Clinical, Hematological, and Pathological Findings in Specific Pathogen-Free Cats Experimentally Infected with Feline Panleukopenia Virus

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ABSTRACT. Clinical, hematological, and pathological studies of experimental feline panleukopenia (FPL) were carried out on 10 specific pathogen-free cats. The main clinical signs of the cats infected with FPL virus were mild diarrhea, mild transient pyrexia, depression, and anorexia. None of the cats died. In many cases, leukopenia involving neutropenia and lymphopenia were observed, though the lowest leukocyte count was approximately 6,000/ μ l. Hypoplastic changes of myeloid and erythroid cells in the aspirated bone marrow, particularly decrease of immature cells, were most common. However, recovery of the cells was very rapid. The main histopathological changes of the experimental cats were observed in lymphatic tissues. Depletion of lymphocytes and activation of the RES in the spleen and mesenteric lymph nodes, atrophy of lobules, and depletion of lymphocytes in the thymus were predominant. In addition, intranuclear inclusion bodies were found in the reticular cells of lymphatic tissue and crypt epithelial cells of the small intestine. These results indicate that the clinical, hematological, and pathological changes of experimental FPL in SPF cats are mild and that recovery from the disease is very rapid.—**KEY WORDS:** feline panleukopenia, SPF cat.

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Feline panleukopenia (FPL), a fatal viral disease of young cats, is usually accompanied by clinical symptoms such as anorexia, diarrhea, pyrexia, dehydration or leukopenia.

These symptoms are seen typically in many cases of conventional cats infected naturally or experimentally with FPL virus. However, mild and non-fatal forms of the disease have been described by several workers including the authors on germ-free and specific pathogen-free (SPF) cats infected with this virus [1, 2, 8, 20, 21]. Our previous reports were concerned principally with a quantitative study on the virological and serological features of the infected animals. The present communication deals with clinical, hematological, and pathological findings of SPF cats infected experimentally with FPL virus.

MATERIALS AND METHODS

Ten SPF cats were obtained from the cat colony which was maintained by a barrier system in the Department of Laboratory Animal Science, Kitasato University (Table 1). They were free from *Toxoplasma gondii*, FPL virus, Reo-3 virus, feline viral rhinotracheitis virus, feline calicivirus, and *Salmonella*. The cats were bred in a completely disinfected isolator and fed sterilized bait and water throughout the observation period.

All the cats were inoculated subcutaneously with 0.5 ml of the Obihiro strain of FPL virus [7] which had a titer of about 10^4 median tissue culture infective dose per ml. Observation of clinical, hematological, and bone marrow changes was regularly performed for 3 to 17 days after inoculation.

Blood was collected from the jugular vein

and 0.2 ml of bone marrow was aspirated from the iliac crest. Hemagglutination inhibition (HI) titer was determined by the method of Goto [7].

For pathological examinations, the cats were sacrificed, and the liver, spleen, kidney, heart, lung, intestine, thymus, bone marrow, and mesenteric lymph node were fixed in 10% buffered formalin. Paraffin sections were made and stained with hematoxylin and eosin (H-E).

RESULTS

Clinical findings:

Table 1. Experimental cats

SPF Cat No.	Age	Sex	Sacrificed days p.i. ^{a)}
1	4 mo.	Male	13
2	4 mo.	Male	17
3	4 mo.	Male	3
4	4 mo.	Male	7
5	3 mo.	Female	11
6	4 mo.	Male	7
7	3 mo.	Female	7
8	2 mo.	Male	6
9	2 mo.	Male	5
10	1.5 mo.	Male	4

a) Inoculated subcutaneously with 0.5 ml of FPLV (10^3 TCID₅₀/0.1 ml).

Clinical symptoms appeared from 2 to 4 days postinoculation (DPI) and continued for 2 to 6 days. Cyanosis of the visible mucous membranes was found in all cases and slight diarrhea-like, soft feces were observed in 90% of the examined cases. Pyrexia (39.3 to 39.5°C) was seen in 40% of the cases, and the transient fever continued for 1 to 2 days. Depression (60%) and anorexia (30%) were also found.

HI titers increased rapidly at 3 to 5 DPI and elevated to a maximum at 7 DPI in almost all the cases examined.

Hematological findings (Table 2):

The mean erythrocyte counts before inoculation was $711 \pm 79 \times 10^4/\mu\text{l}$. It decreased starting the next day after inoculation and showed a minimum value $583 \pm 53 \times 10^4/\mu\text{l}$ on 11th DPI. The values increased rapidly and returned to the preinoculation level on 13th to 15th DPI. Regeneration of erythrocytes accompanied with an increase of reticulocytes was seen in the 7 cases.

