Role of Circulating Antibodies in Feline Infectious Peritonitis after Oral Infection

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ABSTRACT. Apparent disease was experimentally produced by intragastric inoculation with feline infectious peritonitis (FIP) virus in 4 of 20 seropositive kittens, while all of 30 seronegative kittens had no signs of illness except for some enteritis after inoculation. Lesions in visceral organs as well as the serosa seemed to be produced depending upon the presence of high-titered serum antibody, while some other factors should be involved in the disease production. Lesions produced in the serosa and abdominal organs were characterized by fibrinous serositis with necrotic and pyogranulomatous vasculitis and lympho-reticular tissue necrosis. Viral antigen was detected within macrophages in those lesions by immunofluorescence or immunoperoxidase assay.

Pedersen and Boyle [10] and Weiss et al. [13–15] assumed that the primary infection of feline infectious peritonitis (FIP) might cause rather mild lesions in the gut or respiratory tract without any clinical signs. The same authors stated that apparently healthy and seropositive kittens became to have an overt disease after challenge with virulent FIP virus, suggesting that a typical effusive type of FIP might result from a complicated mechanism between the virus and host response within susceptible cells and tissues.

In our previous studies enteritis was experimentally produced in kittens after oral inoculation, suggesting that naturally occurring FIP is transmissible by this route of infection [4]. The purpose of the present study is to see possibility to cause serositis after oral infection and the role of circulating antibody in the disease production.

MATERIALS AND METHODS

FIP virus: Infective materials for inoculation were 10 to 50% (w/v) homogenates of infected mouse brain or feline liver and spleen tissues in phosphate buffered saline (PBS), pH 7.4. The homogenates were centrifuged at 3,000 rpm for 10 min, and 2 ml of the supernatant was introduced either intraperitoneally (i.p.) or into the stomach by a catheter.

Inoculum M was a 10% homogenate of infected suckling mouse brain at the 9th passage level containing approximately 10^4.5 LD_{50} for mice [5]. Inoculum F1 was a 10% homogenate of liver tissue from a cat that had been killed 4 days after i.p. inoculation with inoculum M. Inoculum F2 was a 10% homogenate of spleen tissue from a cat killed 8 days after i.p. inoculation with inoculum F1, while inoculum F3 was a 10% or 50% homogenate of liver tissue from a cat dead at 16...
days postinoculation with inoculum F2.

Animals and inoculation: Conventionally reared kittens 3 to 10 weeks of age weighing 350 to 1,200 g were divided into three groups. Of the first group consisting of 36 kittens being negative for antibodies by immunofluorescence [5], 6 and 30 were inoculated i.p. and intragastrically (i.g.), respectively, with inoculum M, F1, F2 or F3. Six kittens having received inoculum F3 were treated subcutaneously with 50 mg/kg cortisone acetate (Merck Japan, Tokyo).

Of the second group of 15 kittens having FIP-antibody, 2 and 13 were inoculated i.p. and i.g., respectively, with inoculum M, F1 or F3. The third group of 9 kittens having no FIP-antibody received i.p. 25 ml of ascites with an immunofluorescence staining titer of 1:25,600. The ascites was obtained from a natural case of FIP, and centrifuged at 3,000 rpm for 10 min, and the supernatant was heated at 56°C for 30 min. Two days later, 7 of them were inoculated i.g. with inoculum M, while 2 served as non-infected controls. Two days after transfer of the ascites, the recipients were shown to have an antibody titer of 1:25,600.

All cases were examined for histopathology, immunofluorescence and immunoperoxidase assay on 0.5 to 29 days postinoculation.

Histopathology: Tissues from main abdominal organs and the digestive tract were sampled and fixed in formol-PBS (pH 7.0), embedded in paraffin and stained with hematoxylin and eosin (HE).

Immunofluorescence: Cryostat sections were made from selected tissues and fixed in acetone for 10 min and subjected to treating with fluorescein-labelled anti-FIP virus feline antibody [4] at 37°C for 60 min, washed 3 times with PBS (pH 7.4) and mounted in glycerin. Serum antibody titers were measured by an indirect immunofluorescence using target sections of infected suckling mouse brain [5].

