Borna disease virus-induced meningoencephalomyelitis caused by a virus-specific CD4+ T cell-mediated immune reaction

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After intracerebral inoculation of Borna disease virus (BDV), Lewis rats develop a persistent infection of the central nervous system which is pathohistologically represented by perivascular encephalitic lesions predominantly in the grey matter. In previous studies it has been shown that a cell-mediated immune response causes Borna disease (BD). In order to define further the immune cell responsible for this immunopathological disease, a BDV-specific T cell line, NM1, was established and cultured in vitro. Phenotypically this T cell line was characterized by cytofluorometry as CD4-positive (CD4+). Proliferation assays with syngeneic and allogeneic antigen-presenting cells, and blocking experiments with monoclonal antibodies, revealed major histocompatibility complex class II antigens to be restriction elements. After passive transfer of this virus-specific CD4+ T cell into immunosuppressed BDV-infected recipients, full-blown disease could be induced. Immunohistological examination of the cells involved in perivascular inflammatory infiltrates in BDV-infected rats and in recipients of the NM1 T cell line revealed a dominance of macrophages and CD4+ T cells. The presence of these cells in encephalitic lesions strongly suggests a delayed type of hypersensitivity reaction as the pathogenetic mechanism of BD.

Introduction

In general, the outcome of a virus infection depends greatly on the efficiency and speed of the immune system in reacting to the invading agent. Highly cytopathic viruses represent a much greater threat to the life of their hosts than non- or poorly cytoplastic viruses. Therefore the immune system is designed for efficient and rapid elimination of cytopathic viruses in order to reduce tissue destruction. In contrast, a highly efficient immune reaction might have considerable disadvantages for the host if non-cytolytic viruses are involved; the result might be an immune-mediated destruction of tissue. The most thoroughly investigated virus model causing immunopathology in the brain is the lymphocytic choriomeningitis virus (LCMV) of mice. In the LCMV infection the cytotoxic T cell subset has been shown to represent the most important T cell involved in immunopathology (Baenziger et al., 1986; Cole et al., 1972; Doherty et al., 1976) and virus elimination (Byrne & Oldstone, 1984; Lehmann-Grube et al., 1985; Zinkernagel & Welsh, 1976) after intracerebral (i.c.) infection.

In our model, Borna disease virus (BDV) infection in rats, the effect of the cellular immune reaction on the outcome of infection has been elucidated during recent years; antibodies apparently do not play any role in Borna disease (BD) (Herzog et al., 1984; Narayan et al., 1983; Richt et al., 1989). Although the characterization of the virus has not yet been completely achieved, some in vitro and in vivo biological properties, such as replication being exclusive to neural cells (Carbone et al., 1989; Herzog & Rott, 1980; Herzog et al., 1984), axonal transport (Carbone et al., 1987; Krey et al., 1979; Morales et al., 1988), routes of infection (Carbone et al., 1987; Morales et al., 1988), establishment of persistence (Narayan et al., 1983; von Rheinbaben et al., 1985) and the characterization of viral antigens (Ludwig & Becht, 1977), have been investigated.

BDV causes meningoencephalomyelitis preferentially in the grey matter of naturally infected horses and sheep and induces disease in a wide range of animal species after experimental infection. In most infected species disturbances of normal brain function are the consequence of the infection, ranging from severe central nervous symptoms, occasionally resulting in death, to abnormal social behaviour and premature senility (Rott et al., 1988). In this paper we report the establishment of a homogeneous T cell line specific for a 38/39K viral antigen (BDV-Ag), and describe in vivo and in vitro properties of the cell line. We also present data from immunopathological examinations of brains from diseased rats which support the concept that the immunemediated disease caused by BDV infection in rats is based on a delayed type hypersensitivity (DTH) reaction.
Methods

Borna disease virus. The Giessen strain He/80 of BDV, originally obtained from a homogenate of brain from a horse with the natural disease, was used throughout these studies. Brain homogenates were frozen at -70 °C as stock BDV. The stock suspension had a titre of \(6 \times 10^7 \text{ID}_{50}/\text{ml}\) when assayed by fluorescent focus formation in foetal rabbit brain cells (Herzog & Rott, 1980).

