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## **Evaluation of Immunity to Feline Infectious Peritonitis in Cats with Cutaneous Viral-induced Delayed Hypersensitivity**

R.C. WEISS and N.R. COX

*Scott Ritchey Research Program and the Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL 36849 (U.S.A.)*

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### **ABSTRACT**

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Delayed-type hypersensitivity (DTH)-like reactions to feline infectious peritonitis (FIP) virus (FIPV) were induced in the skin of nine cats that were asymptomatic after a previous challenge-exposure with FIPV. Four of the nine previously challenge-exposed cats were negative for virus-neutralizing antibodies against FIPV at the time of intradermal (ID) testing for DTH. Two other cats tested for DTH when acutely ill with clinical FIP did not have cutaneous DTH responses to FIPV.

Gross skin reactions to FIPV injected ID were observed in six of nine asymptomatic cats (67%) at postintra-dermal inoculation hours (PIH) 24, 48, and/or 72. The reactions consisted of focal, 1-5-mm to 2.5-cm diameter indurated or semi-firm, nonerythematous, slightly raised nodules.

Microscopically, DTH-like reactions were observed in biopsies taken from the FIPV-inoculated skin of asymptomatic cats at PIH 24 to 72. The lesions consisted of perivascular and diffuse dermal infiltrations by macrophages, lymphocytes, and polymorphonuclear leukocytes (PMN). The dermal infiltrates, which were maximal at PIH 48 or 72, were predominantly mixed inflammatory cells (five of nine cats) or PMN (four of nine cats) at PIH 24, but later were predominantly mononuclear cells (six of nine cats) or mixed inflammatory cells (two of nine cats) at PIH 72.

Five of nine cats (56%) with positive DTH skin responses had increased survival times after lethal ID challenge-exposure with FIPV compared to mean survival times in FIPV-naive, non-immune control cats that were DTH-negative when ID challenge-exposed. Four of nine DTH-positive cats (44%) resisted an ID challenge-exposure dose of FIPV that was fatal in both control cats, and two of the four remaining DTH-positive cats survived a third challenge-exposure with highly lethal doses of FIPV given intraperitoneally. Four of the six DTH-positive cats (67%) that died after re-challenge and were necropsied had lesions of noneffusive FIP, suggesting that cellular immunity may also be involved in the pathogenesis of noneffusive disease, whereas both control cats and both DTH-negative cats with clinical disease succumbed to effusive FIP. Seemingly,

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DTH responses to FIPV can be associated with an increased level of resistance to disease; however, this state of immunity is variable and apparently can be lost with time in some cats.

## INTRODUCTION

The critical host defensive mechanism(s) that are specifically involved in resistance to feline infectious peritonitis (FIP) are not known. Apparently, humoral immunity by itself is not protective; instead, it may promote development of vascular lesions and cause a fatal immune-mediated disease (Pedersen and Boyle, 1980; Weiss and Scott, 1981a, b, c). Immune complexes of FIP virus (FIPV), anti-FIPV immunoglobulins, and complement (C3) can be demonstrated in lesions, serum, and in the renal glomeruli of cats with FIP (Pedersen and Boyle, 1980; Jacobse-Geels et al., 1980; Weiss and Scott, 1981a; Jacobse-Geels et al., 1982). It is presently believed, but not proven, that cell-mediated immunity (CMI) plays a decisive role in resistance to FIP. This hypothesis is supported by several observations. Firstly, the clinical incidence of FIP is increased greatly by concurrent infection with feline leukemia virus (FeLV), and FeLV is a powerful suppressant of CMI in cats (Hardy, 1982; Rojko and Olsen, 1984). Secondly, there is severe necrosis and depletion of lymphocytes in T-dependent areas, and normal to hyperplastic B cells in spleen and lymph nodes of cats with induced FIP (Weiss and Scott, 1981a, c; Hayashi et al., 1983). Thirdly, there is enhanced dissemination of FIPV in macrophages and a more severe disease in thymectomized vs control kittens infected with FIPV (Hayashi et al., 1983). Also, blastogenesis to T-cell mitogens is suppressed in cats after infection with FIPV (Stoddart et al., 1988), and blastogenic responses to FIPV, or delayed-type hypersensitivity (DTH) responses to FIPV inoculated intraconjunctivally, are increased in some FIPV-recovered cats (Pedersen and Floyd, 1985). Finally, the histopathologic lesions of non-effusive FIP are similar to lesions of tuberculosis or deep mycoses (e.g., histoplasmosis, coccidioidomycosis); and CMI in the latter diseases is involved in both immunity and development of lesions (Beeson et al., 1979; Pedersen and Black, 1983).

In a previous report of two cats, we described strong microscopic DTH-like lesions to FIPV inoculated intradermally (ID) in an FIP-resistant cat, and weak or no DTH responses in a cat with FIP (Weiss and Cox, 1988). We now evaluate DTH skin responses to FIPV and resistance to disease in a group of nine asymptomatic cats previously challenge-exposed with virulent FIPV, in two nonimmune cats without previous exposure to FIPV, and in two cats with induced FIP, and further suggest a role of CMI in FIP disease resistance.

