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ANTIBODY-MEDIATED ENHANCEMENT OF DISEASE IN FELINE INFECTIOUS PERITONITIS: COMPARISONS WITH DENGUE HEMORRHAGIC FEVER*

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Abstract—Non-immune kittens passively immunized with feline serum containing high-titered antibodies reactive with feline infectious peritonitis virus (FIPV) developed a more rapid disease after FIPV challenge than did kittens pretreated with FIPV antibody-negative serum. Antibody-sensitized, FIPV challenged—kittens developed earlier clinical signs (including pyrexia, icterus, and thrombocytopenia) and died more rapidly than did non-sensitized, FIPV-challenged kittens. Mean survival time in sensitized kittens was significantly ($P < 0.05$) reduced compared to non-sensitized kittens (mean \pm SEM, 10.0 ± 0.6 days vs. 28.8 ± 8.3 days, respectively). Lesions induced included fibrinous peritonitis, disseminated pyogranulomatous inflammation and necrotizing phlebitis and periphlebitis. FIPV antigen, immunoglobulin G, complement (C3) and fibrinogen were demonstrated in lesions by immunofluorescence microscopy.

The pathogenesis of dengue hemorrhagic fever (DHF) in persons bears striking resemblance to that of FIP in experimental kittens. In both FIP and DHF, non-neutralizing antibody may promote acute disease by enhancement of virus infection in mononuclear phagocytes or by formation of immune complexes, activation of complement and secondary vascular disturbances.

Key words: Feline infectious peritonitis (FIP), dengue hemorrhagic fever (DHF), immunopathology, immunofluorescence, passive immunity

RÔLE DES ANTICORPS DANS L'AGGRAVATION DE LA PÉRITONITE INFECTIEUX FELINE (COMPARAISONS AVEC UNE FIÈVRE HÉMORRAGIQUE, LA DENGUE)

Résumé—Des chatons passivement immunisés par du sérum félin contenant un titre élevé en anticorps reagissant avec le virus de la péritonite infectieux feline ont développé une maladie plus rapide après injection virale (experimentale) que ceux ayant reçu un sérum dépourvu de ces anticorps. Les chatons sensibilisés par les anticorps et infectés par la virus (PIF) développent les symptômes plus rapidement (notamment de la fièvre, ictère et une thrombocytopenie) et meurent plus vite que les non-sensibilisés. La durée de survie moyenne chez les chatons sensibilisés est réduite significativement ($P < 0.05$) par rapport à celle des non sensibilisés (mean \pm SEM, 10.0 ± 0.6 jours vs. $28.8 \pm$ jours respectivement). Les lésions observées comprennent une péritonite fibrineuse, une inflammation pyogranulomateuse disséminée, et une phlébite et periphlébite nécrosante. L'antigène du FIPV, les immunoglobulines G, le fragment C3 du complément et le fibrinogène ont été révélés dans les lésions par immunofluorescence.

La pathogénie de la fièvre hémorragique dengue (DHF) chez l'homme montre une ressemblance étonnante avec celles de la PIF expérimentale chez les chatons. Dans les deux cas, les anticorps non neutralisants peuvent permettre une maladie plus forte par l'augmentation de l'infection par le virus des cellules phagocytaires, monoclées, par activation du complément et par des perturbations vasculaires secondaires.

Mots-clefs: Péritonite infectieuse feline, la fièvre hémorragique dengue, immunopathologie, immunofluorescence, immunité passive

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INTRODUCTION

Feline infectious peritonitis (FIP) is a contagious, highly fatal coronavirus disease of domestic and exotic cats [1-3]. Most cats naturally exposed to FIP virus (FIPV) develop antibody titers without showing clinical signs. Only a very small number of these infected cats will eventually (weeks to years later) develop a fatal systemic disease characterized by fibrinous peritonitis, pleuritis, or disseminated granulomata [4-6]. The reasons for virus dissemination in these previously exposed cats are unknown. The lesions in FIP are characterized histologically by generalized vasculitis or perivasculitis, fibronecrotizing and pyogranulomatous inflammation of serosal membranes and parenchymal organs, and mesothelial hyperplasia [2, 7-11]. These lesions are believed to result from immunologically-mediated tissue reactions in response to systemic virus infection [1, 2, 12-14]. Spontaneous FIP cases will frequently demonstrate marked hyperproteinemia (greater than 8-10 mg/100 ml), hypergammaglobulinemia, polyclonal gammopathy, and markedly elevated anti-FIPV serum antibody titers [3, 15, 16]. Circulating immune complexes* and renal glomerular deposits of immunoglobulin (Ig)G and complement (C3) [17] can be detected in cats with spontaneous or experimentally induced FIP.

