A Naturally Processed Rat Major Histocompatibility Complex Class I-associated Viral Peptide as Target Structure of Borna Disease Virus-specific CD8⁺ T Cells*

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The first naturally processed peptide synthesized by a virus and recognized by classical CD8⁺ T cells in association with the RT1.A¹ major histocompatibility complex class I molecule of the Lewis rat is reported. Borna disease virus-specific CD8⁺ T cells recognize syngeneic target cells pulsed with peptides extracted from Borna disease virus-infected cells. The predicted peptide sequence AsC14AMTFF derived from the viral p40 protein co-localizes with the cytotoxic T-lymphocyte-reactive fraction was identified among natural ligands by tandem mass spectrometry. Numerous naturally processed peptides derived from intracellular bacteria, viruses, or tumors and recognized by CD8⁺ T cells of man and mice are known, leading to a better understanding of cellular immune mechanisms against pathogens in these two species. In contrast, for the rat little information exists with regard to the function and role of CD8⁺ T cells as part of their cellular immune defense system. This first naturally processed viral epitope in the rat contributes to the understanding of the rat cellular immune response and might trigger the identification of more cytotoxic T-lymphocyte epitopes in this animal.

Major histocompatibility complex (MHC)¹ class I molecules present peptides to CD8⁺ T cells, which recognize this complex by their T cell receptor. Such CD8⁺ cytotoxic T-lymphocytes (CTLs) detect cells infected with viruses or intracellular bacteria and consequently destroy these infected cells by cytotoxic effector mechanisms (1–4). MHC class I molecules are assembled by combining the α-chain and the β₂-microglobulin in association with a peptide derived from cytoplasmic proteins after proteolytic cleavage by proteosomes (5). The resulting peptides are transported into the lumen of the endoplasmic reticulum with the help of a heterodimeric transporter associated with antigen presentation (TAP) (6, 7). The α-chain, β₂-microglobulin, and peptide are assembled in the endoplasmic reticulum, and the mature MHC class I molecule migrates through the Golgi to reach the cell surface. Most of the allelic polymorphism of the α-chain is confined to the α₁,α₂ domains, which form a membrane-distal groove, and specific binding is defined by key amino acids within the peptide, referred as anchor residues (reviewed in Ref. 8). In contrast to humans and mice, in the laboratory rat (Rattus norvegicus) the function of CD8⁺ T cells is poorly understood, and only a few experimental models of intracellular infectious agents are available to analyze T cell functions.

The rat expresses two different types of MHC class I molecules, a classical class Ia, which is responsible for conventional antigen presentation to CTLs, and nonclassical class Ib molecules with unconventional or undefined functions (9, 10). The RT1.A region of the rat MHC encodes for the class Ia molecules, whereas different RT1 regions encode the nonclassical class Ib molecules (11, 12). Furthermore, in rats, unlike humans or mice, two functionally allelic forms of the TAP exist, which are called TAP-A and TAP-B. These molecules can be distinguished by their different peptide transport specificities. In the Lewis rat, RT1.A¹ molecules are linked to TAP-A (reviewed in Ref. 13). To our knowledge, the motifs of only two TAP-A-associated molecules, RT1.A¹ and RT1.A², have been determined (14–16), allowing epitope prediction for these class I molecules. Thus far, only peptides from naturally processed self-proteins are known to bind to RT1.A¹ and RT1.A² molecules, whereas no information on peptides associated with class I molecules from intracellular bacteria or viruses exists.

One of the few experimental infectious models in rats is Borna disease, caused by Borna disease virus (BDV), a noncytopathic single-stranded RNA virus, belonging to the order of Mononegavirales. BDV-induced Borna disease is an encephalomyelitis originally described in horses and sheep (17, 18). In recent years, this viral infection of the central nervous system has been diagnosed in a wide variety of animals including cattle, cats, dogs, and birds (reviewed in Ref. 19). Furthermore, Borna disease virus, its nucleic acid, and specific antibodies were detected in the blood of patients with psychiatric diseases (20–25). However, there is no evidence whether BDV represents the causative agent for any human disorder.

