Adaptation of Borna Disease Virus to the Mouse

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SUMMARY

Borna disease virus has been adapted to the mouse, which required at least three passages in rat brains. Genetic specificity as studied with five inbred mouse strains was not evident. Newborn mice inoculated intracerebrally expressed antigen in neurons and remained persistently infected, with up to $10^7$ infectious units per gram of brain tissue. Animals infected at different ages developed no disease and had high titres of antibodies.

The mouse has been regarded as resistant to Borna disease (BD) virus infection (Traub, cited by Zwick, 1939; Nitzschke, 1963; Heinig, 1969; Ludwig & Becht, 1977). Only two reports described transmission of virus with expressed disease (Nicolau & Galloway, 1927, 1928). Since a mouse model may facilitate study of persistent infection or slow progressing diseases in many ways (Brinton & Nathanson, 1981; Buchmeier et al., 1980; Kimberlin, 1981; Lehmann-Grube et al., 1983; Mahy et al., 1982; Pearson & Mims, 1983; Wege et al., 1982), renewed efforts were made to infect this small animal following our induction of a persistent, tolerant or subacute infection in the rat (Hirano et al., 1983).

Infection of newborn or adult mice with wild-type or rabbit-adapted BD virus had always failed. Preliminary results that mouse neuronal cultures expressed specific antigen after inoculation with rat-adapted virus (Tsukamoto & Ludwig, 1983, and unpublished data) encouraged us to inoculate mice again with a variety of strains (Table 1). Ten% (w/v) brain or eye suspensions were prepared in Dulbecco's modified Eagle's medium and the supernatants (after centrifugation at 1000 g for 10 min) were used for infectivity assays in newborn rabbit brain cells. A fluorescent focus assay (titres given in f.f.u./ml) with cells seeded onto round glass coverslips in 24-well plastic plates (Nunc) was employed (Hirano et al., 1983). Additionally, an immunoassay, based on horseradish peroxidase-coupled antispecies specific immunoglobulin reacted with 3-amino-9-ethylcarbazole (substrate), was introduced for testing infectivity and antibodies. The procedure was as follows. Cells in 24-well or 96-well microtitre plastic plates were infected and 5 days later fixed with 3% formaldehyde in phosphate-buffered saline (PBS). The cells were treated with 1% Triton X-100 in PBS for 0.5 h and after several washes with PBS plus 1% foetal calf serum overlaid with appropriately diluted rabbit anti-BD virus serum (Pauli & Ludwig, 1981) for infectivity assays. For antibody testing the cells were incubated with the diluted mouse serum for 1 h at room temperature, washed three times, overlaid for 1 h with the appropriate dilution of enzyme-linked anti-immunoglobulin (Paesel, Frankfurt, F.R.G), again washed three times, and the enzyme-substrate reaction was performed. Positive foci turned reddish-brown. The serum dilution clearly staining the foci (Fig. 2b) represents the titre. Neutralization titres were determined as outlined previously (Hirano et al., 1983).

In order to study BD in the mouse, newborns were infected intracerebrally (i.c.) with 0.02 ml of various virus-containing suspensions. Strikingly, the fourth and subsequent rat passages initiated infections in mouse brains. This key experiment has been reproduced three times. Further mouse passages resulted in rising virus titres and an appearance of virus in the retina. Surprisingly high titres were detectable after rat to chicken passages (Table 1). Prompted by the initial studies with STU mice (Schäfer, 1979), 1000 f.f.u. (rat passage 5 brain suspension) were inoculated into newborn mice of different inbred strains (received from the Zentralinstitut für
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**Fig. 1.** Virus titres (■) and weight curves of BALB/c mice mock-infected (○) or infected with BD virus (●). Newborns inoculated i.c. with $2 \times 10^3$ f.f.u./animal (5th mouse passage) were sacrificed at the given intervals and the mean infectivity titres of 10% brain suspension from three or four individually tested animals are given. Each point (weight curve) is the mean from 11 infected or 10 mock-infected mice. The experiments have been repeated with similar results.

**Table 1. Response of newborn mice to BD virus infection***

<table>
<thead>
<tr>
<th>Origin of strain</th>
<th>Virus titre† in</th>
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<tbody>
<tr>
<td></td>
<td>Species Code</td>
<td>JKG</td>
</tr>
<tr>
<td>Horse§</td>
<td>NAS</td>
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<tr>
<td>Rabbit†</td>
<td>St. V</td>
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</tr>
<tr>
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<td>Pass. 3 (7C)</td>
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</tr>
<tr>
<td>Rat**</td>
<td>Pass. 4 (7D)</td>
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<tr>
<td>Rat</td>
<td>Pass. 6 (7F)</td>
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<tr>
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<td>Mouse</td>
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<tr>
<td>Chicken‡‡</td>
<td>Pass. 2 (TM2)</td>
<td>5.3</td>
</tr>
</tbody>
</table>

