Pathogenesis of Infectious Bovine Rhinotracheitis Virus Infection in Calves

By MINORU NARITA

The Third Research Division, National Institute of Animal Health
(Yatabe, Ibaraki, 305 Japan)

Infectious bovine rhinotracheitis (IBR) virus was first isolated by Miller in 1955 as an agent of a new respiratory disease of feedlot cattle in Western United States.[11] Thereafter, it was recognized as a bovine herpesvirus of world-wide distribution. The virus causes conjunctivitis,[1,8] vulvovaginitis,[9] meningoencephalitis,[2] and abortion[4] in addition to the respiratory disease. Each of the forms of the disease is generally associated with the upper respiratory form of the disease. This disease was brought into Japan with infected cattle imported from USA and Canada in 1970 and now the IBR virus infection spread throughout the country.[18]

Many of herpesviruses have been known to be neurotropic and capable of establishing a persistent infection in their hosts as a sequel to a primary infection.[5,7] Sensory ganglia have been postulated as the persistent sites of virus.[3,10,17,19] IBR virus also has an ability to cause persistent infection, and recrudescence was induced by administration of synthetic corticosteroides, even for years after primary infection.[6] The virus was recovered from the trigeminal ganglion by explant culture. However, relationships between the pathological changes in the nervous tissues and the persistent infection have not been studied about IBR virus infection in calves.

The present author studied the pathological changes of nervous tissues in calves infected experimentally with the Los Angeles strain of IBR virus, to clarify the pathogenesis of the recurrent infection in the sensory ganglia.[12-16]

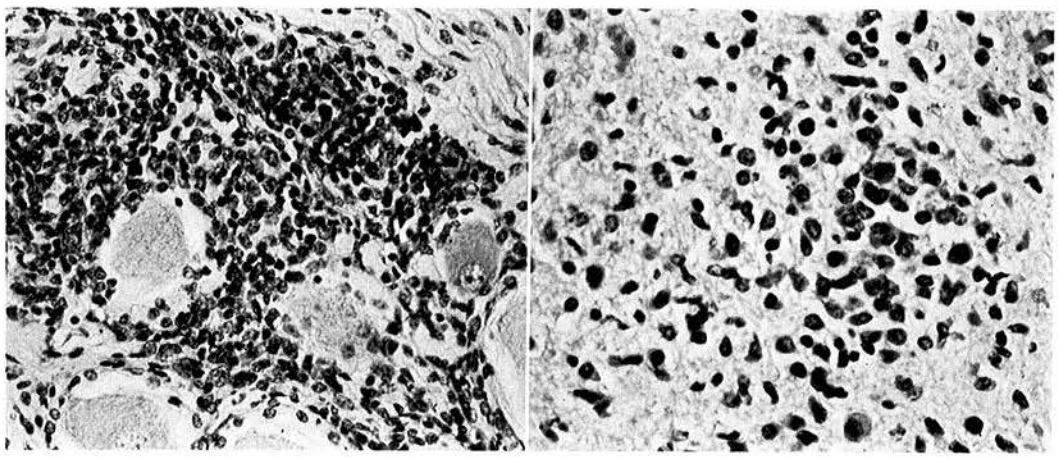

Plate 1. Left: Infiltration of neuroglial cells, and lymphocytes around the trigeminal ganglion cells from a calf 15 days after inoculation. HE staining, ×400
Plate 2. Right: Focal aggregation of neuroglial cells in the nuclei of the trigeminal nerve from a calf 12 days after inoculation. HE staining, ×400

Table 1. Histopathological lesions in calves after intranasal inoculation with IBR virus

Calf No.	Days after inoculation	Trigeminal ganglion	Medulla oblongata	Cerebrum			Cerebellum
				Frontal lobes	Temporal lobes	Occipital lobes	
1	12	++	++	−	−	−	−
2	15	+++	+++	−	−	−	−
3	30	++	++	−	+	−	−
4	57	+	+	−	−	−	−
5	98	+	−	−	−	−	−

Grade of lesion: −, no lesion; +, mild; ++, moderate; +++ severe.

