Interleukin 1 Alpha mRNA-Expressing Cells on the Local Inflammatory Response in Feline Infectious Peritonitis

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ABSTRACT. By *in situ* hybridization with biotinylated human interleukin 1α (IL-1α) cDNA probe, distribution of feline IL-1α mRNA-expressing cells was examined in the tissues from 49 cases diagnosed as feline infectious peritonitis (FIP) by pathological examination. IL-1α mRNA-expressing cells were found in visceral peritoneum, lymphoid organs, liver, kidney, pancreas, digestive tract, lung, pleura, brain, palpebral conjunctiva, and bone marrow. Hybridization signals for IL-1α mRNA were mostly located in the local inflammatory lesions on the serosal surface of various organs and omentum, which were frequently involved in the lesions of FIP (27.8 ± 5.1 cells/mm^2). Morphological examination suggested that they were infiltrated macrophages. However, few IL-1α mRNA-expressing macrophages were in the lesions of other organs. These data suggested that IL-1α produced from macrophages in the local inflammatory sites might participate in the initiation and development of the lesions on the visceral peritoneum in FIP.—KEY WORDS: distribution, FIP, IL-1α, *in situ* hybridization, mRNA.

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Feline infectious peritonitis (FIP), coronavirus infection in cats, can be characterized by severe inflammatory response in various organs. This inflammation is considered to be caused by the deposition of FIP virus (FIPV) antigen-antibody complexes and complement leading to Arthus-type reaction [23, 24, 34, 40, 41]. It is also known that inflammatory cytokines including interleukin 1 (IL-1), IL-6, and tumor necrosis factor (TNF) are essential mediators in the development of various inflammatory response *in vivo* [2, 10, 11, 26, 27, 31–33]. IL-1 can be divided into two forms, IL-1α, and IL-1β, which have similar biological effects and share the same receptor on the target cells [10, 27, 31, 32]. Since IL-1α as well as IL-1β orchestrated immune and inflammatory responses [4, 12, 28], attention was focused on the role of IL-1α in the regulation of immune and inflammatory responses. The previous studies demonstrated the increase of inflammatory cytokines in the serum and ascites in cats affected with FIP [17, 18], and suggested that IL-1α might be associated in the development of inflammatory response in FIP. However, there is little evidence that IL-1α is involved in the development of local inflammatory responses in cats affected with FIP. In the present study, therefore, we demonstrated the distribution of IL-1α mRNA-expressing cells in FIP affected cats by *in situ* hybridization (ISH) with biotinylated human IL-1α cDNA probe to explore the relation of IL-1α to the pathogenesis of FIP.

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MATERIALS AND METHODS

Preparation of biotinylated probes: Human IL-1α cDNA probes (1.6 Kb, GC: 36%, Eco RI to Hind III fragment) [15, 16] kindly provided by Dr. M. Yamada were labeled by photobiotin [14]. Autoclaved (121°C, 60 min) ultra-pure water was treated with 0.01% diethylpyrocarbonate (DEPC) (Sigma, St Louis, MO, U.S.A.) to remove contaminating RNase. This DEPC treated water was used throughout this experiments.

Preparation of tissues and sections: Clean slides were coated with 0.01% poly-L-lysine (Product No. P8920, Sigma, St Louis, MO, U.S.A.) and then heated at 180°C for 3 hr to remove contaminating RNase [20]. Autopsies were carried out in 3 to 24 hr after death. Autopsied all organs of 49 FIP affected cats were obtained during the past 10 years at the Department of Veterinary Pathology, the University of Tokyo. Examined cases were divided into two type of FIP, 45 effusive type of FIP (Abdominal type: 40, Abdominal and thorax type: 5) and 4 non-effusive type of FIP. Control samples were taken from three healthy cats (FIP titer $< 1/160$). All autopsied organs were fixed with neutral buff-

ered formalin (pH 7.2) for 48 to 72 hr, dehydrated, embedded in paraffin, and sectioned at 4 μm for ISH. Slides with sections were heated at 60°C for 12 hr [20].