Immunoperoxidase assay: According to the method described by Nakane and Pierce [8], selected cryostat tissue sections were fixed in acetone for 10 min and treated with a 1:512 dilution of anti-FIP virus feline serum from a natural case showing an immunofluorescence titer of 1:25,600. After incubation at 37°C for 30 min sections were washed 3 times with PBS (pH 7.4) and treated with a 1:16 dilution of peroxidase-conjugated goat antibody against feline IgG (Chapel Laboratories, Cochranville, PA., U.S.A.) at 37°C for 30 min. After washing 3 times with PBS, sections were treated with freshly prepared 0.005% solution of 3,3'-diaminobenzidine plus 0.001% H2O2 in 100 ml 0.005M, pH 7.6 tris-buffer for 5 min, and then they were dehydrated and cleared in xylol and mounted.

Results

As shown in Table 1, 22 of 30 seronegative kittens, which were inoculated i.g. with inoculum M, F1, F2 or F3, had diarrhea 3 to 29 days postinoculation. All of them were shown to have enteric lesions with neither serosal nor visceral lesions. Even in 7 of 8 cases either inoculated with higher dose (50% homogenate) or treated with corticosteroid, no serosal lesions were produced, while they had enteric lesions.

Pathological findings of the enteric lesions in 22 cases were similar to those described previously [4]. In most cases mesenteric lymph nodes were enlarged and intestinal mucosa was edematous and hyperemic with dilated lumen containing mucous or watery contents. Histopathologically the enteric lesions were rather superficial and characterized
Table 1. Inoculation of seronegative kittens

<table>
<thead>
<tr>
<th>Inoculation</th>
<th>No. of cases</th>
<th>No. of cases with enteritis</th>
<th>No. of cases with peritonitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>i.g. 3</td>
<td>3(1)***</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>i.p. 2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>F1</td>
<td>i.g. 6</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>F2</td>
<td>i.g. 4</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>F3</td>
<td>i.g. 9</td>
<td>8(2)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>i.g.*** 6</td>
<td>5(1)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>i.g.*** 2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>i.p. 4</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

* See the text.
** Cortisone acetate (50 mg/kg, s.c.) shortly after inoculation.
*** 50% homogenate of inoculum F3.
**** Number of deaths on days 5 to 12 postinoculation in parenthesis.

by degeneration, hyperplasia and desquamation of epithelial cells with some infiltration of neutrophils, macrophages, lymphocytes and plasma cells in the tunica propia (Fig. 1). By immunofluorescence (Fig. 2) or immunoperoxidase assay, FIP virus specific antigen was detected in the cytoplasm of enterocytes.

The next experiment was conducted to see whether the FIP virus used was still capable of inducing a typical fibrinous serositis after i.p. inoculation. Six seronegative kittens were inoculated i.p. with inoculum M or F3. As shown in Table 1, apparent serositis (4 cases) and enteritis (1 case) were revealed at autopsy on days 4 to 14. Five to 10 ml viscous yellow-colored fluid with some fibrin was accumulated in the peritoneal cavity of the 4 cases. Grossly disseminated grayish white foci were seen on the peritoneum, omentum, mesenterium, liver, spleen and mesenteric lymph nodes.

As previously described [1], histopathology revealed disseminated foci of necrosis with pyogranulomatous inflammation, which were frequently produced in and around small vessels in the serosa, omentum, mesenterium, liver, spleen and mesenteric lymph nodes. The foci were characterized by accumulation of fibrin and necrotized tissue debris as well as vasculitis with infiltration of neutrophils, macrophages, lymphocytes and plasma cells (Fig. 3). By immunofluorescence or immunoperoxidase assay, lesions in the spleen, liver, omentum, mesenterium and mesenteric lymph nodes of all infected cases were positive for FIP virus antigen (Fig. 4).

