Isolation and Identification of Feline Peritoneal Macrophages for In Vitro Studies of Coronavirus–Macrophage Interactions

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Feline peritoneal cells were collected by lavage with isotonic saline without the use of irritants or need for euthanasia of the cats. Macrophages were purified by centrifugation on Percoll followed by selective adherence. Although few macrophages could be obtained from an initial lavage, a second lavage performed on the same cat 9–11 days later yielded six times as many macrophages as the first lavage, providing sufficient numbers of cells for characterization and infection experiments. Macrophages from these subsequent lavages were not more functionally activated in phagocytosis assays than the resident macrophages from the initial lavage, and they were equally susceptible to infection with feline infectious peritonitis virus (FIPV). Infected cultures produced peak titers of $10^{12} \text{TCID}_{50}$ per ml, and FIPV antigen was detected in a small subset (0.1–1.0%) of cells by indirect immunofluorescence. The FIPV-infected cells were identified as macrophages by their characteristic morphology and ability to phagocytize rhodamine-labeled latex beads. The successful isolation of large numbers of unactivated feline macrophages will permit in vitro studies of feline coronavirus–macrophage interactions that otherwise would not have been possible. Such studies will undoubtedly provide valuable insights into the pathogenesis of feline infectious peritonitis, an invariably fatal disease of domestic and exotic cats.

Key words: cats, eosinophils, feline infectious peritonitis

INTRODUCTION

The ability of a virus to infect and replicate within the cells of the mononuclear phagocyte system [32] can be a major factor in the pathogenesis of virus infections because mononuclear phagocytes can facilitate access of viruses to susceptible tissues and organs, thereby hastening the infection process [17–19]. The biological interactions between viruses and macrophages are complex and have numerous possible outcomes [22]. Cytolytic infection of macrophages removes these cells from the mononuclear phagocyte system, permitting infection of other cell types that might otherwise be protected, and noncytolytic infection may result in chronic disease or a virus-carrier state. Abortive or persistent infection can result in macrophage dysfunction, possibly predisposing the host to secondary microbial infections. Even if the macrophage itself cannot support virus replication, immunopathologic disease can result from overzealous destruction of virus-infected cells by macrophage-mediated cytotoxic immune responses. Although the pivotal role of the macrophage in virus pathogenesis was emphasized by Mims [16] nearly 25 years ago, the precise intracellular mechanisms governing macrophage resistance and the outcome of virus infection are only now becoming understood.

Virus–macrophage interactions appear to play a particularly important role in the development of feline infectious peritonitis (FIP), an invariably fatal, immunologically mediated disease of cats [6,7,24,35,38] caused by a coronavirus, feline infectious peritonitis virus (FIPV) [28]. Because the mononuclear phagocyte appears to be the target cell for FIPV infection and dissemination in the cat [8,23,33,36,37], we developed techniques for the collection and cultivation of feline peritoneal macrophages to study interactions between feline macrophages and FIPV in vitro.

The isolation of large numbers of feline macrophages, according to previous reports, has entailed either euthanasia of the cat to obtain alveolar macrophages [2,3,12,14,29,39], bone marrow cells [5], and spleen and lymph node cells [13], or the use of peritoneal irritants such as thioglycolate [4], starch [34], and oyster glycogen [27] to increase the yield of peritoneal macro-

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Lavage, Macrophage culture medium consisted of Leibovitz L-15 medium (GIBCO), 20% heat-inactivated (56°C for 30 min) fetal bovine serum (HyClone Laboratories, Logan, UT), 4 mM L-glutamine (GIBCO) and 100 μg gentamicin sulfate per ml. An isosmotic solution of Percoll (Pharmacia) was made by adding 4 parts Percoll to 1 part (v/v) 1.5 M NaCl (100% Percoll) Percoll for macrophage purification was diluted to 62% in L-15 (final density = 1.076 gm per ml), adjusted to pH 7.2, and sterilized by filtration.

A macrophage counting solution (cetrimide) was prepared by adding 3 g hexadecyltrimethylammonium bromide (Fisher Scientific), 0.85 g NaCl, and 37 mM disodium EDTA to 100 ml distilled water and sterilized by filtration [30]. Growth medium for Crandell feline kidney (CrFK) cells contained Eagle minimum essential medium with 25 mM HEPES buffer and Hanks' saline (GIBCO), 20% L-15, 10% heat-inactivated fetal bovine serum, 3% 0.1 N sodium hydroxide, 2 mM sodium pyruvate (GIBCO), 4 mM L-glutamine, 0.1 mM nonessential amino acids (GIBCO), and 50 μg gentamicin sulfate per ml.

