Pathologic Changes in Closed Porcine Intestinal Loops Inoculated with Aujeszky's Disease Virus

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ABSTRACT. Development of enteric lesions in closed jejunal and ileal loops inoculated with Aujeszky's disease virus (ADV) was examined in four 6-week-old SPF pigs. A large number of ADV antigens were detected first in necrotic foci in the subepithelial areas, and subsequently in the epithelial cells, lymphoid follicles in Peyer's patches and neuronal cells of Meissner's and Auerbach's plexuses.—KEY WORDS: Aujeszky's disease virus, enteric lesion, loop.

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Aujeszky's disease has been recognized as a severe, highly fatal disease of young pigs. Its causative virus commonly affects the central nervous system, producing nonsuppurative encephalomyelitis [1, 3, 7, 8]. Some strains of Aujeszky's disease virus (ADV) reportedly produced characteristic lesions in organs other than the central nervous system [5, 6]. There is little information on gastrointestinal lesions produced by ADV, except those in a few naturally and experimentally infected pigs [2, 4]. In the present studies, ADV was inoculated into ligated closed intestinal loops and the pathogenesis of ADV infection in the small intestine was examined.

Four 6-week-old SPF pigs were injected intramuscularly with 1.0 mg/Kg body weight of xylazine, and then were anesthetized intravenously with 10 mg/Kg body weight of pentobarbital. Laparotomy along the linea alba was performed. In the small intestine, two 10 cm loops were ligated in the middle part of the jejunum and in the distal portion of the ileum. One of the two loops was inoculated with 4 m*l* of $10^{6.0}$ PFU/m*l* of YS-81 strain of ADV, and the other was inoculated with 4 ml of non-infected growth culture medium as control. After that, the abdomen was closed and the animals were observed clinically. During the experiment, the pigs were given milk for food and injected subcutaneously with 200 m*l* of Ringer's solution/day. One pig each was killed daily from 1 to 4 days after inoculation. The loops of each pig were fixed in 10% neutral buffered formalin, embedded in paraffin wax and cut into thin sections that were stained with hematoxylin and eosin.

ADV antigen was demonstrated by the Avidin Biotin Complex (ABC) immunoperoxidase method, using an ABC kit (Vectastain, Vector Laboratories, Burlingame, CA, U.S.A.). Anti-ADV rabbit serum was used as the primary antibody at a dilution of 1:2,048. Sections were counterstained with methyl green. Serum from a non-immunized rabbit was used for the control study. Samples of the jejunum and ileum were fixed in 2.5% glutaraldehyde for 2 hr and 1% osmium tetroxide in 0.1 M phosphate buffer at pH 7.3 for 1 hr, dehydrated in alcohol and infiltrated and embedded in Epon mixture. Ultrathin sections were stained with uranyl acetate and lead citrate and then examined in a JEOL-100CX electron microscope.

Histopathologically, no lesions were observed in a pig killed on post-inoculation day (PID) 1. The villous epithelium was unchanged on PID 2; however, small, necrotic foci first appeared in the subepithelial areas in the jejunum. Marked karyorrhexis was regularly observed within the foci. On PIDs 3 and 4, the necrotic foci developed and gained access to the lymphoid follicles in Peyer's patches in the ileum. Parallel with these changes, some crypt and villous epithelial cells exhibited degeneration, desquamation and a few intranuclear inclusion bodies. Occasionally, slight neuronal degeneration was detected in Meissner's and Auerbach's plexuses in the jejunum and ileum.

Immunohistologically, the presence of ADV antigen correlated closely with the distribution of necrotic foci, which first appeared in necrotic cells distributed among subepithelial cells, and subsequently, in the lymphoid follicles in Peyer's patches and in the degenerating villous epithelial cells (Figs. 1 and 2). Finally, a small number of ADV antigens were found in the neuronal cells of Meissner's and Auerbach's plexuses (Fig. 3).

Electron microscopically, many herpes virus particles were scattered throughout the nuclei and cytoplasm of the degenerating cells. Immature virus particles were arranged in a cluster near the nuclear membrane, and enveloped virus particles were distributed in cytoplasmic vesicles and extracellular spaces in the necrotic areas (Fig. 4). No lesions were found in the closed jejunal and ileal loops inoculated with growth culture medium.

The results of this study indicated that YS-81 strain of ADV replicated in cells distributed in the subepithelial areas, and subsequently in the epithelial cells, lymphoid follicles in Peyer's patches and neuronal cells of Meissner's and Auerbach's plexuses in the jejunum and ileum. The distribution of necrotic foci and the detection of viral antigen and virus particles on PIDs 3 and 4 coincided closely with the observations on suckling pigs with natural and experimentally induced ADV infections [2, 4].

