Persistent, Tolerant or Subacute Infection in Borna Disease Virus-infected Rats

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SUMMARY

The rabbit-adapted Borna disease (BD) virus strain V was passaged by intracerebral infection of 1-day-old Wistar rats. Infectivity titres reached $10^8$ infectious units per gram of brain 4 weeks after infection. No clinical signs were evident. The persistent infection could be induced with adapted or field strains of BD virus. Strains were identified by neutralization tests. The virulence of the rabbit-adapted BD virus for the rat increased with rat passages. The 5th passage induced clinical symptoms in animals infected at 1 week of age or older. Between 20% and 50% of diseased rats died. Virus-specific antigen was detectable immunohistologically in neurons of rats infected at all ages. Animals inoculated at 1 or 2 months of age, but not the neonatal rats, showed signs of inflammation in the brain. Infected rats produced specific antibodies. In the older groups (infected at ages of 1 or 2 months), and especially in surviving animals, occasionally, neutralizing antibodies with high titres were found. Transfer of primed spleen cells resulted in subacute disease. These findings demonstrate that neonatal rats can acquire a persistent, tolerant infection and that expression of disease is mediated by immunological factors.

INTRODUCTION

Elucidation of persistence in viral infections of the nervous system remains a major scientific challenge (Mims, 1974; ter Meulen & Hall, 1978; Johnson, 1982; Mahy et al., 1982), which can successfully be approached by investigating different animal species infected with the same virus. Borna disease (BD), usually associated with encephalomyelitis, represents an excellent example, where the same virus causes various disease manifestations in different animal species (Ludwig & Becht, 1977).

Transmission of the natural agent from diseased horses and sheep to a variety of experimental animals, including the rat, has been achieved (Nicolau & Galloway, 1928; Zwick, 1939; Ludwig et al., 1973; Stitz et al., 1980). Growth of BD virus in tissue culture allowed the quantification of the major specific antigen (Ludwig et al., 1973; Pauli & Ludwig, 1981) and of infectious material (Danner et al., 1978; Herzog & Rott, 1980; Ludwig et al., 1981). The virus itself, however, remains one of the few conventional, unclassified agents (Matthews, 1982).

After adaptation of wild-type virus to the rabbit, this animal was regarded as the most sensitive and suitable model for studying clinical and immunopathological aspects of the disease (Ludwig et al., 1977; Krey et al., 1979, 1981, 1982; Gierend & Ludwig, 1981). In the search for a small-animal model, the mouse generally resisted infection, whereas the rat was susceptible (Nicolau & Galloway, 1928; Zwick, 1939). Extensive studies by Nitzschke (1963) in the rat considerably broadened the existing biological knowledge already gained in the rabbit, and pointed to mechanisms that are nowadays discussed as aspects of persistent infections (Mims, 1982).
In this report the neonatal rat, infected at the age of 1 day, was used for the study of BD. Emphasis was placed on virus growth, on the disease produced and on the identification of passage virus. Efforts were undertaken to convert the persistent infection from the inapparent to the subacute form.

METHODS

Viruses. The rabbit-adapted BD virus, strain V (Nitzschke, 1963; Ludwig et al., 1973), was used. Brain suspensions from two horses, which had recently died with symptoms of BD, served as wild-type virus preparations (strains NAS and KÜNZ).

Animal experiments. Wistar (inbred) rats of different ages and in the late stage of pregnancy were obtained from the breeding colony of the Bundesgesundheitsamt (Berlin, F.R.G.). Pregnant Brown Norway and Lewis rats were kindly supplied by the Deutsche Krebsforschungszentrum (Heidelberg, F.R.G.). The sucklings were nursed by their dams. Six-week-old, outbred, uniformly grey coloured, rabbits were taken from the breeding colony of our own Institute. The animals had free access to commercial food-pellets and water. Virus suspensions were inoculated intracerebrally (i.c.) into the left hemisphere using 0.02 or 0.4 ml of inoculum for rats or rabbits, respectively. Severely diseased animals were exsanguinated and the brain was removed and processed aseptically.

