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Shifts in circulating lymphocyte subsets in cats with feline infectious peritonitis (FIP): pathogenic role and diagnostic relevance

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Abstract

Cats with feline infectious peritonitis (FIP) are usually lymphopenic and have lymphoid depletion evident in spleen and lymph nodes. In particular, the number of $CD4^+$ lymphocytes in tissues decreases during the evolution of FIP lesions. This decrease is most likely due to increased lymphocyte apoptotic rate. In contrast, cats infected with the Feline Coronavirus (FCoV) develop a follicular hyperplasia in the peripheral lymph nodes. The current study was devised to evaluate the possible pathogenic role of shifts in circulating lymphocyte subsets in FIP. Peripheral blood from cats with FIP was evaluated and compared with peripheral blood from clinically healthy cats living in both FCoV-free and FCoV-endemic catteries. Blood from cats with diseases other than FIP was also examined in order to define the diagnostic relevance of the changes. Lymphocyte subsets were analysed by flow cytometry, using a whole blood indirect immunofluorescence technique and mAbs specific for feline CD5, CD4, CD8, CD21. The results of the current study suggest that cats recently infected with FCoV that do not develop the disease have a transient increase in T cells; cats from groups with high prevalence of FIP have a moderate but persistent decrease in T cell subsets; cats with FIP have a very severe decrease in all the subsets of lymphocytes. Moreover, during FIP many lymphocytes do not express any membrane antigen, most likely due to early apoptosis. Cats with diseases other than FIP also had decreased number of lymphocytes: as a consequence, the diagnostic relevance of these findings is very low. Nevertheless, the lack of flow cytometric changes had a high negative predictive value (NPV), thus allowing to exclude FIP from the list of possible diagnoses in cats with normal cytograms.

Keywords: Feline infectious peritonitis; Feline coronavirus; Lymphocytes; Flow cytometry; Diagnosis

1. Introduction

Feline infectious peritonitis (FIP) is a fatal disease of cats caused by Feline Coronavirus (FCoV) strains. Feline Enteric Coronavirus (FECV) is a low pathogenicity FCoV that induces mild enteritis in cats. In contrast, the FCoV strains able to induce FIP are known as FIP virus (FIPV) (Pedersen, 1995). The FIPV arises from the FECV by a series of genomic mutations (Vennema et al., 1995; Rottier, 1999) and acquires the ability to infect, and to replicate within, macrophages. Infected macrophages transport the

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virus through the body (Pedersen, 1995). The frequency of these mutations increases in FCoV-endemic catteries (Heerewegh et al., 1997). Susceptibility to FIP depends also on individual factors such as age, sex and genetic predisposition (Foley and Pedersen, 1996). In a favourable environment, the infection is characterized by a "susceptible–infective–susceptible" (SIS) model: susceptible cats are infected but are not able to mount an immune response and become susceptible again (Foley et al., 1997). Cats that mount a good cell-mediate immune response show a degree of resistance to further infection. The activation of B cells leads to the production of antibodies. In vitro, antibodies increase the viral uptake by macrophages (Hodatsu et al., 1993), while in vivo they induce immune complexes formation and deposition (Jacobse-Geels et al., 1980). During the disease a functional shift of T helper cells from Th1 to Th2 occurs (Gunn-Moore et al., 1998). The number of lymphocytes decreases in lymph nodes (Kipar et al., 2001), in the lesions (Kipar et al., 1998; Paltrinieri et al., 1998) and in blood (Sparkes et al., 1994; Pedersen, 1995). The cause of lymphocyte depletion seems to be lymphocyte apoptosis, most likely due to the release of pro-aptoptotic factors from phagocytes (Haagmans et al., 1996). In contrast, a Th1 cytokine response (Gunn-Moore et al., 1998), and a follicular hyperplasia in lymph nodes (Kipar et al., 1999) have been reported in cats resistant to the infection. Circulating lymphocyte subsets have been analysed only in few cats with FIP and strong individual variations have been reported (Knotek et al., 2000).

This work evaluates the pathogenic role of shifts in circulating lymphocyte subsets in FIP. Blood from FIP-affected cats and from clinically healthy cats living in both FCoV-free and FCoV-endemic catteries has been examined. Also, blood from cats with diseases other than FIP was compared, to define the diagnostic relevance of the lymphocyte subsets changes.

