Hematologic Consequences of Borna Disease Virus Infection of Rat Bone Marrow and Thymus Stromal Cells

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Borna disease virus (BDV) was previously believed to have a strict tropism for the nervous system. BDV has recently been identified by a reverse transcription-polymerization chain reaction-enzyme immunosorbent assay (RT-PCR-EIA) in bone marrow cells and peripheral blood mononuclear cells (PBMC) in BDV-infected Lewis rats. We now report the identification of BDV RNA and infectious virus in thymus cells from rats infected either as neonates (PTI-NB) or as adults (4 weeks of age). Based on in vitro studies, we determined that the BDV-infected cells in bone marrow and thymus tissue are fibroblastic stromal cells. Bone marrow stromal cells are nonhematopoietic, fixed-tissue elements that support hematopoiesis, and, thus, it was not surprising that BDV infection altered the recovery from granulocytopenia and leukocytopenia after myelosuppressive treatment. Notably, unlike other immunotropic and neurotropic viruses, BDV does not appear to infect cells of myeloid or lymphoid lineages. We also report the association between BDV in the thymus with the lack, or loss, of encephalitis in neonatally inoculated rats or adult-inoculated rats during the chronic stage of disease.

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MATERIALS AND METHODS

Virus stock. A stock of BDV was prepared from clarified BDV-infected rat brain homogenate (20% wt/vol) and titrated as previously reported, with a viral titer of $1 \times 10^4$ TCID$_{50}$/mL.

Rats. Male Lewis rats (Charles River Laboratories, Cambridge, DE) were inoculated intracranially at 4 weeks of age (defined as adult) with $1 \times 10^4$ TCID$_{50}$ or within 48 hours of birth with $1 \times 10^3$ TCID$_{50}$ virus stock (PTI-NB). Age-matched uninfected Lewis rats were used as negative controls, receiving an equal volume of viral stock or no treatment.

Tissue/cell collection. Rats were exsanguinated via cardiac puncture after being anesthetized with methoxyfluorane (Pitman-Moore Inc, Mundelein, IL). Brain, thymus, and bone marrow were removed. Portions of these tissues were processed to form single-cell suspensions for studies described below.

Immunofluorescence assays for BDV protein or cell-specific marker detection. Indirect immunofluorescence assays (IFAs) were performed as previously described to detect BDV protein expression in infected cells. After fixation in acetone, cells were treated with polyclonal mouse anti-BDV followed by fluorescein isothiocyanate (FITC)-conjugated goat antimouse Ig (Vector Laboratories, Burlingame, CA). The slides were observed with an Axioshot fluo-
resent microscope (Carl Zeiss Inc, Hanover, MD) to detect the fluorescent signal from BDV antigens in infected cells. An IFA was also performed to identify the localization of BDV protein and cell-specific marker expression. Chamber slides of PTI-NB bone marrow cells and thymus cells were cultured for 1 to 2 weeks and fixed in acetone. After treatment with a blocking solution of 2% normal goat serum (Gibco, Gaithersburg, MD) diluted in phosphate-buffered saline, the cells were incubated with the following mouse monoclonal antibodies (Harlan, Indianapolis, IN) directed against rat cell-specific markers: W3/25 (TH cell antibodies, macrophages), MRC OX-8 (T suppressor/cytotoxic cells, thymocytes), W3/13 (T cells, thymocytes, polymorphonuclear cells), MRC OX-33 (B cells), MRC OX-42 (macrophages, granulocytes, dendritic cells). After washing, the cells were treated with fluorescent-labeled goat antimouse Ig (Vector) and rhodamine-conjugated goat antirabbit Ig (Cappel, Durham, NC). The slides were observed with an Axioplan fluorescence microscope equipped with a rhodamine filter (590 nm) to identify BDV-infected cells and a fluorescein filter (520 nm) to detect cell-specific markers.