Preparation of virus and organ cell suspensions. Organs were stored at −70 °C. Ten per cent suspensions (w/v) in Eagle’s medium, Dulbecco’s modification (EDM) were prepared by sonicating (Branson sonifier, 20 cycles, 40 mA, at 4 °C, 1 min total) of the total brain (rats), pieces of the Ammon’s horn (rabbit, horse), minced organ preparations or of the total retina, collected under the stereomicroscope from the equatorially sectioned eye-ball. After centrifugation (10 min, 1000 g) the supernatant was stored at −70 °C and used for animal or tissue culture inoculation (Ludwig et al., 1973). For transfer experiments with spleen cells, the organisms were removed aseptically, placed in 60 mm plastic Petri dishes containing 2 ml EDM, cut into small pieces and disrupted by grinding with the top of an inverted 50 ml plastic centrifuge tube (Falcon). Repeated pipetting and filtration through sterile gauze pads furnished single cell suspensions. The total, or half spleen cell harvest was immediately inoculated intraperitoneally (i.p.) into rats.

Virus assay. Infectivity tests were performed using the second passage of primary newborn rabbit brain cells, which were grown in EDM, supplemented with 10% inactivated foetal calf serum (Ludwig et al., 1973). The test is a fluorescent focus assay described by Porter et al. (1977). Cells were seeded on round glass coverslips, which were placed in a 24-well plastic plate (Nunc) and incubated with 0.1 ml of 10-fold dilutions of virus suspension, followed by a medium change 1 day later. Five days later, the cells were dried, fixed in acetone and submitted to a standard indirect fluorescence antibody (FA) test (see below). The stained foci were counted per coverslip and the titre was expressed as focus-forming units (f.f.u.) per ml.

Production of antisera. Rats (5 months of age) were hyperimmunized by five i.p. injections at weekly intervals, using 0.5 ml total rat brain suspension (1 × 10^6 f. f.u.) as inoculum. They were exsanguinated 10 days later. A rabbit pool serum (pool A) which recognizes the major BD virus-specific antigen (Pauli & Ludwig, 1981) up to a FA titre of 1 : 128, consisted of five individual rabbit sera. The animals were BD virus-infected and had died 3 to 4 weeks after infection. A rabbit serum (HF1) was available (FA titre 1 : 128) from an animal which had been infected through contact with diseased animals and died after a prolonged disease period at day 180; at day 0 this animal had joined a group of severely sick BD rabbits. Another rabbit serum (no. 11) came from a BD virus-infected animal which had survived (FA titre 1 : 128). Ten sera from horses which had died with symptoms of BD (Ludwig & Thein, 1977) were also included in the studies.

Serological tests. The indirect fluorescence antibody (FA) test was performed as described earlier (Ludwig & Thein, 1977). Rabbit cells (on coverslips) which had been infected with 30 to 50 infectious units (see Virus assay) were used at day 6 post-infection. They were dried, acetone-fixed and stored under vacuum. The anti-species specific fluorescein isothiocyanate (FITC)-coupled antibodies (Behringwerke, Marburg, F.R.G.) were filtered before use (working dilution 1 : 10). The titre is given as the dilution of antibody still showing a clear positive antigen accumulation in the nucleus and cytoplasm of the cells forming a focus.

For neutralization tests the virus was diluted to give 30 to 50 foci per coverslip. It was incubated for 1 h at 37 °C with geometrically diluted inactivated serum in a final volume of 0.1 ml. The incubation mixture was then inoculated for virus assay. The test was performed as a plaque reduction test (Pauli & Ludwig, 1977). The test was evaluated, 5 to 6 days post-infection, with the help of the indirect FA test. The serum dilution leading to 50% focus reduction is given in all neutralization experiments.

Immunohistology and histopathology. Frozen sections (6 μm) were fixed in cold acetone for 5 min, incubated with rabbit pool A serum at 37 °C for 60 min and then overlaid with FITC-coupled goat anti-rabbit IgG. Furthermore, 18 rat brains were fixed by perfusion with Karnovsky’s solution and the brain slices were embedded in paraffin. Brain sections were stained with haematoxylin and eosin (H and E). Antigen was demonstrated on paraffin sections by the peroxidase–anti-peroxidase technique, according to Sternberger (1979).
RESULTS

Establishment of rat-adapted BD virus

In a series of preliminary experiments the optimal age for virus replication in Wistar rats was determined. Infectious virus was first detected (1 week after infection) in animals inoculated at the age of 1 day. Rats inoculated when 1 or 2 months old harboured 100-fold less infectious virus in the brain (Table 1). The rabbit-adapted virus did not induce disease during an observation period of 8 weeks, whatever age group had been inoculated, indicating that it was not virulent for the rat. Additional passages were made in 1-day-old rats. Virus replicated with essentially the same growth patterns, and titres were in the same range as in the initial experiments (Table 1). Interestingly, all the retina suspensions had infectivity titres between $10^3$ and $10^4$ f.f.u./ml, whereas no virus could be detected in organ suspensions of the thymus, spleen, liver and kidneys. The 5th rat brain passage was prepared as seed virus for further experiments. To exclude major genetic restrictions in BD virus growth, 1-day-old Brown Norway or Lewis rats were inoculated. The 3-week-old sucklings had brain titres ranging between $10^5$ and $10^6$ f.f.u./ml. Subsequently, only Wistar rats were used.