## MATERIALS AND METHODS

*Experimental animals*

The source, FIPV exposure history, clinical status, and virus neutralizing serum antibody titers in cats prior to ID tests for DTH are summarized in Table 1. Nine random-source mixed breed healthy adult cats were selected retrospectively from a larger group of cats that had been previously challenge-exposed intraperitoneally (IP) with FIPV. All the cats were FeLV test-negative (by ELISA) and were seronegative for feline coronavirus neutralizing antibodies prior to challenge-exposure with FIPV. The cats were housed separately in cages at the Scott Ritchey Animal Isolation Facilities; housing, care, and testing of cats were in compliance with standards set forth in the "Guide for the Care and Use of Laboratory Animals" (NIH publication no. 85-23). The larger group of FIPV-inoculated cats initially was challenge-exposed with

TABLE 1

Clinical history of cats tested for DTH skin responses to feline infectious peritonitis virus

Cat	Source	Exposure history <sup>a</sup>	Clinical status <sup>b</sup>	VNA
1	SPF	NE	N	Neg
2	SPF	NE	N	Neg
3	R	E	A	480
4	R	E	A	15 360
5	R	E	A	Neg
6	R	E	A	3 840
7	R	E	A	Neg
8	R	E	A	Neg
9	R	E	A	Neg
10	R	E	A	2 560
11	R	E	A	ND
12	R	E	FIP	ND
13	R	E	FIP	640

<sup>a</sup>Cats either were FIPV-naive and nonimmune at time of ID testing for DTH (cats 1 and 2) or were previously inoculated IP with virulent FIPV at 6 weeks (cats 3-9), 5 weeks (cat 10) or 2 weeks (cats 11-13) before evaluation for DTH. All cats were test-negative for FeLV (by ELISA) and for feline coronavirus neutralizing antibodies prior to initial FIPV challenge-exposure.

<sup>b</sup>Clinical condition at time of ID testing for DTH.

Abbreviations: VNA = feline coronavirus neutralizing antibody titer, determined at time of ID challenge-exposure (PIH 0); SPF = specific-pathogen-free cats, which were FIPV-naive and non-immune at time of ID challenge-exposure; R = random source cats, which were healthy and FIPV-seronegative at time of initial IP challenge-exposure; NE = not previously exposed to FIPV; E = challenge-exposed prior to ID challenge-exposure; N = normal; A = asymptomatic; FIP = clinical signs of experimental FIP; Neg = negative at 1:10 serum dilution; ND = not determined.

different concentrations of FIPV in order to establish immunity in some of the cats without inducing fatal disease. Seven of 16 cats given 1–5 cat lethal doses ( $LD_{100}$ ) of FIPV IP survived (cats 3–9) and remained asymptomatic for 8 weeks before ID challenge-exposure. Four of these cats remained seronegative to FIPV; three of the four seronegative cats, however, became febrile 1–2 weeks after IP challenge-exposure. One of 12 cats given 100  $LD_{100}$  of FIPV survived (cat 11) and was asymptomatic for 2 weeks before ID challenge-exposure, and one of four cats given 5000  $LD_{100}$  of FIPV survived (cat 10) and was asymptomatic for 5 weeks. Two other random-source cats (a 5-month-old kitten [no. 12] and an adult cat [no. 13]) were challenge-exposed IP with 100 or 5  $LD_{100}$  of FIPV, respectively; they developed clinical signs of experimental FIP (Weiss and Scott, 1981a) at 2 weeks postinoculation and then were ID tested for DTH. Two healthy 5-month-old specific-pathogen-free (SPF) kittens (FIPV-naive, nonimmune controls) were obtained from a commercial breeder (Liberty Laboratories, Liberty Corners, NJ) and were FeLV test-negative and FIPV antibody-negative at the time of ID challenge-exposure.

### *Virus*

The DF2 strain of FIPV (American Type Culture Collection, ATCC No. VR 2004, Rockville, MD) was used for all challenge-exposures of cats, including ID inoculations, and serological assays. The virus was propagated in Crandell feline kidney (CrFK) cells and passed five times in our laboratory. Cell-free virus was concentrated by ultrafiltration (Minitan<sup>TM</sup>, Millipore, Bedford, MA) and frozen and stored at  $-80^{\circ}\text{C}$  in tissue culture growth medium (1-ml aliquots) consisting of Eagle's minimum essential medium (MEM) in Earle's salt solution supplemented with 10% heat-inactivated fetal bovine serum and antibiotics. The titer of the FIPV in CrFK cells was approximately  $10^7$  tissue culture infective doses ( $TCID_{50}$ )/ml. By previous titration, this inoculum contained approximately  $10^3$   $LD_{100}$  of FIPV/ml.

### *Experimental design*

Nine asymptomatic cats that had survived a previous challenge-exposure with FIPV were inoculated ID with live, cell-free FIPV (200  $LD_{100}$ ) and subsequently were evaluated both grossly and microscopically for induced DTH skin reactions. At 4 weeks after ID challenge-exposure, four of the nine cats were still asymptomatic (nos. 3–6) and received a third challenge-exposure of 100  $LD_{100}$  of FIPV given IP. The cats were monitored daily for fever and other signs of clinical disease. All cats (except cat 7) that died after challenge-exposure with FIPV were necropsied, and their tissues were examined grossly and microscopically for FIP lesions.

Two cats with early signs of induced FIP (nos. 12 and 13) were inoculated

ID with FIPV and tested for DTH. When these cats became moribund, they were euthanatized and necropsied.

