Feline Interleukin 1 Production Induced by Feline Infectious Peritonitis Virus

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ABSTRACT. Feline alveolar macrophages were inoculated with feline infectious peritonitis virus (FIPV). Cultured macrophages produced substantial amounts of interleukin 1 (IL-1) in the supernatants, as determined by C3H/HeJ mouse thymocyte proliferation assay. FIPV by itself did not affect this IL-1 assay system. IL-1 activity in FIPV-inoculated macrophage cultures was partially reduced by heating at 56°C for 30 min, and completely eliminated by heating at 70°C for 30 min. FIPV-induced feline IL-1 was found to have a molecular weight of approximately 15000 daltons and isoelectric points (pI) of 4.1, 4.8, 5.3 and 6.8, as estimated by Sephacryl S-200 gel filtration and chromatofocusing, respectively. These physicochemical properties of FIPV-induced IL-1 were similar to those of lipopolysaccharide (LPS)-induced IL-1 from feline alveolar macrophages. However, IL-1 with pI 6.8 was more prominent in FIPV-induced IL-1 than in LPS-induced IL-1. Taken together with our previous findings that high levels of IL-1 were produced by peritoneal exudate cells from cats with spontaneous effusive FIP, FIPV infection may trigger the IL-1 production in many kinds of macrophages.—KEY WORDS: feline infectious peritonitis, IL-1.


Feline infectious peritonitis virus (FIPV) frequently causes the systemic vasculitis with pyogranulomatous inflammation in feline species. These pathological changes appeared to be indirectly mediated by the inflammatory and immune responses. Humoral FIPV antibodies, which did not neutralize the virus, were proved to induce the development of peritonitis [21]. However, the immunopathological mechanism in the pathogenesis of feline infectious peritonitis (FIP) is not fully understood.

We recently reported the high levels of interleukin 1 (IL-1) production by peritoneal exudate cells from cats with spontaneous effusive FIP [11]. This suggested that several biological activities of IL-1, such as pyrogenicity [7], stimulation of B cells [17, 25] and induction of acute phase protein release [23], might be involved in the pathogenesis of FIP.

In the present study, whether FIPV induces IL-1 release in vitro from alveolar macrophages was examined.

MATERIALS AND METHODS

Virus: The virus was isolated from a 6-month-old female cat with spontaneous effusive FIP, and used for the present study. Feline macrophage-like cell line (fcwf-4) cells were used for the cocultivation of peritoneal exudate cells and for isolation of virus. Isolated virus was morphologically similar to coronavirus in electron microscopy. This isolate was identified to be FIPV using immunofluorescence, as described by Hayashi et al. [14].

Cell preparation: Feline alveolar macrophages were prepared from six healthy adult cats, which were less than 1:25 in anti-FIPV antibody titers, as described previously [10]. The cell preparations consisted of greater than 90% macrophages, as judged by a latex bead ingestion test.

 Supernatant preparation: Feline alveolar
macrophages \( (2 \times 10^6 \text{ cells/ml}) \) were inoculated with \( 5 \times 10^4 \) plaque forming unit viruses. After incubation for 90 min at 37°C, unadsorbed virus was removed by washing with phosphate buffered saline (PBS). The inoculated cells were then cultured for various times at 37°C in a 5% \( \text{CO}_2 \)-humidified atmosphere. In some experiments, a protein synthesis inhibitor, cycloheximide (Nakarai Chemicals, Ltd., Tokyo), was added to the culture at the concentrations of 10 and 50 \( \mu \text{g/ml} \).

**Heat treatment:** Macrophages were inoculated with FIPV for 48 hr. The resulting culture supernatants were heat-treated at 56°C or 70°C for 30 min, and then assayed for IL-1 activity.

**Gel filtration and chromatofocusing:** Molecular weight (m.w.) was analyzed with Sephacryl S-200 (Pharmacia Fine Chemicals, Uppsala, Sweden) gel filtration chromatography [10]. Isoelectric point (pI) was determined with chromatofocusing on Polybuffer Exchanger 94 (PBE94; Pharmacia), as reported previously [10].

