There is increasing evidence that immunological mechanisms are involved in a considerable number of disease processes of the central nervous system (CNS). Thereby persistent viral infections of the brain have gained special interest, e.g., HIV-1 in the AIDS dementia complex (1), measles virus in subacute sclerosing panencephalitis (2), and BK papovavirus in progressive multifocal leukoencephalopathy (3, 4). Borna disease (BD), which we used as a model for a persistent virus infection of the CNS, is caused by a highly neurotropic, but yet only partially characterized virus, which has been shown to spread exclusively by the axonal route. BD naturally occurs endemically in individual horses and sheep as a progressive encephalopathy. After a widely variable incubation time, which can span a few weeks to many months, the disease becomes apparent as disturbances of motility and in sensoric functions followed by paralysis and death. Pathological lesions are restricted to the CNS, consisting of perivascular infiltrations of lymphocytes and other mononuclear cells, predominantly in the grey matter of the brain. A broad spectrum of animals ranging from chickens to primates can be infected experimentally. Depending on the host species and the virus strain used for infection, the disease becomes apparent as paralysis followed by death or the disease remains inapparent or becomes evident only by obesity and disturbance in fertility or behavioral abnormalities (5). With the virus strain used in this study, rats develop an acute encephalitis by day 20, which is followed by an apathic phase that can last life-long. BD virus-specific antibodies have recently been found in sera of psychiatric patients (6). In rats, it has previously been shown that the pathogenesis of BD is due to a virus-induced cell-mediated immune mechanism. Neonatally infected rats or rats immunosuppressed after infection, e.g., with cyclophosphamide, do not develop the disease or histopathological alterations, despite high level of virus persisting in the CNS. Adoptive transfer of cells from the spleen or lymph nodes from diseased rats to the tolerant recipients induced encephalitis and clinical manifestation of the disease (7).

In this article we describe the in vitro and in vivo properties of a T cell line specific for a BD virus antigen and we show its immunopathological relevance.
Materials and Methods

Virus, Rats, and Infection. Throughout the study the Giessen strain HE/80 of BD virus was used (8). Lewis rats purchased from the Zentralinstitut für Versuchstierzucht, Hannover, FRG, were infected intracerebrally with $6 \times 10^4$ ID<sub>50</sub> of BD virus.

Induction and Cultivation of the T Cell Line NMI. For the induction of a BD virus-specific T cell, Lewis rats 8–10 wk old were immunized by inoculation of 60 μg of the purified BD virus-specific 38/39-kD antigen (9) in IFA into the hind footpads. 10 d later cells from the regional lymph node were restimulated in vitro with the same antigen. Restimulations of T cells ($2 \times 10^5$/ml) were performed in the presence of syngenic thymocytes ($2 \times 10^7$/ml) and the specific antigen at intervals of 10 d in order to maintain the cell line. After restimulation T cells were cultured in the presence of IL-2.

Proliferation Assay. Cells ($10^6$) were cultured in round-bottomed 96-well microplates with RPMI 1640, supplemented with 1% Lewis rat serum, l-glutamine, 2-ME, and antibiotics. They were incubated with $10^7$ irradiated thymocytes in the presence or absence of the specific antigen in each microtiter well (0.2 ml) for 72 h at 37°C and 5% CO<sub>2</sub>. [3H]thymidine (0.2 μCi/well) was added 18 h before collection. In proliferation assays establishing the MHC restriction of the antigen-specific proliferation of NMI cells either anti-MHC class I (Ox-18) or class II (Ox-6; Camon, Wiesbaden, FRG) mAbs were added to the test wells.

Cytofluorography. mAbs specific for leukocyte differentiation markers (Camon) as defined (10) were used as primary antibodies and FITC-conjugated goat anti-mouse Ig (Dianova, Hamburg, FRG) as secondary antibody reagent. Viable cells ($2 \times 10^6$) were incubated with the first antibody (1/50) on ice for 30 min. Cells were washed three times with PAB (PBS, 0.2% sodium azide, 1% BSA) and incubated with the second antibody (1/40) for another 30 min on ice. Surface fluorescence of the cells was examined by using the Ortho Cytofluorographic System H 50 (Ortho Diagnostic Systems, Westwood, MA).

