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Humoral immunity in the central nervous system of Lewis rats infected with Borna disease virus

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Abstract

The aim of this study was to investigate the humoral immune response to Borna disease (BD) virus in the brain of experimentally infected Lewis rats. Abundant IgG was detected in BD-rat brain with isotype variation throughout infection. IgG was locally produced as indicated by an intact blood–brain barrier, Ig κ light chain mRNA-containing cells in brain and accumulation of virus-specific antibodies in cerebrospinal fluid. Treatment with BD-rat serum altered viral gene expression in persistently infected cultured rat glioblastoma cells. These data suggest that antibodies, produced in the brain, may influence viral gene expression.

Keywords: Persistent infection; Viral gene expression; Immunoglobulin isotype; Plasma cells; Antibody penetration

1. Introduction

The two principal mechanisms that the immune system uses to control viral infections are destruction of infected cells by cytotoxic T lymphocytes (CTL) and inhibition of viral spread by neutralizing antibodies. In many noncytopathic viral infections, CTL mediated viral clearance is accompanied by a delayed production of neutralizing antibodies that may aid in inhibiting any remaining virus (reviewed in the work of Zinkernagel, 1996).

Antibodies have been suggested to be important for control of viral infections within the central nervous system (CNS). For example, antibodies passively transferred into mice infected with murine hepatitis virus type-4 restrict viral replication to the CNS (Buchmeier et al., 1984). Furthermore, transfer of antibodies into measles virus-infected rats results in limited viral gene expression (Liebert et al., 1990). Finally, transfer of antibodies into rabies virus-infected rats (Dietzschold et al., 1992) or Sindbis virus-infected, severe-combined immunodeficient mice (Levine et al., 1991) results in viral clearance from cells of the CNS. The mechanism by which antibodies interact with infected cells to limit viral expression or clear virus is not known although it appears that this antibody-induced modulation of viral gene expression is not dependent on other immune cells or complement factors (Dietzschold, 1993). In some viral systems, the immunoglobulin appears to enter the infected cells where it may play a role in inhibiting viral expression (Levine et al., 1991; Dietzschold et al., 1992).

Borna disease virus (BDV) is a member of the order *Mononegavirales* with the unique feature of nuclear replication and transcription (reviewed in the work of Schneemann et al., 1995). BDV is neurotropic and causes a noncytolytic infection to persist within the CNS. Infection of immunocompetent hosts typically results in a multiphasic, immune-mediated disease (Narayan et al., 1983). In this classical form of infection known as Borna disease (BD), the predominantly cellular immune response occurs in the acute phase of infection (4–6 weeks post-infection, wks pi). In spite of the robust immune response, BDV is not cleared from the CNS and the animals enter a chronic phase of infection (after 10 wks pi) during which there is a decrease in immune infiltration (Deschl et al., 1990) and an apparent shift from a Th1-like immune response to a

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Th2-like immune response within the CNS (Hatalski et al., 1998) (next paper).

The role of antibodies in the course of BDV infection is not well understood. High titer antibodies against BDV proteins and antibodies with neutralization activity have been identified in BD-rat serum from animals in the chronic phase of infection (Danner et al., 1978; Ludwig et al., 1988; Briese et al., 1995; Hatalski et al., 1995). There is evidence that antibodies to BDV are present in the cerebrospinal fluid (CSF) of infected animals (Ludwig et al., 1977; Ludwig and Thein, 1977); however, neither the specificities of these antibodies nor their role in regulating virus replication or dissemination are known.

Here we show the presence of BDV-specific antibodies in infected rat brain and provide evidence that these antibodies are produced within the brain rather than entering the CNS via a permeable blood–brain barrier (BBB). Although these BDV-specific antibodies do not appear to clear the virus, they may be associated with modulation of BDV gene expression in the CNS.

2. Materials and methods

2.1. BDV infected animals

Four-to-six week old Lewis rats were infected intracranially or intranasally with approximately 5×10^4 focus forming units of BDV strain He/80-1 (Carbone et al., 1987; Schneider et al., 1994). Rats were sacrificed at various timepoints throughout infection including 3.5 wks pi (pre-disease), 4-6 wks pi (acute phase) and 15 wks to 1 year post-infection (chronic phase). Ten-to-twelve week old noninfected Lewis rats were used as controls. At the time of sacrifice, rats were either perfused with calcium–magnesium-free phosphate-buffered saline followed by 4% buffered paraformaldehyde or terminally anesthetized for collection of CSF, serum and brain samples.