Rats. Inbred Lewis rats were provided by the Zentralinstitut für Versuchstierzucht, Hannover, F.R.G.: BS and BN rats were obtained from the Max-Planck-Institut für Immunobiologie, Freiburg, F.R.G.

Infection and immunosuppression. Lewis rats of 4 to 5 weeks of age were inoculated i.c. with 0·1 ml 10\(^{-2}\) brain suspension diluted 1/10 in Dulbecco's medium supplemented with 2% foetal calf serum (FCS) and examined daily thereafter. If indicated, 1 day after infection the rats were immunosuppressed by intraperitoneal application of 150 mg/kg cyclophosphamide (Asta-Werke). At the end of the experiment the animals were deeply anaesthetized with ether, bled by heart puncture and killed by pneumothorax.

Antibody determination. Titres were determined by indirect immunofluorescence as described previously (Narayan et al., 1983).

Antigens. BDV-Ag was extracted from brains of BDV-infected rats and purified as described previously (Haas et al., 1986; Fig. 1). Myelin basic protein (MBP) was dissolved in phosphate-buffered saline (PBS) (1 mg/ml). The purified protein derivative (PPD) of Mycobacterium tuberculosis in PBS (1 mg/ml) was purchased from Statens Seruminstitut, Copenhagen, Denmark.

Immunization. Lewis rats at 8 to 10 weeks old were injected intradermally in each hind footpad with 0·05 ml containing 30 μg purified 38/39K BDV-Ag in PBS, emulsified with equal volumes of complete Freund's adjuvant (CFA) containing 4 mg/ml of M. tuberculosis H₃/7; Ra (Difco).

Establishment of the T cell line. A T cell line reactive to BDV-Ag was established according to the method of Ben-Nun et al. (1981). Briefly, 10 days after immunization the draining popliteal lymph nodes were excised and a single-cell suspension was prepared. The cells were adjusted to 1 \times 10^5 cells/ml and cultured with BDV-Ag (10 μg/ml) in RPMI 1640 (Biochrom) supplemented with 1/50 autologous fresh rat serum, 2-mercaptoethanol (5 × 10\(^{-5}\) M) and antibodies (restimulation medium) in 60 mm Petri dishes (Falcon). After 72 h of incubation at 37 °C in 5% CO\(_2\) and air, the cells were collected and the lymphoblasts were separated by centrifugation using a Ficoll-Hypaque density gradient (Lymphoprep). The lymphoblasts were recovered from the interphase by pipetting, washed twice by centrifugation and propagated in vitro in RPMI 1640 supplemented with 10% FCS, 2-mercaptoethanol (5 × 10\(^{-5}\) M), 15% concanavalin A (Con-A)-stimulated lymphocyte supernatant and antibiotics, without antigen (restimulation medium). The cells were plated in 100 mm Petri dishes (2 \times 10^5 cells/ml) and transferred every 3 to 4 days. In order to propagate the selected lymphocytes as a continuous line, the cells (2 \times 10^5 cells/ml) were stimulated in restimulation medium with BDV-Ag (10 μg/ml) every 9 to 12 days, with 3000 rad-irradiated syngeneic thymocytes (2 \times 10^5 cells/ml) as the antigen-presenting cells (APCs) for proliferation. Astrocytes were also used as APCs. For this purpose, either primary astrocytes isolated from the brain of newborn Lewis rats or an astrocytic cell line (F10; kindly provided by Dr H. Wekerle, Würzburg, F.R.G.) were used. These cells were either used uninfected or persistently BDV-infected for proliferation assays.

