JOURNAL OF VIROLOGY, Feb. 1992, p. 956–965
0022-538X/92/020956-10$02.00/0

Vol. 66, No. 2

Monoclonal Antibodies to the Spike Protein of Feline Infectious Peritonitis Virus Mediate Antibody-Dependent Enhancement of Infection of Feline Macrophages

CHRISTOPHER W. OLSEN,* WAYNE V. CORAPI, CHRISTOPHER K. NGICHABE, JOEL D. BAINES,† AND FRED W. SCOTT

Department of Microbiology, Immunology, and Parasitology and Cornell Feline Health Center, New York State College of Veterinary Medicine, Cornell University, Ithaca, New York 14853

Received 15 August 1991/Accepted 7 November 1991

Antibody-dependent enhancement of virus infection is a process whereby virus-antibody complexes initiate infection of cells via Fc receptor-mediated endocytosis. We sought to investigate antibody-dependent enhancement of feline infectious peritonitis virus infection of primary feline peritoneal macrophages in vitro. Enhancement of infection was assessed, after indirect immunofluorescent-antibody labelling of infected cells, by determining the ratio between the number of cells infected in the presence and absence of virus-specific antibody. Infection enhancement was initially demonstrated by using heat-inactivated, virus-specific feline antiserum. Functional compatibility between murine immunoglobulin molecules and feline Fc receptors was demonstrated by using murine anti-sheep erythrocyte serum and an antibody-coated sheep erythrocyte phagocytosis assay. Thirty-seven murine monoclonal antibodies specific for the nucleocapsid, membrane, or spike proteins of feline infectious peritonitis virus or transmissible gastroenteritis virus were assayed for their ability to enhance the infectivity of feline infectious peritonitis virus. Infection enhancement was mediated by a subset of spike protein-specific monoclonal antibodies. A distinct correlation was seen between the ability of a monoclonal antibody to cause virus neutralization in a routine cell culture neutralization assay and its ability to mediate infection enhancement of macrophages. Infection enhancement was shown to be Fc receptor mediated by blockade of antibody-Fc receptor interaction using staphylococcal protein A. Our results are consistent with the hypothesis that antibody-dependent enhancement of feline infectious peritonitis virus infectivity is mediated by antibody directed against specific sites on the spike protein.

Feline infectious peritonitis virus (FIPV) is a member of the family *Coronaviridae*. Coronaviruses are plus-sense, single-stranded RNA viruses with three major structural proteins, the spike (S), membrane (M), and nucleocapsid (N) proteins (18). FIPV infects domestic as well as exotic cats and produces an ultimately fatal disease called feline infectious peritonitis (FIP) (2, 30, 39). One of the most perplexing aspects of the pathogenesis of FIP is the frequent occurrence of accelerated, more fulminant disease upon FIPV challenge of seropositive as compared with seronegative cats. Accelerated FIP was first documented when Pedersen and Boyle showed that the onset of clinical disease among experimentally infected kittens correlated with the appearance of serum antibodies (31). Weiss and Scott confirmed these results, demonstrating that the onset of viremia, clinical signs, thrombocytopenia, lymphopenia, and the appearance of viral antigen and necrotizing lesions in affected tissues all occurred earlier in seropositive kittens than in seronegative kittens (48, 49). Survival times were also significantly shorter for seropositive kittens (31, 48). In addition, seronegative kittens given immune serum (31, 47) or anti-FIPV immunoglobulin G (IgG) (31) before challenge developed clinical disease in the same manner and over the same time course as did seropositive kittens. The demonstration of immune complex deposition in FIP by Jacobse-Geels et al. (13, 14) initially seemed to explain both the pathologic changes of FIP and the phenomenon of accelerated FIP. However, a new consideration was introduced in 1989 when Stoddart provided evidence for antibody-dependent enhancement (ADE) of FIPV infection of primary feline peritoneal macrophages in vitro (41).

ADE of virus infection occurs when monocytes and macrophages are more efficiently infected by complexes of virus plus antibody (Ab), via Fc receptor-mediated endocytosis, than by virus alone (33). A number of human and animal viruses have been shown to be capable of utilizing this mechanism of infection. These include dengue virus and related flaviviruses (8, 32), respiratory syncytial virus (5, 17), influenza virus type A (26, 27), rabies virus (16), and most recently human immunodeficiency virus type 1 (11, 44), as well as FIPV (41). The FIPV system provides a unique opportunity to investigate ADE of virus infectivity at both the in vitro and in vivo levels. Specific aspects of ADE of FIPV infection need to be elucidated because the immunopathogenesis of FIP has precluded the development of an effective, proven vaccine against FIPV infection (28).

The aims of this work were to confirm that virus-specific feline antiserum can mediate ADE of FIPV infectivity in vitro and to determine whether FIPV-specific murine monoclonal Abs (MAbs) can demonstrate similar in vitro enhancement. A panel of MAbs was evaluated to define which viral protein(s) induces enhancing Abs and to determine whether distinct enhancing epitopes may be involved.

MATERIALS AND METHODS

Cells and viruses. FIPV strains 79-1146 and UCD1 were grown in A72 cells. Virus titers were calculated as 50%

* Corresponding author.
† Present address: Marjorie Kovler Oncology Laboratories, University of Chicago, Chicago, IL 60637.

tissue culture infectious dose ($TCID_{50}$) units by the method of Reed and Muench (34), using Crandell feline kidney cells (CrFKC) as indicator cells.

Polyclonal Ab preparations. Murine anti-sheep erythrocyte (anti-SRBC) serum was obtained from a BALB/c mouse (The Jackson Laboratory, Bar Harbor, Maine) following a series of three intraperitoneal inoculations of 0.5 ml of a 50% suspension of freshly collected, washed SRBC. The serum was heat inactivated by heating to 56°C for 30 min to remove complement activity.

FIPV-specific feline antiserum was kindly provided by Cheryl Stoddart (Stanford University, Palo Alto, Calif.).

MAbs. MAbs to FIPV were produced by using standard techniques (3). Briefly, a semipurified, whole virus preparation of FIPV 79-1146 was used to immunize BALB/c mice (Charles River Breeding Laboratories, Inc., Wilmington, Mass.). Mice were immunized three times and boosted with an intrasplenic injection 3 days prior to fusion. Hybridoma colonies were cloned by limiting dilution, and viral specificity was assessed by indirect immunofluorescent-Ab (IFA) labelling of infected CrFKC. Virus protein specificity was evaluated by radioimmunoprecipitation assay (RIPA) (3), and IgG subclass was determined by using a commercially available kit (Zymed Laboratories, Inc., South San Francisco, Calif.).

Two MAbs raised against the DF2 strain of FIPV and two MAbs raised against the Miller strain of transmissible gastroenteritis virus were kindly supplied by Susan Fiscus (University of North Carolina, Chapel Hill) and Fermenta Animal Health. The viral protein specificity and immunoglobulin subclass of these MAbs have been previously reported (4).

All of the Ab preparations (sera and ascites fluid) for the enhancement assays were heat inactivated prior to use. This was done in order to exclude complement-mediated enhancement of infection, which has been documented in other virus systems (33).

Virus neutralization assay. Each of the MAbs was assayed for virus neutralization activity on CrFKC by using a 96-well plate format. Serial twofold dilutions of heat-inactivated MAb were mixed with an equal volume of either FIPV 79-1146 or FIPV UCD1 containing 100 $TCID_{50}$ of virus. Following a 1-h incubation at room temperature, CrFKC were added and plates were incubated at 37°C for 96 h. Titers are reported as the reciprocal of the highest dilution of Ab that completely inhibited cytopathology.

Collection and purification of primary feline peritoneal macrophages. Macrophages were obtained by saline lavage of the peritoneal cavity (42) of specific-pathogen-free (SPF) cats (Liberty Laboratories, Liberty Corners, N.J.). Before use as a macrophage donor, each cat was confirmed to be coronavirus antibody negative by kinetics-based enzyme-linked immunosorbent assay at the Diagnostic Laboratory at the New York State College of Veterinary Medicine at Cornell University and by serum neutralization testing. The peritoneal lavage technique could be performed on a cat repeatedly at 9- to 12-day intervals without discomfort or other side effects, thus reducing the number of laboratory animals required.

