

Borna Disease: Association with a Maturation Defect in the Cellular Immune Response

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Borna disease virus (BDV) is a negative-strand RNA virus which produces persistent infection in a variety of experimental animals. In the rat, the presence or absence of clinical signs of Borna disease, a characteristic, biphasic neurobehavioral illness, depends on host-related factors. A window of opportunity exists after birth wherein inoculation with BDV produces a persistently infected rat without signs of Borna disease or encephalitis (persistent, tolerant infection-newborn [PTI-NB] rat). Although immunopathological destruction of the nervous system does not occur in the PTI-NB rat, significant alterations in the development of the nervous system were noted, including site-specific lysis of neurons. Unlike the case with other pharmacologically produced, persistent, tolerant BDV infections, adoptive transfer of spleen cells from BDV-infected rats did not produce disease in the PTI-NB rats. PTI-NB rats developed Borna disease after being connected by parabiosis to rats with Borna disease. Bone marrow transplantation experiments revealed that bone marrow cells from PTI-NB rats produced Borna disease in lethally irradiated, BDV-infected recipient rats. Bone marrow from PTI-NB rats contained a complement of inflammatory cells capable of inducing Borna disease. Thus, the loss of BDV-specific cellular immunity appeared to occur after the release of cells from the bone marrow.

Borna disease virus (BDV) is an 8.5-kb negative-strand RNA virus which produces persistent infection in a variety of experimental animals (5, 7, 18). In the rat, the presence or absence of clinical signs of Borna disease, a characteristic biphasic neurobehavioral illness, depends on host-related factors such as strain and age at inoculation (6, 11, 12). Chief among these is the age of the rat when inoculated. A 48-h window of opportunity exists after birth wherein inoculation of BDV produces a persistently infected rat without signs of Borna disease or encephalitis (persistent, tolerant infection-newborn [PTI-NB] rat) (6). Rats infected after the neonatal period (adult-infected [AdI] rats) develop the necrotizing mononuclear cell inflammation in the central and peripheral nervous system associated with the ataxia, hyperactivity, and aggression characteristic of acute Borna disease.

In PTI-NB rats, BDV replicates in extraneural sites, such as the salivary gland, while in AdI rats BDV is believed to be exclusively neurotropic (12). Although PTI-NB rats are often assessed as normal, there are reports of neurobehavioral irregularities elicited by sensitive behavioral testing, such as abnormalities in spacial discrimination learning, increased motor activity, and decreased aversion learning behavior (9). There is one report of induction of Borna disease encephalitis in two PTI-NB rats after adoptive transfer of spleen cells from AdI rats, but the development of disease occurred after an extended period of time (4 to 6 weeks after transfer) and the number of cells required to induce disease was large (one spleen per animal) (13).

Other PTI models of BDV infection have been developed, typically by using a variety of toxic immunosuppressive agents (e.g., cyclophosphamide [Cy] or cyclosporin A [CyA]) (21, 26). Unlike what is observed with the PTI-NB rat

model system, Borna disease can be rapidly and reliably produced in rats inoculated with BDV and Cy (PTI-Cy rats) or with BDV and CyA (PTI-CyA rats) after adoptive transfer of spleen cells from AdI rats. The pharmacologically induced PTI rat models make assessment of virus-specific pathology difficult, however, since the cytotoxic drugs may have systemic effects, such as mucositis-induced weight loss, that are hard to separate from those of the virus (4). In addition, "breakthrough" encephalitis may be seen, depending on variations in dose and the schedule of drug administration, and repeated doses of the pharmacological agent may be required to sustain the effect.

We sought to ascertain whether inoculation of rats with BDV within 48 h of birth would provide a better model to discriminate between the direct cytopathic effects of BDV infection and the immunological response to the BDV-infected cells. The virus-induced alterations on the development of the neonatal rat nervous system were investigated by gross and microanatomical examination. The relationship of disease expression to the immunological response to BDV, or lack thereof in the case of PTI-NB rats, was also explored. By using adoptive transfer experiments, the PTI-Cy and PTI-NB models were compared. We explored other methods of inducing Borna disease in PTI-NB rats, such as parabiosis and bone marrow transplantation (BMT), to suggest potential mechanisms of resistance to Borna disease.

MATERIALS AND METHODS

BDV stock. Litters of newborn Lewis rats were inoculated intracranially (i.c.) with BDV-infected rat brain homogenate, prepared as described previously (5). Three weeks later, the rats were killed and the brains were harvested, homogenized, and sonicated. After clarification by low-speed cen-

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trifugation, the supernatant was collected and frozen at -70°C in aliquots. Virus stock contained 10^6 50% tissue culture infective doses (TCID₅₀) per ml, as detected by the development of BDV antigens in primary fetal rabbit glial cells by indirect immunofluorescence assay (5).

Inoculation of rats. Newborn Lewis rats (PTI-NB) or rats 4 weeks of age (AdI) were inoculated i.c. with 1×10^4 to 5×10^4 TCID₅₀ of BDV. The rats were observed for signs of Borna disease, anesthetized, and exsanguinated at various time points. Some rats were perfused with 4% buffered paraformaldehyde, and nervous tissues were processed and embedded in paraffin. Eight-micrometer-thick sections were cut for histological and immunohistochemical studies. Other rats were perfused with normal saline solution, and the tissues were flash frozen in OCT compound (Ames Co., Elkhart, Ind.). Six- to eight-micrometer-thick sections were then cut on a cryomicrotome for histological and immunohistochemical studies. After perfusion with sterile saline, some nervous system tissues were prepared as described above for assay for infectious virus.

Avidin-biotin immunohistochemistry. Sections of rat brain were treated with a primary antibody (e.g., polyclonal rabbit anti-BDV) and then with secondary biotinylated anti-species immunoglobulin G antibody and avidin-biotin-horseradish peroxidase complex (Vector Laboratories, Burlingame, Calif.) as described previously (5). A hydrogen peroxide-diaminobenzidine solution was added, and the brown precipitate was detected by light microscopy. If needed, a counterstain was applied by brief immersion in hematoxylin.

PTI-NB model assessment. Five PTI-NB rats were weighed at 7 weeks of age. As a control, five age-matched uninfected Lewis rats were weighed. Eleven PTI-NB rats were bled at various intervals, and the anti-BDV titers were measured in an indirect immunofluorescent assay as described previously (5).

Thirty-one PTI-NB and 45 AdI rats were anesthetized, exsanguinated, and perfused with paraformaldehyde at various intervals. One to three rats were killed at each time point. The nervous systems were dissected and examined histologically for inflammation. Neuroanatomical development was compared with that of age-matched normal Lewis rats. Sections were obtained and processed immunohistochemically for major histocompatibility antigen complex (MHC) class I and II by using the monoclonal antibodies OX-18 (MHC class I) and OX-6 (MHC class II, Ia) (Sera Lab, Westbury, N.Y.).

