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Demonstration of Viral Antigen and Immunoglobulin (IgG and IgM) in Brain Tissue of Pigs Experimentally Infected with Haemagglutinating Encephalomyelitis Virus

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Summary

Haemagglutinating encephalomyelitis virus (HEV) was inoculated either orally or intranasally into ten 3-day-old gnotobiotic piglets. All infected pigs showed inappetence and listlessness, but there were no clinical signs of nervous disorder. Severe encephalomyelitis, characterized by neuronophagia, focal gliosis and perivascular cuffing, was observed in the brain stem and cerebral cortex. Nasally infected pigs, in particular, developed lesions in the area of the stria olfactoria and tractus olfactorius. Coincident with the encephalitic changes, HEV antigen was observed first in the trigeminal ganglion cells and then in degenerating neurones. Immunoglobulin (IgG and IgM)-containing cells were also found in perivascular cuffs and glial foci. They appeared at first on PID 7 and after that increased in number. These findings suggest that these encephalitic lesions are a specific immune response to HEV following its multiplication in the central nervous system.

Introduction

The swine coronavirus, haemagglutinating encephalomyelitis virus (HEV), is recognized as being pathogenic for suckling pigs. The characteristic clinical signs include anorexia, depression and vomiting, often followed by nervous signs (Richards and Savan, 1960; Cutlip and Mengeling, 1972; Pensaert and Callebaut, 1974; Werdin, Sorensen and Stewart, 1976; Andries, Pensaert and Callebaut, 1978). The pathogenesis of HEV has been studied in experimentally infected piglets where it was demonstrated that the clinical signs correlated with viral replication (Meyvisch and Hoorens, 1978; Andries and Pensaert, 1980a, 1980b). However, there is little information on the encephalitic lesions associated with virus replication and subsequent immunological response.

In the present study, the distribution of histopathological lesions, HEV antigen and immunoglobulin (IgG and IgM)-containing cells in the brain was investigated with an immunoperoxidase technique in experimentally infected gnotobiotic piglets.

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Materials and Methods

Virus

Strain 67N of porcine HEV (Mengeling, Boothe and Ritchie, 1972) was kindly provided by Dr K. Hirai, Gifu University, Japan, and passaged four times in hybrid cells (established by the fusion of swine kidney line cells and epithelial cells from the small intestine of gnotobiotic piglets) before being used in the present study. The virus stock had a titre of $10^{5.5}$ plaque forming units per ml.

Infection of piglets

Twelve 3-day-old gnotobiotic piglets were used. They were kept at 30°C in individual metal cages with positive pressure ventilation. The pigs were divided into three groups: five pigs (pigs 1 to 5) were inoculated orally with 2 ml of stock virus; five pigs (pigs 6 to 10) were inoculated nasally with 2 ml of stock virus; and two (pigs 11 and 12) were not inoculated and were used as controls.

All pigs were observed at least three times a day for clinical signs. The infected animals were killed by injection of Nembutal on post-inoculation days (PID) 3, 5, 7, 9 and 11, respectively. The two control pigs were killed on 7 (pig 11) and 14 (pig 12) days of life.

Histopathology

Parts of the brain and spinal cord were fixed in 10 per cent neutral buffered formalin, and after fixation they were sliced transversely at selected sites. Each exposed surface was examined for gross lesions. Representative blocks of the brain and spinal cord (cerebral cortex, frontal pole, motor area, occipital pole, corpus striatum, thalamus, colliculus caudalis, cerebral peduncles, pons, cerebellum, medulla oblongata, cervical spinal cord, thoracic spinal cord, lumbar spinal cord, sacrospinal cord and their ganglia) were embedded in paraffin wax, sectioned and stained with hematoxylin and eosin (HE).

Immunohistology

The avidin–biotin complex (ABC) immunoperoxidase method was used to demonstrate HEV antigen, immunoglobulin (Ig) G and IgM. After deparaffinization, endogenous peroxidase activity was blocked by treatment with 0.3 per cent H_2O_2 in absolute methanol. The sections were then incubated in turn with normal goat serum, primary antibody (anti-HEV, anti-porcine IgG or anti-porcine IgM rabbit sera), biotinylated goat anti-rabbit IgG, ABC reagent and 0.05 per cent 3.3-diaminobenzidine tetrahydrochloride and 0.1 per cent H_2O_2 in Tris buffer, pH 7.6 (DAB-H_2O_2). They were then counterstained with methyl green. The anti-HEV rabbit serum was kindly provided by Dr K. Sato, National Institute of Animal Health, Japan and used as primary antibody at a dilution of 1 in 2048. The anti-porcine IgG and anti-porcine IgM rabbit sera were obtained commercially (Miles Lab., Inc) and used at a dilution of 1 in 2560 and 1 in 160, respectively. The secondary biotinylated antibody and ABC reagent were obtained commercially (Vectastain, Vector Lab.). Tissue sections from the two control uninfected animals and serum from a non-immuninized rabbit were used to control the studies.