2.2. Collection of CSF and serum

Rats were sacrificed in the chronic stage of disease, 3–6 months post-infection. At time of sacrifice, rats were terminally anesthetized with methoxyflurane (Metofane, Pitman-Moore) and CSF was withdrawn by ventricular puncture of the fourth ventricle with a 22-gauge needle. The quality of CSF (absence of serum contaminants) was determined by presence of erythrocytes in the CSF. Samples which contained erythrocytes were excluded from evaluation.

2.3. Immunohistochemistry of BD-rat brains stained using anti-IgG and anti-albumin antibodies

Immunohistochemistry was performed using anti-IgG antibodies as previously described (Hickey et al., 1983). Briefly, slide-mounted sections were blocked and incubated with biotinylated goat anti-rat IgG antibodies. After washing and inhibiting endogenous peroxidase activity, the sections were incubated sequentially with avidin-per-

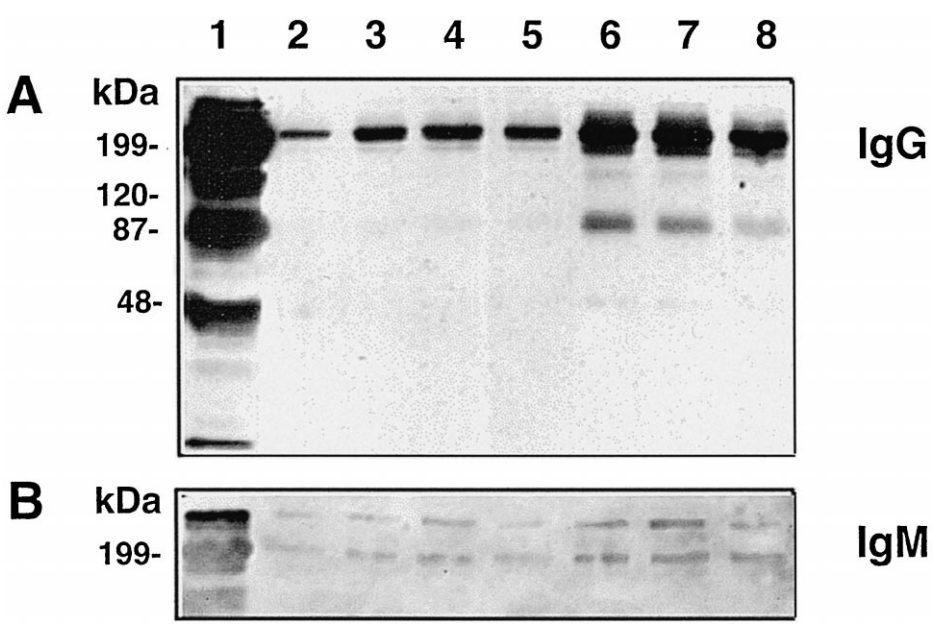

Fig. 1. Western immunoblot analysis of immunoglobulin in BD rat brain homogenates. Rat brain homogenates (20 μg) were size-fractionated by SDS-PAGE (8% gel) under nonreducing conditions and transferred to nitrocellulose membranes. The membranes were bound with goat anti-rat IgG (panel A) or IgM (panel B) antibodies conjugated with horseradish peroxidase and visualized using a chemiluminescent substrate. Samples included: positive control of 0.3 μl of NI rat serum (lane 1); noninfected rat brain (lane 2); acute rat brains, 4–5 wks pi (lanes 3–5); and chronic rat brains, 15 wks pi (lanes 6–8).

oxidase conjugate (Vector Labs) and 3,3-diaminobenzidine. Immunohistochemistry for detection of albumin in brain sections was performed using rabbit anti-human albumin antibodies (American Qualex) diluted 1:100 followed by goat anti-rabbit IgG conjugated with horseradish peroxidase (Sigma). Prior to these experiments, the rabbit anti-human albumin antibodies were shown to cross-react with rat serum albumin by SDS-PAGE immunoblot (Western blot) and enzyme-linked immunosorbent assay (ELISA).

2.4. Western blot of brain homogenate for detection of IgG

Twenty μg of each BD-rat brain homogenate (20% wt./vol. in phosphate buffered saline) were size-fractionated by SDS-PAGE (8% gel) (Laemmli and Favre, 1973) under nonreducing conditions and transferred to nitrocellulose membranes (Schleicher and Schuell) (Towbin et al., 1979). The membrane was bound according to the method described previously (Hatalski et al., 1995) with goat anti-rat IgG (Sigma) or goat anti-rat IgM (Sigma) antibodies (diluted 1:300 and 1:100, respectively) conjugated with horseradish peroxidase and visualized using Lumiglo chemiluminescent substrate (Kirkegaard and Perry Laboratories).

