Immunohistochemical Distribution of Viral Antigens in Pigs Naturally Infected with Porcine Teschovirus

Manabu YAMADA1, Rie KOZAKURA2, Yoshihiro KAKU1,3, Kikuyasu NAKAMURA1, Yu YAMAMOTO1, Masaaki YOSHII1, Ayako MIYAZAKI1, Hiroshi TSUNEMITSU1 and Minoru NARITA1

1/National Institute of Animal Health, Tsukuba, Ibaraki 305–0856, 2/Toubu Livestock Hygiene Service Center, Toyama, Toyama 939–3548 and 3/National Institute of Infectious Disease, Toyama, Shinjuku-ku, Tokyo, 162–8640, Japan

(Received 5 September 2007/Accepted 21 November 2007)

ABSTRACT. A distribution of porcine teschovirus (PTV) antigens in pigs naturally infected with PTV is presented using the method of immunohistochemical examination. In the nervous system, PTV antigens were found in the cytoplasm of neuronal cells and glial cells distributed in the spinal ventral horn and brain stem, and also in the cytoplasm of ganglion cells in the spinal ganglion. No antigens were seen in the cerebral hemisphere. In the nervous system, the distribution of PTV antigens was consistent with lesions characteristic of nonsuppurative encephalomyelitis. In the other examined organ, PTV antigens were observed in bronchiolar epithelial cells in the lung, hepatocytes in the liver, epithelial cells in the tonsils and the myenteric nerve plexus in the small and large intestine.

KEY WORDS: immunohistochemistry, porcine teschovirus, swine.

NOTE

Pathology

Enterovirus encephalomyelitis (previously known as Teschen/Talfan disease) caused by at least 9 serotypes of porcine teschovirus (PTV 1, and 2-6, 8, 12, 13 which are responsible for the milder form of the disease) of the picornaviridae family is considered to be of socioeconomic importance [12, 16]. Infection appears to be selective for specific neuronal populations, resulting in a characteristic clinical syndrome of lower motor neuron paralysis [8]. Infections from PTV are most often asymptomatic, and PTV is still frequently isolated from the faces, tonsils and other non-neural organs of apparently unaffected pigs [10]. There have been many studies of both naturally and experimentally induced PTV infection in pigs [1, 3, 5, 11, 13, 14, 17, 19, 20]. Previously, the organ tropism of PTV infection was examined by virus isolation [1, 20]. However, isolation of viruses is time- and labor-intensive [10] and does not provide any information about the type, proportion, or histological localization of cells containing the virus, or the relationship between the detected virus and the pathogenesis of lesions. Unlike virus isolation, immunohistochemical detection of viral antigens in formalin-fixed paraffin-embedded tissues allows the detection and localization of viral antigens within cells and tissues, and could be useful as a simple and rapid method to study the organ tropism and pathogenesis of PTV. Indeed, the immunohistochemical distribution of PTV antigen has not been described across the entire body of pigs infected with PTV. In this study, we used immunohistochemical methods to examine 7 pigs diagnosed with encephalomyelitis caused by PTV in order to reveal the whole-body distribution of PTV antigens.

Formalin-fixed paraffin-embedded tissue specimens were examined from 7 pigs that had been previously diagnosed with enterovirus encephalomyelitis by virus isolation of PTV 1 and routine histopathological examination [19]. The affected pigs suddenly developed paralysis of the hind limbs and became recumbent at approximately 40 days of age. There were no clinical signs including diarrhea, except for neural signs.

