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Immunohistological detection of bovine viral diarrhoea virus antigen in the central nervous system of persistently infected cattle using monoclonal antibodies

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ABSTRACT

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In a total of 25 cattle persistently infected with bovine viral diarrhoea virus (BVDV) the distribution of viral antigens in the central nervous system was studied. Using a panel of monoclonal antibodies (anti pestivirus C16; anti cytophathic BVDV C38; anti cytopathic and non-cytopathic BVDV C42; anti gp53 BVDV CA-1 and CA-3) and the indirect immunoperoxidase technique, BVDV antigen was located exclusively in neurons. Predilection sites for viral persistence were cerebral cortex and hippocampus. Morphological cellular alterations were not seen. Reactive perivascular lymphocytic infiltrations were occasional findings.

INTRODUCTION

Persistent infection with bovine viral diarrhoea virus (BVDV) may occur if the bovine fetus is transplacentally infected with non-cytopathic BVDV at a time when it is still immunologically immature (Coria and McClurkin, 1978; Liess et al., 1984; McClurkin et al., 1984; Roeder et al., 1986). Fetal infection during the first 4 months of gestation results in immunological tolerance to the homologous virus. Cattle persistently infected with BVDV are permanently viraemic and lack detectable levels of neutralizing antibodies. BVDV has a marked tropism for lymphoid and certain epithelial tissues, especially the mucosae of the digestive tract (Bielefeldt Ohmann, 1982; Baker, 1987; Bielefeldt Ohmann et al., 1987). However, in cattle persistently infected with

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non-cytopathic BVDV, established by fetal infection during the first 4 months of gestation, BVDV may replicate in certain non-lymphoid and non-epithelial tissues including the central nervous system (CNS) (Meyling, 1970; Cutlip et al., 1980; Liess et al., 1983, 1987). So far, only polyclonal antisera have been used for the immunocytochemical detection of BVD viral antigen in tissues of cattle persistently infected (Bielefeldt Ohmann, 1988; Fernandez et al., 1989). In the present study the distribution of viral antigens in the CNS of cattle with persistent BVDV infection was examined applying immunoperoxidase techniques and a panel of monoclonal antibodies.

MATERIAL AND METHODS

Cattle

A total of 25 Holstein-Friesian cattle persistently infected with BVDV were examined. All animals were field cases. At necropsy, they were between 4 and 36 months old, with a mean of 20 months. Over the life-span the animals were viraemic; that is ncp BVDV was repeatedly demonstrated on peripheral blood leucocytes. Serologically, all animals remained negative for neutralizing antiviral antibodies. Virological techniques used have been described elsewhere (Orban et al., 1983).

Tissue samples

At necropsy, tissue samples of a total of 18 locations of the brain and spinal cord, trigeminal ganglia, pituitary gland, and eyes were collected and processed. For immunoperoxidase techniques cryostat and paraffin sections of ethanol and formalin-fixed tissues were used. Processing of tissues has been described previously (Fernandez et al., 1989). Hematoxylin and eosin (HE) stains were prepared from formalin-fixed tissues.

Antibodies and immunohistological methods

The specificities of monoclonal antibodies (MAbs) used for the detection of BVD viral antigens are depicted in Table 1. Production and in vitro reactivity of MAbs with different BVDV and hog cholera virus (HCV) strains have been reported elsewhere (Peters et al., 1986; Moennig et al., 1987).

BVD viral antigens were detected by the indirect immunoperoxidase technique using MAbs and and a peroxidase-conjugated rabbit anti-mouse IgG (Dakopatts, Glostrup, Denmark) in optimal working dilutions determined by titration. Cryostat sections were fixed in acetone (5 min at RT). Paraffin sections were deparaffinized. Tissues that had been fixed in formalin for several days were pretreated for 60 min with 0.25% trypsin (Fluka Co., Buchs, Switzerland), pH 7.6, at 37°C. Endogenous peroxidase activity was blocked with methanol–H_2O_2. After three washes with tris buffered saline (TBS) pH 7.6, sections were preincubated with normal swine serum for 20 min at RT.

