JOURNAL OF VIROLOGY, Jan. 1991, p. 457–460
0022-538X/91/010457-04$02.00/0

Vol. 65, No. 1

Atypical Dissemination of the Highly Neurotropic Borna Disease Virus during Persistent Infection in Cyclosporine A-Treated, Immunosuppressed Rats

LOTHAR STITZ,[1]* DOROTHEA SCHILKEN,[1] AND KNUT FRESE[2]

Institut für Virologie[1] and Institut für Veterinär-Pathologie,[2] Justus-Liebig-Universität, D-6300 Giessen, Federal Republic of Germany

Received 26 June 1990/Accepted 2 October 1990

In adult rats infected with Borna disease virus, the virus was found exclusively in the brain, whereas in cyclosporine A-treated rats, infectious virus was also detected in peripheral nerve fibers and, unexpectedly, in adjacent organ-specific cells. In contrast to untreated virus-infected rats, no major histocompatibility complex class II expression was found in the brain of cyclosporine A-treated animals.

We are using Borna disease virus (BDV) infection in rats as a model of a persistent virus infection of the central nervous system. Previous studies have shown that BDV is a highly neurotropic virus that replicates in adult rats exclusively in cells of the central nervous system and migrates intra-axonally (2, 4–6, 11). Disturbances of normal brain functions, usually appearing as a severe central nervous system disease, occasionally results in the death of experimentally infected animals of various species (7–9, 11, 16–18). The most prominent feature after BDV infection in vivo is a cell-mediated immune response dominated by macrophages and $CD4^+$ T cells (13), which supports the view that Borna disease is based on a delayed-type hypersensitivity reaction.

In a previous study (17), we were able to inhibit the development of an inflammatory reaction in the brain of BDV-infected Lewis rats and consequently development of Borna disease by the administration of cyclosporine A (CSA). In this report, we describe an altered tissue distribution of infectious virus during persistent BDV infection in rats treated with CSA.

Throughout this study, the Giessen strain He/80 of BDV was used as reported earlier (17). Female Lewis rats (Zentralinstitut für Versuchstierzucht, Hannover, Federal Republic of Germany) were infected intracerebrally with 10^4 50% tissue culture infectious doses ($TCID_{50}$) at 4 to 5 weeks of age. CSA was injected subcutaneously in daily doses of 25 mg/kg diluted in neutral lipid (Myglyol 812). Treatment was started 1 day before intracerebral infection and was continued until day 28 postinfection. Rabbit embryonal brain cells served as indicator cells for the determination of infectious virus in tissue homogenates as described earlier (15, 17). In addition, the presence of virus-specific antigen was demonstrated in tissue homogenates by Western immunoblot analyses (17). BDV-specific antigen and major histocompatibility complex (MHC) class II antigen was detected by means of the peroxidase-antiperoxidase (PAP) method, using a BDV-specific monoclonal mouse antibody (MAb) and MAb Ox-6.

In CSA-treated, virus-infected rats, which showed neither encephalitic lesions nor disease, we tested the brain and other organs for the presence of virus. Testing of tissue homogenates by Western blot analysis and immunohistological screening of tissue sections revealed the presence of virus-specific antigen in several organs (Fig. 1). To determine whether merely viral antigen or infectious virus was present in organs, infectivity assays were performed; the results revealed relatively low but significant virus titers in all peripheral organs tested except the kidneys (Fig. 1). The results obtained by Western blotting, virus titrations, and immunofluorescence correlated well with each other. The highest titer (2×10^3) of infectious virus in peripheral organs was found in the liver, where both viral antigens (38 to 39 kDa and 24 kDa) were detectable by Western blot analysis; the result of immunohistological examination with a BDV-specific MAb was also clearly positive. No viral antigen was detectable in Western blots, and no infectious virus ($<10^1$) was found in organs other than the brain of untreated, BDV-infected control rats. The infectivity titer in the brain of all experimental rats was 10^6.