The mean thrombocyte count before inoculation was $29 \pm 5 \times 10^4/\mu\text{l}$. It showed a minimum value ($17 \pm 5 \times 10^4/\mu\text{l}$) on 9th DPI and then it returned to the normal range.

The mean leukocyte count before inoculation was $17,033 \pm 3,445/\mu\text{l}$. The value decreased from the 3rd DPI in all the cases and showed a minimum value

Table 2. Hematological findings of SPF cats inoculated with FPLV

Days p.i.	Erythrocyte ($\times 10^4/\mu\text{l}$)	Thrombocyte ($\times 10^4/\mu\text{l}$)	Leukocyte ($/\mu\text{l}$)	Differential leucocyte count ($/\mu\text{l}$)			
				Neutrophil	Lymphocyte	Eosinophil	Monocyte
0(10) ^{a)}	$711 \pm 79^b)$	29 ± 5	17033 ± 3445	9651 ± 2486	3618 ± 1846	2797 ± 1607	398 ± 277
1(10)	677 ± 120	34 ± 15	17043 ± 5613	11546 ± 4199	2734 ± 1473	2212 ± 1097	531 ± 188
3(10)	660 ± 103	26 ± 12	12940 ± 6098	8879 ± 4686	1114 ± 729	1347 ± 1164	248 ± 209
5(8)	640 ± 78	22 ± 9	10403 ± 2126	8533 ± 2440	1049 ± 546	596 ± 419	290 ± 143
7(6)	618 ± 57	24 ± 8	15479 ± 6424	12745 ± 7348	2360 ± 1301	797 ± 425	694 ± 288
9(3)	598 ± 123	17 ± 5	20133 ± 6195	16499 ± 4976	2128 ± 953	936 ± 240	740 ± 498
11(3)	583 ± 53	21 ± 4	22033 ± 1793	17672 ± 1162	3142 ± 936	891 ± 697	735 ± 425
13(2)	674 ± 45	35 ± 10	23000 ± 6300	19900 ± 2527	3575 ± 820	469 ± 322	712 ± 503
15(1)	743	34	23200	17914	4100	823	627

a) Number of cats tested in parenthesis.

b) Mean $\pm$ S.D.