Induction of Borna disease in PTI rats. (i) Adoptive transfer of spleen cells. On day 0, 13 8-week-old syngeneic Lewis rats were inoculated intraperitoneally (i.p.) with Cy (100 mg/kg of body weight; Mead-Johnson, Syracuse, N.Y.) (Cy rats), and 8 of these rats were also inoculated i.c. with BDV (PTI-Cy rats). On day 10, four PTI-NB, three Cy, and three PTI-Cy rats were infused intravenously with spleen cells from five AdI rats infected 2 weeks earlier (ratio, one spleen to two recipients). The rats were observed for signs of Borna disease. Fifteen days later, the rats were sacrificed and perfused with paraformaldehyde and the tissues were processed for histological and immunohistochemical evaluation. In a separate experiment, three PTI-Cy rats were killed and the brains were harvested for quantitation of infectious virus, as described earlier.

(ii) Parabioc rats. Nine size-matched syngeneic Lewis rats were anesthetized and paired surgically through the peritoneal cavities to form parabioc rat pairs to allow communication of the immune systems. Five pairs each consisting of a PTI-NB rat and a normal rat were formed,

and two pairs of normal rats were joined. One week after surgery, one normal rat from each of these seven pairs was inoculated i.c. with 5×10^4 TCID₅₀ of BDV to form AdI-PTI-NB and AdI-normal pairs. Two parabioc pairs of PTI-NB rats were also formed. The animals were observed for signs of Borna disease, anesthetized at various time points, exsanguinated, and perfused with either paraformaldehyde or saline, and tissues were harvested for histopathology and immunohistochemical staining as described above.

(iii) Cohabitation of infected and uninfected rats. Eight AdI rats were housed with 13 normal rats. The normal rats were sacrificed 52 or 90 days later and were examined for the presence of infectious BDV, BDV antigen in the brain, and anti-BDV antibody in the serum.

(iv) Reinoculation of BDV into the PTI-NB. Eighteen PTI-NB and five AdI rats were inoculated i.c. At 30 days of age, eight of the PTI-NB rats were reinoculated i.c. with BDV. All the rats were observed for signs of Borna disease.

Fourteen newborn Lewis rats were inoculated with 5×10^4 TCID₅₀ in the subcutaneous tissue of the footpad (PTI-NB/FP rats). Forty-two days later, seven rats were reinoculated i.c. with BDV. Both groups of rats were observed for 75 days for signs of disease.

BMT. Syngeneic Lewis rats were used for BMT, as described previously (28). The donor rats consisted of normal, PTI-NB, and AdI rats. Bone marrow was harvested from the femur and inoculated intravenously (donor-to-recipient ratio, 1:2.5 to 1:3) into PTI-NB or AdI rats infected 1 week before or 2 weeks after BMT or into normal rats. Four AdI rats from the donor group did not undergo transplantation and served as controls. All recipient rats received lethal doses of gamma irradiation one day prior to transplantation (1,050 rads). The rats were observed for onset of Borna disease, sacrificed at various time points, and perfused with paraformaldehyde as described above. The nervous system tissues were examined histologically for inflammation and MHC class II expression.

RESULTS

Characterization of the PTI-NB rat model. Although previously described as normal in appearance, PTI-NB rats differed significantly from uninfected rats. The PTI-NB rats had normal body shape and proportion but were overall much smaller. PTI-NB rats weighed an average of 116 g (range, 100 to 140 g), while age-matched normal Lewis rats had an average weight of 174 g (range, 140 to 200 g) ($t = 4.08$, $df = 7.14$, $P < 0.01$ by Student's t test). The PTI-NB rats were screened for development of antibodies to BDV, since the apparent lack of cellular response to BDV is associated with a similar absence of B-cell response to viral antigens (11). The anti-BDV antibody titer in PTI-NB rats was $<1:10$ until day 133 ($n = 5$). By day 144, the animals had an average titer of 1:20 ($n = 2$); by day 300, the average titer had increased to 1:100 ($n = 4$).

Direct viral cytopathic effects of BDV infection on neural cells have been difficult to ascertain in the past. In the absence of inflammation or toxic drugs, neuronal dropout in the PTI-NB rat reflected virus-induced cytolysis. In addition, inoculation of virus at an early stage of neural development illustrated the effects of BDV infection on the ability of neural structures to develop via cell migration and differentiation. In PTI-NB rats, a severe gliosis which persisted through the last time point, 44 weeks, was noted (Fig. 1C and F). The dentate gyrus, which was intact at day 21, began to involute by day 47 and was completely degenerated by 19