2.5. Determination of relative amounts of antibodies, albumin and neutralization activity in CSF and sera

A modified ELISA was performed to determine the relative amounts of IgG, IgM and albumin in the heat-inactivated CSF and sera. Multi-well plates were coated overnight at 37°C with serial dilutions (0.2 μl to 1×10^{-4} μl per well) of sera or CSF in borate buffer (100 mM boric acid, 50 mM sodium borate and 75 mM sodium chloride, pH 8.4). After washing thrice with wash buffer (0.05% Tween-20 in phosphate buffered saline), the wells were blocked for 1 h at 37°C using 0.01% nonfat dry milk in wash buffer. Antibodies were bound for 2 h at 37°C. The antibodies used were anti-rat IgG (whole molecule, Sigma), anti-rat IgM (μ heavy chain, Sigma) and anti-human albumin (American Qualex); all antibodies were conjugated with horseradish peroxidase and diluted 1:1000 in wash buffer containing 0.01% nonfat dry milk. After washing five times, the plates were developed using 3,3′,5,5′-tetramethylbenzidine. The CSF and serum titers were determined for each animal as the endpoint dilutions that yielded an equal optical density at 450 nm within a linear optical density range (0.1–0.4).

ELISA was performed as previously described (Briese et al., 1995) for detection of IgGs in CSF and serum

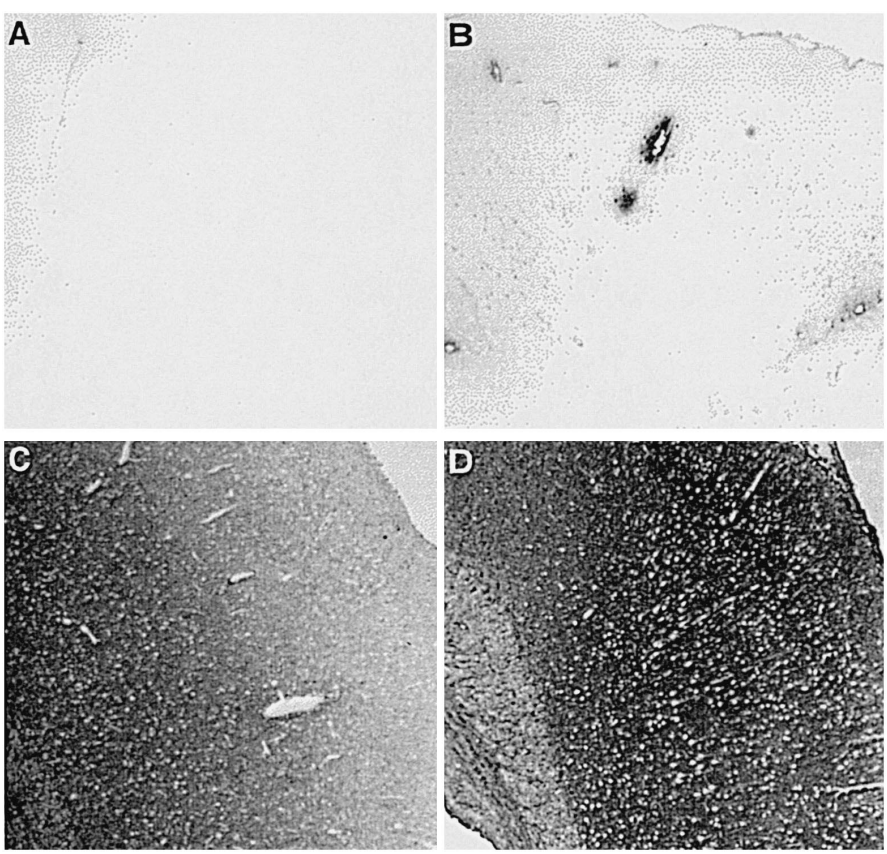

Fig. 2. Immunohistochemical analysis of IgG in BD-rat brains. BD-rat brain sections from rats at different times post infection were bound with anti-rat IgG antibodies conjugated with horseradish peroxidase and stained with 3,3-diaminobenzidine. Representative fields from parietal cortex are shown for noninfected rat (A), or BD-rats from 3.5 wks pi (B), 5 wks pi (C), 15 wks pi (D).

samples that bound to BDV proteins (N, P or gp18). Serum and CSF titers were measured simultaneously for individual animals. Neutralization assays were performed as previously described (Hatalski et al., 1995). Briefly, serially-diluted sera or CSF were incubated with virus prior to inoculation of susceptible cells. The neutralization titer for each CSF and serum sample was defined as the reciprocal dilution that inhibited viral infectivity by 50%.