Neutralization test

The virus neutralization test employed was a virus dilution plaque technique carried out on monolayers of hybrid cells. Undiluted sera collected from pigs at necropsy were used. The titre was expressed as $\log_{10}$.

Results

Clinical observations

Following an incubation period of 4 to 6 days the piglets appeared inappetent and listless. No vomiting, respiratory distress or nervous signs were observed.

Pathological changes

No macroscopic lesions were observed in any infected pigs. Microscopic lesions were confined to the central nervous system (CNS). Lesions were those of an encephalomyelitis and trigeminal ganglionitis characterized by neuronal degeneration, neuronophagia, focal gliosis and perivascular cuffing by mononuclear cells. No inclusion bodies were found. The distribution of the encephalitic changes differed slightly between the orally and nasally infected pigs (Table 1).

In the orally infected pigs (pigs 1 to 5), cell infiltration and ganglion cell degeneration were found in the trigeminal ganglion in a pig killed on PID 5. After that, encephalitic lesions, composed of neuronophagia, focal gliosis and perivascular cuffing, in the medulla oblongata, pons, midbrain and thalamus were observed. Lesions were most frequently seen in the nucleus tractus spinalis nervi trigemini and nucleus solitarius in the medulla oblongata, the nucleus tractus spinalis nervi trigemini, nucleus motoris nervi trigemini and nucleus dorsalis corporis trapezoidei in the pons and the nucleus tractus mesencephalicus nervi trigemini in the midbrain.

In the nasally infected pigs (pigs 6 to 10), slight trigeminal ganglionitis was also detected in a pig killed on PID 3. After that, the lesions were found in the brain stem and cerebral cortex. The distribution of the lesions in the brain stem closely resembled those seen in the orally infected cases. Moreover, the focal gliosis and perivascular cuffing in the cerebral cortex occurred in the area of the stria olfactoria lateralis and stria olfactoria medialis in the lobus frontalis, the tractus olfactorius in the lobus temporalis and the corpus amygdaloideum in the lobus parietalis.

No macro- or microscopic lesions were found in either of the two control pigs.

Immunohistology

A positive reaction indicating the presence of HEV, IgG or IgM consisted of a characteristic dark brown deposit on the slide.

HEV antigen was present in the cytoplasm of the trigeminal ganglion cells on PID 3 (pig 6) and the neurones on PID 5 (pigs 2 and 7), but not in the nuclei (Figs 1 and 2). After that, the viral antigen was detected in the cytoplasm of degenerating neuronal cells and accumulating neuroglial cells (Figs 3 to 5). The distribution of viral antigen correlated with the neuronal degeneration and focal gliosis (Table 1).

The IgG- and IgM-containing cells were present both in the perivascular cuffs and in the glial foci (Figs 6 to 8). They were detected first in pigs killed on

Table 1.
The distribution of encephalitic lesions and HEV antigen in piglets orally or nasally inoculated with 67N strain

Location	*Oral infection*					*Nasal infection*				*Control*	
Pig No.	1	2	3	4	5	6	7	8	9	10	11	12
PID	3	5	7	9	11	3	5	7	9	11		
Trigeminal ganglion		1+/−*	2+/−					1+/−	2+/−	1+/−		
Medulla oblongata			3+/+	1+/+			1+/+	3+/++	1+/+	1+/−		
Pons			3+/++	2+/−	1+/−		−/+	3+/++	2+/+	1+/−		
Midbrain			3+/++	1+/+	2+/−		1+/++	3+/++	2+/++	2+/−		
Thalamus			1+/−	1+/−	1+/−			2+/++	1+/−	1+/−		
Cerebrum												
Frontalis				1+/−				2+/+	1+/−	1+/−		
Temporalis				1+/+				2+/+	1+/−	1+/−		
Parietalis				2+/+	2+/−			3+/++	1+/−	1+/−		
Occipitalis												
Cerebellum												
Cervical spinal cord			1+/+	1+/+	1+/−			1+/−		1+/−		
Thoracic spinal cord				1+/−				1+/+		1+/−		
Lumbar spinal cord												
Sacrospinal cord												

*Histopathological lesion/HEV antigen by immunoperoxidase.
Degree of lesion: −, negative; 1+, slight; 2+, moderate; 3+, severe.
Granules: +, small number; ++, moderate number.
No entry indicates that neither lesion nor antigen was present.