Previous experiments in our laboratory demonstrated marked acceleration of disease in clinically normal, FIPV antibody-positive kittens after challenge with FIPV [12, 14]. Many of these kittens died after developing acute disseminated intravascular coagulation (DIC) and severe systemic vasculitis; FIP viral antigen and immunoglobulin G were demonstrated in hepatic lesions [12]. Additional studies demonstrated that healthy non-viremic seropositive kittens developed viremia and vascular lesions earlier and died more rapidly after FIPV challenge than did seronegative kittens [14]. These studies showed that FIPV persists in circulating mononuclear cells of infected kittens despite high levels of anti-FIPV antibodies. Other investigators recently reported enhancement of disease in FIPV-challenged kittens possessing naturally acquired or passively administered antibodies against FIPV [13]. These studies demonstrated FIPV, virus-bound IgG, and complement (C3) within macrophages in lesions, hypocomplementemia, and the occurrence of non-neutralizing serum antibodies in infected cats.

The immunopathological features of FIP in cats and the proposed pathogenesis of dengue hemorrhagic fever (DHF) in persons show striking similarities. The majority of cases of DHF occur as secondary infections in patients with pre-existing serum antibody to dengue viruses [18, 19]. It is postulated that upon reinfection with a heterologous serotype of dengue virus, the patient with DHF rapidly develops high titers of cross-reacting but non-neutralizing antibodies [20]. The formation of large amounts of virus-antibody complexes may then lead to activation of complement and a hemorrhagic shock syndrome [21, 22]. In DHF, as in FIP, virus replicates and persists in mononuclear phagocytes in the presence of non-neutralizing antibodies [23, 24]; circulating immune complexes [25, 26] and decreases in serum complement levels [21, 27] may occur; virus, antiviral antibody, and complement components are demonstrated within blood vessels or lesions [28]; and DIC may develop in certain cases [21, 29].

Although our previous experiments demonstrated enhanced disease in FIPV antibody-

* Horzinek, M. C. Pathogenesis of feline infectious peritonitis, seminar presented at Cornell University, Ithaca, New York, 16 June, 1980

positive cats re-exposed to FIPV, it still was necessary to show that this phenomenon is mediated by serum factors, i.e. antibodies reacting with FIPV. The purpose of the present study therefore was two-fold: to demonstrate an antibody-mediated enhancement of disease in experimental FIP, and to compare these findings with the immunopathologic features of DHF.

MATERIALS AND METHODS

Experimental design

Kittens were divided into three experimental groups as follows: one group of three kittens received cat serum containing a high titer of anti-FIPV antibodies prior to FIPV infection (antibody-sensitized group); a second group of four kittens received cat serum not containing anti-FIPV antibodies prior to infection (non-sensitized group); and a third group of two kittens received the FIPV antibody-positive serum but were not challenged with FIPV (serum controls). Infected and control kittens were monitored daily for fever and clinical signs. Infected kittens were bled 24 hr prior to infection and at 3–4 day intervals after infection for complete blood count (CBC), platelet count, bilirubin, and serum enzymes. FIPV serum antibody titers were determined for all kittens prior to infection and on postinoculation day (PID) 1, 7, 10, 14, and 21. Postmortem or necropsy examinations were performed on all infected and control kittens. Tissues were processed for histopathology and immunofluorescence microscopy.

Animals. Twelve-week-old specific-pathogen-free (SPF) kittens were obtained from a commercial breeder (Liberty Laboratories, Liberty Corners, New Jersey). The kittens were housed in standard Horsfall isolation cages and maintained at the Cornell Laboratory Animal Isolation Facility. Infected cats and non-infected controls were housed in separate isolation units.

Virus. The UCD-1 strain of FIPV provided by Dr. N. Pedersen, University of California at Davis, was passed three times in SPF cats in our laboratory and stored at -70°C as 1 ml aliquots of 50% liver homogenate. By previous titrations it was determined that each ml yielded approximately 100 cat infectious doses (ID_{100}). Cultures of liver homogenates for bacteria, mycoplasma, feline leukemia virus and other cytopathic feline viruses were negative.

Preparation of immune and non immune serum. A healthy 5-month-old kitten with an FIPV serum antibody titer greater than 1:1600 served as the immune serum donor. This animal was an SPF kitten who had seroconverted by natural exposure to FIPV-shedding contact cats and had remained asymptomatic. An SPF kitten whose serum was negative for FIPV antibodies served as the non-immune donor. Both kittens were bled at 2 week intervals. Blood was collected by jugular venepuncture into sterile glass tubes without anticoagulant and centrifuged at 700g for 10 min. The serum was withdrawn with a disposable pipette and stored at -70°C in sterile glass vials until use.

Passive transfer of serum and experimental infection. After mild sedation of all kittens with ketamine hydrochloride (Vetelar; Parke, Davis and Co., Detroit, Michigan, 10 mg/kg, given im) and atropine sulfate (Sussex Drug Co., Edison, New Jersey, 0.04 mg/kg, given im) the external jugular vein was catheterized with a 23-gauge needle (Butterfly Infusion Set, Abbott Hospitals Inc., North Chicago, Illinois). Serum was administered by slow intravenous injection and given at a dosage of 11 ml/kg per cat. Six hr after administration

of serum, the experimental kittens were inoculated intraperitoneally with 0.5 ml of FIPV-infected liver homogenate (approximately 50 ID₁₀₀). Control kittens were inoculated similarly with liver homogenates from an FIPV-negative cat.