Two SPF kittens (nos. 1 and 2; FIPV-naive, nonimmune controls) were inoculated ID with FIPV and then evaluated for induced skin changes from the FIPV (or medium) alone. Determination of survival times also was made in nonimmune cats after primary challenge-exposure with FIPV given ID. The two control kittens subsequently were euthanatized and necropsied 1–2 months later when moribund with FIP. Survival times and mortality after challenge-exposure(s) with FIPV, the presence or absence of induced FIP, and the clinical form of FIP produced (effusive or noneffusive) were evaluated in all cats.

### *Intradermal skin tests*

Prior to ID skin inoculations, each cat was mildly sedated with ketamine hydrochloride (Vetalar, Parke Davis) given intramuscularly (11 mg/kg), and the hair over the left and right lateral flanks was clipped. The skin was scrubbed with disinfectant soap (Betadine, Purdue Frederick), rinsed in water and wiped with 70% ethyl alcohol. The FIPV-DF2 was diluted 1:5 in growth medium, and a sample subsequently was saved for virus titration. Tests for DTH were performed by injecting either 0.1 ml of FIPV (containing approximately  $10^6$  TCID<sub>50</sub>) or 0.1 ml of growth medium (medium control) ID into each of 10 skin sites on the left or right flank, respectively, using a 1-ml tuberculin syringe attached to a 26-gauge needle. At postintradermal inoculation hour (PIH) 10–12, 24, 48, or 72, each cat was sedated as described before, the skin injection sites were examined, and visible reactions were measured and recorded in mm of diameter. The skin was wiped with 70% alcohol and duplicate biopsies were taken both from the FIPV-inoculated and media control skin using either a scalpel or a 6-mm biopsy punch (Baker's biopsy punch, Key Pharmaceuticals, Florida). The skin incision was closed with 2–0 nylon sutures. The biopsy samples were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 6  $\mu$ m, stained with hematoxylin and eosin, and examined under a light microscope. Photomicrographs of selected sections were taken with an automated camera system (PM-10ADS, Olympus) using 35-mm black and white film [Panatomic X (ASA 32), Kodak] and neutral density and green contrast filters.

### *Serology*

Virus neutralizing antibody (VNA) assays were performed on serum samples (heat-inactivated at 56°C for 0.5 h) using a standard infectivity inhibition-type assay in feline embryo (fcwf-4) cells. This assay was performed essentially as described by Pedersen and Black (1983) except that 50 TCID<sub>50</sub> of

FIPV-DF2 were incubated with each dilution of serum (starting at a 1:10 dilution) and viral cytopathic effects (CPE) were scored at 48 h. Serum VNA titers were recorded as the reciprocal of the highest dilution of serum that completely inhibited CPE in 50% of test wells. The titer of the FIPV (TCID<sub>50</sub>/0.05 ml) was determined by endpoint dilution in CrFK cells, using the method of Reed and Muench (1938).

## RESULTS

### *Delayed-type hypersensitivity skin responses*

*FIPV-naive, nonimmune control cats.* Gross reactions in the FIPV- or medium-inoculated skin were not observed at PIH 10–12, 24, 48, or 72 (Table 2). Microscopically, the FIPV-inoculated skin at PIH 10–12 either was normal (cat 1), or it had edema and occasional vasculitis characterized by mild accumulations of neutrophils in the deep dermis (cat 2) (Table 3). At PIH 24, 48, or 72, there were mild infiltrations by neutrophils or mixed inflammatory cells and

TABLE 2

Gross skin reactions after intradermal injection of FIPV in FIPV-naive, nonimmune cats or in FIPV challenge-exposed asymptomatic or FIP-symptomatic cats<sup>a</sup>

Cat	Time after injection (h)			
	10–12	24	48	72
<b>FIPV-naive, nonimmune</b>				
1	Neg	Neg	Neg	Neg
2	Neg	Neg	Neg	Neg
<b>FIPV challenge-exposed, asymptomatic</b>				
3	Neg	+	Neg	Neg
4	Neg	Neg	++	Neg
5	Neg	Neg	Neg	Neg
6	Neg	Neg	+++	++++
7	Neg	Neg	Neg	Neg
8	Neg	Neg	++	++
9	Neg	+	++	++
10	Neg	Neg	+	Neg
11	Neg	Neg	Neg	Neg
<b>FIP-symptomatic</b>				
12	Neg	Neg	Neg	Neg
13	Neg	Neg	Neg	Neg

<sup>a</sup>Reactions at injection site characterized by focal induration with or without swelling (edema), graded in severity as follows: diameter of lesion, + 1–5 mm; ++ 5–10 mm; +++ 10–15 mm; ++++ > 15 mm. Injection of virus diluent (growth media) into control skin did not produce gross reactions at 10–12, 24, 48 or 72 h. Neg=no reactions observed.

TABLE 3

Microscopic skin responses after intradermal injection of FIPV in FIPV-naive, nonimmune cats or FIPV challenge-exposed asymptomatic or FIP-symptomatic cats<sup>a</sup>

Cat	Time after injection (h)			
	10-12	24	48	72
FIPV-naive, nonimmune				
1	Neg	++	++	++
2	++	++	++	++
FIPV challenge-exposed, asymptomatic				
3	++	+++	++++	++
4	++	+++	++++	++++
5	+++	+++	++++	+++
6	++	++	+++	++++
7	+	+++	+++	+++
8	+	++	+++	+++
9	+	+++	+++	+++
10	Neg	++	++++	++++
11	++	+++	++++	++++
FIP-symptomatic				
12	+	+	+	+
13	ND	Neg	Neg	+

<sup>a</sup>Perivascular, perifollicular and diffuse dermal infiltrates of macrophages, lymphocytes and polymorphonuclear cells, graded in severity as follows: + edema with or without occasional inflammatory cells; ++ edema plus mild increase in inflammatory cells; +++ edema plus moderate increase in inflammatory cells; ++++ edema plus marked increase in inflammatory cells. Neg = no abnormal findings; ND = not determined. Injection of growth media into control skin resulted in a neg or + response in all cats, except for cat 11, which showed a ++ response at 24 h.

edema within the dermal interstitium or perivascularly (Fig. 1). The medium-inoculated control skin had mild edema and occasional inflammatory cells at PIH 10-12 and 24; no reactions were observed at PIH 48 or 72.

