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Some aspects of humoral and cellular immunity in naturally occuring feline infectious peritonitis

S. Paltrinieri[a,*], M. Parodi Cammarata[b], G. Cammarata[b], S. Comazzi[a]

[a] *Istituto di Patologia Generale Veterinaria, Via Celoria 10, I-20133, Milan, Italy*
[b] *Istituto di Anatomia Patologica Veterinaria e Patologia Aviare, Via Celoria 10, I-20133, Milan, Italy*

Abstract

Haematology, antibody titers and serum protein electrophoresis from 48 cats (34 effusive and 14 noneffusive forms) affected with feline infectious peritonitis (FIP) were studied and compared with those of 20 healthy cats. In the effusive form, antibody titers and protein electrophoresis in the effusions were analyzed. The distribution of the immune cells and of the virus in FIP lesions were also investigated immunohistochemically with the avidin–biotin complex (ABC) method, using antibodies against the FIP virus (FIPV), myelomonocytic (MAC387) and lymphoid (CD3, CD4 and CD8 for T-cells and IgM and IgG for B-cells) antigens. Seropositive animals (antibody titer>1:100) were present among both the FIP infected cats (73%) and the healthy cats (70%). Cats with effusive FIP had neutrophilic leukocytosis ($P>0.05$), lymphopenia ($P<0.01$) and eosinopenia ($P<0.001$). In both effusive and noneffusive forms decreased albumin/globulin ratio ($P<0.001$) with hypoalbuminemia ($P<0.001$), hyperglobulinemia ($P<0.001$) and increased α_2- ($P<0.05$), β- ($P<0.05$) and γ-globulins ($P<0.001$) were found. Hypergammaglobulinemia was not related to the antibody titers, suggesting the presence of other proteins with γ-motility (e.g. complement fractions). The electrophoretic pattern of the effusions was always similar to that of the corresponding serum. Antibody titers higher than those of the corresponding serum were often detected in the effusions. Immunohistochemical findings were not related to the antibody titers, but they were related to the histological aspect of the lesions. In cellular foci of FIP lesions many virus-infected macrophages and few lymphocytes, mainly $CD4^+$, were found. Extracellular viral and myelomonocytic antigens were also detectable in the foci with intercellular necrosis. Only few FIPV-infected cells were present at the periphery of the larger necrotic foci: in these lesions $MAC387^+$ cells were mainly neutrophils, with many $MAC387^-$ macrophages, probably due to their activated state; a small number of lymphocytes, with an increasing percentage of $CD8^+$ cells was present. Lymphocytes were more abundant when cellular foci and FIP-infected macrophages were centered around neoformed vessels. IgM and IgG exposing B-cells were always few and scattered. In conclusion the simultaneous analysis of body fluids and of the cellular composition of the lesions showed a

* Corresponding author. Tel.: +39 2 26680443; fax: +39 2 2364470; e-mail: savepal@unimi.it

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complex immune status, on which type III and type IV hypersensitivity could coexist.

Keywords: Feline infectious peritonitis; Pathogenesis; Hypersensitivity; Protein electrophoresis; Serology; Immunohistochemistry

1. Introduction

Feline infectious peritonitis (FIP) is a disease of felids due to coronaviruses. The FIP virus (FIPV) originates from the less pathogenic feline enteric coronavirus (FECV) by a minor mutation (Pedersen and Floyd, 1985), and acquire the ability to pass through the epithelium into the lymph and to replicate in the cells of the monocytic–macrophagic line (Stoddart and Scott, 1989). Within the monocytes, FIPV spreads throughout the body to target organs. The two feline coronaviruses (FCoV) have slight and poorly understood differences in their genoma (Vennema et al., 1995), but they are morphologically and antigenically identical (Boyle et al., 1984). This leads to some problems in both diagnosis and understanding of the pathogenesis of the disease.

The appearance of the disease and the different clinical forms (effusive or noneffusive) depends on the cellular immune efficiency: cats with a strong cellular immunity may not develop the disease, while those with a weak cellular immunity have the noneffusive FIP and those without a cellular reaction have the effusive form (Pedersen and Black, 1983; Pedersen, 1995a, 1987.

Humoral immunity, in contrast, seems to increase the probability of getting the disease. Even if antibody-enhanced infection has been recently questioned (Olsen et al., 1992, 1993; Addie et al., 1995), many experimental results demonstrate that anti-FCoV antibodies facilitate the uptake of the virus by the macrophages (Hayashi et al., 1983; Stoddart and Scott, 1989; Barlough and Stoddart, 1990; Hohdatsu et al., 1994; Pedersen, 1995a), and that immunocomplexes lead to a type III hypersensitivity reaction with disseminated intravascular coagulation and fibrinoid necrosis of the vessel's walls, responsible for the effusions (Hayashi et al., 1977, 1978; Pedersen and Boyle, 1980; Weiss et al., 1980; Jacobse-Geels et al., 1980; Weiss and Scott, 1981; Fenner, 1987; Pastoret and Bourtonboy, 1991; Pedersen, 1995a). This hypothesized immune-mediated pathogenesis is also supported by epidemiologic considerations (Pedersen and Floyd, 1985; Addie et al., 1995) and by other immunopathological findings, such as the fluctuations of antibody titers and complement fractions during the course of the infection (Pedersen and Boyle, 1980; Jacobse-Geels et al., 1982).

