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Borna disease virus-infected astrocytes function in vitro as antigen-presenting and target cells for virus-specific CD 4-bearing lymphocytes*

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Summary. Astrocytes isolated from the brain of newborn Lewis rats and an astrocytic cell line were susceptible to infection with the neurotropic Borna disease virus in vitro. Since astrocytes also have been found to be infected in vivo it seemed appropriate to test this cell type for interaction with a Borna disease virus-specific CD 4^+ T cell line. Borna disease virus-infected astrocytes were found to be capable of presenting virus-specific antigen to virus-specific T cells in vitro. However, the response was significantly enhanced if the purified 38/39 kDa Borna disease virus-specific protein was added exogenously to the cultures. Beside the function as antigen-presenting cells for various antigens including virus-specific protein and myelin basic protein, persistently infected astrocytes were also found to act as target cells for a CD 4^+ T cell line as shown in conventional ^{51}Cr release assays after induction of MHC class II expression by gamma interferon. Infection of astrocytes alone did not cause expression of this self antigen. It could be shown that the ability of CD 4^+ BDV-specific T cells to mediate lysis was in part dependent on the stage of activation. Lymphocytes "activated" before testing exerted high lysis after only 4 h of coincubation with target cells, whereas "resting" T cells did not cause significant lysis until 12 h of coincubation. The dependence of the interaction between effector and target cells on MHC class II antigen was demonstrated by the finding that antibodies to Ia antigens reduced lysis of target cells.

Introduction

Borna disease is a subacute neurological disease of horses and sheep in Europe and is caused by the Borna disease virus (BDV) [28, 43]. BDV-specific cDNA

* Dedicated to Prof. Dr. Rudolf Rott on the occasion of his 65th birthday.

clones have been recently isolated and BDV has been characterized as an RNA virus [10, 25, 41, 52].

Adult rats develop an infection exclusively in the nervous system which is followed by an acute necrotizing meningo-encephalomyelitis that results in ataxia, aggressive behavior and frequently in death [35]. Both the lesions and the disease do not occur after infection of athymic rats [20] and can be prevented by treatment of adult infected rats with immunosuppressive drugs, such as cyclophosphamide and Cyclosporine A (CSA) [35, 47]. Drug-induced tolerance can be overcome by adoptive immunization of the animals with spleen and lymph node cells from acutely infected syngeneic adult rats, while virus specific antibodies apparently do not play a role in the pathogenesis of Borna disease [35, 47]. Newborn and CSA-treated, immunosuppressed rats inoculated with BDV develop a life-long, productive and tolerant infection in the nervous system and in peripheral organs without clinical signs of BD [19, 49].

Thus, this unique neurological disease is not caused by the virus itself, but is rather the result of a cell-mediated immune response to BDV-specific antigens. In order to further characterize the immunological basis of the disease, the BDV-specific T cell line NM1 had been developed after immunization of adult Lewis rats with the virus-specific 38/39 kDa antigen. These cells express the CD 4-phenotype, proliferate in an MHC class II-dependent manner and cause disease after adoptive transfer into virus carrier recipients [39, 40].

Studies on the pathogenesis of experimental allergic encephalomyelitis (EAE) have shown that astrocytes can present the autoantigen myelin basic protein (MBP), a major brain protein involved in autoimmune reactions in the central nervous system, efficiently to MBP-specific T cells [13] and that these T cells can lyse MBP-pulsed astrocytes [51] and brain endothelial cells [42, 44]. EAE can be induced by the transfer of MBP-specific $CD4^+$ T cells which are cytotoxic and may recognize myelin protein(s) on astrocytes [4, 51].

Since astrocytes are one of the cell types in the brain that contain BDV-specific antigen [8, 11, 29] we decided to determine whether such cells could present viral antigen and whether they also could serve as targets for lysis by BDV-specific T cells. In this report we show that astrocytes are susceptible to infection with BDV in vitro and are capable of presenting BDV-antigen as well as other antigens to $CD4^+$ T cells and simultaneously act as target cells for lysis by pathogenic BDV-specific $CD4^+$ T cells.

The possible importance of our observation with regard to the induction of the immunopathological reaction after BDV infection and the resolution of encephalitic lesions during the late phase of the infection are discussed.

