Experimental Inoculation of Cats with Human Coronavirus 229E and Subsequent Challenge with Feline Infectious Peritonitis Virus

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ABSTRACT

Minimal-disease cats exposed to live human coronavirus 229E developed homologous antibody responses that suggested little or no replication of the virus in inoculated animals. Oronasal and subcutaneous inoculation of coronavirus 229E did not elicit an antibody response by heterologous (transmissible gastroenteritis virus, canine coronavirus) neutralization or by heterologous (transmissible gastroenteritis virus) kinetics-based enzyme-linked immunosorbent assay. No clinical signs attributable to coronavirus 229E were seen in inoculated cats. Although the number of animals in each of the five experimental groups was small (n = 2), antibodies produced in response to the virus did not appear to sensitize cats to subsequent feline infectious peritonitis virus challenge, but neither did they cross-protect cats against the challenge dose.

Key words: Human coronavirus 229E, transmissible gastroenteritis virus, canine coronavirus, feline infectious peritonitis virus, cats, challenged.

RÉSUMÉ

L'inoculation du coronavirus humain vivant 229E à des chats "minimal-disease" provoqua le développement d'un faible taux d'anticorps à son endroit, indice d'une réplication faible ou nulle. L'inoculation oronasale ou sous-cutanée du dit virus ne stimula pas la production d'anticorps contre le virus de la gastro-entérite transmissible, ni contre le coronavirus canin, comme le démontra l'épreuve de séroneutralisation; une épreuve immunoenzymatique, basée sur la cinétique ne réussit pas non plus à mettre en évidence des anticorps contre le virus de la gastro-entérite transmissible. Les chats expérimentaux ne manifestèrent pas de signes cliniques attribuables au coronavirus 229E; bien que chacun des cinq groupes de chats expérimentaux n'en comptait que deux, les anticorps produits à la suite de l'inoculation du virus précité ne semblèrent pas sensibiliser ces chats à une infection de défi ultérieure avec le virus de la péritonite infectieuse féline, ni leur conférer une immunité croisée contre cette infection.

Mots clés: coronavirus humain 229E, virus de la gastro-entérite transmissible, coronavirus canin, virus de la péritonite infectieuse féline, chats, infection de défi.

INTRODUCTION

The coronaviruses are a large and widely distributed family of single-stranded ribonucleic acid viruses and are important causes of respiratory and enteric disease, vasculitis, serositis, hepatitis and encephalomyelitis in several species of birds and mammals (1). Feline infectious peritonitis virus (FIPV), transmissible gastroenteritis virus (TGEV), canine coronavirus (CCV) and human respiratory coronaviruses of the 229E group (HCV 229E) together comprise an antigenic cluster of viruses within the Coronaviridae (2). In fact, the major structural polypeptides of FIPV, TGEV and CCV are so similar antigenically that some regard these agents as host-range mutants rather than as individual virus species (3). In domestic and exotic cats, FIPV produces a progressive and lethal immunologically mediated disease that is characterized by fibrinous serositis, perivasculitis and formation of disseminated pyogranulomas (4). Current treatment regimens for feline infectious peritonitis (FIP) are purely palliative and a safe and effective vaccine is unavailable.

Most cats afflicted with FIP have serum coronavirus antibodies (often of high titer) that can be detected by a variety of serological techniques, using FIPV itself as target antigen (homologous assays)(4,5). However, the fastidious growth requirements of this virus (6,7) have spurred development of heterologous assays using other members of the FIPV antigenic cluster that are more amenable to routine propagation in cell culture (primarily TGEV and CCV)(5,8,9). Seroepizootiological surveys using both homologous and heterologous techniques have subsequently demonstrated that, in addition to those with active FIP, many healthy cats and many cats with diseases other than FIP are also coronavirus antibody-positive, suggesting that many natural infections with FIPV must result in seroconversion without progression to fatal FIP (4,8-10).

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This work was supported by unrestricted contributions to the Cornell Feline Health Center and was performed in partial fulfillment of the requirements for a Ph.D. degree from Cornell University by the senior author.

Submitted August 8, 1984.