The morphology of the lesions, similar to those of other immunological granulomas, such as the tubercular ones (Pedersen, 1987; Paltrinieri et al., 1998) and the detection of a cutaneous FIPV-induced delayed-type hypersensitivity-like reaction in cats (Weiss and Cox, 1988), suggests that type IV hypersensitivity reactions could be involved in the pathogenesis of focal lesions (Pedersen, 1987).

To further understand the pathogenesis of the disease, parameters indicative of the involvement of humoral immunity (total and fractioned proteins and antibody titers in

serum and in effusions), and the distribution of viral antigen and immune cells in the lesions were studied in cats with spontaneous FIP.

2. Material and methods

2.1. Animals

Forty eight cats of different sex, age and breed and with clinical signs of spontaneous FIP submitted by clinicians were examined: 34 cats had the effusive form, while the remaining 14 had noneffusive form of the disease. The clinical diagnosis was confirmed by necropsy, histology and by the immunohistochemical detection of the FIP antigen in the lesions. A blood sample of 2 ml was taken from the cephalic or jugular vein of each cat: 1 ml was put in a EDTA-coated tube for hematology and 1 ml in a tube without anticoagulant for serum.

From the cats with effusive FIP, 1.0 ml of ascitic fluid or thoracic effusion was also withdrawn by paracentesis before the cats were euthanatized or immediately after death and collected in EDTA-coated tubes. Hundred μl of each effusion were cytocentrifuged in a multiwell cytocentrifuge (Bioprobe). The slides were routinely stained with May Grünwald–Giemsa. The remaining amount of the effusions was centrifuged and the supernatants separated to perform both protein analysis and serology. The sera or the effusions were aliquoted and stored at -20°C.

2.2. Hematology

A complete hemogram in an automatic counter (Seac Hemat 8) and a differential leukocyte count on a May Grünwald–Giemsa stained smear were carried out. The percentages of reticulocytes were evaluated in cresyl brillant blue-stained smears.

2.3. Protein analysis

Total proteins in serum and effusions were measured by a discrete autoanalyzer (Abbott VP) using the colorimetric biuret method (Abbott). Serum protein electrophoresis was carried out by the semimicro-method, using cellulose polyacetate strips (Gelman) in a barbitone and Tris buffer (Helena Lab.). Undiluted serum or effusions were applied on the strips and run for 40 min, 150 V in the same buffer. Then the strips were stained for 15 min in Red Ponceau (0.5 g in 100 ml of 5% trichloroacetic acid), destained in a 5% acetic acid and cleared (Helena Lab.) The gels were scanned in a densitometer (Gelman DCD16).

2.4. Serology

Antibody titers were evaluated using a commercially available ELISA kit (Diagnostica 2000). Serial 2-fold dilutions in phosphate buffered solutions (PBS) were prepared and 100 μl of each dilution were put in a microtiter plate coated with polyclonal FIP proteins.

After incubation (60 min at 37°C) 100 µl of anti-feline horseradish peroxidase-conjugated antibody were added. After 60 min of incubation at 37°C, 100 µl of substrate buffer solution were added for 20 min. The reaction was stopped with 50 µl of stop solution. The multiwell microtiter plates were then read at 450 nm in an automatic ELISA analyzer (Dasit Multiskan). Negative and positive controls were included in each plate.

2.5. Histology

From each affected organ, including the central nervous system (CNS), when nervous symptoms were present, and kidney, even without any lesions, two samples (approximately 1 cm^3) were taken, one was fixed in buffered 10% iso-osmotic formalin and embedded in paraffin, the other was frozen. One of the cryostatic sections and one of the formalin-fixed, paraffin-embedded sections were used to confirm the diagnosis by hematoxylin–eosin stain. The histological lesions were recorded and a comparison with the results of body fluid analysis was carried out.

2.6. Immunohistochemistry

A monoclonal antibody against the FIP (kindly provided by Prof. N.C. Pedersen, Davis, USA) was used to detect the virus in lesions. Myeloid cells were identified using the monoclonal antibody MAC387 (Dako). A polyclonal antibody against CD3 antigen (Dako) and monoclonal antibodies against CD4 and CD8 antigens (both of these kindly provided by Dr. Willet, Glasgow, UK), were used to identify T-lymphocytes and their subpopulations. Polyclonal antibodies were employed to detect IgG- and IgM-expressing B-lymphocytes.