Prevalence of BDV-infected cells in the immune system. Bone marrow tissues (n = 10), thymus tissues (n = 8), and PBMC (n = 3) from 2- to 3-month-old PTI-NB rats were examined for infectious BDV by cocultivation with a BDV-susceptible indicator cell line (C6 or PC-12) as described previously. In addition, serial half log dilutions of intact bone marrow cells (n = 5), thymus (n = 3), and PBMC (n = 2) from PTI-NB rats (2 months postinoculation [pi]) were cocultivated with either C6 or PC-12 cells. After 1 week, the monolayers were fixed in cold acetone, and an IFA was performed to detect BDV antigens as described above.

Alkaline phosphatase enzyme activity. BDV-infected bone marrow and thymus cells from three PTI-NB rats were examined for alkaline phosphatase enzyme activity, a marker for fibroblastic stromal cells. The cells were fixed in citrate-acetone-formaldehyde solution and stained using an alkaline phosphatase enzyme detection kit (Sigma, St. Louis, MO). Negative controls were also included in the assay by boiling slides of bone marrow and thymus cells in water for 1 minute to inactivate the alkaline phosphatase enzyme before the assay. BDV protein expression in these cultures was examined by IFA as described above. For double-label BDV antigen/alkaline phosphatase enzyme activity colocalization studies, Triton X-100 (0.1%) was included in all solutions to facilitate entry of anti-BDV antibodies into the cells.

Reverse transcription-polymerization chain reaction–enzyme immunoassay (RT-PCR-EIA) for BDV RNA. An RT-PCR-EIA was performed using oligonucleotide primers specific for BDV ORF 1 as performed previously. RNA was extracted from brain and thymus tissue from 15 PTI-NB rats, six adult-inoculated rats in the acute stage of disease (less than 2 months pi), and four adult-inoculated rats during the chronic stage of disease (=2 months pi). RNA was synthesized from ethanol-precipitated RNA via reverse transcription. PCR amplification followed, using primers constructed from cDNA clone pAB5, a partial open reading frame for the p34/40 BDV protein. The PCR product was detected via an enzyme immunoassay using a biotinylated DNA probe synthesized from pAB5 using nested oligonucleotide primers. A sample was considered positive if the mean fluorescent value was greater than 3 SD from the mean of negative control values (greater than 35 fluorescent units). As negative controls, reactions were performed using duplicate aliquots of samples without reverse transcriptase or with the full protocol using tissues from eight uninfected rats. Additionally, RNA samples underwent PCR using primers derived from housekeeping gene rat glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) to control for RNA presence and integrity in each sample.

Long-term bone marrow and thymus cell cultures. Long-term bone marrow and thymus cell cultures were developed from three PTI-NB and two uninfected rats using a modified version of the Whittlock culture method. The resulting monolayers were designated Carbine-Rubin rat stroma (CRRS)-M (of uninfected bone marrow origin), CRRS-M-BV (of BDV-infected bone marrow origin), CRRS-T (of uninfected thymus origin), and CRRS-T-BV (of BDV-infected thymus origin). Baseline and post-cyclophosphamide peripheral white blood cells counts in BDV-infected and uninfected rats. Baseline complete peripheral white blood cell counts with differentials were obtained via tail bleeds from four 5-week-old uninfected rats and four aged-matched PTI-NB rats. In two separate experiments, peripheral WBC counts were examined in uninfected and PTI-NB rats after cyclophosphamide treatment. Uninfected 4-week-old (n = 11) or PTI-NB (n = 13) Lewis rats were bled (day 0) and then inoculated intraperitoneally with 50 mg/kg of cyclophosphamide (Adria, Dublin, OH). At various time points up to 21 days post-cyclophosphamide inoculation (n = 5 to 7 rats per time point; total of 135 samples examined), blood was collected in Microtainer tubes containing EDTA (Becton Dickinson, Franklin Lakes, NJ) from the cyclophosphamide-treated groups and from 11 age-matched uninfected rats that did not receive cyclophosphamide.