ISH: Feline IL-1α mRNA was stained by a method as previously described [20]. In brief, sections which had been deparafinized and rehydrated with RNase-free reagents treated with DEPC were digested with proteinase K (50 μg/m*l*) (Sigma, St Louis, MO, U.S.A.) for 30 min at 37°C, and then treated with 0.3% H_2O_2 in methanol for 30 min at 25°C. Each individual section was incubated with 20 to 50 μl of hybridization buffer containing 50% deionized formamide, 10% dextran sulfate, 5x standard saline citrate (SSC, 1xSSC is 0.15 M NaCl plus 0.015 M sodium citrate), 300 μg/m*l* of salmon testis DNA and 200–400 ng/m*l* of biotinylated probe DNA. Slides were incubated at 80°C for 15 min within a moist box, and subsequently hybridized at 37°C for 18 hr. After hybridization, slides were treated with 5 min wash with 2xSSC at 25°C, 10 min wash with 2xSSC at 37°C, 5 min wash with 2xSSC at 25°C, 2 min wash with 0.1% Triton X-100 in phosphate buffered saline (PBS, pH 7.4) and finally rinsed with PBS. Detection was carried out with streptavidin biotinylated peroxidase complex. Sections were counterstained with methylgreen, covered with coverslide. All sections were examined by a light microscope. Number of IL-1α mRNA-expressing cells per square millimeter was counted with the england finder. As a control experiment, before hybridization serial sections were treated with 50 μg/m*l* RNase A and 1 μg/m*l* RNase T_1 in 2xSSC for 30 min at 37°C and hybridized by a same procedure as described above.

RESULTS

IL-1α mRNA-expressing cells were detected in 42 of the 45 effusive type of FIP (Abdominal type: 37 cases, Abdominal and thorax type: 5 cases) and in 3 of the 4 non-effusive type of FIP. There was no significant difference on the distribution of IL-1α mRNA-expressing cells between effusive and non-effusive type of FIP. Frequent hybridization signals could be found in the inflammatory lesions on the serosal surface of varied organs and omentum, which were frequently involved in the lesions of FIP (Table 1). As shown in Fig. 1, infiltrated macrophages expressing IL-1α mRNA were localized in the inflammatory response on the mesenterium. A

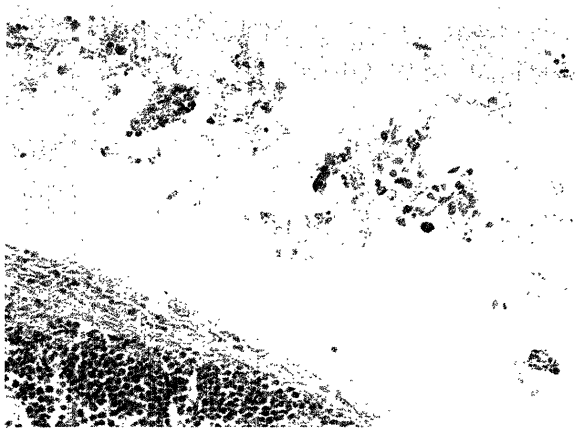

Fig. 1. *In situ* hybridization analysis of feline IL-1α mRNA expression on the mesenterium. Higher number of IL-1α mRNA-expressing cells is localized in the inflammatory reaction on the mesenterium. IL-1α mRNA is in the cytoplasm of infiltrated macrophages (×40).

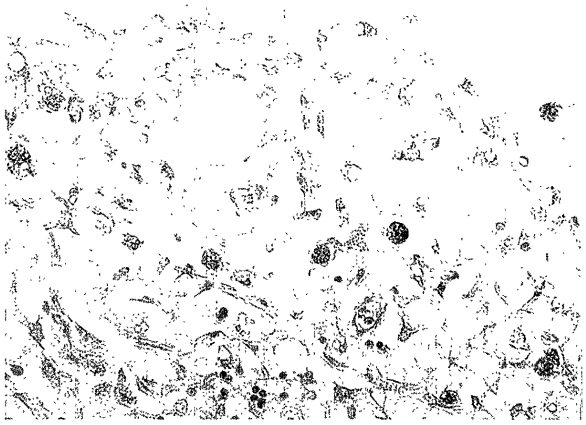

Fig. 2. *In situ* hybridization analysis of feline IL-1α mRNA expression on the omentum. A large number of IL-1α mRNA-expressing cells can be seen in the inflammation on the omentum. Most hybridization signals are localized in the cytoplasm of infiltrated macrophages (×100).

