Pathology of Non-Effusive Type Feline Infectious Peritonitis and Experimental Transmission

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Abstract. Seven cases of feline infectious peritonitis (FIP) clinically diagnosed as non-effusive type, were examined histopathologically. Most lesions were similar to those of effusive type of FIP, showing necrosis with vasculitis and granulomatous inflammation in the kidneys, liver, mesenteric lymph nodes, lungs, brain and eyes. After inoculation with emulsion of materials from one of the natural cases, effusive and non-effusive types of FIP were produced in 10 and 2 kittens, respectively. Histopathology and electron microscopy of the naturally occurring and experimentally induced cases revealed viral growth in large mononuclear cells at early stages of infection prior to formation of necrotic lesions.

The disease entity of feline infectious peritonitis (FIP) was first established by Wolfe and Griesemer [18] in 1966. Since then, the etiology has been studied by many workers, and a coronavirus was closed up as a causative agent [4–6, 9–11, 14, 15, 17]. Pathologically, there have been many reports on the classical effusive type of FIP, but the pathogenesis remains still unclear [12]. On the other hand, the non-effusive type of FIP, which had poor or no accumulation of ascites and/or thoracic exudate, was described by Montali and Strandberg [8] and Pedersen [13]. Montali and Strandberg [8] made transmission experiments of this type in cats, revealing the production of effusive type. No report has dealt with the process of the formation of local lesions in non-effusive type of FIP.

This paper deals with pathological observations of 7 natural cases of non-effusive type of FIP, and of 12 experimental cases which were produced by inoculation with materials from one of the natural cases.

Materials and Methods

Seven cases of non-effusive type of FIP were obtained from veterinary practitioners working in Tokyo area (Table 1) and used for this study. The kidney of Case 5 was aseptically sampled and stored at -70°C for 1 month. Then, the sample was emulsified in 3 volumes of phosphate buffered-saline, pH 7.4 (PBS), containing penicillin (100 units/ml) and streptomycin (100 μg/ml), and 0.5 ml of the emulsion was inoculated intraperitoneally into 5 littermate kittens 2 weeks of age (Cases A1–A5, 164 to 178 g) and 3 kittens 2 months of age (Cases A6–A8, 840 to 956 g). At 14 days postinoculation (p.i.) the liver was harvested from Case A4, and 0.5 ml of 1:3 emulsion was inoculated intraperitoneally into 2 kittens 2 weeks of age (Cases B1 and B2, 186 and 194 g), and then 0.5 ml of 1:3 emulsion of the liver from Case B2 sampled at 9 days p.i. was inoculated intraperitoneally into 2 kittens 2 months of age (Cases C1 and C2, 948 and 952 g).

Both natural and experimental cases were autopsied immediately after death or killing, and main organs were fixed in 10% buffered formalin, pH 7.0. Paraffin sections were made by a routine procedure and stained with hematoxylin and eosin. Some tissues from the mesenteric lymph nodes, liver and spleen of experimental cases were fixed in 5% glutaraldehyde, postfixed 1% osmium tetroxide and embedded in Epon 812. Ultra-thin sections were
Table 1. Clinical symptoms of natural cases

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Breed</th>
<th>Age (months)</th>
<th>Sex</th>
<th>Major presenting signs</th>
<th>Duration (months)</th>
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<tr>
<td>1</td>
<td>Mongrel</td>
<td>8</td>
<td>M</td>
<td>Ophthalmitis, Renal masses*, Jaundice, Leukocytosis, Anemia, Hypergamma-globulinemia</td>
<td>1</td>
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<tr>
<td>2</td>
<td>Mongrel</td>
<td>8</td>
<td>F</td>
<td>Renal masses*, Hepatic masses*, Hypergamma-globulinemia</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>Persian</td>
<td>13</td>
<td>F</td>
<td>Ophthalmitis, Leukocytosis, Hypergamma-globulinemia, Nervous disorders</td>
<td>7</td>
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<tr>
<td>4</td>
<td>Mongrel</td>
<td>7</td>
<td>F</td>
<td>Renal masses*, Anemia, Leukocytosis</td>
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<tr>
<td>5</td>
<td>Siamese</td>
<td>7</td>
<td>M</td>
<td>Renal masses*, Jaundice, Hypergamma-globulinemia</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>Siamese</td>
<td>10</td>
<td>M</td>
<td>Renal masses*, Anemia, Jaundice, Nervous disorders</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>Siamese</td>
<td>7</td>
<td>M</td>
<td>Ophthalmitis</td>
<td>1</td>
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* Determined by palpation or radiography.

made and stained with uranyl acetate and lead nitrate. They were observed by electron microscopes, Hitachi HU-12 at 75 Kv and JEM 100S at 80 Kv.