Hematologic evaluations. For the CBC, platelet count, and serologic tests, 2.7 ml blood were collected into vacuum-evacuated tubes containing 0.06 ml 7.5% EDTA (K₃) or no anticoagulant (Vacutainer tubes, Becton Dickinson, Rutherford, New Jersey). Total erythrocyte and leukocyte counts were performed on an electronic cell counter (Coulter Counter Model Zf, Coulter Electronics, Hialeah, Florida). Packed cell volume was determined by the microhematocrit method. Differential leukocyte counts were made on Wright's stained blood smears. Platelet counts were performed by the direct count method with a commercially available blood dilution pipette (Unopette, Becton Dickinson and Co., Columbus, Nebraska) and phase contrast microscopy. Total bilirubin was determined by semi-automated methods (Coulter Chemistry, Coulter Electronics, Hialeah, Florida); direct and indirect bilirubin determinations were made by the unmodified Evelyn-Malloy procedure [30]. Serum activity of alanine aminotransferase (ALT) was determined by a commercially available kinetic photometric technique (Fisher Diagnostic, Fair Lawn, New Jersey). Serum activity of aspartate aminotransferase (AST) was determined colorimetrically with prepared substrates (Trans Ac Test Kit, General Diagnostics, Morris Plains, New Jersey). FIPV serum antibody tests were performed by the heterologous indirect fluorescent antibody test which uses the cross-reacting transmissible gastroenteritis virus of pigs as antigen [31].

Pathologic and immunopathologic studies. Complete post-mortem examinations were made on all infected kittens; control cats were necropsied on PID 28. Representative sections of all organs were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 6 µm, and stained with hematoxylin and eosin. Sections of liver, lung, mesenteric lymph node, omentum, and spleen were frozen by immersion in liquid nitrogen and stored at -70°C until they were cryostat-microtome sectioned and processed for immunofluorescence microscopy. Indirect fluorescent antibody techniques for detection of FIP viral antigen were performed essentially as described by Pedersen [5] except fluorescein isothiocyanate (FITC)-conjugated Staph Protein A (Pharmacia Fine Chemicals, Piscataway, New Jersey) replaced the FITC-conjugated rabbit anti-cat immunoglobulin G (IgG). The former binds specifically to the Fc region of IgG of a number of species [32]. For demonstration of IgG, complement (C3), and fibrinogen, acetone-fixed sections of each sample were overlaid with FITC-conjugated Staph Protein A, FITC-conjugated goat anti-dog C3 (lot No. 10183, Cappel Laboratories, Inc., Cochranville, Pennsylvania), or FITC-conjugated goat anti-human fibrinogen (lot No. S379, Miles Laboratories, Inc., Elkhart, Indiana), respectively. Previous pilot studies in our laboratory have demonstrated by immunofluorescence microscopy a cross-reactivity between canine and feline C3, and between human and feline fibrinogen. Companion samples of those stained with Protein-A were washed for 10 min in 0.1 M glycine-HCl buffer (pH 2.2) prior to staining. After incubation for 60 min at 37°C, the slides were washed three times in phosphate-buffered saline solution (PBSS) (0.1 M, pH 7.2) and counterstained for 10 min in PBSS containing a 1 to 500 dilution of Evans Blue. After staining, the slides were examined with an epifluorescent ultraviolet microscope (Dialux Microscope, Wetzler, Ernst Leitz Ltd., Midland Ontario, Canada).

Statistical analyses. Differences in survival times between antibody-sensitized and non-

sensitized kittens were tested for significance by the Mann-Whitney test (non-parametric method). Mean differences between the two groups' platelet counts were tested for significance by the Student's *t* test. Values were accepted as significant when $P < 0.05$.

RESULTS

Clinical signs

Antibody-sensitized kittens developed an initial rise in body temperature of 40.5 to 41.5°C on PID 1 (Fig. 1). The temperature remained elevated above 40°C for 7–9 days and dropped to subnormal temperatures of 37°C or less within 12–24 hr preceding death. Two non-sensitized kittens developed transient temperature elevations of 39.5–40.0°C on PID 2 and 8. Persistent temperatures of 39.7–40.0°C in non-sensitized kittens first occurred on PID 8 to 12. The persistent fevers were accompanied by anemia, icterus, dehydration, depression, weight loss, and pendulous abdomen. Icterus first occurred on PID 5 in sensitized kittens and PID 9 in non-sensitized kittens. Most infected kittens developed enlarged mesenteric lymph nodes and mild to moderate ascites. Mortality rate in both groups of infected kittens was 100%. Mean survival times were significantly ($P < 0.05$) decreased in sensitized kittens compared to non-sensitized kittens. Mean survival times \pm SEM were 10.0 ± 0.6 (range 9–11) days in sensitized kittens vs 28.3 ± 8.3 (range, 14–52) days in non-sensitized kittens. Control kittens did not develop fever or other clinical symptoms.

