Effect of Borna Disease Virus Infection on Athymic Rats

By S. HERZOG, K. WONIGEIT, K. FRESE, H. J. HEDRICH and R. ROTT

Institut für Virologie and Veterinär-Pathologie, Justus-Liebig-Universität Giessen, D-6300 Giessen, Klinik für Abdominal- und Transplantationschirurgie and Zentralinstitut für Versuchstierzucht, D-3000 Hannover, Federal Republic of Germany

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SUMMARY

Homozygous athymic nude rats (rnu/rnu) infected intracerebrally with Borna disease virus produced relatively high titres of infectious virus in the central nervous system. However, no clinical signs of disease or pathological alterations could be found during a 100 day observation period. In contrast, heterozygous euthymic albino littermates (rnu/+) which were used as controls, reacted in a similar manner to immunocompetent Lewis rats. They developed behavioural alterations which coincided with encephalitis and retinitis. The results obtained confirm our previous concept that the genesis of Borna disease, at least in rats, is attributed to a cellular immune response.

INTRODUCTION

Studies on the pathogenesis of Borna disease (BD) virus infection in rats have revealed that the cause of the disease is a virus-induced immunopathological process. After intracerebral (i.c.) infection of immunocompetent rats the virus is strictly neurotropic and the resulting disease is characterized by a particular pattern of neurological disorders. Histologically, a meningoencephalitis and retinitis can be seen with infiltrations of mononuclear cells. If the immune response is inhibited by cyclophosphamide, BD virus-infected animals do not develop any signs of clinical disease or pathological reactions in spite of extensive virus replication. Similarly, in rats infected neonatally, the virus replicates persistently and productively in the central nervous system (CNS). These animals did not develop encephalitis or retinitis during the observation period of several months (Narayan et al., 1983a, b). When immune spleen cells are transferred to cyclophosphamide-treated animals, pathological changes comparable to those occurring in normal rats can be seen. In contrast, injection of immune serum does not induce the disease (Narayan et al., 1983a, b; Hirano et al., 1983; S. Herzog et al., unpublished results). Therefore, it is reasonable to assume that BD depends on a virus-specific cell-mediated immunopathological mechanism.

To confirm this assumption, we studied the pattern of BD virus infection in athymic nude rats (rnu/rnu). These animals do not possess a functional thymus, and the T-cell-dependent areas of their lymph nodes, spleen and Peyer's patches are depleted of lymphocytes. They are highly immunodeficient and can accept skin allotransplants (Festing et al., 1978; Brooks et al., 1980). It will be shown that the nude rat reacts analogously to immune-depressed animals, i.e. virus replicates in the CNS to high titres without inducing the disease.

METHODS

Virus. The Giessen strain He/80 of BD virus, which was originally isolated from a brain homogenate of a naturally diseased horse, was used. The virus was passaged twice in rabbit by i.c. infection, three times in cultural foetal rabbit brain cells and twice in newborn Lewis rats.

Animals. Homozygous, athymic nude rats (rnu/rnu) and their heterozygous littermates (rnu/+ ) of the Rowett rat strain as well as Lewis rats were bred in the Zentralinstitut für Versuchstierzucht in Hannover. The nude rats and their littermates are from an outbred colony. The animals were infected i.c. with about 10^4 ID_{50} (50% infective dose) per ml at the ages indicated in Results. They were examined 20, 35 and 100 days after infection. The rats...
were anaesthetized, and killed by heart puncture for serum collection. Brain samples and eyes were processed for infectivity tests and histological examinations as described by Narayan et al. (1983b).

**Infectivity test.** Samples for infectivity assay were collected on Glasgow modified MEM with 10% foetal bovine serum (FBS). Tissues were minced with scissors, sonicated, and clarified by centrifugation at 1000 g for 10 min. Tenfold dilutions of the homogenates were prepared in MEM plus 10% FBS and were mixed with equal volumes of freshly dispersed rabbit glia cells and transferred onto chamber slides (Lab-Tek). After incubation for 10 days at 37 °C the cell monolayers on these slides were fixed in acetone at -20 °C and inoculated with a rabbit antiserum to BD virus. After incubation for 30 min at 37 °C the cells were washed in phosphate-buffered saline (PBS) and allowed to react with fluorescein-labelled goat antiserum to rabbit IgG (Behringwerke AG, Marburg, F.R.G.).

**Antibody determination.** Twofold dilutions of sera were incubated with acetone-fixed preparations of MDCK cells persistently infected with BD virus (Herzog & Rott, 1980). After an incubation period of 30 min at 37 °C the cells were washed in PBS and reacted with fluorescein isothiocyanate-conjugated goat anti-rat IgG (Miles-Yeda, Rehovot, Israel) for 30 min.

**Virus neutralization.** Equal volumes of stock virus containing 100 ID₅₀ and twofold dilutions of post-infectious sera were incubated for 1 h at 37 °C and further incubated overnight at 4 °C. A similar preparation of virus mixed with normal rat serum was used as a control. The mixtures were tested for infectivity as described.