In the subsequent experiment, 13 and 2 kittens having anti-FIP virus antibody were challenged i.g. and i.p., respectively, as shown in Table 2. After i.g. challenge, 6 of 13 cases developed gross enteric lesions with diarrhea on days 4 to 21, and 2 of them had also apparent serosal as well as parenchymatous lesions with 5 to 10 ml ascites. Immunofluorescence and immunoperoxidase assay revealed virus antigen within enterocytes as well as macrophages in the lesions except for one case. One of the 2 seropositive kittens exposed to i.p.challenge was found to have peritonitis with a small amount of ascites as well as superficial enteric mucosal lesions on day 7. After i.p. inoculation abdominal lesions were similar to those produced in the seronegative and i.p. inoculated cases, and histopathologically, the enteric mucosa was also affected.

To see possible role of antibodies in producing FIP-specific lesions, 7 kittens received i.p. injection of 25 ml ascites showing an immunofluorescence antibody titer of 1:25,600. They were then exposed to i.g. challenge infection with inoculum M, 2 days after antibody administration. Two kittens showed both peritonitis and enteritis and became depressive and emaciated with anorexia and diarrhea, and one of them died on
Table 2. Challenge inoculation of seropositive or antibody transferred kittens

<table>
<thead>
<tr>
<th>Inoculum*</th>
<th>Serum antibody tier at challenge</th>
<th>No. of cases</th>
<th>No. of cases with enteritis</th>
<th>No. of cases with peritonitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naturally seropositive</td>
<td>i.g.</td>
<td>1:400</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Naturally seropositive</td>
<td>i.g.</td>
<td>1:1,600</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Naturally seropositive</td>
<td>i.g.</td>
<td>1:1,600</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Naturally seropositive</td>
<td>i.p.</td>
<td>6:400</td>
<td>2</td>
<td>1(1)***</td>
</tr>
<tr>
<td>F1</td>
<td>i.g.</td>
<td>1:400</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>F1</td>
<td>i.g.</td>
<td>1:1,600</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>F3</td>
<td>i.g.</td>
<td>1:100</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Antibody transferred</td>
<td>i.g.</td>
<td>1:25,600**</td>
<td>7</td>
<td>3(1)</td>
</tr>
</tbody>
</table>

* See the text.
** Titer of transfused ascites (25 ml).
*** Number of dead cases on day 7 or 19 postinoculation in parenthesis.

day 19 (Table 2). Three kittens had enteric lesions, and 4 cases were shown to have neither enteric nor visceral lesions, when examined at 6 to 15 days postinoculation.

Gross and microscopic lesions of the cases with peritonitis were of typical fibrinous FIP having about 10 ml ascites. There was diffuse fibrinous exudate on the serosal surfaces of liver, spleen, and other abdominal organs. The intestinal mucosa was anemic and the lumen was empty. Histopathologically the enteric as well as abdominal lesions were similar to those observed in seropositive kittens after i.g. inoculation (Figs. 5, 6 and 7). FIP virus antigen was detected within enterocytes and macrophages by immunofluorescence or immunoperoxidase assay (Figs. 8 and 9). Two non-infected controls showed neither enteric nor abdominal lesions.

**DISCUSSION**

In experimental studies on FIP, the i.p. route of inoculation has been conventionally adopted. This route of infection, however, is hardly considered as that occurring in natural transmission of the disease among cats. In the present study experimental designs were focussed on oral inoculation which might reflect the port of entry in naturally occurring FIP infection.

After oral inoculation in seronegative kittens, viral antigen was detectable in enteric lesions of about 70% of infected cases. In these seronegative and orally infected cases, neither serosal nor visceral lesions were produced as described previously [4]. Association of corticosteroid or inoculation with high-titered virus failed to produce visceral lesions in seronegative kittens. In contrast, after i.p. inoculation, serositis as well as enteritis were readily produced in seronegative kittens, suggesting that the FIP virus used retained its tropism not only to enterocytes but also to macrophages and mesothelial cells [2, 9, 12, 16].