Each blood sample was analyzed by the Johns Hopkins Hematology Laboratory (Baltimore, MD) for total WBCs per microliter and percentage of monocytes, neutrophils, and lymphocytes. The absolute number of each cell type per microliter was calculated by multiplying total WBC count by the percentage of each cell in the differential analysis. For all samples, the mean value and SEM (95% confidence limit) was determined for each group of rats and each cell type. Data between groups were compared using the Student’s t test. For each time point, all samples from treated and untreated control rats were evaluated on the day of sampling. For the purposes of standardization, the data from cyclophosphamide-treated rats at each time point were normalized to the data from untreated control rats from the same time point. Analyzing the data as percentage of normal values allowed for an easier comparison of changes in blood cell counts sampled over several days during the course of the study. In addition, normalization of the data for each time point minimized the day-to-day technical variations in data collected during the 3-week study (eg, each sample was normalized to the controls analyzed on the same day, then the normalized samples taken on different days were compared).

RESULTS

In vitro analysis of BDV-infected bone marrow cells. In 2- to 3-month-old PTI-NB rats (infected at birth), infectious BDV was recovered both from the bone marrow (9 of 10 rats) and PBMC (two of three rats) via coculture with C6 or PC-12 cells. The prevalence of BDV-infected cells in these tissues was 1 in 10^6 (three of five rats) to 1 in 10^5 (five of five rats) in bone marrow cells, and 1 in 5 × 10^6 in PBMC (one of two rats). When bone marrow cells were placed in tissue culture for 1 week (without C6 cells), cells were identified that expressed BDV proteins as detected by IFA (Fig 1A and C). Consistent with these findings, bone marrow cells cultured from uninfected rats (CRRS-M) were permissive to BDV infection in vitro (data not shown). A double-label IFA was performed to examine the possibility of colocalization of leukocyte markers with BDV antigens (Fig 1A through D). Although the antileukocyte antibodies demonstrated the
Fig 1. Antibodies directed against BDV and against lymphoid/myeloid cell-specific markers do not colocalize in bone marrow or thymus cells in vitro by double-label IFA. Bone marrow cells (A through D) and thymus cells (E and F) from PTI-NB rats were placed in culture for 1 to 2 weeks and doubly stained for BDV proteins and lymphoid/myeloid cell-specific markers. (A, C, and E) Stained with rabbit anti-BDV sera followed by rhodamine-conjugated antirabbit Ig and showing intranuclear BDV protein expression (arrows). (B) Same field as panel A, stained for T cells and thymocytes using mouse monoclonal antibody (MoAb) W3/13 followed by FITC-conjugated antimouse Ig. (D) Same field as panel C, stained for macrophages, granulocytes, and dendritic cells using mouse MoAb OX-42 followed by FITC-conjugated antimouse Ig. (F) Same field as panel E, stained for monocytes and macrophages using mouse MoAb ED-1 followed by FITC-conjugated antimouse Ig. Asterisks in panels B, D, and F represent cells staining positively for the lymphoid/myeloid markers. Note that these same cells identified by asterisk in corresponding panels A, C, and E do not express BDV proteins. Due to overlap in emission spectra, the BDV antigens with rhodamine label are seen with rhodamine (A, C, E) and fluorescein (B, D, F) filters. None of the BDV-infected bone marrow or thymus cells were doubly stained with W3/13 (B), OX-42 (D), or ED-1 (E). Original magnification (OM), ×400.

presence of thymocytes (Fig 1B), granulocytes, and macrophages/monocytes (Fig 1D) in these cultures, none of these cell types coexpressed BDV proteins. None of the BDV protein-expressing cells were recognized by any of the cell-specific antibodies. Due to overlap in the emission wavelengths of rhodamine and fluorescein, the foci of intranuclear rhodamine fluorescence (representing BDV protein expression) can be visualized both with the rhodamine (Fig 1A and C) and fluorescein (Fig 1B and D) filters.