TABLE 1

Specificities of monoclonal antibodies used for the detection of BVDV antigen

Designation	Specificity	Virus-specific polypeptide[1]
C 16	Pestivirus-specific (BVDV and HCV strains)	(p125/p80)
C 38	cp BVDV strains	(p125/p80)
C 42	cp and ncp BVDV strains	(p125/p80)
CA-1 CA-3	major glycoprotein of BVDV	(gp53)
HC 34	HCV glycoprotein	(gp53)

[1]According to nomenclature proposed by Collett et al. (1989).
BVDV = bovine viral diarrhoea virus; HCV = hog cholera virus; cp = cytophatic; ncp = non-cytopathic.

Sections were incubated with MAbs for 45 min at 37 °C, washed and exposed to the peroxidase conjugated antiserum for 60 min at RT. The peroxidase activity was visualized using a standard solution of 3,3-diaminobenzidine (DAB) substrate. After rinsing the slides were counterstained with hematoxylin and mounted.

RESULTS

The distribution of BVD viral antigens in 18 different locations of the central nervous system (CNS) was studied using the indirect immunoperoxidase technique on cryostat and paraffin tissue sections.

In 24 of 25 cattle examined viral antigen was exclusively detectable in neurons. Immunoperoxidase staining of antigen in neurons was seen either in a limited area in the cytoplasm or in the entire perikaryon, while the nucleus was always negative. Viral antigen was also detected in dendrites and axons of nerve cells. In the leptomeninges, and in other cellular elements such as astrocytes, oligodendrocytes, microglial cells or vascular cells viral antigen was never seen. In one case, however, the ependymal cells lining the fourth ventricle were antigen-positive. Predilection sites for BVD viral antigen specific staining are the cerebral cortex and the hippocampus. In both locations about 90% of neurons of the different cell layers were positive (Fig. 1, 2). In other areas such as diencephalon (thalamus, hypothalamus), mesencephalon (corpus quadrigeminus, nucleus ruber and niger), rhombencephalon (pons, medulla oblongata), cerebellum, and spinal cord BVD viral antigen was detectable only in single neurons or in small groups of neurons (Fig. 3, 4). In