Material and methods

Virus and viral antigen

For infection of cells the Giessen strain He/80 of BDV was used [18]. This isolate originated from the brain of a horse with BD and was passaged twice in rabbits, thereafter in rabbit fetal brain cells and finally twice in newborn Lewis rats. BDV specific antigen was obtained

from brain homogenates of infected rats by means of affinity chromatography using a mouse mAb specific for the 38/39 kDa protein [16].

Reagents and monoclonal antibodies

MBP was derived by HPLC purification of spinal cord homogenates from guinea pigs (kindly provided by Dr. H. Wekerle, Munich, Federal Republic of Germany). Concanavalin A (Con A) and Ovalbumin (OVA) were purchased from Sigma Chemicals Company (St. Louis, Mo., U.S.A), polyclonal antibodies to glial fibrillary acidic protein (GFAP) from Dakopatts (Hamburg, Federal Republic of Germany) and the mAb to rat MHC class I [14] and class II [33] antigens were purchased from Camon, Wiesbaden, Federal Republic of Germany. mAb to BDV were derived from supernatant of the BDV-specific hybridoma line #18 [16].

Cells

Primary astrocyte cultures were prepared from the brain of neonatal Lewis rats. Minced brain tissue, trimmed free of adherent meninges [32] was rinsed for 10 min at 37 °C in PBS without Ca/Mg and then incubated 3 times each for 20 min at 37° C with trypsin, using the following solution: 2 ml of trypsin (2.5%), 2 ml of EDTA (1 mg/ml) in 100 ml of PBS without CA/Mg, supplemented with 20 mg DNAse (Boehringer, Mannheim, Federal Republic of Germany). The dissociated cell population was pooled and passaged through nylon wool (Fenwal Laboratories, Ill., U.S.A.) and seeded in RPMI 1640 supplemented with penicillin/streptomycin (1% v/v), L-glutamine (1% v/v) and 10% fetal calf serum (FCS; Biochrom KG, Berlin, Federal Republic of Germany). After 8–14 days the cells were trypsinized and an aliquot was seeded on Lab-Tek chamber slides for further characterization. Primary astrocyte cultures were passaged weekly. The homogeneous astrocyte line F 10, cloned from a primary astrocyte culture by limiting dilution was provided by Dr. H. Wekerle, Munich. Astrocytes used as stimulator or target cells in appropriate assays were incubated with MHC class II-inducing recombinant mouse interferon gamma (rIFN-γ) for 3 days prior to testing. The establishment of the T cell line NM 1 specific for the 38/39 kDa protein of BDV has been described previously [39]. Antigen-specific restimulation of this T cell line was performed in medium supplemented with 2-mercaptoethanol (5×10^{-5} M; Merck, Darmstadt, Federal Republic of Germany), Nystatin (1×10^4 I.U./ml), 1% autologous rat serum and 10–20 µg/ml BDV-specific 38/39 kDa protein. After 3 days of antigen-specific restimulation, viable cells were purified by centrifugation over a Ficoll-Hypaque gradient and washed extensively thereafter. These freshly activated cells are referred to as "activated" T cells. These activated cells were then maintained for 10 days in medium supplemented with 10–15% supernatant from ConA-stimulated mouse spleen cells as a source for IL-2. Beyond 8 days of propagation in IL-2-conditioned medium these cells were referred to as "resting" T cells. The MBP-specific T cell lines Z 82 and S 19 (kindly provided by Dr. H. Wekerle) were cultured in vitro in the presence of MBP [51].

Proliferation assay

Astrocytes (1×10^4/well), pretreated for 3 days with 20 U/ml rIFN-γ and irradiated with 6000 rad were cultured in flat-bottom 96-well microtiter plates with RPMI 1640 supplemented with 1% autologous rat serum, 2-mercaptoethanol, glutamine and antibiotics. "Resting" T cells were added in the presence or absence of the specific antigen (20 µg/ml) for 72 h at 37 °C and 5% CO_2. [^{3}H]thymidine (0.2 µCi/well; spec. activity 1 mCi/ml; Amersham Buchler, Braunschweig, Federal Republic of Germany) was added for the final 18 h before cell harvest. All cultures were tested in triplicate. The cells were harvested with a Skatron Titertek Cell Harvester and [^{3}H]thymidine incorporation was measured using a

beta scintillation counter (Packard). The results of these tests were expressed as the stimulation index (S.I.), calculated by the formula:

$$\text{S.I.} = \frac{\text{cpm in the presence of astrocytes with BDV antigen}}{\text{cpm in the presence of astrocytes without BDV antigen}}.$$