Recently, added complexity has been contributed to interpretation of feline coronavirus antibody titers by reports that at least three other coronaviruses, all serologically cross-reactive with FIPV, are also infectious for cats. These viruses are: TGEV, which produces an asymptomatic infection and can be excreted in feces for as long as three weeks postexposure (11-14); partially characterized feline enteric coronaviruses (FECVs), possible variants of FIPV (or vice versa), which can produce a range of effects from asymptomatic infection of the gastrointestinal tract to severe enteritis (15,16); and CCV, which produces an asymptomatic infection and can be excreted from the oropharynx for as long as one week postexposusre (17,18). Thus the serodiagnostic potential of commercially available feline coronavirus antibody assays (i.e. their ability to identify cats with active FIP and/or potential virus carriers/excretors) is limited not only by the widespread distribution of serum coronavirus antibodies in the feline population but also by the possibility that non-FIPV coronaviruses may be responsible for some of the seroconversions they detect.

It was therefore of interest to us to learn whether HCV 229E also might induce antibody responses in exposed cats that could be detected with assays routinely employed in research and clinical practice. In addition, because development of a safe and effective FIPV vaccine has to date remained elusive (4), it was important to determine whether exposure to live HCV 229E might cross-protect cats against challenge with virulent FIPV.

MATERIALS AND METHODS

VIRUSES AND CELLS

Human coronavirus 229E was propagated at 33° C in human diploid fetal tonsil (HFT) cells (both from Dr. O.W. Schmidt, University of Washington, Seattle, Washington) (19,20) using Eagle minimum essential medium (with Earle's salts and L-glutamine) (GIBCO Laboratories, Grand Island, New York) supplemented with 10% heat-inactivated fetal bovine serum (GIBCO), 50 μg/mL gentamicin sulfate (Schering Corporation, Kenilworth, New Jersey) and 2.5 μg/mL amphotericin B (E.R. Squibb & Sons, Princeton, New Jersey). The DL/Miller isolate of TGEV (from the New York State Diagnostic Laboratory, Cornell University) and the Karbatsch isolate of CCV (from Dr. L.E. Carmichael, James A. Baker Institute for Animal Health, Cornell University) were propagated in canine A-72 cells (catalog no. CRL 1542, American Type Culture Collection, Rockville, Maryland) (21) as described previously (22). The UCD-1 strain of FIPV (from Dr. N.C. Pedersen, School of Veterinary Medicine, University of California, Davis, California) was prepared as a 50% liver homogenate after passage through minimal-disease cats (23).

PREPARATION OF INOCULA

For preparation of HCV 229E inocula, flasks containing infected HFT cells were subjected to one freeze-thaw cycle approximately four days after virus adsorption. The cell culture fluids were then centrifuged at 600 x g for 15 min at 4° C and the supernatants were pooled and stored in aliquots at -80° C. Control cells were treated in an identical manner except that virus was omitted. For preparation of FIPV challenge inocula, samples of infected liver homogenate were thawed and centrifuged at 500 x g for 10 min at 4° C and the supernatants were pooled and stored in aliquots at -80° C.

SEROLOGICAL ASSAYS

Antibody titers to HCV 229E were determined by indirect immunofluorescence using human fetal lung (L132) cells persistently infected with HCV 229E (HCV 229E/HV-1)(24). Briefly, monolayers of infected cells were prepared in 8-well slide chambers (Miles Scientific, Napierville, Illinois), then fixed in acetone and stored at -20° C until required. The indirect immunofluorescence assay described by Pedersen *et al* (2) was used to first screen sera at dilutions of 1:20 and then to titrate all positive samples (1:20 through 1:160). Controls were included with each test series and consisted of homologous and heterologous positive standards (absorbed guinea pig anti-HCV 229E hyperimmune and convalescent anti-FIPV sera, respectively), and negative, non-immune sera and/or phosphate-buffered saline. Fluorescein conjugates were obtained commercially (Zymed Laboratories, San Francisco, California) and used at recommended dilutions. All test sera were coded and the code was not broken until all testing had been completed. Slides were examined for specific immunofluorescence using a Leitz Laborlux II epifluorescent microscope.

Neutralization assays using either DL/Miller TGEV or Karbatsch CCV were performed as described previously (22). Coronavirus antibody titers were also determined using DL/Miller TGEV in a computer-assisted, kinetics-based enzyme-linked immunosorbent assay (KELA) as described previously (25).

MINIMAL-DISEASE CATS

Eight 14 week old coronavirus antibody-negative minimal-disease kittens were obtained from a commercial breeding colony (Liberty Laboratories, Liberty Corner, New Jersey) and housed singly in negative-pressure fiberglass isolation cages (Germfree Laboratories, Inc, Miami, Florida) that were specially equipped for maximal air exchange (26,27). In addition, two adult coronavirus antibody-positive minimal-disease cats were obtained from the barrier-maintained Cornell feline breeding colony (Division of Laboratory Animal Services, New York State College of Veterinary Medicine, Cornell University) and housed singly in a separate isolation room. Cats in this colony routinely become coronavirus antibody-positive at five to eight weeks of age and remain seropositive indefinitely (26,27). Strict isolation procedures were followed in the care of cats throughout this experiment. To further minimize the possibility of cross-contamination between groups of animals, virus-inoculated and control cats were cared for on alternate days.