Proliferative response of the T cell line. Cells were cultured in triplicate in round-bottomed microtitre wells (Nunc) with 0·2 ml restimulation medium. Each well contained 1 \times 10^4 lymphocytes and 1 \times 10^6 accessory cells (3000 rad-irradiated) with antigens at appropriate concentrations. The following antigen concentrations were used: BDV-Ag, 8 to 30 μg/ml; MBP, 20 μg/ml; PPD, 10 μg/ml; Con A, 2·5 μg/ml. The proliferative response was determined by measuring the incorporation of [\(^3\)H]thymidine (0·2 μCi/well, sp. act. 2 Ci/mmol, Amersham), added for the last 18 h of a 72 h culture. The cultured cells were then harvested on fibreglass filters and the incorporation of [\(^3\)H]thymidine was measured in a liquid scintillation counter. The proliferative response was expressed in c.p.m.

Phenotyping of the cell line. Immunofluorescence was used to assess surface markers of T cells, using anti-rat monoclonal antibodies (MAbs) (Camon) as the first antibody and fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG (Dianova) as the second antibody. Viable lymphocytes (2 \times 10^5) were incubated with the MAb (1/50) on ice for 30 min, washed three times with PAB (PBS, 0·2% sodium azide, 1% bovine serum albumin) and incubated for another 30 min on ice with the FITC-conjugated antibody (1/40), again followed by washing with PAB. Surface fluorescence was examined using ortho-cytofluorographic system H 50.

Passive transfer of T cell lines. Lewis rats were inoculated intravenously (i.v.) with various numbers of cells of lines that were selected for reactivity to BDV or PPD and propagated in vitro for a number of months. Briefly, the cell lines were stimulated with 10 μg/ml BDV-Ag or PPD in the presence of irradiated syngeneic thymocytes as accessory cells. The cells were harvested 3 days later, washed with HEPES-buffered Dulbecco's medium and injected i.v. The injected rats were observed for the development of clinical signs and prepared for histological studies.

Fig. 1. SDS-PAGE analysis of purified 38/39K BDV-specific antigen. Lane 1, Mₐ markers; lane 2, BDV-specific antigens; lane 3, purified 38/39K BDV-specific antigen.
**Histology and immunohistology.** Brain tissue was obtained immediately after killing of the animals and sections were frozen or formalin-fixed. For the characterization of cells present in perivascular infiltrates, the peroxidase-anti-peroxidase (PAP) method employing MAbs specific for the respective cell subsets was used.

**Results**

**Establishment of a virus-specific T cell line**

Adult Lewis rats were immunized in the hind footpads with 60 μg affinity chromatographically purified virus-specific 38/39K antigen (Haas et al., 1986) in CFA. The popliteal lymph nodes were removed 10 days after immunization and the cells were cultured in vitro in the presence of the same antigen. Rats which had been immunized by this protocol did not show a BD-specific clinical manifestation, but testing of the sera revealed a specific antibody response to the 38/39K antigen as shown by Western blot analysis and immunofluorescence (data not shown). To maintain the cell line, called NM1, the cells had to be restimulated at intervals of about 10 days using irradiated (3000 rad) syngeneic thymocytes in the presence of the specific 38/39K antigen. Repeated stimulation of cultured cells revealed an increased reactivity in parallel with the number of restimulations (Table 1).

**Specificity of restimulation responses**

In order to define the specificity of the cell line cultured and restimulated in vitro, unrelated antigens were also tested for their capacity to induce proliferation. For example, the cell line NM1 reacted with neither MBP, PPD nor a preparation from the brain of uninfected rats which had been processed in the same way as the preparation containing the 38/39K-specific antigen (Fig. 2). MBP was chosen as a control antigen because of its appearance in the brain and its involvement in autoimmune diseases of the nervous system, such as experimental allergic encephalitis (EAE; Levine, 1974). PPD has been shown to be the specific antigen for T cells stimulated by *M. tuberculosis* and is a component of CFA. Furthermore, titrations employing various amounts of the NM1 cell line-specific antigen were carried out and revealed that as little as 1 μg of the 38/39K antigen was able to induce a significant proliferation of the cell line. Consequently, increasing doses of the antigen revealed a parallel increase in the