The best investigated animal model for the pathogenesis of BDV infection is the Lewis rat. After intracerebral infection, the animals develop an encephalomyelitis in which the infiltrating cells have been characterized as CD4⁻ and CD8⁺ T...
cells and macrophages (26, 27). BDV-specific CD8+ T cells represent the effector cell population during the acute phase of the disease and significantly contribute to the destruction of virus-infected brain cells in vivo. Moreover, evidence has been presented that this T cell population also participates in the degenerative encephalopathy resulting in a severe cortical brain atrophy in the chronic phase of the disease (28–30). Besides their role in immunopathology, however, BDV-specific CD8+ T cells were also known to eliminate the virus, without causing disease. BDV-specific CD4+ T cells given prior to infection induce CD8+ T cells, which eliminate the virus without causing significant cell damage (31).

The nucleoprotein (p40) and phosphoprotein (p24) of the virus are most abundantly synthesized during BDV infection and represent the main targets for the immune system. Recently, we have shown that the nucleoprotein is a major target for CTL in the Lewis rat model (32). In this report, we describe the characterization and quantification of a naturally processed RT1A1 ligand from the nucleoprotein of BDV. This peptide is recognized by classical CD8+ T cells.

**EXPERIMENTAL PROCEDURES**

**Virus and Experimental Animals**—The Giessen strain He/80 of BDV was used for this study (33). Female Lewis rats were purchased from the central breeding facilities of the Federal Research Center for Viral Diseases of Animals in Tu¨ bingen. At an age of 5 weeks the animals were infected intracerebrally in the left brain hemisphere with 0.05 ml of BDV corresponding to 5 x 107 focus-forming units.

**Cell Lines and Cell Collection**—Skin homogenates were obtained from 2-week-old Lewis (LEW), Brown Norway (BN), and Louvain (LOU) rats and cultured for more than 8 years in our laboratory (28). F10 (Lewis astrocytes) cells were originally obtained from Dr. H. Wekerle, Munich. OX 18 hybridoma cells, secreting antibodies directed against RT1A1 were purchased from ATCC and cultured in our laboratory. In addition, LEW and F10 cells were persistently infected with BDV (BDV-LEW and BDV-F10), and persistent infection was controlled on a routine basis by immune response or fluorescence-activated cell sorter analysis. For peptide elution BDV-LEW or BDV-F10 cells were cultured in a spin bottle system using Cultisphere™ microcarrier (Integra, Fernwald, Germany). This system allowed the production of 1–2 x 109 cells/liter. OX18 monoclonal antibody was produced in 350-ml CELLine™ flasks (Integra, Fernwald, Germany).

**Preparation of BDV-specific T Cells**—Lymphocytes from the brains of BDV-infected rats isolated by a method previously described (34) and modified for the BDV infection of rats (28). Twenty days after BDV infection rats were anesthetized with ketamine hydrochloride and perfused with balanced salt solution. The brain tissue was carefully homogenized through a stainless steel mesh and collected in balanced salt solution containing collagenase D (0.05%), trypsin inhibitor (TLCK; 0.1 µg/ml), DNase I (10 µg/ml), and EDTA (10 mM). The cell suspension was stirred at room temperature for 1 h and allowed to settle for 30 min. The supernatant was pelleted at 200 x g for 5 min. The pellet was resuspended in 10 ml of calcium-magnesium-free phosphate-buffered saline. Five ml of the suspension were layered on top of 10 ml of a modified RPMI medium-Ficol gradient and centrifuged at 500 x g for 30 min. The pellet containing the lymphocytes was resuspended in IMDM with 5% rat serum and 5% Con A supernatant and cultured overnight. The next day, the cells were counted and used for further use.