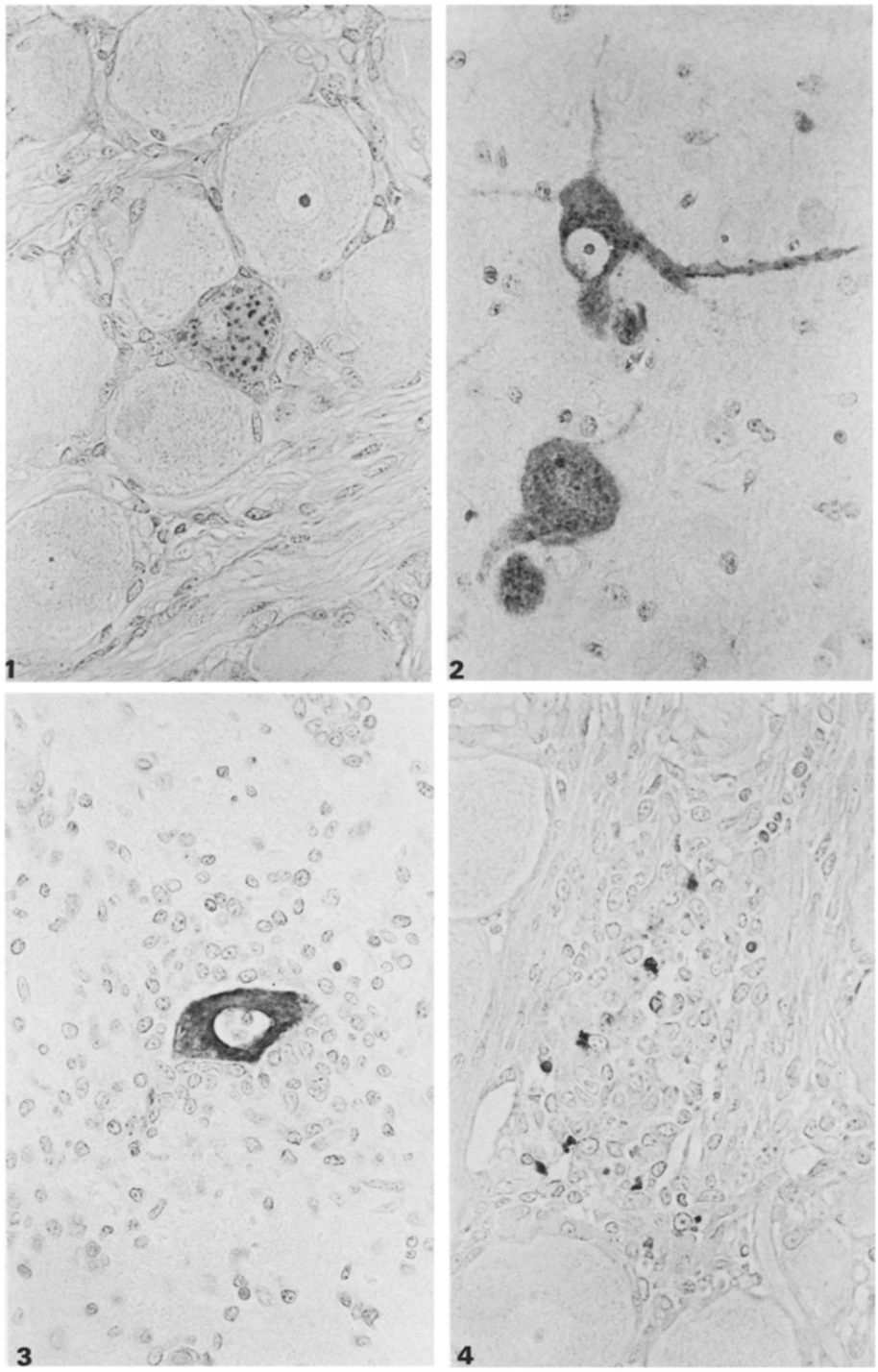

Fig. 1. HEV antigen in a trigeminal ganglion cell of an orally infected pig killed on PID 3. Immunoperoxidase (IP) staining, × 400.

Fig. 2. HEV antigen in neurones in the brain stem of a nasally infected pig killed on PID 5. IP staining, × 400.