![Graph](image)

*Fig. 2. Specificity of T cell line NM1 for BDV antigens. The experimental set-up was as described in Methods. Cocultivation was carried out in the presence of MBP (20 μg/ml), PPD (10 μg/ml), a preparation from the brain of uninfected rats (40 μg/ml), BDV-specific purified 38/39K antigen (30 μg/ml) or Con A (2.5 μg/ml).*

<table>
<thead>
<tr>
<th>Control medium</th>
<th>Concana avalin A</th>
<th>BDV-specific antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30 μg/ml</td>
</tr>
<tr>
<td>C.p.m. (SI)</td>
<td>C.p.m. (SI)</td>
<td>C.p.m. (SI)</td>
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<td>----------------</td>
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</tr>
<tr>
<td>NM1 2nd restimulation</td>
<td>110 (1)</td>
<td>9124 (83)</td>
</tr>
<tr>
<td>NM1 4th restimulation</td>
<td>153 (1)</td>
<td>3447 (23)</td>
</tr>
<tr>
<td>NM1 10th restimulation</td>
<td>73 (1)</td>
<td>16579 (227)</td>
</tr>
<tr>
<td>Control (lymph node cells)</td>
<td>1026 (1)</td>
<td>32801 (32)</td>
</tr>
</tbody>
</table>

*SI, stimulation index.*

Table 1. *Proliferative response of the T cell line NM1 cultured with thymocytes in the presence of antigen*
proliferative response. An optimal dose (30 μg/ml) of the BDV-specific purified antigen was used in all subsequent proliferation assays.

Characterization of T cell line NM1

The phenotypic characterization of the BDV-specific cell has been reported in a recent publication and revealed the phenotype Ox-1+, W3/13+, W3/25+, Ox-6−, Ox-8− (Richt et al., 1989). This pattern is characteristic for T cells mediating graft-versus-host (GvH) reactions, or which are involved in helper activities, for some cytotoxic T cells and cells reactive in DTH reactions. In the present study we were able to confirm this finding even in long-term cultured NM1 cells, thus demonstrating the stability of this BDV-specific T cell line. We extended our analysis to some other markers which were judged to be of importance for the characterization of this T cell line. Of particular interest was the MAb Ox-22, which reacts with a marker which is related to the function of CD4+ effector T cells (Spickett et al., 1983). For this purpose, cells taken from cultures soon after the establishment of BDV specificity as well as from long-term cultures were employed. However, independent of the time of culturing in vitro, no Ox-22-positive cells were found in this cell line. NM1 cells were also shown to carry IL-2R (MAb ART 18) and were transferrin receptor-positive (MAb Ox-26) (data not shown).

Functional characterization and major histocompatibility complex (MHC) restriction of the NM1 T cell line

After defining the NM1 cell line as one composed of CD4+ T cells, this finding was verified by functional assays, including an inhibition assay of T cell proliferation using MAbs directed against MHC antigens and employing antigen-presenting cells from various haplotypes. As depicted in Fig. 3, proliferation of NM1 cells is completely inhibited by MAb Ox-6, specific for a monomorphic determinant of rat Ia (RT1.B; equivalent to I-A). Even at a dilution of 10−4 the MAb reduced the proliferative response to 50% of that of the control (data not shown). MAbs Ox-17 and Ox-18, directed against monomorphic determinants of rat MHC class II (RT1.B; equivalent to I-E) and MHC class I antigen (RT1.A), respectively, did not inhibit the specific proliferative response of equivalent NM1 T cells at all (Fig. 3).

MHC restriction elements were also determined in proliferation assays using APCs from syngeneic or allogeneic donor strains (Fig. 4). Presentation of the specific antigen by APCs from the rat strain BS, sharing MHC class I (RT1.A) and MHC class II antigens (RT1.B) with Lewis rats but with a different genetic background, was comparable. In contrast, thymocytes from allogeneic BN rats (Wekerle & Wonigeit, 1984)
induce proliferation of NM1 cells in vitro. However, this was only possible if the cells had been preincubated with interferon (IFN-γ) prior to cocultivation with NM1 T cells.