For histopathological examination, the formalin-fixed paraffin-embedded tissue samples, including liver, spleen, kidney, heart, lung, trachea, salivary gland, tongue, esophagus, stomach, small and large intestine, gallbladder, bladder, genital system, pancreas, adrenal gland, thyroid gland, hypophysis, thymus, tonsil, lymph node, cerebral hemisphere, mesencephalon, pons, medulla-oblongate, spinal cord, spinal ganglia, trigeminal ganglia, peripheral ganglia and nerve fibers, eyes, skin, and skeletal muscle, were sectioned at 4 µm. Dewaxed sections were stained with hematoxylin and eosin (HE). For immunohistochemistry (IHC), dewaxed sections were subjected to the streptavidin-biotin complex peroxidase (SAB-PO) method using a HISTOFINE SAB-PO kit (Nichirei, Japan) [18]. As pretreatment, heat denaturation was used for antigen retrieval [15, 18]. The antibodies used in this study were monoclonal anti-PTV 1 Talfan strain [No. 9, 1:128, NIAH, Japan] [9, 18]. A negative control of each section used sera from a non-immunized mouse instead of the primary antibody. A negative control of each section leave out the secondary antibody was also prepared. Samples were counterstained with hematoxylin.

Histological findings, which have already been reported [19], are summarized briefly. Lesions were limited to the central nervous system (CNS) and peripheral nerve fibers. All clinically affected pigs had similar histological changes. The changes observed were those of nonsuppurative encephalomyelitis, characterized by perivascular cuffing of the mononuclear cells, focal gliosis, neuronal necrosis and neuronophagia. The spinal cord was severely affected and the lesion was seen along the full length of the spinal cord.
In the ventral horns, nerve cells were degenerated to varying degrees up to, and including, necrosis accompanied by neuronophagia, inflammatory or glial nodules, hemorrhage, and a rather diffuse infiltration of mononuclear cells (Fig. 2). In addition to the infiltrative changes, severe vacuolar changes and axonal swelling were observed in the white matter of the spinal cord. Infiltration of mononuclear cells was observed in the dorsal root ganglia, spinal nerves, and sciatic nerves. The cerebellar nuclei and the gray matter of the brainstem were also severely affected (Fig. 1). In the cerebral hemisphere, only slight perivascular cuffing was present.

Immunohistochemically, we found that the PTV antigen was mainly localized to the nervous system. In the cerebellar nuclei, the gray matter of the brain stem, and the ventral horn of the spinal cord of all examined pigs, PTV antigens were detected in the cytoplasm of large nerve cells and glial cells (Figs. 3 and 4). The positive reaction was strongest in the ventral horn of the spinal cord. The cytoplasm of endothelial cells also showed a positive reaction in those locations. In the spinal ganglia, PTV antigen was strongly detected in the cytoplasm of ganglion cells (Fig. 5). In the nervous system, the distribution of PTV antigen was consistent with the lesion distribution. In the lesion, no antigens were seen in the central severe area. Antigens were mainly seen in the peripheral of the severe lesion, especially in slight to mild areas around perivascular cuffing.

PTV antigens were also detected in the cytoplasm of the majority of bronchiolar epithelial cells in the lung (Fig. 6), the cytoplasm of some hepatocytes in the liver (Fig. 7), and the cytoplasm of some epithelial cells in the tonsil in all examined pigs. In the small and large intestine, PTV antigens were detected in the myenteric nerve plexus (Fig. 8). However there were no PTV antigens in the intestinal epithelium of any examined pigs (Fig. 8). No PTV antigen was detected in any sections of negative control stained with normal rabbit sera. The negative control section leave out the secondary antibody also did not show PTV antigens. No PTV antigens were detected in any other organs including the cerebral hemisphere and peripheral ganglia and nerve fiber.