Cats were sedated by intramuscular injection of ketamine-HCl (Fort Dodge Laboratories, Fort Dodge, Iowa), 15 mg/kg, and acepromazine (The Butler Company, Rochester, N.Y.), 0.15 mg/kg. Peritoneal exudate cells were then obtained by lavage of the peritoneal cavity with 300 ml of sterile phosphate-buffered saline (PBS), at room temperature, containing 200 μg of gentamicin sulfate (GIBCO Laboratories, Grand Island, N.Y.) per ml. Macrophages were concentrated by centrifugation over a 62% Percoll (Pharmacia Fine Chemicals, Piscataway, N.J.) cushion and then pelleted, washed, and resuspended in complete macrophage medium (CMM; L-15 [GIBCO], 20% heat-inactivated, characterized fetal bovine serum [Hyclone Laboratories, Logan, Utah], 4 mM added glutamine [GIBCO], 20 μg of gentamicin [GIBCO] per ml) to a concentration of 7.5×10^5 cells per ml. Macrophages were cultured on eight-chamber glass Lab-Tek slides (Nunc, Inc., Naperville, Ill.) at a concentration of 1.5×10^5 cells per chamber. Macrophage cultures were maintained at 37°C in the absence of CO_2. Cell chambers were washed 7 h postseeding with PBS to remove nonadherent cells, after which fresh medium was added. Adherent cells were characterized as macrophages on the basis of morphologic appearance, demonstration of nonspecific esterase activity (Sigma kit 90; Sigma Chemical Co., St. Louis, Mo.), and demonstration of functional Fc receptors in an Ab-coated SRBC phagocytosis assay (see below).

Ab-coated SRBC phagocytosis assay. An Fc receptor-mediated phagocytosis assay was conducted as previously described (42). Fresh SRBC were pelleted and washed three times and then resuspended to 5% (vol/vol) in PBS. Equal volumes of this 5% SRBC solution and either rabbit anti-SRBC serum (1:128) (Organon Teknika, Durham, N.C.), murine anti-SRBC serum (1:128) (see above), or PBS were incubated at 37°C for 30 min. The erythrocyte-Ab complexes were then pelleted and washed three times in PBS and resuspended to 1% (vol/vol) in L-15 medium. Macrophages in eight-chamber Lab-Tek slides were inoculated with the 1% SRBC solutions (0.1 ml per chamber) and incubated for 60 min at 37°C. The slides were then rinsed in PBS, dipped in distilled water, and stained with May-Grünwald-Giemsa stain.

In vitro assay for enhancement of FIPV infectivity. The assay for enhancement of virus infectivity was adapted from the method described by Stoddart (41). Macrophages were infected with virus 22 to 24 h after having been seeded onto Lab-Tek slides. Each MAb (as ascites fluid) or antiserum sample was initially evaluated at a dilution (in CMM) of 1:64 and serial twofold dilutions from 1:512 to 1:16,384. FIPV UCD1 stock virus was diluted to 2×10^6 $TCID_{50}$/ml in CMM. Equal volumes of diluted virus plus Ab (or plus CMM for the wells to be infected by virus alone) were mixed and incubated at 37°C for 30 min. Macrophage cultures in triplicate wells were then washed once with PBS and inoculated with 400 μl of preincubated virus plus Ab or virus plus medium. Inoculation with CMM alone and mixtures of MAb or antiserum plus medium served as negative controls. To rule out enhancement of virus infection by nonspecific serum factors, heat-inactivated preimmune feline serum as well as coronavirus-negative murine serum was incubated with FIPV as described above, and the level of infection achieved was compared with that obtained with virus alone.

Following inoculation, macrophages were cultured for 60 min at 37°C, after which the Lab-Tek chambers were thoroughly washed with PBS to remove nonadsorbed virus and replenished with 300 μl of CMM. At 10.5 h after infection, the Lab-Tek slides were disassembled and washed with 0.1% bovine serum albumin (BSA) (Sigma) in PBS. Cells were fixed by sequential immersion in methanol and acetone at −20°C for 10 min, washed in 0.1% BSA, dried, and stored at −70°C to await indirect IFA labelling.

Ab preparations found to enhance FIPV UCD1 infection at any of the initial screening dilutions were further evaluated for enhancement of both FIPV UCD1 and FIPV 79-

1146, using serial twofold dilutions from 1:32 to 1:1,048,576. Because of the limited number of primary macrophages available from a single peritoneal lavage procedure, evaluation of the two strains of virus was usually conducted by using macrophages collected on different days. Inoculation of virus plus enhancing FIPV-specific feline antiserum served as a positive control for enhancement and as a control for day-to-day variation in the macrophages in all assays.

Indirect IFA labelling. Indirect IFA labelling of infected macrophages or CrFKC was conducted as previously described (42), with slight modification. Briefly, slides were incubated with a 1:2,000 dilution (in PBS) of FIPV-specific, hyperimmune feline antiserum in a humidified chamber for 60 min at room temperature. The slides were then thoroughly washed with 0.1% BSA in PBS and gently blotted dry. Slides were subsequently incubated with a 1:50 dilution (in PBS) of fluorescein isothiocyanate-conjugated goat anti-feline IgG (Organon Teknika) for 30 min at 37°C, washed with 0.1% BSA, and counterstained with 0.002% Evans blue stain.

Quantitation of infection enhancement. Following indirect IFA labelling, each slide was evaluated for the number of infected (IFA-positive) cells per well. The degree of FIPV infection enhancement caused by each dilution of antiserum or MAb (enhancement factor [EF]) was calculated as follows: the mean number of infected cells per well for the wells infected in the presence of a given dilution of Ab was divided by the mean number of infected cells per well for the wells infected with virus alone (41).

Staphylococcal protein A blockade of the Fc portion of enhancing Abs. Parallel enhancement assays were conducted in the presence or absence of soluble staphylococcal protein A (P 6650; Sigma) as previously described (41). Protein A was added to preincubated virus-Ab mixtures (1:512 dilution of antiserum, 1:1,024 dilution of ascites fluid) to a final concentration of 200 μg/ml. Virus-Ab mixtures were made in media without fetal bovine serum to avoid competition for protein A binding. The protein A-containing mixtures were incubated for an additional 60 min at 37°C before inoculation of macrophages.

Construction of RPV-S. A recombinant raccoonpox virus expressing the S protein of FIPV 79-1146 (RPV-S) was constructed (25) essentially as described by Mackett et al. (19, 20). Briefly, a full-length clone of the S gene of FIPV 79-1146, kindly supplied by M. C. Horzinek (Utrecht, The Netherlands) as a *Bam*HI fragment in pGS20 (46), was inserted into the *tk* gene of raccoonpox virus. Recombinant viruses were selected by plaque titration in TK^- 143B cell monolayers under overlay medium containing 30 μg of bromodeoxyuridine (Boehringer Mannheim, Indianapolis, Ind.) per ml. Presence of the FIPV S gene in the recombinant virus was determined by dot blot hybridization (21). S protein expression was determined by immunoblotting (29), RIPA (40), and indirect IFA labelling.

CrFKC were grown to confluence on eight-chamber Lab-Tek slides and infected with RPV-S at a multiplicity of infection of 3. Twenty-four hours after infection (at the time of optimal plaque formation), the slides were processed for indirect IFA labelling using each of the MAbs assayed for enhancement.