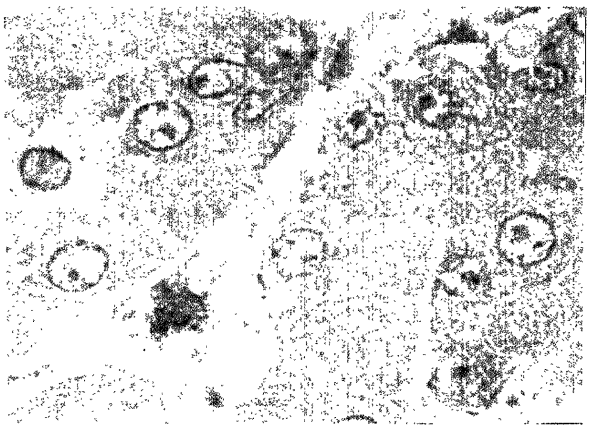

Fig. 3. IL-1α mRNA-expressing Kupffer cells is observed in the liver in cats affected with FIP (×250).

Table 1. Feline IL-1α mRNA-expressing cells in FIP

Tissues	Signals	Number of positive cells (Mean±S.E./mm^2)	IL-1α mRNA-expressing cells
Brain	+[a)]	6.4±2.2	Vascular endothelium*, Macrophages on the meninges, Ependymocytes
Pituitary	−[b)]	0.0±0.0	
Eye	+	3.4+1.9	Epithelium of palpebral conjunctiva
Thyroid	−	0.0±0.0	
Pleura and lung	+	0.8±0.3	Infiltrated macrophages
Heart	−	0.0±0.0	
Liver	+	1.0±0.5	Kupffer cells*
Spleen	+	0.5±0.2	Reticuloendothelial cells and macrophages in the red pulp*
Lymph nodes	+	0.9±0.3	Reticuloendothelial cells and macrophages in the sinuses*
Pancreas	+	0.9±0.5	Infiltrated macrophages
Kidney	+	0.4±0.2	Infiltrated macrophages
Adrenal	−	0.0±0.0	
Uterus	−	0.0±0.0	
Digestive tract	+	1.0±0.5	Infiltrated macrophages
Visceral peritoneum including omentum	+	27.8±5.1	Infiltrated macrophages, Vascular endothelium
Bone marrow	+	0.3±0.3	Interstitial cells

a) +=Detected. b) −=Not detected. *=Detected in healthy control.

large number of IL-1α mRNA-expressing macrophages were also detectable in the inflammatory lesions on the omentum (Fig. 2). However, few IL-1α mRNA-expressing cells could be observed in the pyogranulomatous lesions with many degenerated neutrophils on the visceral peritoneum. Additionally, no hybridization signals were localized in the cytoplasm of infiltrated neutrophils.

Few IL-1α mRNA-expressing cells could be found in the other organs. Infrequent positive hybridization was associated with microvascular endothelial cells in the omentum with thick layer of fibrin adherent and severe inflammation. In the liver, hybridization signals were found in some Kupffer cells (Fig. 3). Hybridization signals were located in the cytoplasm of reticuloendothelial cells and macrophages in the red pulp of spleen, and in the sinuses of lymph nodes. In the kidney, positive infiltrated macrophages were found in the medullary and/or cortical areas, whereas no hybridization signals were observed in the mesangial cells. In severe granulomatous lesions on the serosa and parenchyma of pancreas, hybridization signals could be seen in the foamy macrophages and mesothelial cells. In the digestive tract, IL-1α mRNA-expressing cells were found in the mucosal layer and/or muscular layer, and serosal surface. Very few positive cells could be seen in the visceral pleura. In the brain, hybridization signals were localized in the cytoplasm of vascular endothelium. In addition, IL-1α mRNA was found in the ependymocytes and macrophages on the meninges. There were IL-1α mRNA-expressing cells in the epithelium layer at palpebral conjunctiva. IL-1α mRNA-containing interstitial cells were also located in bone marrow. Table 1 summarizes the distribution of IL-1α mRNA-expressing cells in tissues from cats with FIP. IL-1α mRNA-expressing cells observed in healthy cats were indicated by * in Table 1.