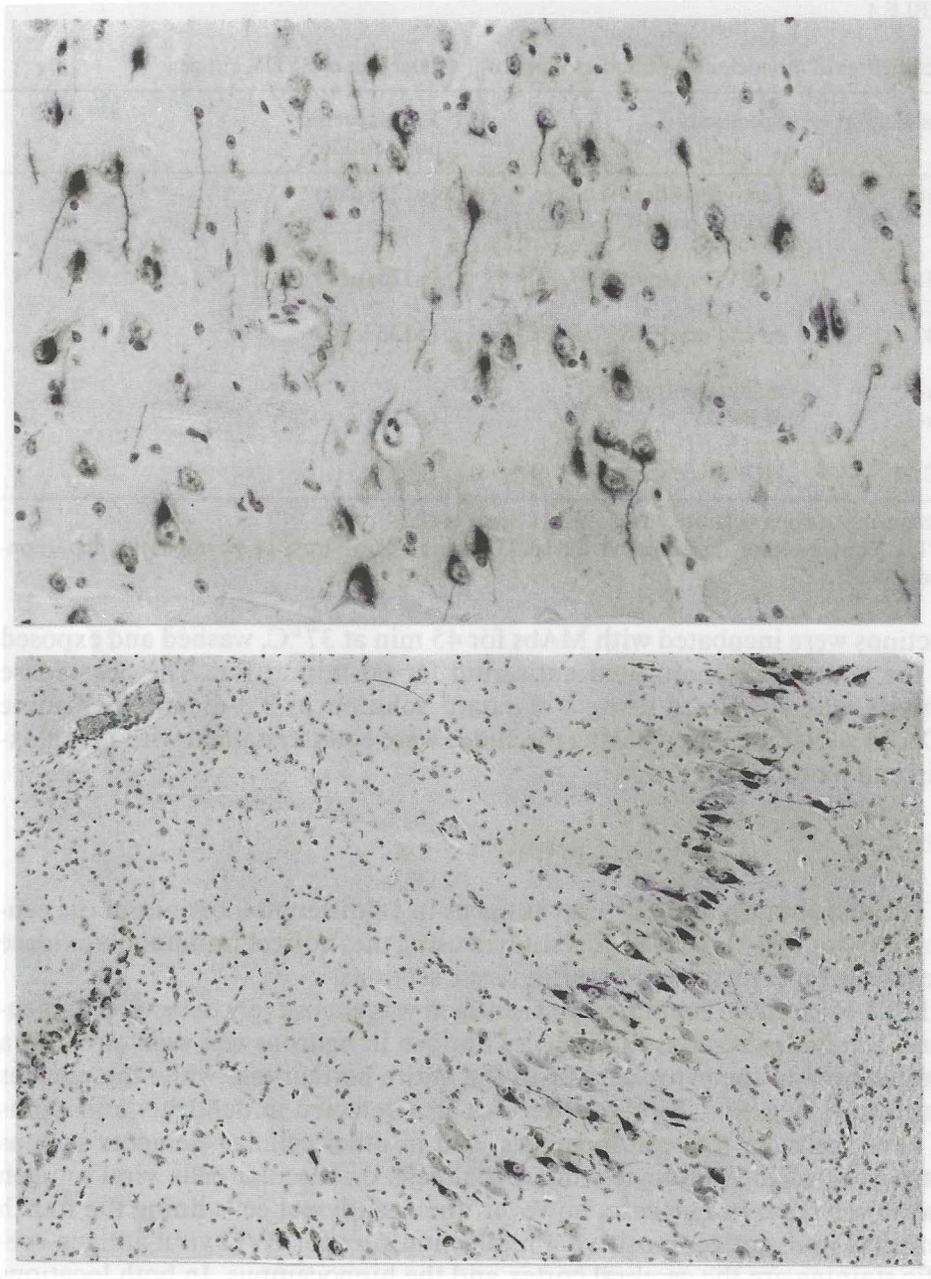

Fig. 1. Cerebral cortex, detection of cytophathic BVD viral antigen (MAb C38). Antigen-specific staining of perikaryon and cellular processes of neurons (16.705).

Fig. 2. Hippocampus, antigen-specific staining of neurons of polymorphous cell layer (16.535). MAb C42 detects cytopathic and non-cytophathic BVD viral antigen.