Many investigators have suspected that the intestinal epithelium is the primary site of the attachment and

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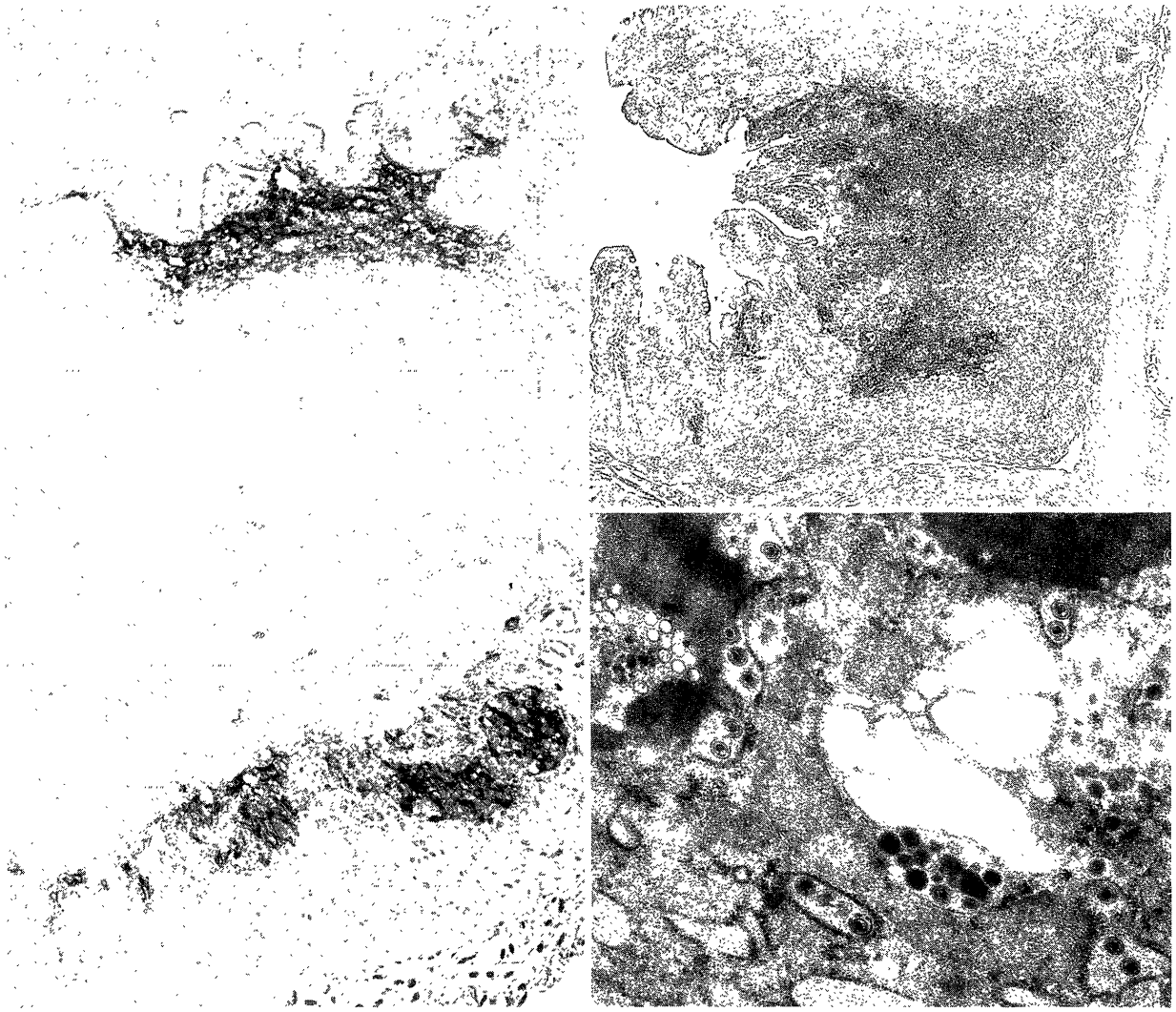

Fig. 1. ADV antigen in the subepithelial area in the jejunum of a pig killed on PID 2. Immunoperoxidase stain. × 200.
Fig. 2. ADV antigen in degenerating epithelial cells and the lymphoid follicles of Peyer's patches in the ileum of a pig killed on PID 4. Immunoperoxidase stain. × 50.
Fig. 3. ADV antigen in the neuronal cells in Auerbach's plexus. Immunoperoxidase stain. × 400.
Fig. 4. Many enveloped virus particles in the degenerating cells within the center of the necrotic foci in the subepithelial area of a pig killed on PID 3. × 20,000.

replication of ADV [3, 6, 8]. In the present study, a large number of ADV antigens were detected first in a pig killed on PID 2 in necrotic foci in the subepithelial areas, but not in the epithelium. These findings corresponded well with those of ADV infection in the tonsils [5]. Therefore, we assumed that the primary target of ADV infection in the intestines is also the cells distributed in the subepithelial area of the jejunum and ileum. The question of how ADV penetrates the intestinal barrier is still unclear.

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REFERENCES

1. Corner, A. H. 1995. *Res. Vet. Sci.* 6: 337–347.
2. Ezura, K., Usami. Y., Tajima, K., Komaniwa, H., Nagai, S., Narita, M., and Kawashima, K. 1995. *J. Vet. Diagn. Invest.* 7: 451–455.
3. McFerran, J. B. and Dow, C. 1965. *Am. J. Vet. Res.* 26: 631–635.
4. Narita, M., Kubo, M., Fukusho, A., Haritani, M., and Moriwaki, M. 1984a. *Vet. Pathol.* 21: 450–452.
5. Narita, M., Imui, S., and Shimizu, Y. 1984b. *Am. J. Vet. Res.* 45: 247–251.
6. Narita, M., Imada, T., Haritani, M., and Kawamura, H. 1989. *Am. J. Vet. Res.* 50: 1940–1945.
7. Sabo, A., Rajcani, J., and Blaskovic, D. 1968. *Acta Virol.* 12: 214–221.
8. Sabo, A., Rajcani, J., and Blaskovic, D. 1969. *Acta Virol.* 13: 407–414.

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