2. Materials and methods

2.1. Animals and study design

The present study was performed on 48 blood samples collected from cats of different breed, age, and sex. Based on the remote anamnesis and on the clinical findings the cats were grouped as follows (Table 1):

Group 1 (*negative controls*). 9 clinically healthy cats from two catteries (sub-groups: 1A, $n = 4$; 1B, $n = 5$) on which no cases of FIP were recorded in the past 3 years.

Table 1
Breed, sex and age of the sampled cats[a]

Group 1 (controls)	Group 2 (FCoV exposed)	Group 3 (FIP)	Group 4 (other diseases)
Sub-group 1A	Sub-group 2A	(21) DSH, F, Unk	(38) DSH, M, 5 y
(1) P, M, 9 y	(10) P, M, 12 y	(22) P, M, 3 y	(39) DSH, F, Unk
(2) P, F, 11 m	(11) P, F, 7 y	(23) P, M, 10 y	(40) DSH, M, Unk
(3) P, M, 3 y	(12) P, F, 1 y	(24) P, F, 10 m	(41) DSH, M, Unk
(4) P, F, 4 y	(13) P, F, 10 m	(25) DSH, M, 13 y	(42) DSH, F, Unk
Sub-group 1B	Sub-group 2B	(26) DSH, M, 5 y	(43) DSH, M, 9 m
(5) DSH, M, 9 y	(14) P, F, 3 y	(27) DSH, M, 15 m	(44) DSH, M, 5 y
(6) DSH, F, 7 m	(15) DSH, M, 3 y	(28) Unk, Unk, Unk	(45) DSH, M, 1 y
(7) DSH, F, 3 y	(16) P, M, 8 y	(29) DSH, F, 12 y	(46) P, M, 4 y
(8) DSH, F, 2 y	Sub-group 2C	(30) DSH, F, 7 m	(47) Unk, Unk, Unk
(9) DSH, F, 6 m	(17) S, F, 1 y	(31) DSH, M, 1 y	(48) Unk, M, 2 y
	(18) S, F, 2 y	(32) S, M, 3 m	
	(19) S, M, 2 y	(33) Unk, Unk, Unk	
	(20) P, M, 2 y	(34) P, F, 1 y	
		(35) DSH, F, 1 y	
		(36) Unk, Unk, 1 y	
		(37) DSH, F, 8 m	

[a] P: persian, DSH: Domestic Shorthair, S: siamese, Unk: unknown, F: female, M: male, m: months and y: year.

Group 2 (*FCoV-exposed cats*). 11 cats coming from three catteries (sub-groups: 2A, $n = 4$; 2B, $n = 3$; 2C, $n = 4$) on which recent cases of FIP were diagnosed: in particular, during the past 3 years, nine cases of FIP were recorded in group 2A (last case: 3 months before this study), four cases in group 2B (last case: 6 months before this study) and one case in group 2C (1 week before the present study).

Group 3 (*FIP-affected cats*). 17 cats referred to this department from private vets and affected by effusive FIP, diagnosed by post-mortem examinations ($n = 12$) or by serum protein electrophoresis, cytology, and immunofluorecence test against FCoV performed on the effusion ($n = 5$).

Group 4 (*non-FIP cats*). 11 cats referred to this department from private veterinarians and presenting one or more clinical signs consistent with FIP (fever, anaemia, cavitary effusions, lymphopenia, increased serum globulins and/or neurologic signs). A disease other than FIP was diagnosed in these cats, based on the follow up, on the post-mortem examination or on other clinico-pathological findings.

Three millilitre of blood were withdrawn from the jugular vein of each cat: 1 ml was put in EDTA-coated tubes to be used for haematology and flow cytometry, while the remaining blood was put in tubes without anticoagulant. Serum was obtained by centrifugation (10 min, $450 \times g$) and frozen at $-20\,^{\circ}C$ before to perform serology.

In order to check any possible change due to the occurrence of FIP cases in the cattery, cats of Group 2 were also sampled every 30 days for two (Group 2C) or three times (Groups 2A and 2B). Cats from Group 1 were also sampled monthly for three times, in order to exclude that changes eventually detected in Group 2 might be due to repeated sampling. In contrast, it was not possible to repeat the sampling from cats with FIP because they were euthanasized or died few days after bleeding.

2.2. Serology

The possible presence of antibodies against feline immunodificiency virus (FIV) and of feline leukaemia virus (FeLV) circulating antigens was investigated on serum using commercially available ELISA kits (Snap test, IDEXX Lab, Westbrook, MA). In contrast, a latex agglutination test (Eiken Chemical, Tokyo, Japan) was used to identify antibodies against Toxoplasma gondii.