RESULTS

ADE of FIPV infectivity by virus-specific feline antiserum. ADE of FIPV infection of primary feline peritoneal macrophages was demonstrated by using serum obtained from an SPF cat 22 days after infection with FIPV UCD1 (Fig. 1). The peak level of enhancement of strain UCD1 occurred at a serum dilution of 1:2,048. At this dilution of antiserum, there was a 56-fold increase in the number of macrophages infected compared with macrophages infected with virus alone. The peak EF for strain 79-1146 was 34 and occurred at a serum dilution of 1:8,192. Each data point represents the mean EF calculated from triplicate wells. (The remainder of the enhancement data [Fig. 4 through 10] is presented in similar graphic form, although the scales on the axes vary to accommodate the data.) Infection was not detected among macrophages inoculated with medium alone or with antiserum plus medium. In addition, no effect on the level of infection was noted in the presence of preimmune (coronavirus antibody-negative) serum.

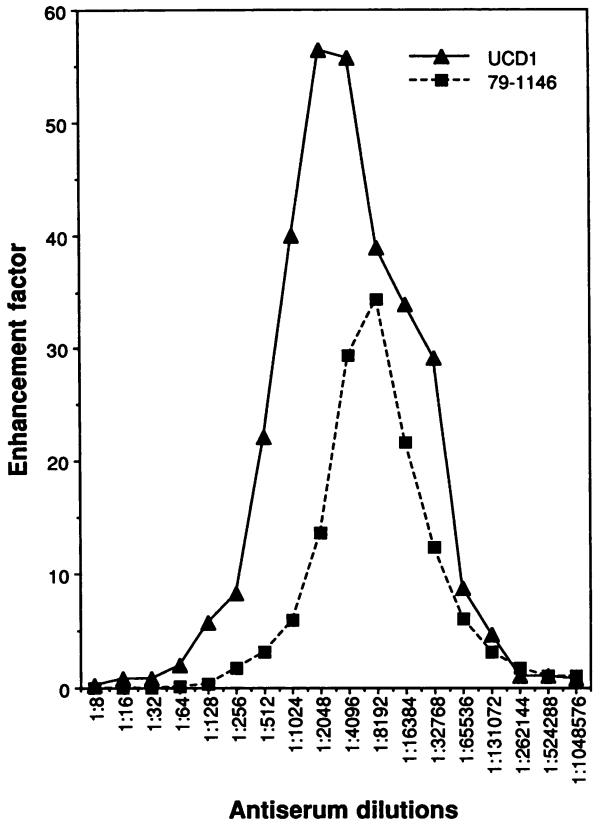

FIG. 1. ADE of FIPV (strains 79-1146 and UCD1) infection of primary feline peritoneal macrophages by FIPV-specific feline antiserum. Primary feline peritoneal macrophages were cultured on eight-chamber glass Lab-Tek slides (Nunc) and infected with FIPV 79-1146 or FIPV UCD1 in the presence or absence of serial dilutions of FIPV-specific feline antiserum. EF values were calculated as the mean number of infected cells per well ($n = 3$) for wells infected in the presence of FIPV-specific Ab divided by the mean number of infected cells per well ($n = 3$) for the wells infected with virus alone.

To reduce the inherent variability involved in using primary macrophages, all of the data contained in Fig. 1 were derived by using macrophages obtained from a single donor cat. However, the ability of polyclonal feline antiserum to enhance FIPV infectivity was confirmed by using a variety of antisera and using macrophages from several SPF cats (data not shown).

Murine immunoglobulin and feline Fc receptor compatibility. Before attempting to document ADE by using MAbs, it

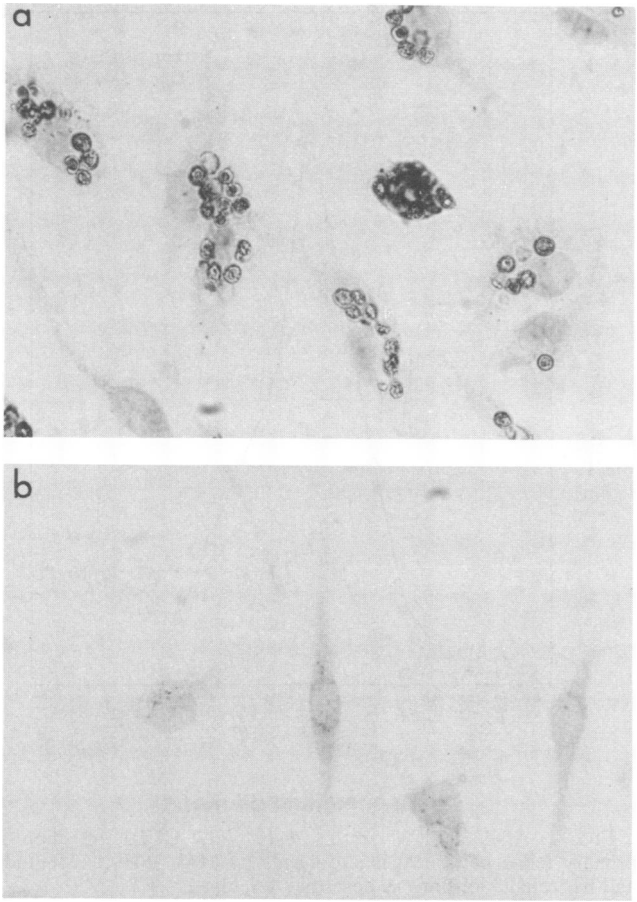

FIG. 2. SRBC phagocytosis assay. Primary feline peritoneal macrophages, cultured on eight-chamber glass Lab-Tek slides, were inoculated with a 1% suspension of murine anti-SRBC-coated SRBCs (a) or uncoated, fresh SRBCs (b). The functional interaction of the Fc portion of murine immunoglobulin with the Fc receptors present on feline macrophages is demonstrated by the prominent erythrophagocytosis of the Ab-coated SRBC (a).

was necessary to determine whether murine immunoglobulins are functionally compatible with the Fc receptors present on feline macrophages. Such compatibility was demonstrated by using murine anti-SRBC serum in an Ab-coated SRBC phagocytosis assay. Primary feline macrophages phagocytosed murine anti-SRBC-coated SRBC (Fig. 2a) but did not engulf uncoated SRBC (Fig. 2b).

ADE of FIPV infectivity by virus-specific murine MAbs. Each MAb was assayed by indirect IFA labelling of FIPV-infected CrFKC before evaluation for enhancement ability. Only those MAbs demonstrating strong IFA signals to both FIPV 79-1146 and UCD1 were selected for further evaluation. The characteristics of the 37 MAbs used in this study are shown in Table 1.

The MAbs produced during this study were initially assayed by RIPA to determine FIPV protein specificity. Many of the M- and S-specific MAbs were found to precipitate both the M and S glycoproteins. The addition of various reducing agents to the reaction mixture did not enhance our ability to distinguish specificity to these two proteins by RIPA. To confirm which MAbs were S protein specific, the MAbs were tested by indirect IFA labelling of RPV-S-infected CrFKC. Those MAbs which reacted with the poxvirus plaques (Fig. 3) were designated as S protein specific, and those that did not were considered to be M protein specific. Membrane protein-specific MAbs provided by S. Fiscus served as controls for this experiment.