*Asymptomatic cats with previous exposure to FIPV.* Gross reactions in FIPV-inoculated skin were observed in two of nine cats at PIH 24, in five cats at PIH 48, and in three cats at PIH 72 (Table 2). Reactions were not observed in virus-inoculated skin at PIH 10-12, or in medium-inoculated skin at PIH 10-12, 24, 48, or 72. The skin reaction consisted of a focal, indurated or semi-firm, slightly raised nodule (Fig. 2); erythema or pruritus was not observed and pain responses were not elicited by palpation of the lesion. The skin reactions ranged in size from 1-5 mm to 2.5 cm in diameter and were maximal at PIH 48-72. The lesions were mild-to-moderate and transient in three cats and occurred at PIH 24 (cat 3) or PIH 48 (cats 4 and 10); in three other cats (nos. 6, 8, and



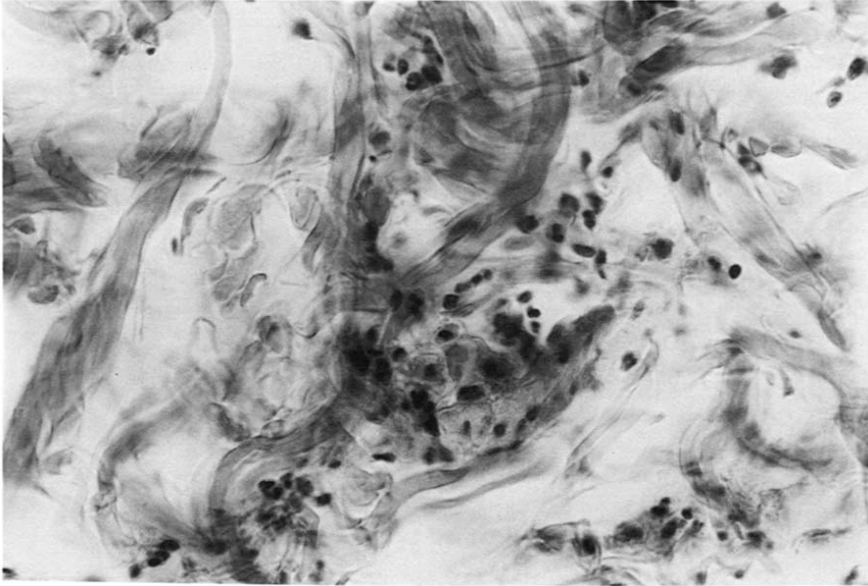


Fig. 1. Skin biopsy taken at PIH 72 from nonimmune control cat 2. FIPV-inoculated skin has a mild diffuse inflammatory cell infiltrate in the dermis. H & E stain;  $\times 421$ .

9), the reactions were moderate-to-severe at PIH 48 or 72 and persisted for 24 h or more.

Microscopically, ID responses to FIPV at PIH 10–12 generally were mild and consisted only of edema, or edema with a few inflammatory cells; the responses at PIH 24 were moderate; and the responses at PIH 48–72 were moderate-to-severe (Table 3). Cat 5 developed a moderate inflammatory reaction consisting of perivascular infiltrations of mixed inflammatory cells (predominantly neutrophils) at PIH 10–12. In the other cats, responses at PIH 10–12 ranged from edema with or without occasional inflammatory cells (four of nine cats) to edema with mild infiltrations by mononuclear cells and neutrophils (in four cats). There were perivascular (predominantly perivenular) and perifollicular cellular infiltrates in three cats. Medium-inoculated control skin at PIH 10–12 only had edema with or without occasional inflammatory cells (nine of nine cats).

At PIH 24, there were mild (in three cats) or moderate (in two cats) cellular infiltrates in the FIPV-inoculated skin. In six of nine cats, there was some perivascular and/or perifollicular orientation of inflammatory cells. The infiltrates consisted of neutrophils, macrophages and lymphocytes (mononuclear cell: neutrophil ratio approximately 1:1 to 2:1) in four cats, or they were predominantly neutrophils in four cats. The sections from cat 6 contained increased numbers of eosinophils in addition to neutrophils, macrophages, and lymphocytes. Medium-inoculated skin at PIH 24 had edema with or without