EXPERIMENTAL DESIGN

Minimal-disease cats were divided into five experimental groups as shown in Table I. Inoculated and control cats were monitored daily for clinical signs, and blood samples for coronavirus antibody titer determinations were obtained weekly or biweekly by jugular venipuncture. Tissues from

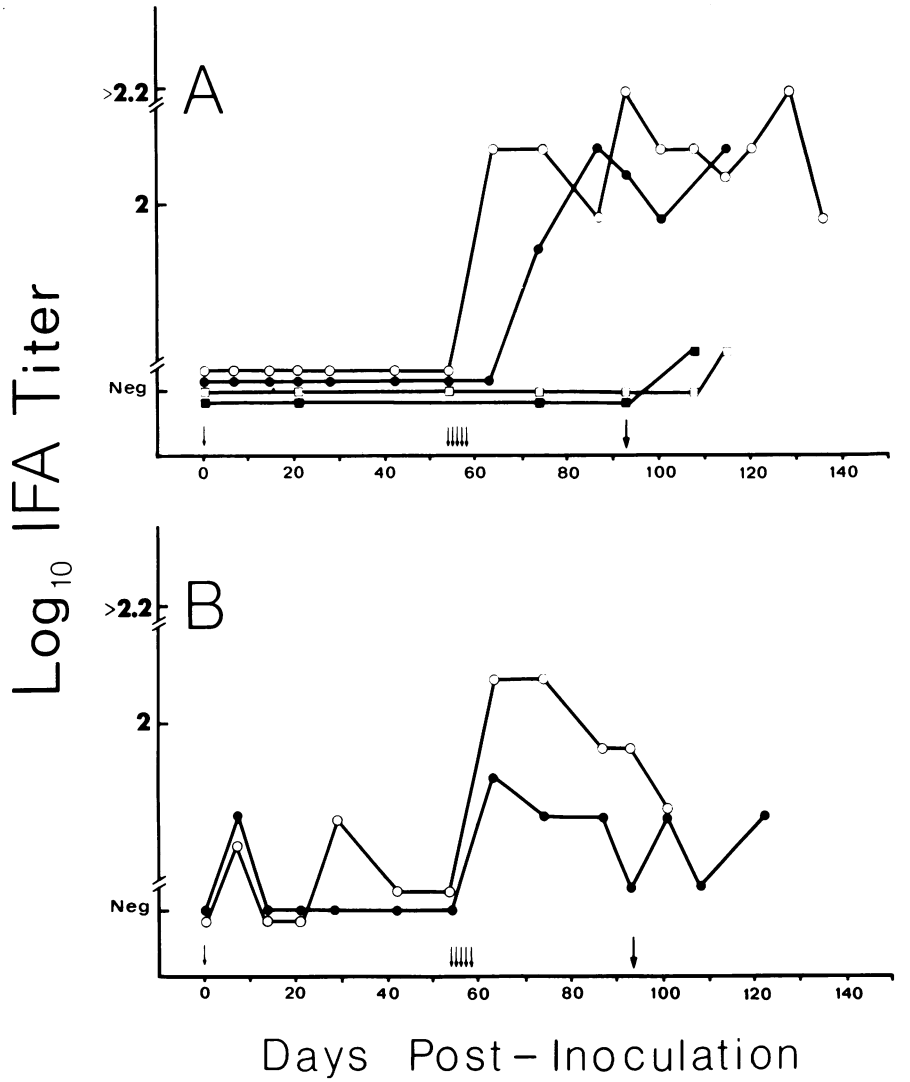

Fig. 1. Homologous immunofluorescent antibody responses of HCV 229E-exposed and unexposed cats. Time scale is calculated from the day of the first HCV 229E inoculation (day 0). A, Cats exposed by the oronasal route; B, Cats exposed by the subcutaneous route. *Symbols*: In A: ●,○, antibody responses of cats given HCV 229E (group 1); ■,□, antibody responses of cell culture control cats (group 3). In B: ●,○, antibody responses of cats given HCV 229E (group 2). Smaller arrows indicate inoculations with HCV 229E (days 0 and 54 through 58); larger arrows indicate aerosol challenge with UCD-1 FIPV (day 93).

cats given FIPV were collected for histopathological examination after natural death in order to confirm the clinical diagnosis. Clinical observation of room control cats was continued for several months after termination of the experiment.