Recently it has been shown that infection of astrocytes with the murine coronavirus JHM induces the expression of MHC class II antigens (Massa et al., 1987a). Therefore we infected astrocyte cultures with BDV and tested at various times after infection whether this self-antigen was also expressed on the surface of BDV-infected cells. However, neither immunofluorescent nor immunohistological examination of infected cells revealed any evidence for virus-induced MHC class II expression. In contrast, treatment of BDV-infected astrocytes with recombinant IFN-γ (20 units/ml) readily resulted in the appearance of MHC class II gene products as detected by MAb Ox-6 (data not shown).

Furthermore, and presumably more relevant to the in vivo situation, we tested whether those astrocytes infected with BDV can function in antigen presentation. However, even after induction of MHC class II antigen by IFN-γ, BDV-infected astrocytes were not functionally antigen presenting. This was again only achieved if the 38/39K BDV-Ag was added exogenously to the cultures (Fig. 5). A comparison between astrocytes and thymic cells in their capacity to present BDV-Ag to NM1 T cells revealed a higher efficiency for thymocytes (Fig. 5).

**In vivo activity of NM1 T cells**

After the specificity, MHC restriction pattern and the phenotype of the NM1 cell line had been established, it was of interest to investigate the relevance of this BDV antigen-specific T cell for the pathogenesis of this disease. Therefore, based on earlier findings (Narayan et al., 1983) that the disease can be evoked in BDV-infected but otherwise healthy immunosuppressed animals by adoptive transfer of immune cells from diseased animals, the NM1 T cells were passively transferred into cyclophosphamide-treated rats. Firstly, the potency of NM1 cells to induce disease symptoms and their specificity were tested. As a relevant control a PPD-specific T cell line was adoptively transferred into rats treated in exactly the same way as recipients of NM1 cells. As demonstrated in Table 2, uninfected immunosuppressed rats did not develop the disease after transfer of NM1 cells or of PPD cells (Table 2, groups 2 and 3). In contrast, BDV-infected immunosuppressed rats, which never became ill without further treatment (group 5), did show clinical disease symptoms after the transfer of 2.5 x 10⁶ NM1 cells but not after transfer of 6 x 10⁶ PPD-specific cells (groups 6 and 7). These experiments clearly showed that NM1 cells are able to cause BD in infected immunosuppressed recipients.

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**Astrocytes functioning as BDV-APCs**

In line with our efforts to define cells relevant for the in vivo situation in BD, we tested whether astrocytes were capable of presenting BDV-Ag to NM1 cells rather than employing the generally used thymocytes. Previously, immunohistological examinations of the brain of BDV-infected rats revealed the presence of viral antigen in astrocytes (Carbone et al., 1989). First, astrocytes freshly isolated from the brain of newborn Lewis rats or an astrocytic cell line (F10) were tested for their capacity to present exogenously added 38/39K BDV-Ag to NM1 cells. As can be seen in Fig. 5, astrocytes were able to primed with the 38/39K antigen in vitro and used as APCs, did not induce proliferation of NM1 cells.
Table 2. *Transfer of BDV-specific T cell line NM1 into BDV-infected immunosuppressed rats*

<table>
<thead>
<tr>
<th>Group no.</th>
<th>BDV infection</th>
<th>Immunosuppression</th>
<th>Specificity of cells</th>
<th>Clinical signs</th>
<th>Encephalomyelitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>+</td>
<td>BDV (lt/4)</td>
<td>0/4</td>
<td>0/4</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>+</td>
<td>PPD (i)</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>0/3</td>
<td>0/3</td>
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<td>6</td>
<td>+</td>
<td>+</td>
<td>BDV (lt/4)</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>+</td>
<td>PPD (i)</td>
<td>0/3</td>
<td>0/3</td>
</tr>
</tbody>
</table>

* 2.5 × 10^6 BDV-specific NM1 cells or 6 × 10^6 PPD-specific T cells (BS.PPD) were passively transferred i.v. immediately after the last restimulation.
† No histological changes were found in the brain of this rat; clinical signs and death observed cannot be related to BDV as they were non-specific, most probably due to immunosuppression by cyclophosphamide.