Primary infection

1) Intranasal inoculation

Five calves were intranasally inoculated with IBR virus. The clinical response in calves was characterized by a rise in body temperature, depress and discharging nasal mucus. The virus was isolated from nasal secretion of all inoculated animals during 1 to 11 days after inoculation. Its titre ranged from $10^{0.5}$ to $10^{7.5}$ $TCID_{50}$/*ml*. The significant histopathological changes were non-suppurative inflamation of the bilateral trigeminal ganglia and the central nervous system (CNS) (Table 1). Bilateral trigeminal ganglionitis was composed of the proliferation of neuroglia cells (Plate 1). In the CNS, the lesions composed of focal aggregation of glial cells and lymphocytic perivascular cuffing were localized in the main sensory and spinal tract nuclei of the trigeminal nerve in the medulla oblongata (Plate 2). These lesions existed until 98 days after inoculation. IBR virus was recovered from the cerebrum, medulla oblongata and trigeminal ganglion of calves necropsied at 12 and 15 days after inoculation. By the fluorescent antibody (FA) techniques, the virus antigen was also detected in the satellite cells around the trigeminal ganglion cells, Schwann cells and neuroglia cells in the brain.

These findings suggested that the virus is capable of travelling in the nerve fibre from the nasal mucosa to the trigeminal ganglion.

2) Intravaginal inoculation

Six calves intravaginally inoculated with

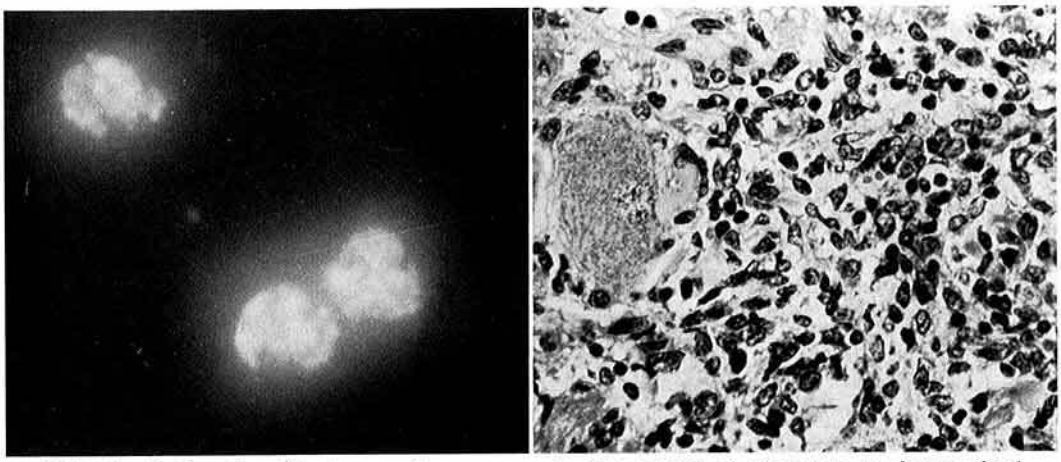

Plate 3. Left: Specific immunofluorescence in the nuclei of moderate to large sized mononuclear cells in the blood from a calf 3 days after inoculation. FA staining, ×800

Plate 4. Right: Inflammatory changes in sacral spinal ganglia from a calf 18 days after inoculation. HE staining, ×400

Table 2. Histopathological lesions in calves after intravaginal inoculation with IBR virus

Calf No.	Days after inoculation	Trigeminal ganglion	Medulla oblongata	Cerebrum	Medulla spinalis							
					CSC	Gan	TSC	Gan	LSC	Gan	SSC	Gan
21	12	−	−	−	−	−	−	−	+	−	++	+
22	18	−	−	−	−	−	−	−	−	−	++	+
23	18	+	+	−	−	−	−	−	−	+	++	+
24	24	−	−	−	−	−	−	−	−	−	−	+
25	30	+	+	+	−	−	−	−	−	−	+	−
26	35	++	++	+	−	−	−	−	−	+	+	+

CSC: cervical spinal cord, Gan: ganglion, TSC: thoracic spinal cord, LSC: lumbar spinal cord, SSC: sacral spinal cord. Grade of lesion: −, no lesion; +, mild; ++, moderate.