DISCUSSION

In the present study, the distribution of feline IL-1α mRNA in FIP affected cats was demonstrated by ISH with biotinylated cDNA probes. The positive cells containing hybridization signals could be detected on the tissues which were preserved for 10 years as paraffin blocks (5 of 7 cases). Hankin *et al.* [19] also reported that hybridized signals of mRNA from 10-year-archival paraffin blocks were detectable by ISH with biotinylated probes. These evidences suggested that fixed RNA was stable in the paraffin-embedded tissues at least for 10 years. Many IL-1α mRNA-expressing cells were found on various organs in cats affected with FIP as compared to healthy cats, indicating that IL-1α may contribute to the development of the disease or the augmentation of immune reaction under the diseased

condition [4, 10, 12, 26–28, 33]. In healthy control cats, few IL-1α mRNA-expressing cells were located in the brain, liver, spleen, and lymph nodes (* in Table 1). The number and distribution of positive cells were not significantly different in the liver, spleen, and lymph nodes, between FIP affected cats and healthy cats.

Hybridization signals detected in this experiment was limited, since hybridization signals might be influenced by the various factors, such as time from death to autopsy (3 to 24 hr in this study), temperature and time of fixation, sensitivity of proteinase K on each specimens, and the amount of IL-1α mRNA [3, 19, 20, 22, 31, 32, 36, 42]. However, quite similar pattern of the distribution for IL-1α mRNA was obtained in all autopsy specimens. Most positive cells were localized in the inflammatory response on the visceral peritoneum, whereas few IL-1α mRNA was found in the other organs. These findings were not surprising, because visceral peritoneum is frequently involved in the lesions of FIP, and previous reports demonstrated that IL-1 was a essential mediator in the initiation and development of inflammatory response [7, 10, 11, 26, 27, 31–33, 38, 39]. Some investigators also demonstrated by *in vivo* and *in vitro* study that IL-1α might be involved in the regulation of immune and inflammatory reaction [4, 6, 12, 28, 39]. These results indicated that IL-1α produced by macrophages in the local inflammatory sites might participate in the development of inflammatory response. The possible explanations for the role of IL-1α are as follows; 1) IL-1α may be an important mediator to activate vascular endothelium [5, 35], 2) IL-1α may regulate the production of chemotactic cytokines, such as IL-8 [10, 11, 27, 29, 31–33, 37], and may be an important chemotactic factor [10, 11, 25, 30–33], 3) IL-1α may activate mononuclear cells by more secretion of cytokines such as IL-6 and colony stimulating factor to develop the inflammatory reaction in the lesions [1, 2, 10, 11, 26, 31–33, 35].

IL-1 has been generally involved in the vasculitis [35] and all stages of inflammatory reaction [8, 10, 11, 26, 31–33]. However, few positive cells expressing IL-1α mRNA could be found in the lesions of vasculitis and chronic inflammation such as granulomatous reaction in the liver and kidney (data not shown). This different resolution may be due to the short life of IL-1 mRNA (< 24 hr) [9, 13, 21]. Since IL-1α might be synthesized in the limited period, IL-1α mRNA-expressing cells could not be found in these lesions. IL-1α might have a minor role in the maintenance of vasculitis and chronic inflammation in FIP [7, 8, 38].

The comprehension of the role of inflammatory cytokines should help us to understand the pathogenesis of FIP.

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