CD3 and MAC387 antibodies were tested in formalin-fixed and paraffin-embedded sections after antigen unmasking by microwave pre-treatment (Cattoretti et al., 1993); IgG and IgM expressing B-lymphocytes were investigated in formalin-fixed and paraffin-embedded sections after antigen unmasking using a proteolytic pretreatment (protease XXIV, Sigma); CD4 and CD8 antibodies were detected in frozen sections.

Immunohistochemistry was performed with the Avidin Biotin Complex (ABC) method with a commercially available kit (Vectastain Elite, Vector laboratories, CA, USA), as previously described (Hsu et al., 1981). Briefly, the formalin-fixed and paraffin-embedded sections were deparaffinated and rehydrated in xylene and ethanol, endogenous peroxidase were inhibited with H_2O_2 (1%) in methanol and antigen unmasking was carried out using the protease XXIV (0.5% in prewarmed Tris-buffered solution, pH 7.7, for 15 min at 37°C) or microwave pretreatment (2 cycles of 5 min in citrate-buffered solution, 0.01 M, pH 6). In cryostatic sections, endogenous peroxidase was inhibited with H_2O_2 (1%) in 0.1% sodium azide. The blocking sera (20 min at room temperature) were obtained from goats (for CD3), from rabbits (for IgG and IgM) or from horses (for monoclonal antibodies). Then the primary antibodies were applied overnight at 4°C. After three washes in Tris-buffered solution (pH 7.7) the biotinylated secondary antibodies (30 min at room temperature), and finally the ABC complex (30 min at room temperature) were added. Diaminobenzidine tetrahydrochloride or 3-amino-9-ethyl-

carbazole served as chromogen for the reaction. The reaction was blocked by washing in running tap water and the slides were counterstained with Mayer's haematoxylin and then the coverslips were mounted.

Some sections of each sample were used as negative controls, with the primary antibody substituted by Tris-buffered solution.

2.7. Statistical analysis

The results obtained from hematology and protein analysis in effusive and noneffusive FIP-infected cats were compared with those obtained from the controls using an one-way ANOVA test. The nonparametric Spearman correlation test was employed to plot the antibody titers against total and fractioned protein concentrations.

Protein analysis of the serum were compared with those of the corresponding effusions by a *t*-test for dependent samples. The nonparametric Wilcoxon matched paired test was employed to compare the antibody titers in serum and in corresponding effusions.

3. Results

3.1. Diagnostic features

All 48 cats had pathological features of FIP, consistent with those described in literature (Wolfe and Griesemer, 1971; Montali and Strandberg, 1972): 34 cases had a yellowish, dense, and fibrinous effusion in the abdomen (79.4%), in the chest (17.6%) or in both the cavities (2.9%). Perivisceral fibrin was present on the omentum and on all the peritoneal or pleural surfaces; small nodular foci were detectable under the intestinal serosa, and in the liver and kidneys. Larger nodular foci were often detectable in the kidneys. The involvement of the central nervous system was characterized by a fibrinous meningitis.

Histology of the lesions showed the presence of perivisceral fibrin with few scattered inflammatory cells or with subperitoneal or subpleural cellular foci: these larger foci contained central necrosis with peripheral fibrosis. A lymphohistiocytic perivasculitis was often detectable, as well as intraparenchymatous pyogranulomatous hypercellular lesions with necrotic centers. Fibrinous and lymphohistiocytic meningitis was found in the CNS.

The remaining 14 cases were the noneffusive form, with nodular lesions (diameter: 0.3–1 cm) in the kidneys or in the lymph nodes or, less frequently, in other organs. These lesions were characterized by a central necrosis surrounded by a pyogranulomatous reaction similar to those detectable in the larger and intraparenchymatous lesions of the effusive forms.

3.2. Hematology

Means and standard deviations observed in controls and in FIP-infected cats are reported in Table 1. All the FIP cats were anemic, with a decreased number of

Table 1
Means±standard deviations of the haematological parameters in FIP cats and in the controls

	Controls	Effusive FIP	Non-effusive FIP		
Erythrocytes$\times 10^6$ µl	7.74±1.35	5.73±1.60	5.94±2.18	***	C vs. E, NE
Reticulocytes/µl	21867±25844	15487±17739	18052±15516	ns	–
Hb (g/dl)	12.7±2.7	8.9±2.3	9.2±2.9	***	C vs. E, NE
PCV (%)	35.5±8.8	27.0±6.9	28.9±8.4	**	C vs. E, NE
Leucocytes/µl	11319±4025	17884±8949	14228±9407	*	E vs. C
Segmented Neutrophils/µl	7112±3092	14794±8121	10695±7915	***	E vs. C
Band Neutrophils/µl	412±350	1297±1488	1132±1155	*	E vs. C
Eosinophils/µl	760±629	77±154	163±242	***	E vs. C
Lymphocytes/µl	2578±2234	1283±866	1695±1512	*	E vs. C
Monocytes/µl	393±320	417±383	521±297	ns	–