2.6. Detection of Ig κ light chain mRNA

Northern blot hybridization and nonradioactive in situ hybridization analysis were performed as described previously (Lipkin et al., 1990). Probes were generated from plasmids containing fragments of Ig κ light chain and IgG heavy chain cDNAs (Schwemmle and Lipkin, unpublished data).

2.7. Isotype of serum and brain immunoglobulin

Radial immunodiffusion (RID) assays were performed using Bind-a-RID rat isotyping kits (The Binding Site) to quantitate levels of IgA, IgM, IgG, IgG1, IgG2a, IgG2b and IgG2c in sera from rats at different stages of BD. In addition, brain homogenates from rats at different stages of disease were used to isolate all immunoglobulins using the T-gel purification kit (Pierce). Following purification, samples were concentrated using Centricon columns (Amicon) and assayed for IgG subclass in the Bind-a-RID isotyping system.

2.8. Anti-sera treatment in vitro and analysis of viral gene expression

C6BDV cells (persistently-infected rat glioblastoma cells, Carbone et al., 1993) were treated every 6 h for 48 h with medium containing either pooled, heat-inactivated, noninfected rat sera (NIsera) diluted 1:100 or pooled, heat-inactivated sera from chronically-infected rats (BDsera) diluted 1:100. To determine if activity was removed by treatment with protein-A, each pooled serum was diluted 1:33 in PBS and bound with 0.2 g of protein-A–Sepharose (Sigma) overnight at 4°C. This mix was further diluted with concentrated culture medium to 1:100 and used to treat cells. Rabbit anti-N, rabbit anti-P and rat anti-nonglycosylated gp18 monospecific sera were each also used for treatment at 1:50 dilution. After treatment, the cells were harvested and RNA was extracted (Chomczynski and Sacchi, 1987). Northern hybridization analysis was performed using double-stranded, [^{32}P]-labeled DNA

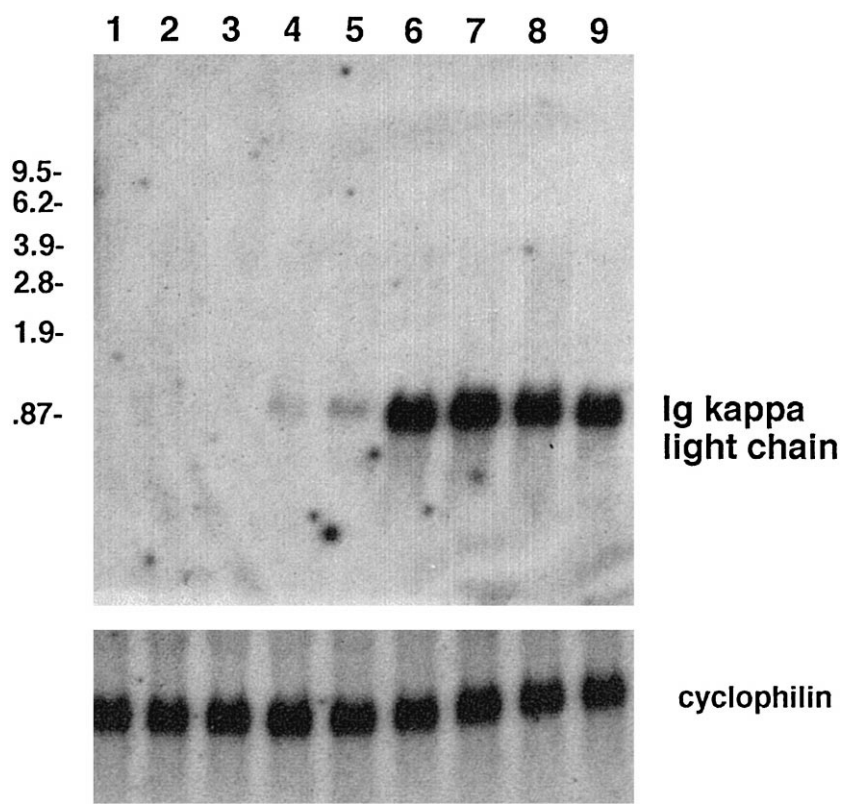

Fig. 3. Northern blot hybridization analysis of Ig κ light chain mRNA in BD rat brain samples. A total of 10 μg of total cell RNA from brains of noninfected rats (lane 1), or BD-rats 4 wks pi (lanes 2–3), 5 wks pi (lanes 4–5) and 15 wks pi (lanes 6–9) were hybridized with a double-stranded cDNA probe for rat Ig κ light chain mRNA. Blots were stripped and rehybridized with a probe for rat cyclophilin mRNA to control for RNA quantity and integrity.

probes to fragments of the BDV N and P ORFs (Lipkin et al., 1990). These probes hybridize to both viral genomic RNA and viral subgenomic mRNAs.