**In Vitro Cytotoxicity Assay and Peptide Labeling**—Effector T cells were used in a concentration of 5 x 103 cells/ml or 106 cells/ml IMDM, 2% fetal calf serum. Persistently infected BDV-treated LEW (BDV-LEW) were labeled with 0.2 mCi of ³⁵Cr at 37 °C for 1 h, washed three times with balanced salt solution, and used as target cells. Dried HPLC peptide fractions were resuspended in a standard volume of 150 µl of phosphate-buffered saline. For titration, 50 µl of each fraction either undiluted or diluted 1:10 or 1:100 was added to 106 untreated LEW cells in 50 µl of IMDM, 2% fetal calf serum for 90 min at 37 °C. Thereafter, 100 µl of effector cells (effector to target ratio of 30:1 or 10:1) were added and incubated for 4 h at 37 °C. Synthetic peptides were dissolved in Me₂SO in a concentration of 1 µg/ml. For peptide titration, either 5 or 1 µl of the different peptides and 1:10 and 1:100 dilutions in a volume of 50 µl of IMDM, 2% fetal calf serum were used to pulse 106 LEW cells in 50 µl of IMDM, 2% fetal calf serum for 90 min at 37 °C. Effector cells were used as described above. For effector cell titration, the standard peptide ASYAQMTTY was used in a concentration of 20 µM.

**Extraction of MHC Class I Molecules and Isolation of Viral Peptides from Infected Cells**—2.5 x 1010 virus-infected and infected LEW or F10 cells were resuspended in 5 ml of lysis buffer (phosphate-buffered saline, 10 mM CHAPS, 0.1 mM phenylmethylsulfonyl fluoride, 1 mM fluoride, protease inhibitor mixture tablets (Roche Molecular Biochemicals)) and disrupted using a handheld glass homogenizer and sonication. The suspensions were stirred at 4 °C for 1 h before centrifugation at 4000 rpm for 10 min. The supernatants were spun in an ultracentrifuge at 40,000 rpm for 1 h and passed through prefilters before loading onto a cyanogen bromide-activated Sepharose 4B column as a preclearing step. The MHC I molecules were then purified by immunofinity chromatography using monoclonal antibody OX18 coupled to cyanogen bromide-activated Sepharose 4B. After elution of the RT1A1 complexes using 0.1% trifluoroacetic acid (pH 2), the eluted material was filtered through a Centricon 10 and concentrated to 0.5 ml by vacuum centrifugation.

**Fractionation by HPLC**—Peptide separations were carried out on a reversed-phase precapped column (C2/C18, 2.1 x 100 mm; Amersharm Pharmacia Biotech) using the Amersham Pharmacia Biotech SMART system. Samples were injected in a volume of 500 µl. The following elution procedure was used: solvent A, 0.1% trifluoroacetic acid in H₂O; solvent B, 0.081% trifluoroacetic acid in 80% acetonitritile; 0–10 min, 30% B; 10–25 min, linear increase to 20% B; 25–45 min, 1%/min increase to 40% B; 45–55 min, 2%/min increase to 60%; 55–60 min, linear increase to 75% B; and 60–65 min, constant 75% B. The flow rate was 150 µl/min. Fractions were collected by time fractionation (1–10 min, 450 µl/min; 10–65 min, 150 µl/min), and elution was monitored by measuring UV light absorption at 214 nm in a continuous flow detector. Acetonitrile was removed from eluted material by vacuum centrifugation before samples were made up to a standard volume of 150 µl using phosphate-buffered saline and stored at −80 °C. For the coelution experiments, 1 µg of synthetic ASYAQMTTY diluted in 0.1% trifluoroacetic acid was injected in a total volume of 500 µl and separated using the same conditions as described above.