Field strains of BD virus originating from the horse with titres of $5 \times 10^3$ (strain NAS) or $1 \times 10^4$ (strain KÜNZ) f.f.u./ml, respectively, replicated to titres of $8 \times 10^4$ or $3.2 \times 10^4$ f.f.u./ml of rat brain suspension and $10^2$ or $1.5 \times 10^2$ f.f.u./ml of retina suspension, when tested in 1-day-old rats and assayed 3 weeks later. This demonstrates the universality of BD virus growth in the neonatally infected rat.

In further experiments the response of 1-day-old rats to different inoculum doses was tested (Table 2). BD virus replicated to a titre of $10^6$ f.f.u./ml rat brain suspension, regardless of whether 10000 or 1 infectious unit had been injected. This demonstrates that the 1-day-old rat

<table>
<thead>
<tr>
<th>Passage*</th>
<th>Age</th>
<th>Infectivity at weeks post-infection†</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1 day</td>
<td>2.4  5.2  6.0</td>
</tr>
<tr>
<td>1</td>
<td>1 month</td>
<td>&lt;1.0  4.5  4.0</td>
</tr>
<tr>
<td>2</td>
<td>1 month</td>
<td>&lt;1.0  2.0  4.0</td>
</tr>
<tr>
<td>3</td>
<td>1 day</td>
<td>3.1  5.4  6.4</td>
</tr>
<tr>
<td>4</td>
<td>1 day</td>
<td>NT†  5.4  6.3</td>
</tr>
<tr>
<td>5</td>
<td>1 day</td>
<td>NT†  5.6  6.0</td>
</tr>
</tbody>
</table>

* Passage 0; $1 \times 10^3$ f.f.u. of rabbit-adapted virus were used for inoculation into rats.
† Geometric means of titres determined by tissue culture assays (see Methods) and given as log_{10} f.f.u./ml; standard deviation $\leq 0.2$. Three infected brains were titrated individually. The titrations have been repeated with similar results.
‡ NT, Not tested.

<table>
<thead>
<tr>
<th>Inoculum (f.f.u.)</th>
<th>Infectivity at weeks post-infection*</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^4$</td>
<td>5.0  5.0  6.0</td>
</tr>
<tr>
<td>$10^3$</td>
<td>5.2  6.0  6.0</td>
</tr>
<tr>
<td>$10^2$</td>
<td>3.8  5.7  6.1</td>
</tr>
<tr>
<td>$10^1$</td>
<td>3.3  5.3  6.0</td>
</tr>
<tr>
<td>$10^0$</td>
<td>&lt;1.0  5.3  6.2</td>
</tr>
</tbody>
</table>

* Titrations as given in legend to Table 1. This experiment has been repeated with similar results. In control experiments 1-day-old rats were inoculated with normal rat brain suspension and no BD virus could be demonstrated.
Table 3. Response of rats inoculated at different ages to infection with rat-passaged virus*

<table>
<thead>
<tr>
<th>Age</th>
<th>CNS/tested</th>
<th>Mortality</th>
<th>Brain titre</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 day</td>
<td>0/20‡</td>
<td>0/20‡</td>
<td>5.8§</td>
</tr>
<tr>
<td>1 week</td>
<td>5/20</td>
<td>2/20</td>
<td>5.4</td>
</tr>
<tr>
<td>2 weeks</td>
<td>20/20</td>
<td>2/20</td>
<td>5.4</td>
</tr>
<tr>
<td>3 weeks</td>
<td>20/20</td>
<td>4/20</td>
<td>4.5</td>
</tr>
<tr>
<td>4 weeks</td>
<td>20/20</td>
<td>3/20</td>
<td>4.5</td>
</tr>
</tbody>
</table>

* Inoculum dose: $1 \times 10^4$ f.f.u. of passage 5; animal observations were made over 4 weeks. The repetition of this experiment gave similar results.