3. Results

3.1. Analysis of BD-rat brains for the presence of immunoglobulin

Western immunoblot analysis of brain homogenates was performed for detection of IgGs in BD-rat brains and to compare the relative abundance of this signal in brain samples from different times post-infection. Immunoblots were bound with antibodies to detect IgG or IgM. Although IgG was detected in normal rat brain samples, there was a marked increase in IgG signal from 4–5 to 15 wks pi (Fig. 1A). There was no apparent difference between brain IgM levels in normal rats and BD-rats at 4–5 wks pi; however, there was a slight increase in IgM in brain homogenates from BD-rats at 15 wks pi (Fig. 1B).

To determine the distribution of immunoglobulin in BD-rat brains, immunohistochemical analyses were performed using anti-rat IgG antibodies on rat brain sections from different times post-infection. No staining was detected in normal rat brain (Fig. 2A). At 3.5 wks pi, faint signal was detected around blood vessels (Fig. 2B). By 5–6 wks pi, there was heterogeneous signal for IgG in many gray matter regions (including cerebral cortex, thalamus, basal ganglia; Fig. 2C). In the chronic phase of disease (after 10 wks pi) all rats showed intense staining for IgG throughout the brain (Fig. 2D). This staining pattern was observed as late as 1 year post-infection, the latest timepoint examined (data not shown). The concentration of IgG was most intense in gray matter regions and, consistent with findings of Deschl et al. (1990), appeared to be predominantly intercellular or on the cell surface.

3.2. Analysis of BBB integrity

To determine whether the immunoglobulin detected in the CNS was due to leakage of the BBB, analysis of BBB integrity was performed by measuring the amount of albumin in the CSF and the serum. The ratio of albumin in the CSF relative to the serum was determined for 11 chronically-infected BD-rats and four noninfected controls. There was no significant difference in the CSF to serum ratio of albumin between the infected and noninfected rats (3.42 ± 0.78 vs. 2.65 ± 0.37, respectively), indicating that the BBB in the chronically infected animals was similar in integrity to that of the noninfected animals. Furthermore, in all rats, consistent with previous reports for normal rats (Westergren and Johansson, 1991), the CSF to serum ratio for albumin was less than 5. This analysis was only performed for rats in the chronic phase of infection, when immune infiltration had diminished. To assess BBB integrity in acute infection, thin sections of rat brain from different times post infection were stained using anti-albumin antibodies as a secondary measure for permeability. Consistent with previous results (Deschl et al., 1990), no albumin immunoreactivity was detected in the brain parenchyma (data not shown).

3.3. Analysis of BD-rat brains for the presence of Ig mRNA

To determine the timecourse and sites for synthesis of IgG in the BD-rat brain we performed Northern blot

