

## Presence of CD4+ and CD8+ T Cells and Expression of MHC Class I and MHC Class II Antigen in Horses with Borna Disease Virus-Induced Encephalitis

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Tissues from 9 horses and 1 donkey suffering from natural Borna disease were investigated immunomorphologically. Lymphocytic inflammatory reactions and increased expressions of MHC class I and class II antigen were found in the brain as well as in the trigeminal and olfactory system. Perivascular inflammatory infiltrates were dominated by CD4+ T cells, whereas the majority of CD8+ T cells were disseminated intraparenchymally. No evidence of inflammation was found in the retina. Borna disease virus proteins and nucleic acids were present in the hippocampus, thalamus and medulla oblongata in all 10 animals, in the cerebral cortex, retina, trigeminal ganglion and nerve in 9, in the olfactory epithelium in 6 and in roots and proximal parts of large peripheral nerves in 3. No evidence of infection was found in the autonomic nervous system, lung, heart, liver, kidney or gut. BDV- proteins and nucleic acids were even more abundant in the trigeminal system than in the olfactory system, suggesting that infection may have occurred via the trigeminal nerve.

### Introduction

Borna Disease (BD) is a meningoencephalomyelitis due to infection with Borna disease virus (BDV; 30), a highly neurotropic nonsegmented negative-strand

RNA virus (5, 7, 8, 26). Natural infection has been found in horses, sheep (reviewed in 15, 24), and cattle (3, 6) and possibly cats (17) and birds (18). Furthermore, evidence has been provided that BDV might be a human pathogen (4, 13, 25). A variety of species ranging from birds to primates can be experimentally infected but the most commonly used species for experimental infection is the rat. In the rat, the manifestations of infection can vary from subclinical disease with only subtle disturbances in cognitive function to severe disturbances of movement and behavior (9, 11, 20). Critical factors in disease include host strain, host age, route of infection and virus strain. The pathogenesis of severe BD is based on a T-cell-mediated immune reaction in which both CD4+ and CD8+ T cells participate (reviewed in 27, 28). BD in horses is characterized by deafness, hypo- and hyperkinesia, hyperaesthesia, excitation, colics, and finally blindness and paralysis (16). Similar to infected immunocompetent rats, infected horses have a severe meningoencephalitis with marked pathology in the limbic system and the cerebral cortex. Inflammation is dominated by lymphocytes and by microglia, whereas plasma cells are less prominent (10).

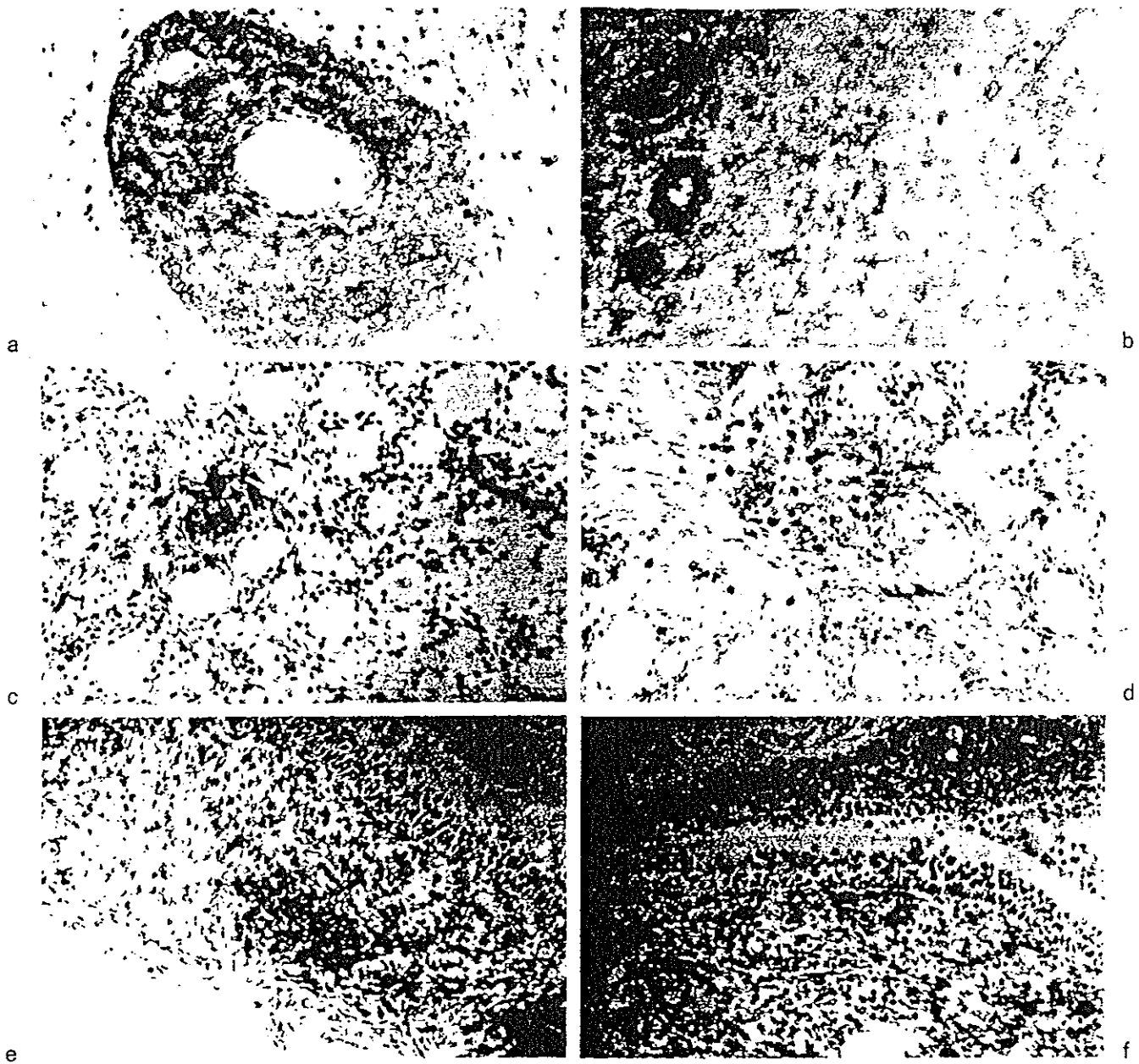
In this study, we characterize inflammatory cell infiltrates and cellular expression of MHC class I and class II antigens in brains of 9 naturally infected horses and 1 donkey in an effort to compare the immunopathology of BD in the natural host with the experimental model of BD in the Lewis rat.