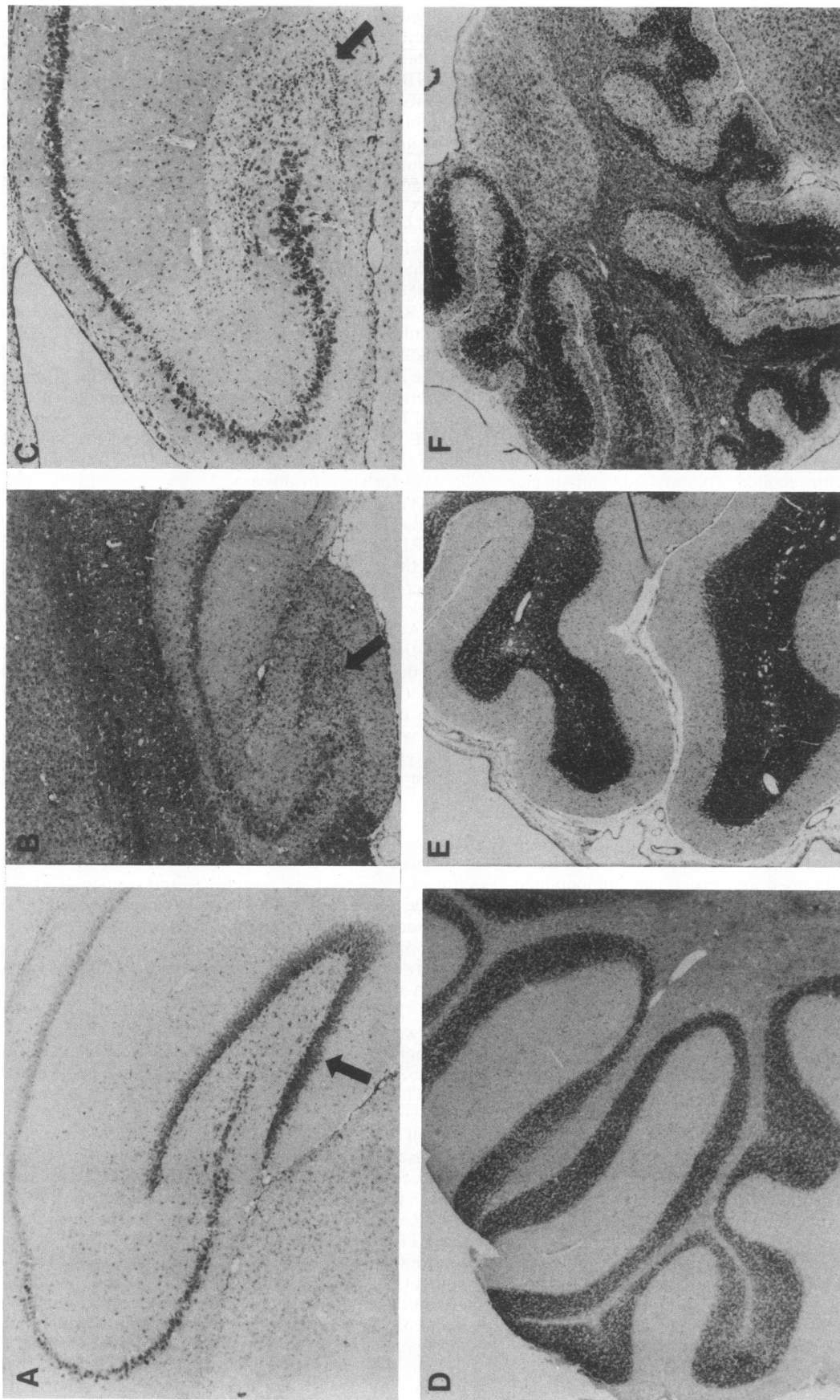


FIG. 1. Neuroanatomical abnormalities in PTI-NB and AdI rats. (A) Hippocampus from AdI rat 47 days old, 15 days after inoculation with BDV. This rat is age matched with the PTI-NB rat of panel C. The dentate gyrus is intact (arrow). (B) Hippocampus from AdI rat 79 days old, 47 days p.i. This rat is matched by days p.i. with the PTI-NB rat of panel C. The dentate gyrus has been largely destroyed (arrow). (C) Hippocampus from PTI-NB rat 47 days old, 47 days p.i. Involution of dentate gyrus (arrow) in the PTI-NB rat and replacement with reactive glial cells, indicative of selective viral cytopathic injury of a discrete neuroanatomical region, are evident. (D) Cerebellum from AdI rat 47 days old, 15 days p.i., age matched to the PTI-NB rat of panel F. (E) Cerebellum from AdI rat 79 days old, 47 days p.i., matched by days p.i. with the PTI-NB rat of panel F. (F) Cerebellum from PTI-NB rat 47 days old, 47 days p.i. The cerebellum from the PTI-NB rat was smaller than that of the AdI rats. Layers of the cerebellum, including the granule cell layer, were disorganized. All panels are stained with hematoxylin and eosin. Magnification, $\times 40$ (A, B, and C) and $\times 50$ (D, E, and F).

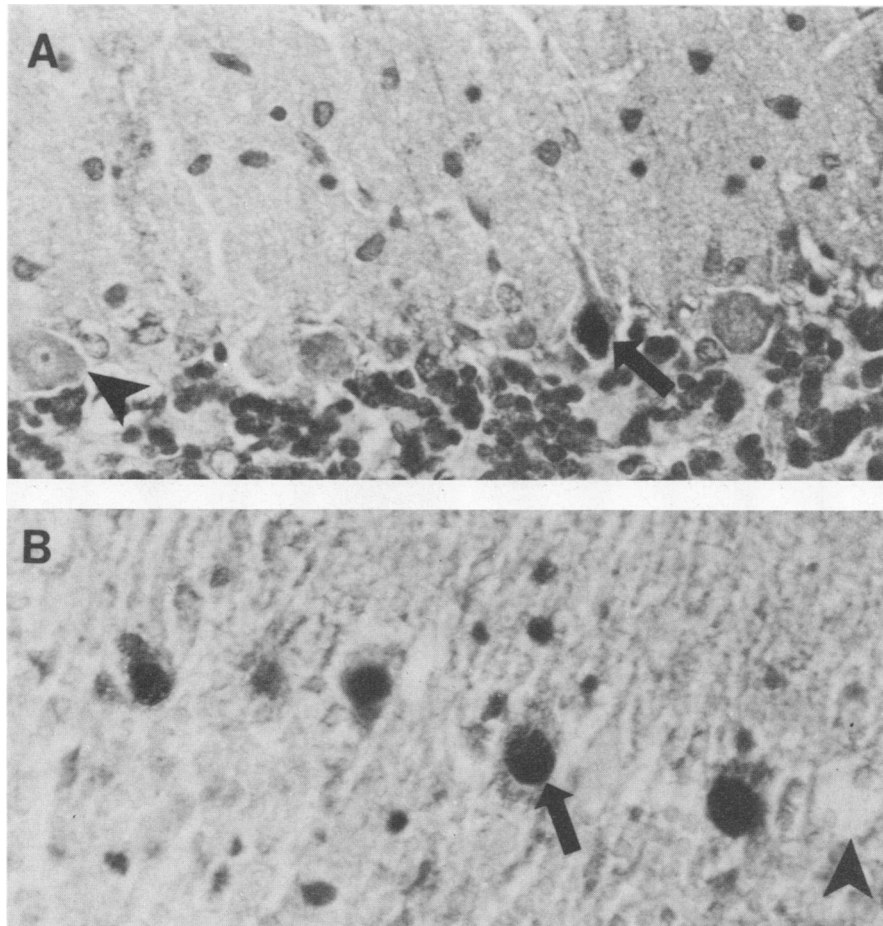


FIG. 2. BDV antigen distribution in the cerebellum. (A) Cerebellum from AdI rat 4 weeks p.i. Immunohistochemical staining for BDV antigens revealed one Purkinje cell with internuclear BDV proteins in the field (arrow). The remainder of the Purkinje cells are uninfected (arrowhead). Hematoxylin counterstain was used. Magnification, $\times 580$. (B) Cerebellum from PTI-NB rat 4 weeks p.i. Immunohistochemical stain revealed BDV proteins in most Purkinje cells in the field (arrow); rarely, a Purkinje cell did not contain BDV antigens (arrowhead). No counterstain was used. Magnification, $\times 580$.

weeks (Fig. 1C). Another striking neuroanatomical abnormality was the disorganized, hypoplastic cerebellum seen at all time points (Fig. 1F). The PTI-NB rats had no difficulty ambulating, however, and showed no overt signs of cerebellar dysfunction.

Pathological sections from AdI rats matched to PTI-NB rats by age or days postinoculation (p.i.) were also examined. At 47 days of age, 15 days after inoculation with BDV, the dentate gyrus was intact (Fig. 1A) in AdI rats. In contrast, by 79 days of age and 46 days p.i., the dentate gyrus was largely destroyed in the AdI rats (Fig. 1B). The cerebellums of both rats matched by age (47 days old, 15 days p.i.) and rats matched by days p.i. (79 days old, 47 days p.i.) showed much less structural disorganization than those of the PTI-NB rats (Fig. 1D, E, and F).

The nervous system inflammatory response in the PTI-NB rats was rated 0 to 4+. There was no evidence of encephalitis (Fig. 1C and F), with the exception of 1+ perivascular inflammatory infiltrates in one rat at day 21 (data not shown).