In order to increase the number of animals tested and, additionally, to determine the number of cells necessary for the induction of disease, different numbers of NM1 cells were transferred. It can be seen from Table 3 that as few as 1 × 10^6 NM1 cells induced disease, whereas even 8 × 10^6 cells transferred into uninfected immunosuppressed animals had no effect. Disease symptoms such as loss of weight, somnolence, apathy and pareses, comparable to BDV-infected adult rats, were observed as early as 5 to 10 days after passive transfer of NM1 cells. It should be mentioned that the clinical signs observed after transfer were not only seen earlier but were much more intensive than in adult untreated rats infected with BDV.

Taken together, these results strongly suggest that the BDV-specific NM1 T cell line represents the cell type responsible for the induction of BD.

Immunohistological demonstration of immune cells in BD encephalitic lesions

To demonstrate further the importance of CD4+ T cells in the pathogenesis of BD, cells found in encephalitic lesions of diseased rats were defined immunohistologically.

Using MAbs directed against the total peripheral T cell population (Ox-19), T suppressor/cytotoxic and natural killer (NK) cells (Ox-8) or T helper cells (W3/25) on frozen tissue sections, it could be clearly shown that the majority of Ox-19+ cells accumulating perivascularly in the grey matter (Fig. 6a) were also W3/25+ (Fig. 6b), whereas Ox-8+ cells were found only occasionally (Fig. 6c). Additionally, staining of cells involved in the perivascular infiltrates was performed with MAbs directed against macrophage markers on formalin-fixed sections. As shown in Fig. 6(d), considerable numbers of cells in these infiltrates carried the macrophage marker ED1. It should be stressed that the kinetics of appearance of the immune cells reacting with the above mentioned markers were different but that macrophages and T helper cells represented the most prominent cell type in the early phase after i.c. infection. At 10 days post-infection (p.i.) more than 60% of total T cells were of the CD4 phenotype. When BD became manifest at about 20 days p.i., more than 80% of T cells found in encephalitic lesions were CD4+ cells. Considerable

Table 3. *Induction of Borna disease after transfer of various numbers of BDV-specific T cells*

<table>
<thead>
<tr>
<th>BDV infection</th>
<th>Immunosuppression</th>
<th>Number of BDV-specific T cells transferred*</th>
<th>Clinical symptoms</th>
<th>Encephalomyelitis</th>
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<tr>
<td>-</td>
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<td>8 × 10^6</td>
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<td>+</td>
<td>8 × 10^6</td>
<td>3/3</td>
<td>3/3</td>
</tr>
</tbody>
</table>

* NM1 cells were passively transferred i.v. immediately after the last restimulation in vitro.
All animals were killed at day 15 p.i., i.e. 5 days after transfer.
Fig. 6. Immunohistological characterization of cells in perivascular infiltrates of BDV-infected rats. Frozen tissue sections from brains were reacted in a PAP reaction with MAbs Ox-19 (total T lymphocyte population; a), W3/25 (helper T cells; b), Ox-8 (cytotoxic/suppressor T cells; c) or formalin-fixed sections with MAb ED1 (monocyte/macrophage; d).

numbers of ED1+ cells were detected in inflammatory infiltrations at day 10 p.i. and the maximum number of macrophages was found around day 20 p.i. Ox-8+ cells, representing populations of cytotoxic T cells and NK cells, were present in the perivascular infiltrations but at no time exceeded 20% of the total W3/13+ cells.