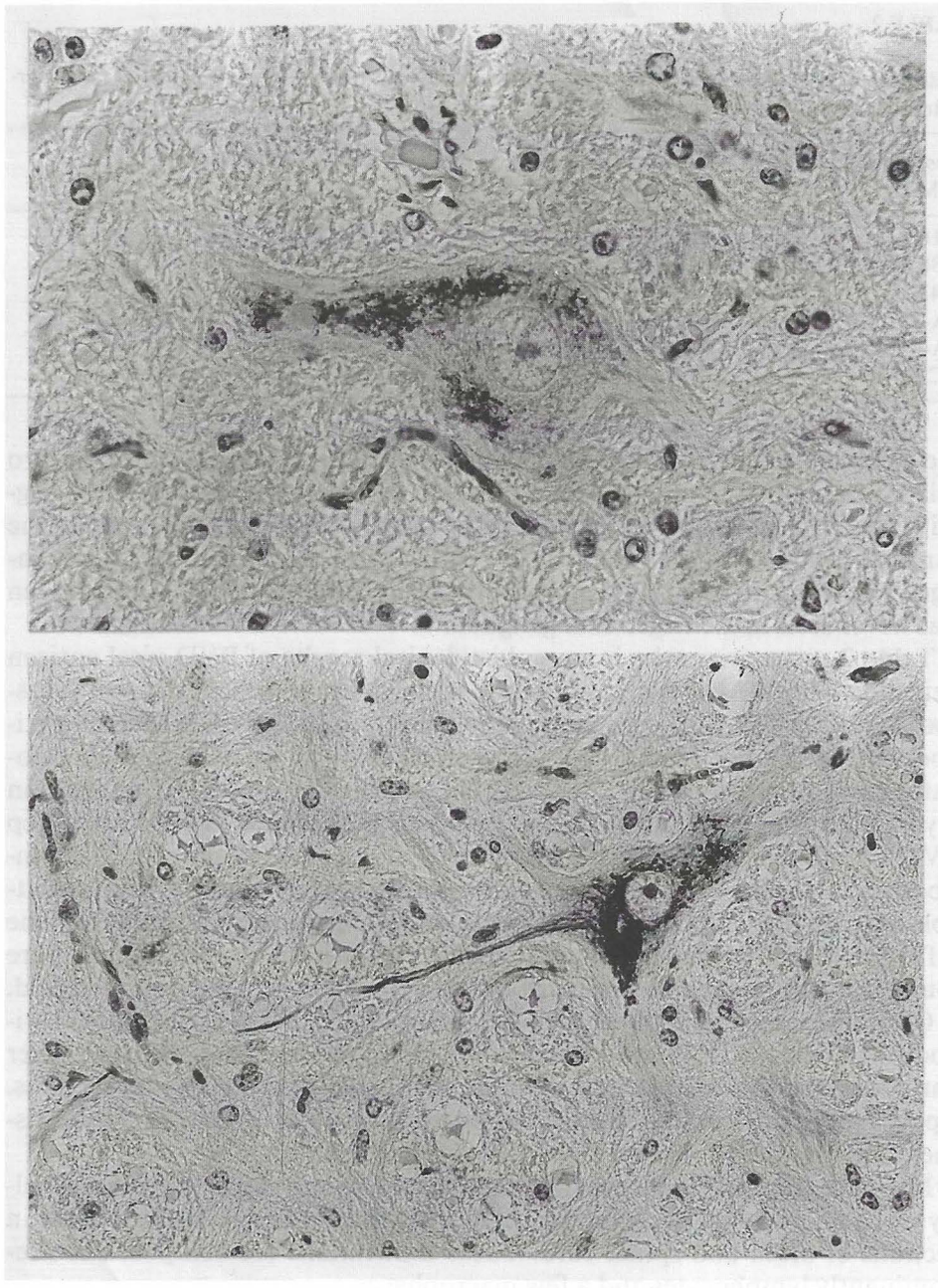

Fig. 3. Rhombencephalon (pons), antigen-specific staining of perikaryon of a single large neuron (16.515). MAb C42.

Fig. 4. Rhombencephalon (medulla), BVD viral antigen in perikaryon and cellular processes of a large neuron (16.522). MAb C42.

TABLE 2

Detection of BVDV antigen in cryostat sections of the central nervous system of 17 cattle persistently infected with MAbs

Designation of MAb	Result	No. of cases (positive/studied)
C 16	+	(17/17)
C 38	+	(15/17)
C 42	+	(17/17)
CA-1	+	(10/17)
CA-3	+	(12/17)
HC 34	–	(17/17)

the cerebellum only the large and small Golgi cells and the so-called Lugaro cells contained viral antigen, while Purkinje cells and granule cells were negative in all cases studied. In the eyes single positive neurons were found in the ganglion cell layer of the retina. In one of the cases examined, BVD viral antigen was found in cells of the adenohypophysis of the pituitary. Viral antigen was never seen in the trigeminal ganglion.