BDV-infected cells were identified by immunohistochemical staining for BDV antigens. The general anatomical sites of infected neural cells resembled those described earlier for

AdI animals, with some notable exceptions. Overall, there appeared to be a more diffuse involvement of the nervous system, with fewer isolated clusters of infected cell groups. Whether this reflected a failure of migration of infected cells into normal anatomical structures or whether BDV was infecting more widely was not clear. For example, virtually every Purkinje cell in the cerebellums of the PTI-NB rats was infected (Fig. 2B), versus scattered infection of a few Purkinje cells in the AdI rats (Fig. 2A). The entire hippocampus (CA1 to CA4) was infected in the PTI-NB rat soon after inoculation, compared with a preference for the CA3-CA4 region in the AdI rat (data not shown).

MHC antigen expression. MHC antigens are necessary for antigen presentation to T lymphocytes but are not present in normal rat brain tissue (29). In the AdI rat, BDV infection is associated with inflammation and MHC class II expression (5, 8). We sought to evaluate the MHC class I and II expression in PTI-NB rats in the setting of BDV infection without inflammation and to compare these findings with those for the AdI rat (Fig. 3). No MHC class I expression was detected at any time point in normal or PTI-NB rats (data not shown).

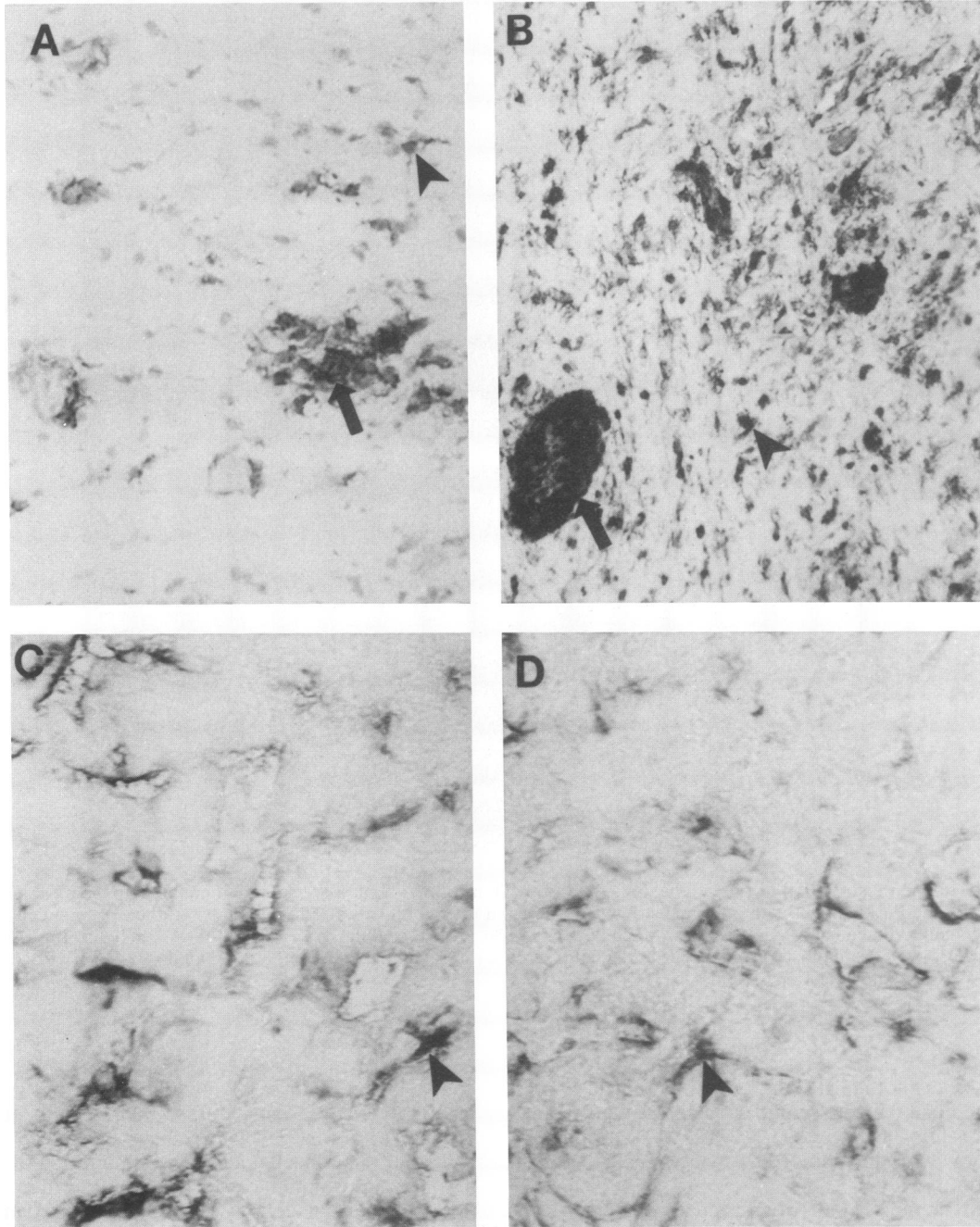


FIG. 3. MHC class I and II antigens in the brains of AdI rats. (A) On day 33 p.i., MHC class II-expressing cells were in the perivascular cuff (arrow) and in the parenchyma (arrowhead). Cells in the parenchyma appear to be microglial cells or stellate astrocytes. No counterstain was used. Magnification, $\times 250$. (B, C, and D) MHC class I expression at 42 (B), 86 (C), and 300 (D) days p.i. MHC class I expression is initially intense in the perivascular cuffs (arrow) and the cells in the parenchyma (arrowheads). MHC class I-expressing cells in the parenchyma persist through day 300. Immunohistochemical staining was with OX6 (MHC class II) and OX18 (MHC class I); no counterstain was used. Magnification, $\times 250$ (B) and $\times 500$ (C and D).

In AdI rats, MHC class I was first noted on day 5 in the meningeal infiltrate (1+). By day 13, MHC class I was seen in the meningeal infiltrate (3+) and the perivascular cuffs (3+), with barely detectable expression in cells in the parenchymal tissue (1+). From day 20 through day 28, the intensity of the MHC class I expression increased in parallel with the increasing meningeal infiltrate. By day 34, the signal

was strong in cells in the meningeal (4+) and perivascular (4+) infiltrate, and there was development of 4+ parenchymal expression as well. This intensity of MHC class I expression remained constant through day 55. Between days 84 and 300, the inflammatory cell-associated MHC class I expression decreased, concurrent with the loss of perivascular and meningeal infiltrates (0 to 1+), while MHC class I

staining persisted in the parenchyma (2+ to 3+). The cells in the parenchyma expressing MHC class I morphologically resembled microglia cells or stellate astrocytes.

PTI-NB rats showed no evidence of MHC class II in the brain. Adoptive transfer of spleen from a BDV-infected rat into PTI-NB rats produced a few MHC class II positive inflammatory cells in the meninges on day 4 through day 40 posttransfer (data not shown).