*=P<0.05; **=P<0.01; ***=P<0.001.
ns=not significant; C=controls; E=effusive FIP; NE=non-effusive FIP.

erythrocytes (P<0.001), haemoglobin concentration (P<0.001) and PCV (P<0.01). No change in reticulocyte number was detected between the groups. Leukocytosis (P<0.05) with increased number of segmented (P<0.001) and band (P<0.05) neutrophils and decreased number of eosinophils (P<0.001) and lymphocytes (P<0.05) were present in cats with effusive FIP.

3.3. Serum protein analysis

The total protein concentration (Table 2) in cats with effusive FIP (73.5±18.1) and in those with noneffusive FIP (80.2±14.5) was similar to those observed in controls (70.1±9.4 g/l). The FIP cats demonstrated a decrease of albumin/globulin (a/g) ratio (P<0.001), due to decreased albumin (P<0.001) and increased globulin (P<0.001) concentrations. Hypoalbuminemia was greater in the effusive than in the noneffusive

Table 2
Means±standard deviations of the protein analysis in FIP cats and in the controls

	Controls	Effusive FIP	Non-effusive FIP		
Serum total proteins (g/l)	70.1±9.4	73.5±18.1	80.2±14.5	ns	–
Albumin (g/l)	36.5±7.4	19.6±5.5	26.1±6.8	***	C vs. E, NE; E vs. NE
Globulins(g/l)	33.6±6.7	54.0±16.2	54.1±12.6	***	C vs. E, NE
α-Globulins (g/l)	12.4±3.4	16.9±5.7	17.3±5.2	**	C vs. E, NE
α1-Globulins (g/l)	4.2±1.7	6.1±4.8	4.1±3.4	ns	–
α2-Globulins (g/l)	7.6±3.5	10.4±4.3	11.0±5.8	*	E vs. C
β-Globulins (g/l)	7.5±1.8	14.8±12.2	9.4±2.4	*	E vs. C
γ-Globulins (g/l)	13.7±4.0	22.3±13.1	27.4±11.5	***	C vs. E, NE
a/g Ratio	1.2±0.4	0.4±0.1	0.5±0.2	***	C vs. E, NE

*=P<0.05; **=P<0.01; ***=P<0.001.
ns=not significant; C=controls; E=effusive FIP; NE=non-effusive FIP.

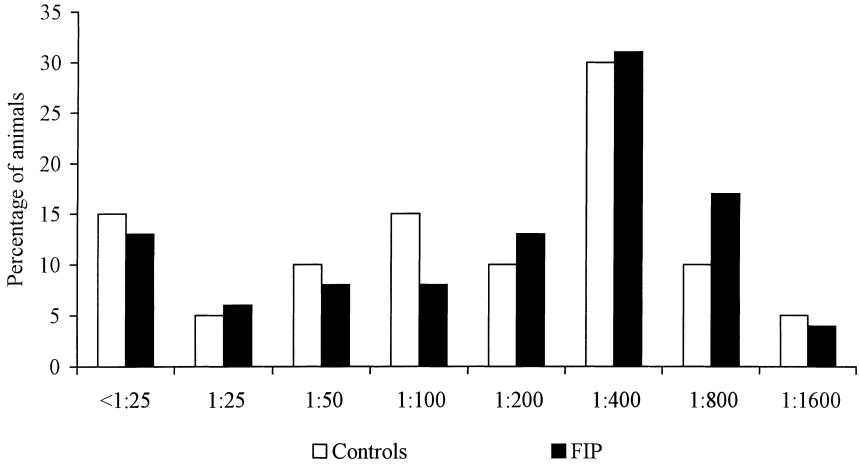

Fig. 1. Distribution of FCoV antibody titers in serum of the controls and of the FIP-affected cats.

form ($P<0.001$). All globulin fractions increased in FIP cats, but the increase was lower in β- ($P<0.05$) than in α- ($P<0.01$) and in γ- ($P>0.001$) globulins; the increase of α-globulins was due mainly to an increase in the α_2 fraction ($P<0.05$).

3.4. Serology

Antibody titers higher than 1:100 were present in healthy cats (70%) and FIP-infected cats (73%) (Fig. 1). The prevalence of seropositivity was higher in the effusive (79%) than in the noneffusive form (57%) (Fig. 2). No correlations were found between the antibody titers and total or fractioned protein concentrations.