Using these markers we determined the phenotype of cells appearing in perivascular infiltrations after transfer of the NM1 T cell line. The immunological evaluation of those sections revealed a strong positive reaction for W3/13+ and ED1+ cells, but only a weak reaction with Ox-8 (data not shown). However, we were not able to use the W3/25 MAb because, in contrast to the other MAbs used, it did not stain cells on formaldehyde-fixed sections. From this we conclude that the majority of W3/13+ T cells were of the CD4 phenotype.

Presence of MHC class II antigen in the brains of BDV-infected rats

Finally, we investigated whether the local immune response during the course of BD is accompanied by the expression of MHC class II antigen in the brain. For this purpose immunohistology was carried out on formalin-fixed brain sections employing MAb Ox-6. Using the PAP technique we were able to demonstrate the presence of MHC class II antigen in the brain 10 days after i.c. infection. Thereafter, an increasing number of cells were found to be positive, reaching a maximum around day 20 p.i. At this time, full-blown BD was also observed. This situation did not change significantly until day 70 p.i. when the experiments were terminated. MHC class II-expressing cells were found in the vicinity of perivascular infiltrates (Fig. 7) but, most interestingly, also in the parenchyma and on vascular surfaces in the absence of perivascular infiltrates (data not shown).

Discussion

Our experiments further elucidate the pathogenetic pathway of BDV infection in rats. Immunization of uninfected Lewis rats with a highly purified virus-specific 38/39K antigen (Haas et al., 1986) led to the isolation of the homogeneous BDV-specific T cell line NM1. The fluorometric characterization revealed that NM1 cells were of the CD4 phenotype, representing a T cell type involved in helper and cytotoxic functions and GvH or DTH reactions (type IV). Proliferation assays with syngeneic and allogeneic APCs and blocking experiments with MAbs to respective restriction elements showed an MHC class II restriction (I-A) for the NM1 T cell line. Experiments to transfer this CD4+ T cell line into healthy immunosuppressed BDV-infected recipients were carried out in order to define whether this T cell is of any relevance in the pathogenesis of BD. This question was answered by the fact that full-blown BD was induced by relatively small numbers of transferred cells. Whether the cells transferred into recipient animals are identical to those present in perivascular infiltrations after passive transfer was not determined. Alternatively and supported by results from other disease models, the injected cells might act on other cell(s) which in turn induce the BDV-specific inflammatory reaction dominated by CD4+ T cells.

Unlike coronavirus- and measles virus-induced encephalomyelitis, which represent models for virus-induced autoimmune diseases (Liebert & ter Meulen, 1987; Massa et al., 1986, 1987a, b) and in which CD4+ cells also have been shown to be of immunopathological rel-
evance, BD is an example of immunopathology in the brain resulting exclusively from an immune reaction to viral antigen(s). This has now been definitely shown by passive transfer experiments which revealed that the BDV-specific CD4+ T cell line NM1 is not encephalitogenic in uninfected recipients.

Previously, our group had shown in different experiments that the cell-mediated immune response to BDV plays the crucial role in the disease process. It could be demonstrated that infection of cyclophosphamide-treated (Narayan et al., 1983), cyclosporin A (CSA)-treated (Stitz et al., 1989) or athymic rats (Herzog et al., 1985) does not lead to typical encephalitic lesions and development of the disease. However, after passive transfer of spleen cells from diseased rats into these immunosuppressed recipients, pathological alterations in the brain and concomitant disease were observed. On the other hand, by transfer of immune sera into immunosuppressed recipients, it could be shown that BDV-specific antibodies do not participate in the immunopathological disease process (Narayan et al., 1983). Basically the same results were obtained from experiments with rats infected i.c. within 24 h of birth. These rats, infected in the state of immunoincompetence, became tolerant and did not develop disease or meningoencephalitis (Narayan et al., 1983). However, a late onset of virus-specific antibody response was observed. These (endogenously produced) antibodies, however, were of no pathogenetic importance because these animals stayed healthy throughout their lives. In further experiments employing the immunosuppressive drug CSA which acts mainly on T cells (Shevach, 1985), indirect proof for the action of T cells as the most likely effector mechanism was obtained. Rats treated with CSA developed neither inflammatory reactions in the brain nor disease, but were fully susceptible to BDV after the transfer of immune lymphocytes (Stitz et al., 1989). Furthermore, in CSA-treated rats the irrelevance of antiviral antibodies in the pathogenesis of BD was again demonstrated (Stitz et al., 1989).