In AdI rats, MHC class II expression was present as early as day 4 in the inflammatory cells in the meninges (1+) and in the choroid plexus cells (2+) (Fig. 3). MHC class II in perivascular cuffs were noted at day 11 (2+) along with a few cells in the parenchyma (1+). MHC class II expression continued to increase, peaking at approximately day 34 in mononuclear cells in the perivascular cuffs and meningeal infiltrate and in cells in the parenchyma which resembled microglia (all 4+). As the inflammatory response receded, the MHC class II expression in perivascular cuffs and meningeal infiltrate also declined, so that by day 55 the expression in those areas was greatly reduced (1+). The cells in the parenchyma continued to express at high levels (4+). At day 90, the parenchymal expression was decreased (2+ to 3+), and by day 300 the parenchymal expression of MHC class II was barely detectable (0 to 1+).

Induction of Borna disease in PTI-NB rats. (i) **Adoptive transfer of spleen cells from AdI rats.** Adoptive transfer of spleen cells from AdI rats rapidly induces Borna disease in PTI-Cy rats. We attempted to use the same techniques to produce Borna disease in the PTI-NB rats. Two of two PTI-Cy rats tested had mild to no signs of inflammation (0 to 2+) and no signs of Borna disease. Adoptive transfer of spleen cells from AdI rats produced Borna disease and 3+ to 4+ meningitis and encephalitis in three of three PTI-Cy rats (data not shown). None of four PTI-NB rats which received aliquots of the same spleen cells as the PTI-Cy rats showed signs of Borna disease or encephalitis on histological examination (data not shown). Immunohistochemical stains revealed a few inflammatory cells in the meninges of the PTI-NB rats after transfer, as mentioned earlier. Infectious BDV in PTI-Cy rats ($n = 2$) and PTI-NB rats ($n = 30$) was 10^6 to 10^7 TCID₅₀ per g.

(ii) **Parabiotic rats.** Traditional adoptive transfer experiments which produced Borna disease in PTI-Cy rats were not effective in PTI-NB rats. Possible explanations included BDV-antigen-specific immunosuppressive activity in PTI-NB rats and the transfer of insufficient numbers or types of the critical cells necessary to induce disease in PTI-NB rats. Finally, the absence of a necessary, noncellular factor in the transferred material could not be excluded. Parabiotic joining of syngeneic rats allowed a prolonged, comprehensive sharing of both cellular and humoral elements of the immune system (25). Parabiosis between AdI and PTI-NB rats would then result either in the suppression of Borna disease in the AdI rat by PTI-NB-supplied suppressor elements or in the induction of Borna disease in the PTI-NB rat by immunological components supplied by the AdI rat. The AdI rats were infected with BDV after parabiosis in order to expose the PTI-NB rat to early immunological responses of the AdI partner.

Two of three PTI-NB rats paired with AdI rats exhibited signs of Borna disease by day 15 p.i. (Table 1). In the PTI-NB rats, severe neuritis (3+ to 4+) was present as well as mild to moderate meningitis and encephalitis (1+ to 2+) (Fig. 4 and 5). By day 15 p.i., three of three AdI rat partners had 2+ meningitis and one of three had 2+ encephalitis. The AdI partners of PTI-NB rats developed Borna disease in the

TABLE 1. Presence of Borna disease and inflammation after parabiotic union^a

Combination	Days p.i.	No. of rats showing signs of Borna disease/no. in group	Presence of inflammation
AdI-PTI-NB	13, 15	1/3, 2/3	+, +
	48	2/2, 2/2	+, +
AdI-normal	13, 18	2/2, 0/2	+, -
PTI-NB-PTI-NB	19 ^b	0/1, 0/1	-, -
	45 ^b	0/1, 0/1	-, -

^a In pairs of data in the last three columns, the first value refers to rats of the first type listed in the combination (e.g., AdI rats in the AdI-PTI-NB combination) and the second value refers to rats of the second type.

^b Day after parabiotic union at which rat was infected as a neonate.

same time frame as single AdI rats (days 15 to 22 p.i.). Two of two PTI-NB-PTI-NB pairs showed no evidence of Borna disease or inflammation. Two of two normal rats paired with AdI rats developed no Borna disease or inflammation. MHC class II expression in the PTI-NB rats from PTI-NB-AdI pairs was found only on inflammatory cells in perivascular locations (Fig. 4).

(iii) **Reinoculation with BDV.** In the parabiotic setting, the PTI-NB rats may have been exposed to a second inoculum of virus, since the AdI rats were inoculated with BDV after being joined to the PTI-NB rats. We examined whether reexposure to BDV was sufficient to induce Borna disease in a PTI-NB rat. First, to determine whether merely cohabiting in the same cage was enough to transmit BDV, uninfected and infected rats were housed together for up to 3 months. The 13 uninfected rats which cohabited with AdI rats did not acquire BDV infection, as determined by BDV antigen or infectious BDV in the nervous system or BDV-specific antibodies in the sera.

Since parenteral BDV inoculation was required to reliably infect the animals, PTI-NB rats were exposed to a second i.c. BDV challenge at the age of 4 weeks. The majority (nine of ten) of these reinoculated PTI-NB rats showed no signs of Borna disease. The same finding was observed for the PTI-NB rat littermates (seven of eight rats) which were not reinoculated with BDV. Normal rats inoculated at the same time became ill with Borna disease beginning on day 22 p.i.

(iv) **Development of tolerance to Borna disease after peripheral inoculation.** Development of immune tolerance to a virus which replicates in the nervous system may reflect the timing of the delivery of the viral antigens to the immune system rather than the timing of the infection of the nervous system. Since a large part of the i.c. inoculum is probably delivered systemically (19), we attempted to induce the PTI-NB state by peripheral inoculation. Footpad inoculation with BDV at birth (PTI-NB/FP rats) also resulted in tolerance to Borna disease in 13 of 13 rats. At day 42 p.i., seven PTI-NB/FP rats were reinoculated i.c. with BDV (PTI-NB/FP + BDV rats). One PTI-NB/FP + BDV rat showed signs of Borna disease; the remaining rats were clinically normal.

(v) **BMT.** The parabiotic rat data suggested that the PTI-NB rat's resistance to the development of Borna disease was not due to active BDV-specific immunosuppression but to a lack of essential cells or factors. When supplied with these elements by parabiosis to an AdI rat, the PTI-NB rats developed Borna disease; further experiments suggested that simple reinoculation with BDV was not responsible for the development of Borna disease and that tolerance was a function of the delivery of virus to the extraneural system.