Consequently, the kinetics of the appearance of viral antigen and infectious virus were determined. No infectivity was found in organs other than the brain of CSA-treated,

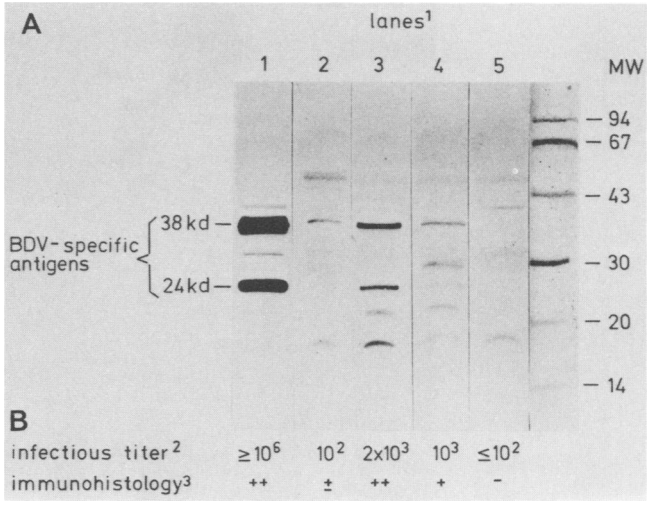

FIG. 1. Infectious virus and viral antigen in organs of CSA-treated, BDV-infected rats. (A) Western blot analyses of the brain (lane 1), lung (lane 2), liver (lane 3), spleen (lane 4), and kidney (lane 5) from CSA-treated rats. (B) Infectious virus titers and results of immunohistological examination. kd, Kilodaltons.

* Corresponding author.

TABLE 1. Presence of BDV antigens or infectious virus in the organs of CSA-treated rats and untreated controls

Organ	Presence of viral antigen at given time (wk) postinfection											
	CSA-treated rats										Untreated rats[a]	
	4		8		10		15		23			
	Western blot	$TCID_{50}$[b]	Western blot	$TCID_{50}$	Western blot	$TCID_{50}$	Western blot	$TCID_{50}$	Western blot	$TCID_{50}$	Western blot	$TCID_{50}$
Lung	−	$<10^2$	−	$<10^2$	+	$<10^2$	+	10^2	+	10^2	−	$<10^2$
Liver	−	$<10^2$	−	$<10^2$	−	$<10^2$	+	10^2	+	2×10^3	−	$<10^2$
Spleen	−	$<10^2$	+	$<10^2$	+	10^2	+	10^3	+	10^3	−	$<10^2$
Kidney	−	$<10^2$	−	$<10^2$	−	$<10^2$	−	$<10^2$	−	$<10^2$	−	$<10^2$
Brain	+	10^6	+	10^6	+	10^6	+	10^6	+	10^6	+	10^6

[a] Values for 4 to 23 weeks postinfection.
[b] Determined by titration on rabbit embryonal cells.

BDV-infected rats earlier than 10 weeks postinfection, whereas viral antigen and infectious virus was detectable in the brains of all infected animals as early as 4 weeks postinfection (Table 1). Even small amounts of viral antigen were detected by the Western blot method before the detection of infectious virus. Again, in none of the abdominal organs of BDV-infected rats did we find any evidence for infectivity or viral antigen. Immunohistological examination of organ sections revealed that viral antigen was detected mainly in autonomous nerves of the organs and occasionally in foci of parenchymal cells adjacent to nerve fibers, e.g., hepatocytes in the liver (Fig. 2).

Finally, we looked for MHC class II expression as a possible consequence of local lymphokine production in the brains of CSA-treated and untreated infected animals. Immunohistological examination of the brain from untreated, BDV-infected rats revealed a clear positive reaction, whereas no staining reaction was observed in CSA-treated, infected animals (Fig. 3 and 4).

Our data provide evidence for a dissemination and unusual tissue distribution of a highly neurotropic virus during persistent infection of the brain under immunosuppression by CSA. In contrast to the results for untreated, BDV-infected Lewis rats, in which the virus was found exclusively in the brain, we detected viral antigen and considerable titers of infectious virus in several organs, especially the liver and spleen. In vitro data provide strong evidence that the observed dissemination of BDV in CSA-treated rats is not due merely to an enhanced spread of virus resulting from a direct effect of CSA on virus-infected cells (unpublished observation). Therefore, we assume that the induced unresponsiveness of the immune system upon treatment with CSA (1, 3, 12, 14) is responsible for the observed phenomenon. We suggest a contribution of the immune response: locally produced lymphokines controlling virus spread in untreated infected rats that are not operative in CSA-treated animals. The action of lymphokines in the brain of untreated BDV-infected rats has been implicated by the demonstration of