Results

1. Natural cases

Gross lesions: The kidneys were enlarged bilaterally in 6 of 7 cases examined, showing numerous greyish-white and firm nodules protruding on the surface and extending to the medulla. In these cases the renal capsules were difficult to separate from the parenchyma. In Case 6 the cortex was mostly replaced by nodular lesions and necrotic foci were scattered in the medulla, representing a more advanced phase of the disease.

Some white nodular lesions sized a pin-head or soy-bean were present in the liver of 4 cases, the mesenteric lymph nodes of 5 cases and the lungs of 3 cases. In Case 2 the entire lobes of the liver were occupied by fused greyish-white nodular lesions.

Ocular lesions which were bilaterally recognized in 3 cases, were characterized by clouding of the cornea and accumulation of greyish-white or red-brown exudate in the anterior and posterior chambers. Considerable dilatation of cerebral ventricles was seen in Cases 3 and 4. In these cases the wall of ventricles appeared granular and the ventricles were filled with viscid fibrinous exudate.

Histopathology: In every organ examined essential changes were necrosis and granuloma formation with various degrees of infiltration of neutrophils, large mononuclear cells, lymphocytes and plasma cells.

The cortex of the kidneys was occupied by granulomatous lesions with necrosis (Fig. 3), and sometimes thrombosis was seen in the stellate veins. The granulomas were composed of large mononuclear cells and fibroblasts with infiltration of a considerable number of neutrophils, lymphocytes, plasma cells and fibrin. Necrosis was seen at the center of some granulomatous lesions. There existed cystic dilatation and regeneration of the tubules, and interstitial fibrosis.

In the liver were disseminated similar granulomatous lesions with necrosis, most of which were located in hepatic lobules and portal areas. In Case 2 most normal tissues disappeared being replaced by extensively fused lesions. Granulomatous lesions with necrosis were recognized in the mesenteric lymph nodes, particularly in paracortical or medullary regions. They
ventral leptomeninges, ependyma and choroid plexus of 4th ventricle in Cases 6 and 7.

Similar changes as in the brain were seen in the inner layer of the cornea, iris, ciliary bodies, choroid membrane and sometimes in the retina (Cases 1, 3, 5, 6 and 7). In 3 of these cases, the anterior chamber was filled with a large amount of cellular exudate (Fig. 6).

The distribution of histopathologic lesions are presented in Table 2.

2. Experimental cases

Gross lesions: Ten of 12 experimental cases (Cases A1-A7, B1, B2 and C1) were effusive type and died or moribund 3 to 19 days p.i. These cases had 5 to 40 ml of ascites which was greyish-white, transparent, viscous and coagulated when exposed to air. There were fibrin deposits on the surface of the peritoneum and visceral organs, especially the spleen and liver. Other lesions were almost similar to those of natural cases [3].

On the other hand, Cases A8 and C2 which were moribund 24 and 29 days p.i., respectively, were non-effusive type. While Case A8 had some lesions on the serosa of the visceral organs, Case C2 had no serosal

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Table 2. Distribution of histopathologic lesions in natural cases

<table>
<thead>
<tr>
<th>Organ</th>
<th>Case No.</th>
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<td>Lung</td>
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* —, negative; +, slight; ++, moderate; ++++, severe.

Fig. 1. Distribution of histopathologic lesions in the brain and spinal cord (Case 3).
- Dilatation of ventricles and aqueducts.
- Cellular infiltration.
lesions.

Histopathology: All cases of the effusive type had necrotic and granulomatous lesions with a considerable amount of fibrin deposit on the peritoneum and omentum, while Case A8 of non-effusive type had only some necroses on the serosa with infiltration of a few inflammatory cells.

In the liver of all cases of both types, there existed small focal aggregations of large mononuclear cells and neutrophils. There were granulomas which were composed of large mononuclear cells with infiltration of neutrophils, lymphocytes, plasma cells, and fibrin. Often the center of granulomas was necrotic (Fig. 7). The proliferation of fibroblasts which was observed in natural cases was not seen. The similar lesions were also present in interlobular or central veins. Electron microscopy revealed a large number of virus particles in the cytoplasm of large mononuclear cells in the lesions (Figs. 8 and 9).

In the spleen of all cases of both types, aggregations of neutrophils and large mononuclear cells and granulomatous lesions with necrosis were found around the central arteries and red pulp. In the affected splenic follicles degeneration of large mononuclear cells was frequent and there was lymphoid cell depletion (Fig. 10). Viral particles were abundant in the cytoplasm of large mononuclear cells (Figs. 11 and 12).