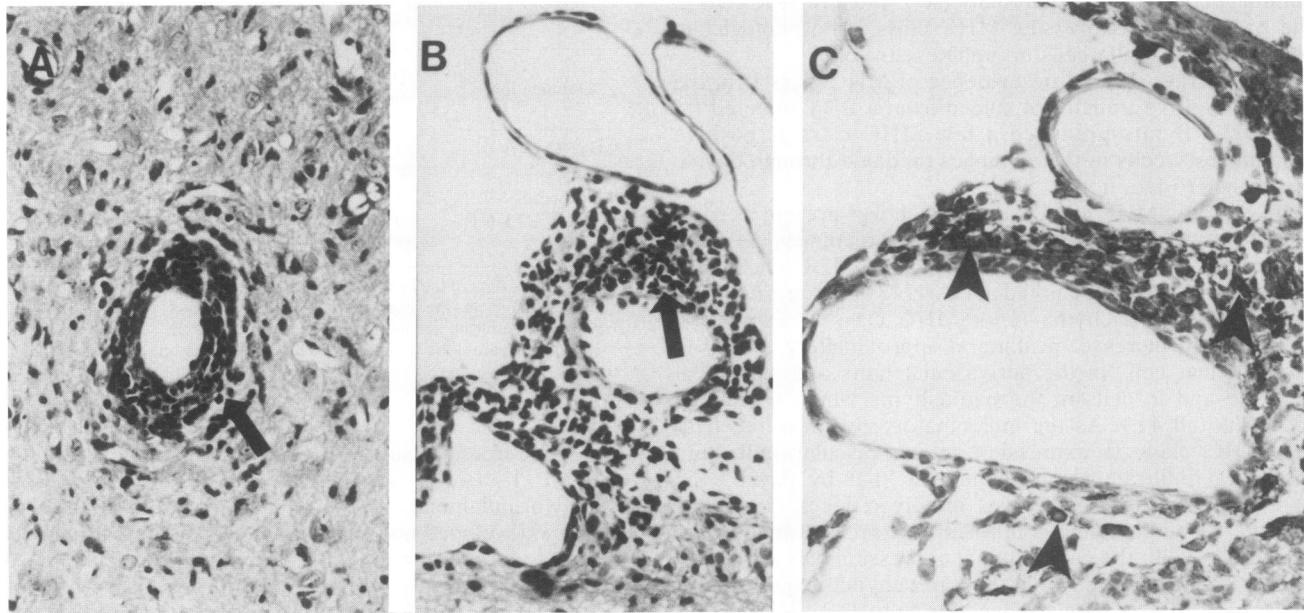


FIG. 4. Brain tissue from parabiotic rats. (A) Tissue from AdI rat, parabiotic partner of PTI-NB rat. Mononuclear encephalitis is indicated by the arrow. Hematoxylin and eosin stained; magnification, $\times 280$. (B) Tissue from PTI-NB rat, partner of the AdI rat of panel A. Perivascular mononuclear cell inflammatory infiltrate is indicated by the arrow. Hematoxylin and eosin stained; magnification, $\times 280$. (C) Section from the PTI-NB rat of panel B. Immunohistochemical staining for MHC class II shows positive cells in the perivascular region (arrowheads). Hematoxylin counterstain; magnification, $\times 280$.

The mechanism of tolerance to BDV seemed to be similar to that of the development of tolerance to MHC (self) antigens, that is, by clonal deletion or anergy of antigen-specific immune cells during maturation (16, 17, 27). BMT experiments enabled us to test this hypothesis. If tolerance to Borna disease occurred during the maturation of the immune system, then the immature cells in the bone marrow of PTI-NB donor rats might still contain a repertoire of inflammatory cells capable of producing Borna disease. Similarly, one would expect no BDV-specific cells from the AdI donor marrow to mature to functional anti-BDV status in the PTI-NB recipient rats.

The five normal lethally irradiated rats which received bone marrow either from normal rats or from AdI rats showed no signs of Borna disease or inflammation when they were killed at day 52 (Table 2). The seven lethally irradiated PTI-NB rats which received normal, PTI-NB, or AdI marrow showed no signs of Borna disease or inflammation of the nervous system on histological examination (Fig. 6). Six of six lethally irradiated AdI rats (infected either 7 days before or 14 days after transplant) developed signs of Borna disease after receiving normal, PTI-NB, or AdI bone marrow. Histological examination revealed signs of encephalitis (2+ to 3+) and meningitis (3+) in the AdI recipient group of rats. Thus, the PTI-NB donor marrow contained cells which produced inflammation and Borna disease in AdI rats, while the PTI-NB recipients did not develop Borna disease or inflammation regardless of the source of the donor marrow.

Three out of four normal rats which received the PTI-NB marrow developed signs of Borna disease by day 48, with 3+ encephalitis and 3+ meningitis, indicating that infectious BDV was located in the bone marrow cells in the PTI-NB rats.

DISCUSSION

The PTI-NB rat is a good model of resistance to disease after inoculation with an immunopathogenic virus, with virtually no encephalitis or Borna disease in the face of high titers of infectious virus. Despite the absence of necrotizing encephalitis seen in AdI rats, neuropathological disruption of the central nervous system in PTI-NB rats was still observed. Neuroanatomical abnormalities of PTI-NB rats, presumably due to direct virus effect, included a diffuse, persistent gliotic reaction in the brain and gradual involution of the neurons of the dentate gyrus, leaving behind astrocytes and microglial nodules. The loss of the dentate gyrus at about 6 weeks p.i. occurred in both PTI-NB and AdI rats, suggesting that these neurons suffered a direct cytopathological insult from BDV infection. Why the dentate gyrus neurons are specific targets of BDV is not known. The abnormal hippocampal development was consistent with the behavioral abnormalities found with detailed testing of PTI-NB (9).

The neuroanatomical abnormalities associated with neonatal inoculation of BDV are likely to be due to the direct effect of the virus infection on neural cells rather than a byproduct of an inflammatory response. As has been suggested for other viruses, BDV infection may interfere with "luxury functions" of neural cells and might hinder the normal formation of intercellular connections and migration (22). In the PTI-NB cerebellum, one major abnormality seemed to be in cell organization; however, interneural connections appeared to be formed appropriately despite the disorganized cerebellar arrangement, as demonstrated by the animals' normal coordination and motor skills. These neuroanatomical and behavioral abnormalities correlated with the loss of cells which are largely produced after birth,