TABLE 1. MAbs tested for ADE FIPV infection of primary feline peritoneal macrophages

MAb	Immunizing virus[a]	FIPV protein specificity	Virus neutralization titer[b]	ADE[c]
2A10	FIPV 79-1146	S	<10	−
3G7	FIPV 79-1146	S	2,560	+
1A4	FIPV 79-1146	S	<10	−
1A11	FIPV 79-1146	M	<10	−
3H8	FIPV 79-1146	S	<10	−
3C3	FIPV 79-1146	S	<10	−
1.2	FIPV 79-1146	S	<10	−
2.1	FIPV 79-1146	S	<10	−
3C7	FIPV 79-1146	S	<10	−
15A9.9	FIPV 79-1146	M	<10	−
16C11.3	FIPV 79-1146	N	<10	−
16F5.11	FIPV 79-1146	S	<10	−
16F7.2	FIPV 79-1146	S	<10	−
17B7.1	FIPV 79-1146	N	<10	−
17D7.4	FIPV 79-1146	S	10	+
17E1.6	FIPV 79-1146	S	10	+
17H12.1	FIPV 79-1146	S	<10	−
18A7.4	FIPV 79-1146	S	2,560	+
18A9.4	FIPV 79-1146	S	40	+
18E7.6	FIPV 79-1146	S	<10	−
18F2.11	FIPV 79-1146	S	10	+
18G1.7	FIPV 79-1146	S	<10	−
18H9.1	FIPV 79-1146	S	<10	+
19A7.6	FIPV 79-1146	N	<10	−
19G11.10	FIPV 79-1146	S	20	+
20A5.4	FIPV 79-1146	S	<10	−
20C6.10	FIPV 79-1146	S	15	+
23A1.8	FIPV 79-1146	S	10	+
23B4.9	FIPV 79-1146	S	<10	−
23C1.2	FIPV 79-1146	M	<10	−
23F4.5	FIPV 79-1146	S	2,560	+
23F8.1	FIPV 79-1146	S	320	+
24H5.4	FIPV 79-1146	S	80	−
52D5[d]	FIPV DF2	N	<10	−
G4.1[d]	FIPV DF2	M	<10	−
G7.1[d]	TGEV Miller	N	<10	−
H11.1[d]	TGEV Miller	M	<10	−

[a] Strain of virus used to immunize mice for MAb induction. TGEV, transmissible gastroenteritis virus.

[b] Assayed on CrFKC and expressed as the reciprocal of the highest dilution of MAb (as ascites fluid) that completely inhibited cytopathology. A value of <10 indicates that there was no evidence of virus neutralization at the highest concentration of MAb tested. [c] +, ability of a given MAb to induce ADE of FIPV infection (EF of >2); −, lack of such ability (EF of <2).

[d] Kindly supplied and characterized (immunoblotting in place of RIPA) by S. Fiscus, Fermenta Animal Health.

Only 12 of the 37 MAbs tested were found to be capable of mediating ADE of FIPV infectivity (ADE+; Table 1). All of the enhancing MAbs were S protein specific, and all but one (18H9.1) of the enhancing MAbs had a virus neutralizing titer of ≥10. Interestingly, MAb 18H9.1 was the least enhancing and the only MAb to demonstrate enhancement of only one of the two strains of FIPV tested. While the peak EF of 18H9.1 was only 5, this degree of enhancement was consistent in a repeated experiment. Conversely, all but one (24H5.4) of the neutralizing MAbs were also found to be enhancing. All of the MAbs defined as nonenhancing had an EF of <2.0 (with the majority of them having an EF of <1.2)

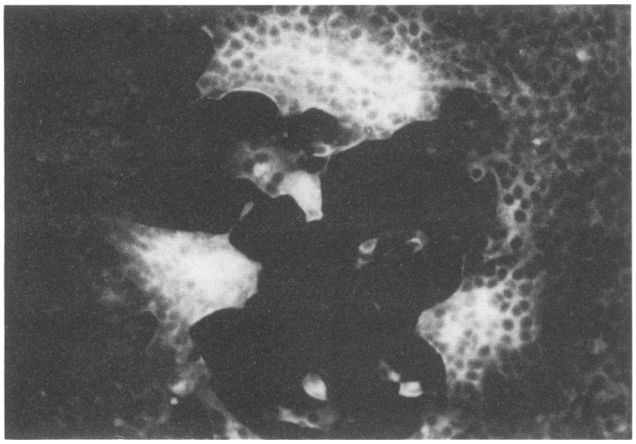

FIG. 3. CrFKC infected with a recombinant racoonpox virus (RPV-S) expressing the S protein of FIPV 79-1146. CrFKC were grown to confluence on eight-chamber glass Lab-Tek slides, infected with RPV-S (multiplicity of infection of 3), and fixed 24 h after infection. The poxvirus plaque shown was labelled by indirect IFA using MAb 23F4.5 and is representative of results obtained with the S protein-specific MAbs.

at all dilutions tested. There was no correlation between neutralization or enhancement and IgG subclass (data not shown).

Seven of the twelve enhancing MAbs were completely characterized in enhancement assays over 16 serial dilutions of ascites fluid, using both FIPV 79-1146 and FIPV UCD1 (Fig. 4 through 10). As in Fig. 1, all of the data contained in Fig. 4 through 10 were derived by using macrophages from the same donor cat in order to reduce macrophage variability. To ensure that these MAbs were not uniquely enhancing for macrophages from this one particular cat, each of the enhancing MAbs was tested at a single dilution of 1:4,096 (near peak EF for each) for its ability to enhance FIPV UCD1 infection of macrophages which were collected and processed the same day from two different SPF cats. When the results from these two cats were compared, the absolute levels of enhancement for a given MAb were different, but the relative pattern of enhancement among the MAbs was consistent—MAb 18H9.1 was the least enhancing, 3G7 and 17E1.6 were moderately enhancing, and 18A7.4, 23A1.8, 23F4.5, and 23F8.1 were strongly enhancing.

Infection in the presence of those MAbs showing the highest EFs (18A7.4, 23A1.8, 23F4.5, and 23F8.1) also led to the formation of dramatic syncytia among infected macrophages. Syncytia were most prevalent under conditions of near-maximal enhancement, suggesting that polykaryon formation may simply have been due to the presence of a large number of infected macrophages in close proximity to one another. An epitope-specific basis for syncytium formation cannot be ruled out, however, since occasional syncytia were found in the presence of these MAbs at lower levels of enhancement, whereas no syncytia were formed among macrophages infected in the presence of the other enhancing MAbs or in the absence of any Ab.

Enhancement is blocked in the presence of staphylococcal protein A. Results of parallel infections carried out in the presence or absence of staphylococcal protein A are shown in Table 2. The presence of protein A did not adversely affect the infectivity of FIPV itself. However, protein A dramatically reduced the level of FIPV infectivity in the presence of enhancing Ab, often to below even the level obtained with virus alone. This finding indicated that the enhancement of infection seen in the presence of Ab was Fc mediated.

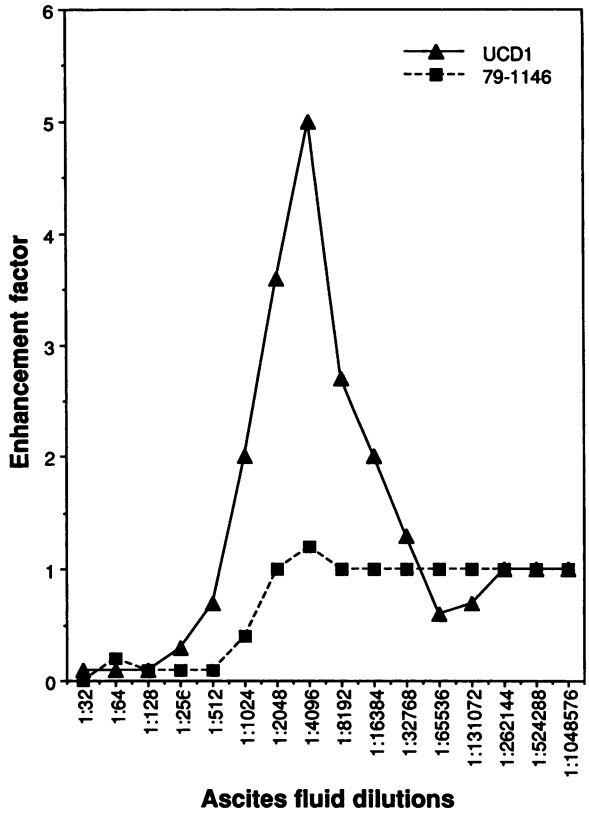

FIG. 4. ADE of FIPV (strains 79-1146 and UCD1) infection of primary feline peritoneal macrophages by MAb 18H9.1. Methods and EF calculations are as described for Fig. 1.