2.3. Haematology and flow cytometry

On each blood sample, a complete blood cell count by mean of an automatic cell counter (Hemat 8, SEAC, Firenze, Italy) was performed, followed by the differential leukocyte count on May Grünwald-Giemsa (MGG) stained smears.

The analysis of lymphocyte subsets was performed by using a whole blood indirect immunofluorescence technique, previously adapted to feline blood (Rocchi et al., 1992). The following mAbs, provided by Prof. P.F. Moore (Davis, CA) and specific for feline leukocyte markers, were used: FE1.B11 (CD5), FE1.7B12 (CD4), FE110E9 (CD8), CA2.1D6 (CD21). As a negative control, an irrelevant antibody (mouse IgG control, Becton Dickinson) was used. In the second staining step, the F(ab')2 antibody binding fragment of goat anti-mouse phycoerythrin-conjugated immunoglobulins (DAKO A/S, Glostrup, Denmark) was used. The stained cells were analysed by using the FACSort flow cytometer (FACSort, Becton Dickinson, San Jose, CA), equipped with the Cell Quest software.

Lymphocytes were gated and the percentage of the lymphocyte subsets was calculated. These values were utilized to calculate the $CD4^+/CD8^+$ ratio and the absolute numbers of the subsets using WBC absolute and differential counts. The percentage of $CD5^+ + CD21^+$ and of $CD4^+ + CD8^+$ cells was also calculated, as suggested by the guidelines for the flow cytometric immunophenotyping standardization (Reichert et al., 1991; Calvelli et al., 1993; Byrne et al., 2000). In healthy individuals, a 85–95% $CD5^+ + CD21^+$ range and a $CD4^+ + CD8^+$ value close to the percentage of $CD5^+$ cells $\pm 10\%$ is expected (Reichert et al., 1991).

2.4. Analysis of the effusions

Approximately 2 ml of fluid were withdrawn from each cat with abdominal or thoracic effusion, and put in a EDTA-coated tube. The protein content was evaluated in an automated analyser (EOS BRAVO, Hospitex, Firenze, Italy) by the biuret method using a commercial kit (Hospitex, Firenze, Italy). Then 50–100 μl of fluid were cytocentrifuged (Cytospin 2,

Shandon Scientific, Runcorn, Cheshire, UK) at $130 \times g$ for 10 min. Two slides were obtained from each sample: one was routinely stained with MGG, while the other was used to perform a direct immunofluorescence (DIF) tests as previously described (Paltrinieri et al., 1999), using a feline polyclonal fluorescein-conjugated antiserum detecting both FCoV biotypes I and II (VMRD, Pullman, WA).

2.5. Post-mortem examinations

Tissue samples were fixed in buffered 10% iso-osmotic formalin and embedded in paraffin. Microthomic sections (5 μm) were stained by haematoxylin-eosin and by immunohistochemistry using a mAb against the FCoV (kindly provided by Prof. N.C. Pedersen, Davis, USA). The Avidin Biotin Complex (ABC) method with a commercially available kit (Vectastain Elite, Vector Laboratories, Burlingame, CA) was used to detect the positive reaction (Hsu et al., 1980), after inhibition of the endogenous peroxidase (H_2O_2 1% in methanol) and antigen unmasking using microwave pretreatment (2 cycles of 5 min in citrate-buffered solution, 0.01 M, pH = 6.2). 3-Amino-9-ethyl-carbazole or diaminobenzidine served as chromogen for the reaction, then the slides were counterstained with Mayer's hematoxylin. Some sections of each sample were used as negative controls, with the primary antibody substituted by normal mouse serum (DAKO A/S, Glostrup, Denmark).

2.6. Statistical analysis

Statistical analysis was done with a specific software (Statsoft Inc., Tulsa, OK). Data from sub-groups 1A and 1B were compared each other by a Student's *t*-test for independent samples, or when a normality test showed that data did not have a normal distribution, by the corresponding non-parametric Mann–Whitney *U*-test.

Data from sub-groups 2A, 2B and 2C were compared each other using one-way ANOVA followed by the Tukey Honest Significant Difference (HSD) test, or using the non-parametric Kruskall–Wallis test.

Data from each cattery of Group 2 and from Groups 3 and 4 were compared with those of Group 1 using a one-way ANOVA followed by the LSD (planned comparison test), or using the Kruskall–Wallis test.

Results from repeated samplings from each cattery of Groups 1 and 2 were compared each other using an ANOVA test for repeated measurement or the corresponding Friedmann test.