Collection of Peritoneal Cells

Peritoneal cells were obtained by lavage of the peritoneal cavity with sterile saline. Cats were anesthetized by intramuscular injection of 0.6 mg acepromazine maleate per kg and 28 mg ketamine hydrochloride per kg, and their abdomens were shaved and thoroughly scrubbed with surgical soap. With the cat lying flat on its back, a sterile 5-cm-long, 18-gauge catheter (Sovereign canula, indwelling catheter, Monoject, Sherwood Medical, St Louis) was inserted near the umbilicus into the peritoneal cavity with sterile saline. Cats were anesthetized by a) an intramuscular injection of 0.6 mg acepromazine maleate per kg and 28 mg ketamine hydrochloride per kg, and 300 ml of saline solution at room temperature was administered using a 60-ml syringe. The catheter was removed and the abdomen was vigorously massaged for 5 min. The cat was then placed on its right side and a new catheter was inserted between the right caudal teats. Lavage fluid was retrieved by applying gentle pressure on the abdomen while carefully maneuvering the catheter through the peritoneal wall and allowing the fluid to flow freely out of the catheter into sterile 50-ml polystyrene centrifuge tubes that were immediately placed on ice.

Macrophages from different cats were not pooled; each experiment was performed with cells from an individual animal. Cats were allowed to recover from anesthesia and could therefore provide a continuing supply of macrophages.

Macrophage Purification

Lavage fluid was centrifuged (200 g for 10 min), and cell pellets were resuspended in 6 ml culture medium, layered over 5 ml of 62% Percoll in a 15-ml polystyrene centrifuge tube, and centrifuged (400 g for 20 min) without abrupt acceleration or braking.

Materials and Methods

Cats

Six specific pathogen-free cats (four females, two males) were purchased from a commercial source (Liberty Laboratories, Liberty Corner, NJ) and were housed individually in isolation cages. Cats from this breeding colony are free of serum coronavirus antibodies, feline leukemia virus, and other feline virus infections. Macrophages were collected when cats were six months to two years old.

Media and Solutions

A sterile isotonic solution consisting of 0.1 M phosphate-buffered saline (PBS) (pH 7.0) and 200 μg gentamicin sulfate (GIBCO Laboratories) per ml was used for peritoneal lavage. Macrophage culture medium consisted of Leibovitz L-15 medium (GIBCO), 20% heat-inactivated (56°C for 30 min) fetal bovine serum (HyClone Laboratories, Logan, UT), 4 mM L-glutamine (GIBCO) and 100 μg gentamicin sulfate per ml. An isosmotic solution of Percoll (Pharmacia) was made by adding 4 parts Percoll to 1 part (v/v) 1.5 M NaCl (100% Percoll) Percoll for macrophage purification was diluted to 62% in L-15 (final density = 1.076 gm per ml), adjusted to pH 7.2, and sterilized by filtration.

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The band of cells at the interface and the upper 3 ml of Percoll were removed with a Pasteur pipette, washed once in 50 ml L-15 (250 g for 10 min), and resuspended in 5 ml culture medium. Except for the Percoll centrifugation step, cells were kept in polypropylene tubes to minimize adherence (and subsequent loss) of cells to tube walls.

Culture Conditions

Cells (150,000 per well) were seeded into either 8-chamber Lab-tekss (Miles Scientific) or 96-well tissue culture plates (Costar) and incubated at 37°C in humid air with no added CO₂. Macrophage monolayers were washed 5 h after seeding with PBS to remove nonadherent cells, and cultures were processed for enzyme histochemistry and phagocytosis assays or inoculated with FIPV 24 h after seeding. Adherent macrophages in representative wells were counted by releasing the nuclei with cetrimonide.

Cell Identification

Resuspended cells were adjusted to 2 × 10⁶ per ml in culture medium, and cell preparations were made in a cytocentrifuge (Shandon Southern). Slides were air dried, fixed in methanol, and stained with May-Grünwald-Giemsa.

Differential counts were also performed on monolayers in Lab-tekss 5 h and 24 h after seeding; plastic chambers and gaskets were removed, and slides were rinsed in PBS or 5 min and fixed and stained as previously described.

