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Preliminary Studies on the Biology of Borna Disease Virus

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SUMMARY

Borna disease virus (BDV) is an unclassified agent that causes neurological disease in a wide range of animal species and possibly in humans. The infectious nature of BDV has been long established but, despite extensive progress on the pathogenesis of the infection, the aetiological agent is still uncharacterized. Recent studies have shown that BDV replicates productively in cultures of foetal rabbit glial cells (FRG) which produce a virus-specific protein that is easily detected immunocytochemically. This provides a marker for BDV infectivity. This cell culture system was used to investigate the replication cycle of BDV. The agent required at least 1 h to bind to and penetrate the cells and the antigen was detected 24 h later. Cycloheximide and actinomycin D inhibited production of the antigen in inoculated cells, indicating that both protein synthesis and a DNA-dependent function were required for the production of viral antigen. Cocultivation of BDV-infected FRG cells with Vero cells resulted in a persistent productive infection in the latter. Use of these cells showed that the infectious agent matured exclusively in the cytoplasm and within the plasma membrane of the cell. Antigen-laden nuclei did not have infectivity. These studies showed that BDV has the physical and replicative properties typical of conventional viruses but its mechanism of replication and site of morphogenesis may be unique.

Borna disease virus is the aetiological agent of a number of animal encephalopathies whose clinical manifestations include paralysis and death (Ludwig et al., 1988; Heinig, 1969; Waelchli et al., 1985), hyperactive and aggressive behaviour (Narayan et al., 1983a), listlessness and apathy and subtle changes in behaviour affecting social interactions (Sprankel et al., 1978). Recent reports suggest that a BDV-like agent is associated with certain types of schizophrenia in humans (Amsterdam et al., 1985; Rott et al., 1985). Experimentally, BDV causes infection optimally when exposed to nerve endings, after which it is conveyed by axoplasmic transport throughout the nervous system (Carbone et al., 1987; Morales et al., 1988; Krey et al., 1979). Replication occurs mainly in neurons (Narayan et al., 1983a; Gosztonyi & Ludwig, 1988; Ludwig et al., 1988), except in neonatal animals in which the infection spreads to foci of non-neural cells in visceral organs (Herzog et al., 1984). BDV-specific antigen(s) are produced in the nuclei and cytoplasm of infected cells (Wagner et al., 1968) and all animals infected with the agent produce antibodies to this antigen (Gosztonyi & Ludwig, 1984). These antibodies lack protective or neutralizing properties (Narayan et al., 1983b).

BDV replicates at a minimally productive rate in astrocytic and neuronal cell cultures after inoculation with homogenates of infectious brain material (Herzog & Rott, 1980). The agent is non-cytopathic and strictly cell-associated. Infection is detected in the cells by development of BDV antigens in the cell or by the ability of lysates of antigen-positive cells to cause encephalitis in rats. Infectivity rarely exceeds 1 ID_{50} per cell. Cultures of non-neural cells are poorly susceptible to infection with BDV and presumably lack surface receptors for the agent. However, infection can be established in cell lines of non-neural cells by cocultivation with infected glial cells. A portion of the cell population becomes infected during this period and
these antigen-positive cells can be selected so that 100% of the cells show infection. This mechanism has been used to derive persistently infected cell lines such as BDV-infected MDCK cells described by Herzog & Rott (1980).

Despite its unique biology, BDV is uncharacterized structurally. It has never been visualized by electron microscopy (Anzil & Blinzinger, 1972) and its site of morphogenesis and its mechanism of replication are not yet understood. Moreover, it is not clear whether the BDV antigen, identified previously as a 38K non-glycosylated protein (Haas et al., 1986), is a structural component of the agent. In this report, using Vero cells persistently infected with BDV, we show that the agent is located almost exclusively within the plasma membrane of the infected cell, but only negligible infectivity was present in the antigen-laden nuclei. Foetal rabbit glial (FRG) cultures inoculated with lysates of infected Vero cells developed BDV antigens 24 h after inoculation. At least 60 min was required to accomplish entry of the agent into the cell, after which both DNA-dependent functions and new protein synthesis were essential for synthesis of the antigen.

Vero cells (ATCC) were propagated in minimal essential medium and 10% foetal bovine serum (FBS) and, while still at a subconfluent stage, inoculated with 1 × 10⁶ TCD₅₀ of BDV. The inoculum was an infectious brain homogenate derived from rats inoculated with a Giessen strain of BDV, kindly provided by Professor R. Rott, Institute for Virology, Giessen, F.R.G. The inoculum was maintained in the culture for 1 week, after which the cells were subcultured and examined for infection. BDV antigen was not identified in any of the cells. A suspension of 1 × 10⁵ Vero cells was then added to a culture of infected FRG cells and separated by gentle trypsinization 1 week later. Examination of these Vero cells showed that approximately 20% had now acquired BDV antigens. This ratio was maintained through several subcultivations. Cells from this mixed culture were then cloned in a 96-well plate and six clones expanded blindly into new stocks. Three of these new stocks had no antigen and three had BDV antigens in 100% of the cells. Although the sample size was small, the all-or-none segregation between infected and non-infected cells suggested that this cell-associated agent does not spread only from cell to cell, like other known cell-associated viruses, but that the agent spreads from parent to progeny.