† No. of cases showing CNS symptoms typically for BD/no. tested.
‡ No. of dead rats/no. tested, 4 weeks post-infection.
§ Titrations as given in legend to Table 1; three individual brains of the surviving rats were titrated 4 weeks after infection.

represents a highly sensitive system for the assay of BD virus, since 1 infectious unit can not be detected by tissue culture assay, and does not kill the rabbit (T. Leiskau & H. Ludwig, unpublished).

Response of different age groups to rat-passaged virus

Since older rats were reported to be more susceptible to BD (Nicolau & Galloway, 1928; Zwick, 1939; Nitzschke, 1963), animals ranging between 1 day and 4 weeks of age were infected using the 5th rat passage virus (Table 3). At 2 weeks post-infection, rats of all age groups (except the 1-day-old group) became sensitive to sound and light and some of them exhibited clinical symptoms consisting of abnormal movements, ataxia and convulsions. In the following 2 weeks, disease symptoms increased and 100% of the rats inoculated at an age of 2 or more weeks developed central nervous system symptoms; some animals died. The surviving rats harboured virus in the brain (Table 3). In contrast to the original virus (rabbit-adapted strain) used for passaging in rats, the fifth passage was able to induce disease. This indicates a gain of virulence for the rat.

In more detailed studies three groups of rats, inoculated at 1 day, 1 month and 2 months of age, were monitored over several months for expression of disease, for infectious virus and virus-specific antigen.

After 3 to 4 weeks post-infection, all the 1-month-old rats and approx. 50% of the 2-month-old animals developed clinical signs. These were more severe in the 2-month-old than in the 1-month-old group. Some of the severely diseased animals appeared to be blind, as was reported for rabbits (Krey et al., 1979) and approx. 20% of the rats of each group died from encephalitis. However, all the neonatally infected rats were free of any symptoms, even with high virus titres in the brain.

The mortality rates estimated 4 months post-infection were approx. 20% and 50% in animals which had been infected at the age of 1 month or 2 months, respectively. It is of considerable interest that about 30% of the animals (both groups) developed an obesity syndrome (our unpublished observation). No clinical signs or deaths were observed in neonatally infected rats.

Growth curves of BD virus in these three rat groups show that virus titres reached a plateau 3 or 4 weeks post-infection, when the 1-month- and 2-month-old groups developed clinical signs. Titres remained constant over 3 months (Fig. 1). They reflect the dependence on the age of the host. Seven months post-infection, average titres of $10^4$ and $10^2$ f.f.u./ml were still detectable in the healthy neonatally infected rats and the survivors of the group infected when 1 month old, respectively. No virus was found in rats inoculated at an age of 2 months (data not shown).

To find the replication sites of virus in the brain, immunohistological studies were done during the exponential growth phase (at 2 weeks post-infection), and late (4 weeks post-infection) in five animals per age group. Early after infection, specific antigen of granular or spherical appearance, located only in the nucleus, was present in neurons of the cortex, hippocampus, in the choroid plexus and ependyma. Diffusely spread antigen was prominent in the cytoplasm, dendrites and axons of neurons at late stages (Fig. 2). Animals inoculated with
Persistent infection by Borna disease virus

Fig. 1. Borna disease virus growth in the brains of rats inoculated i.c. with the 5th rat passage virus (10^4 f.f.u./rat) when 1 day (●), 1 month (○) and 2 months (■) old. At the given intervals the mean infectivity titre of a 10% brain suspension from three or four individually assayed animals of each age group was determined. The experiments have been repeated with similar results; in one experiment maximum titres of 7 × 10^6 f.f.u./ml were reached.

Fig. 2. Borna disease virus-specific antigen appears in spherical form in the nucleus (small arrows) and diffuse in the perikaryon (large arrows) and dendrites of brain neurons. Rat infected at 1 month of age and investigated 4 weeks later. Cells of the pyramidal cell layer (CA3) in the hippocampus are shown. Rabbit pool A serum (dilution 1:100; see Methods) was used in the peroxidase-anti-peroxidase technique. Magnification ×425.

normal rat brain suspension were negative. Conventional histological studies made from animals 12 weeks after infection revealed that the rats inoculated at 1 or 2 months of age showed the typical alterations described for horses (Seifried & Spatz, 1930) or rabbits (Roggendorf et al., 1983). These are microglial proliferation and inflammatory haematogenous infiltrations in the
meninges, in the perivascular and sub-ependymal areas (Fig. 3a, b) and neuronophagia. There were no signs of demyelination. The picture clearly contrasted with those of neonatally infected animals, where, as a rule, no inflammatory response could be detected.