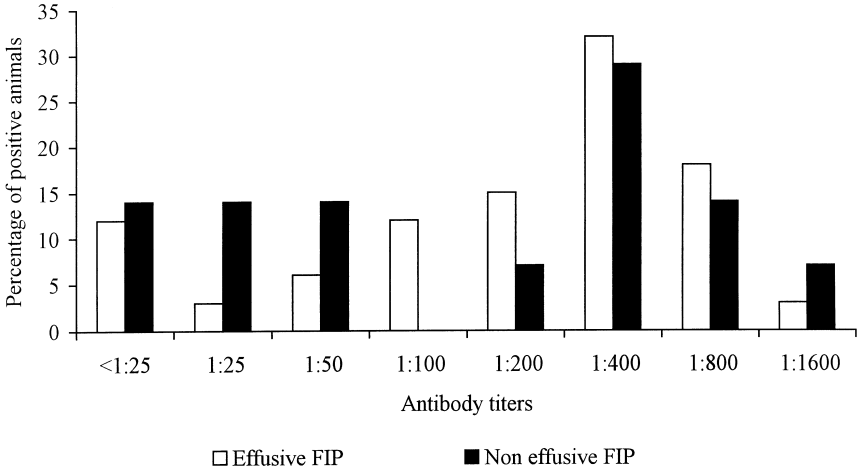

Fig. 2. Distribution of FCoV antibody titers in serum of cats with effusive and noneffusive FIP.

Table 3
Means±standard deviations of the protein analysis in serum and in effusions for cats with effusive FIP

	Serum	Effusion		Effusion/serum ratio
Total proteins (g/l)	73.5±18.1	61.7±16.1	***	0.86±0.17
Albumin (g/l)	19.6±5.5	18.5±7.0	ns	0.97±0.30
Globulins(g/l)	54.0±16.2	43.3±15.2	***	0.81±0.18
α-Globulins (g/l)	16.9±5.7	12.1±3.8	***	0.77±0.30
α1-Globulins (g/l)	6.1±4.8	3.7±2.6	***	0.65±0.33
α2-Globulins (g/l)	10.4±4.3	8.0±3.7	**	0.78±0.38
β-Globulins (g/l)	14.8±12.2	12.3±11.8	ns	0.99±0.68
γ-Globulins (g/l)	22.3±13.1	18.6±13.3	ns	1.39±2.93
a/g Ratio	0.4±0.1	0.5±0.3	*	–

*=$P<0.05$; **=$P<0.01$; ***=$P<0.001$.
ns=not significant.

3.5. Analysis of the effusions

Thirty of the 34 examined effusions (88.2%) had cytologic findings consistent with the clinical diagnosis of FIP, according to Cowell et al. (1989), with neutrophils, mesothelial cells and a proteinaceous background and without any bacteria or noninflammatory cells.

Most (32 of 34) effusions (94.1%) had a protein content higher than 35 g/l, a value considered diagnostic for FIP (Sparkes et al., 1991). The mean protein concentration (61.7±16.1 g/l) was lower than those observed in the serum ($P<0.001$) and the mean ratio between protein content in the effusions and sera was 0.86±0.17 (Table 3). The electrophoretic pattern of the effusions was similar to those of the corresponding serum. No differences were found between the albumin concentrations in serum and in effusions, with an effusion/serum ratio of 0.97±0.30. The concentrations of total and α-globulins were higher in the serum (P>0.001 for both parameters). These differences were higher for α_1- than α_2-globulins ($P<0.001$ and $P<0.01$, respectively). All of these parameters had the lowest effusion/serum ratio (see Table 3). Mean values of β- and γ-globulins concentrations were similar in serum and in the corresponding effusions, may be due to the high variability, with many cats that had higher values of these compounds in the effusions than in the serum (effusion/serum ratio 0.99±0.68 and 1.39±2.93, respectively).

The percentage of animals in which the effusions had antibody titers higher than 1:100 (79.4%) was similar to those observed in the serum (Fig. 3). No correlation was found between the titers observed in serum and in the corresponding effusions. 47% of the animals had higher titers in the effusions than in serum.

3.6. Immunohistochemistry

Each examined cat had FIP viral antigen in the lesions. The amount and the localization of viral antigen was very variable between the lesions, without any relationship to the clinical form, antibody titer or the size of the foci. In contrast, the