**Immunohistology.** Tissue specimens were coated with OTC-R compound (Miles Laboratories) and frozen on dry ice. Sections (5 μ) were cut in a Jung Frigocut (Heidelberg, F.R.G.) and fixed with acetone at -20 °C. The slices were stained with the indirect immunoperoxidase method using rabbit antisera to BD virus according to Burns (1975).

**RESULTS**

In a given host, clinical manifestations and pathological alterations induced by BD virus may vary depending on the origin of the virus preparation (S. Herzog et al., unpublished results). Therefore, the inoculum used in the series of experiments described here was re-examined using Lewis rats, to ensure that the effects of the virus corresponded to those previously described (Narayan et al., 1983a, b).

It was found that the animals showed an analogous reactivity after i.c. infection with the inoculum applied as described before (Table 1). At day 35 after i.c. inoculation infectious virus with an ID₅₀/ml of about 10⁸ or 10⁴ was found in the brain and retina, respectively. Using indirect immunofluorescence, high antibody titres could be detected in the serum, but even sera with fluorescent antibody titres of 1:2560 did not reduce infectivity in cell cultures. The rats developed clinical signs of disease such as hyperactivity, aggressiveness and mass mobility, starting at 20 days after infection. The onset of disease coincided with the appearance of encephalitis and retinitis. Besides perivascular, parenchymal and meningial infiltrations of mononuclear cells an extensive loss of neurons in the fascia dentata of the hippocampus, as well

<table>
<thead>
<tr>
<th>Animal</th>
<th>Clinical disease†</th>
<th>Antibody titre</th>
<th>ID₅₀/ml in Brain</th>
<th>Retina</th>
<th>Brain</th>
<th>Retina</th>
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<tbody>
<tr>
<td>rnu/rnu</td>
<td>0</td>
<td>0</td>
<td>6 × 10⁸ 2 × 10⁵</td>
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<td>0</td>
<td>2 × 10⁸ 6 × 10⁵</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>rnu/rnu</td>
<td>0</td>
<td>0</td>
<td>6 × 10⁸ 6 × 10⁵</td>
<td>0</td>
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<tr>
<td>rnu/+</td>
<td>+</td>
<td>1:2560</td>
<td>6 × 10⁸ 6 × 10³</td>
<td>++ +</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>rnu/+</td>
<td>+</td>
<td>1:2560</td>
<td>6 × 10⁵ 6 × 10³</td>
<td>++ +</td>
<td>+</td>
<td>+</td>
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<td>6 × 10⁶ 6 × 10⁴</td>
<td>++ +</td>
<td>+</td>
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</tr>
<tr>
<td>Lewis</td>
<td>+</td>
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<td>6 × 10⁶ 6 × 10⁴</td>
<td>++ +</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Lewis</td>
<td>+</td>
<td>1:5120</td>
<td>6 × 10⁶ 6 × 10³</td>
<td>++ +</td>
<td>+</td>
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</tr>
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* The rats were infected at 4 weeks of age and the different parameters of response were determined 35 days after infection.
† 0, No clinical disease, no antibody production, no inflammation observed; +, animals became sick.
‡ ++, ++ +, +++ +, Increasing degrees of inflammation.

Table 1. Reactions of athymic homozygous (rnu/rnu) and euthymic heterozygous (rnu/+ ) Rowett rats and Lewis rats following infection with Borna disease virus*
Fig. 1. Histology and immunohistology of brain and retina of athymic homozygous nude rats (rnu/rnu) after i.c. infection with BD virus. (a) Section of brain 100 days after infection without inflammatory lesions (haematoxylin and eosin, × 12). (b) BD virus antigen in hippocampal neurons of the same rat (indirect immunoperoxidase, × 230). (c) Control reaction of (b) with normal rabbit serum (× 230). (d) Retina 100 days after infection showing no lesions: 1, ganglionic layer; 2, inner nuclear layer; 3, outer nuclear layer; 4, photoreceptor layer; 5, pigment epithelium (haematoxylin and eosin, × 225). (e) Retina 100 days after infection. BD virus antigen was found in the ganglionic layer (1) and the inner (2) and outer (3) nuclear layers of the retina. 4, photoreceptor layer (indirect immunoperoxidase, × 275).
Fig. 2. Inflammation and viral antigen in brain and retina of euthymic heterozygous rats (rnu/+ ) after i.c. infection with BD virus. (a) Massive encephalitis with perivascular and parenchymal mononuclear infiltration in the cerebral cortex 35 days after infection (haematoxylin and eosin, ×135). (b) Intranuclear BD virus antigen in cortical neurons of the same rat (indirect immunoperoxidase, ×500). (c) Retinitis with mononuclear infiltration of the ganglionic layer, complete loss of the photoreceptors and partial reduction of the nuclear layers 35 days after infection (haematoxylin and eosin, ×420). (d) Retina 35 days after infection. BD virus antigen was found in cells of the inner (1) and outer (2) nuclear layers; 3, photoreceptor layer (indirect immunoperoxidase, ×480).
BD virus infection in athymic rats

Fig. 3. (a) Hydrocephalus ex vacuo in a heterozygous (rnu/+ ) Rowett rat 100 days after infection (haematoxylin and eosin, ×10). (b) Chronic retinitis with progressive loss of the photoreceptors and nuclear layers in a heterozygous (rnu/+ ) Rowett rat 100 days after infection; 1, remnants of the retina; 2, sclera (haematoxylin and eosin, ×300).