IBR virus showed a pyrexia and severe pustular vulvovaginitis which was composed of small white pustules about 1 to 3 mm in diameter. Specific antigen with immunofluorescence was observed in the nuclei of the blood mononuclear cells between 3 and 4 days after inoculation of the virus (Plate 3). Thereafter, the virus was recovered from nasal secretions of 3 of the 6 calves examined. At the early stage of infection, focal gliosis and perivascular cuffing were found in the sacral spinal cords and sacro-lumbar spinal ganglia (Plate 4). At the late stage, a slight trigeminal ganglionitis was also observed in 3 of the 6 calves (Table 2). The results showed that the lesions in the sacro-lumbar spinal cord and ganglia indicated a close association with viral replication in the vaginal mucosa. Moreover, the occurrence of transitory haematogeneous phase of infection was also suggested in this experiment.

3) Intraconjunctival inoculation

Six calves were given the IBR virus by instillation into the conjunctival sacs. IBR virus caused severe conjunctivitis with lachrymation and a slight rhinitis. Recovery of the virus from ocular and nasal secretions was generally coincidental with the extent of clinical signs. In the conjunctiva, the most marked changes were ulceration and lymphocytic infiltration in the lamina propria (Plate 5). In the peripheral and CNS, focal gliosis and perivascular cuffing were extensively and consistently observed in the trigeminal ganglia and medulla oblongata (Table 3). The CNS changes were mostly located in 2 specific sites; the tractus solitarius (Plate 6) and the main sensory and spinal tract nuclei of the trigeminal nerve. Findings of this experiment may suggest that the IBR virus is able to spread up from the nasal mucosa and conjunctiva to the CNS via two sensory pathways, one the trigeminal and the other the lacrimal nerve.

Recurrent infection

1) Recurrent respiratory infection

Five months later, the intranasally infected calves were injected intravenously for 5 consecutive days with a daily dose of 0.1 mg of dexamethasone (DM)/kg body weight. The virus appeared in nasal secretion of the calves from the 4th day after the initiation of DM treatment until the 9th day (Table 4). The clinical signs of recurrent infection appeared as a slight nasal discharge. The most significant neural changes was trigeminal ganglionitis with neuronophagia (Plates 7 and 8), which was observed from the 3rd to the 11th day (Table 5). Significantly, the extent of changes in the trigeminal ganglion and medulla oblongata corresponded to the amount of DM administered. The IBR virus antigen was first observed in the trigeminal ganglion cells (Plate 9), and after that, it was detected in the Schwann cells, satellite cells, neuroglial cells (Plate 10) and nasal mucosa until the 10th day. These observations indicated that

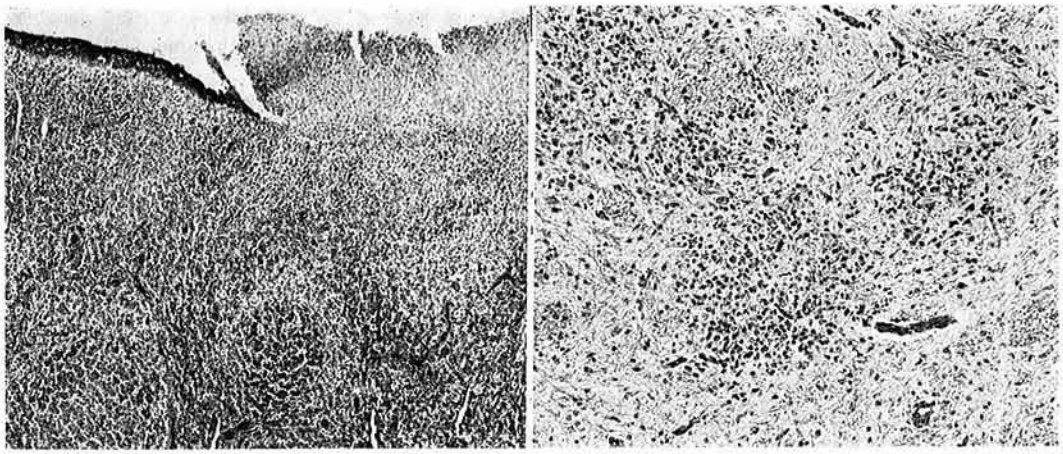

Plate 5. Left: Ulceration and marked lymphocytic infiltration in the lamina propria of the conjunctiva from a calf 5 days after inoculation. HE staining, ×130