**IL-1 assay:** For IL-1 determination, the thymocyte costimulation assay was carried out by using C3H/HeJ mouse thymocytes and 1 \( \mu \text{g/ml} \) of phytohemagglutinin (PHA; Wellcome Foundation, Ltd., Beckenham, England), as described previously [10]. In certain experiments, protease inhibitors, aprotinin (Sigma Chemical Co., St. Louis, MO) and soybean trypsin inhibitor (SBI Sigma), were added to murine thymocyte cultures, to avoid some possible effects of proteases on thymocyte proliferation. The results were expressed as the mean of count per minute \( \text{(cpm)} \) of triplicate cultures.

**Interleukin 2 (IL-2) assay:** IL-2 levels were determined with murine IL-2 dependent T cell line (CTLL-2) cells, as described previously [8, 9].

### RESULTS

The kinetics of IL-1 production by FIPV-inoculated macrophages is given in Fig. 1. Moderate levels of IL-1 activity were observed in the supernatants after incubation for 24 to 48 hr. High levels of activity were sustained until 96 hr. The accumulation of IL-1 activity in FIPV-inoculated macrophage cultures was completely inhibited by the supplementation with 10 \( \mu \text{g/ml} \) of cycloheximide.

In addition, the culture supernatants of FIPV-inoculated macrophages were tested for their sensitivity to protease inhibitors. As shown in Table 1, neither aprotinin (100
Table 2. Effects of heat treatment on FIPV-induced feline IL-1 activity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$^3$H-TdR incorporation$^a$ (cpm)</th>
</tr>
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<tbody>
<tr>
<td>Untreated</td>
<td>9910</td>
</tr>
<tr>
<td>Heated at 56°C for 30min</td>
<td>2764</td>
</tr>
<tr>
<td>Heated at 70°C for 30min</td>
<td>856</td>
</tr>
</tbody>
</table>

a) $^3$H-TdR incorporation (mean cpm) by thymocytes cultured with 4-fold diluted culture supernatants of FIPV-inoculated feline alveolar macrophages in the presence of PHA for 72hr is given. Background $^3$H-TdR incorporation induced by PHA alone was 967 cpm.

Fig. 2. Gel filtration on Sephacryl S-200 of culture supernatants of feline alveolar macrophages inoculated with FIPV. Two milliliters of 10-fold concentrated samples were applied to the column which had been calibrated with the following molecular weight markers; bovine serum albumin (67K), ovalbumin (43K), chymotrypsinogen (25K), myoglobin (17K) and cytochrome C (12.5K). Fractions of 4 ml were collected, and assayed for IL-1 activity at a 1:4 dilution in the presence of PHA.

![Graph showing gel filtration results](image)

induced PHA-stimulated mouse thymocyte proliferation.

As shown in Table 2, IL-1 activity in culture supernatants of FIPV-inoculated macrophages was reduced by heat-treatment at 56°C for 30 min, and completely eliminated by heat-treatment at 70°C for 30 min.

Size exclusion chromatography was performed using Sephacryl S-200 column. Fig. 2 indicated a peak of IL-1 activity in the fraction with a m.w. ranging from 12000 to 20000 daltons. Sephacryl S-200 column-purified IL-1 was analyzed with chromatofocusing. This partially purified IL-1 consisted of four different isoelectric points; 4.1, 4.8, 5.3 and 6.8 (Fig. 3). These fractions showed no IL-2 activity, as determined by the CTLL-2 cell proliferation assay.

DISCUSSION

It has been suggested that viruses as well as bacteria induce IL-1 production, since viral and bacterial infections frequently cause fever. However, only a few studies on virus induced IL-1 production have been reported [18, 24]. The results presented in
this report showed that FIPV is an efficient IL-1 inducer in feline alveolar macrophage cultures. These results also extended our previous observations that peritoneal exudate cells from cats with FIP spontaneously released high levels of IL-1 [11].