The morphology of BDV-infected bone marrow cells became uniform after 4 to 6 weeks in culture, and nearly 100% of these cells expressed BDV antigens (Fig 2A). The cells from long-term culture (CRRS-M-BV) consisted of uniform cells of flattened rectangular, fibroblastic appearance with many cytoplasmic extensions. Alkaline phosphatase, an enzyme characteristic of stromal cells not found in macrophages or macrophage-like stromal cells, was colocalized to cells expressing BDV proteins in the CRRS-M-BV culture (Fig 2B and C). The morphology, absence of leukocyte markers, and presence of alkaline phosphatase activity all suggested that the BDV-infected cell type in these cultures was a fibroblastic stromal cell.

Baseline and recovery of peripheral blood leukocytes after cyclophosphamide treatment. As bone marrow stromal cells support hematopoietic activity, we investigated the baseline peripheral WBC counts in 4-week-old uninfected (n = 5) and age-matched PTI-NB (n = 4) rats. The blood cell count data are presented as mean number of cells per
Fig 2. BDV protein and alkaline phosphatase expression in long-term bone marrow cultures. (A) Field of BDV-infected bone marrow stromal cells as detected by IFA using polyclonal rabbit anti-BDV sera followed by FITC-conjugated antirabbit Ig (OM, ×100) with inset (a) showing these cells at a greater magnification (OM, ×400). Arrows show intranuclear expression of BDV proteins. (B and C) Colocalization of BDV protein (B) and alkaline phosphatase expression (C) in bone marrow stromal cells. Arrows show cells that express both BDV proteins and alkaline phosphatase activity. BDV proteins were detected by IFA using polyclonal rabbit anti-BDV sera followed by FITC-conjugated antirabbit Ig, and alkaline phosphatase activity was detected using an alkaline phosphatase detection kit and visualized by light microscopy. Panels B and C are identical fields (OM, ×800). Note: In panel B, the IFA signal in some cells is partially blocked by the opaque black precipitate formed after the alkaline phosphatase stain.

microliter (SEM at a 95% confidence limit) from uninfected normal rats: total WBCs: 13,864 (1,491) versus 11,200 (438), P < .10; neutrophils: 2,182 (411) versus 1,540 (292), P < .23; lymphocytes: 10,692 (1,108) versus 9,165 (621), P < .24; and monocytes: 953 (353) versus 386 (116), P < .14. Thus, there is no significant difference in peripheral WBC counts between uninfected and BDV-infected rats.

Pathologic consequences of viral infections of the bone marrow may be worsened by a second insult to the bone marrow,27,30 thus, we investigated blood cell replication and differentiation in uninfected and PTI-NB rats after a myelotoxic insult. After cyclophosphamide treatment of 11 uninfected and 13 PTI-NB rats, the suppression and recovery of peripheral blood lymphocytes and neutrophils were measured. The mean values for one of the two representative experiments are shown in Fig 3. The data from cyclophosphamide-treated rats was normalized to the untreated control rat specimens taken and evaluated on the same day, and the mean values are graphically illustrated as percentage of normal values. The normalized data avoids the day-to-day variation in blood cell count analysis between time points and allows presentation of the data as suppression stage (days 0 to 4 posttreatment) and recovery stage (days 5 to 21 posttreatment) relative to normal values, as described by Wheeler et al.26 The error bars represent the calculated SEM (95% confidence limit) for the values from cyclophosphamide-treated rats.