Hematologic and serologic changes

Sequential changes in CBC, platelet counts, total bilirubin, and serum enzymes of infected kittens are presented (Tables 1 and 2). Infected kittens developed a progressive,

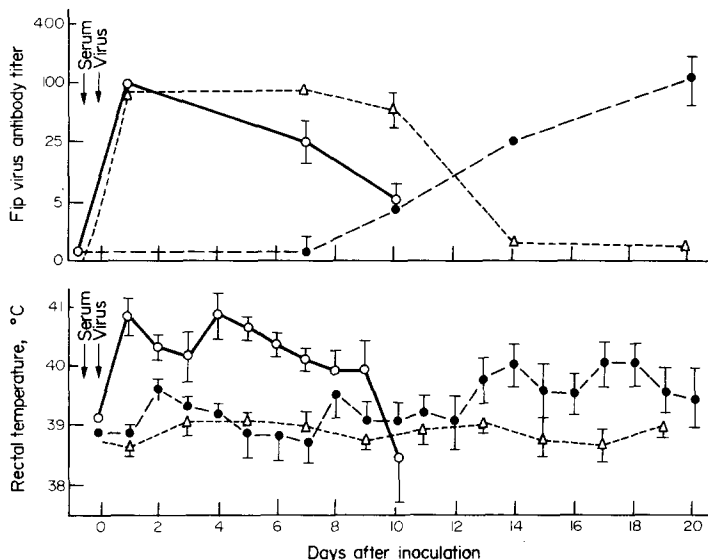


Fig. 1. Mean values (\pm SEM) of antibody titers (top graph) and rectal temperatures (bottom graph) in kittens inoculated with feline infectious peritonitis (FIP) virus. Kittens were given either FIP virus immune serum $\circ - \circ$ or non-immune serum $\bullet - \bullet$ 6 hr before virus inoculation. Control kittens $\triangle - \triangle$ were given immune serum but were not challenged with virus.

Table 1. Hematologic changes in kittens inoculated with feline infectious peritonitis virus

Day	Packed cell volume (%)	Hemoglobin (g/dl)	Erythrocytes ($10^6/\mu\text{l}$)	Leukocytes ($10^3/\mu\text{l}$)	Lymphocytes (%)	Platelets ($10^5/\mu\text{l}$)
Passively immunized kittens ($N=3$)						
0	29.3±0.7	10.3±0.2	7.3±0.4	26.4±2.5	46.3±10.2	3.8±0.3
3	24.3±1.3	8.4±0.5	5.9±0.6	8.9±2.4	26.7±3.0	2.6±0.3
7	22.0±1.2	7.6±0.3	5.4±0.5	4.5±1.1	13.3±4.1	1.1±0.2
10	24.3±0.7	7.6±0.1	5.7±0.3	8.0±1.8	5.0±0.6	0.9±0.4
Non-immunized kittens ($N=4$)						
0	28.3±0.9	9.6±0.2	7.3±0.4	24.1±4.0	36.5±8.5	4.4±0.7
3	24.5±1.2	8.3±0.3	6.0±0.3	14.7±2.1	13.0±4.5	4.9±0.1
7	24.0±2.2	8.3±0.5	5.8±0.5	6.2±1.4	31.0±6.6	3.1±0.5
10	20.3±1.3	6.9±0.3	4.9±0.3	5.4±0.7	22.5±10.1	2.3±0.7
14	19.5±2.0	6.6±0.6	4.7±0.4	7.5±1.8	9.8±4.2	3.4±1.4
17*	17.3±2.8	5.9±0.7	4.2±0.5	9.5±2.4	17.0±8.1	3.3±1.0
21*	18.0±3.0	6.3±0.7	4.0±0.6	8.1±3.7	7.0±6.0	2.0±0.7
Normal range†						
	24.0-45.0	8.0-15.0	5.0-10.0	5.5-19.5	20.0-55.0	3.0-8.0

Values expressed as the mean ± SEM.

* $N=3$.

† From Schalm, O. W., Jain N. C., *et al.* Veterinary Hematology, 3rd ed. Lea and Febiger, Philadelphia, 1975, p. 109.

Table 2. Serologic changes in kittens inoculated with feline infectious peritonitis virus

Day	Total bilirubin (mg/dl)	AST (Trans Ac Units)	ALT (I.U./l)
Passively immunized kittens ($N=3$)			
0	0.2±0.1	65.3±4.3	25.0±3.5
3	0.2	64.0±9.2	34.7±4.3
7	2.5±0.8	206.0±42.7	55.3±17.0
10	3.1±0.5	377.0±80.3	ND
Non-immunized kittens ($N=4$)			
0	0.2	84.8±11.9	51.3±22.8
3	0.2	91.8±14.4	59.3±21.1
7	1.0±0.2	264.8±22.8	164.0±25.6
10	1.3±0.4	344.5±77.0	179.3±42.3
14	1.5±0.8	323.0±163.8	134.3±12.9
17*	1.1±1.0	122.3±0.7	89.0±12.0
21*	1.3±0.5	211.7±63.2	93.7±23.0
Normal range†			
	0.2-0.4	32-58	10-50

Values expressed as the mean ± SEM.

* $N=3$.

† Range of values in random group ($N=100$) of clinically normal cats presented to the Small Animal Clinic, N.Y.S. College of Veterinary Medicine, Cornell University.

AST=aspartate aminotransferase.

ALT=alanine aminotransferase.