The lowest and the highest values recorded in Group 1 were used to establish the reference range of each parameter as suggested by Lumsden (2000) for small groups of animals. Results from each sampling of Groups 2 and 4 were compared with the reference range: cats that had values lower than the reference range were considered false positive, and those with values within the reference range were considered true negative. In contrast cats from Group 3 with values within the reference range were considered as false negative, and those with values lower than the reference range as true positive. Sensitivity, specificity and positive and negative predictive values (PPV and NPV, respectively) were then calculated as suggested by Jacobson (1991).

3. Results

3.1. Clinical and pathological findings

Cats from Groups 1 and 2 had negative FIV, FeLV and *T gondii* serology and did not show clinical symptoms consistent with FIP or with other disease. However, a case of FIP was diagnosed in sub-group 2B 20 days after the first sampling. This cat was sampled at the appearance of the symptoms and included in Group 3 (cat no. 24).

The diagnosis of FIP was confirmed in all the cats from Group 3: electrophoretic and cytological findings from the effusions were always consistent with FIP, and FCoV positive cells were always detected in the effusions. Furthermore gross and histological findings, when available, were consistent with FIP, and FCoV positive cells were always detected within the lesions by immunohistochemistry.

In contrast, the follow up or the pathological and immunohistochemical findings excluded the diagnosis of FIP in all cats from Group 4. The final diagnoses for the cats included in this group were FIV (cat nos. 41, 44, 45, 48), hepatic carcinoma with abdominal effusions (cat nos. 38 and 42), *Candidatus Mycoplasma haemofelis* infection (cat nos. 39 and 40), gastroenteritis due to parvoviral infection

Table 2
Flow cytometric results (mean ± S.D.) in the different groups of cats

	Control Group 1	FCoV-exposed cats			FIP Group 3	Non-FIP Group 4
		Group 2A	Group 2B	Group 2C		
Lymphocytes × 10^3/μl	2.52 ± 1.25	2.44 ± 0.87	1.32 ± 0.33	4.07 ± 2.37	0.76 ± 0.66***	1.21 ± 1.01*
$CD5^+$ × 10^3/μl	1.55 ± 0.54	0.96 ± 0.71	0.90± 0.42	2.60 ± 1.41	0.51 ± 0.48***	0.62 ± 0.51***
$CD4^+$ × 10^3/μl	0.86 ± 0.29	0.47 ± 0.30*	0.60 ± 0.31	1.26 ± 0.87	0.35 ± 0.40**	0.33 ± 0.33**
$CD8^+$ × 10^3/μl	0.66 ± 0.24	0.42 ± 0.34	0.28 ± 0.12*	1.38 ± 0.88*	0.12 ± 0.10***	0.25 ± 0.19***
$CD21^+$ × 10^3/μl	0.65 ± 0.35	0.75 ± 0.57	0.30 ± 0.09	0.92 ± 0.39	0.22 ± 0.24**	0.42 ± 0.47
$CD5^+ + CD21^+$ (%)	89.9 ± 8.3	71.4 ± 38.2	89.6 ± 6.7	92.7 ± 18.6	69.5 ± 26.5*	86.1 ± 16.8
$CD4^+/CD8^+$	1.35 ± 0.38	1.25 ± 0.60	2.09 ± 0.21*	1.05 ± 0.44	3.29 ± 2.90	2.03 ± 2.90

* $P < 0.05$ vs. controls.
** $P < 0.01$ vs. controls.
*** $P < 0.001$ vs. controls.

(cat no. 43), uroperithoneum (cat no. 46), FELV (cat no. 47).

3.2. Haematology and flow cytometry

No significant differences were found between Groups 1A and 1B (data not shown). These two catteries were thus considered as an unique control group (Group 1).

In contrast, the comparison of the results from sub-groups 2A, 2B and 2C showed that the $CD4^+/CD8^+$ ratio was higher in sub-group 2B than in 2C ($P < 0.05$). As a consequence, differences among these three groups and controls were detectable (Table 2). Specifically, the number of $CD4^+$ lymphocytes recorded in cats from sub-group 2A were lower that those recorded in controls; the $CD4^+/CD8^+$ ratio recorded in sub-group 2B was higher than in controls, most likely due to the significant decrease of $CD8^+$ cells in sub-group 2B compared to controls; the number of $CD8^+$ cells was higher in sub-group 2C than in controls.