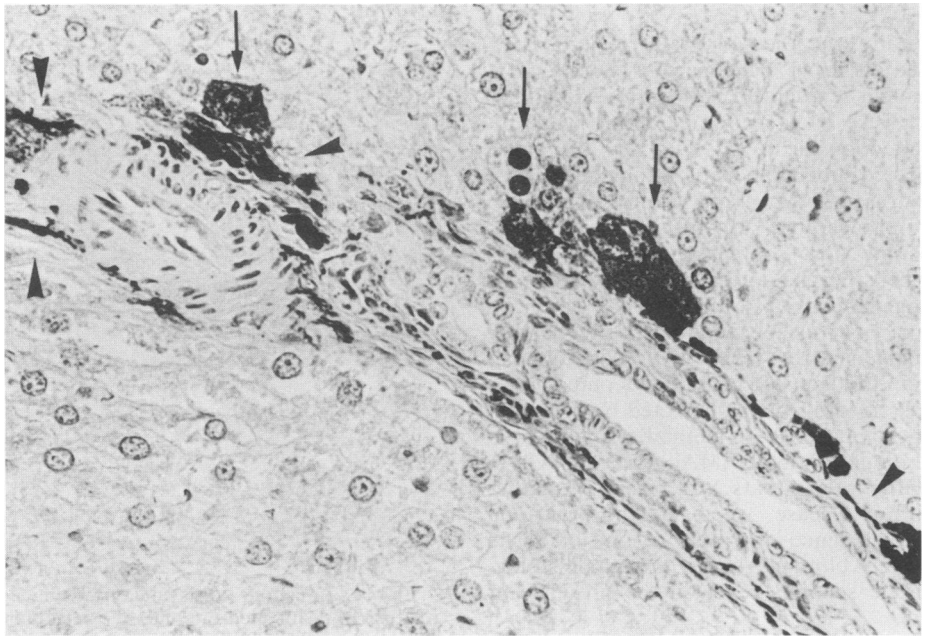

FIG. 2. Liver section from a CSA-treated, BDV-infected rat showing BDV antigen in perivascular nerves (arrowheads) and in single hepatocytes (intranuclear, or cytoplasmatic, or both; arrows). PAP technique counterstained with hematoxylin.

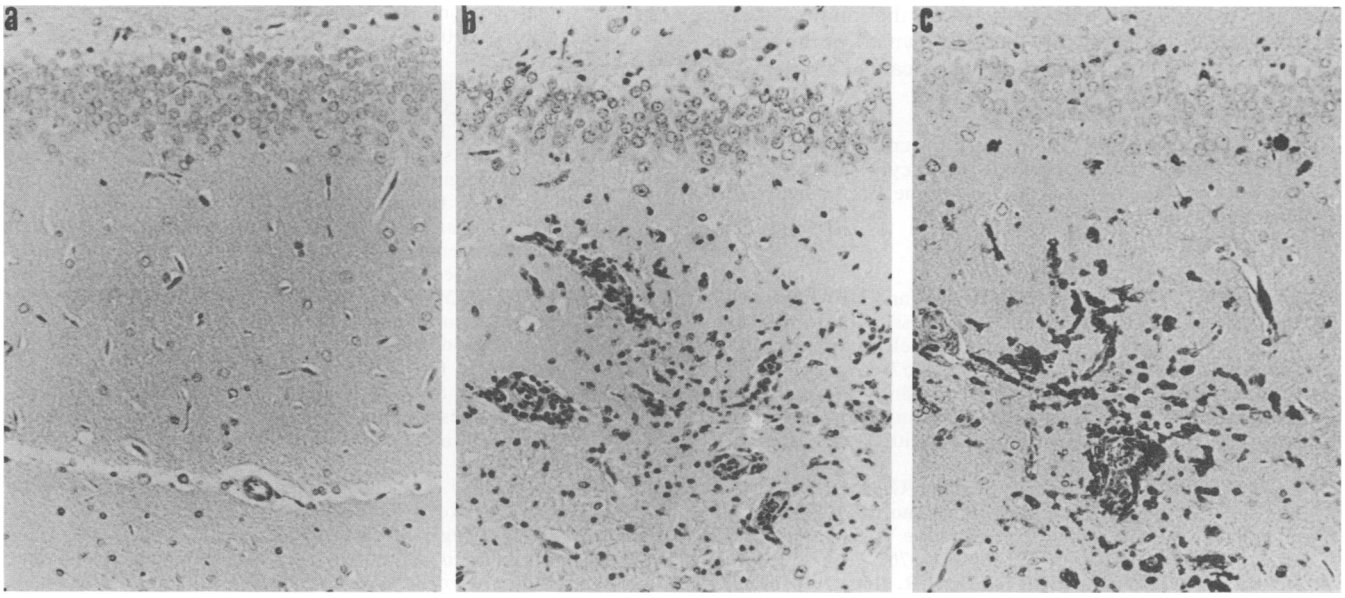

FIG. 3. Brain section from the hippocampus of CSA-treated (a) or untreated (b and c) BDV-infected Lewis rats. CSA-treated rats exhibit no histological lesions (a), whereas extensive perivascular and parenchymal mononuclear infiltrations (b) and MHC class II antigen (c) are present in untreated BDV-infected rats. (a and b) Hematoxylin-eosin stain; (c) MHC class II expression with MAb Ox-6 (PAP technique).