Plate 6. Right: Focal aggregation of neuroglial cells in the tractus solitatius in the medulla oblongata from a calf 12 days after inoculation. HE staining, ×160

Table 3. Histopathological lesions in calves after intraconjunctival inoculation with IBR virus

Calf No.	Days after inoculation	Trigeminal ganglion	Medulla oblongata	Cerebrum			Cerebellum
				Frontal lobes	Temporal lobes	Occipital lobes	
96	5	+	−	−	−	−	−
97	9	++	−	−	−	−	−
98	12	++	++	+	+	+	−
99	16	++	++	−	−	−	−
100	21	++	++	−	−	−	−
101	26	++	++	++	+	−	−

Grade of lesion: −, no lesion; +, mild; ++, moderate; ++, severe.

Table 4. Recovery of IBR virus from nasal secretions of infected calves after Dexamethasone (DM) treatment

Calf No.	Days after start of DM treatment										
	1*	2*	3*	4*	5*	6	7	8	9	10	11
Treated Infected Calves											
86	—										
87	—	—	—								
88	—	—	—	$10^{1.5}$							
89	—	—	—	—	$10^{2.5}$						
90	—	—	—	$10^{3.75}$	$10^{7.5}$	$10^{7.0}$					
91	—	—	—	—	$10^{0.25}$	$10^{4.0}$	$10^{5.0}$				
92	—	—	—	$10^{3.5}$	$10^{6.75}$	$10^{6.25}$	$10^{5.5}$	$10^{1.75}$			
93	—	—	—	$10^{2.5}$	$10^{1.25}$	$15^{2.0}$	$10^{3.0}$	$10^{4.25}$	$10^{1.5}$	$10^{2.0}$	
94	—	—	—	—	$10^{0.25}$	$10^{1.25}$	$10^{1.0}$	$10^{1.5}$	$10^{1.5}$	—	—
Nontreated Infected Calves (Controls)											
30	—	—	—	—	—	—	—	—	—	—	—
31	—	—	—	—	—	—	—	—	—	—	—
Treated Calves (Not Inoculated) (Controls)											
186	—	—	—	—	—	—	—	—	—	—	—
196	—	—	—	—	—	—	—	—	—	—	—

*: Day of DM-treatment. Data are expressed as $TCID_{50}/ml$. —: No viral recovery.

Table 5. Histopathological lesions in calves treated with Dexamethasone (DM)

Calf No.	Days after start of DM treatment	Trigeminal ganglion	Medulla oblongata	Pons	Cerebrum	Cerebellum	Spinal cord	Spinal ganglia
Treated infected calves								
86	1*	—	—	—	—	—	—	—
87	3*	###	+	—	—	—	—	—
88	4*	###	+	—	—	—	—	—
89	5*	###	+	—	—	—	—	—
90	6	###	+	—	—	—	—	—
91	7	+	+	—	+	—	—	—
92	8	###	+	+	+	—	—	—
93	10	+	+	+	+	—	—	—
94	11	###	+	+	—	—	—	—
Nontreated infected calves (Controls)								
30		—	—	—	—	—	—	—
31		—	—	—	—	—	—	—
Treated calves (Not inoculated) (Controls)								
186	11	—	—	—	—	—	—	—
196	11	—	—	—	—	—	—	—

*: Day of DM-treatment. Grade of lesion: —, no lesion; +, mild; ++, moderate; ###, severe.