ND=not determined.

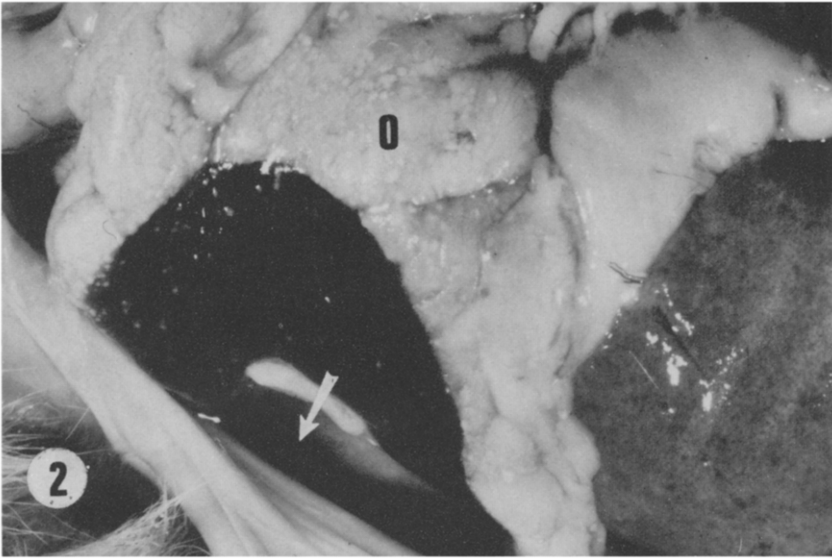


Fig. 2. Gross lesions in abdomen of a passively immunized kitten 9 days after inoculation with FIP virus. There are foci of necrosis in the spleen and omentum, marked ascites (arrow), and thickening of the omentum (O).

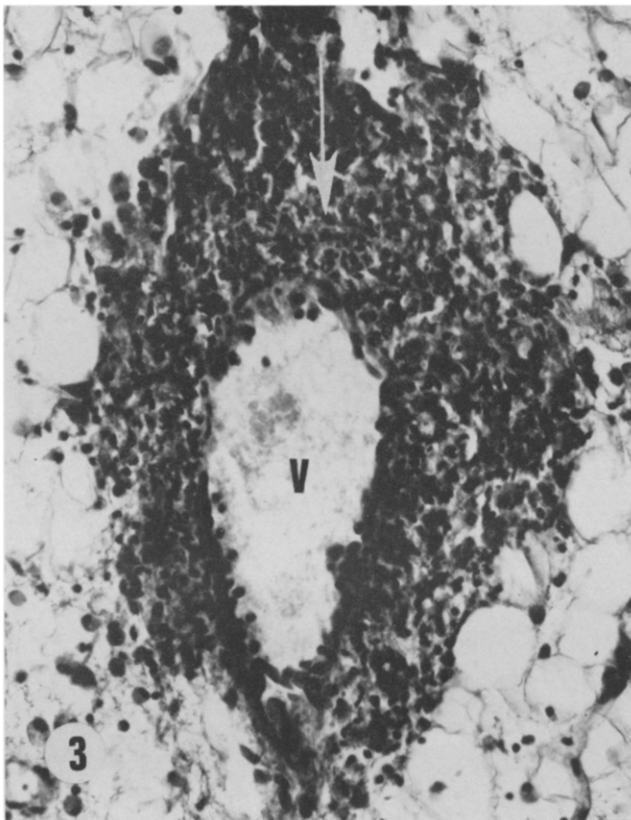


Fig. 3. Focal necrotizing phlebitis in the omentum of a passively immunized kitten on postinoculation day (PID) 9. There is marked infiltration by mixed inflammatory cells and foci of necrosis (arrow) in the wall and perivascular tissue of an omental vein (v) (hematoxylin-eosin, $\times 300$).

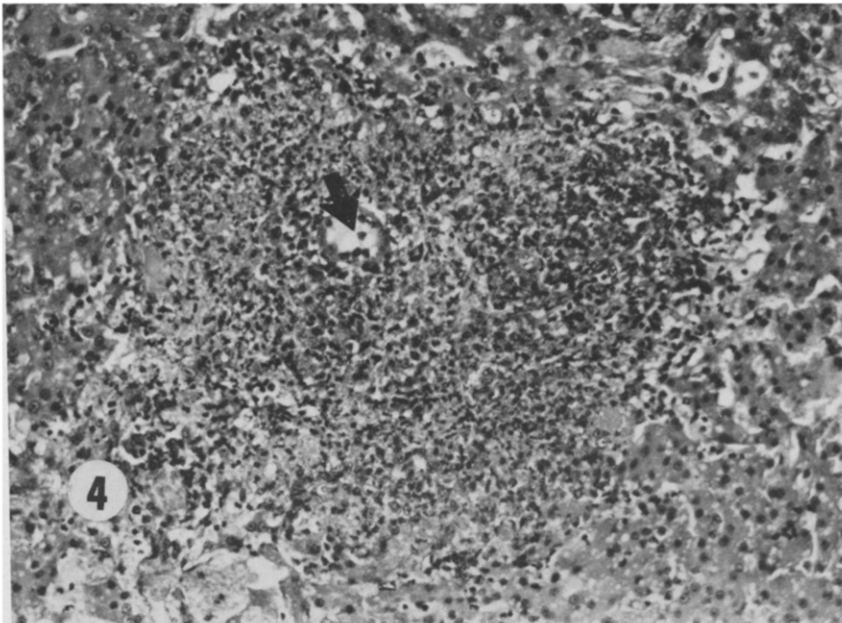


Fig. 4. Liver of passively immunized kitten on PID 9. Severe necrosis and inflammatory cell infiltration occur around an hepatic vein (arrow) (hematoxylin-eosin, $\times 180$).