The cyclophosphamide-induced lymphopenia and neutropenia were similar in magnitude in uninfected and infected rats during the suppression stage (day 0 to day 4 post-cyclophosphamide treatment); however, there appeared to be a trend towards a more profound lymphopenia during the recovery stage (day 5 to day 21 posttreatment) in the PTI-NB rats as compared with the uninfected rats (Fig 3A). Com-
Days post cyclophosphamide treatment

Stage of immune system response to cyclophosphamide treatment

Days post cyclophosphamide treatment

Stage of immune system response to cyclophosphamide treatment

Days post cyclophosphamide treatment

Stage of immune system response to cyclophosphamide treatment

Fig 3. Change in peripheral blood neutrophil and lymphocyte counts after cyclophosphamide treatment of PTI-NB (II) and uninfected (O) rats. Mean cell counts for cyclophosphamide-treated rats are expressed as percentage of normal controls. Error bars show calculated SEs. One of two replicate experiments is shown in panels A and C; combined data from two experiments summarized in panels B and D. (A) Peripheral blood lymphocyte counts over a 3-week period after cyclophosphamide treatment (n = 2 to 3 rats per group at each time point). (B) Combined data from two experiments (normal rats, n = 11; BDV-infected rats, n = 13) showing lymphocyte counts after cyclophosphamide treatment during the suppression stage (days 0 to 4 posttreatment, *P < .003) and recovery stage (day 5 to 21 posttreatment, **P < .006). (C) Peripheral blood neutrophil counts over a 3-week period after cyclophosphamide treatment (n = 2 to 3 rats per group at each time point). (D) Combined data from two experiments (normal rats, n = 11; BDV-infected rats, n = 13) showing neutrophil counts after cyclophosphamide treatment during the suppression stage (days 0 to 4 posttreatment; P < .34) and recovery stage (days 5 to 21 posttreatment; **P < .006). Note that during the recovery stage lymphocytes are depressed and neutrophils are enhanced in PTI-NB rats as compared with uninfected rats. All error bars represent a 95% confidence limit.

BDV dissemination to the thymus after intracranial inoculation. The pronounced lymphopenia after cyclophosphamide treatment led us to examine thymus tissue, an organ important in lymphocyte maturation, as another possible site of BDV replication (Table 1). After intracranial inoculation of BDV, BDV RNA was identified by RT-PCR-EIA in the thymus of PTI-NB rats (two of three) at the first time point (3 days pi) and in all subsequent thymus samples up to the last time point (3 months pi). In contrast, BDV RNA was detected in a minority of adult-infected rats during the acute stage of disease (one of six) and in the majority of rats during the chronic stage of disease (three of four). Brain samples from all the BDV-inoculated rats contained BDV RNA (data not shown). All samples were positive by RT-PCR for GAPDH mRNA, indicating the presence of RNA even in BDV-negative samples (data not shown).

Identification of the BDV-infected thymus cell. Infectious BDV was recovered from the thymus tissues of 2- to 3-month-old PTI-NB rats (six of eight) by coculture tech-
BDV INFECTION OF STROMAL CELLS

Table 1. Detection of BDV RNA in the Thymus of Neonatally and Adult-Inoculated Rats

<table>
<thead>
<tr>
<th>Time pi</th>
<th>PTI-NB</th>
<th>Adult*</th>
</tr>
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<tbody>
<tr>
<td>3 d</td>
<td>194 ± 0.5</td>
<td>82 ± 0.3</td>
</tr>
<tr>
<td>14 d</td>
<td>1,046 ± 46</td>
<td>4 ± 0.1</td>
</tr>
<tr>
<td>2 mos</td>
<td>815 ± 23†</td>
<td>1,119 ± 193</td>
</tr>
<tr>
<td>3 mos</td>
<td>1,530 ± 58‡</td>
<td>1,310 ± 48</td>
</tr>
<tr>
<td>Normal rat§</td>
<td>9 ± 0.1</td>
<td>728 ± 0</td>
</tr>
</tbody>
</table>

Values represent fluorescent units (fu) as determined by EIA detection of RT-PCR products: greater than 25 fu, positive (in bold); ± 3 SD above control mean. Each value represents one rat unless otherwise noted.