DISCUSSION

Although the immunologic aspects of FIP in vivo have been well documented since 1980 (13, 49), ADE of FIPV infection of macrophages in vitro has been investigated only recently (41). The experiments reported here were designed to address this in vitro phenomenon more completely and to begin to define the specific viral components responsible for the induction of enhancing ABs.

ADE of virus infectivity in other virus systems has been quantified by using several different methods, including determination of the number of cells infected by IFA labelling (44) or infectious center assays (37) or by titration of the amount of virus (38), antigen (44), or reverse transcriptase activity (10) generated. We chose to evaluate the number of macrophages infected during a single cycle of infection. In doing so, we are evaluating primarily the enhancing effect of Ab at the level of virus uptake and initiation of infection. In contrast, if levels of total virus output from populations of cells infected in the presence or absence of Ab are compared, the effect of Ab both at the cell surface and throughout virus replication must be considered. Previous reports of ADE of virus infectivity have not provided a consensus as to whether measurement of the number of cells infected and the titer of virus produced lead to the same assessment of enhancement (12, 36).

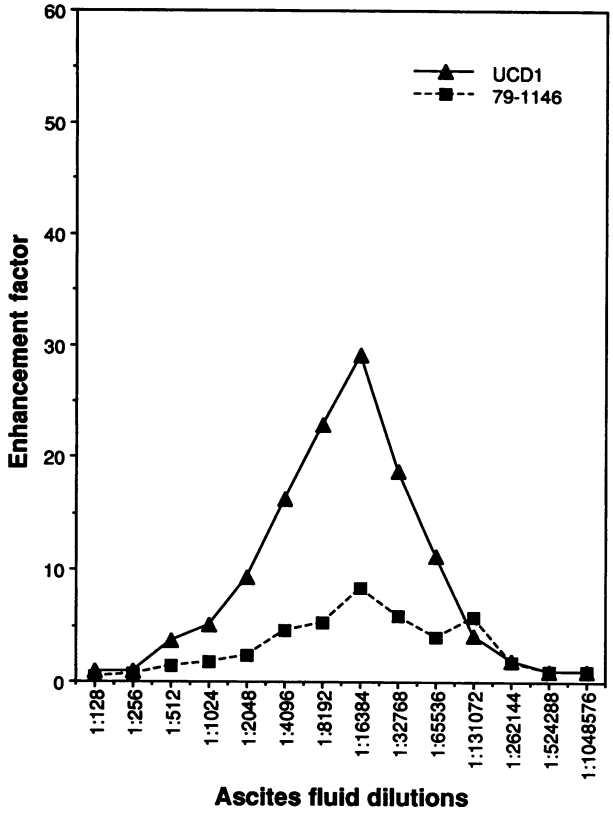

FIG. 5. ADE of FIPV (strains 79-1146 and UCD1) infection of primary feline peritoneal macrophages by MAb 3G7. Methods and EF calculations are as described for Fig. 1.

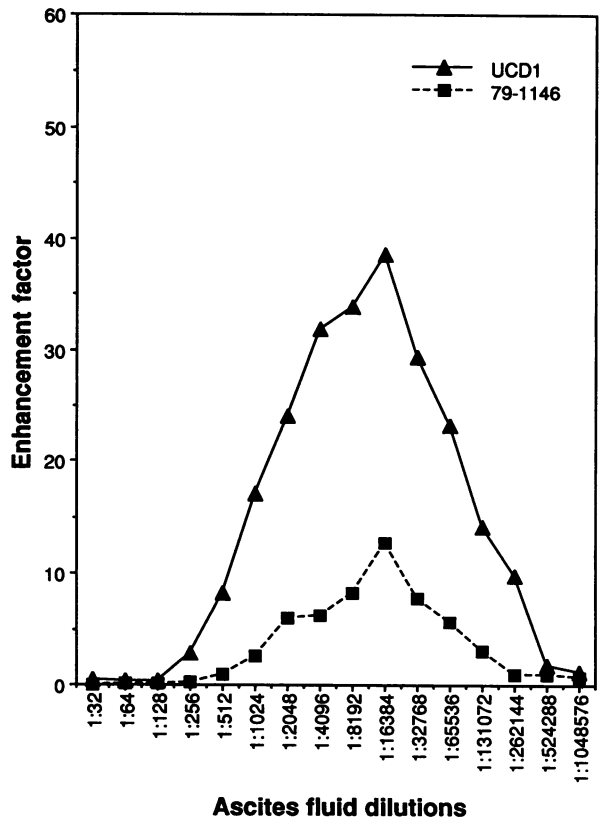

FIG. 6. ADE of FIPV (strains 79-1146 and UCD1) infection of primary feline peritoneal macrophages by MAb 17E1.6. Methods and EF calculations are as described for Fig. 1.

We began by examining the ability of FIPV-specific feline antiserum to mediate ADE of infection. Our experiments, using the same assay but different virus stocks, different antisera, and different macrophage donor cats, confirm previous results (41) and demonstrate that ADE of FIPV infectivity is a real and reproducible phenomenon. The results of our study of MAb-dependent enhancement of FIPV infectivity clearly confirm the hypothesis that enhancement is mediated by Abs to specific sites on the S protein of FIPV. These data also provide the first in vitro correlate to the results of a previous in vivo study in which vaccination of cats with a recombinant vaccinia virus expressing the S protein of FIPV 79-1146 caused ADE of disease (45). MAbs have previously been used to define the viral proteins responsible for inducing enhancing Abs in other virus systems (17, 24, 35). While it is generally assumed that ADE of virus infectivity is mediated by Ab to surface proteins, it was important to test MAbs not only to the S and M proteins but also to the N protein of FIPV, since enhancement of dengue virus infectivity has been demonstrated with a MAb specific for a nonenvelope protein (9).

The overall bell-shaped distribution of enhancement factor relative to dilution of antiserum or ascites fluid is consistent with the results of other studies of ADE (6, 23, 26, 41). Our results also show that in some instances, low dilutions/high concentrations of Ab can actually reduce the level of infection to below that obtained with virus alone, resulting in an EF of <1. This pattern is most easily seen in Fig. 4 (MAb 18H9.1) but was also evident with polyclonal feline antiserum (Fig. 1) and with MAbs 3G7, 17E1.6, 18A7.4, and 23F4.5 (Fig. 5 through 8, respectively). This phenomenon at low dilutions of Ab has also been documented in prior studies of ADE of virus infectivity (6, 23, 41, 44). FIPV is typical of other virus systems in that Ab-mediated virus uptake and infection occur via an Fc receptor-mediated endocytosis pathway which can be blocked by lysomotrophic amines (41). During endocytosis, there are both uptake and fusion phases of the internalization process. The bell-shaped enhancement profiles may reflect a need to optimize virion and Ab molecule concentrations for complex formation before uptake can occur. At high concentrations of Ab, the virions may be so coated with Ab that they are unable to approach and fuse with the endosome membrane due to steric hindrance (7, 8, 27), thus resulting in a level of infection below that seen with virus alone. Although this prozonelike effect at high concentrations of Ab may reflect virus neutralization, we have avoided that term since it is possible that the inhibition of virus infectivity by Ab in the endosome of a macrophage and virus neutralization in the more traditional sense may occur by different mechanisms (7).