### Materials and Methods

**Animals and tissue preparation.** Within a two year period, 5 male horses, 4 female horses and one male donkey suffering from severe BD were euthanized for the reason of animal welfare. All animals were from an endemic zone for BD southeast of Munich, Germany. The horses ranged in age from 3 to 16 years; the donkey was seven years old. Samples collected from brain (Tab. 1), lung, heart, liver, kidney and gut were either frozen at -150°C in isopentane or fixed in 4% buffered paraformaldehyde. Two horses with traumatic spinal ataxia were used as a negative control.

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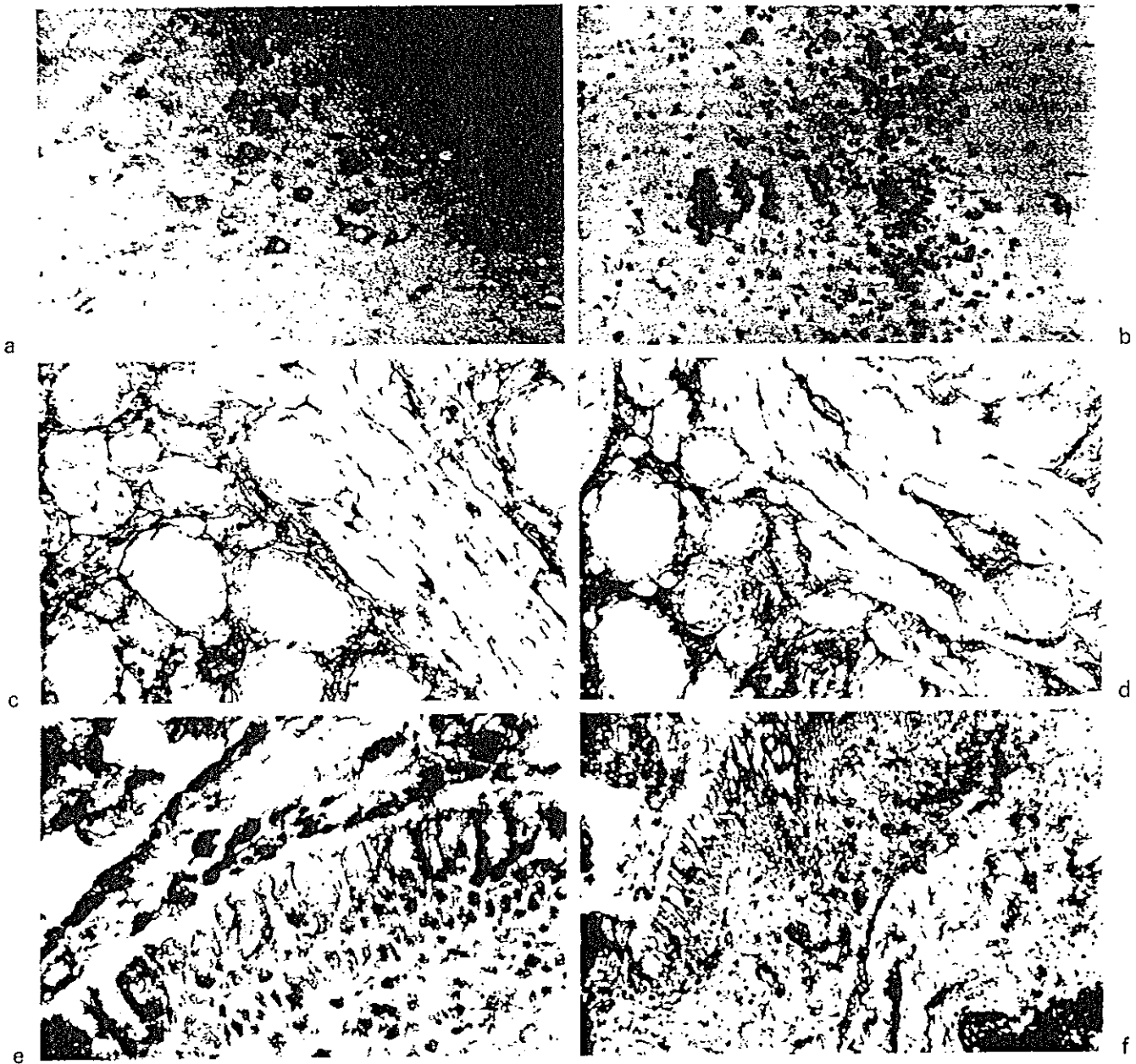