- * Rats inoculated at 4 weeks of age.
- † Pooled sample of two rats.
- ‡ Pooled sample of seven rats.
- § Representative of normal rat controls.

We determined the prevalence of BDV-infected thymus cells in 2-month-old PTI-NB rats to be 1 per 10^6 cells (one of three rats) to 1 per 10^7 cells (two of three rats) cells. In culture, BDV-infected thymus cells did not express the cell-specific leukocyte markers noted earlier (Fig 1E and F) but did contain alkaline phosphatase enzyme activity (data not shown). Long-term thymus cell cultures were developed from uninfected (CRRS-T) and PTI-NB rats (CRRS-T-BV) that appeared morphologically identical to the CRRS-M and CRRS-M-BV cultures (data not shown).

DISCUSSION

In reports published before 1993, infectious virus, viral proteins, and BDV-specific RNA were not detected in bone marrow, thymus, or blood of rats infected with BDV as neonates or adults. Recently, semiquantitative RT-PCR-EIA demonstrated that BDV RNA can be found in cells of the immune system using cell-specific leukocyte markers noted earlier (Fig 1E and F) but did contain alkaline phosphatase enzyme activity (data not shown). Long-term thymus cell cultures were developed from uninfected (CRRS-T) and PTI-NB rats (CRRS-T-BV) that appeared morphologically identical to the CRRS-M and CRRS-M-BV cultures (data not shown).

In the in vitro work presented here, we show, by double-label IFA studies, that the BDV-infected cells in bone marrow, thymus, and PBMC were not neutrophils, lymphocytes, dendritic cells, or macrophages. These findings are supported by observations that the separation of B cells, T cells, and macrophages (Immunal cell separation kit, Biotex Laboratories Inc, Houston, TX) from BDV-infected bone marrow cells did not localize BDV-RNA to any specific myeloid or lymphoid cell type (A.M.S.-H. and K.M.C., unpublished data, September 1992). Additionally, several reports have demonstrated the inability to recover BDV-infected macrophages/monocytes from adult-infected rats and the resistance of macrophages to infection with BDV in vitro.

Long-term culture of bone marrow and thymus cells from BDV-inoculated rats yielded monolayers of BDV-infected cells, named CRRS-M-BV and CRRS-T-BV, respectively. In this type of culture, myeloid and lymphoid cells are depleted, but stromal cells survive. Both the morphology and alkaline phosphatase enzyme activity studies suggested that BDV-infected cells in the bone marrow were of the fibroblastic stromal cell subtype. Notably, fibroblastic reticular stromal cells in human bone marrow express nerve growth factor (NGF) receptors, and NGF has been shown to enhance BDV RNA and protein expression in neural cells. Thus, our finding that BDV replicates in bone marrow and thymus fibroblastic stromal cells as well as neural cells is conceptually consistent with the tropism of many neuroviruses for both the nervous and immune systems.

Stromal cells constitute less than 25% of all cells in the bone marrow. Furthermore, it has been shown that only 6% of these cells survive recovery from the bone marrow when maintained in vitro. Thus, the predicted number of bone marrow stromal cells expected to survive in culture (up to one stromal cell per 13,800 bone marrow cells) is similar to the recovery of BDV-infected bone marrow cells in our studies (one BDV-infected cell per 10,000 bone marrow cells). Interestingly, the circulation of stromal cell precursors in the bloodstream has been suggested. Thus, it is tempting to speculate that the minute fraction of BDV-infected cells recovered from PBMC (1 in 5 × 10^5 PBMC) might represent small numbers of circulating BDV-infected stromal cell precursors in the peripheral blood.

In the bone marrow, stromal cells provide the microenvironment for hematopoietic stem cell growth and differentiation of monocytes, neutrophils, lymphocytes, erythrocytes, and platelets. Viral infections of the bone marrow stromal cells, eg, cytomegalovirus, human herpes virus-6, and human immunodeficiency virus, are associated with myelosuppression, thrombocytopenia, and anemia. To determine if BDV infection of the bone marrow was associated with hematopoietic abnormalities, we examined baseline peripheral blood leukocyte concentrations in uninfected and PTI-NB rats. The BDV-infected rats had total WBC, neutrophil, and monocyte counts that were not significantly different from uninfected rats. Thus, baseline hematopoiesis appeared normal in BDV-infected rats.