Table 3. Changes of myelogram in SPF cats inoculated with FPLV

Days p.i.	0(10) ^{a)}	1(10)	3(10)	5(8)	7(6)	9(3)	11(3)	15(1)
Total nucleated cells ($\times 10^3/\mu l$)	146 $\pm$ 38 ^{b)}	167 $\pm$ 96	90 $\pm$ 42	44 $\pm$ 8	149 $\pm$ 74	142 $\pm$ 66	174 $\pm$ 62	160
Myeloblasts	0.73 $\pm$ 0.23	0.66 $\pm$ 0.40	0.60 $\pm$ 0.38	0.20 $\pm$ 0.16	3.70 $\pm$ 3.55	0.62 $\pm$ 0.41	0.46 $\pm$ 0.01	0.64
Promyelocytes	1.61 $\pm$ 1.27	1.38 $\pm$ 1.78	0.52 $\pm$ 0.24	0.35 $\pm$ 0.24	0.95 $\pm$ 0.91	0.56 $\pm$ 0.07	0.87 $\pm$ 0.16	0.64
Mytocytes	15.4 $\pm$ 14.4	14.9 $\pm$ 8.90	7.11 $\pm$ 3.00	4.00 $\pm$ 3.07	28.7 $\pm$ 24.8	11.2 $\pm$ 1.19	12.6 $\pm$ 3.86	9.64
Neutrophils	49.9 $\pm$ 12.1	58.8 $\pm$ 34.9	34.3 $\pm$ 14.8	23.6 $\pm$ 6.24	62.1 $\pm$ 2.41	58.1 $\pm$ 16.0	59.3 $\pm$ 9.56	87.7
Eosinophils	6.87 $\pm$ 4.66	4.13 $\pm$ 1.80	2.12 $\pm$ 2.50	1.41 $\pm$ 1.65	1.71 $\pm$ 0.67	2.62 $\pm$ 0.28	3.61 $\pm$ 2.02	1.93
Basophils	0.36 $\pm$ 0.23	0.82 $\pm$ 0.76	0.26 $\pm$ 0.10	0.13 $\pm$ 0.14	0.37 $\pm$ 0.24	0.62 $\pm$ 0.30	1.72 $\pm$ 1.55	1.92
Proerythrobl.	1.04 $\pm$ 0.76	1.88 $\pm$ 1.82	0.74 $\pm$ 0.64	0.22 $\pm$ 0.20	3.64 $\pm$ 3.31	0.83 $\pm$ 0.41	1.91 $\pm$ 1.38	1.93
Erythrobl. Baso.	4.76 $\pm$ 1.99	8.19 $\pm$ 7.37	2.72 $\pm$ 1.82	1.18 $\pm$ 0.48	9.80 $\pm$ 7.09	1.06 $\pm$ 0.86	8.36 $\pm$ 3.84	7.39
Erythrobl. Poly. & Ortho.	36.0 $\pm$ 10.5	43.2 $\pm$ 23.0	29.6 $\pm$ 19.1	8.30 $\pm$ 4.47	41.3 $\pm$ 18.9	54.5 $\pm$ 35.7	54.5 $\pm$ 34.6	32.4
M/E ratio	2.01 $\pm$ 0.69	1.55 $\pm$ 0.36	1.72 $\pm$ 0.72	4.27 $\pm$ 2.50	1.41 $\pm$ 0.70	2.16 $\pm$ 1.30	1.55 $\pm$ 0.62	2.45
Lymphocytes	23.7 $\pm$ 9.45	32.2 $\pm$ 24.6	10.5 $\pm$ 3.49	4.36 $\pm$ 2.22	14.2 $\pm$ 3.53	15.4 $\pm$ 8.37	17.3 $\pm$ 12.7	15.4
Mitotic cells	0.39 $\pm$ 0.08	0.95 $\pm$ 0.46	0.37 $\pm$ 0.08	0.07 $\pm$ 0.06	1.20 $\pm$ 1.13	1.84 $\pm$ 1.13	0.82 $\pm$ 0.11	0.38
Reticulum cells	0.15 $\pm$ 0.11	0.53 $\pm$ 0.35	0.54 $\pm$ 0.30	1.37 $\pm$ 0.79	1.01 $\pm$ 0.53	0.64 $\pm$ 0.29	0.37 $\pm$ 0.18	0.37
Degenerated & Unclassified cells	0.32 $\pm$ 0.28	0.34 $\pm$ 0.43	0.26 $\pm$ 0.09	0.15 $\pm$ 0.14	0.21 $\pm$ 0.14	0.33 $\pm$ 0.10	0.08 $\pm$ 0.13	0

a) No. of cats tested in parenthesis.

b) Mean $\pm$ S.D.

Table 4. Frequency of Lesions in 10 SPF cats Inoculated with FPLV

Pathology	Frequency
Small intestine	
Degeneration of villi	10% (1/10)
Dilatation of crypt	30% (3/10)
Inclusion body	40% (4/10)
Mesenteric lymph node	
Depletion of lymphocytes	100% (10/10)
Dilatation of sinus	100% (10/10)
Activation of RES	100% (10/10)
Inclusion body	60% (6/10)
Spleen	
Depletion of lymphocytes	100% (10/10)
Activation of RES	100% (10/10)
Inclusion body	50% (5/10)
Bone marrow	
Depletion of nucleated cells	20% (2/10)
Inclusion body	20% (2/10)
Thymus	
Atrophy of lobules	80% (8/10)
Depletion of lymphocytes	80% (8/10)
Inclusion body	30% (3/10)

($10,403 \pm 2,126/\mu\text{l}$) on the 5th DPI. However, it increased from 5th to 9th DPI, and leukocytosis was observed at 11th to 15th DPI. None of the cats showed leukocyte count less than $6,000/\mu\text{l}$ in all the cases.

Leukopenia in the present experimental cats was caused mainly by a decrease in the number of lymphocytes and neutrophils. Eosinophils and monocytes were also decreased. The mean neutrophil count before inoculation was $9,651 \pm 2,486/\mu\text{l}$. It revealed a minimum value ($8,533 \pm 2,440/\mu\text{l}$) on 5th DPI followed by a rapid increase with clear neutrophilia on 7th DPI. Lymphocyte count before inoculation was $3,618 \pm 1,846/\mu\text{l}$ in mean value. It showed a minimum value ($1,049 \pm 549/\mu\text{l}$) on 5th DPI and then returned to the normal range. Eosinophils and monocytes showed minimum values on 3rd to 5th DPI, respectively. However, basophils did not show significant changes. *Findings on aspirated bone marrow (Table 3):*

Fig. 1. Ileum (Case No. 9). Slight cryptitis and intranuclear inclusion bodies in cryptal epithelial cells are seen. H-E, $\times 396$.