Enzyme Histochemistry

Lab-tekss and cytospin preparations were stained for α-naphthyl acetate esterase, using α-naphthyl acetate and α-st blue RR salt (Sigma Chemical, procedure no. 90); acid phosphatase, using naphthol AS-BI phosphate and redish diazotized fast garnet GBC salt (Sigma, procedure no. 387); and peroxidase, using 0.1% 3,3 diaminobenzidine tetrahydrochloride (Sigma) and 0.1% hydrogen peroxide in 0.05 M Tris buffer. Some slides were stained in the presence of 7 mM tartrate (to inhibit acid phosphatase) or 10 mM sodium fluoride (to inhibit acid phosphatase and esterase). All slides were counterstained with hematoxylin-Gill no. 3 (Sigma), rinsed in tap water, and mounted with glycerol-gelatin (Sigma).

FIPV-Infected Macrophages

Sheep blood diluted 1:2 in Alsever’s solution was washed three times in PBS (1,250g for 10 min at 4°C), and RBC were resuspended in PBS at a concentration of % (v/v) and added to an equal volume of a subagglutinating dilution (1:128 in PBS) of rabbit anti-sheep RBC IgG (Cordis Laboratories, Miami) [1]. The RBC-antibody mixture was incubated at 37°C for 30 min, washed three times in PBS, and resuspended in L-15 at a concentration of 1%. Medium was removed from Lab-tekss, 0.1 ml of 1% untreated RBC or IgG-coated RBC was added to each well, and macrophages were incubated 5–10 min (for rosette formation) or 1 h (for phagocytosis) at 37°C. Wells were rinsed in PBS, and slides for phagocytosis assays were dipped in distilled water for 5 s to lyse extracellular RBC.

Virus and Cell Culture

A virulent strain of FIPV (FIPV-79-1146) [15,25] was provided by N.C. Pedersen, School of Veterinary Medicine, University of California, Davis, and propagated in CrFK cells obtained from J.W. Black, Specialized Assays Inc., Nashville, TN. The virus was plaque cloned once, and stock FIPV was prepared in confluent CrFK cells. Virus titers were calculated using the accumulative 50% endpoint method of Reed and Muench. The titer of stock FIPV-79-1146 was 10⁷.⁶ TCID₅₀ (mean tissue culture infective doses) per ml.

Inoculation of Macrophages With FIPV

Medium was removed from the 96-well plates, and wells were inoculated at an input multiplicity of infection (ratio of virus particles to cells) of 0.1 with stock FIPV diluted in culture medium. Macrophages were incubated with virus at 37°C for 1 h, each well was rinsed five times with PBS to remove unadsorbed virus, fresh medium was added, and cultures were incubated at 37°C. Control wells were given medium alone. A portion of culture medium was removed at various intervals after inoculation for virus titration in CrFK cells.

Macrophages in Lab-tekss were inoculated in a similar manner, but at an input multiplicity of infection of 100. Monolayers were fixed in acetone at −20°C 10–12 h after inoculation for immunofluorescence microscopy.

Phagocytosis of Latex Beads by FIPV-Infected Macrophages

Infected macrophages in Lab-tekss were assayed for their ability to phagocytize 4.5-μm-diameter rhodamine-labeled latex beads (Fluoresbrite microspheres labeled with YO dye, Polysciences, Warrington, PA) 10 h after inoculation with FIPV. Beads were washed three times in macrophage culture medium (1,200g for 30 min) and resuspended in medium at a final concentration of 10⁷.⁰ beads per ml. Medium was removed from wells, and beads were incubated with the macrophages for 1 h at 37°C. Monolayers were vigorously washed with PBS, fresh medium was added, and incubation at 37°C was continued for 1 h to allow macrophages to ingest the beads completely.

Immunofluorescence Microscopy

Slides were incubated for 1 h at 37°C in a humidified chamber with a 1:2,000 dilution (in PBS) of anti-FIPV
Fig. 1. Cell composition of peritoneal lavage fluid obtained 10 days after a previous lavage. Macrophages (MΦ) and neutrophils (N) were the predominant cell types; small percentages of lymphocytes (L), eosinophils (E), and mast cells (M) were also present (cytospin; May-Grünwald-Giemsa).

Fig. 2. Cell composition of peritoneal lavage fluid obtained at various intervals after a previous lavage, demonstrating the influx of cells into the peritoneal cavity in response to lavage procedure. We chose an interval of 9–11 days between lavages for obtaining macrophages for all subsequent experiments (arrow). Each data point represents the results from one lavage performed on one cat; lavages were performed on all six cats.