MAbs 23A1.8 (Fig. 9) and 23F8.1 (Fig. 10) typify the remaining seven enhancing MAbs in this study which did not show a prozonelike effect to dilutions of 1:32. Enhancement assays using these seven MAbs were extended to a dilution of 1:8. At this dilution, all of the MAbs except 23F8.1 still induced substantial enhancement. (The level of infection in the presence of a 1:8 dilution of MAb 23F8.1 was similar to that seen with virus alone.) It is unclear whether these MAbs

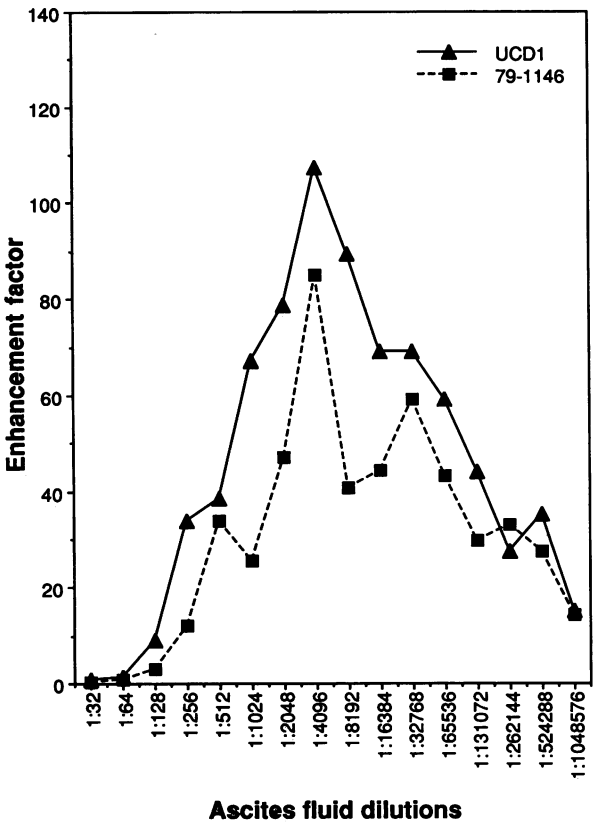

FIG. 7. ADE of FIPV (strains 79-1146 and UCD1) infection of primary feline peritoneal macrophages by MAb 18A7.4. Methods and EF calculations are as described for Fig. 1.

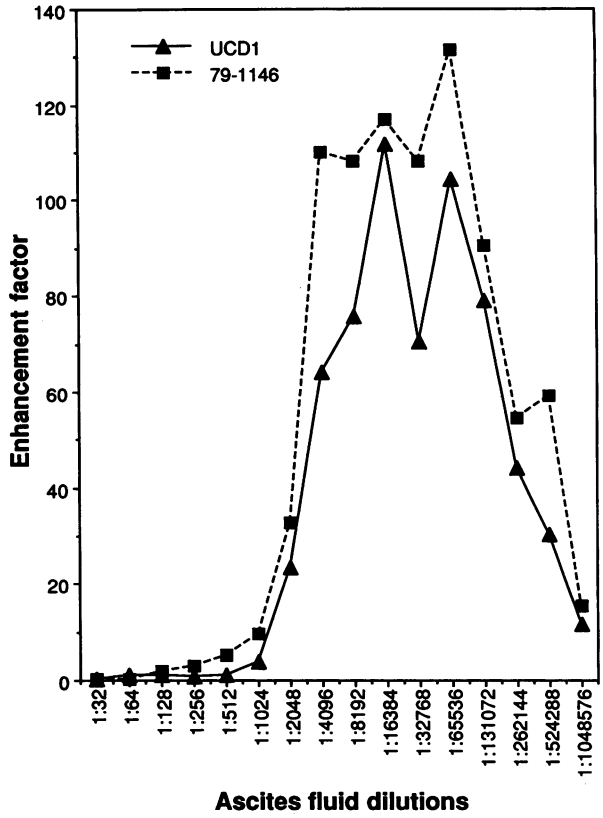

FIG. 8. ADE of FIPV (strains 79-1146 and UCD1) infection of primary feline peritoneal macrophages by MAb 23F4.5. Methods and EF calculations are as described for Fig. 1.

are functionally different from the others, are specific for a different epitope(s), or are different only by virtue of their concentration in the ascites fluid or their avidity for FIPV. It is interesting, however, that enhancement by these MAbs was also relatively less efficiently blocked by protein A (MAbs 23A1.8 and 23F8.1; Table 2), suggesting a functionally different basis for their infection-enhancing effect.

Inspection of the enhancement profiles also reveals that FIPV strains 79-1146 and UCD1 are enhanced to different degrees by most of the Abs evaluated. Strain variation has previously been demonstrated for enhancement of dengue virus infection with MAbs (8, 22, 24) and for enhancement of FIPV infection with antiserum (41). Morens et al. have debated whether such strain variation in ADE may reflect a basic difference between each strain's ability to infect macrophages in the absence of Ab (23, 24). Although strain variation in the ability of FIPV 79-1146 and UCD1 to infect macrophages has previously been documented (43), our results over repeated experiments did not demonstrate a significant difference in the ability of FIPV UCD1 and FIPV 79-1146 to infect macrophages in the absence of Ab (data not shown). In addition, the pattern of strain variation of ADE was not consistent among all of the enhancing MAbs. Four MAbs enhanced UCD1 more than 79-1146 (MAbs 18H9.1, 3G7, 17E1.6, and 18A7.4; Fig. 4 through 7, respectively), while two MAbs enhanced 79-1146 more than UCD1 (MAbs 23F4.5 and 23F8.1; Fig. 8 and 10, respectively). It is therefore likely that the strain variation in enhancement seen here is related to the specific epitopes recognized by the enhancing Abs and the binding avidity of each Ab for the two strains of FIPV.

Perhaps the most paradoxical of our results is the strong correlation between a MAb's ability to mediate both neutralization and ADE of FIPV infectivity. It is important to emphasize that the neutralization ability of the MAbs in this study was defined in a standard virus neutralization assay using CrFKC and not as the prozonelike effect that occurred at high concentrations of Ab in the macrophage assay system. As such, it is likely that the ability of Ab to mediate both neutralization and enhancement is dependent on the specific epitope involved and not simply the concentration of Ab. Previous investigations of ADE have also shown that a given Ab can both neutralize and enhance virus infectivity. The most exhaustive data come from work done with dengue virus (8, 23). Halstead et al. examined neutralization by plaque reduction neutralization in LLC-MK2 cells and enhancement in the murine macrophage cell line P388D1, using seven different strains of dengue 2 virus and five MAbs (8). While certain MAb and strain combinations demonstrated only neutralization or enhancement (similar to MAbs 18H9.1 and 24H5.4; Table 1), most MAbs could both neutralize and enhance virus infectivity (8).

This description of ADE of FIPV infectivity, like that of ADE in other virus systems, does not resolve the question of how Ab binding to a particular site on the virion induces an overall increased efficiency of infection. Among other theories, antibody binding may facilitate uncoating (15), protect against pH- or protease-initiated damage during endolysosomal transit, or enhance conformational changes required

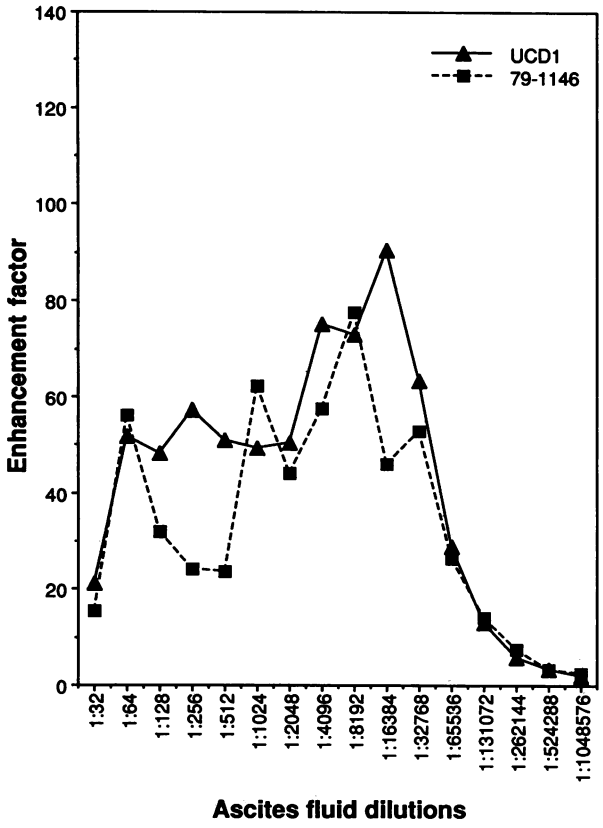

FIG. 9. ADE of FIPV (strains 79-1146 and UCD1) infection of primary feline peritoneal macrophages by MAb 23A1.8. Methods and EF calculations are as described for Fig. 1.