**Epitope Prediction**—Potential RT1A1-presented peptides were selected by epitope prediction as described (35). Briefly, nonamer peptides accessed from the sequence of p40 (Swiss-Prot accession number Q001529) and other Borre disease virus proteins were selected using a matrix pattern suitable for the calculation of peptides fitting to the RT1A1 peptide motif. The peptide motif and epitope predictions are available on our web page, where additional information can be obtained.

**Synthetic Peptides**—Peptides were synthesized in an automated peptide synthesizer 432A (Applied Biosystems, Weiterstadt, Germany) following the Fmoc (N-(9-fluorenylmethoxycarbonyl)Val) strategy. After removal from the resin by treatment with trifluoroacetic acid/pheno- nethanethiol/thiouisolate/water (90:3.75:1:25:2.5:2.5 by volume) for 1 h or 3 h (arginine-containing peptides), peptides were precipitated from methyl tert-butyl ether, washed once with methyl tert-butyl ether and twice with diethyl ether, and resuspended in water prior to lyophilization. The products were analyzed by HPLC (Hewlett-Packard, Germany) and matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry (see below). Peptides of less than 80% purity were purified by preparative HPLC.

**Mass Spectrometry**—For MALDI-TOF MS 0.5 µl of sample was mixed with 0.5 µl of dihydroxyacetoneophenone matrix (20 mg of 2,5-dihydroxyacetonephenone, 5 mg of ammonium citrate in 1 ml of 80% 2-propanol) on a gold target and analyzed on a Hewlett-Packard G2025A instrument (Hewlett-Packard, Waldbronn, Germany) at a vacuum of 10−6 torr (1 torr = 133 pascals). For signal generation, 50–150 laser shots were added up in the single shot mode.

**Nanocapillary HPLC-MS and MSMS of synthetic and naturally processed peptides were done as described (36) by coupling a reversed-phase HPLC system (AB1 140D, Applied Biosystems) to a hybrid quadrupole orthogonal acceleration time of flight tandem mass spectrometer (Q-TOF, Micromass, Manchester, United Kingdom) equipped with an electron spray ionization source. As a modification of the described setup, loading of typical sample volumes of 100 µl was achieved by preconcentration on a 300-µm x 5-mm C18 µ-precolumn (LC Packings, San Francisco, CA). A syringe pump (PHD 2000, Harvard Apparatus Inc., Holliston, MA) was equipped with a gas line filled with a gas of argon (1710 RNR, Hamilton, Bonaduz, Switzerland), which was used to deliver solvent and sample at a flow rate of 2 µl/min. A blank run was performed prior to any HPLC-MS run to ensure that the system was free of any residual synthetic peptide.

**For nanocapillary HPLC-MSMS experiments, fragmentation of the parent ion was achieved at the given retention time by collision with**
argon atoms. Q1 was set to the mass of interest ± 0.5 Da, and an optimized collision energy was applied. Fragmentation was completed after 60 s.

**RESULTS**

**Recognition of Synthetic Peptides by BDV-specific T Cells**—Peptide motifs and anchor residues of the RT1.A1 molecule have been published previously (15). Therefore, the five entries of Borna disease virus proteins contained in the Swiss Protein Database, release 39 (nucleoprotein, phosphoprotein, matrix protein, glycoprotein, and the L-polymerase of BDV), were screened for RT1.A1 motifs using the database SYFPEITHI. Peptides optimal for presentation by RT1.A1 are nonamers carrying Phe or Tyr in position 3 and large hydrophobic residues in position 9. According to this prediction, several sequences were synthesized from each viral protein (Table I). Fibroblast cells from the Lewis rat (LEW, RT1.A1) were loaded with peptides because no TAP-deficient cell lines are available that express the RT1.A1 class I molecule.