RESULTS

Collection and Composition of Peritoneal Lavage Fluid

We routinely retrieved 250 ml of the 300 ml (83%; instilled into the peritoneal cavity, and the cats exhibited no ill effects from the procedure. The lavage fluid contained a highly variable number of RBC and a mixed population of leukocytes (Fig. 1) that were identified according to standard criteria [9]. An average of 2.5 × 10^6 resident macrophages (sufficient for the proper seeding of only 15 wells) could be obtained from an initial lavage, an inadequate amount for most of the infection experiments we had planned. We discovered, however, that subsequent lavages performed on the same cat one to two weeks after an initial lavage yielded many more macrophages.

We then studied the influx of cells into the peritoneal cavity in response to the procedure as a function of time between lavages by collecting cells from each of six cats at intervals from 1 to 32 days after a previous lavage (Fig. 2). From this study we determined that the optimal interval between lavages was 9 to 11 days. At that interval, an average of six times as many macrophages could be collected as compared to the initial lavage, and the relative percentages of the different cell types obtained from initial and subsequent lavages were identical (45% macrophages, 50% neutrophils, 4% lymphocytes, and 1% eosinophils and mast cells). There was little variation in the relative percentages of cells among the six cats, demonstrating that this is a reliable procedure for obtaining large numbers of macrophages from the cat.

Macrophage Purification

Centrifugation of the lavage fluid on 62% Percoll produced a band of cells at the interface containing mostly macrophages and a cell pellet consisting of RBC and neutrophils. A mixture of macrophages and neutrophils was present in the Percoll layer, so to maximize the macrophage yield we collected the top 3 ml of Percoll in addition to the cells at the interface. The collected fluid contained an average of 83% macrophages and 15% neutrophils, and the final macrophage yield was typically 65%. We could obtain higher yields by collecting more

hyperimmune cat serum, washed three times in PBS, and stained with a 1:30 dilution of fluorescein isothiocyanate conjugated rabbit anti-cat IgG (Cappel, Cooper Biomedical, West Chester, PA) for 1 h at 37°C. Diluted reagent were filtered through 0.2-μm filters (Gelman Sciences Ann Arbor, MI) before use. After staining, slides were washed twice in PBS, counterstained for 10 min in 0.002% Evans blue in PBS, mounted with 50% glycerol-PBS, and examined with an epifluorescent ultraviolet light microscope.
of the Percoll layer, but only at the expense of greater neutrophil contamination. Cultures were purified to >90% macrophages by washing, and by 24 h after seeding the percentage of adherent cells was >95% macrophages.

**Macrophage Morphology and Identification**

Macrophages adhered to culture vessels soon after settling, and after 5 h of incubation a majority of the cells were adherent and spreading. Cytochemical analysis revealed that most of these cells possessed α-naphthyl acetate esterase and acid phosphatase activity, but no apparent peroxidase activity (Fig. 3). Acid phosphatase activity was reduced approximately 50% by tartrate and abolished by sodium fluoride, whereas esterase activity was unaffected by sodium fluoride. The amounts of both enzymes varied remarkably between cells.

After 24 h of cultivation, the macrophages appeared round, elongated, or stellate and differed dramatically in absolute amount of cytoplasm and degree of cytoplasmic spreading (Figs. 4a, 5). Macrophage nuclei were often eccentric and kidney-shaped with a prominent nuclear membrane, small nucleoli, and sparsely distributed chromatin (Fig. 4b). Large perinuclear vacuoles were evident in many cells, and an occasional vacuole contained phagocytized granulocyte nuclei or RBC. The macrophages did not appear to divide in culture.

Macrophages displayed Fc-mediated rosetting and phagocytosis of IgG-coated sheep RBC (Fig. 6a,b). After 1 h of incubation, 25% of macrophages had phagocytized no RBC, 45% had phagocytized 1–5 RBC, and 30% had phagocytized >5 RBC; noncoated RBC neither formed rosettes nor were phagocytized by the macrophages. Resident peritoneal macrophages obtained from an initial lavage phagocytized sheep RBC in an identical manner. Macrophages phagocytized the latex beads somewhat more avidly; 10% of macrophages contained no beads, 25% contained 1–5 beads, and 65% contained >5 beads after incubation.