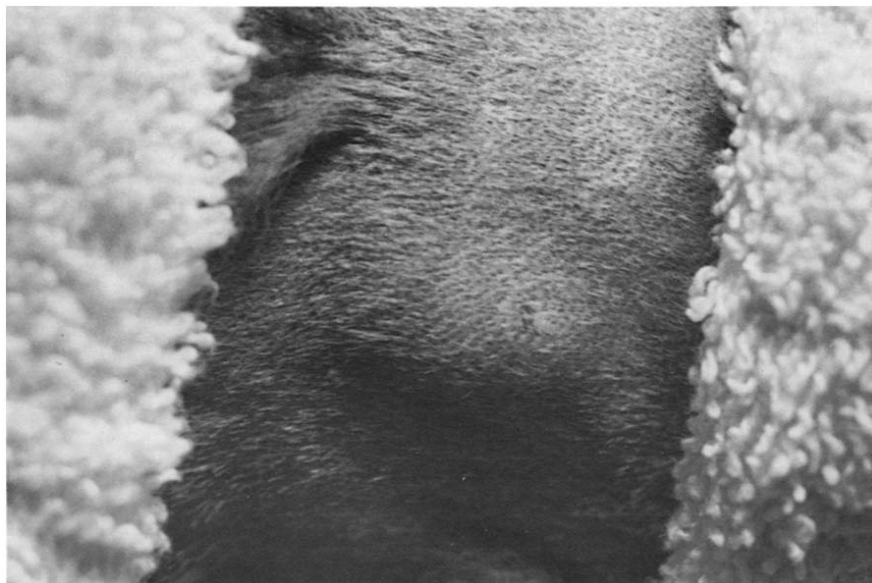


Fig. 2. Gross skin reaction at PIH 72 in asymptomatic cat 6 inoculated ID with FIPV. The lesion was about 2.5 cm in diameter. This cat originally had been challenge-exposed IP with FIPV 6 weeks prior to skin inoculation, had shown only a transient fever that lasted 5 days, and had seroconverted.

rare inflammatory cells in eight of nine cats; in cat 11, there was a mild increase in inflammatory cells.

At PIH 48, FIPV-inoculated skin had moderate (in four cats) or marked (in five cats) cellular infiltrates adjacent to blood vessels and hair follicles or other dermal adnexa. In cats 3 and 6, the lesions involved blood vessel walls but fibrinoid necrosis was not observed. The infiltrates consisted predominantly of mixed inflammatory cells (mononuclear cell: neutrophil ratio of 1:1) in five cats, neutrophils in two cats, and mononuclear cells in two cats. Three cats had increased numbers of eosinophils at the injection site. Medium-inoculated skin either had no reactions or had edema with or without rare inflammatory cells.

At PIH 72, FIPV-inoculated skin had moderate dermal infiltrates in three cats or marked infiltrates in four cats. Cat 6, which had shown a marked reaction at PIH 48, showed a mild reaction at PIH 72. The cellular infiltrates in most cats were associated with blood vessels and were diffuse in the dermal interstitium or were around hair follicles and other adnexa. Three cats still had mixed inflammatory cell responses (Fig. 3) whereas six cats now had predominantly mononuclear perivascular reactions (Fig. 4). Cat 3 had focal areas of necrosis and polymorphonuclear leukocytes (PMN) associated with perivas-

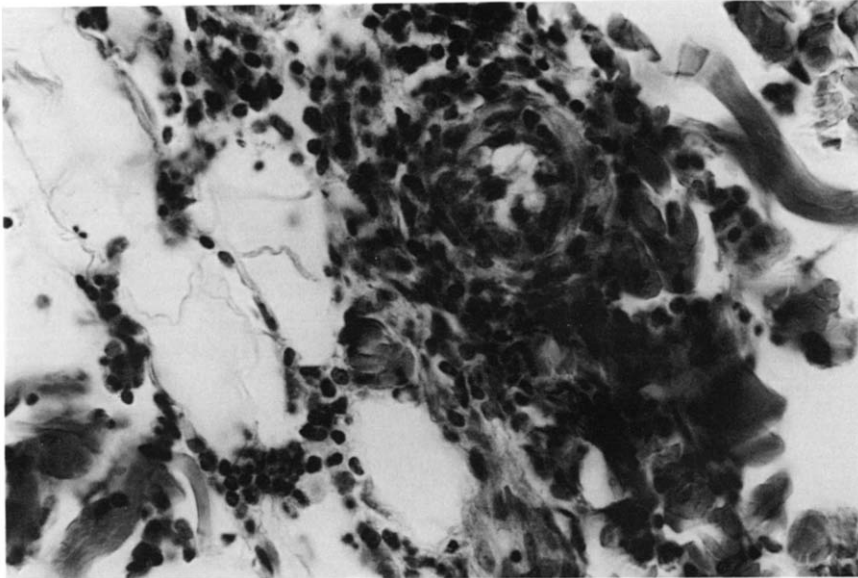


Fig. 3. Biopsy of FIPV-inoculated skin taken at PIH 72 from asymptomatic cat 11. There is a DTH-like reaction of mixed mononuclear cells and PMN within and around the wall of a blood vessel in the deep dermis. H & E stain;  $\times 421$ .