**Figure 2** CD 4 + and CD8+ T cells in natural Borna disease of horses. Severe lymphocytic infiltrations of the hippocampus (a, b), the trigeminal ganglion (GASSERI, c, d) and olfactory mucosa (e, f) of animal no. 8. CD4+ T cells were more concentrated perivascularly or focal (a, c, e), CD8+ T cells were often found dispersely spread within the parenchyma (b, d, f). a-f: x150

2a); 20 to 30% were CD8+, many of them disseminated within the neuropil (Fig. 2b); some did not react with either mAb HB61A (CD4+), or mAb HT14A (CD8+). Cells of the macrophage/monocyte type and many "stellate cells", presumably activated microglia, were identified morphologically; MHC class I and MHC class II antigens were found on inflammatory cells, but also on blood vessels, ependymal cells and on brain cells (Fig. 3 a, b). As described (1,2, 22), it can be difficult to identify individual MHC-expressing cells within the neuropil on cryostat sections. There was often no clear correlation between the

extent of infection and intensity of inflammation (see Fig. 1). For example, medulla oblongata, and retina had strong expression of virus antigens and nucleic acids yet only minimal or no evidence of inflammation.

**Inflammation outside the brain.** Moderate to strong lymphocytic inflammatory reactions were found in all animals with infection in the trigeminal nerve and ganglion (Fig. 2c, d), and in the olfactory epithelium (Fig. 2e, f; Tab. 2). MHC class I and MHC class II antigens were found in neural structures outside the brain such as Schwann cells of the



**Figure 3** Expression of MHC class I and class II antigen in natural Borna disease of horses. Presence of MHC class I (a, c, e) and MHC class II (b, d, f) antigen is shown in the brain (a, b), trigeminal ganglion (c, d) and olfactory mucosa (e, f) of animal no. 9. Although it can be difficult to determine individual cells that express MHC molecules - especially in the brain - , it can be seen that they are widespread in all three locations and are present on parenchymal cells. Large ganglion cells are negative (c, d), olfactory epithelial cells positive (e, f). Moderate lymphocytic infiltration of the olfactory mucosa can be seen in f. a, b: x60; c, d, f: x180; e: x360.

trigeminal nerve, satellite cells in the trigeminal ganglion (Fig. 3c, d) and olfactory epithelial cells (Fig. 3e, f). No inflammation (Fig. 1f) and only weak sporadic expression of MHC class I and class II antigen were found in the 9 animals with infected retinae (Tab. 1 and 2). This finding is of special interest, since blindness is one of the main clinical findings in the final stage of BD in horses.

#### Discussion

Detailed studies on the pathogenesis of the

progressive encephalopathy induced after infection with BDV have previously been conducted only in experimentally infected rats. The objective of this study was to determine the extent to which the T cell-mediated immunopathological reaction in rats represented BD in naturally infected horses. We studied 9 horses and 1 donkey to determine the distribution of virus, viral antigens, viral nucleic acids and analysed inflammatory cell infiltrates and MHC antigen expression in neural tissues. Immunohistochemistry with mAbs directed against