Data obtained from immunohistological examinations, especially the presence of CD4+ T cells together with the presence of macrophages in the relative absence of CD8+ and NK cells in encephalitic lesions, makes a DTH reaction the prime candidate for the underlying immunopathological mechanism. Our effort to define CD4+ NM1 cells by MAb Ox-22 as a cell belonging to the subset of inflammatory cells involved in DTH was not successful. This negative result, however, is not conclusive because the expression of this marker on CD4+ T cells of the rat is not stable and evidence was found that cells originally Ox-22+ become and remain Ox-22− after activation (Powrie & Mason, 1988).

With regard to the immunopathological mechanism, several additional findings might be of importance. Since CD4+ T cells are restricted for MHC class II antigens, we have looked in the brain of diseased animals for the expression of this self-antigen which is not detectable in the brain of apparently healthy animals (Wong et al., 1985). After BDV infection, we found a positive reaction around encephalitic lesions. MHC class II antigen was detected also in areas where no inflammatory reactions took place, arguing for a general MHC class II induction and expression in the brain of BDV-infected rats. Furthermore, in CSA-treated rats which do not show a cellular infiltration despite the presence of infectious virus in the brain, no expression of MHC class II antigen is observed (L. Stitz, D. Schilken & K. Frese, unpublished results). The enhancement of MHC class II expression alone cannot be regarded as an indicator of an ongoing MHC class II-restricted immune response because enhanced expression of MHC class II antigens has been found in a variety of chronic infectious and non-infectious encephalitis. However, together with other findings (Deschl et al., 1990), these observations fit very well with the concept of the action of MHC class II-restricted virus specific T cells in the pathogenesis of BD.

Although we favour a DTH reaction elicited by virus-specific CD4+ T cells as the basis of immunopathology in BD, another component might be important in the pathogenetic pathway. We cannot estimate the role of the presence of CD8+ T cells, which are found on a much smaller scale than all other cell types detected in encephalitic lesions, in the pathogenesis of BD at present. Recent work by our group (L. Stitz & B. Fleischer, unpublished results), employing splenic lymphocytes as well as lymphocytes isolated from the brain or cloned lymphocytes from the cerebrospinal fluid of BDV-infected Lewis rats, did not reveal evidence of BDV-specific cytotoxic T cells.

Previous work by others has shown that astrocytes represent an important host cell in the brain of BDV-infected rats (Carbone et al., 1989) and are known to be highly efficient APCs (Fontana et al., 1984). However, it is an apparent paradox that BDV-infected astrocytes do not trigger proliferation of the CD4+ T cell line NM1 in vitro, even after induction of MHC class II antigens by IFN-γ. BDV-specific antigens obviously are not recognized on infected astrocytes by the BDV-specific T cell. Presently we do not have a conclusive explanation of why only the addition of the purified 38/39K antigen results in the induction of a specific proliferative response. These data can possibly be explained by considering that recently, although not demonstrated with astrocytes as APCs, it has been reported that endogenous antigen can be recognized by MHC class I- but not necessarily by MHC class II-restricted T cells (Sweester et al., 1989). In this context it again becomes rather important to focus future work on the MHC class I-restricted T cell response in the course of BD.
The results presented here, defining the cultivated T cell as CD4+ and demonstrating that BD can be induced after passive transfer of the NM1 cell line, together with the above-mentioned findings, directly indicate the immunopathological relevance of this T cell subset in BD. Ongoing studies are aimed at elucidating the mechanisms by which CD4+ T cells cause the disease.

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References


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