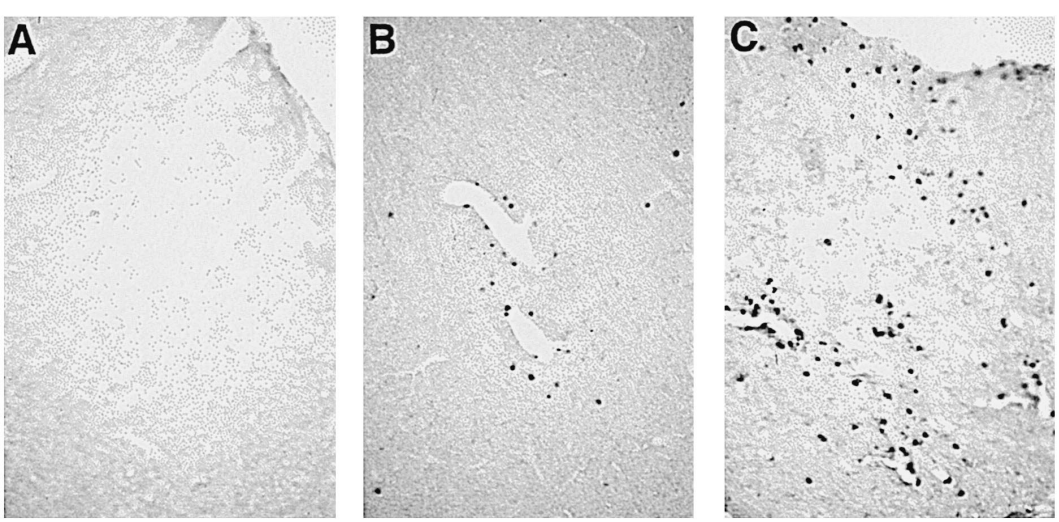

Fig. 4. Detection of cells containing Ig κ light chain mRNA. Nonradioactive in situ hybridization was performed using a digoxygenin-labeled RNA probe complementary to a fragment of the Ig κ light chain mRNA. Following hybridization, tissue was bound with anti-digoxygenin alkaline phosphatase conjugated antibody and developed using NBT/BCIP substrate. Photomicrographs of piriform cortex taken at 25 $\times$ magnification are shown for (A) noninfected rat brain, (B) BD-rat brain at 5 wks pi, and (C) BD-rat brain at 15 wks pi.

Table 1
Mean CSF to serum antibody ratio

	Nonspecific IgM	Nonspecific IgG	BDV			
			N	P	gp18	Neutralizing
Noninfected	0.00286 ± 0.001313	0.00276 ± 0.00142	n.d.	n.d.	n.d.	n.d.
Chronic BD-rat	0.00531 ± 0.00115	0.00531 ± 0.00115	0.07 ± 0.013	0.1611 ± 0.0524	0.0667 ± 0.0176	0.0996 ± 0.0221

Antibody CSF to serum ratio is defined as the antibody titer from the CSF sample divided by the antibody titer from the serum sample from the same animal. The mean of at least four animals per group.
n.d. = Not detectable.

hybridization and in situ hybridization for detection of Ig mRNA. Northern blot hybridization analyses were performed on BD-rat brain samples from the acute and chronic phases of infection using a probe to detect Ig κ light chain mRNA (Fig. 3). No Ig κ light chain mRNA was detected in brains of noninfected rats or rats at 4 wks pi. At 5 wks pi, Ig κ light chain mRNA was present at low levels with a marked increase by 15 wks pi. Northern blot hybridization analysis using a probe to detect IgG heavy chain mRNA gave similar results (data not shown).

Nonradioactive in situ hybridization analysis was performed to identify the cells in the BD-rat brain that express Ig κ light chain mRNA. In noninfected rat brain, rare labeled cells were detected in the meninges, but not in the brain parenchyma (Fig. 4A). In contrast, labeled cells with morphology consistent with plasma cells were detected in the meninges, perivascular infiltrates and brain parenchyma at 5 wks pi (Fig. 4B). The abundance of labeled cells increased at 6–7 wks pi, and remained at high levels through 15 wks pi (Fig. 4C). Although labeled cells were detected in most gray matter regions of the brain, these cells were most abundant in the areas of the olfactory tubercle, piriform cortex and amygdala in both the acute and the chronic phases of infection.

3.4. Production of BDV-specific antibodies in the CNS

Previous results suggest that BDV-specific antibodies are present in the CSF of naturally and experimentally-infected animals (Ludwig et al., 1977; Ludwig and Thein, 1977). CSF and sera were analyzed for BDV immunoreactivity to determine if the antibodies produced within the CNS were specific for viral antigens. The ratio of antibodies in the CSF relative to the serum was determined for nonspecific IgG and IgM, the BDV N, P and gp18 proteins, and the virus neutralization activity (Table 1). The CSF to serum ratios for antibodies to BDV proteins and neutralization activity were at least five-fold greater than for nonspecific IgG or IgM indicating that BDV-specific antibodies are produced in the CNS.

3.5. Characterization of immunoglobulin isotypes

The isotypes of total serum immunoglobulins were determined for rats in the acute (5 wks pi) and chronic (15 wks pi) phases of infection by RID (Table 2). Acute infection with BDV resulted in increased levels of serum IgA, IgM, IgG2b and IgG2c. Transition to the chronic phase of disease was associated with a reduction in IgA, and elevations of IgM, IgG (in particular IgG2a and IgG2b subtypes); IgG2c remained constant throughout the course of the disease.

The profiles of IgG isotypes isolated from the CNS differed from those in sera. CNS levels of IgG, IgG1, IgG2b and IgG2c were each elevated in the acute phase of infection and continued to increase into the chronic phase of infection (Table 3). In contrast, IgG2a in the CNS was elevated in the acute phase but declined in the chronic phase of infection.