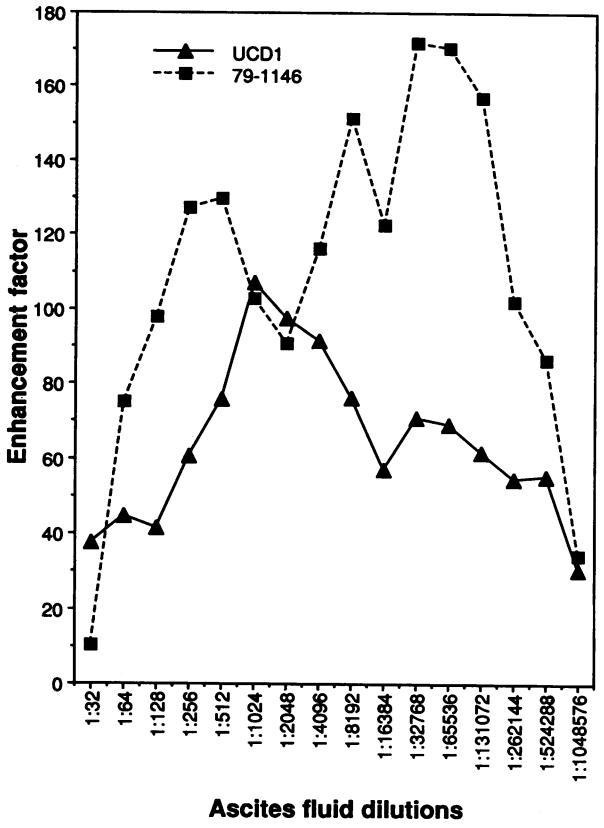

FIG. 10. ADE of FIPV (strains 79-1146 and UCD1) infection of primary feline peritoneal macrophages by MAb 23F8.1. Methods and EF calculations are as described for Fig. 1.

for virus fusion to the endosomal membrane. FIPV is unlike many of the other coronaviruses because its S protein is not cleaved during synthesis in routine cell culture (1, 18). However, a trypsin-sensitive cleavage site has been biochemically identified for FIPV 79-1146 and FECV 1683 (1), and cleavage of these S proteins by exogenously supplied trypsin has been shown to enhance FIPV infection of primary feline macrophages (1). Since the S protein is important for viral attachment and membrane fusion of coronaviruses, it is possible that antibody binding to a critical site on the S protein may facilitate cleavage or stabilize a subsequent conformational change required for uptake of FIPV.

TABLE 2. Effects of preincubation of virus-Ab complexes with staphylococcal protein A on ADE of FIPV infectivity

Inoculum	Mean no. of macrophages infected/Lab-Tek well	
	− Protein A[a]	+ Protein A[b]
FIPV UCD1	56	67
FIPV UCD1 plus:		
Feline antiserum[c]	872	15
3G7[d]	507	20
17E1.6[d]	825	3
18A7.4[d]	4,197	46
18H9.1[d]	90	12
23A1.8[d]	4,110	1,546
23F4.5[d]	247	24
23F8.1[d]	6,833	1,479

[a] Macrophages were infected with FIPV UCD1, with or without Ab, in the absence of protein A.

[b] Macrophages were infected with FIPV UCD1, with or without Ab, after preincubation with protein A at a final concentration of 200 μg/ml for 60 min at 37°C.

[c] FIPV-specific feline antiserum dilution = 1:512.

[d] Ascites fluid dilution = 1:1,024.

The results of this work indicate that ADE of FIPV infection of primary feline macrophages can be demonstrated by using both virus-specific feline antiserum and murine MAbs. The characteristics of ADE of FIPV infectivity outlined here parallel those of previously defined systems such as dengue virus. In view of these results and the well-documented occurrence of ADE of FIP in vivo, FIPV may serve as a useful model for future investigations into the mechanism of ADE of virus infectivity and the correlation between enhanced infection in vitro and enhanced disease in vivo.

ACKNOWLEDGMENTS

This work was supported by a resident research grant from Solvay Animal Health, Inc., and by private donations to the Cornell Feline Health Center. A portion of the MAbs listed in Table 1 was produced with the support of a grant from Rhone Merieux.

We gratefully acknowledge the technical support of C. Geissenger, R. Adams, and M. Brush and are grateful to Jeffrey Barlough and Margaret Barr for reviewing the manuscript. We also thank Cheryl Stoddart for many helpful discussions.