Table 6. Recovery of IBR virus from vaginal secretions of infected calves after Dexamethasone (DM) treatment

Calf No.	Days after start of DM treatment										
	1*	2*	3*	4*	5*	6	7	8	9	10	11
Treated infected Calves											
140	—	—	$10^{1.5}$	$10^{2.5}$							
146	—	—	$10^{1.5}$	$10^{3.5}$	$10^{3.5}$	$10^{3.5}$					
164	—	—	$10^{1.5}$	$10^{1.5}$	$10^{3.5}$	$10^{3.5}$	$10^{4.0}$	$10^{3.75}$	$10^{2.5}$		
108	—	—	—	—	$10^{2.5}$	$10^{2.5}$	$10^{2.5}$	$10^{2.5}$			
Nontreated infected calves (Controls)											
134	—	—	—	—	—	—	—	—	—	—	—
Treated calves (Not inoculated) (Controls)											
254	—	—	—	—	—	—	—	—	—	—	—
264	—	—	—	—	—	—	—	—	—	—	—

*: Day of DM-treatment. Data are expressed as $TCID_{50}$/m*l*. —: No viral recovery

the IBR virus is capable of producing a persistent infection in the trigeminal ganglion and that trigeminal ganglionitis may be a characteristic lesion due to the reactivation of latent IBR virus.

2) Recurrent genital infection

Three months later, the intravaginally infected calves were given 5 consecutive daily dose of 0.1 mg of DM/kg body weight. The virus was first recovered from the vaginal secretions on the 3rd day after the initiation of DM treatment (Table 6). However, the