Table 2 summarizes the immunohistological results of BVD viral antigen detection with MAbs on cryostat sections. In all cases studied the pestivirus-specific MAb C16 showed a positive reaction, while the HCV-specific antibody HC34 did not react. MAb C42, which in vitro reacts with both cytopathic and non-cytopathic (ncp) BVDV strains detected viral antigen on cryostat sections of all cases studied. Antibody C38, which in vitro detects cp BVDV strains, reacted positive in the majority of cases examined. With antibodies CA-1 and CA-3 the major glycoprotein (gp53) of BVDV was detectable in more than 50% of cases studied. Comparing the staining pattern of the different MAbs within the tissue locations examined, no differences were found concerning the cellular distribution of the BVD viral antigens detected.

On paraffin sections of ethanol-fixed tissues the pestivirus-specific antibody C16, and the HCV-specific antibody HC34 did not react, whilst all other antibodies used showed antigen-specific staining as seen on cryostat sections. Applying the different antibodies on paraffin sections of formalin-fixed tissues, only MAb C42 showed a positive staining reaction.

Morphological tissue lesions were not seen. The only significant abnormality was an occasional perivascular cellular infiltration in HE-stained paraffin sections of brain and spinal cord. These infiltrations are composed of predominantly lymphocytes and a few macrophages.

DISCUSSION

The presence of BVD viral antigens in the CNS of persistently infected cattle was demonstrated by applying MAbs with specific in vitro reactivities and immunoperoxidase techniques on tissue sections. The reactivity of both MAbs C42 and C38, suggests that nerve cells of persistently infected young and adult

animals are infected with cytopathic (cp) and/or non-cytopathic (ncp) BVD viruses. However, these results must be interpreted with caution, since the specific reaction pattern of the antibodies with the BVD viral biotypes was established in vitro using cultured bovine cells. Whether it corresponds to the expression of cp and ncp BVD virus in vivo is not yet clear. With MAbs CA-1 and CA-3 the major glycoprotein (gp53) of BVDV was demonstrable in more than 50% but not in all cases studied. This corresponds to their specificities found in vitro (Moennig et al., 1987) where not all BVDV strains are detectable by these antibodies. Further similarities between the in vitro specificities and the staining reaction on tissue sections were found for MAbs C16 and HC34. The pestivirus-specific antibody C16 showed a positive immunoperoxidase staining reaction, whilst the MAb against HCV never reacted. The finding that on paraffin sections of formalin-fixed tissues BVD viral antigens were only detectable with MAb C42 can probably be attributed to the denaturation of viral epitopes during the process of tissue fixation with formalin. This corresponds to in vitro results on BVDV-infected cultured cells (Mateo, 1988). There is no explanation, however, why with MAb C16 on paraffin sections of ethanol-fixed tissues viral antigen was not detectable, whilst this was possible with all other BVDV-specific antibodies tested.

Comparing the immunohistological results of this study with previous results obtained with a polyclonal antiserum (Fernandez et al., 1989), no obvious differences were found concerning the distribution of BVD viral antigen in the CNS of persistently infected cattle. In 24 of 25 cases studied viral antigen was exclusively seen in neurons and their cellular processes, while positive staining reactions were never found in other cellular elements. Recently, a single receptor specific for BVDV could be identified on the surface of cultured bovine cells (Moennig et al., 1988). Our results may indicate that neurons in the CNS have a specific receptor for BVDV on their cytoplasmic membranes. In vitro experiments to detect such a virus-specific receptor on cultured bovine brain cells are under way. It appears that BVDV infection of neurons does not cause obvious morphological alterations, and that only weak focal immune reactions due to the long persistence of BVDV in the CNS eventually occur.

Our study shows that antigens of both cp and ncp biotypes of BVDV seem to persist in neurons of cattle persistently infected. The possible pathogenic relevance of this finding with respect to the development of clinical fatal mucosal disease, however, is not understood.

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