Table 2. Responses of athymic homozygous (rnu/rnu) and euthymic heterozygous (rnu/+ ) Rowett rats of different ages to infection with Borna disease virus

<table>
<thead>
<tr>
<th>Animal</th>
<th>Age of animal (months)</th>
<th>Clinical disease</th>
<th>Antibody production</th>
<th>Virus replication in</th>
<th>Histopathological changes in</th>
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</thead>
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<td>rnu/rnu</td>
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<td>0/2*</td>
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<td>2/2</td>
<td>0/2</td>
</tr>
<tr>
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<td>4/4</td>
</tr>
<tr>
<td>rnu/rnu</td>
<td>5</td>
<td>0/5</td>
<td>0/5</td>
<td>5/5</td>
<td>0/5</td>
</tr>
<tr>
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<td>5</td>
<td>4/4</td>
<td>4/4</td>
<td>4/4</td>
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</tr>
</tbody>
</table>

* No. positive/no. infected.

as in the cerebral cortex, was observed. In later stages hydrocephalus ex vacuo developed. The eyes showed a non-purulent retinitis with a progressive loss of photoreceptors and cells of the inner and outer nuclear layers.

In contrast, homozygous athymic rats (rnu/rnu) did not show any clinical reaction, when infected i.c. at 4 weeks of age with the same virus preparation. No signs of inflammation could be seen either in the brain or in the retina in spite of virus replication in these organs as shown by immunohistological examinations (Fig. 1). Infectivity titres in the brain and in the retina were similar to those found with Lewis rats (Table 1). As expected, no virus-specific antibodies could be demonstrated by immunofluorescence during a 35 day observation period.

It has been shown that at the age of about 3 months T-lymphocytes appear in the lymph nodes but not in the spleen of nude rats (Wonigeit & Hedrich, 1982; Schwinzer et al., 1984). Therefore, animals at the age of 1 and 5 months were infected i.c. with BD virus in order to determine whether these T-cells would influence the outcome of the disease. These animals were observed over a period of 100 days after infection. As shown in Table 2, nude rats of both age groups did not develop virus-specific antibodies, symptoms or pathological alterations in spite of virus replication.

However, when heterozygous euthymic albino littermates (rnu/+ ) were used for infection, the disease followed its regular course irrespective of age (1 or 5 months) of the animals. Like
Lewis rats, these animals developed encephalitis and retinitis (Fig. 2) which led to hydrocephalus (Fig. 3) and loss of cells of the nuclear layers. The neurological symptoms, as evidenced by changes in behaviour and blindness, corresponded to those described for Lewis rats. The heterozygous rats produced infectious virus in the CNS and virus-specific serum antibodies (Fig. 2, Tables 1 and 2). Despite high antibody titres, the sera did not neutralize the infectivity of BD virus. These findings show that the heterozygous (rnu+/+) Rowett rat is comparable to the Lewis rat with respect to its susceptibility to BD virus infection, and therefore represents a suitable control for the experiments with their nude littermates.

**DISCUSSION**

All the data presented here are in accordance with the previously outlined concept (Narayan et al., 1983a, b) that BD virus in rats represents an immunopathological phenomenon mediated by T-lymphocytes. The nude rats are naturally deficient in the T-lymphocyte system (Festing et al., 1978; Brooks et al., 1980) and therefore offer the advantage that no drug must be applied which may cause various side effects in addition to immunosuppression. We have confirmed that the absence of T-cells obviates the outbreak of any signs of disease. However, later in the life span of the nude rats some lymphocytes appear which can be identified as T-cells by the presence of specific markers on their surface (Wonigeit & Hedrich, 1982; Schwinzer et al., 1984). They are obviously not capable of producing the disease, and are therefore either functionally deficient or are present in insufficient amounts.

Since heterozygous littermates, which possess a functional thymus, developed the same kind of disease as Lewis rats, it ensured that the rat strain used for the experiments was potentially susceptible to BD virus. Therefore, resistance of nude rats is due to T-cell deficiency and not to the genetic background of the outbred Rowett rat strain.

It is not surprising that homozygous nude rats did not produce any antibodies in spite of the presence of B-cells, because it should be expected that T-cell helper functions are needed for antibody production. It has already been conclusively shown that antibodies cannot play an essential role in the pathogenesis of BD, since injection of antiserum has no effect in immunosuppressed animals, in contrast to the transfer of lymphocytes (Narayan et al., 1983a, b). The definition of the T-cell subpopulation which is responsible for initiating the pathological events awaits further studies.

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**REFERENCES**


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