Immunosuppression and Adoptive Transfer. Lewis rats infected intracerebrally with BD virus were given a single dose of cyclophosphamide (150 μg/kg; Endoxan Asta, Bielefeld, FRG) intraperitoneally 1 d later. Immunosuppressed, infected rats do not show clinical or histopathological signs of Borna disease, apparently due to the suppression of immunopathologically relevant immune cells (7). For adoptive transfer studies, $1 \times 10^6$ to $8 \times 10^6$ of the BD virus-specific NMI cell line or as a control a PPD-specific T cell line was injected intravenously into infected immunosuppressed rats 10 d after infection.

Results and Discussion

Immune cells were obtained from the regional lymph node of a rat immunized with the purified BD virus 38/39-kD antigen, which regularly can be found in the nucleus and the cytoplasm of BD virus-infected cells (9). After restimulation in vitro with the same antigen a homogeneous cell line, designated NMI, could be maintained. The NMI cells responded specifically to the viral antigen in lymphocyte proliferation assays as documented in Table I. The stimulation indices were in the same range as those after cultivation in the presence of Con A. On the other hand, virus unrelated antigens such as myelin basic protein (MBP), purified protein derivative of mycobacteria (PPD), or a homogenate from an uninfected brain (NL-brain) prepared in a similar way as the viral antigen did not stimulate the NMI cells. Cytofluorographic studies with mAbs against leukocyte differentiation markers showed a staining pattern characteristic for T helper/inducer (CD4<sup>+</sup>) cells. The marker for the CD8 antigen gave no staining reaction (Fig. 1). In experiments with different rat strains as donors of APCs (data not shown) and by inhibition experiments with mAbs against respective restriction elements, the established cell line proved to be MHC class II restricted (Fig. 2).

The BD virus-specific NMI cells were able to cause meningoencephalitis and the
TABLE I
Specific Proliferative Response of T Cell Lines Cultured in the Presence of Different Antigens

| Antigens    | BD virus-AG | NL-brain | MBP | PPD | Con A |
|-------------|-------------|----------|-----|-----|-------|
| cpm         | cpm         | SI       | cpm | SI  | cpm   |
| Control     | 73          | 12,073   | 165 | 105 | 1     |
| NM1         | 54          | 61       | 165 | 1   | 1     |
| Z82         | 83          | 52       | ND  | 44  | 1     |

Values represent counts per min and the stimulation indices (SI) of the T cell lines NM1, specific for BD virus, and Z82, specific for MBP (12), cocultured with syngeneic irradiated (3,000 rad) thymocytes. Each probe was measured in triplicates. The standard deviation ranges below 20%. Antigen concentrations: 2.5 µg/ml Con A, 10 µg/ml PPD, 20 µg/ml MBP, 30 µg/ml BD virus-specific antigen (BDV-AG), 40 µl/ml noninfectious brain homogenate (NL-Brain). ND, not done.

FIGURE 1. Cytographic histograms of the T cell line NM 1. Surface phenotype of the T cell line NM 1 was assessed by immunofluorescence using mouse anti-rat mAbs specific for leukocyte differentiation markers (Camon) as defined in (11) as primary antibodies and FITC-conjugated goat anti-mouse Ig (Dianova) as secondary antibody reagent. Viable cells (2 × 10⁶) were incubated with the first antibody (1/50) on ice for 30 min. Cells were washed three times with PAB (PBS, 0.2% sodium azide, 1% BSA) and incubated with the second antibody (1/40) for another 30 min on ice. Surface fluorescence of the cells was examined by using the Ortho Cytographic System H 50.
development of BD after adoptive transfer into immunosuppressed BD virus infected Lewis rats even if as few as $1 \times 10^6$ cells were applied (Table II). The pathological alterations consist of perivascular and parenchymal infiltrations mainly by mononuclear cells and by scattered neutrophilic granulocytes preferentially found in the meninges and the grey matter (Fig. 3). The clinical signs became manifest already 5 d after transfer. The animals showed loss of weight, exsiccosis, apathy, weakness, and somnolence. The hyperactive phase, which can be observed in the usual course of the disease, and the production of virus-specific antibodies (7) did not appear in animals that had received the NM1 cells. Injection of these cells into noninfected immunosuppressed rats had no effect. Similarly, a CD4+ cell line specific for PPD, but irrelevant for BD virus, did not result in the establishment of disease (Table II).