To test whether the predicted peptides are recognized by BDV-specific CD8\(^+\) T cells, Lewis rats were infected by the intracerebral route, and 19 days later lymphocytes were isolated from the brain. As shown in Table I, these T cells recognized only 1 of the 16 predicted peptides when pulsed on LEW cells. The sequence ASYAQMTTY is located within the nucleoprotein of BDV. After loading LEW cells with different concentrations of peptide, a cytotoxicity assay using BDV-specific T cells as effector cells was performed. As shown in Fig. 1A, the titration experiment indicated the highest specific lysis with 5 pg of peptide; half-maximal recognition was observed with 50 pg of peptide. When the amino acid tyrosine at position 9 was changed to a glycine, the peptide was still recognized to a lower extent (Fig. 1B), whereas after replacement of the tyrosine at position 3 by a glycine, cell lysis was only observed with the highest peptide concentration (Fig. 1C).

The RT1.A1 restriction of BDV-specific T cells was demonstrated by loading fibroblast cell lines from the Brown Norway rat (RT1.A1) and from the Louvain rat (RT1.Au) with peptide and using them as target cells. As shown in Table II, BDV-specific T cells are unable to recognize peptide-labeled BN or LOU cells, whereas peptide-loaded LEW cells as well as infected cells were killed. In addition, T cells from BDV-infected rats were unable to kill YAC cells, demonstrating the absence of NK cell activity (Table II). These results indicate that the synthetic peptide ASYAQMTTY is recognized in combination with the RT1.A1 class I molecule by BDV-specific T cells from the Lewis rat.

**T Cell Recognition of Naturally Processed Peptides from BDV-infected Cells**—Persistently BDV-infected LEW cells (2.5 \(\times\) 10\(^{10}\)) were lysed, and the RT1.A1 complexes were purified.

### Table I

| Position\(^a\) | MW  | Peptide\(^b\) | Specific Lysis\(^c\) |
|-------------|-----|--------------|---------------------|
| p40         |     | DAMEQDLY     | 0/2/1               |
| 12 - 20     | 1098| PTEMLIETF    | 0/6/8               |
| 18 - 26     | 1004| NQFLNIPFL    | 1/5/6               |
| 24 - 33     | 1057| VSYLNHTTI    | 2/n.d./n.d.         |
| 122 - 131   | 1219| DSFVINLDP    | 0/4/4               |
| gp45        | 1101| HVFTYCL      | 6/10/17             |
| 440 - 448   | 1084| KEFQDSALL    | 10/7/10             |

\(^a\) Position is the amino acid position of the respective protein.

\(^b\) Peptide concentration was 1 mg/ml.

\(^c\) Effector:target ratio was 30:1; numbers indicate lysis with 5-\(\mu\)g/0.5-\(\mu\)g/50-ng peptide to label the target cells.

**Fig. 1.** BDV-specific T cells were isolated from the brains of BDV-infected rats (see *Experimental Procedures*). LEW cells were used as target cells and labeled with peptide ASYAQMTTY (A), peptide ASYAQMTTG where position 9 was replaced by a glycine (B), or ASGAQMTTY where position 3 was replaced by a glycine (C) in different amounts as indicated. An effector to target ratio of 30:1 was used. After 10 h 50 \(\mu\)l of the supernatant were harvested and counted in a Packard gamma counter, and percent specific lysis was determined. Spontaneous release in this experiment was 28%. The experiment with peptide A was repeated four times; experiments with peptide B and C were done twice.
fied by immunoaffinity chromatography using the MHC class I-specific monoclonal antibody OX18. The peptides were eluted from the RT1.A complexes and fractionated by HPLC. In control experiments, RT1.A-bound peptides from uninfected LEW cells were eluted (Fig. 2B and data not shown). Thereafter, uninfected LEW cells were incubated with aliquots of the different HPLC fractions and were tested for recognition by BDV-specific T cells. Significant BDV-specific lysis was found with the HPLC fraction 24 and to a lower extent with fraction 23 (Table III, Fig. 2C). Similar results were obtained when RT1.A molecules from $2.5 \times 10^{10}$ persistently BDV-infected F10 cells were immunoprecipitated and fractionated by HPLC (data not shown). As a control, LEW cells were incubated with HPLC fractions 23 and 24 of the peptide mixture eluted from a column that contained glycine instead of the monoclonal antibody OX18. No lysis of target cells was observed, indicating that HPLC fractions 23 and 24 of persistently BDV-infected LEW cells contained peptides that are recognized by BDV-specific T cells (Table III). Furthermore, no specific lysis was found when target cells were incubated with HPLC fractions of RT1.A molecules from $2.5 \times 10^{10}$ uninfected LEW or F10 cells (data not shown).