Cytotoxicity assay

Astrocytes (1×10^6), untreated or treated with rIFN-γ (10 U/ml) were labelled with 100 µCi of ^{51}Cr, incubated for 90 min and washed three times. Labelled astrocytes were coincubated with BDV-specific NM 1 T cells at effector/target ratios of 30 : 1, 20 : 1 or 10 : 1 and twofold dilutions of the original concentrations in a final volume of 200 µl/well. All tests were performed either in the presence or absence of the specific antigen (20 µg/ml) added 1 h before cocultivation and/or mAb directed against MHC class I or class II determinants in round bottom microtiter plates. After various periods of time (4–20 h) 50 µl of supernatant was collected and counted in a Packard gamma counter. The percentage of ^{51}Cr release was calculated according to the formula:

$$\frac{\text{Test release} - \text{spontaneous release}}{\text{Max. release} - \text{spontaneous release}} \times 100 = \% \text{ specific release}$$

in which test stands for the release in the presence of effector cells, spontaneous release is in the presence of medium alone and max. is the maximal release by 1 N HCl.

Immunofluorescence

To test astrocytes for expression of BDV, GFAP and MHC class II antigens, the indirect immunofluorescence technique was employed. Cells were fixed with acetone for 20 min and for 10 min in chloroform at room temperature. Nonspecific staining was blocked by incubation with undiluted swine serum (Gibco, Eggenheim, Federal Republic of Germany) for 30 min. After several washings with PBS the primary antibodies were incubated for 60 min at RT in the following dilutions in PBS: polyclonal rabbit anti-GFAP 1 : 200; mAb Ox 6 (MHC class II) 1 : 50; mAb #18 (BDV) 1 : 100. After several washes FITC or TRITC labelled secondary antibodies (Sebak, Aidenbach, Federal Republic of Germany) were added for 60 min at RT at concentrations of 1 : 50 or 1 : 100 in PBS. The slides were finally mounted with PBS/glycerin solution.

Results

Antigen expression by astrocytes

More than 95% of the cultured brain cells were positive for the glial fibrillary acid protein (GFAP) during the first 5 subcultures. Incubation of these cells with 20 U/ml rIFN-γ for 72 h induced expression of MHC class II antigens as detected by using Ox 6, a mouse mAb to rat MHC class II antigen (data not shown).

Since astrocytes harbor virus-specific antigen throughout the course of BDV-induced encephalopathy, these cells may be important in triggering immune reactions in the brain. To determine whether astrocytes are susceptible to BDV infection in vitro, primary cultures and the cloned astrocytic cell line F 10 were infected repeatedly with infectious rat brain homogenate containing 1×10^5 $TCID_{50}$/ml of BDV. The cells were shown to express BDV-specific antigen as

detected by immunofluorescence using polyclonal rat sera and mouse mAb (Fig. 1 A). No cytopathic effect was observed in infected cultures, a finding in keeping with other reports on the interaction of BDV with cells [18]. We then asked whether BDV-infection of astrocytes results in the expression of MHC class II antigen as reported for other neurotropic viruses such as coronavirus and measles virus [30, 31]. However, no spontaneous expression of this antigen was found on persistently BDV-infected astrocytes by immunofluorescence (data not shown). In contrast, treatment with rIFN-γ (20 U/ml for 72 h) was able to induce Ia antigen expression in BDV-infected astrocytes (Fig. 1 B).