The mean total nucleated cell count was $146 \pm 38 \times 10^3/\mu\text{l}$. It showed a minimum value ($44 \pm 8 \times 10^3/\mu\text{l}$) on 5th DPI. After that its rapid recovery was observed. Myeloid cells also showed a minimum value as did total nucleated cells on the same day. These findings were characterized especially by decrease of myeloblasts and promyelocytes. From 7th DPI, however, the number of these cells increased rapidly and returned to the normal range. In the erythroid series, decrease of immature cells was noted. It showed a minimum value on 5th DPI: thereafter, it should a recovery pattern. Marked decrease of lymphocytes as low as $4.36 \pm 2.26 \times 10^3/\mu\text{l}$ was recognized on 5th DPI in contrast to the preinoculation value of $23.7 \pm \times 10^3/\mu\text{l}$. After that a recovery pattern was observed.

Fig. 2. Mesenteric lymph node (Case No. 3). Marked decrease of lymphocytes in lymph follicle and intranuclear inclusion bodies in proliferating reticulum cells are seen. H-E, $\times 396$.

Fig. 3. Bone marrow (Case No. 9). Intranuclear inclusion bodies in reticulum cells. H-E, $\times 990$.

Pathological findings:

Atrophy of the thymus, swelling of the mesenteric lymph nodes and spleen were observed in almost all the cases investigated. No gross findings were recognized in other organs.

Histopathological changes and their frequencies are summarized in Table 4. The changes were recognized especially in lymphoid tissues, such as the mesenteric lymph nodes, thymus, and spleen, and also in the small intestines and bone marrow of some cases.

In small intestine, mild focal cryptitis in the jejunum and ileum was observed in 3 cases (Case Nos. 6, 9 and 10). In the affected crypt, the lumen was dilated and filled with necrotic debris. The crypt epithe-

lium was swollen and intranuclear inclusion bodies were often recognized (Case Nos. 3, 8, 9 and 10) (Fig. 1). In these cases, intranuclear inclusion bodies were also found in Peyer's patches.

In mesenteric lymph node, dilatation of lymph sinuses and depletion of lymphocytes were observed in every case. The dilated sinuses were filled with macrophages. Depletion of lymphocytes was observed in the area from follicular center to marginal zone. activation of the reticuloendothelial system (RES) was found in lymphatic follicles (Fig. 2) and intranuclear inclusion bodies were also observed in the cells in 6 cases (Case Nos. 3, 5, 6, 8, 9 and 10).

Depletion of splenic lymphocytes and activation of the RES in the white pulp were noted. Intranuclear inclusion bodies were

Fig. 4. Thymus (Case No. 1). Atrophy of lobules is marked and border between cortex and medulla is obscure. H-E, $\times 100$.

found in reticular cells of the white pulp in 5 cases (Case Nos. 3, 6, 8, 9 and 10).

Two cases (Case Nos. 2 and 3) showed a slight decrease of nucleated cells in the bone marrow. Intranuclear inclusion bodies were demonstrated in reticulum cells of 2 cases (Case Nos. 3 and 10) (Fig. 3).

Atrophy of thymic lobules was observed in 8 cases. In these cases, the border between cortex and medulla became obscure and marked depletion of lymphocytes in the cortex was observed (Fig. 4). Intranuclear inclusion bodies were also identified in reticular cells of 3 cases (Case Nos. 1, 9 and 10).

The findings described above were predominant, especially in the cases sacrificed on 7th DPI. The cells possessing acidophilic

intranuclear inclusion bodies had swollen and deformed nuclei.

No particular changes were recognized in other organs.

DISCUSSION

Severe clinical symptoms with high mortality as described in conventional cats [4-6, 9, 11-13, 19] were not observed in the present experimental FPL cats. Only slight soft feces and transient pyrexia were observed on 3rd to 7th DPI in some cases.

It was reported that hematological changes in the conventional cats infected with FPL virus were characterized by severe leukopenia, mainly granulocytopenia and lymphopenia [4, 5, 9, 12, 13, 17, 18]. In addition, thrombocytopenia was also reported by some workers [10, 11]. In the present study, slight leukopenia and thrombocytopenia, and decrease of erythrocytes were observed. Leukocytes started to decrease on the 3rd DPI and showed a minimum value on the 5th DPI. Leukopenia was caused by a decrease of granulocytes and lymphocytes.

Rohovsky *et al.* [20] suggested that lymphocytes may be the target cells for the virus in the experimental FPL of germfree cats, on the basis of the fact that lymphopenia occurred before neutropenia.