**Coelution of the Synthetic Peptide ASYAQMTTY with the CTL-reactive Fraction**—One μg of the synthetic peptide ASYAQMTTY was analyzed by HPLC using identical conditions as for the separation of RT1.A ligands from BDV-LEW. As shown in Fig. 2A, an intense UV signal is visible in the HPLC profile at fraction 24. Additional peaks, particularly in fractions 13, 28, and 47, result from medium contents, because the peptide had been dissolved in IMDM before it was diluted in 0.1% trifluoroacetic acid. Fraction 24 and fraction 23 were analyzed by MALDI-TOP mass spectrometry. Only one m/z signal corresponding to the molecular mass of ASYAQMTTY (MH+ 1035) was detected, indicating that ASYAQMTTY coeluted with the naturally processed peptide recognized by BDV-specific T cells (data not shown). Furthermore, when fractions 23 and 24 were used to label target cells, these cells were lysed by BDV-specific T cells (Table III). Dilution experiments indicated that the majority of ASYAQMTTY peptide eluted in fraction 24. Moreover, Table III suggests that in fraction 24 of the BDV-LEW, the OX18 HPLC run contained less copies of the peptide than the respective fraction of the HPLC run performed with the synthetic peptide.

**Characterization and Quantification of the Natural RT1.A Ligand from Borna Disease Virus p40 on BDV-LEW Cells**—Although CTL recognition after coelution experiments indicated the presence of ASYAQMTTY in fraction 24 of the RT1.A ligand separation from infected LEW, the amino acid sequence of this naturally processed peptide was confirmed by nanocapillary liquid chromatography-MSMS analysis (Fig. 3). Comparison of the liquid chromatography-MS signal intensities of the naturally processed peptide and 2 pmol of coeluting synthetic peptide indicated that a total of 3.7 pmol of naturally processed ASYAQMTTY had been isolated from $2.5 \times 10^{10}$ BDV-infected LEW cells (data not shown). This corresponds to $\sim 350$ copies/cell, assuming an overall yield of 25% after peptide extraction and HPLC (36).

**TABLE II**

| Target cells | Percent specific lysis* |
|--------------|-------------------------|
| BDV-LEW (RT1.A) | 50/20/3 |
| LEW-peptide (RT1.A) | 60/36/12 |
| BN-peptide (RT1.A) | 0/0/0 |
| LOU-peptide (RT1.A) | 0/0/0 |
| YAC | 3/0/0 |

* Effector:target ratio, 10:1/3:1/1.

**DISCUSSION**

In the present communication, we identified and characterized the first rat MHC class I ligand derived from an infectious agent and recognized by CD8+ T cells. The peptide was isolated from BDV-infected cells upon MHC immunoprecipitation and purified by HPLC. The peptide is recognized by BDV-specific T cells. Furthermore, following peptide prediction, a synthetic peptide is recognized in combination with the RT1.A class I molecule of the Lewis rat by BDV-specific T cells. Replacing an amino acid either in position 3 or position 9 results in clearly lower lytic activity, where position 3 seemed to be more decisive for an efficient binding of the peptide to MHC class I than did position 9. The peptide is located within the nucleoprotein (p40) of the virus. After HPLC fractionation of the synthetic peptide ASYAQMTTY, we found that this synthetic peptide coelutes with the naturally processed peptide recognized by BDV-specific T cells. Amino acid sequence by nanocapillary HPLC-MSMS analysis confirmed that ASYAQMTTY is also a naturally processed peptide. Furthermore, after quantification of the peptide, we were able to show that $\sim 350$ copies of this peptide are complexed with MHC class I molecules on the surface of a BDV-infected cell.