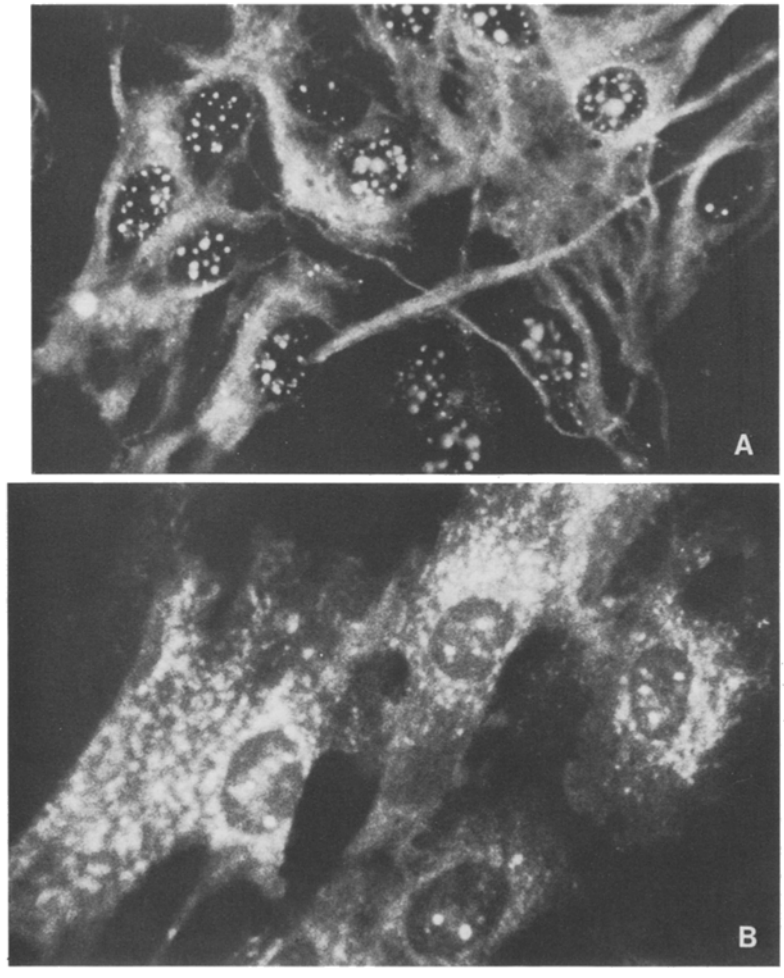

Fig. 1. Immunofluorescence of astrocytes persistently infected with BDV. **A** Cells were stained with antibodies directed against glial fibrillary acid protein (GFAP) as a marker for astrocytes (FITC) and with serum antibodies from BDV-infected rats (TRITC). Note the characteristic intranuclear staining of BDV-specific antigen. **B** Ia expression of astrocytes after treatment with gamma interferon. Cells were stained with Ox 6 mAb (anti-Ia; FITC) and with serum antibodies from BDV-infected rats (TRITC)

Astrocytes as antigen-presenting cells

Earlier reports had shown that astrocytes are capable of presenting MBP to $CD4^+$ MBP-specific T cell lines [13]. Accordingly, the MBP-specific T cell line Z 82 was incubated with rIFN-γ treated syngeneic irradiated astrocytes and lymphocyte proliferation was determined in the presence or absence of the specific antigen MBP. The results clearly confirmed this antigen-specific proliferation as shown in Table 1, but there was no response either to BDV-antigen nor to purified protein derivative (PPD) of *Mycobacterium tuberculosis* (data not shown).

Using the proliferation assay we then asked whether uninfected astrocytes pretreated with rIFN-γ were also capable of presenting exogenously added 38/39 kDa BDV-specific antigen to BDV-specific T cells. The results in Table 2 show that indeed the NM 1 cells proliferated specifically in response to BDV antigen.

Having established that uninfected astrocytes can present BDV-specific antigen or MBP to T cells we asked whether persistently BDV-infected cells could also function as antigen presenting cells (APC) and whether the BDV-specific 38/39 kDa protein was one of the antigens present on infected astrocytes. Again, first the ability of BDV-infected astrocytes to present exogenously added MBP to the MBP-specific T cell line was tested. As shown in Table 1 MBP-specific T cells proliferated in an antigen specific manner to MBP, whereas PPD or BDV-antigen did not induce specific proliferation (data not shown). This clearly shows that BDV-infected astrocytes can function as APC.