Pedersen et al. described [11] that daily feeding of FIP virus resulted in disease production in seronegative kittens and that typical effusive type of FIP was seen 20% of cases at about 5 to 14 weeks postinfection. In those cases, diseased kittens invariably had antibodies at the time of clinical onset. In our experi-
mament, some of the seronegative and i.g. inoculated kittens were shown to have antibodies at 2 or 3 weeks postinfection (data not shown). Furthermore, of naturally infected seropositive cases or antibody transferred cases only some cases developed typical effusive FIP after oral challenge of the virus, probably because the observation time in the present study might be too short to see the production of peritoneal lesions after i.g. inoculation. Even in antibody negative cases serosal lesions may have occurred after a long latent period as suggested by Pedersen et al. [11].

A marked disease enhancement has been shown after aerosol or i.p. challenge in antibody positive kittens but not in antibody negative kittens [10, 13–15], suggesting that other antibodies than neutralizing ones can be responsible for promoting infection of macrophages and vascular lesions in some seropositive or antibody-transferred cases. In these cases complexes of inoculated virus and antibody might be formed shortly after oral infection, causing generalized necrotizing and pyogranulomatous lesions being specific for FIP. In the effusive type FIP the presence of granular deposits of FIP virus antigen, IgG and C3 was demonstrated in macrophages possibly causing tissue damages in the serosa and organs [10, 13–15]. The IgG and C3 were previously shown to be precipitated in the glomeruli causing nephritis [3, 6, 7], but not in the present studies, while only FIP virus antigen was demonstrated in the cytoplasm of macrophages.

In conclusion, fibrinous serositis was produced in 4 of 20 seropositive or antibody transferred kittens which had been inoculated i.g. with FIP virus, suggesting that the circulating antibodies might play an important role in production of the serositis as well as parenchymatous organ lesions.

References


**EXPLANATION OF FIGURES**

**Figs. 1. and 2.** Seronegative and i.g. inoculated case killed on day 7 postinoculation.
Fig. 1. Desquamation of epithelial cells at the tip of fusing jejunal villi with mild polymorphonuclear and mononuclear cell infiltration in the tunica propria. HE stain. ×620
Fig. 2. Virus antigen within the cytoplasm of jejunal epithelial cells. Immunofluorescence. ×930.
Figs. 3 and 4. Seronegative and i.p. inoculated case killed on day 14 postinoculation.
Fig. 3. Necrotic foci and diffuse fibrin deposit with infiltration of polymorphonuclear and mononuclear cells on the capsule of the spleen. HE stain. ×620.
Fig. 4. Virus antigen within the cytoplasm of macrophages on the capsule of the spleen. Immunofluorescence. ×930.
**Figs. 5–9.** Antibody transferred and i.g. inoculated case dead on day 19 postinoculation.
Fig. 5. Desquamation of cecal epithelial cells with severe infiltration of polymorphonuclear and mononuclear cells. HE stain. ×930.
Fig. 6. Cellular infiltration and proliferation of fibroblasts on the cecal serosa. HE stain. ×620.
Fig. 7. Severe coagulative necrosis, focal infiltration of inflammatory cells and proliferation of fibroblasts in the liver. HE stain. ×620.
Figs. 8 and 9. Virus antigen within the cytoplasm of macrophages accumulated in and around an interlobular artery. Immunofluorescence (Fig. 8) and immunoperoxidase assay (Fig. 9). ×930.

**要 約**

経口感染によるネコ伝染性腹膜炎の病理発生における循環抗体の役割：林俊春、渡部嘉範、竹之内俊、藤原公策（東京大学農学部家畜病理学教室）——ネコ伝染性腹膜炎（FIP）ウイルスを抗体陰性ネコ30例の胃内に接種したときは全例に病変をみとめなかったが、抗体陽性ネコに接種すると、20例中4例に腹膜炎および腹腔内臓器病変がみられた。腹腔臓器および腹膜には線維素性腹膜炎、壊死性～化膿性～肉芽腫性血管炎およびリンパ細胞浸潤組織の壊死がみとめられ、病巣のマクロファージ細胞質には蛍光抗体法および酵素抗体法によってウイルス抗原が検出された。
ROLE OF ANTIBODY IN PATHOGENESIS OF FIP