**Replication of FIPV in Macrophages**

Virus was first detected in macrophage culture supernatants 8 h after inoculation and reached peak titers of $10^{5.0}$ TCID$_{50}$ per ml 2 days after inoculation. Virus titers remained constant until 4 days after inoculation and then steadily declined until 8–10 days after inoculation when most macrophages had detached or were dead (in both inoculated and control wells) and no infectious virus could be recovered. Virus was not detected in uninoculated control wells.

Immunofluorescence staining revealed that 0.1–1.0% of macrophages in different experiments were positive for FIPV antigen 10–12 h after inoculation at an input multiplicity of infection of 100 (Fig. 7a). Culture supernatants from these wells contained $10^{4.0}$--$10^{5.0}$ TCID$_{50}$.
Fig. 4. Morphology of feline macrophages after 24 h of cultivation. Typical macrophage monolayer at low magnification (a) and characteristic morphologic features of individual macrophages (b), one of which contains a phagocytized granulocyte nucleus (arrowhead) (May-Grünwald-Giemsa).

Fig. 5. Phase contrast image of viable unfixed feline macrophages cultivated for 48 h shows compact arrangement of phase dense secondary lysosomes and refractile lipid droplets around the central nucleus, thinly spread cytoplasm, and characteristic cytoplasmic ruffling (arrowhead).

Macrophages obtained from an initial lavage and inoculated with FIPV in an identical manner produced FIPV titers and immunofluorescence staining patterns identical to those described above for macrophages obtained 9–11 days after a previous lavage. The variation in the number of FIPV-antigen positive cells in different experiments did not depend on the individual cat from which the macrophages were obtained.

Identification of FIPV-Infected Cells

The FIPV antigen-positive cells possessed characteristic macrophage features and were morphologically indistinguishable from neighboring antigen-negative cells (Fig 7b). In addition, a majority of the FIPV-infected cells as well as uninfected cells were capable of phagocytizing the latex beads (Fig. 8a). In May-Grünwald-Giemsa stained preparations, the phagocytized beads were contained within the margins of the cells and were not merely adherent to the outer cell membrane (Fig. 8b).

DISCUSSION

Lavage of the peritoneal cavity with isotonic saline 9–11 days after a previous similar lavage, purification of the lavage fluid by centrifugation on Percoll, and removal of nonadherent cells by washing was a successful method for obtaining 95% pure cultures of viable, functionally normal feline macrophages in quantities sufficient for virus infection experiments. Saline lavage provides an inherently milder stimulus than that produced by injection of irritants such as thioglycolate, fetal bovine serum, mineral oil, and starch. Although it might be preferable...
Isolation of Feline Peritoneal Macrophages

**Fig. 6.** Fc-mediated rosetting (a) and phagocytosis (b) of IgG-coated sheep RBC by feline macrophages cultivated for 24 h (May-Grünwald-Giemsa).

**Fig. 7.** Immunofluorescence staining of feline peritoneal macrophages 12 h after inoculation with FIPV-79-1146. Although few cells were susceptible to virus infection (a), the infected cells possessed characteristic macrophage morphology (b).

- To study resident rather than elicited macrophages when seeking in vitro correlates to in vivo phenomena, we found that some elicitation was necessary to obtain non-activated feline macrophages in sufficient numbers without sacrificing the host.

  The purified lavage cells adhered to the substrate, did not divide in culture, displayed morphologic features characteristic of macrophages, possessed acid phosphatase and α-naphthyl acetate esterase activity, bore Fc receptors, and were capable of avid phagocytosis. We identified these cells as macrophages because they met more than three of the essential criteria set forth by van Furth [31]. Cultures of feline macrophages infected with virulent strain of FIPV produced infectious virus titers of $10^{5.0}$ TCID$_{50}$ per ml, yet immunofluorescence microscopy revealed that few (0.1–1.0%) of the macrophages were susceptible to infection in vitro.

  It was initially difficult to retrieve more than 100 ml of lavage fluid, but with practice we could retrieve more than 250 ml of the original 300 ml. Proper placement of the catheter tip away from the omentum in an area of the peritoneal cavity where the lavage fluid had pooled was crucial for maximum fluid retrieval. The lavage fluid was typically clear and contained few RBC; when heavier RBC contamination did not occur, however, centrifugation on 62% Percoll separated more than 90% of the RBC from the macrophages. The cats exhibited no ill effects from repeated lavages, and they were often continually lavaged every 9–11 days for two to three months at a time. This was not only a cost-effective approach to
macroiphage collection, but also allowed whole series of experiments to be performed with cells from just a few animals, minimizing potential variability between experiments caused by genetic differences between individual cats.