significant MHC class II expression, whereas CSA-treated rats lack this reaction. If an altered pathogenesis of viruses (10) is a general feature after CSA treatment, the described phenomenon may have different consequences in vivo. On the one hand, CSA treatment could contribute to a spread of (persistent) virus, which might lead to an unpredictable immune response at atypical sites of virus replication, thereby possibly promoting a disseminated immunological disease. On the other hand, distribution of the virus throughout the body may prevent the immune-mediated disease by dispersing potential effector cells. This could lead to local immune responses at multiple sites, causing a relative deficit at the pathogenetically critical site.

The results presented here suggest the need for further investigations of the effects of CSA on the biological properties of microorganisms involved in persistent or chronic

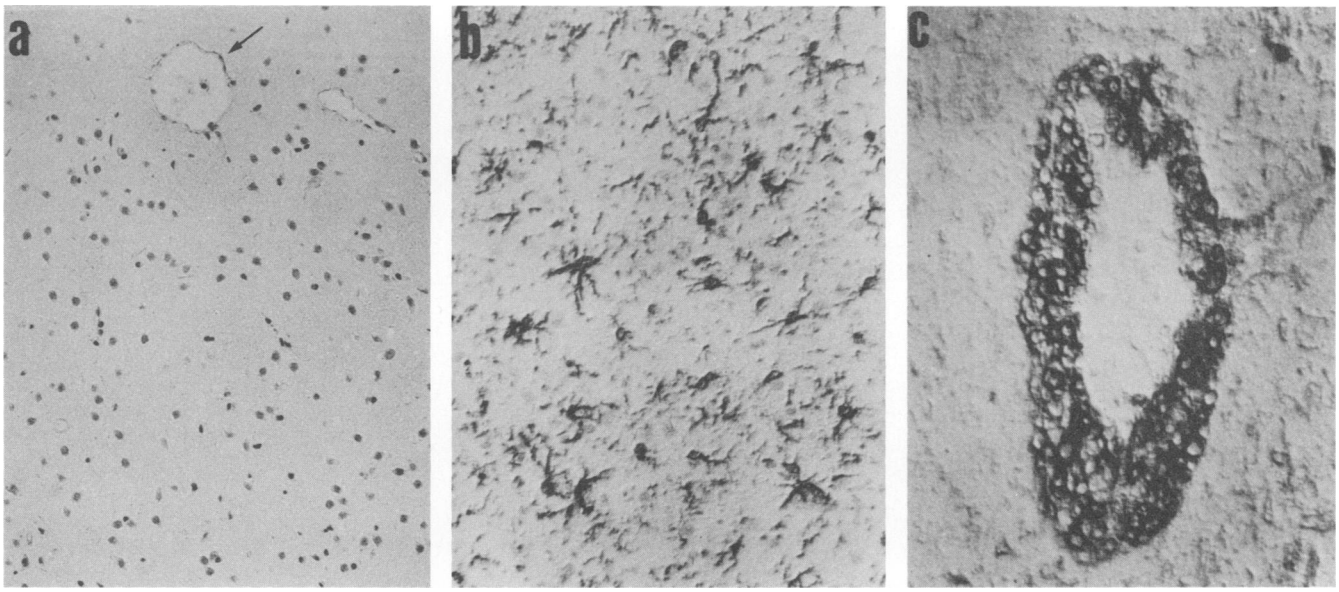

FIG. 4. MHC class II expression in the brain of CSA-treated (a) and untreated (b and c) BDV-infected rats. CSA-treated rats (a) exhibit no MHC class II expression either in the parenchyma or perivascularly (arrow); nuclei were counterstained with hematoxylin. In sections of the same area (thalamus) of untreated BDV-infected rats (b), a strong reaction is found in multiple stellate cells, presumably activated microglia. Panel c shows massive perivascular MHC class II expression of untreated BDV-infected rats. All sections were incubated with MAb Ox-6 and treated by the PAP technique.

infections for which a balance between the immune system and the infectious agent has been shown to be important. Furthermore, our results may be significant with respect to the clinical use of CSA.