* Experiments have been repeated at least once with similar results.
† Titrations were all done in JKG (Junges Kaninchen Gehirn) cells; geometric means of titres determined by tissue culture assays and given as $\log_{10}$ f.f.u./ml; three or more brains were titrated individually; standard deviation $\leq0.2$.
‡ Brain or eye suspensions of mice killed 2 weeks after infection were titrated.
§ Wild-type strain (Ammonshorn preparation; Gosztonyi & Ludwig, 1984).
†† Rabbit-adapted strain originating from a horse (Zwick et al., 1929); unknown passage number.
ND, Not done.
** Rat-adapted strain (Hirano et al., 1983).
‡‡ Mouse passages (all done in BALB/c) originating from the rat-adapted strain 7F.
+++ Strain originating from the rat-adapted strain 7F.
Fig. 2. (a) Mouse occipital cortex, 10 days after i.c. inoculation of BD virus. Several strongly immunoreactive neurons may be seen. (b) Culture of primary newborn rabbit brain cells (JKG cells) infected with BD virus and showing an immunoreactive focus. (c) Electron micrograph of the cytoplasm of a neuron with stacks of filaments (arrows); the upper one is attached to a cytoplasmic cistern. (d) Cross-section of the retina showing immunoreactive neurons in the ganglion cell layer (arrowheads) and internal granular layer (empty arrowheads). Due to the rich chromatin content of the external granular cells the few antigen-containing nuclei are not distinguishable; rods and cones (small arrows) are filled with antigen. (e) Gasserian ganglion. Several heavily labelled sensory ganglion cells and short, positively labelled axonal segments (arrows) may be seen. Bar markers represent (a, b) 50 μm, (c) 100 nm, (d, e) 25 μm.
Versuchstiere, Hannover, F.R.G.). Two weeks later, the animals had the following virus titres: DBA/2J Han, $2 \times 10^4$ f.f.u./ml; C3H/He Han, $3 \times 10^4$ f.f.u./ml; C57BR/Cd Han, $3 \times 10^4$ f.f.u./ml; BALB/cAn Han, $5.9 \times 10^4$ f.f.u./ml; BALB/c and STU, $9 \times 10^4$ f.f.u./ml. These data indicate no genetic specificity. Subsequently, all mouse passages were done in BALB/c mice. A total of 300 animals were inoculated as newborns or in age groups at 1, 2, 3 or 4 weeks after birth; none of them ever acquired disease. At present the observation time covers 10 months.

Infectious virus appeared early after inoculation, reaching a maximum 2 to 3 weeks after infection. The titre remained constant over some months (Fig. 1). It was of interest that the weight increase of the infected animals slowed down 3 weeks after birth, but later on paralleled the weight of control mice, although at a slightly lower level (Fig. 1).

Antibodies were present in 95% of the mice checked at approx. 4 weeks or later. These antibodies stained intranuclear and intracytoplasmic structures of tissue culture cells (Fig. 2b), but did not neutralize. Maximal titres of $1:40000$ could be reached as early as 4 months post-infection. Only 8 to 10% of the mice harboured neutralizing antibodies (150 animals tested). Titres of $1:10$ (at approx. 3 months), $1:40$ (at approx. 5 months) and in two cases $1:320$ (at 8 months) were measured.

Neuropathological investigations followed perfusion fixation (Karnovsky, 1965; Somogyi & Takagi, 1982). The central nervous system (CNS) and eyes were embedded in paraffin and Araldite and processed for electron microscopy (EM). Immunohistology was performed on paraffin sections using the biotin–avidin system (Vectastain, Vector Laboratories, Burlingame, Ca., U.S.A.). A rabbit hyperimmune serum directed against the major BD virus-specific antigen (Pauli & Ludwig, 1981) served as primary antibody. Antigen could be demonstrated extensively in the neurons of numerous brain areas and in the retina (Fig. 2). It was localized in the nucleus, perikaryon, dendrites and axons of neurons and appeared in the retina (Fig. 2d) and cerebellum later in the course of infection than in the cerebral hemispheres and trigeminal ganglia (Fig. 2a, e), which supports the hypothesis of a neural (axonal) spread of BD virus inside the CNS (Krey et al., 1979). EM examination of the brain revealed small accumulations of fine filaments with a diameter of 3 to 5 nm associated preferentially with cytoplasmic cisternae (Fig. 2c). Similar structures have been observed in brain cells of experimentally infected rats and rabbits as well as naturally infected horses (Gosztonyi & Ludwig, 1984; S. Sasaki, unpublished results). By light microscopy no inflammatory signs were seen in the brains or eyes of the majority of the infected animals (25 mice tested) and only 3% of the animals showed a few small perivascular lymphomonocytic infiltrates and a slight leptomeningitis.

These experiments on BD virus infection in the mouse have revealed three important new findings: (i) adaptation of the virus to newborns, (ii) induction of persistent infection without signs of disease, whatever age group is infected, and (iii) demonstration of an immune response including the production of neutralizing antibodies. Obligatory passage of this virus through another rodent, the rat (Hirano et al., 1983), may explain why transmission of this virus to the mouse had failed earlier. The mouse model parallels in some aspects infections of the young rat (Nitzschke, 1963; Hirano et al., 1983; Narayan et al., 1983) and the adult hamster (Anzil et al., 1973). In these animals, however, no adaptation of the virus was required. In contrast to these reports, we continuously found relatively high titres of neutralizing antibodies in the rat and now in some of the infected mice as well, although the significance of this antibody in the pathogenesis of the infection is not clear.

The adaptation of BD virus to the mouse enables us to exploit the potential of this genetically well-defined host together with its immunological capabilities. Observations on this basis will certainly provide interesting data regarding the mechanisms of tolerant infection within the CNS.

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REFERENCES

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