The kinetics of IL-1 elaboration by FIPV-inoculated macrophages revealed that IL-1 activity was detected at 48 hr and sustained a high level until 96 hr later. This kinetics profile was slightly different from the result of macrophages incubated with LPS in which macrophages stimulated with LPS showed maximum IL-1 activity between 12 and 24 hr [10]. The IL-1 production induced by FIPV was completely inhibited with cycloheximide, indicating that de novo protein synthesis was required for this IL-1 production.

Several serine proteases have been reported to mimic the thymocyte proliferation promoting activity of IL-1 [15]. In this experiment, however, protease inhibitors did not affect the thymocyte proliferative response to FIPV-inoculated macrophage culture supernatants. This finding suggests that thymocyte proliferation observed is not mediated by proteases.

The m.w. of FIPV-induced feline IL-1, estimated by Sephacryl S-200 gel filtration, was approximately 15000 daltons. This result was similar to the values of IL-1 released from LPS-stimulated feline alveolar macrophages [10] and from peritoneal exudate cells of cats with spontaneous effusive FIP [11]. Heat sensitivity of FIPV-induced IL-1 resembled the IL-1 described previously [10, 11].

Chromatofocusing of column-purified FIPV-induced feline IL-1 showed four different isoelectric points; 4.1, 4.8, 5.3 and 6.8. The pH 6.8 IL-1 was more prominent in FIPV-induced feline IL-1 than in LPS-induced feline IL-1. In human and murine IL-1, two distinct genes encoding IL-1α (pI 5) and IL-1β (pI 7) were identified [2, 12, 19]. The pI 4.1, 4.8 and 5.3 IL-1 elicited by FIPV seemed to be identical to human and murine IL-1α which often show charge microheterogeneity [20]. The pI 6.8 IL-1 might be identical to human IL-1β. IL-1α and IL-1β are different in activities to resorb bone tissues [26], to activate T cells [1] and to induce platelet activating factors [6]. Furthermore, IL-1α and IL-1β are also different in localization in the cell [5] and in genomic expression by messenger RNA [16]. However, it is not fully understood whether IL-1α and IL-1β have different pathophysiological activities.

Macrophages are the major target of FIPV infection [27] and are thought to play an important role in the pathogenesis of FIP. Thus, it appears that the local production of IL-1 by macrophages infected with FIPV may be associated with the development of FIP. Biological effects of IL-1 on connective tissues and endothelial cells were already known [3, 4, 22], and these IL-1 activities might be critical in the development of inflammation. Continued efforts to elucidate the role of IL-1 in the development of vasculitis are necessary to understand more details of pathogenesis in FIP.

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IL-1 PRODUCTION INDUCED BY FIPV


要約

ネコ伝染性腹膜炎ウイルスによって誘発されたネコのインターロイキン1の産生：後藤孝俊・恩田千景・廣田好和・長谷川篤彦・友田勇（東京大学農学部家畜内科学教室）－ネコ伝染性腹膜炎（FIP）ウイルスに曝露されたネコ肺胞マクロファージ培養上清中のインターロイキン1（IL-1）活性をマウス胸腺細胞の増殖反応を用いて検索したところ、培養開始後48時間から96時間にかけて高い活性が認められた。IL-1は35℃、30分の熱処理で活性が低下し、Sephacryl S-200を用いたゲル通過で分子量約15000 daltonであることが明らかになった。これらの物理化学的性状はlipopolysaccharide（LPS）で誘発されたネコIL-1のそれと類似していた。クロマトフォーカシング法を用いて検索した結果、FIPウイルス誘発のネコIL-1の等電点は、4.1, 4.8, 5.3および6.8であり、前3者はヒトのIL-1αに、等電点6.8のIL-1はIL-1βに相当すると考えられた。FIPウイルス誘発のネコIL-1は、LPS誘発のIL-1に比べ、等電点6.8のIL-1の比率がより大であった。