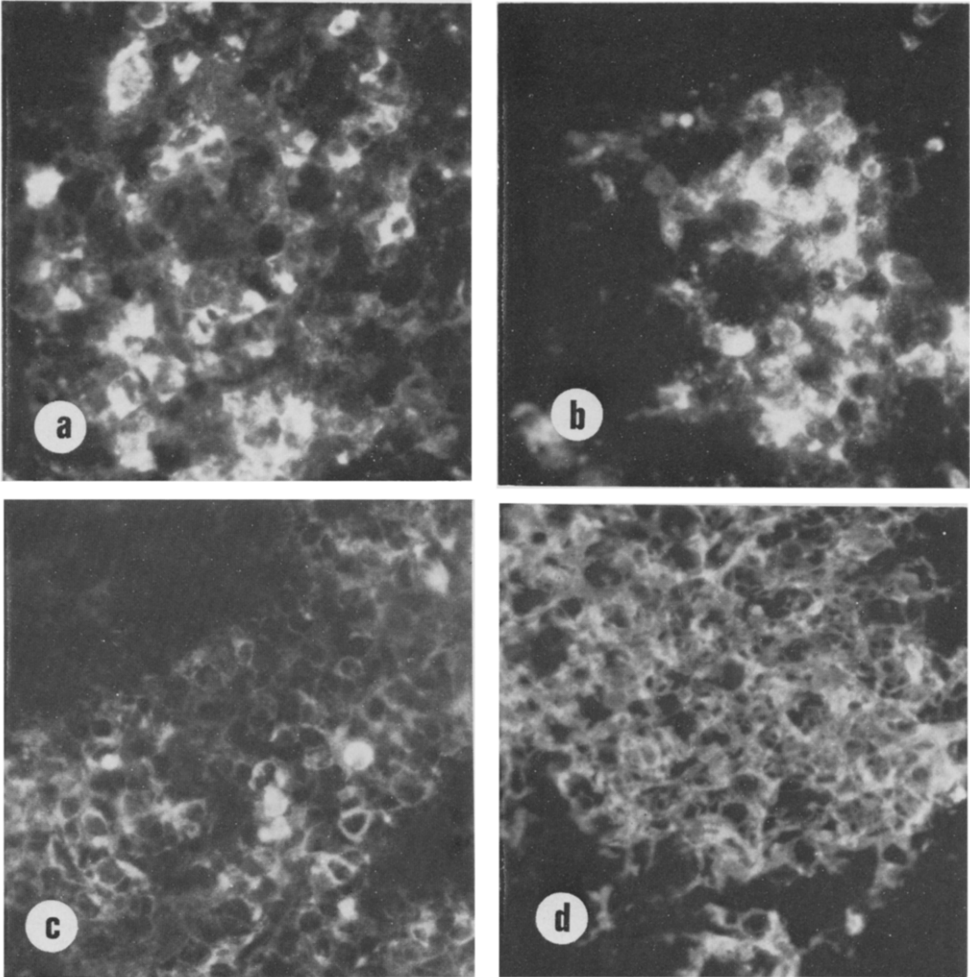


Fig. 5. Immunofluorescent (IF) staining of acetone-fixed serial sections of spleen from a passively immunized kitten 9 days after inoculation with FIP virus. (a) Indirect IF staining with FIP virus hyperimmune serum demonstrates FIP viral antigen in cytoplasm of mononuclear cells present in a focal necrotic lesion. (b) Direct IF staining of similar section with fluorescein isothiocyanate (FITC)-conjugated Staph Protein A demonstrates immunoglobulin G in cytoplasm of cells. (c) Direct IF staining of similar section with FITC-conjugated goat anti-dog complement (C3) demonstrates complement protein in cytoplasm of cells. (d) Direct IF staining of similar section with FITC-conjugated goat anti-human fibrinogen demonstrates marked extracellular deposition of fibrinogen in lesion (Evan's Blue counterstain, $\times 500$).

normochromic, normocytic anemia with moderate poikilocytosis. Total leukocyte counts decreased to 30% of preinoculation levels on PID 7, and there was an absolute and relative decrease in the numbers of lymphocytes. Reductions in platelet numbers first occurred on PID 3 in sensitized kittens and on PID 7 in non-sensitized kittens. Significant ($P < 0.025$) differences in mean platelet counts between sensitized and non-sensitized kittens occurred on PID 7. Sensitized kittens in general developed a more severe thrombocytopenia, and demonstrated platelet counts of less than $95,000 \text{ cells/mm}^3$ on PID 7.