Fig. 3. HEV antigen in a degenerating neurone in the brain stem of an orally infected pig killed on PID 7. IP staining, × 400.

Fig. 4. HEV antigen in accumulating neuroglial cells in the trigeminal ganglion of an orally infected pig killed on PID 9. IP staining, × 400.

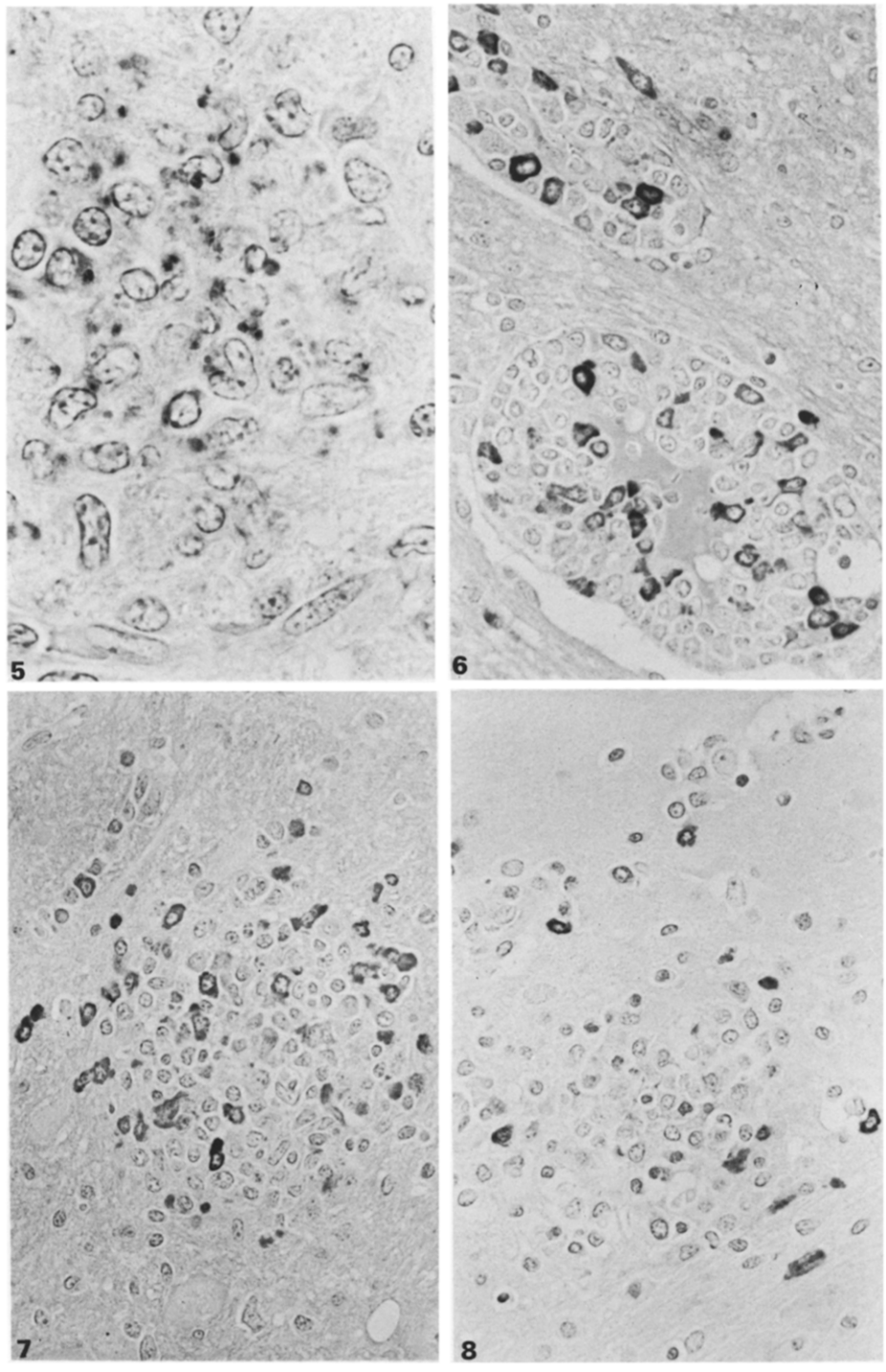

Fig. 5. HEV antigen in accumulating neuroglial cells in the brain stem of an orally infection pig killed on PID 9. IP staining, × 1000.

Fig. 6. IgG containing cells in a perivascular cuff in the brain stem of a nasally infected pig killed on pid 7. IP staining, × 400.