![Figure 2](attachment:image.png)

**Figure 2.** Inhibition of antigen-specific proliferation by mAbs specific for MHC class I or class II antigens. Irradiated syngeneic thymus cells ($10^6$ cells/well) were incubated with the appropriate dilution of α-class I (Ox-18) or α-class II (Ox-6) mAbs (Camon) in 96-well round-bottomed microtiter plates and cocultivated with NM 1 cells ($10^4$ cells/well) and BDV-Ag (15 μg/ml) to give a final volume of 200 μl/well. Cells were incubated for 72 h at 37°C, 0.2 μCi/well [3H]thymidine was added 18 h before the cells were harvested. Controls were cultures incubated with (+) or without (−) BD virus-specific antigen not treated with the Ox-6 or Ox-18 mAbs.

### Table II

**Adoptive Transfer of CD4+ T Cell Lines into Rats**

| BDV-infected | Immunosuppressed | Specificity of transferred cells | Number of transferred cells | Clinical manifestations* | Meningoencephalitis* |
|--------------|------------------|---------------------------------|-----------------------------|--------------------------|-----------------------|
| −            | +                | BD virus                        | $8 \times 10^6$             | 0/4                      | 0/4                   |
| −            | +                | BD virus                        | $1 \times 10^6$             | 0/3                      | 0/3                   |
| −            | +                | PPD                             | $6 \times 10^6$             | 0/3                      | 0/3                   |
| +            | −                | −                               | 0                           | 0/3                      | 0/3                   |
| +            | +                | BD virus                        | $1 \times 10^6$             | 2/2                      | 2/2                   |
| +            | +                | BD virus                        | $4 \times 10^6$             | 3/3                      | 3/3                   |
| +            | +                | BD virus                        | $8 \times 10^6$             | 3/3                      | 3/3                   |
| +            | +                | PPD                             | $6 \times 10^6$             | 0/4                      | 0/4                   |

T cell lines ($2 \times 10^5$/ml specific for BD virus [NM 1] or PPD [BS PPD, reference 13]) were incubated with 10 μg/ml of the specific antigen and cocultivated with syngeneic irradiated (3,000 rad) thymocytes ($2 \times 10^7$/ml). After 72 h the cells were collected and washed three times in Hepes-buffered DME. The cells were transferred intravenously into Lewis rats, which were infected intracerebrally with BDV into the left brain hemisphere and immunosuppressed 1 d later by intraperitoneal injection of a single dose of 150 mg/kg cyclophosphamide as indicated in the table. The transfer was performed 10 d after infection and 9 d after immunosuppression. (*) Number of rats showing clinical or pathohistological disorders versus total numbers of animals.
These data provide direct evidence that MHC class II-restricted BD virus-specific cells with the T helper/inducer phenotype represent the T cell subset relevant to the immunopathological reaction. Since classical cytotoxic CD8+ cells have never been detected in BD virus-infected animals (our unpublished results), the pathogenesis of BD differs from that of lymphocytic choriomeningitis (LCM), which represented for a long time the paradigm for a virus-induced immunopathological reaction. In LCM class I-restricted, virus-specific CD8+ cells are the only cell population found to be essential for the immunopathological event leading to the fatal disease (11). Furthermore, in contrast to MBP-specific CD4+ T cells found in experimental autoimmune encephalomyelitis (12) cellular infiltrates were found predominantly in the grey matter after transfer of the BD virus-specific CD4+ T cells. Thus, BD is likely to be a unique virus-induced immunopathological disorder affecting the CNS. Additionally, BD lends itself exquisitely to investigate the course and consequence of a virus infection of the CNS, where intensive contact with the immune system is greatly avoided by strategies of replication and spread of the virus exclusively in neu-
ronal cell tissue of the infected host; this is especially true for the presumable intranasal natural infection. The results presented and the finding that cellular infiltrates in the brain of diseased rats have been characterized immunohistologically mainly as CD4+ T cells, macrophages, and late B cell infiltration (Richt, J. A., manuscript in preparation) suggests that the pathogenesis of BD is based on a delayed-type hypersensitivity reaction (DTH).

Summary

A homogeneous T cell line NM1 with Borna disease (BD) virus reactivity could be established. The NM1 cells have been characterized as CD4+ T cells. Adoptive transfer revealed that this MHC class II-restricted immune cell is responsible for the immunopathological effect leading to BD, a progressive meningoencephalomyelitis.

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