped host cell RNAs to prime transcription of viral mRNAs.\textsuperscript{22} Though there are no definitive data to indicate that BDV uses host cell RNAs to prime transcription, support for such a mechanism comes from the studies in which actinomycin D, which interrupts host transcription, prevented expression of BDV proteins.\textsuperscript{23}

**BDV morphology**

BDV morphology remains to be determined. Virus release preparations that contain the 8.5 kb negative polarity RNA also contain 42 nm spherical structures that appear to be enveloped (Lipkin and Ribak, unpublished data). The size of the particles and the putative envelope is consistent with the biology of BDV (8.5–10.5 kb single-stranded RNA genome, sensitivity to detergents); however, particles could not be labeled with antibodies. Because we do not have antibodies that neutralize BDV, failure to immunostain does not necessarily imply that these structures are not viral particles. To establish the identity of these particles without neutralizing antibodies, we have initiated electron microscopic studies using \textit{in situ} hybridization for detection of BDV genomic RNA.

**Conclusions and future directions**

BDV is no longer refractory to characterization. Though BDV is not yet classified, its genome appears to be a negative-strand RNA which is transcribed in the cell nucleus. Molecular analysis of BDV is already providing insight into virus evolution and the
structure and function of RNA polymerases. The explosion of information on the biology of BDV makes it practical to consider exploiting the neurotropism of this agent for anatomy, cell biology and possibly, targeted drug delivery. Finally, tools are now in place to determine whether BDV or a related agent can be implicated in human neuropsychiatric diseases.

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References