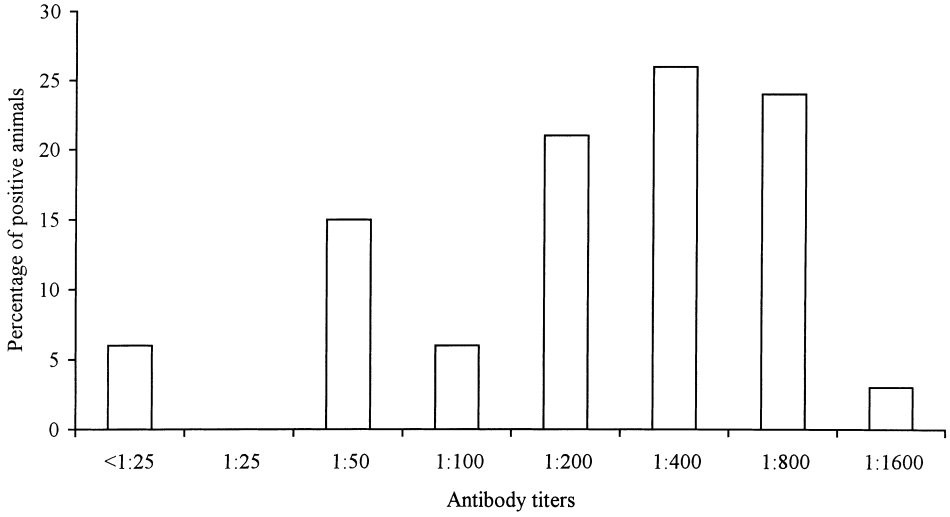

Fig. 3. Distribution of FCoV antibody titers in the effusions from cats with effusive FIP.

distribution of the FIP in each focus seemed to be related to the pattern of the lesion: in cellular foci, many of the histiocytic cells were $FIPV^+$, at the periphery of necrotic foci. In some cases, viral antigen was detectable in dendritic-like cells into germinal centers of lymph nodes draining the affected organs. Antigen was never detected where immune complexes may be deposited (vessel's walls or the glomeruli).

The number of $MAC387^+$ myeloid cells was high within cellular lesions. Many of these cells near necrotic foci were granulocytes. No differences in the distribution of the $MAC387^+$ and $MAC387^-$ myeloid cells were found between cats with different antibody titers or protein concentrations.

$CD3^+$ lymphocytes were randomly distributed within fibrin and small cell foci; their numbers increased in the larger organized lesions where they were concentrated around the periphery of necrotic foci. $CD3^-$ cells were also present, mainly in more organized lesions. No differences in the localization and in the number of $CD3^+$ lymphocytes were found between cats with different antibody titers or protein concentrations.

IgG^+ and IgM^+ lymphocytes and plasma cells were detectable in FIP lesions, but their number was always low and their distribution very variable among the cases. No relationship with the histological pattern of the lesions, or with the humoral findings of the cats has been detected.

The number of $CD4^+$ and $CD8^+$ lymphocytes increased with the age/size of the FIP lesion. In the small foci, $CD4^+$ cells predominates, whereas in the larger necrotic foci, the number of $CD8^+$ cells were increased. The $CD4^+$ lymphocytes were uniformly distributed throughout the lesions, whereas the $CD8^+$ lymphocytes were at the periphery of the foci in each stage of the granuloma. All these findings were detectable both in the cats with high antibody titers and in those with low antibody titers.

4. Discussion

No changes indicative of immune system dysfunction were detectable by the hemograms: nonregenerative anemia, neutrophilic leukocytosis and lymphopenia are well-recognized hematologic findings in FIP (Jain, 1986, 1993; Pedersen, 1995a). In other immune-mediated diseases such as leishmaniasis (Carvalho et al., 1992; Bourdoiseau et al., 1997), canine blastomycosis (Legendre and Becker, 1982) and feline immunodeficiency virus (Lin et al., 1990) or feline leukemia virus infections (Hoover et al., 1987), lymphopenia has been interpreted as a sign of reduced cell-mediated immunity. This conclusion was based upon a reduced lymphocyte blastogenesis (Legendre and Becker, 1982; Lin et al., 1990; Bishop et al., 1992; Carvalho et al., 1992) or a low CD4/CD8 ratio (Bishop et al., 1992; Lawrence et al., 1995; Hoffmann-Fezer et al., 1996). This study gives only a quantitative evaluation of lymphocytes, and no information about blood lymphocyte subpopulations or functions in FIP are available in literature. Thus, although an impairment of lymphocyte functions can not be excluded, the neutrophilia and eosinopenia only in the cats with the acute effusive form suggest a stress leukogram (Jain, 1986, 1993).

Hyperproteinemia has been reported in FIP (Sparkes et al., 1991, 1994; Pedersen, 1995a), but was not observed in spite of the increase of total globulins, because of the decrease in serum albumin levels. Hypoalbuminemia was more evident in the effusive form perhaps due to the loss of albumin in the effusions. In fact no differences were found between albumin concentration in serum and in effusions, with high effusion/serum ratios.