The lavage procedure elicited an influx of macrophages, neutrophils, and, most notably, eosinophils, into the peritoneal cavity (Fig. 2). Because histamine appears to play the key role in chemotaxis and tissue localization of eosinophils [9], their dramatic influx was most likely the result of mast cell degranulation caused by physical agitation of the peritoneal cavity during the lavage procedure. The collection of peritoneal cells one to two days after a previous lavage thus could be used to obtain large numbers of feline eosinophils for other in vitro studies. We considered the macrophages obtained 9–11 days after a previous lavage to be suitable for further study because they were not more functionally activated in phagocytosis assays than resident macrophages and were similarly susceptible to FIPV infection (in both percentage of susceptible cells and maximum virus titers produced). We chose an interval of 9–11 days between lavages to minimize eosinophil contamination while maximizing total macrophage yield and to allow the cats to completely recover from the procedure.

The acid phosphatase and α-naphthyl acetate esterase of human monocytes and macrophages are inhibited by tartrate and sodium fluoride, respectively [10]. We found the acid phosphatase of feline macrophages to be partially tartrate-resistant and the α-naphthyl acetate esterase to be completely fluoride-resistant. This may reflect the presence of isozymes different from those reported in human cells [9]. Fewer than 3% of the macrophages were peroxidase-positive, and their nuclei were typically round; these more mature cells probably represent a transitional form between exudate and resident macrophages [31].

Only a small subset (0.1%–1.0%) of the macrophages was susceptible to infection with FIPV in vitro, even when inoculated at a high multiplicity of infection. The FIPV antigen-positive cells were morphologically indistinguishable from neighboring antigen-negative cells, possessed characteristic macrophage morphologic features, and, most importantly, were avidly phagocytic. We have demonstrated, therefore, that the cells susceptible to FIPV infection are indeed macrophages and are not a contaminating cell type present in very low numbers.

In most studies of the in vitro susceptibility of macrophages to virus infection, only a subset (3–20%) of macrophages is reported to become infected, a phenomenon attributed to the inherent heterogeneity of most macrophage populations. The origins of this diversity remain an enigma, but may be related to cell cycle, stages of differentiation, maturation from monocyte to macrophage, macrophage sublines, or environmental factors [22]. Culture conditions did not appear to be a crucial factor in feline macrophage susceptibility to FIPV because cultivation of cells in a variety of media supplemented with 20% fetal bovine serum did not affect virus titers produced after inoculation (data not shown).

Feline macrophages infected with FIPV exhibited no cytopathic effects and continued to produce infectious virus for at least 6–8 days after inoculation. Noncytolytic infection of macrophages with FIPV has important implications for the pathogenesis of FIP and the production
and maintenance of a virus carrier-state in vivo. Cats exposed to virulent FIPV can harbor the virus asymptotically for at least four months after initial exposure [26]. The macrophage, therefore, may not only serve to spread FIPV systemically, but may also sequester the virus and form the basis for viral persistence.

The limited permissiveness of macrophages for most viruses has been established experimentally and supports the concept of intrinsic macrophage resistance, wherein mononuclear phagocytes serve as ubiquitous virus-resistant cells that can adsorb, phagocytize, and destroy viruses, thereby reducing the amount of infectious virus and impeding their systemic dissemination [21]. Using macrophages obtained as outlined in this report, we have subsequently demonstrated a correlation between virulence (FIP-inducing capacity) and the ability of feline coronavirus strains to infect and replicate in feline macrophages (C.A. Stoddart and F.W. Scott, submitted for publication). In these studies, avirulent coronaviruses infected fewer macrophages, produced lower virus titers, and were less able to sustain their replication and spread to other susceptible macrophages than were virulent FIPV inducing strains. Furthermore, infection of macrophages by virulent coronavirus strains was enhanced when coronavirus antibody was added to the culture.

The ability to study feline macrophage permissiveness and feline coronavirus-macrophage interactions in vitro, made possible by the methods outlined here, has permitted detailed investigations of the role of macrophages in the pathogenesis of FIP. As a tool for obtaining enhanced numbers of functionally normal macrophages, it will have important applications for studies of other feline viruses and macrophages from many species.

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