In persistently infected rats, antigen could be detected in all layers of the retina, with the highest amounts in the ganglion cell layer (Fig. 4). In older rats which had shown clinical signs,
Persistent infection by Borna disease virus

Fig. 4. Borna disease virus-specific antigen in the retina of the rat. The three inner layers are represented. Antigen-containing cells are shown (arrows) in the ganglion cell layer. The peroxidase-anti-peroxidase technique was used (conditions as in Fig. 2). A persistently infected rat was investigated 6 weeks after infection. Magnification × 605.

Table 4. Response of 6-week-old rabbits (one litter) to rat-passaged and rabbit-passaged BD virus*

<table>
<thead>
<tr>
<th>Virus (f.f.u.)</th>
<th>CNS/tested†</th>
<th>Mortality‡</th>
<th>Virus isolation§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat passage 5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 × 10⁵</td>
<td>2/2</td>
<td>2/2 (17, 19)</td>
<td>2/2</td>
</tr>
<tr>
<td>10³</td>
<td>2/2</td>
<td>2/2 (23, 24)</td>
<td>2/2</td>
</tr>
<tr>
<td>10¹</td>
<td>1/2</td>
<td>1/2 (28)</td>
<td>1/2</td>
</tr>
<tr>
<td>Rabbit passage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 × 10⁵</td>
<td>2/2</td>
<td>2/2 (18, 25)</td>
<td>2/2</td>
</tr>
</tbody>
</table>

* Inoculum: 0.5 ml, animal observations were made for 4 weeks.
† No. cases showing CNS symptoms/no. tested.
‡ No. dead rabbits/no. tested (days of animal death after inoculation).
§ No. positive/no. tested in tissue culture. In none of the individual total rabbit brain suspensions did the infectivity titres exceed 10⁴ f.f.u./ml. The one surviving rabbit had < 10¹ f.f.u./ml in the brain. The efficiency of virus assay in vitro is the same for both rat- and rabbit-passaged virus.

the retina was heavily damaged and infiltrated by inflammatory cells. The histological picture was similar to that of BD virus-infected rabbits (Krey et al., 1979).

Characterization of the rat-passaged virus

Pathogenicity studies revealed that the rat virus kept its virulence for the rabbit. Nevertheless, the same infectious rat material inoculated either into rabbits or rats showed a different effect. Ten f.f.u. did not induce signs of BD virus infection in all the rabbits, nor was virus replicated to measurable titres (Table 4). In the neonatal rat, however, 1 f.f.u. always replicated to high virus titres (Table 2).

Since identity of passaged virus with wild-type virus or field virus remained a major concern in experimentation with this unclassified agent, cross-neutralization experiments were per-
formed. Heat-inactivated rat sera (no. 4 and 9) with FA titres of 1:40 and 1:80 neutralized rat virus at dilutions of 1:400 and 1:600, and horse virus at dilutions of 1:300 and 1:800, respectively. Furthermore, two rabbit sera (HF1 and no. 11) neutralized rat virus at dilutions of 1:256 and 1:1800. Finally, one out of 10 sera derived from naturally infected horses neutralized rat virus at a dilution of 1:500.

**Antibody response and disease**

Since earlier reports had demonstrated that rats produced BD virus-specific antibodies (Nitzschke, 1963; Ludwig et al., 1973) it was of major interest to determine whether the neonatally infected animals would produce a humoral immune response. From 35 sera collected 2 to 3 months after infection, 50% had FA titres ≤ 1:64. Five of these animals exhibited neutralizing serum titres of 1:8 to 1:16. In contrast, all 12 rat sera, obtained from surviving rats 5 to 8 months after infection of 1- or 2-month-old animals, showed FA titres between 1:128 and 1:256, and neutralizing titres up to 1:400 in individual animals from the 20% positively reacting rats. Five rats infected as adults (3 to 5 months) and killed 18 and 30 months after infection harboured serum neutralizing titres of 1:200 to 1:800. Rat hyperimmune sera (four animals) reached titres of 1:2000.

To clarify the influence of antibodies and immune cells on disease, three groups of five rats infected at day 1 after birth received either 2 ml each of rabbit serum no. 11 (neutralizing titre 1:1800) or rabbit pool A serum (neutralizing titre < 1:4), or normal rabbit serum (neutralizing titre < 1:4), at 2-week intervals beginning 4 weeks post-infection. None of the animals acquired disease during an observation period of 2 months.