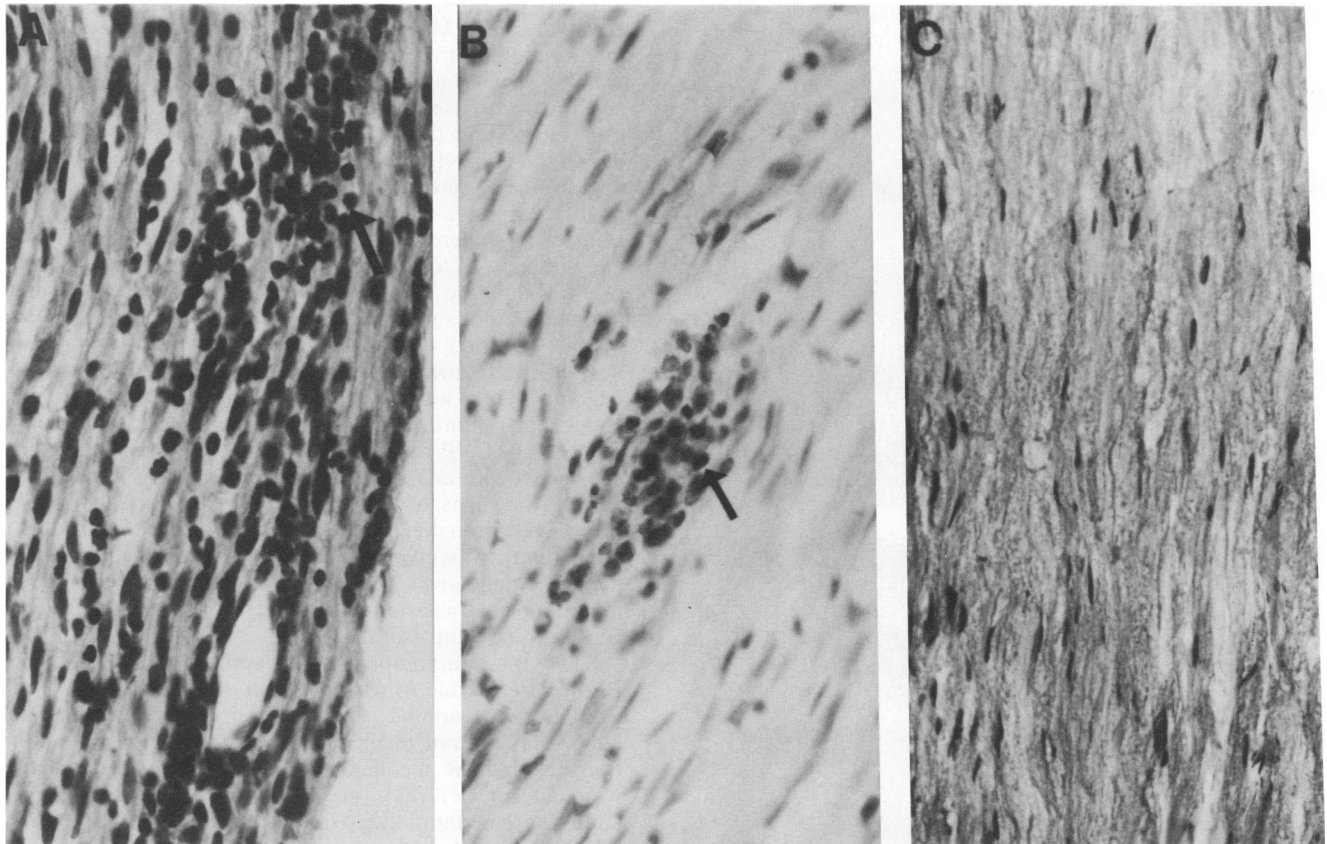


FIG. 5. Sciatic nerve tissue from parabiotic rats. (A) Tissue from PTI-NB rat, parabiotic partner of AdI rat. Intense mononuclear cell inflammation in the nerve is indicated (arrow). (B) Tissue from AdI rat, partner of the PTI-NB rat of panel A, with inflammation in the sciatic nerve (arrow). (C) Sciatic nerve from PTI-NB rat, partner of a PTI-NB rat. No inflammation of the nerve is evident. Hematoxylin and eosin stained; magnification, $\times 480$ (A and B) and $\times 310$ (C).

e.g., the granule cells of the hippocampus and cerebellum (2). In the cerebellum, for example, disruption of the granule cell layer development may lead to hyperactivity, not ataxia. A disorganized, hypoplastic cerebellum, as seen in the PTI-NB rat, has been described for several viruses administered in utero or perinatally (20, 24). All of these viruses

may influence the normal development and migration of neural cells.

The weak antibody response to BDV in the PTI-NB rats suggests an absent or limited cellular immune response to BDV. While the late rise in antibody titer was still not equivalent to the titers of 1:2,500 seen in AdI rats (5), the development of anti-BDV antibodies over time suggested either a reduction of the immunological tolerance as the animal aged and an increased T-cell responsiveness to BDV over time or the development of T-cell-independent antibody production. The BMT experiments suggested that unresponsiveness to Borna disease is maintained for at least a year, the age of the oldest PTI-NB rat undergoing BMT.

Coincident with the lack of inflammatory response and Borna disease, MHC class I or II in the brains of PTI-NB or normal rats was not detected. In AdI rats, MHC class I was noted very soon after infection, peaked by day 34, and maintained a high degree of expression through day 55. The subsequent decrease in MHC class I expression coincided with the loss of the inflammatory cell infiltrate. A significant amount of MHC class I expression persisted even after the loss of the inflammatory cell infiltrate; however, this expression was seen largely in cells in the parenchyma which resembled microglial cells or stellate astrocytes.

The MHC class II expression in the AdI rats followed a similar pattern, as noted previously (8), with the exception that by day 90 there was a marked decrease in MHC class II

TABLE 2. BMT of BDV-infected and uninfected rats

Recipient	Donor	BDV inoculation of recipient ^a	No. of rats contracting Borna disease/no. in group	No. of rats with inflammation/no. in group
Normal	Normal	None	0/4	0/4
		D-7	2/2	2/2
	AdI	D+14	1/1	1/1
		None	0/1	0/1
	PTI-NB	D-7	2/2	2/2
		D+14	2/2	2/2
PTI-NB	Normal	None	3/4	3/4
		D-7	2/2	2/2
	AdI	D+14	3/3	3/3
		*	0/1	0/1
	PTI-NB	*	0/4	0/4
		*	0/2	0/2

^a D-7, infected 7 days before transplant; D+14, infected 14 days after transplant; *, infected at birth.

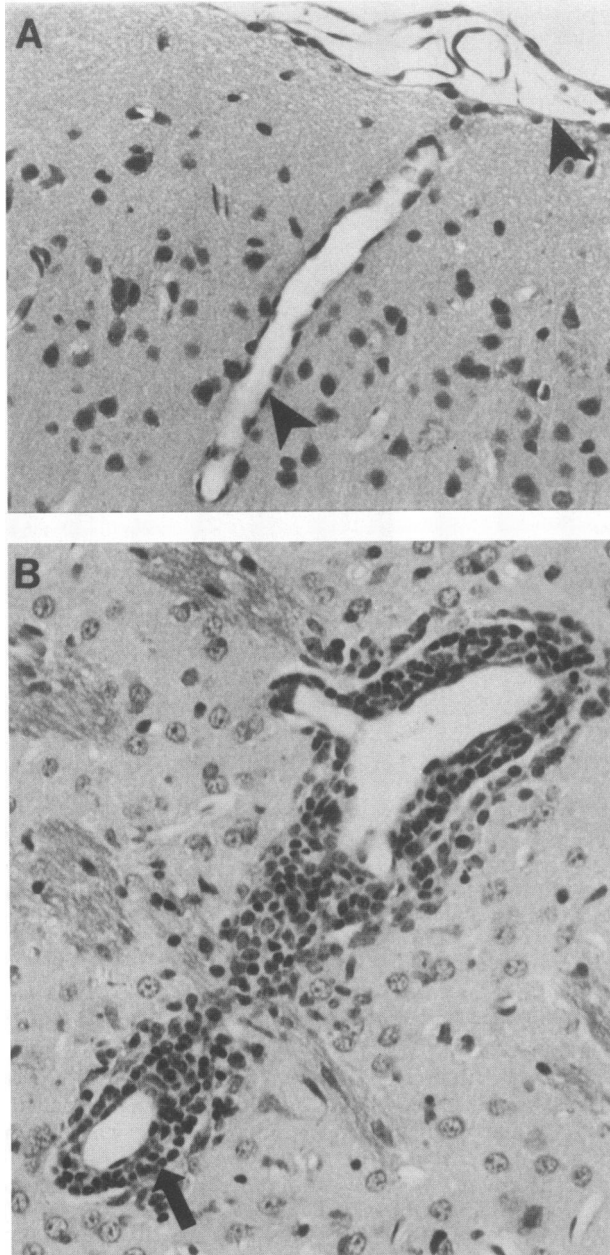


FIG. 6. Brain tissue from BDV-infected rats after BMT. (A) Tissue from PTI-NB recipient transplanted with AdI donor bone marrow. Encephalitis and meningitis are not present (arrowheads). Hematoxylin and eosin stained; magnification, $\times 580$. (B) Tissue from AdI recipient transplanted with PTI-NB rat donor bone marrow. Intense perivascular mononuclear inflammatory cell infiltrate is evident (arrow). Hematoxylin and eosin stained; magnification, $\times 580$.