In the mesenteric lymph nodes of all cases of both types examined, were recognized granulomatous lesions of early phase as well as those with necrosis in the cortical nodules, paracortical areas and medulla (Fig. 13). The sinuses and medullary cords had infiltration of neutrophils, macrophages, lymphocytes and plasma cells. A large number of virus particles were detected in large mononuclear cells (Figs. 14 and 15). The bone marrow of 5 cases (Cases A3, A6–A8 and C2) (Figs. 16 and 17) and the thymus of 6 cases (Cases A1, A4, A6, A7, B1 and B2) had also granulomatous lesions of early phase (Fig. 18) and those with necrosis.

In the kidney of Case C2 there were intima aggregation of neutrophils and large mononuclear cells and granulomatous lesions with necrosis in stellate and arcuate veins suggesting a perivascular development of lesions (Figs. 19, 20 and 21). In the omentum of 10 cases there appeared granulomatous lesions of early phase and granulomatous lesions with necrosis. The same lesions were also observed in the capillaries and adventitia of small blood vessels running in the omentum (Cases A1, A2 and A5) (Figs. 22 and 23), and mesenterium (Cases A1 and B2) (Figs. 24 and 25). In Case A5 necrotic lesions were developed to the whole areas of the omentum.

The lungs were also affected in Cases A7 and C2, showing granuloma formation in the intralobular venous adventitia. In Case C2 granulomatous lesions were seen also around small blood vessels in the brain and leptomeninges (Fig. 26).

The relationship between the distribution of histopathologic lesions and p.i. time period is presented in Table 3. On 3 days p.i. (Case B1) some small foci of large mononuclear cell aggregations with a small number of neutrophils appeared in the serosa and some parenchymatous organs. Multiple large-sized lesions with some central necrosis were shown to be produced 7 days p.i. (Case A1). On 9 days or more p.i. various degrees of inflammatory responses were prominent as seen in natural cases.
Table 3. Distribution of histopathologic lesions in experimental cases

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<td>Thymus</td>
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<td>Bone marrow</td>
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<td>Brain</td>
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</table>

* Case No., encircled cases are non-effusive type. ** Days after inoculation. *** —, negative; ±, very slight; +, slight; +, moderate.

Fig. 2. Schematic presentation of histopathogenesis of granulomatous lesion.

Discussion

Except for absence of ascites and/or hydrothorax, the present natural cases of non-effusive type of FIP were shown to have clinical signs similar to those of effusive type, that is, hypergamma-globulinemia, neutrophilia, icterus and anemia as shown in Table 1. The pathological findings were also similar to those of the effusive type except for absence of severe serosal lesions. Granulomatous lesions with necrosis which were common to both natural and experimental cases might be considered to be formed as presented in Fig. 2. Aggregations of large mononuclear cells having virus particles appeared first focally in the serosa and parenchymatous organs with a small number of neutrophils, and later, they were increased in size turning to have a central necrosis, to which inflammatory responses would occur. Ward et al. [16] observed a viral growth followed by necrosis after inoculation of FIP virus by different routes. The exudate was principally composed of fibrin and inflammatory cells with a mixed population of large mononuclear cells, neutrophils, lymphocytes and plasma cells. The severity of necrosis or exudation and the predominant type of cells varied in each lesion. Sometimes there was proliferation of fibroblasts.
In both natural and experimental cases, the reticuloendothelial system (RES) was found to be extensively affected. This and the presence of many viral particles in large mononuclear cells suggest that FIP virus has a tropism not only to mesothelial cells [4] but also to RES.

Systemic vascular lesions described previously in cases of effusive type by the present authors [3], were also demonstrated frequently in the present cases of non-effusive type. In the mesenterium, omentum, lungs and brain, vasculitis was developed from the adventitia to either surrounding tissues or intima, resulting in a formation of adventitial granuloma in which adventitial mesenchymal cells seem to be targets for virus having passed through the vascular wall. In the kidney and liver, the intima seems to be affected first and then the lesions might develop to outside of the vessels, suggesting that virus-carrying macrophages were fixed onto the intima forming intimal granulomas. The lesions were formed at very early phase of infection, for example 7 days p.i. (Case A1), and they might result directly from viral multiplication.

In non-effusive type of FIP, the kidneys, lungs, brain and eyes were reported to be affected at higher incidence than in effusive type of FIP [8, 13]. This was confirmed in the present cases (Table 2), suggesting that FIP virus might be distributed through blood circulation causing systemic infection.

As described by Fankhauser and Fatzer [2] and Krum et al. [7], internal hydrocephalus was observed in 2 of the present cases. Accumulated liquor was considered to be of inflammatory responses because of high protein content and other characteristics. Such hydrocephalus might have resulted from vascular lesions, choroiditis and ependymitis causing obstruction of ventricles [7]. Severe exudative changes in the anterior chamber of the eye seem to be due to local vasculitis [1].