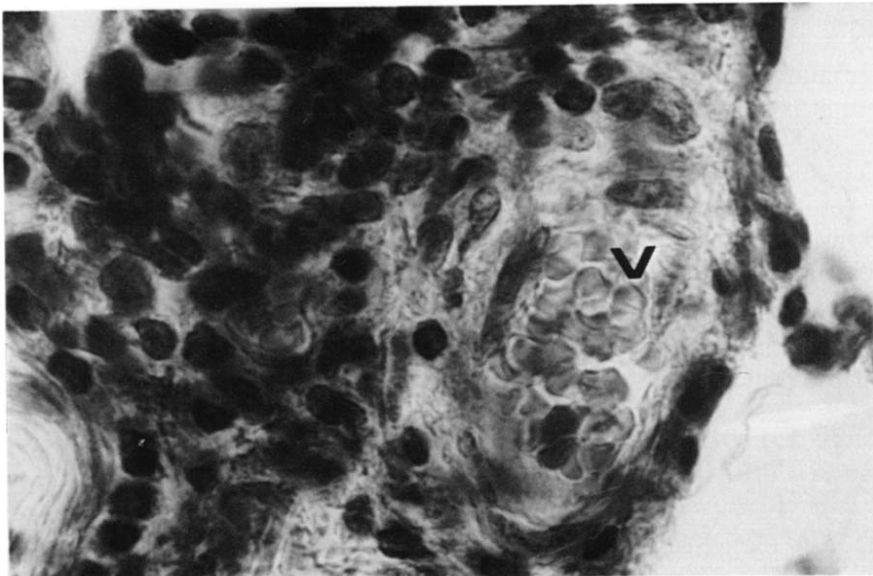


Fig. 4. Skin biopsy taken at PIH 72 from asymptomatic cat 5. There is a DTH-like reaction consisting of a heavy mononuclear cell infiltration by macrophages and lymphocytes within and around the wall of a dermal blood vessel (V). H & E stain;  $\times 1053$ .

cular reactions and hair follicles. Medium-inoculated skin either had no reactions or had edema with or without occasional inflammatory cells.

*FIP-symptomatic cats.* Gross reactions were not observed either in FIPV- or medium-inoculated skin at PIH 10–12, 24, 48, or 72 (Table 2). Microscopically, there was edema without noticeable inflammatory cell infiltration in the FIPV- or medium-inoculated skin of cat 12 at PIH 10–12 through 72. Rarely, a few inflammatory cells were within or around blood vessel walls in the FIPV-inoculated skin (Fig. 5). In cat 13, reactions were not observed in the FIPV-inoculated skin at PIH 24 or 48, and there were only occasional mild perivascular foci of mixed inflammatory cells at PIH 72. Lesions were not observed in the medium-inoculated skin.

### *Survival studies*

Survival times and postmortem findings in cats challenge-exposed one or more times with lethal doses of FIPV are shown in Table 4. Survival times after ID challenge-exposure with highly lethal doses of FIPV were increased in five of nine DTH skin test-positive cats (56%) compared to mean survival times in nonimmune control cats (Table 4). Four of nine DTH-positive cats (44%) resisted two challenge-exposures with lethal amounts of FIPV, and two

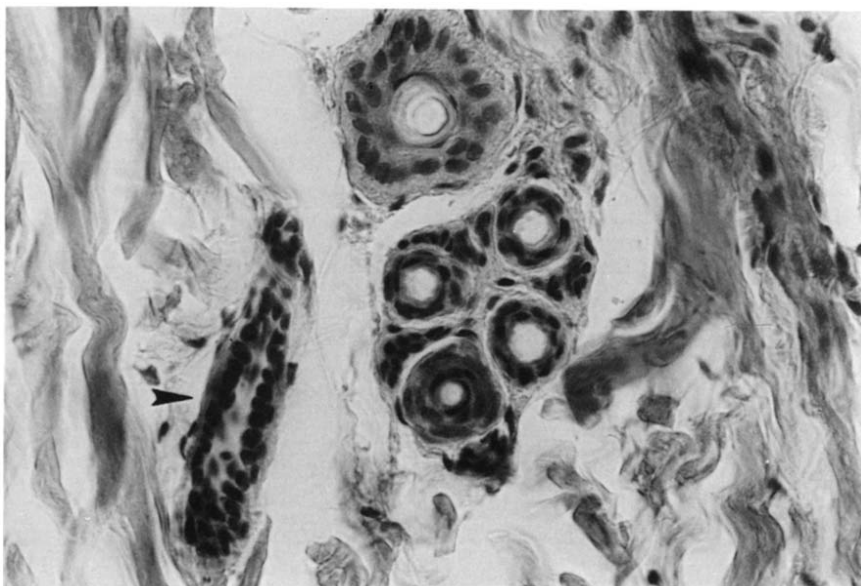


Fig. 5. Skin biopsy taken at PIH 72 in FIP-symptomatic cat 12. There is a mild vascular reaction characterized by a focal inflammatory cell cuff (arrow) involving a blood vessel in the dermis of FIPV-inoculated skin. H & E stain;  $\times 421$ .

TABLE 4

Skin biopsy results, survival times and postmortem findings in cats after one or more challenge-exposures with FIPV

Cat	DTH <sup>a</sup>	FIPV challenge-exposure			Days survived (PID) <sup>d</sup>	Postmortem FIP diagnosis
		1st	2nd <sup>b</sup>	3rd <sup>c</sup>		
FIPV-naive, nonimmune						
1	No	ID/200 <sup>e</sup>	NA	NA	59	effusive
2	No	ID/200	NA	NA	27	effusive
FIPV challenge-exposed, asymptomatic						
3	Yes	IP/1-5	+	+	>300 <sup>f</sup>	NA
4	Yes	IP/1-5	+	+	>300 <sup>f</sup>	NA
5	Yes	IP/1-5	+	+	122	noneffusive
6	Yes	IP/1-5	+	+	51	noneffusive
7	Yes	IP/1-5	+	NA	30	ND
8	Yes	IP/1-5	+	NA	23	noneffusive
9	Yes	IP/1-5	+	NA	40	mixed
10	Yes	IP/5000	+	NA	153	noneffusive
11	Yes	IP/100	+	NA	30	effusive
FIP-symptomatic						
12	No	IP/100	+	NA	4	effusive
13	No	IP/5	+	NA	3	effusive

<sup>a</sup>Presence or absence of DTH in skin biopsy sample.