Elevated serum levels of bilirubin and increased activities of ALT and AST were first detected on PID 7 in both groups of kittens (Table 2). Hyperbilirubinemia was more pronounced in the sensitized kittens and occasionally exceeded 4 mg/dl. Serum activity of AST in infected kittens increased four-to-five-fold by PID 7 and 10; ALT activity rose two-to-three-fold on PID 7.

Kittens given FIPV immune serum intravenously developed passive antibody titers of 1:100 within 24 hr (Fig. 1). The antibody titers in virus-challenged, passively immunized kittens decreased to 1:25 and 1:5 on PID 7 and 10, respectively. Kittens given non-immune serum and challenged with virus remained seronegative until PID 10. These kittens developed elevated FIPV antibody titers during the following 2 weeks and demonstrated maximal titers of 1:400 on PID 24. Non-challenged control kittens given FIPV immune serum demonstrated mean passive antibody titers of 1:100 on PID 1 and 7, 1:50 on PID 10, and less than 1:5 on PID 14 and 20.

Pathologic changes

The characteristic gross and microscopic lesions in experimental FIP were detailed in a previous report [12] and will only be summarized here. Gross lesions usually appeared as disseminated 0.2–2.0 mm grey-white to yellow foci in the parietal and visceral peritoneum, pleura, pericardium, omentum and mesentery, liver, spleen, kidney, pancreas, diaphragm, and abdominal and thoracic lymph nodes. Petechial hemorrhage and fibrin were commonly associated with these foci. Abdominal and sternal lymph nodes were enlarged. Clear yellow, viscous peritoneal fluid containing fibrin strands developed in excess of 50 to 75 ml in most cats (Fig. 2). Microscopic lesions consisted of disseminated foci of necrosis and pyogranulomatous inflammation frequently oriented around small veins and venules in serosal membranes, omentum (Fig. 3), liver (Fig. 4), spleen, intestines, lung, kidney, eyes, and brain. These foci were characterized by perivascular and vascular infiltrations by macrophages, neutrophils, lymphocytes, fibrin and necrotic debris. Affected veins frequently demonstrated degeneration and necrosis of cells in the media and adventitia and perivascular edema. Fibrin thrombi were occasionally present, especially in pulmonary and hepatic veins. Reticuloendothelial hyperplasia, lymphoid hyperplasia or depletion, and necrosis occurred in lymphoreticular organs such as spleen, thymus and lymph nodes. Marked erythrophagia by macrophages was frequently observed in sinuses of spleen and lymph nodes. Mesothelial hyperplasia was commonly present in serosal membranes. Lesions were not observed in the tissues from control kittens.

Immunofluorescence microscopy

Serial sections from lesions in spleen, liver, lung, omentum or mesenteric lymph node stained positively for FIP viral antigen, IgG, C3, and fibrinogen. Lesions were positive on

PID 9, 14, and 28. Specific fluorescence for FIP viral antigen, IgG, and C3 was observed as a granular or diffuse staining of the cytoplasm within mononuclear inflammatory cells (Fig 5a, b and c). Anti-fibrinogen immunofluorescence was observed predominantly as an extracellular deposit of strands and fibers investing cells within lesions (Fig. 5d) or as dense deposits within vascular lumen. Washing of slides with acid-glycine buffer prior to staining for IgG resulted in a reduction of specific anti-IgG fluorescence. Specific fluorescence for FIPV, IgG, C3, or fibrinogen was not observed in tissues from control cats.

DISCUSSION

Non-immune kittens passively immunized with serum containing a high titer of antibodies reactive against FIPV developed an enhanced disease when later challenged with virus. Compared to kittens pretreated with non-immune serum, the FIPV antibody-sensitized kittens developed clinical signs earlier and died more rapidly after infection. Non-sensitized kittens did not show clinical signs or die of FIP until an active antibody response was demonstrated. These findings confirmed earlier observations made by Pedersen and Boyle [13].

Examination of lesions from infected kittens showed widespread necrosis, vascular inflammation, and evidence of marked changes in vascular permeability, i.e. perivascular edema and effusion of transudate fluids into body cavities. Severe inflammation of veins in multiple organs suggested a systemic Arthus-type reaction. The latter, a local inflammatory lesion of venules characterized by granulocytic infiltration, edema, necrosis and hemorrhage, is produced experimentally by local injection of antigen into a previously immunized animal; complement activation secondary to local immune complex formation within venules leads to inflammation and changes in vascular permeability [33].