RESULTS

HOMOLOGOUS (HCV 229E) ANTIBODY RESPONSES

Cats given HCV 229E by the oronasal route (group 1) developed detectable homologous antibody titers only after at least two doses of virus (Fig. 1A). These titers remained elevated for the remainder of the experiment, rising slightly after FIPV challenge. By contrast, cats given HCV 229E by the subcutaneous route (group 2) developed low homologous antibody titers following a single dose of virus (Fig. 1B). These titers then declined, but were boosted to high levels by subsequent reexposure to virus. Cats inoculated by the oronasal route with uninfected cell culture supernatants (group 3) did not develop antibodies against HCV 229E until they were challenged with FIPV, after which a very low cross-reactive response was observed (Fig. 1A).

HETEROLOGOUS (TGEV, CCV) ANTIBODY RESPONSES

Cross-reacting antibodies to DL/Miller TGEV were not detected by either neutralization or KELA following exposure of cats to HCV 229E, nor was a cross-reacting neutralization response to Karbatsch CCV observed. Following FIPV challenge, however, a single cat from group 1 (cat B, Table I) that survived for an extended period of time (47 days) developed low KELA titers approximately 36 days postexposure ($\log_{10}$ KELA titer 1.20 on day 36; $\log_{10}$ KELA titer 1.60 on day 43), a delay reflecting the swiftness of the experimental disease (29) and the heterologous nature of both of the assays employed (22,28,29). As expected, antibody-sensitized control cats (group 4) experienced a modest anamnestic response following FIPV challenge (Table II). Room control cats (group 5) remained seronegative in all assays throughout the course of the experiment.

CLINICAL SIGNS

No signs of illness were noted in any cats following HCV 229E inoculations. However, all eight cats that received FIPV developed clinical signs characteristic of FIP. Cats given HCV 229E did not appear to be sensitized to subsequent FIPV challenge, but neither were they protected against the challenge dose (Table I). All eight cases of clinically diagnosed FIP at death demonstrated gross pathological and histopathological lesions characteristic of the disease (4,6,23). Room control cats (group 5) did not develop fevers and remained clinically normal throughout the course of the experiment.

DISCUSSION

In this paper we report that neither oronasal nor subcutaneous inoculation of minimal-disease cats with HCV 229E was able to elicit a serological response by heterologous (TGEV, CCV) neutralization or by heterologous (TGEV) KELA. Although the number of cats in each of the five experimental groups was small (n = 2), the anamnestic responses seen after FIPV challenge in cats given HCV 229E by the oronasal route (group 1,

TABLE I. Evolution of FIP in HCV 229E-exposed and Unexposed Minimal-disease Cats

Group No. and Designation	Inoculum Contents[b]	Cat[c]	Challenged[d]	Initial Rise of Rectal Temperature[a]	Survival Time
				Days Postchallenge	
1 — HCV 229E-exposed (oronasal)	HFT cell culture fluid + HCV 229E[e]	A	Yes	12	23
		B	Yes	25	47
2 — HCV 229E-exposed (subcutaneous)	As above	C	Yes	15	30
		D	Yes	9	20
3 — Cell culture controls (oronasal)	HFT cell culture fluid	E	Yes	11	35
		F	Yes	12	22
4 — Antibody-sensitized controls[f]	Not inoculated	G	Yes	2	9
		H	Yes	3	11
5 — Room controls	Not inoculated	I	No	NE[g]	NE
		J	No	NE	NE

[a]Temperature greater than 39°C
[b]Each inoculum consisted of 1 mL of cell culture fluid supernatant administered by either the oronasal or subcutaneous route. Inoculations were performed on days 0 and 54 through 58 (the latter in order to mimic exposure of cats living in close contact with an infected human being)
[c]Two cats per experimental group
[d]On day 93, cats were exposed inside a semi-closed glass anesthetic chamber to approximately 3 mL of nebulized FIPV-infected 50% liver homogenate supernatant. Nebulization was performed with a fine-particle nebulizer (Hoechst-Roussel Pharmaceuticals, Inc., Somerville, New Jersey) at 25 psi for 10 min for each cat
[e]$10^{6.33}$ $TCID_{50}$ HCV 229E/mL
[f]Coronavirus antibody-positive cats obtained from the Cornell University barrier-maintained feline breeding colony. When challenged with FIPV, such cats develop a more rapid and fulminating form of FIP than do seronegative cats similarly challenged (see Discussion)
[g]NE = No effect; room control cats did not develop coronavirus antibody titers or clinical signs of disease