The electrophoretic pattern of FIP cats was consistent with those reported in literature (Sparkes et al., 1991, 1994; Pedersen, 1995a). The changes in α- and β-globulins are well documented in experimental infections by Stoddart et al. (1988) and characterized by a biphasic acute phase plasma protein response with increases of α_1-orosomucoid, α_2-haptoglobin and β_2-transferrin levels. The same author stated that γ-globulins increases as the antibody titers begin to mount. In the spontaneous disease the early phases of antibody production are not detectable: however the occurrence of animals with high antibody titers without symptoms and of symptomatic cats with low antibody titers have been reported (Pedersen, 1976, 1977, 1995a, b; Reynolds et al., 1977; Vanden Bossche, 1990; Sparkes et al., 1992a, b). Based upon the pattern of antibody and γ-globulin levels, four groups of cats were identified (Fig. 4):

1. Cats with low antibody titers and low γ-globulin concentrations: these cats were mainly those of the control group;
2. Cats with high antibody titers and low γ-globulin concentrations: these were cats with effusive FIP or control cats with FCoV antibodies: the latter occurs in FECV-endemic environments (Pedersen, 1995b);
3. Cats with low antibody titers and high γ-globulin concentrations: all of these cats were FIP affected, with either the effusive or noneffusive forms. On these animals, the low antibody titers result from the formation of immune complexes (Pedersen, 1987);
4. Cats with high antibody titers and high γ-globulin concentrations: they were symptomatic cats with effusive or noneffusive FIP.

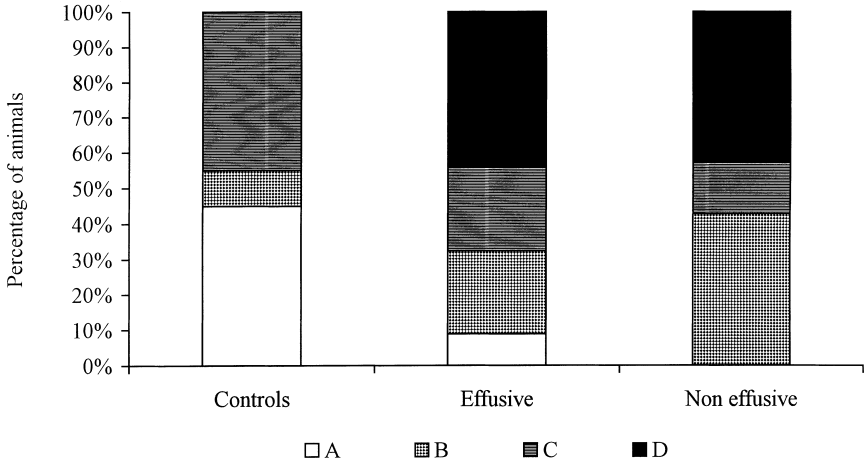

Fig. 4. Percentage of animals with different antibody titers and γ-globulin concentrations (A=low titer, low γ-globulins; B=low titer, high γ-globulins; C=high titer, low γ-globulins; D=high titer, high γ-globulins) in the controls and in cats with effusive and noneffusive FIP

Furthermore, cats with the same antibody titer but with different β-, and γ-globulin levels were detected (Fig. 5), suggesting that some of the detected proteins are not immunoglobulins. Many of complement fractions or immune complexes have similar motility. The C1q subunit, involved in immune complex disease, migrates with the β-globulins (Yamada and Hirayama, 1983). A C3 activating factor (C3AF) (Arroyave et al., 1976) and the C1r subunit (Ziccardi and Cooper, 1976) have a γ-globulin motility (Roberts et al., 1981). The changes in the level of these proteins support the previous studies on the fluctuation of complement fractions (Jacobse-Geels et al., 1982) and further confirm the role of type III hypersensitivity in FIP. These changes were present in

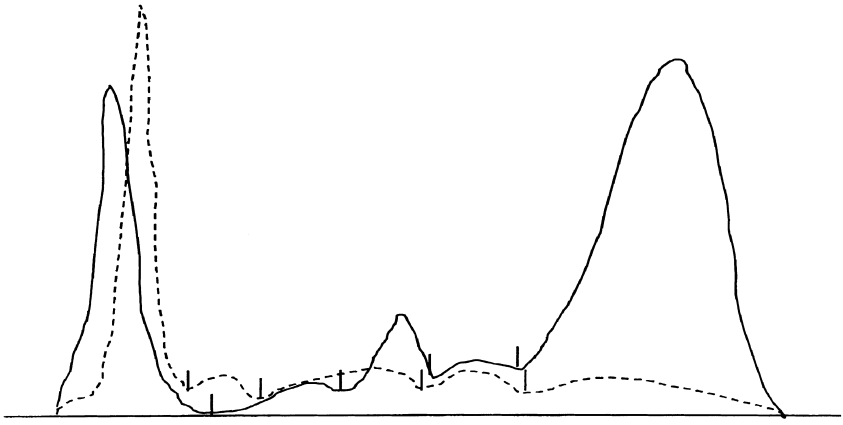

Fig. 5. Serum protein electrophoresis in cats with the same antibody titers: dashed line=control cat, titer 1:25, γ-globulins 12.7 g l; solid line=FIP cat, titer 1:25, γ-globulins 75.9 g l.

both the effusive and noneffusive forms, suggesting a similar involvement of the immune system.