Spleen cell preparations of 5-month-old rats which had been infected at 1 month of age were inoculated i.p. into persistently infected rats (animals infected neonatally and treated 4 weeks later). The two rats, which had each received the cell equivalent of a total spleen, showed typical progressing clinical disease 4 to 5 weeks later. Four rats which had received half this number of cells became clinically ill approx. 6 weeks later. The spleen cell-treated rats harboured lymphocytic infiltrations in the brain (compare Fig. 3a). Such lesions were absent in rats treated with antibodies or receiving corresponding amounts of control spleen cells. These animals had remained without signs of disease.

**DISCUSSION**

The establishment of persistent tolerant BD virus infection using the neonatal rat as animal model extends and revises the limited studies of Nicolau & Galloway (1928) and the broad investigations of Nitzschke (1963), who could not reliably measure virus titres at that time, and did not identify rat-adapted strains. All these BD virus strains have been lost (E. Nitzschke, 1980, personal communication).

Our present knowledge on BD together with the published work of others (Zwick, 1939; Nitzschke, 1963) point to analogies between this disease and lymphocytic choriomeningitis (LCM) of the mouse (Traub, 1936; Rowe, 1956; Hotchin et al., 1962; Fenner et al., 1974). Like LCM virus in the mouse, BD virus remains persistent in the rat infected soon after birth, although with certain exceptions (Hotchin et al., 1962; Oldstone & Dixon, 1969; Lehmann-Grube, 1982). None of the hundreds of rats infected with BD virus, and observed in the course of persistent infection, acquired clinical disease during an observation period of 8 months, provided that the animals had been inoculated within the first 24 h after birth. Furthermore, the age dependency of BD and LCM is striking. Similarities can be seen in pathology, although the types of BD illness following different routes of inoculation in a defined host, like the rat, have to be investigated before further parallels with LCM can be drawn (Wallnerova & Mims, 1971; Lehmann-Grube, 1982). BD virus shows no cross-reactivity with LCM virus, since FA tests and neutralization experiments with a LCM virus-specific rabbit antiserum (kindly supplied by Drs M. Bruns and F. Lehmann-Grube) were negative (H. Ludwig & T. Leiskau, unpublished results).

The major dissimilarities are that BD virus *in vivo* can only be found in neural tissue and organs derived from the ectoderm. This was established initially by Nicolau & Galloway (1928)
and is supported by these rat experiments, and earlier rabbit experiments. Only brain and retina, but no other organ or lymphocytes, harbour virus (Krey et al., 1979; Ludwig et al., 1981; Gierend et al., 1982). The strong cell association of BD virus in vitro and in vivo, with no signs of cell alterations (Danner et al., 1978; Ludwig et al., 1981), which is unique for this agent, discriminates BD virus from LCM virus or other virus groups causing persistent infection (Mahy et al., 1982).

The most prominent parallels between infections by BD virus in the rat and LCM virus in the mouse are found in the immune response. Although rats infected neonatally with BD virus never showed clinical symptoms, they produced antibodies and even low titres of neutralizing antibodies. This is reminiscent of the situation in mice persistently infected with LCM virus, a finding which challenged the phenomenon of immunological tolerance (Buchmeier et al., 1980). Our experiments with spleen cell transfer and antibody treatment indicate the importance of cell-mediated immunity for expression of the disease. The lack of inflammatory infiltration in the brain of immunosuppressed rabbits (Gierend & Ludwig, 1981) or rhesus monkeys (Cervós-Navarro et al., 1982) demonstrated the immunological character of BD for the first time. In the rhesus monkey at least, disease could be correlated with the presence of cytotoxic T cells (Stitz et al., 1980). The results of the cell transfer experiments in persistently infected rats, which subsequently became ill, point in the same direction.

With the introduction of a small-animal model for studies of BD, the possibility of immunological studies in syngeneic animals becomes available. There seems to be no obvious genetic resistance in rat strains. BD virus infections in Lewis rats (O. Narayan, personal communication) support our findings. All the data collected up to now suggest that BD is a process where immunological factors are of paramount importance in tissue destruction and manifestation of disease. The neonatally infected rat has demonstrated persistent infection. Within the limitations previously discussed for LCM by Buchmeier et al. (1980) this can also be regarded as a tolerant infection. Studies on the mechanisms of persistence, still unsolved in better defined systems (Mims, 1982), can now be approached.

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