<sup>b</sup>Inoculated ID with a total of 200 LD<sub>100</sub> of FIPV at 8 weeks (cats 3-9), 5 weeks (cat 10) or 2 weeks (cat 11) after 1st challenge-exposure with FIPV.

<sup>c</sup>Inoculated IP with 100 LD<sub>100</sub> of FIPV at 4 weeks after 2nd challenge-exposure with FIPV.

<sup>d</sup>Total number of days survived after challenge-exposure ID with FIPV.

<sup>e</sup>Route/dose (LD<sub>100</sub>) of FIPV.

<sup>f</sup>Alive at time of report.

Abbreviations: DTH = delayed-type hypersensitivity; ID = intradermal; IP = intraperitoneal; PID = postintra-dermal injection day; NA = not applicable; ND = not determined.

of these cats succumbed to FIP only after a third highly lethal challenge-exposure with FIPV was given IP. Two DTH-positive cats (22%) completely resisted a total of three separate challenge-exposures with FIPV. The mean survival time ( $\pm$  SEM) after ID challenge-exposure in the seven DTH-positive cats that eventually succumbed to FIP – each of which received a total of two or more challenge-exposures with lethal doses of FIPV – was  $64.1 \pm 19.5$  (range, 30–146) days. The total mean survival time after an initial IP challenge-exposure with FIPV in these cats was  $109.4 \pm 16.6$  (range, 42–188) days.

Four of the six DTH-positive cats (67%) in which necropsies were performed predominantly had lesions of noneffusive FIP. One cat (17%) had lesions both of effusive and noneffusive FIP (mixed type), and one cat (17%) had lesions only of effusive FIP.

In the two cats with FIP that were DTH skin test-negative, the mean sur-

vival time after challenge-exposure ID with FIPV was  $3.5 \pm 0.5$  (range, 3–4) days. The total mean survival time after initial challenge-exposure IP was  $17.5 \pm 0.5$  (range, 17–18) days. Both of the cats had lesions of effusive FIP.

The mean survival time after challenge-exposure ID with FIPV in the two FIPV-naive, nonimmune control cats was  $43.0 \pm 11.4$  (range, 27–59) days. Both of the cats, which were DTH-negative at the time of challenge-exposure, had lesions of effusive FIP.

Three of four DTH-positive cats (75%) that had lesions of noneffusive FIP also had increased survival times compared to mean survival times in controls. In contrast, the two DTH-positive cats that either had effusive- or mixed-type FIP lesions did not have increased survival times.

## DISCUSSION

Classical DTH skin reactions to common ID sensitizing antigens [e.g., bovine serum albumin, bovine gamma globulin, egg albumin, bacille Calmette Guerin (BCG)] used in other species are difficult to demonstrate in cats and can be transient (Schultz and Adams, 1978; Legendre et al., 1979; Aiken and McCusker, 1969). In our study, transient gross skin reactions to ID-injected FIPV occurred in three of nine cats (33%) at PIH 24 or 48; reactions persisted for 24 h or more in three cats (33%); and reactions were not observed in three cats (33%). Histologically, the classic DTH (i.e., tuberculin-induced) skin reactions described in persons and guinea pigs are maximal at PIH 24–48, predominantly perivascular (perivenular), and have a mononuclear:PMN ratio of 2:1 or greater (Sell, 1975); stromal edema generally is mild because DTH reactions in these species do not result in marked changes in vascular permeability (Greene et al., 1984). In contrast, DTH responses to ID-injected antigens in mice (Crowle, 1975) or cats (Schultz and Adams, 1978; Legendre et al., 1979) have a more pronounced PMN component, although contact sensitization of the skin of cats and topical challenge with the hapten, 1-chloro,2,4-dinitrobenzene, can produce more classical DTH responses with predominantly mononuclear (lymphocytic) infiltrates (Schultz and Maguire, 1982).

The feline DTH skin lesions to ID-injected FIPV that we described, like the lesions described previously in cats injected ID with sensitizing antigens such as BCG, had a substantial PMN component (approximately 30–50% of cells in infiltrates), especially during the acute phase of the reaction; stromal edema and vasomotor changes, viz. dilation of veins, also were present. Possibly, the histopathology of DTH lesions in cats can be related to certain characteristics of the feline inflammatory response per se. Macrophages are attracted to sites of immunologic activity by lymphokines that are produced and secreted specifically by antigen-primed T lymphocytes (Greene et al., 1984). The recruited macrophages subsequently can release inflammatory mediators, including leukotrienes (LT), prostaglandins (PG) and complement (C) components which

are strongly chemotactic for PMN and which can mediate vascular permeability and vasomotor changes (Parker, 1984; Piper, 1984). Potentially, feline macrophages are activated during CMI reactions and can produce physiologically active amounts of chemotactic molecules and other inflammatory mediators. Indeed, significant increases in plasma or serum LTB<sub>4</sub>, PGE<sub>2</sub>, or C3 are found in cats with induced FIP (Jacobse-Geels et al., 1982; Weiss et al., 1988); and C3b complexes are bound to macrophages in FIP lesions (Pedersen and Boyle, 1980; Weiss and Scott, 1981a). It is not known whether activated macrophages of cats produce relatively greater amounts of chemotactic molecules than do macrophages of other species such as humans or guinea pigs, or whether biochemical differences in the secretory products of effector cells are responsible for interspecies variation in the DTH lesion.