Compared to controls, cats with FIP had severe lymphopenia (Table 2). In three cats lymphopenia was so severe (43, 45 and 112 lymphocytes/μl) that flow cytometric evaluation of lymphocyte subsets could not be performed. Moreover, in cats with FIP the number of all the lymphocyte subsets and the percentage of $CD5^+ + CD21^+$ were significantly lower than in controls. The total lymphocyte number and the number of all the T cell subsets were significantly lower in cats from Group 4 than in controls (Table 2).

3.3. Fluctuation of lymphocyte subset in cats from Groups 1 and 2

In order to determine whether the differences recorded among the three catteries of Group 2 were due to the different prevalence of FIP, to the appearance of FIP cases in the cattery, or to normal fluctuations

Table 3
Flow cytometric results (mean ± S.D.) of the repeated samplings of cats from Group 2B

	Day 0	Day 30	Day 60	Day 90
Lymphocytes × 10^3/μl	1.32 ± 0.33	3.60 ± 0.78**	1.73 ± 1.42	2.37 ± 1.03
$CD5^+$ × 10^3/μl	0.90 ± 0.42	1.70 ± 0.05*	1.00 ± 1.08	1.65 ± 0.97
$CD4^+$ × 10^3/μl	0.60 ± 0.31	1.16 ± 0.08*	0.76 ± 0.94	1.01 ± 0.64
$CD8^+$ × 10^3/μl	0.28 ± 0.12	0.37 ± 0.07	0.37 ± 0.47	0.50 ± 0.37
$CD5^+ + CD21^+$ (%)	89.6 ± 6.7	73.4 ± 11.4	75.5 ± 21.8	93.2 ± 4.1
$CD21^+$ × 10^3/μl	0.30 ± 0.09	0.93 ± 0.66	0.46 ± 0.44	0.55 ± 0.05**
$CD4^+/CD8^+$	2.09 ± 0.21	3.17 ± 0.50*	2.11 ± 0.16	2.31 ± 1.15

* P<0.05 vs. Day 0.
** P<0.01 vs. Day 0.

Table 4
Diagnostic relevance for FIP of flow cytometric changes

Flow cytometric change	Sensitivity	Specificity	PPV[a]	NPV[b]
Decreased lymphocytes $\times 10^3$/μl	82.4	70.7	45.2	93.2
Decreased $CD5^+ \times 10^3$/μl	85.7	55.2	31.6	94.1
Decreased $CD4^+ \times 10^3$/μl	85.7	63.8	36.4	94.9
Decreased $CD8^+ \times 10^3$/μl	100.0	62.1	38.9	100.0
Decreased $CD21^+ \times 10^3$/μl	78.6	81.0	50.0	94.0
Decreased $CD5^+ + CD21^+$ (%)	50.0	84.5	43.8	87.5
Decreased $CD4^+/CD8^+$	14.3	87.9	22.2	81.0
Increased $CD4^+/CD8^+$	64.3	77.6	40.9	90.0

[a] Positive predictive value.
[b] Negative predictive value.

of lymphocyte subset number, cats of these groups and of the control group were repeatedly sampled.

No significant changes among the different samplings were detectable in the control group as well as in sub-groups 2A and 2C. In contrast just after the appearance of a case of FIP in the cattery, cats from sub-group 2B (Table 3) showed a significant increase of total lymphocytes, of the number of $CD5^+$ and $CD4^+$ subsets, and of the $CD4^+/CD8^+$ ratio. B cell number increased but only 3 months after the first sampling this increase was statistically significant.

3.4. Diagnostic relevance of the changes in circulating lymphocyte subset

The decreased number of T cell subsets had a high sensitivity but a low specificity (Table 4). Only the decrease of $CD4^+/CD8^+$ ratio had a good specificity. PPV was always low. In contrast all the changes observed had a high NPV; in particular, the decreased number of $CD8^+$ cells was characterized by the highest NPV.

4. Discussion

Results from control group were in agreement with reference ranges (Clinkenbeard and Meinkoth, 2000) and with the recommendations of guidelines for cytometric test standardization (Calvelli et al., 1993; Byrne et al., 2000). These findings, together with the lack of any positive serology for feline infectious diseases and the lack of any clinical symptom during the study period, confirmed that the results of Group 1 can be considered adequate control values. Although no data about the presence of FCoVs in control group were available, our knowledge about FCoV epidemiology strongly suggests that this group was not FCoV-endemic (Addie and Jarrett, 1995; Kass and Dent, 1995; Addie et al., 2000). All the cats from Group 1 had only sporadic contact with other cats and no cases of FIP were detected in their environment.