The finding of FIP viral antigen, IgG, and C3 in lesions of FIPV-infected kittens in this study and in previous investigations [12, 13] suggests that complement activation secondary to antigen-antibody reactions may indeed be a central mechanism in the pathogenesis of FIP. Complement activation in FIP may result from intravascular or perivascular deposition of immune complexes, or possibly from cytotoxic, complement-fixing antibodies directed against virus-infected cells. Antibody-mediated or immune complex-mediated immunopathologic reactions in or around small blood vessels may result in endothelial injury, platelet aggregation, microthrombi formation and disseminated intravascular coagulation (DIC); several cases of DIC have been induced in FIP antibody-positive kittens experimentally infected with FIPV [12]. FIPV-infected kittens in the present study developed severe phlebitis, occasional thrombosis, and marked deposition of fibrinogen within veins and in lesions. An interesting observation is the earlier and more severe thrombocytopenia in the antibody-sensitized vs non-sensitized kittens. This finding may reflect early immunologic reactions in veins with perhaps two consequences of intravascular complement activation: immune complex vasculitis with platelet clumping at sites of endothelial injury, or complement-mediated platelet aggregation [33]. Alternatively, there may be an accelerated (antibody-mediated?) lysis of platelets in sensitized kittens.

All kittens given FIPV-immune serum developed fevers above 40.5°C within 24 hr after virus infection. In contrast, similar temperatures were not observed in kittens given non-immune serum until after PID 8 to 12; two non-sensitized kittens developed mild transient

fever on PID 2. The onset of clinical signs in non-sensitized kittens coincided closely with seroconversion and the appearance of the second temperature spike. The latter may represent systemic immunopathological disease (e.g. immune complex hypersensitivity) or perhaps late (secondary) viremia, whereas the mild transient temperature rise on PID 2 in two non-sensitized kittens suggests a primary viremia. The earlier and persistent fever in seropositive kittens, in contrast, may reflect enhanced (antibody-mediated?) viral infection, resulting in more rapid viremia and immunopathological disease. Indeed, previous studies in our laboratory have shown earlier cell infection, viremia, and vasculitis in FIPV-challenged seropositive kittens compared to seronegative kittens [14]; it was suggested that FIPV antibody may be involved not only in immunopathologic disease (i.e. immune complex disease or complement-mediated cytotoxicity) but also in *in vivo* antibody-mediated enhancement of virus infection in macrophages. The latter phenomenon has been reported in both *in vitro* and *in vivo* studies of dengue virus infection in monkeys [34, 35].

The more rapid decline in passive antibody titers of FIPV-challenged kittens as compared to unchallenged controls in the present study suggests that FIPV antibodies are actively consumed during the course of disease in addition to spontaneous degradation. The former could represent binding of free antibody with virus as immune complexes prior to an active primary humoral immune response. These complexes may then circulate free in the serum, deposit in blood vessels, or be phagocytized by mononuclear phagocytes in blood or around blood vessel walls.

The antibody-mediated enhancement of disease demonstrated in laboratory kittens infected with FIPV resembles certain immunopathological features of dengue hemorrhagic fever (DHF) in persons. Patients with preinfection heterotypic immunity to one of the four known serotypes of dengue virus occasionally develop a severe shock syndrome when infected by a different serotype [19]. This syndrome is characterized by fever, marked changes in vascular permeability, hypovolemic shock, thrombocytopenia and hemorrhagic diathesis [36]. Several theories are proposed to explain the occurrence of DHF. In one view, complement activation by immune complexes is the major initiating factor [21]. According to this theory, patients with preinfection immunity against a single dengue virus serotype make an anamnestic antibody response when infected with a second serotype. These antibodies are non-neutralizing yet cross-reactive with the heterologous virus; circulating immune complexes are formed which may deposit in blood vessels, activate complement and result in a shock syndrome. In support of this are the findings in DHF patients of lowered serum complement levels [21, 27], circulating immune complexes [25, 26], and deposition of dengue virus, IgM, and β -1c globulin in or around blood vessels in lesions [28]. A second theory argues against immune complex disease *per se* and proposes instead that non-neutralizing antibody opsonizes virus and leads to an immunologic enhancement of virus infection in mononuclear phagocytes and possibly complement activation via the alternative pathway [37]. An accelerated, severe viral disease would thus occur in patients with preinfection heterotypic immunity prior to the appearance of neutralizing antibodies against the infecting virus. In support of this view, several studies have demonstrated antibody-mediated enhancement of dengue virus infection in monkeys [34] and in cultured primate leukocytes [35].

The present study shows that in FIP, as in DHF, an antibody-mediated enhancement of disease occurs. Passively administered antibodies reactive with FIP virus do not neutralize parentally inoculated virus; these antibodies, in fact, somehow sensitize the recipient, and a

more acute and fulminating disease occurs. The enhanced infection of FIPV in macrophages of seropositive kittens [14, 38] may, as in dengue virus infection, reflect the opsonic activity of antibodies which are non-neutralizing for but reactive with the challenge virus. It is presently unclear whether non-neutralizing FIP antibodies are heterotypic (as in DHF) or homotypic (as in Aleutian disease of mink [39]). Although the existence of different FIPV serotypes is not yet established, differences in virus neutralization of various FIPV isolates against a cross-reacting porcine coronavirus suggest that more than one FIPV serotype may occur [31]. If this is the case, a situation similar to DHF in persons could develop in seropositive cats acutely infected with a heterologous FIPV serotype (or perhaps other cross-reacting coronavirus) i.e. high titers of non-neutralizing, cross-reacting antibodies, rapid formation of virus-antibody complexes, facilitated virus replication and persistence in mononuclear phagocytes, and widespread complement activation.

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