REFERENCES

1. **Baines, J. D.** 1988. Ph.D. thesis. Cornell University, Ithaca, N.Y.
2. **Barlough, J. E., and C. A. Stoddart.** 1990. Feline coronaviral infections, p. 300–312. *In* C. E. Greene (ed.), Infectious diseases of the dog and cat. W. B. Saunders Co., Philadelphia.
3. **Corapi, W. V., R. O. Donis, and E. J. Dubovi.** 1990. Characterization of a panel of monoclonal antibodies and their use in the study of the antigenic diversity of bovine viral diarrhea virus. Am. J. Vet. Res. **51**:1388–1394.
4. **Fiscus, S. A., and Y. A. Teramoto.** 1987. Antigenic comparison of feline coronavirus isolates: evidence for markedly different peplomer glycoproteins. J. Virol. **61**:2607–2613.
5. **Gimenez, H. B., H. M. Keir, and P. Cash.** 1989. In vitro enhancement of respiratory syncytial virus infection of U937 cells by human sera. J. Gen. Virol. **70**:89–96.
6. **Gollins, S. W., and J. S. Porterfield.** 1984. Flavivirus infection enhancement in macrophages: radioactive and biological studies on the effect of antibody on viral fate. J. Gen. Virol. **65**:1261–1272.
7. **Gollins, S. W., and J. S. Porterfield.** 1986. A new mechanism for the neutralization of enveloped viruses by antiviral antibody. Nature (London) **321**:244–246.
8. **Halstead, S. B., C. N. Venkateshan, M. K. Gentry, and L. K. Larsen.** 1984. Heterogeneity of infection enhancement of dengue 2 strains by monoclonal antibodies. J. Immunol. **132**:1529–1532.
9. **Henchal, E. A., J. M. McCown, D. S. Burke, M. C. Sequin, and W. E. Brandt.** 1985. Epitopic analysis of antigenic determinants on the surface of dengue-2 virions using monoclonal antibodies. Am. J. Trop. Med. Hyg. **34**:162–169.
10. **Homsy, J., M. Meyer, and J. A. Levy.** 1990. Serum enhancement of human immunodeficiency virus (HIV) infection correlates with disease in HIV-infected individuals. J. Virol. **64**:1437–1440.
11. **Homsy, J., M. Meyer, M. Tateno, S. Clarkson, and J. A. Levy.** 1989. The Fc and not CD4 receptor mediates antibody enhancement of HIV infection in human cells. Science **244**:1357–1360.
12. **Hotta, H., A. S. Wiharta, and S. Hotta.** 1984. Antibody-mediated enhancement of dengue virus infection in mouse macrophage cell lines, Mk1 and Mm1 (41802). Proc. Soc. Exp. Biol. Med. **175**:320–327.
13. **Jacobse-Geels, H. E., M. R. Daha, and M. C. Horzinek.** 1980. Isolation and characterization of feline C3 and evidence for the immune complex pathogenesis of feline infectious peritonitis. J. Immunol. **125**:1606–1610.
14. **Jacobse-Geels, H. E., M. R. Daha, and M. C. Horzinek.** 1982. Antibody, immune complexes, and complement activity fluctuations in kittens with experimentally induced feline infectious peritonitis. Am. J. Vet. Res. **43**:666–670.
15. **Jolly, P. E., D. L. Huso, D. Sheffer, and O. Narayan.** 1989. Modulation of lentivirus replication by antibodies: Fc portion of immunoglobulin molecule is essential for enhancement of binding, internalization, and neutralization of visna virus in macrophages. J. Virol. **63**:1811–1813.
16. **King, A. A., J. J. Sands, and J. S. Porterfield.** 1984. Antibody-mediated enhancement of rabies virus infection in a mouse macrophage cell line (P388D1). J. Gen. Virol. **65**:1091–1093.
17. **Krilov, L. R., L. J. Anderson, L. Marcoux, V. R. Bonagura, and J. F. Wedgewood.** 1989. Antibody-mediated enhancement of respiratory syncytial virus infection in two monocyte/macrophage cell lines. J. Infect. Dis. **160**:777–782.
18. **Lai, M. M. C.** 1990. Coronavirus: organization, replication and expression of genome. Annu. Rev. Microbiol. **44**:303–333.
19. **Mackett, M., G. L. Smith, and B. Moss.** 1982. Vaccinia virus: a selectable eukaryotic cloning and expression vector. Proc. Natl. Acad. Sci. USA **79**:7415–7419.
20. **Mackett, M., G. L. Smith, and B. Moss.** 1984. General method for production and selection of infectious vaccinia virus recombinants expressing foreign proteins. J. Virol. **49**:857–864.
21. **Mackett, M., G. L. Smith, and B. Moss.** 1985. The construction and characterization of vaccinia virus recombinants expressing foreign genes, p. 191–211. *In* D. M. Glover (ed.), DNA cloning, vol. II. IRL Press, Oxford.
22. **Morens, D. M., and S. B. Halstead.** 1987. Disease severity-related antigenic differences in dengue 2 strains detected by dengue 4 monoclonal antibodies. J. Med. Virol. **22**:169–174.
23. **Morens, D. M., and S. B. Halstead.** 1990. Measurement of antibody-dependent infection enhancement of four dengue virus serotypes by monoclonal and polyclonal antibodies. J. Gen. Virol. **71**:2909–2914.
24. **Morens, D. M., C. N. Venkateshan, and S. B. Halstead.** 1987. Dengue 4 virus monoclonal antibodies identify epitopes that mediate immune infection enhancement of dengue 2 viruses. J. Gen. Virol. **68**:91–98.
25. **Ngichabe, C. K., C. W. Olsen, and F. W. Scott.** Unpublished data.
26. **Ochiai, H., M. Kurokawa, K. Hayashi, and S. Niwayama.** 1988. Antibody-mediated growth of influenza A NWS virus in macrophagelike cell line P388D1. J. Virol. **62**:20–26.
27. **Ochiai, H., M. Kurokawa, Y. Kuroki, and S. Niwayama.** 1990. Infection enhancement of influenza A H1 subtype viruses in macrophage-like P388D1 cells by cross-reactive antibodies. J. Med. Virol. **30**:258–265.
28. **Olsen, C. W., and F. W. Scott.** 1991. Feline infectious peritonitis vaccination-past and present. Feline Health Top. **6**:1–4, 8.
29. **Panicali, D., S. W. Davis, R. L. Weinberg, and E. Paoletti.** 1983. Construction and characterization of live virus vaccines by using genetically engineered poxviruses: biological activity of recombinant vaccinia virus expressing influenza virus haemagglutinin. Proc. Natl. Acad. Sci. USA **80**:5364–5368.
30. **Pedersen, N. C.** 1983. Feline infectious peritonitis and feline enteric coronavirus infections. Part 2. Feline infectious peritonitis. Feline Pract. **13**:5–20.
31. **Pedersen, N. C., and J. F. Boyle.** 1980. Immunologic phenomena in the effusive form of feline infectious peritonitis. Am. J. Vet. Res. **41**:868–876.
32. **Peiris, J. S. M., S. Gordon, J. C. Unkeless, and J. S. Porterfield.** 1981. Monoclonal anti-Fc receptor IgG blocks antibody enhancement of viral replication in macrophages. Nature (London) **289**:189–191.
33. **Porterfield, J. S.** 1986. Antibody-dependent enhancement of viral infectivity. Adv. Virus Res. **31**:335–355.
34. **Reed, L. J., and H. Muench.** 1938. Simple method of estimating 50 per cent endpoints. Am. J. Hyg. **27**:493–497.
35. **Robinson, W. E., T. Kawamura, M. K. Gorny, D. Lake, J.-Y. Xu, Y. Matsumoto, T. Sugano, Y. Masuho, W. M. Mitchell, E. Hersh, and S. Zolla-Pazner.** 1990. Human monoclonal antibodies to the human immunodeficiency virus type 1 (HIV-1) transmembrane glycoprotein gp41 enhance HIV-1 infection in vitro. Proc. Natl. Acad. Sci. USA **87**:3185–3189.
36. **Schlesinger, J. J., and M. W. Brandriss.** 1981. Antibody-mediated infection of macrophages and macrophage-like cell lines with 17D-yellow fever virus. J. Med. Virol. **8**:103–117.
37. **Schlesinger, J. J., and M. W. Brandriss.** 1981. Growth of 17D yellow fever virus in a macrophage-like cell line, U937: role of Fc and viral receptors in antibody-mediated infection. J. Immunol. **127**:659–665.
38. **Schlesinger, J. J., and M. W. Brandriss.** 1983. 17D Yellow fever virus infection of P388D1 cells mediated by monoclonal antibodies: properties of the macrophage Fc receptor. J. Gen. Virol. **64**:1255–1262.
39. **Scott, F. W.** 1986. Feline infectious peritonitis and other feline coronaviruses, p. 1059–1062. *In* R. W. Kirk (ed.), Current veterinary therapy IX. W. B. Saunders Co., Philadelphia.
40. **Smith, G. L., M. Mackett, and B. Moss.** 1983. Construction and characterization of an infectious vaccinia virus recombinant that expresses the influenza virus haemagglutinin gene and induces resistance to influenza virus infection in hamsters. Proc. Natl. Acad. Sci. USA **80**:7155–7159.
41. **Stoddart, C. A.** 1989. Ph.D. thesis. Cornell University, Ithaca, N.Y.
42. **Stoddart, C. A., and F. W. Scott.** 1988. Isolation and identification of feline peritoneal macrophages for in vitro studies of coronavirus-macrophage interactions. J. Leukocyte Biol. **44**:319–328.
43. **Stoddart, C. A., and F. W. Scott.** 1989. Intrinsic resistance of

feline macrophages to coronavirus infection correlates with in vivo virulence. J. Virol. **63**:436–440.

44. **Takeda, A., C. U. Tuazon, and F. A. Ennis.** 1988. Antibody-enhanced infection of HIV-1 via Fc receptor-mediated entry. Science **242**:580–583.
45. **Vennema, H., R. J. deGroot, D. A. Harbour, M. Dalderup, T. Gruffydd-Jones, M. C. Horzinek, and W. J. M. Spaan.** 1990. Early death after feline infectious peritonitis challenge due to recombinant vaccinia virus immunization. J. Virol. **64**:1407–1409.
46. **Vennema, H. L., L. Heijnen, A. Zijderveld, M. C. Horzinek, and W. J. M. Spaan.** 1990. Intracellular transport of recombinant coronavirus spike proteins: implications for virus assembly. J. Virol. **64**:339–346.
47. **Weiss, R. C., and F. W. Scott.** 1981. Antibody-mediated enhancement of disease in feline infectious peritonitis: comparisons with dengue hemorrhagic fever. Comp. Immunol. Microbiol. Infect. Dis. **4**:175–189.
48. **Weiss, R. C., and F. W. Scott.** 1981. Pathogenesis of feline infectious peritonitis: nature and development of viremia. Am. J. Vet. Res. **42**:382–390.
49. **Weiss, R. C., and F. W. Scott.** 1981. Pathogenesis of feline infectious peritonitis: pathologic changes and immunofluorescence. Am. J. Vet. Res. **42**:2036–2048.