The present pigs had been diagnosed with enterovirus encephalomyelitis by virus isolation of PTV 1 and routine histopathological examination [19]. It was also confirm nonsuppurative encephalomyelitis appeared in examined pigs were caused by PTV by immunohistochemical detection of PTV antigens in the lesion in this study. In this study, we present a detailed distribution of PTV antigens in pigs naturally infected with PTV. We report that in addition to the nervous system, PTV antigens were also observed in the lung, liver, tonsil and myenteric nerve plexus in the small and large intestine. In the nervous system, the distributions of lesions and PTV antigens in the nervous system were same as those of our previous study that examined pigs experimentally infected with PTV. We report that in addition to the nervous system, PTV antigens were also observed in the lung, liver, tonsil and myenteric nerve plexus in the small and large intestine. In the nervous system, the distributions of lesions and PTV antigens in the nervous system were same as those of our previous study that examined pigs experimentally infected with PTV 1 Talfan strain [18], although the degree of the positive reaction have some differences. PTV antigens were strongly seen in the ventral horn of the spinal cord in the present study. On the other hand, the positive reaction was strongest in the medulla-oblongata and pons in the previous experimental study [18]. In that previous study, PTV antigens were detected only in the nervous system, especially in the brainstem and there were no PTV antigens in the other organs including the lung, intestine, liver and tonsil [18]. The reason that PTV antigen was limit in the nervous system in the previous cases might be that the previous experimental cases were intravenously inoculated and necropsyed until postinoculation day.
Fig. 3. Medulla oblongata of a pig naturally infected with PTV. PTV antigen is detected in the cytoplasm of nerve cells. IHC, Bar =500 µm.

Fig. 4. Ventral horn of the spinal cord of a pig naturally infected with PTV. PTV antigen is detected in the cytoplasm of nerve cells. IHC, Bar =200 µm.

Fig. 5. Spinal ganglion of a pig naturally infected with PTV. PTV antigen is detected in the cytoplasm of ganglion cells. IHC, Bar =200 µm.

Fig. 6. Lung of a pig naturally infected with PTV. PTV antigen is detected in the cytoplasm of bronchiole epithelial cells. IHC, Bar =200 µm.

Fig. 7. Liver of a pig naturally infected with PTV. PTV antigen is rarely detected in the cytoplasm of hepatocytes. IHC, Bar=200 µm.

Fig. 8. Duodenum of a pig naturally infected with PTV. PTV antigen is detected in the myenteric nerve plexus. No PTV antigen is seen in the intestinal epithelium. IHC, Bar=400 µm.
The difference of the whole-body distribution of PTV antigens may be related to the difference of the infectious stage or the infectious route. Although PTV infection is most frequently asymptomatic [4], some strains have been associated with a wide variety of clinical symptoms including female reproductive disorders [2], enteric disease [7], pneumonia [14], pericarditis and myocarditis [11], and polioencephalomyelitis [5, 17]. Histopathological changes have also been described in the kidneys, liver, and spleen [14], and in the adrenal and thyroid glands [6]. In this study, PTV antigens were detected in the liver, lung, tonsil and myenteric nerve plexus in the small and large intestine in addition to the nervous system, but not in the genital system, heart, kidney, spleen, and adrenal and thyroid glands. There were no lesions in the organs that tested positive for PTV antigens in this study, except for the nervous system. Each strain or serotype of PTV may have its own different organ tropism and pathogenesis. It is interesting the presence of viral antigens in the myenteric nerve plexus in this study. The movement of PTV through the myenteric nerve plexus was unclear because other intraabdominal ganglions and plexus were not examined for immunohistochemistry in this study. The effect of PTV to the myenteric nerve plexus was also unclear. The presence of viral antigen in the myenteric nerve plexus and that effect for the intestinal system should be substantiated with further study.

Current concepts of the pathogenesis of PTV infection envision three phases: (1) local replication in the gut, mucosal lymphoid tissues (tonsils, Payer’s patches), and the local lymph nodes; (2) viremia; and (3) central nervous system (CNS) invasion [8]. However, the local lymph nodes, mucosal lymphoid tissues, and epithelium of the gut did not show positive reactions for PTV antigens in the present study. The tested animals may have been in the late stages of infection and PTV antigens may have already disappeared from these tissues, although the movement of the virus population in the host is still unclear. It is also currently unclear how PTV travels from the periphery to the CNS in pigs. Further studies are necessary to clarify the mechanisms of PTV neural infections that cause encephalitis in pigs.

ACKNOWLEDGMENTS. We thank Mr. M. Kobayashi and Miss. M. Shimada for preparing the pathology sections and Dr. Y. Ando and Mr. T. Fujisawa for preparing the photographs.

REFERENCES