Generally, the characteristic reaction times (24–72 h), gross lesions and the dermal histopathology which we observed after ID inoculation of FIPV in asymptomatic FIPV challenge-exposed cats were compatible with the feline DTH response described after ID sensitization of cats with BCG (Legendre et al., 1979), in contrast to immediate-type (atopic), Arthus-type or Jones-Mote (cutaneous basophil hypersensitivity) lesions (Sell, 1975; Kier et al., 1988). In FIPV, nonimmune control cats, the FIPV inoculum itself caused a persistent but mild neutrophilic perivascular response at PIH 10–12, 24, 48, and 72. In contrast, perivascular dermal responses in the asymptomatic cats were moderate at PIH 24 and were marked with more mononuclear cells at PIH 48 or 72. Other than edema and occasional inflammatory cells, skin lesions were not induced by injection of medium ID in the control cats or by ID injection of medium in cats that had been injected IP once before with the media. It seemed likely that a component of the edema probably was poor fluid resorption following injection of hypertonic solution and bovine serum protein.

The apparent lack of Arthus-type (antibody-mediated) lesions in the FIPV-inoculated skin of five cats that were seropositive was surprising because the vascular lesions in FIP are believed to result from Arthus-type immunopathology (Pedersen and Boyle, 1980; Weiss and Scott, 1981a,c). Perhaps the serum antibodies detected by virus neutralization in these cats were not complement-fixing; or, cats may not respond dermatologically to FIPV antigens with classic Arthus-type reactions. Another possibility was that the FIP vasculitis was due primarily to cellular rather than humoral mechanisms (Pedersen, 1985). Interestingly, four asymptomatic seronegative cats had prominent perivascular DTH-like skin responses to FIPV.

Natural resistance to FIP is believed to be dependent on an effective CMI (Pedersen, 1983; Pedersen and Black, 1983). Apparently, cats that develop strong antiviral CMI, with or without humoral immune responses, are resistant to fatal FIP, whereas cats which lack CMI or develop poor CMI responses and respond humorally develop effusive FIP; noneffusive FIP probably is an intermediate stage between immunity and effusive disease (Pedersen, 1983;

Pedersen and Black, 1983). Depending on the CMI response of the cat, low but infectious challenge-exposure dosages of FIPV either can induce protective immunity, or can promote noneffusive FIP (Pedersen, 1983). In our study, seven of nine asymptomatic DTH skin test-positive cats originally were challenge-exposed with low dosages of FIPV. Four of the cats given low dosages remained VNA-seronegative for 8 weeks before they were ID-tested for DTH; three of those seronegative cats originally had transient fevers within 1–2 weeks after primary challenge-exposure with FIPV (data not shown), suggesting acute infection without seroconversion. Perhaps a low challenge-exposure dose did stimulate CMI (preferentially?) in the seronegative cats that had strong DTH skin responses to FIPV. In three DTH-positive cats that responded to a low FIPV dose with strong antibody responses, a protective (CMI?) response undoubtedly was also stimulated, because two of these cats (nos. 3 and 4) survived two additional challenge-exposures with highly lethal doses of FIPV, and the third cat (no. 6) successfully resisted an additional challenge-exposure with lethal amounts of FIPV but succumbed eventually to a third dose. A new and significant finding in this study was the apparent loss of protective immunity with time in some of the DTH-positive cats that had initially resisted lethal challenge-exposure(s) with FIPV.

The systemic lesions of noneffusive FIP are characterized by perivascular granulomatous or pyogranulomatous inflammation (Montali and Strandberg, 1972; Hayashi et al., 1980). Seemingly, these DTH-like lesions may represent an ineffectual CMI response to FIPV disseminated perivascularly in macrophages (Weiss and Scott, 1981b; Pedersen, 1983; Pedersen and Black, 1983). Our findings support this concept because four of six cats (67%) with induced DTH reactions that eventually died and were necropsied also had noneffusive FIP, and another DTH-positive cat had lesions both of noneffusive and effusive FIP. The one DTH-positive cat (no. 11) that died from effusive FIP had neutrophilic dermal infiltrates in skin biopsies at PIH 48 and mixed mononuclear cells and PMNs at PIH 72. Perhaps the lesions in this cat reflect a mixture of cellular and humoral immune responses to FIPV that were not protective. Unfortunately, there seems to be no reliable way to predict histologically – by differences in maximal reaction times, lesion scores or infiltrating cells – which DTH lesions in biopsies indicate complete immunity or which ones indicate partial responses.

Additional *in vitro* studies of T-cell functions in DTH skin test-positive and negative cats are needed to evaluate more specifically the relationship between viral-induced skin lesions, T-cell immunity, and resistance to FIP. In our study, the two cats with FIP selected for testing lacked or had very poor DTH responses to FIPV. Some cats with induced FIP have depressed lymphocyte blastogenesis to FIPV or to feline T-cell mitogens (Stoddart et al., 1988). Additional *in vitro* studies of T-cell functions in cats challenge-exposed with FIPV, moreover, will be necessary to characterize the potential immunosuppressive effects of FIPV on CMI.



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