expression and by day 300 the brain expressed a barely detectable level of MHC class II, a clear difference from the MHC class I expression. These findings are consistent with the notion that the MHC class II-restricted CD4 response is important in the immunopathogenesis of Borna disease, since the loss in MHC class II expression coincided with the loss of inflammation (23). Borna disease development in the PTI-NB parabiotic partners of AdI rats did not appear to be

linked to the development of parenchymal MHC expression, however, since MHC class II was seen only on the inflammatory cell infiltrate.

PTI-Cy rats, but not PTI-NB rats, developed encephalitis and Borna disease after the transfer of spleen cells from AdI rats, suggesting that there were significant differences between the PTI-NB and the PTI-Cy rats in the mechanism or degree of responsiveness to BDV. The absence of inflammatory response and Borna disease following the adoptive transfer of spleen cells in the PTI-NB rats might have been due either to a "dose effect," wherein insufficient numbers of BDV-immune cells were transferred to produce inflammation, or to a suppressor cell or factor in the PTI-NB rat.

In order to try to distinguish between these two possibilities, PTI-NB and AdI rats were connected by parabiosis, which provided an opportunity for the exchange of humoral and cellular immune components for extended periods of time. All PTI-NB rats paired with AdI rats developed Borna disease, severe neuritis, and mild encephalitis. The AdI rats showed no signs of suppression of disease from elements transferred from their PTI-NB partners. Therefore, when the opportunity was given for an intensive communication between immune systems, the AdI animal apparently supplied immunological assistance to the PTI-NB rat to respond to BDV and develop Borna disease.

PTI-NB rat parabiotic partners apparently did not develop Borna disease due to reinoculation or transmission of virus from the AdI animal. Cohabitation of infected and uninfected rats did not result in transmission of the virus infection, leaving the mechanisms of virus transmission in the wild unclear. PTI-NB rats reinoculated *i.c.* with BDV as adults were no more likely to develop Borna disease than the PTI-NB controls.

Inoculation of newborn Lewis rats in the footpad produced a persistent infection, with no clinical signs of Borna disease. Reinoculation of the PTI-NB/FP rats with BDV did not induce clinical signs of Borna disease. A common theme to both *i.c.* and footpad inoculation of neonatal rats is the delivery of substantial amounts of BDV to the extraneural compartment, e.g., the thymus, bone marrow, and lymphatic system (19). In previous work, a minimum delay of 2 days occurred from footpad inoculation to transmission and infection in the nervous system (5). Thus, in the PTI-NB/FP rat, virus replication in the nervous system may begin after the critical 48-h postnatal period for immune tolerance. Our data suggest that hyporesponsiveness to BDV may be a function of the delivery of virus antigens to the extraneural system and may not be dependent on viral replication.

The bone marrow cells of all rats, including the PTI-NB rats, were able to induce Borna disease when transplanted into a lethally irradiated AdI rat. None of the transplanted marrows resulted in Borna disease or inflammation in the PTI-NB recipients. Thus, hyporesponsiveness to BDV appeared to occur during immune cell maturation in the PTI-NB recipient rat. These data are consistent with the hypothesis that the Borna disease-inducing inflammatory cells in the parabiotic pairs may have originated from the AdI rat or may have been PTI-NB cells that circulated through and matured in the AdI rat and reentered the PTI-NB rat.

Within the limits of the timing of transplantation and virus inoculation, we did not see a recapitulation of unresponsiveness to Borna disease develop in the AdI recipient rats. In the neonatal rat, the timing of immune system development and BDV inoculation are critical. If there was a similar window of opportunity for the AdI recipients, it was missed

by our inoculation schedule. The identification of suppressor cells can be dependent on time elapsed after transplantation, so it is possible that part of the mechanism of tolerance to Borna disease in PTI-NB rats involved a suppressor cell. The parabiotic experiments are not consistent with the presence of suppressor cells, however, since the AdI partners of the PTI-NB rats still developed Borna disease.

The onset of encephalitis is typically associated with the development of Borna disease. PTI-NB parabiotic rats paired with AdI rats had severe Borna disease with only mild encephalitis, and thus the association between encephalitis and the severity of Borna disease was not constant. One possible explanation is that some systemic factors released by the AdI rats may have induced or triggered Borna disease in the PTI-NB partners, indicating that Borna disease may not be simply a local response to inflammation in the central nervous system. This conclusion is supported by recent data on host genetics and Borna disease showing that some BDV-infected Lewis/Black-hooded hybrid rats with encephalitis show no signs of Borna disease (11) and by our observations of milder disease manifestations in rat strains differing immunologically from Lewis rats (22a). In the parabiotic model system, however, the influence of genetic effects on susceptibility to Borna disease are avoided by the use of syngeneic rats. Therefore, the resistance of PTI-NB rats to Borna disease is developmental and is not a function of genetic variation.

Although BDV is generally considered to be an infection exclusively in the nervous system, extraneural sites of BDV replication have been found in PTI-NB rats (12). Three of the four normal rats which received PTI-NB bone marrow developed BDV infection and Borna disease, demonstrating that infectious BDV resides in cells in bone marrow. Previous work has been unable to demonstrate BDV infection in macrophages *in vitro* or *in vivo*, but these studies could be extended by using PTI-NB bone marrow cells (5). In lymphocytic choriomeningitis virus (LCMV), neonatal inoculation and tolerance is associated with the development of a lymphotropic LCMV variant (1). A similar event may occur in the PTI-NB rat, although the rats which received the infected PTI-NB marrow clearly developed symptomatic, immunopathological Borna disease.

BMT experiments in mice with LCMV suggest that the virus inhibits the development of the donor marrow (14). In the BDV system, infection of the recipient rats before or after transplantation gave the same results. The infection of viruses in the nervous system is often accompanied by the infection of immune system cells, and vice versa (e.g., measles virus, human immunodeficiency virus, cytomegalovirus) (3, 10, 15, 30). The role of a lymphotropic BDV in the PTI-NB rat's hyporesponsiveness to Borna disease remains to be explored.

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