Descriptions concerning the erythrocytic changes in FPL virus infection of cats have been very few until now. Hammon and Ender [10] and Horvath *et al.* [11] reported a decrease of erythrocytes during the recovery stage and in chronic cases, and Riser [19] reported that leukopenia was followed by a decrease of erythrocytes. In the present study, erythrocytes decreased gradually from the first DPI and showed a minimum value on 11th DPI. Moreover, increase of reticulocytes was observed from 5th to 7th DPI. From the above findings, the authors determined that erythrocytes decrease, but

that the decrease coexists with a regenerative change characterized by an increase of reticulocytes, in FPL virus infection of SPF cats.

The hematological changes described above were also found by examination of the bone marrow. There have been only two reports by Lawrence *et al.* [17] and Ichijo *et al.* [12] on changes in aspirated bone marrow. Lawrence *et al.* [17] described a decrease and degeneration of myeloid cells and Ichijo *et al.* [12] clarified the hypoplasia of the myeloid cells, megakaryocytes, and erythroid cells in this disease. In the present study, hypoplasia of the bone marrow, mainly involving the myeloid and erythroid cells, was observed. However, the recovery was very rapid with an increase in HI titer. On the basis of the above findings, it is clear that leukocytic and erythrocytic cells are target cells for FPL virus in SPF cats.

On the other hand, it is known that degenerative change with intranuclear inclusion bodies in the small intestine especially in the jejunum and ileum, and hypoplasia of hematopoietic organs such as the bone marrow, mesenteric lymph nodes, spleen, and thymus are noted in histopathological examination in FPL virus infection of conventional cats.

Also in the present study using SPF cats, decrease and hypoplasia of lymphocytes, and appearance of intranuclear inclusion bodies in reticulum cells were recognized in lymphoid tissues, such as the spleen, mesenteric lymph nodes and thymus. However, only mild cryptitis and intranuclear inclusion bodies in the cryptal epithelium of small intestine were recognized in some cases autopsied on 7th DPI. The intestinal lesions in SPF cats were very mild compared with those of conventional cats [4-6, 9-13].

It is suggested that severity of intestinal alterations plays an important role in worsening the clinical symptoms and is a major cause of death in FPL virus infection

of conventional cats [13]. In addition, Rohovsky *et al.* [20, 21] and Johnson *et al.* [14] suggested that secondary infection with enterobacter was important for appearance and progress of intestinal changes in experimental FPL virus infection of germfree cats, since characteristic alteration by FPL virus was observed in lymphoid tissues but not in the intestine.

Carlson *et al.* [1-3] reported that intestinal alterations were very slight in germfree cats. Furthermore, Kirk [15] reported that cases with decrease of leukocytes less than 1,000/ μ l revealed severe secondary infection, while decrease of leukocytes in germfree cats were 3,000 to 4,000/ μ l.

Ichijo *et al.* [12, 13] had already made it clear that experimental FPL virus infection in conventional cats showed severe clinical, hematological, and pathological changes similar to those of natural infection cases. In the present study, FPL virus was isolated with a high frequency from the mesenteric lymph nodes, bone marrow, and small intestine. Hematological and intestinal changes, however, were slight and the recovery was rapid without notable clinical symptoms. These findings indicate that sensitivity for FPL virus in SPF cats is lower than that of conventional cats. Also, in conventional cats, the existence of pathogenic enterobacter may be one of the worsening factors for clinical symptoms and intestinal lesions.

Further studies are needed to reveal the relationship of pathogenic enterobacter and FPL virus infection.

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要 約

ネコ汎白血球減少症ウイルス接種 SPF ネコにおける臨床・血液・病理学的所見：細川 暁，一条 茂，後藤 仁¹⁾（帯広畜産大学獣医学科家畜内科学教室，¹⁾帯広畜産大学獣医学科家畜微生物教室）——10頭の SPF ネコにネコ汎白血球減少症ウイルスを接種し，臨床・血液・病理学的検索を実施した。ウイルス接種後の主な臨床症状は，可視粘膜のチアノーゼ，軟便，一過性の軽度な発熱，元気・食欲の減退などであったが，いずれも軽症で，死亡例はなかった。血液所見では，主としてリンパ球と好中球の減少を認めたが，白血球数の最低値は 6,000/ μ l 以上であった。骨髓液の所見では，顆粒球系細胞と赤芽球系細胞の低形成が認められ，とくに幼若細胞の減少が特徴的であった。病理組織学的には，リンパ系組織の変化が明瞭で，脾臓・腸間膜リンパ節におけるリンパ球の減少と網内系の活性化，および胸腺における萎縮とリンパ球の減少が特徴的であった。核内封入体は，リンパ系組織の細網細胞と小腸の陰窩上皮細胞に認められた。