We then tested whether these BDV-infected astrocytes could also present antigen to the T cell line NM 1 specific for the 38/39 kDa BDV antigen. In-

Table 1. Proliferation of MBP-specific T cell line Z 82 cocultured with various syngeneic antigen presenting cells

Antigen presenting cell	[^{3}H]thymidine incorporation					
	No antigen		MBP		ConA	
	cpm	S.I.	cpm	S.I.	cpm	S.I.
Uninfected astrocytes	70 ± 23	1	1309 ± 135	19	8730 ± 790	125
BDV-infected astrocytes	128 ± 15	1	1190 ± 122	9	6093 ± 637	48
Thymocytes	83 ± 12	1	3766 ± 384	45	6091 ± 493	73

1×10^5 T lymphocytes were cocultivated with irradiated (6000 rad) antigen presenting cells in the presence or absence of the specific antigen MBP. Three days before the addition of T cells astrocytes were seeded at a concentration of 1×10^4 cells/well into micotiter plates and 20 U/ml IFN-γ was added. Thymocytes were used at a concentration of 1×10^6 cells/well without further treatment

Table 2. Proliferation of BDV-specific T cell line NM 1 cocultured with various syngeneic antigen presenting cells

Antigen presenting cell	[^{3}H]thymidine incorporation					
	No antigen		BDV-antigen		ConA	
	cpm	S.I.	cpm	S.I.	cpm	S.I.
Uninfected astrocytes	*133 ± 21*	1	1493 ± 109	11	8510 ± 693	64
BDV-infected astrocytes	458 ± 109	1	1631 ± 131	4	20425 ± 1350	45
S.I. based on stimulation by uninfected astrocytes[a]		*3*		*12*		*153*
Thymocytes	73 ± 20	1	4956 ± 393	68	16579 ± 850	227

The proliferation assay was performed as described in Table 1 except the presence of 20 µg/ml purified BDV-specific antigen

[a] In order to discriminate between the capacity of BDV-infected astrocytes to present endogenous BDV-antigen or exogenously added BDV-specific protein, the stimulation index was additionally based on the background proliferation of uninfected astrocytes without antigen (133 ± 21 cpm)

cubation of NM 1 cells with rIFN-γ induced, Ia-expressing infected astrocytes resulted in a moderate specific proliferation (S.I. of 3) when compared with the uninfected control. However, addition of BDV-specific antigen exogenously resulted in a significant specific proliferation comparable to the stimulation by uninfected astrocytes in the presence of virus-specific antigen (Table 2). This shows that BDV-infected astrocytes can efficiently present exogenous BDV-antigen but have rather weak antigen presenting capacity for endogenous proteins.

Astrocytes as targets for cytotoxic T cells

Sun and Wekerle [51] found that astrocytes which are able to stimulate MBP-specific T cells can also be lysed by these CD4$^+$ T cells. This cytolytic function was shown to be antigen-specific and MHC class II-restricted.

We then analyzed the T cell-dependent destruction of BDV-infected vs. uninfected astrocytes in conventional cytotoxicity assays. MHC class II expressing BDV-infected or uninfected astrocytes were used as target cells for BDV-specific effector cells, which were either in an "activated" stage (i.e., immediately after antigenic stimulation for 3 days with thymocytes as source of APC) or in a "resting" stage (i.e., kept for more than 8 days in IL-2 containing medium without restimulation).

Examination of the T cell dose response of astrocyte lysis revealed optimal

effector/target cell ratios (E/T ratio) of 5:1 or 10:1 using both "activated" or "resting" T cells. However, the kinetics of lysis exerted on target cells was considerably different in so far as freshly "activated" NM 1 cells caused specific lysis after only 4 h of coincubation, whereas "resting" T cells did not cause specific lysis of targets after that short of an incubation time, even at high E/T ratios of 20:1 (Fig. 2 and data not shown). Significant killing by "resting" effector cells started after 12 h of incubation. Infected astrocytes not treated with rIFN-γ prior to incubation with "activated" effector T cells were not lysed at 4 h of cocultivation (E:T ratio 10:1 = 1.7% from untreated vs. 25.3% from r-IFN-γ treated BDV-infected astrocytes; untreated, uninfected cells: 2.3%). However, after 12 h of cocultivation an increase of lysis by "activated" effector cells was observed in untreated as well as in rIFN-γ induced target cells (E:T ratio 10:1 = 22.1% from untreated vs. 42.7% from rIFN-γ treated BDV-infected astrocytes; untreated, uninfected cells: 16.0%). Astrocytes inoculated with "resting" T cells were never lysed significantly without prior rIFN-γ induction.

Furthermore, it was tested whether uninfected astrocytes inoculated exogenously with BDV-specific antigen can act as targets for cytotoxic BDV-specific T cells. Figure 3 shows the result from such an experiment revealing that astrocytes pulsed with BDV antigen before T cell-astrocyte interaction were lysed by virus-specific T cells in an antigen-specific manner.