Fig. 7. IgG containing cells in a glial focus in the brain stem of an orally infected pig killed on pid 7. IP staining, × 400.

Fig. 8. IgM containing cells in a glial focus in the brain stem of an orally infected pig killed on pid 7. IP staining, × 400.

Table 2
Immunoglobulin-containing cells in the brain tissue and neutralizing antibody in piglets infected with HEV

Antibody		*Oral infection*					*Nasal infection*					*Control*	
	Pig No.	1	2	3	4	5	6	7	8	9	10	11	12
	PID	3	5	7	9	11	3	5	7	9	11		
IgG		−	−	+ +	+ + +	+ +	−	−	+ +	+ + +	+ +	−	−
IgM		−	−	+	+	+	−	−	+	+	+	−	−
Antibody		0*	1·5	3·3	3·4	3·9	0·1	1·3	3·4	4·0	4·4	0	0

Degree: − = negative; + = small number; + + = moderate number; + + + = large number
* Neutralization log-index.

PID 7 (pigs 3 and 8), after which they increased in number. The number of IgG-containing cells was generally much greater than the number of IgM-containing cells (Table 2).

No positive reaction for HEV, IgG or IgM was detected in the brain tissue of the two control pigs.

Virus neutralization

As shown in Table 2, neutralizing antibody in the serum was first detected on PID 5. No antibody was detected in the pigs killed on PID 3 or in the two uninfected control pigs.

Discussion

Although vomiting did not develop in any of the infected pigs, characteristic encephalomyelitis accompanied by neuronal degeneration, neuronophagia, focal gliosis and perivascular cuffing was observed in the brain stem and cerebral cortex. These findings have also been described in previous reports of experimentally and naturally infected pigs (Richards and Savan, 1960; Cutlip and Mengeling, 1972; Meyvisch and Hoorens, 1978).

Immunohistologically, HEV antigen was demonstrated in the cytoplasm of trigeminal ganglion cells on PID 3, degenerating neuronal cells on PID 5 and then in neuroglial cells associated with lesions. Thus, it is suggested that during the course of the encephalitis HEV multiplied within the neurones.

The pathway of the virus to the CNS from the naso-oral mucosa has been investigated with herpes simplex virus (Goodpasture, 1925; Cook and Stevens, 1973), semliki forest virus (Kaluza, Lell, Reinacher, Stitz and Willems, 1987), infectious bovine rhinotracheitis virus (Narita, Inui, Namba and Shimizu, 1976) and pseudorabies virus (Sabo, Rajcani and Blaskovic, 1968, 1969; Field and Hill, 1974). These viruses appear to migrate along certain peripheral nerves towards the associated peripheral ganglia and to proceed to the corresponding segment of CNS. Andries and Pensaert (1980a, 1980b) demonstrated the neurotropism of HEV in pigs and showed three pathways by which it could reach the CNS. The first was via the trigeminal ganglia, the second was via the inferior vagal ganglia and the third was from the intestinal plexus to the spinal cord. In the present study, lesions in the CNS associated with HEV antigen were observed in the trigeminal ganglion, nucleus spinalis nervi trigemini and nucleus solitarius. Moreover, a characteristic feature following nasal infection was the occurrence of focal gliosis and the presence of viral antigen in the stria olfactoria lateralis, stria olfactoria medialis, tractus olfactorius and corpus amygdaloideum in the cerebral cortex. Thus, it would seem that HEV is able to spread from the nasal and oral mucosae to the CNS via two sensory pathways: the trigeminal nerve and the olfactory nerve.

It is generally accepted that the inflammatory cells appearing in the brain parenchyma and perivascularly during the acute stage of a viral encephalitis are derived from circulating mononuclear leucocytes (Kitamura, 1975; Fujita and Kitamura, 1976; Wolinsky, Jubelt, Burke and Narayan, 1982). Gehard and Koprowski (1977) demonstrated the virus reactive memory B cell, a clone

of antiviral-antibody-producing plasma cells, in the brains of mice injected intracerebrally with the 6/94 strain of para-influenza type 1 virus. In the present study, HEV produced severe perivascular cuffing and focal gliosis, which contained both neuroglial cells and IgG- and IgM-containing cells. This finding indicated that the cells infiltrating the CNS are actually migrating mononuclear leucocytes and lymphocytes and that cells of haematogenous origin also contribute to the focal gliosis. Moreover, the appearance of immunoglobulin-containing cells coincided with the detection of specific neutralizing antibody against HEV. Thus, it seems likely that the immunoglobulin-containing cells represent a specific antiviral immune response.

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