It remains unknown why both effusive and non-effusive types have been produced in the present experimental cases. However, the non-effusive type was observed rather in older animals (Cases A8 and C2) or those in which the time period from inoculation to onset was longer. This had been indicated by Montali and Strandberg [8]. Observations of Case A8 suggest that, when acute phase of the disease passes in comparatively mild form after invasion of the virus to mesothelial cells, non-effusive type may occur with prominent affection of parenchymatous organs [8].

References


要　約

ネコ伝染性腹膜炎非渇出型の病理組織学と伝達試験：林　俊春・内海文枝・高橋令治・藤原公策（東京大学農学部家畜病理学教室）—臨床および病理学的に非渇出型の伝染性腹膜炎（FIP）と診断されたネコ7例について病理組織学的に検索した。病理組織学的变化は渇出型と同じで、主として胃、肝、腸間腺リンパ節、肺、膵、眼に見られる血管炎および壊死をともなう肉芽腫性炎であった。非渇出型自然例のうち1例の腎由来の材料を接種した12例の仔ネコには、渇出型（10例）、非渇出型（2例）の両型が発現した。自然例および伝達例の観察から、壊死型に先行して初期には大単核細胞におけるウィルス増殖が注目された。
Explanation of Figures

Fig. 3. Kidney of natural Case 5. Extensive necrosis with dense cellular infiltration and proliferation of fibroblasts in the cortex and medulla, corresponding to a protruded greyish-white spot in gross observation. ×310.

Fig. 4. Marked dilatation of lateral and 3rd ventricles, and some areas with inflammatory exudate, in natural Case 3. ×1.8.

Fig. 5. Higher magnification of Fig. 4. Desquamation of ependymal cells with subependymal infiltration of large mononuclear cells, lymphocytes, plasma cells and a few neutrophils, and proliferation of fibroblasts, and fibrin deposit on the ependyma. ×620.

Fig. 6. Accumulation of exudate and inflammatory cells in the anterior eye chamber and posterior surface of cornea in natural Case 3. ×620.

Figs. 7–15. An experimentally infected case killed at 11 days p.i. (Case A2).

Fig. 7. Early granulomatous lesions with or without necrosis in hepatic lobules. ×620.

Fig. 8. Electronmicroscopy of a granulomatous lesion with infiltration of large mononuclear cells and neutrophils in the liver. ×5,000.

Fig. 9. Corona virus particles in a large mononuclear cell in Fig. 8. ×20,000.

Fig. 10. Fibrin deposit on the splenic capsule and granulomatous lesions of early phase in the red pulp and around the central arteries. ×310.

Fig. 11. Electronmicroscopy of infected and degenerated cells in the splenic red pulp. ×6,000.

Fig. 12. Higher magnification of Fig. 11, showing coronavirus particles in a large mononuclear cell. ×20,000. Budding process (arrow) in the inserted. Bar=100 nm.

Fig. 13. Mesenteric lymph node. Many markedly swollen reticulum cells with karyorrhexis in the cortical nodules and early granuloma with some karyorrhexis in the paracortical and medullary zones. ×930.

Fig. 14. Electronmicroscopy of infected and degenerated mononuclear cells in the medulla of a mesenteric lymph node. ×8,000.

Fig. 15. Higher magnification of Fig. 14, showing many coronavirus particles in a large mononuclear cell. ×20,000.

Figs. 16–18. An experimentally infected case dead at 15 days p.i. (Case A7).

Fig. 16. Granulomatous lesions of early phase in the bone marrow. ×930.

Fig. 17. Large granulomatous lesion with central necrosis in the bone marrow. ×620.

Fig. 18. Granuloma of early phase in the thymus cortex. ×930.

Figs. 19–21. An experimentally infected case killed at 29 days p.i. (Case C2).

Fig. 19. Intima granuloma of early phase in a stellate vein of the kidney. ×1240.

Fig. 20. Lesions extended to surrounding tissues from a stellate vein of the kidney. ×620.

Fig. 21. Intima granuloma of early phase in an arcuate vein of the kidney. ×620.

Fig. 22. Granuloma of early phase with a capillary at the center (arrow) in the omentum of an experimentally infected case (Case A2) 11 days p.i. ×320.

Fig. 23. Adventitial granuloma in the omentum of an experimentally infected case (Case A5) 16 days p.i. showing desquamation of endothelial cells and degeneration of the media. ×620.

Fig. 24. Venulitis in the intestinal serosa of an experimentally infected case (Case A1) 7 days p.i. ×310.

Fig. 25. Adventitial granuloma of early phase in the intestinal serosa of an experimentally infected case (Case B2) 9 days p.i. ×310.

Fig. 26. Granulomatous lesions with necrosis in the cerebral gyrus leptomeninges of an experimentally infected case (Case C2) 29 days p.i. ×310.