The experiment suggested also that the cocultivation of the Vero cells with the infected glial cells resulted in infection by fusion, overcoming an apparent lack of receptors on Vero plasma membranes.

Using lysates of infected Vero cells, we next determined sensitivities of the agent to physicochemical denaturing procedures to obtain preliminary information on essential structural components of the particles. Sonicated lysates of 1 × 10⁶ infected Vero cells had an infectivity titre of approximately 1 × 10⁶ TCD₅₀ in FRG cultures. Treatment of this material with 250 µg trypsin for 15 min at 37°C, u.v. light (22 J/m²/s) for 30 min or 0.5% NP40 for 60 min all resulted in loss of infectivity to < 1 × 10³ TCD₅₀ per ml (99.9% loss of infectivity). This suggested that the agent has essential proteins, nucleic acid and lipid. This sensitivity of BDV to lipids solvents had been observed more than 70 years ago (Nicolau & Galloway, 1928); we confirmed this. We next sought to determine whether infectious particles were present on the external portion of the plasma membrane of the infected Vero cells. Treatment of a suspension of infected, intact Vero cells with 250 µg/ml trypsin for 15 min at 37°C followed sequentially by quenching of the enzyme with serum, sonication of the preparation and assay in FRG cultures, showed that there was no reduction in infectivity of the lysate compared to the titre in a lysate of untreated infected cells; identical titres of infectivity of 1 × 10⁶ TCD₅₀ were obtained. These data suggested that only negligible infectivity was present on the external portion of the plasma membrane. Further, intact plasma membranes of the infected cells protected the agent in the cell against proteolysis.

In order to investigate further the intracellular location of the agent, we subjected suspensions of persistently infected Vero cells to three cycles of freezing and thawing, followed by Dounce homogenization and sedimentation of nuclei by centrifugation at 1000 g for 10 min. The supernatant fluid containing the enucleated cells and cell fragments was sonicated and assayed for infectivity. Fig. 1 shows that all of the nuclei had BDV antigen but sonicated lysates of these structures had < 10³ TCD₅₀ per ml. Nearly all of the infectivity (1 × 10⁶ TCD₅₀) was located in
Fig. 1. Immunofluorescence using rabbit anti-BDV antibodies and fluorescein isothiocyanate-conjugated goat anti-rabbit IgG. (a) BDV-infected foetal rabbit glial cells, (b) uninfected Vero cells, (c) BDV-infected Vero cells, (d) BDV-infected Vero cell nuclei.

the enucleated cell fraction (Fig. 2). This experiment suggested that maturation of the BDV particle occurs mainly in the cytoplasm. These data also support previous findings that the pathognomonic BDV-induced nuclear inclusion bodies recognized in histological sections and by immunocytochemical staining procedures do not represent aggregated virus particles (Joest & Degen, 1909; Anzil & Blinzinger, 1972; Blinzinger & Anzil, 1973). Whether the antigen is a precursor structural protein or a non-structural protein of the agent or even an aberrant virus-specified host protein is not known.

Assuming that the synthesis of the antigen is an integral part of the virus life cycle, we next performed experiments to determine the kinetics of entry of the agent into glial cells and some of the biochemical events essential for production of the antigen. Using trypsin protection...
Short communication

BDV/Vero cell culture

- Supernatant: < 10 TCD₅₀/ml
- Sonicated portion: 1 x 10⁶ TCD₅₀/ml
- Cell suspension: 1 x 10⁶ cells/ml
- Hypotonic buffer

- Dounce homogenization: 1000 g for 10 min
- Sonicate
- Enucleated cells: 1 x 10⁶ TCD₅₀/ml
- Nuclei: 100% have BDV antigen

Fig. 2. Localization of BDV infectivity in persistently infected cells. The flow chart correlates infectivity with different compartments of infected cells and shows that it localizes exclusively in the enucleated cell fraction.

In summary, the preliminary experiments described in this report show that BDV has many properties in common with conventional viruses. These include the physical components of the agent, a suggestion for the requirement of cellular receptors and dependence on the biosynthetic machinery of the cell for replication. The strict cytoplasmic site of assembly of the infectious
particle with minimal budding from plasma membranes, the lack of infectivity in antigen-laden nuclei and the suggestion that the agent spreads only from parent to progeny cell are properties that have been observed individually among other viruses. However, their combined expression in the life cycle of BDV makes this a unique agent. These properties probably contribute to the remarkable neurotropic nature of the virus and its propensity for spread from neuron to neuron via interconnecting cytoplasmic (dendritic) processes.

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REFERENCES


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