Fig. 1A) and in antibody-sensitized control cats (group 4, Table II); the brief, low-level immunofluorescence titers to HCV 229E seen in cell culture control cats (group 3, Fig. 1A) following FIPV challenge; and the delayed heterologous KELA response detected finally in the single long-surviving cat from group 1 (cat B, Table I), nevertheless served to confirm the antigenic cross-reactivity among the four presently accepted members of the FIPV antigenic cluster (2). However, any ability for HCV 229E exposure of cats to produce cross-reacting responses in heterologous coronavirus antibody assays commonly utilized in research (neutralization, KELA) or in clinical practice (KELA) could not be demonstrated.

Subcutaneous inoculation of cats with HCV 229E elicited an initial, low-level homologous response that was boosted considerably by subsequent reexposure (Fig. 1B). No initial response was detected in cats exposed by the oronasal route, but reexposure resulted in an anamnestic response (Fig. 1A). It would thus appear from these data that little or no replication of HCV 229E occurred in inoculated cats, since a steady rise in antibody titer following initial exposure, characteristic of *in vivo* amplification (30), could not be demonstrated.

The recognized antigenic cross-reactivity between FIPV and HCV 229E prompted examination of the potential of the latter agent for sensitization or protection of cats following virulent FIPV challenge. Previous studies (12,15,17,31-36) have shown that some cats with actively or passively acquired coronavirus antibody titers, when challenged with FIPV, develop a more rapid and fulminating form of the disease than do seronegative cats similarly challenged. Seropositive cats develop fever and cell-associated viremia within 48 h of exposure to FIPV aerosols and die one to two weeks postinoculation; by contrast, seronegative cats become febrile only after ten to 14 days and die approximately three to five weeks postinoculation. An antibody-mediated state of hypersensitivity thus exists in certain coronavirus antibody-positive cats, representing an obvious impediment to development of an effective vaccine (15,31,32,35). Until recently it had been assumed that coronavirus antibodies in sensitized cats were the result exclusively of prior exposure to FIPV; however, newer information suggests the existence of at least one other sensitizing agent, the partially characterized FECV from California (15,31). Additionally there is some evidence that TGEV strains may occasionally sensitize (12). In other cases, as with the Cornell feline breeding colony, the identity of the sensitizing coronavirus remains undetermined (26,27). Although there has seemingly been some slight success in attempts to immunize cats against FIP (31), more often the reports have indicated that vaccination with either FIPV or FECV has sensitized vaccinees to a more precipitous disease course upon challenge (15,29,31). Neither has TGEV (12-14) nor CCV (17) afforded protection, although in most cases they have not sensitized. In the experiment reported here, antibodies produced in response to HCV 229E did not appear to sensitize cats to subsequent FIPV challenge, but neither did they protect cats against the challenge dose. However, the relatively lengthy survival of cat B (group 1) might reflect some degree of partial cross-protection provided by oronasal HCV 229E immunization. Alternatively, the relatively small number of cats used per experimental group (n = 2) is probably insufficient to detect subtle differences even in such a stereotyped challenge system as the FIPV system (17,23,32-36). Further work in this

TABLE II. Heterologous (TGEV) KELA Responses to FIPV Challenge in Antibody-sensitized Control Cats (group 4)[a]

Cat	Day Postchallenge	Log_{10} KELA Titer (TGEV)
G	0[b]	1.43
	3	2.53
H	0	2.17
	3	2.77

[a]Neutralization assays (TGEV, CCV) uniformly negative
[b]0-day serum samples drawn prior to nebulization with FIPV

area using larger numbers of experimental animals may be helpful in this regard.

In view of the recognized cross-relationship between FIPV and HCV 229E in the feline host, it could be of considerable interest to determine whether a similar situation might exist in the human host. Such studies would also serve to further elucidate, on the one hand, the relationship between FIPV and HCV 229E/HV-1 (the *in vitro* persistent HCV 229E), while on the other hand would also provide important information on the relationships of both of these viruses to standard "wild-type" HCV 229E strains. Possibly the use of HCV 229E/HV-1 as the oronasal immunogen in cats would be more effective in protecting against FIPV.

ACKNOWLEDGMENTS

The authors acknowledge the expert technical assistance of Giovanna Sorresso, Patricia Lad and Cathy Shea.

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