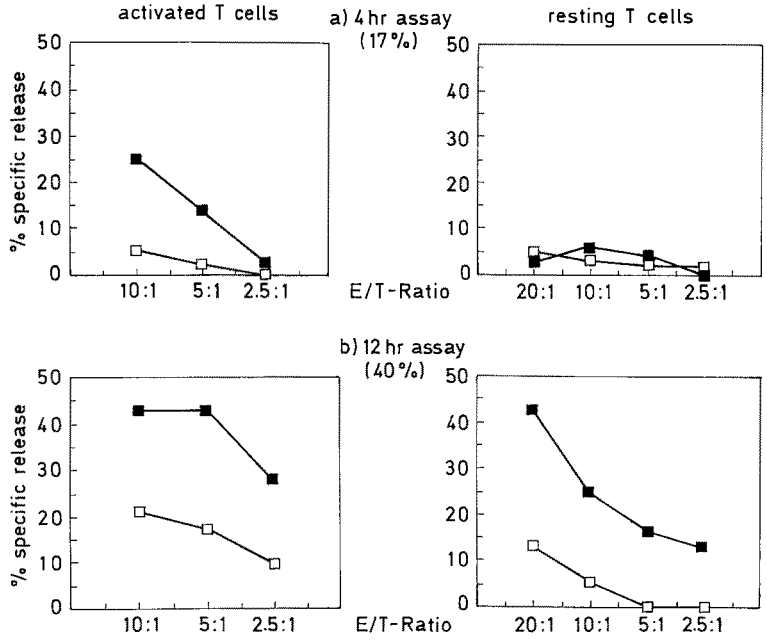

Fig. 2. "Activated" or "resting" BDV-specific NM 1 T cells were tested for cytotoxicity on ^{51}Cr-labeled BDV-infected (■) or uninfected (□) astrocytes in an 4 h assay (spontaneous release 17%) or an 12 h assay (40%) at the indicated effector/target (E:T) ratios. Target cells were pretreated with rIFN-γ (10 U/ml) for 72 h prior to testing

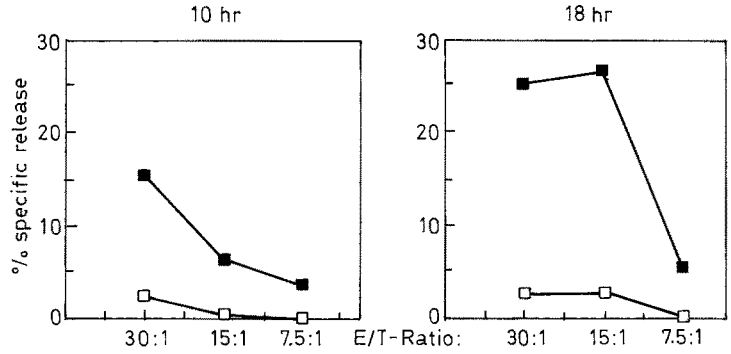

Fig. 3. "Activated" BDV-specific NM 1 T cells were assayed on ^{51}Cr-labeled uninfected astrocytes which had been cultured for 72 h in the presence of the BDV-specific 38/39 kDa protein (15 µg/ml) and rIFN-γ (■). □ No antigen. The figure shows the result from an experiment, where the assays were terminated after 10 h (spontaneous release 29%) or after 18 h (47%)

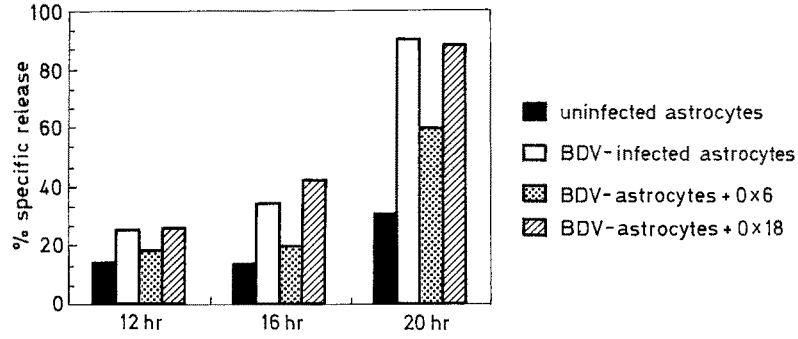

Fig. 4. "Activated" NM 1 T cells were assayed on ^{51}Cr-labeled BDV-infected astrocytes in presence or absence of mAb (1 : 200) directed against MHC class II (Ox 6) or class I (Ox 18) antigen at an E/T ratio of 10 : 1. The assay was terminated after 12 h (spontaneous release 34%), after 16 h (46%) and after 20 h (55%)