3.6. Antibody-mediated alteration in BDV gene expression

In order to determine whether the presence of anti-BDV antibodies might influence production of viral gene products, persistently-infected C6BDV cells were treated with pooled sera from chronically-infected rats (3–6 mo pi, BDsera) and analyzed for the presence of viral nucleic

Table 2
Comparison of antibody isotypes in sera from rats at different times post-infection

Rat sera	IgA	IgM	IgG	IgG1	IgG2a	IgG2b	IgG2c
Noninfected	34	224	7660	931	1535	1415	137
Acute (5 wks pi)	124 ± 46	1284 ± 94	10,186 ± 2343	931 ± 301	1550 ± 454	2833 ± 538	476 ± 37
Chronic (15 wks pi)	9 ± 0	1804 ± 47	17,000 ± 2368	846 ± 0	2443 ± 473	5423 ± 732	478 ± 61

Mean antibody isotype (mg/l) in serum ± standard error of the mean ($n = 3$ for each acute and chronic groups).
Noninfected values from a single animal are consistent with published reports (Kinoshita and Ross, 1993).

Table 3
Comparison of antibody isotypes in brain from rats at different times post-infection

Rat brain sample	IgG	IgG1	IgG2a	IgG2b	IgG2c
Noninfected	38	n.d.	n.d.	3	n.d.
Acute (5 wks pi)	242	30	31	26	1.3
	246	28	32	30	1.3
Chronic (15 wks pi)	271	38	19	115	4.39
	257	45	20	120	2.57

Data presented as μg antibody isotype per gram of brain (data shown for $n = 1$ noninfected and $n = 2$ each acute and chronic).
n.d. = Not detectable.

acids. For comparison, C6BDV cells were also treated with pooled sera from noninfected rats (NIsera) or sera that had been incubated with protein A–Sepharose to remove IgG. Following treatment of cells, RNA was extracted and analyzed by Northern hybridization for the presence of BDV genomic and anti-genomic RNAs (8.9 kb) and two subgenomic viral transcripts (0.8 and 1.2 kb). Levels of BDV RNAs were lower in C6BDV cells treated with BDsera than in parallel cultures treated with NIsera (Fig. 5). RNA levels were reduced 27%, 53% and 47% for the 8.9 kb, 1.2 kb and 0.8 kb RNAs, respectively. Incubation of BDsera with protein-A prior to its application to C6BDV cells abrogated these effects. Treatment of C6BDV cells with monospecific sera directed against N, P or nonglycosylated gp18 did not alter level of BDV RNAs (data not shown).

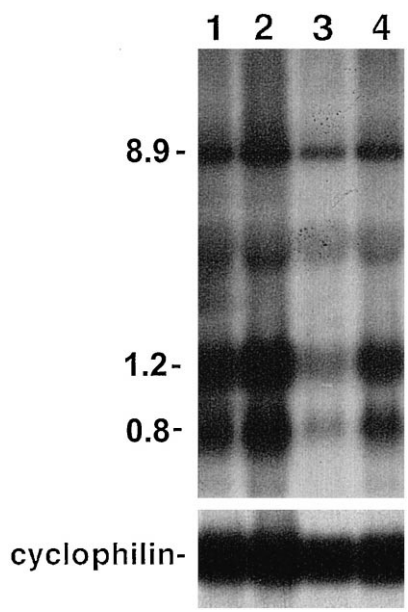

Fig. 5. BD-rat anti-sera modulate viral gene expression in vitro. Northern hybridization analysis of viral nucleic acid from persistently-infected C6 (C6BDV) cells treated with pooled noninfected rat sera (lanes 1 and 2) or pooled chronic BD-rat sera (lanes 3 and 4). To determine if immunoglobulin or another serum component was responsible for this effect, sera were pretreated with protein-A Sepharose to remove IgG prior to addition to cells (lanes 2 and 4). Blots were hybridized with double-stranded, [^{32}P]-labeled DNA probes to fragments of the BDV N and P ORFs to detect the 1.2 and 0.8 kb RNAs, respectively as well as the 8.9 kb RNA.

4. Discussion

In the Lewis rat model system of BDV infection, the chronic phase of disease is associated with high titer serum antibodies to BDV proteins including antibodies with neutralizing activity (Hatalski et al., 1995). The experiments described here were performed to determine (i) the time-course and distribution of antibodies to BDV in brains of BD-rats, (ii) whether the source of these antibodies is local synthesis or transport into the CNS from the periphery, and (iii) the potential effects of these antibodies on viral gene expression.