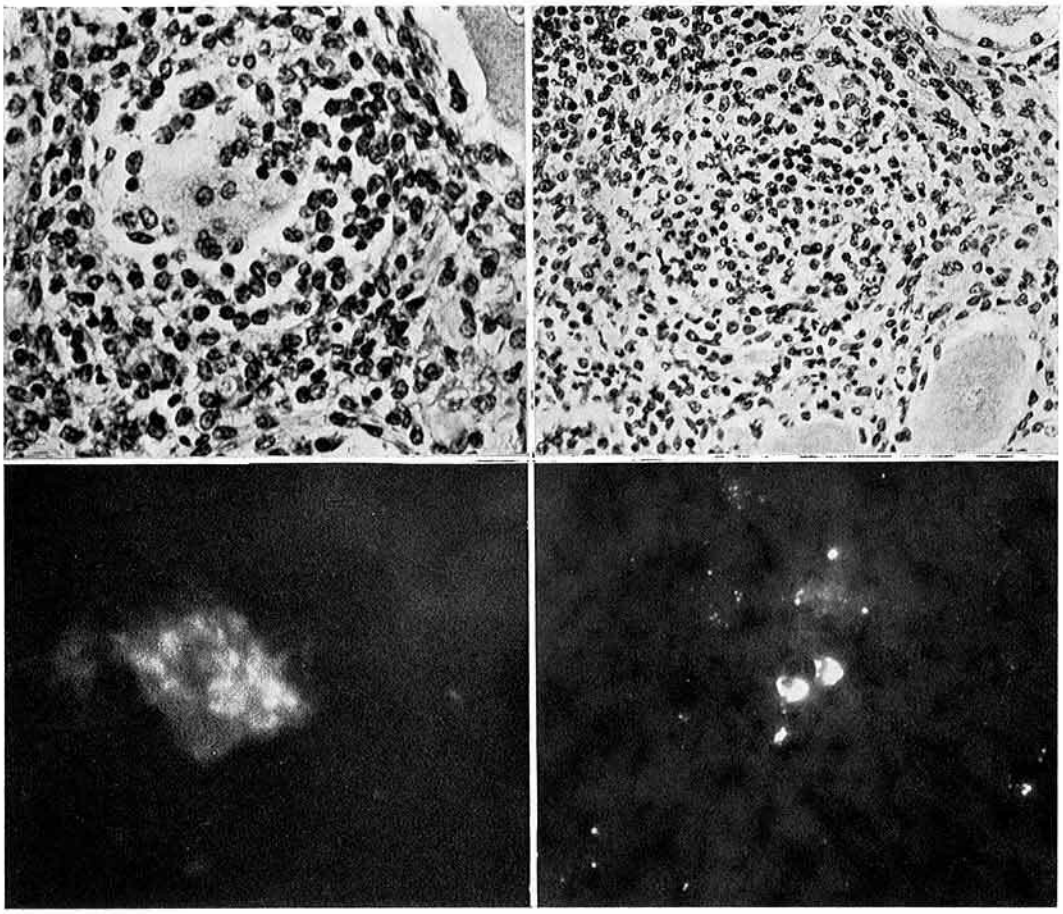

Plate 7. Top-left: Neuronophagia on the trigeminal ganglion on the 3rd day of DM treatment (Calf 87). HE staining, ×400

Plate 8. Top-right: Neuronophagic nodules and accumulation of inflammatory cells in the trigeminal ganglion on the 4th day of DM treatment (Calf 88). HE staining, ×200

Plate 9. Bottom-left: Specific immunofluorescence of a ganglion cell in the trigeminal ganglion on the 3rd day of DM treatment (Calf 87). FA staining, ×480

Plate 10. Bottom-right: Specific immunofluorescence of neuroglia cells in the medulla oblongata on the 5th day of DM treatment (Calf 89). FA staining, ×480

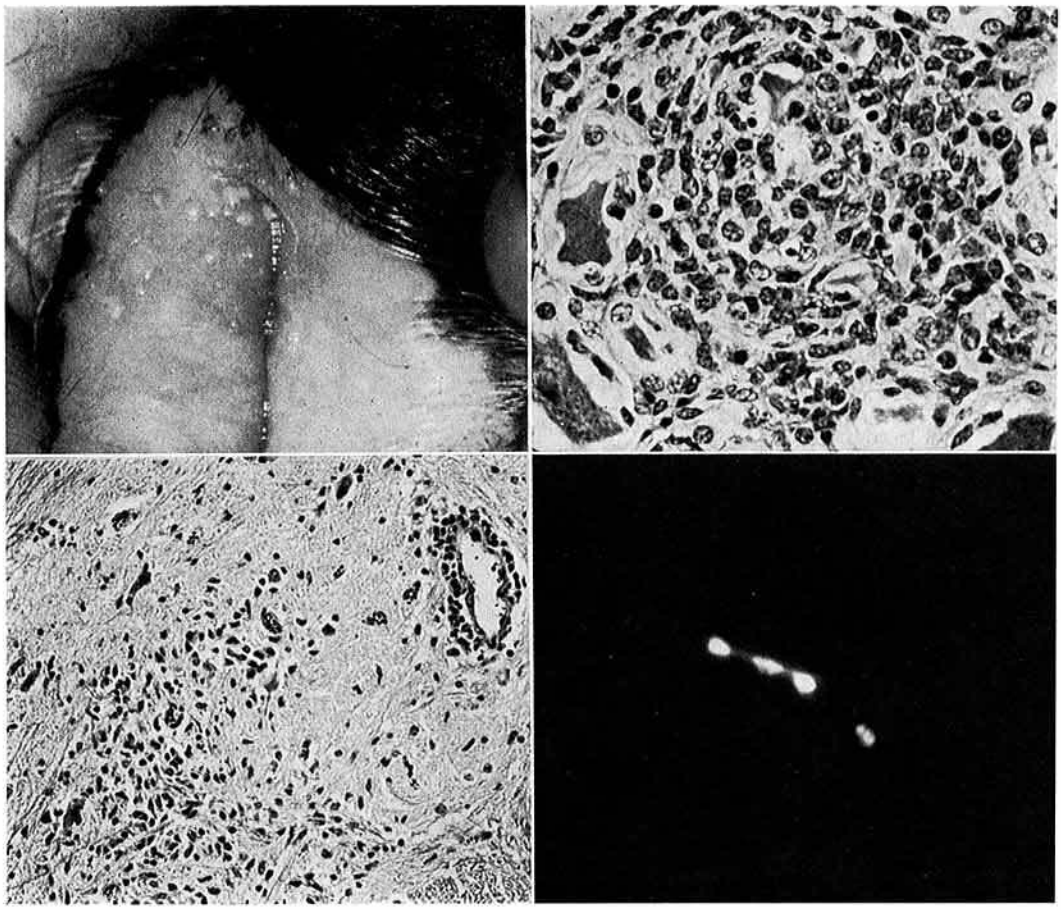

Plate 11. Top-left: Small pustules on the mucosal surface of the valva on the 6th day of DM treatment (Calf 164).