Inhibition of lytic activity by mAb to MHC antigens

Previous experiments have shown that the T cell line NM 1 expresses the markers W 3/13 (CD 5) and W 3/25 (CD 4), but not Ox 8 (CD 8) and Ox 22 (CD 45R), which characterizes this cell as T helper/inflammatory lymphocyte [39]. Thus, these cells recognize their antigen only in context with syngeneic MHC class II antigens. In order to prove that the expression of MHC class II antigen was also essential for lysis, cytotoxicity assays were performed with rIFN-γ treated BDV-infected astrocytes in the presence of antibodies to MHC class I (Ox 18) and class II (Ox 6) antigens. The results presented in Fig. 4 show that NM 1 cells again are able to lyse infected astrocytes in a time dependent manner, and furthermore, lysis was not inhibited by the mAb Ox 18. In contrast, antibodies against MHC class II (Ox 6) restriction elements suppressed cytolysis signifi-

cantly. The results obtained by using mAb Ox 6 were not consistent in all experiments performed, sometimes showing just a slight inhibition. This may have occurred because we did not preincubate the target cells with the appropriate antibody as proposed in an earlier report [26].

Discussion

The present in vitro study shows the role of astrocytes in antigen presentation and as target cells for lysis by a BDV-specific CD 4$^+$ T cell. It could be demonstrated that infection of astrocytes with BDV does not induce the expression of MHC class II as demonstrated recently for other neurotropic virus infections in vitro [30, 31], and that persistently infected astrocytes retained their MHC inducibility and antigen-presenting ability.

The expression of CD 4 or CD 8 antigens was initially thought to be directly related to their function, where CD 4$^+$ lymphocytes were described as non-cytotoxic helper/inducer T cells and CD 8$^+$ T cells were shown to exert cytotoxic or suppressor activity [38]. More recently, however, it has become evident that there is not necessarily a correlation between phenotype and function; it appears that the expression of CD 4 or CD 8 antigens on T cells is linked to their restriction by MHC class I or class II antigens, respectively, rather than to T lymphocyte function [50].

In order to define a cell type in the CNS which might be relevant to the in vivo situation in BD, we tested possible functional interactions between CD 4$^+$ BDV-specific T cells and astrocytes in vitro. Previous studies have shown that astrocytes are one of the target cells for BDV infection in the rat brain [8, 11, 29]. Furthermore, astrocytes are potent antigen presenting cells [13], suggesting an involvement in immunopathology after viral infections or in autoimmune disorders of the central nervous system.

In a previous report it was shown that astrocytes isolated from the brain of newborn Lewis rats were able to present exogenously added BDV-specific antigen to the T cell line NM 1 although less efficient than thymocytes as a source of APCs [40]. In the present study it is shown that persistently BDV-infected astrocytes only marginally induce an antigen-specific proliferation of NM 1 cells, whereas the presence of purified BDV-specific antigen triggered a stronger response. Furthermore, persistently infected astrocytes were as efficient in presenting MBP to specific T cells as their uninfected counterparts. These data confirm other reports and indicate that astrocytes persistently infected with the neurotropic BDV retained their full ability to present foreign antigen, i.e., that BDV infection does not interfere with essential cell functions.

Based on these data and considering the fact that necrosis and loss of neurons can be observed in the brain of infected rats despite the absence of cytopathogenicity of BDV [18], we reasoned that there might be a cytolytic reaction against infected brain cells either mediated by antibodies or immune cells. Since no evidence for a pathogenetic role of antibodies has so far been found [35, 47] this possibility seems unlikely. However, in the past it was never possible

to demonstrate BDV-specific cytotoxic T cells in preparations of spleen cells, brain lymphocytes or cloned lymphocytes from the cerebrospinal fluid of BDV-infected rats (L. Stitz and B. Fleischer, unpubl. results). In these cytotoxic assays, however, either MHC class I-matched target cells which did not express MHC class II antigens or target cells which were not treated with rIFN-γ to induce MHC class II expression, were used.