Abundant IgG was found in the brain homogenates of chronically-infected rats. Localization of this IgG in the brain showed intense staining throughout the gray matter and was not confined to perivascular regions. Two lines of evidence indicate that this IgG was produced in the CNS rather than in the periphery. First, the BBB appears to be intact in BD. Albumin immunohistochemistry and CSF to serum ratios were similar in infected and noninfected rats. These results confirm and extend studies of other investigators who found evidence for BBB integrity through albumin histochemistry (Deschl et al., 1990) and Evan's blue dye perfusion studies (Ludwig and Thein, 1977). Second, in situ hybridization confirmed the presence of plasma cells in the BD-rat brain parenchyma.

Analysis of antibodies found in the CNS revealed specificity for BDV. Comparison of the titers of antibodies in CSF and sera for binding to BDV proteins and neutralization activity suggests that BDV-specific antibodies are concentrated in the CNS relative to nonspecific IgG (Table 1). Titers were highest to P protein although antibodies with neutralization activity were also prominent. No direct data were uncovered to indicate the target antigens for parenchymal brain IgG; however, the high concentration of BDV-specific antibodies in the CSF suggests that parenchymal IgG are directed against viral proteins.

Serum isotype analysis showed an increase in all isotypes from the acute to the chronic phase of infection (Table 2). This result is consistent with elevated levels of BDV-specific antibodies throughout the course of BD (Briese et al., 1995). Analysis of immunoglobulin isotypes in the CNS also revealed that most CNS immunoglobulin levels increased between the acute and chronic phases of infection. This increase was coincident with increased numbers of Ig κ mRNA-containing cells (plasma cells) in brain during the chronic phase of infection (Fig. 4). IgG2a was the only isotype that was detected at lower levels in the CNS in the chronic phase of infection (Table 3). This finding is consistent with a shift from a Th1-like immune response (acute phase) followed by a Th2-like immune

response (chronic phase), a phenomenon that may facilitate reduction of immunopathology in the face of persistent CNS infection (Hatalski et al., 1998). This alteration in the immune response may be specific for the CNS as serum IgG2a levels are elevated in the chronic phase of infection (Table 2).

The presence of BDV-specific antibodies in the CNS suggests that antibody-mediated mechanisms may contribute to cell death in the BD-rat brain. Effector cells in antibody-dependent cell-mediated cytotoxicity (ADCC), such as NK cells, macrophages and activated microglia, are present in the brains of BD-rats (Deschl et al., 1990) (unpublished observations). Dietzschold et al. (1995) have shown that complement system components are present in BD rat brain. The antibody isotypes IgG2a and IgG2b participate ADCC and complement mediated lysis. Thus, it is conceivable that antibody-mediated mechanisms of cell lysis such as ADCC and complement-mediated lysis contribute to the neuropathology associated with BD.

In vitro studies presented here suggest the potential of virus-specific antibodies in the CNS to modulate viral gene expression. BDV genomic and subgenomic RNA levels in C6BDV cells were reduced following treatment with BD-rat sera (Fig. 5). The reduction was more marked for viral transcripts (0.8 and 1.2 kb RNAs) than the genomic and anti-genomic RNAs (8.9 kb) suggesting that, for this treatment paradigm, the antibodies may modulate abundance of viral gene products. We were unable to discern the specificities of the antibodies responsible for altered BDV gene expression. Experiments to define the target antigen(s) responsible using monospecific sera were unsuccessful. Rabbit monospecific anti-sera to N or P did not alter viral gene expression; it was unclear whether this reflects a species difference, or implies that antibodies to N and P are not responsible for the effect. In other systems, antibodies to viral glycoproteins are often responsible for clearance or altered gene expression (Schneider-Schaulies et al., 1992; Dietzschold, 1993). The antiserum to gp18 that was used for this assay was generated against nonglycosylated, recombinant protein. Thus, it is difficult to assess its failure to modulate levels of BDV RNAs. Definition of the epitopes and mechanisms responsible for antibody-induced modulation of BDV gene expression in vitro will require studies with antibodies to native gp18 as well as the G, pol and X proteins of BDV.

The in vivo significance of the humoral immune response to BDV for control of viral gene expression and pathogenesis remains to be determined. Furthermore, there is a modest decrease in viral gene expression in the chronic phase of infection relative to the acute phase (Hatalski and Lipkin, unpublished observations), which is not inconsistent with the time course for the appearance in the CNS of high levels of antibodies specific for BDV. However, it is not clear if these changes are important for controlling the virus, influencing host cell survival or affecting other aspects of the immune response. Nonetheless, the concentration of IgG within the CNS of chronically-infected rats suggests that the humoral immune response is likely to influence pathogenesis and may play a role in modulation of viral gene expression.

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