The knowledge of antigen processing and presentation in rats is fragmentary compared with what is known in humans and mice. For the laboratory rat (R. norvegicus) only the peptide motifs of RT1.A1, RT1.A4, RT1.A5, and RT1.A8 are known, showing a restricted preference for peptides of 9–12 amino acids.
Borna disease virus is found in a wide variety of mammals including man (reviewed in Ref. 19). The best investigated experimental model of Borna disease, a virus-induced immunemediated encephalomyelitis, is the infection of the Lewis rat. The knowledge of a defined CTL epitope of BDV will help to further characterize the immunopathological mechanisms in more detail. An earlier study showed that only target cells infected with a recombinant vaccinia-BDVp40 construct were recognized by BDV-specific CTL, whereas target cells infected with vaccinia virus carrying the phosphoprotein, the matrix protein, or the glycoprotein were not recognized (32). Because the peptide ASYAQMTTY is recognized by CTL most efficiently, one might assume that this peptide represents an immunodominant trait. However, we cannot exclude the existence of other, subdominant, nucleoprotein-specific CTL epitopes. Because no NK cell activity was found in brain lymphocyte preparations, NK-specific killing directed against the peptide ASYAQMTTY can be excluded. This finding is supported by an earlier report in which CD8+ T cell-mediated, MHC class I-restricted lysis of BDV-infected target cells, but no killing of NK-sensitive YAC cells, was found (28).

The quantification of the natural RT1.A1 ligand ASYAQMTTY from persistently BDV-infected cells showed that ~350 copies/cell were present. This copy number is similar to those reported from other viral epitopes associated with MHC class I molecules from humans and mice (41). In persistently BDV-infected cells, only a very few infectious viral particles can be found (42, 43). Because BDV is a negative-stranded RNA virus, one might speculate that high copy numbers of p40 are required for RNA stabilization and consequently virus replication. Therefore, our data provide additional information for the biology of BDV and a better understanding of the poorly understood mechanism of replication. MHC-restricted cytoxic T cells recognize virus-specific peptides in combination with MHC class I. This can take place relatively early after infection in the absence of an infectious virus (44). Recently it was shown that translation of RNA by ribosomes into protein can result in defective ribosomal products, leading to an early recognition of the virus-infected cell by the immune system, whereas the foreign proteins are still being produced (45, 46). These findings support the earlier investigations by Zinkernagel and Doherty (1, 44) and also suggest that the numbers of copies found for a peptide must not correlate with the amount of protein made and needed for virus replication. On the other hand, because the gene encoding for the nucleoprotein is located at the 3' end of the antigenome (open reading frame I), and therefore viral transcription and translation of this protein occur very early, the nucleoprotein is a good candidate for an early immunodominant CTL response of the host against BDV. This hypothesis is supported by the findings that p40 is the first BDV-specific protein detectable in infected cells and tissue and that BDV-specific CD8+ T cells are directed against the nucleoprotein (32, 47).

During the last 15 years BDV was repeatedly found in patients with psychiatric disorders (20, 21, 23). Nevertheless, it is not clear if BDV is the causative agent of these disorders or if it is simply a secondary infection. Antibodies in humans were found to be predominantly directed against the nucleoprotein, the phosphoprotein, and the matrix protein (25, 48, 49). The role of the cellular immune response against BDV in man is still unknown. With our data obtained in the BDV model system and with the help of epitope prediction and transgenic mouse models, one might be able to define BDV-specific HLA-restricted CTL epitopes to investigate a possible CTL response in man.

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