Some viral infections of the bone marrow are relatively asymptomatic in the normal host until an additional myelotoxic challenge is encountered, eg, bone marrow transplant and cytomegalovirus-induced pancytopenia. To determine whether BDV infection altered the bone marrow’s ability to recover from a myelosuppressive insult, uninfected and PTI-NB rats were given a myelotoxic dose of cyclophosphamide, and the peripheral blood leukocyte concentrations were measured. PTI-NB rats were used in these experiments in preference to adult-infected rats for two reasons: (1) The extraneural dissemination of BDV to bone marrow occurs sooner after infection in neonatally inoculated rats as compared with rats inoculated as adults (S.A.R. and K.M.C., unpublished data, November 1993), and (2) neonatally infected rats have demonstrated viral replication and immune response to BDV, allowing the study of WBC production and measurement of hematopoietic recovery without the complication of cellular-mediated immune-induced lysis of BDV-infected bone marrow cells.
After cyclophosphamide treatment of uninfected and PTI-NB rats, peripheral blood lymphocyte recovery was depressed in the BDV-infected rats as compared with uninfected rats. On the other hand, neutrophil recovery and rebound granulocytosis were actually enhanced in the PTI-NB rats. Notably, lymphocytes and neutrophils originate from distinct lines of progenitor cells.40 The discrepancy in post-cyclophosphamide recovery of cells from two separate lineages suggested a differential influence of BDV infection of stromal cells on specific progenitor cell types in the bone marrow. Furthermore, because BDV infects thymus stromal cells as well as bone marrow stromal cells, the reduction in post-cyclophosphamide lymphocyte recovery may have been affected either by changes in lymphocyte precursor production (in the BDV-infected bone marrow) or by the effects on maturation of lymphocytes (in the BDV-infected thymus).

As seen in LCMV infection, virus infection in the thymus can correlate with the maintenance of cellular immune tolerance to virus antigens. We noted that the rapid appearance of BDV in the thymus of neonatally infected rats (less than 72 hours pi) was temporally associated with the apparent state of cellular immune nonresponsiveness to BDV. It is commonly believed that bone marrow-derived macrophage and dendritic-like stromal cells play the role in tolerance induction in the thymus, while fibroblastic thymic stromal cells play a major role in early T-lymphocyte support.50-55 In the work presented here, the BDV-infected thymus cell was identified as a fibroblastic stromal cell. Therefore, it may be important to consider the possible direct or indirect contribution of thymus fibroblastic stromal cells to the cellular immune response, based on our findings.

In adult-inoculated rats with encephalitis, BDV RNA was detected in the thymus only in a minority of rats with acute disease. However, in adult-inoculated rats with chronic Borna disease, BDV RNA was commonly recovered from the thymus. Notably, BDV-specific, cellular-mediated immune responses seen during the acute stage of disease are lost during the chronic stage of disease, indicated by the disappearance of BDV encephalitis13,14 and by the reduction of lymphoproliferative responses to immunoaffinity-purified BDV antigens.15 Thus, the loss of BDV-specific, cellular-mediated immune responses in adult rats during the chronic stage of disease is also temporally associated with BDV replication in the thymus.

Over the past 10 years, important new information about BDV infection and the immunopathogenesis of Borna disease has been presented. However, none of the previously published work has addressed the potential effects of BDV infection of the immune system on the immunopathogenesis of Borna disease. The data presented in this report open for consideration a new area of study in this persistent viral infection, that of the contribution of BDV-infected bone marrow and thymus stromal cells to the immunobiology of Borna disease.

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