In the present study we report on experiments which were carried out to evaluate cytolytic activity of a BDV-specific CD4$^+$ T cell line, a subset of T lymphocytes of decisive importance in the pathogenesis of BD [11, 40]. This CD4$^+$ T cell line was able to specifically kill syngeneic, rIFN-γ treated persistently infected astrocytes and lysis was significantly reduced by antibodies directed toward MHC class II antigens.

These results confirm studies in several other viral infections, although not of the CNS, which have revealed the existence of specific MHC class II-restricted cytotoxic T cells [6, 21, 22, 24, 26, 53]. Antiviral activity of classical MHC class I restricted virus-specific T cells has been exemplified in numerous reports [2, 27, 45, 55]. However, in none of the virus infections in which MHC class II-restricted CTL activity was elicited in vitro, any direct evidence has been presented for an antiviral effector mechanism in vivo resulting in virus elimination. In the probably best studied LCMV infection in mice, a potent CD8$^+$ T cell response resulting in both virus elimination [7, 34, 54] and immunopathology [3, 9] has been shown. In BDV infection of rats, the pathogenic importance of virus-specific CD4$^+$ T cells has been demonstrated recently [11, 39, 40], while in the present report evidence for the cytolytic activity of a BDV-specific CD4$^+$ T cell line in vitro is presented.

Other significant findings of the present work are the kinetics of lysis by BDV-specific MHC class II-restricted effector cells as compared to MHC class I-restricted CTL and the different kinetics of lysis exerted by "activated" vs. "resting" T cells. Regarding the first point, lysis mediated by MHC class I-restricted T cells is fast and mostly linear, in contrast to the rather prolonged period of effector/target incubation necessary to obtain significant lysis by MHC class II-restricted T cells. Another factor important for the outcome of cytotoxicity assays with CD4$^+$ T cells is related to the question of whether T cells are "activated" or "resting". It was clearly demonstrated that "activated" NM 1 cells cause much faster kinetics of lysis of target cells than "resting" NM 1 cells. This phenomenon seems to be dependent on the induction of MHC class II antigens on the respective target cells, since non-induced astrocytes are not lysed significantly at 4 h of cocultivation. However, after longer incubation periods untreated target cells were also lysed by "activated" T cells, although less effectively as compared to MHC class II-induced target cells. In contrast, "resting" T cells were not able to exert cytotoxic activity on untreated astrocytes. This latter finding underlines the MHC-dependence of the NM 1-mediated cytotoxic reaction.

The fact that persistently BDV-infected astrocytes without exogenously

added antigen act as relatively poor APC and that the same cells functioned efficiently as target cells are not necessarily contradictory. It has recently been shown that about 200–300 MHC class II/peptide complexes are needed to obtain a stimulatory effect of responder cells [17]. However, the number of such complexes on cells susceptible to lysis by cytotoxic T cells has never been determined. Therefore, these differences may simply reflect a quantitative problem.

The data presented show that BDV-infected astrocytes may play a role as APC as well as target cells in BD; however, the antigen-presenting capacity of these cells without addition of exogenous antigen seems to be rather low. Since BDV is a noncytolytic virus, we cannot exclude that the late onset of immune-mediated disease after BDV-infection might be caused by the low stimulatory activity of infected astrocytes. On the other hand, it is possible that the cellular immune response might be strongly influenced by BDV-antigen which is released from infected cells after immune-mediated lysis. In both cases the presentation of foreign antigen in association with class II MHC antigen might be possible, since alternative pathways of antigen presentation (endogenous vs. exogenous) have been suggested recently [5]. Firstly, it has been demonstrated in several virus infections that protein processed by the endogenous pathway can not only be presented by MHC class I antigen, but also by class II MHC antigen [23, 36, 37]. Secondly, it has been shown that exogenously added protein can associate both with class I [12, 46, 48] and class II [1, 15] molecules for presentation to T cells.

Our findings might also provide an explanation for the significant decrease of encephalitic lesions in the late phase of disease, resulting in a complete absence of cellular infiltrates in the brain, beside high titers of infectious virus. If BDV-infected astrocytes are also lysed in vivo as demonstrated in vitro by BDV-specific cytotoxic T cells, this might limit and ultimately terminate the stimulation of BDV-specific T cells, and consequently the immunopathological reaction.

Acknowledgement

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