**Table 2** Inflammatory reactions and expression of MHC class I molecules in horses suffering from natural BD

Location	Animal number												Inflammation	MHC I			
	1	2	3	4	5	6	7	8	9	10	11	12					
Cerebral cortex	+++*	++ +**	+++ +	- +	++ -	+++ +	+ +	+++ +	++ +	+	+	-	-	+	-	+	+
Thalamus (incl. Thal. opticus)	+ +	+ +	++ +	+ +	+ +	++ +	+ +	++ +	+ +	+	+	-	-	-	-	-	-
Hippocampus	+++	+++ +	+++ +	+ +	+++ +	+++ +	++ +	+++ +	+++ +	++ +	+	-	-	+	-	+	+
Medulla oblongata	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	++ +	+ +	+	-	-	+	-	+	+
Retina	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
Trigeminal nerve	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	- +	- +	- +	- +	- +	- +	- +	- +
Ganglion Gasseri	++ +	++ +	++ +	++ +	++ +	++ +	+ +	++ +	++ +	- +	- +	- +	- +	- +	- +	- +	- +
Olfactory epithelium	+ +	+ +	+ +	- +	- -	- -	+ -	+ +	+ +	- +	- +	- +	- +	- +	- +	- +	- +

\*+ - +++: degree of inflammation, score see "Materials and methods"  
 \*\*+ or -: presence or absence of MHC class I antigen on brain cells

the putative M protein (gp18), P protein (p24) and N protein (p38/39) of BDV revealed the presence of viral proteins in the brains of all 10 animals. The distribution of viral proteins was similar to that found by Gosztonyi and Ludwig (10) using polyclonal antibodies. BDV antigen was preferentially expressed in neurons and astrocytes of the medial neocortex, allocortex including hippocampus formation and the basal ganglia, but also in the optic, trigeminal and olfactory systems. However, the distribution of virus-specific antigen showed some heterologies: M protein could be found in all 10 animals in most of the neural tissues and locations investigated, whereas N and P proteins were less expressed. Similarly, nucleic acid could not always be demonstrated in all locations tested. Interestingly, apart from locations where both virus-specific antigen and nucleic acid were present or absent, some patterns of mixed reactions were found. The presence of antigen in the absence of RNA might be due to the persistence of antigen after virus clearance. Alternatively, it is conceivable that viral antigen is specifically transported within the central nervous system; however, there is no present evidence to support this possibility. In addition, we also found some examples where nucleic acid was demonstrated but antigen was lacking. This finding indicates that either the genetic information is not translated properly in some parts of the brain or that the amount of antigen present was below the

threshold for detection by the methods used. The different patterns observed might also indicate that the virus spreads within the nervous system of naturally infected horses by different routes. Finally, we cannot exclude the possibility that the mAbs used did not recognize low levels of wild-type BDV-strains. However, analysis of coding sequences for the p24 (P) protein and the p38/40 (N) protein from field and experimental isolates of BDV revealed a degree of sequence conservation unusual for negative-strand RNA-viruses (26).

The character of the immunopathological reactions in the brains of these naturally infected animals is similar to that found in experimentally infected Lewis rats. In both equine species and rats, CD4+ and CD8+ T cells as well as macrophages/microglia are involved in the encephalitic reaction. Furthermore, MHC class I antigen was expressed in brains of all animals at high levels. This finding supports the hypothesis that CD8+ cells and the presence of MHC class I antigen on brain cells including neurons play a role in the pathogenesis of BD (2, 22, 23, 29, 31). Interestingly, there was no simple correlation between extent of infection and the degree of inflammation in infected equines. We detected large concentrations of BDV nucleic acids and antigen in the retina, but no sign of inflammation. In contrast, Lewis rats develop severe retinitis within 4 weeks of intracerebral infection, approximately 2 weeks after the appearance of cerebral dysfunction. The retina

becomes atrophic, presumably as a consequence of a vigorous immunopathological reaction in the eye (21). The absence of MHC antigen expression in the equine retina may play a role in downregulating inflammation. Since there is also no sign of a progressive retinal degeneration, the "blindness" regarded as one of the cardinal features of terminal BD in horses cannot be explained by severe inflammation or retinal destruction. It is possible that the loss of vision is due to the central inflammatory alterations e.g. in the thalamus opticus rather than to a retinopathy. Another difference between natural BD in equines and experimental BD in rats, is the minor evidence of neurodegeneration in the former. This may simply reflect the fact that naturally infected horses are euthanized early after diagnosis, at a stage in which neurodegeneration may not have occurred. To resolve this question it will be necessary to investigate horses in a late stage of BD. The finding that virus-specific antigen and information was present in the trigeminal ganglion and nerve of almost all infected animals might indicate the trigeminal nerve as a natural route of infection. It can be speculated that microlesions in the oral or nasopharyngeal mucosa allow the entry of the agent and access to the brain via the trigeminal system.

The results of the present study confirm the findings of experimental BD and underline the importance of the Lewis rat as a model of natural disease in various animals and probably in man.

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