We thank R. Rott and R. Zinkernagel for critical comments and J.-F. Borel, Sandoz, for his generous gift of cyclosporine A.

This work was supported by the Deutsche Forschungsgemeinschaft (Ro 202/7-1).

REFERENCES

1. **Bunjes, D. C., C. Hardt, M. Röllinghof, and H. Wagner.** 1981. Cyclosporine A mediates immunosuppression of primary cytotoxic T cell responses by impairing the release of interleukin 1 and interleukin 2. Eur. J. Immunol. **11**:657–661.
2. **Carbone, K. M., B. D. Trapp, J. W. Griffin, C. S. Duchala, and O. Narayan.** 1989. Astrocytes and Schwann cells are virus-host cells in the nervous system of rats with Borna disease. J. Neuropathol. Exp. Neurol. **48**:631–644.
3. **Espevik, T., I. S. Figori, M. R. Shalaby, G. A. Lackides, G. D. Lewis, H. M. Shepard, and M. A. Palladino, Jr.** 1987. Inhibition of cytokine production by Cyclosporine A and transforming growth factor β. J. Exp. Med. **166**:571–576.
4. **Herzog, S., C. Kompter, K. Frese, and R. Rott.** 1984. Replication of Borna disease virus in rats: age dependent differences in tissue distribution. Med. Microbiol. Immunol. **173**:171–177.
5. **Herzog, S., and R. Rott.** 1980. Replication of Borna disease virus. Med. Microb. Immunol. **168**:153–158.
6. **Hirano, N., M. Kao, and H. Ludwig.** 1983. Persistent, tolerant or subacute infection in Borna disease virus infected rats. J. Gen. Virol. **64**:1521–1530.
7. **Kao, M., H. Ludwig, and G. Gosztonyi.** 1984. Adaptation of Borna disease virus to the mouse. J. Gen. Virol. **65**:1845–1849.
8. **Ludwig, H., L. Bode, and G. Gosztonyi.** 1988. Borna disease. A persistent virus infection of the central nervous system. Prog. Med. Virol. **35**:107–151.
9. **Ludwig, H., V. Koester, G. Pauli, and R. Rott.** 1977. The cerebrospinal fluid of rabbits infected with Borna disease virus. Arch. Virol. **55**:209–213.
10. **Markham, D. F., B. P. Griffith, E. Lerner, H. L. Lucia, and F. J. Bia.** 1987. Effects of Cyclosporine on chronic cytomegalovirus infection in the guinea pig. Intervirology **28**:171–180.
11. **Narayan, O., S. Herzog, K. Frese, H. Scheefers, and R. Rott.** 1983. Pathogenesis of Borna disease in rats: immune-mediated viral ophthalmoencephalopathy causing blindness and behavioral abnormalities. J. Infect. Dis. **148**:305–315.
12. **Palacios, R., D. Martinez-Maza, and M. De Ley.** 1983. Production of human interferon (huIFN-j) studied at the single cell level: origin, evidence for spontaneous secretion and effect of Cyclosporine A. Eur. J. Immunol. **13**:221–225.
13. **Richt, J. A., L. Stitz, H. Wekerle, and R. Rott.** 1989. Borna disease, a progressive meningoencephalitis as a model of CD4′ T cell-mediated immunopathology in the brain. J. Exp. Med. **170**:1045–1050.
14. **Shevach, E.** 1985. The effect of Cyclosporine A on the immune system. Annu. Rev. Immunol. **3**:397–423.
15. **Stitz, L., H. Hengartner, A. Althage, and R. Zinkernagel.** 1988. An easy and rapid method to screen large numbers of antibodies against internal cellular determinants. J. Immunol. Methods **106**:211–216.
16. **Stitz, L., H. Krey, and H. Ludwig.** 1980. Borna disease in rhesus monkeys as a model of uveo-cerebral symptoms. J. Med. Virol. **6**:333–340.
17. **Stitz, L., D. Soeder, U. Deschl, K. Frese, and R. Rott.** 1989. Inhibition of immune-mediated meningoencephalitis in persistently Borna disease virus infected rats by Cyclosporine A. J. Immunol. **143**:4250–4256.
18. **Waelchli, R. O., F. Ehrensperger, A. Metzler, and C. Winder.** 1985. Borna disease in sheep. Vet. Rec. **117**:499–507.