In contrast, cats from Group 2 were most likely living in FCoV-endemic groups. The repeated or the recent appearance of clinical forms of FIP in these catteries suggested the endemic presence of FCoVs and of persistent or recurrent faecal shedders of the virus (Addie and Jarrett, 1995; Kass and Dent, 1995; Heerewegh et al., 1997; Addie and Jarrett, 2001). The simple contact with the virus does not necessarily mean that the cats become infected: nevertheless, electrophoretic changes and fluctuations of some acute phase proteins (Giordano et al., in press), histological changes in lymphoid organs (Kipar et al., 1999) and increases of B-cell stimulating cytokines (Gunn-Moore et al., 1998) have been reported in cats that do not develop the disease. This suggests that these cats may come into contact with the virus, most likely due to the shedding of FCoV by the affected cat or to increased fecal shedding among the cats (Heerewegh et al., 1997; Addie and Jarrett, 2001). The analysis of lymphocyte subsets supports this hypothesis. Although of variable severity, changes in total and differential lymphocyte counts were detectable in FCoV-exposed cats. These findings were most

likely due to the different prevalence of FIP in the groups. Cats form sub-group 2A (high prevalence of FIP) had a moderate but persistent decrease in T cell subsets, more evident for $CD4^+$. In sub-group 2B (mild prevalence of FIP), T cell subsets, and in particular $CD8^+$ cells, were also reduced. However, in this group all lymphocyte subsets were transiently increased after the appearance of a case of FIP inside the cattery. In sub-group 2C (low prevalence of FIP but recent diagnosis of a case of FIP) the numbers within all lymphocyte sub-populations, and in particular $CD8^+$ cells, were higher than in controls. These findings confirm that the appearance of a clinical form of FIP in the group is followed by increased number within all T cell subsets in the cats that did not develop the disease. This is in agreement with the recent detection of FCoV-specific $CD4^+$ and $CD8^+$ cells in cats with prolonged survival times after FCoV infection (de Groot-Mijnes et al., 2002). In contrast, the low T cell counts detected in catteries with high prevalence of FIP suggests that the decreased number of these cells might be involved in differences in disease susceptibility among cats. This hypothesis is supported by the results recorded in cats with FIP, which had extensive lymphopenia with decreased numbers within all the lymphocyte subsets, in agreement with previous reports on blood (Knotek et al., 2000) and on tissues, where advanced FIP lesions were found to have less $CD4^+$ lymphocytes than recent ones (Paltrinieri et al., 1998). Compared to controls, the percentage of $CD5^+ + CD21^+$ cells was significantly lower in cats with FIP, while the percentage of $CD4^+ + CD8^+$ cells was close to the percentage of $CD5^+$ cells. Although the decreases in $CD5^+ + CD21^+$ cells was not significant (high S.D.), a similar decrease was also recorded in non-symptomatic cats from Group 2A, in which the prevalence of FIP was very high, and in cats from Group 2B after the appearance of a case of FIP in the cattery. This finding has been previously interpreted as a consequence of early apoptotic changes, which might alter membrane antigens, by a yet unidentified mechanism (Potter et al., 1999). Apoptosis of lymphocytes has been reported to occur during FIP, most likely due to the release of pro-apoptotic factors from phagocytes (Haagmans et al., 1996). It is possible that early apoptotic changes might be responsible for this finding in FIP-affected cats. However, the possibility that in FIP-affected cats the percentage of non-T–non-B lymphocytes increases cannot be excluded. These findings, together with previously reported defects of T cell function (Gunn-Moore et al., 1998; Knotek et al., 2000) might play an important role in the development of FIP.

A decrease of T cell subsets, however, was also detected in cats with symptoms consistent with FIP but affected by diseases other than FIP, most likely due to the presence, in this group, of cats with viral diseases (FIV, FeLV, parvovirus) which are known to reduce the number of circulating T cells, or with diseases that can be associated with a stress-induced lymphopenia (Cowell and Decker, 2000). This might reduce the diagnostic relevance of changes in circulating lymphocyte subsets in cats with FIP. Nevertheless, the decrease in T cell counts had a high sensitivity, thus reducing the possibility to have false negative results, but a low specificity, as usually occurs for changes with high sensitivity (Jacobson, 1991). ‘‘False positive’’ cats (cats without FIP with decreased T cell subset counts) might thus be detected. Moreover, the low PPV recorded does not allow cats with reduced lymphocyte subsets as being affected by FIP. On the contrary, the high NPV associated with a normal flow cytometry (and in particular with regards to $CD8^+$ counts) may identify cats that are not affected by FIP.

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