The observed protein concentrations and the electrophoretic pattern agree with those reported in literature (Jain, 1986; Shelly et al., 1988; Sparkes et al., 1991, 1994). In the effusive form, β- and γ-globulin concentrations in the effusions were often higher than those of the corresponding serum. In immune complex disease, low antibody titers are expected. In this study, the effusions often had antibody titers higher than the corresponding serum (Jacobse-Geels et al., 1982).

Despite the variability in total and fractioned protein concentrations and antibody titers, no differences were found between the forms in the distribution of the lesions. Fibrinous perivisceral lesions were not seen in the noneffusive cases. The histologic pattern of the foci was similar in effusive and in noneffusive forms: three different patterns of the lesions were identified: (a) cellular infiltrates without necrosis; (b) cellular infiltrates with scattered necrotic intercellular debris; (c) large foci with a central necrosis.

The distribution of the virus and of the immune cells were not influenced by the antibody titers or by the total and fractioned protein concentration. The distribution of antigen and of immune cells varied depending upon the type of lesion identified:

In the first many $FIPV^+$ cells were distributed across the lesions, with many $MAC387^+$ macrophages, lymphocytes $CD3^+$, many of which $CD4^+$ and rare $CD8^+$, IgG^+ and IgM^+ lymphocytes were detectable. These foci were considered as acute lesions. $CD3^+$ and $CD4^+$ lymphocytes were often detectable near vessels. The $MAC387^+$ macrophages have recently been identified as blood-origin macrophages (Poston and Hussain, 1993) typical of perivascular acute inflammations (Bhardwaj et al., 1992). Expression of the MRP14 protein, the target antigen of this antibody (Goebeler et al., 1994), is largely absent in activated tissue macrophages (Lagasse and Weissman, 1992).

In the foci with scattered necrosis, viral and myeloid antigen was present in the necrotic debris, most likely due to the lysis of virus-infected macrophages. Many of the $MAC387^+$ cells in these lesions were granulocytes. A moderate number of $CD3^+$ and $CD4^+$ lymphocytes, were diffusely distributed across the lesions, while the distribution and the number of $CD8^+$, IgG^+ and IgM^+ lymphocytes were similar to those of the cellular foci.

When a central necrosis was present, necrotic debris was diffusely and weakly positive for myeloid antigen. Viral antigen was detectable only in the cells and in the intercellular necrotic debris around the necrotic center. In these lesions $MAC387^+$ cells were mainly granulocytes, and $MAC387^-$ activated macrophages (Bhardwaj et al., 1992; Lagasse and Weissman, 1992). The latter are likely involved in immune-cell down-regulation, as demonstrated in other granulomas, such as those of human tuberculosis and sarcoidosis, or in hypersensitivity reactions, such as contact hypersensitivity (Roitt et al., 1996). $CD3^+/CD4^+$, IgG^+ and IgM^+ lymphocytes were scattered over the cells around the necrosis while the number of peripherally distributed $CD8^+$ lymphocytes appeared to be greater than those observed in the foci without necrosis.

These results agree with those reported in previous studies on effusive FIP lesions (Paltrinieri et al., 1998) and with those on other immunological granulomas, in which T-lymphocytes (mainly $CD4^+$) and macrophages, with few IgM-exposing B-lymphocytes

are present in each stage of the lesion and fewer numbers of $CD8^+$ cells was detectable at the periphery of the larger lesions (Modlin et al., 1984; Momotani et al., 1989; Scanziani et al., 1990).

5. Conclusion

The results of this study strongly support the role of type III hypersensitivity (Hayashi et al., 1977, 1978; Pedersen and Boyle, 1980; Weiss et al., 1980; Weiss and Scott, 1981; Jacobse-Geels et al., 1980; Fenner, 1987; Pastoret and Bourtonboy, 1991; Pedersen, 1995a). In particular, the differences between globulin fractions and antibody titers suggest either the presence of immune complexes or the presence of complement fractions in FIP affected cats. These changes occur in both the effusive and in noneffusive forms.

In contrast, the results regarding the distribution of the virus and of the immune cells in lesions support the role of type IV hypersensitivity in their pathogenesis as suggested by others (Pedersen, 1987, 1995a; Paltrinieri et al., 1998). In particular advanced lesions, with central necrosis, activated macrophages, few and scattered IgG and IgM positive B-lymphocytes and peripherally distributed CD8 T-lymphocytes, were very similar to those observed in other granulomatous diseases (Scanziani et al., 1990).

The concurrent detection of humoral and cellular findings consistent with both the pathogenetic mechanisms indicates a complex involvement of the immune system. A better understanding of the pathogenesis of this disease may derive from the study of others and more sensitive indicators of the immune status such as the cytokines.

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