Plate 12. Top-right: Neuronophagic nodules in the sacrospinal ganglia on the 4th day of DM treatment (Calf 140). HE staining, ×400

Plate 13. Bottom-left: Focal gliosis and perivascular cuffing in the sacrospinal cords on the 6th day of DM treatment (Calf 146). HE staining, ×160

Plate 14. Bottom-right: Specific immunofluorescence of the Schwann cells in the peripheral nerve fibers of the vagina on the 6th day of DM treatment (Calf 146). FA staining, ×400

Table 7. Histopathological lesions in calves treated with Dexamethasone (DM)

Calf No.	Days after start of DM treatment	Trigeminal ganglion	Medulla oblongata	Cerebrum	Medulla spinalis							
					CSC	Gan	TSC	Gan	LSC	Gan	SSC	Gan
Treated infected calves												
140	4*	−	−	−	−	−	−	−	+	−	++	+
146	6	−	+	−	−	−	−	−	++	+	+++	++
164	9	−	++	−	+	−	+	+	++	+	+++	+
108	11	+	++	−	−	−	−	−	++	+	+++	+
Nontreated infected calves (Controls)												
134		−	−	−	−	−	−	−	−	−	−	−
Treated calves (Not inoculated) (Controls)												
254	11	−	−	−	−	−	−	−	−	−	−	−
264	11	−	−	−	−	−	−	−	−	−	−	−

*: Day of DM-treatment. Grade of lesion: −, no lesion; +, mild; ++, moderate; +++, severs.

virus was not recovered at all from nasal secretions or from cerebrospinal fluid of the DM treated calves. The clinical signs of recurrent infection first appeared as a mild hyperaemia and submucosal oedema of the vagina. After that, dissemination of the small pustules (1 to 2 mm in diameter) was found on the mucosal surface (Plate 11). The significant neural changes in all recurrent infected calves were non-suppurative poliomyelitis in the lumbo-sacral spinal cords and their ganglia (Plates 12 and 13), and were severer in the sacrospinal cords than in other parts of the spinal cord (Table 7). The virus antigen was detected in the sacrospinal ganglia and peripheral nerve fibres in the submucosa of the vagina on the 4th and 6th days after start of DM treatment (Plate 14). These observations indicated that the non-suppurative poliomyelitis may be a characteristic lesion in the recurrent genital infection of IBR virus. The sacrospinal cords and their ganglia are considered as latent site of IBR virus.

Conclusion

The pathogenesis of primary and recurrent infections with IBR virus was studied on experimentally infected calves.

In the primary infection, the IBR virus produced severe rhinitis, vulvovaginitis or conjunctivitis according to the site of infection. Each lesion was accompanied with focal gliosis and perivascular cuffing in the regional central nervous tissue. The location of pathological changes which is related to the site of infection suggested the possibility that the virus travels centripetally from the mucous membrane to the regional CNS through sensory nerves and gives rise to non-suppurative sensory ganglionitis and encephalitis.

In the recurrent infection induced by the DM treatment, the calves in latent condition excreted the virus from nasal and vaginal secretions in spite of the fact that the animals had neutralizing antibody, and showed trigeminal ganglionitis and sacro-spinal ganglionitis with neuronophagia. The distribution of these lesions was mostly similar to that of the calves in the primary infection. The virus antigen was also first detected in the ganglion cells and satellite cells. Therefore, it was considered that the sensory ganglia are a latent site of IBR virus, and that the virus reactivated by such immunosuppression as the DM treatment seems to travel centrifugally through the nerve fibres to the mucous membrane, and the virus is no longer accessible to the humoral antibodies.

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