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Feline Infectious Peritonitis Virus

II. Propagation in Suckling Mouse Brain

By

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With 3 figures and one table

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Introduction

Feline infectious peritonitis (FIP) virus was shown to possess many of the physical properties of Coronaviridae family members (2, 5, 10). Support for this tentative classification has been obtained recently from neutralization and immunofluorescence studies showing an antigenic relationship between FIP virus and the coronavirus causing transmissible gastroenteritis (TGE) of swine (6, 8, 13). Numerous attempts to isolate FIP virus in chicken embryos (11, 14), in primary feline cells (1, 3, 7, 12) and continuous lines (7) have failed; in vitro virus growth could be demonstrated so far only in cultures of cells derived from the peritoneal exudates of kittens after experimental infection with FIP virus (7). The present report describes the successful propagation of FIP virus in the brain of one-day-old mice. This system was selected since coronaviruses of such diverse species as man, mouse, rat and chicken have been shown to grow in suckling mouse tissues (for review see 4).

Material and Methods

Virus

For mouse inoculation experiments, material from the third cat passage of the DAHLBERG strain (2) was used as a 10 % (w/v) homogenate of infectious liver tissue in phosphate buffered saline (PBS). The material was stored at − 70 °C.

Sera

For virus identification using the indirect immunofluorescence test (IFT), the following sera were employed:

— paired pre- and postinoculation sera of an experimental FIP case (DAHLBERG strain) from our laboratory (Utrecht).

— paired pre- and postinoculation sera from an SPF kitten, kindly provided by Dr. M. C. PEDERSEN, Davis. Cal.

— 20 field sera from randomly selected cats in The Netherlands; their antibody titers had been determined in heterologous indirect immunofluorescence tests published previously (6): 13 of them had high (anti-TGE-virus) titers and 7 were negative (< 10).

— 20 sera from SPF cats from a barrier-contained colony (Centraal Proefdierenbedrijf TNO, Zeist, The Netherlands) in which no antibodies to TGE virus had been found (6).

Animal inoculation experiments

Mice

Specified pathogen-free mice (strain CPB SE) were obtained from the breeding colony mentioned above (CPB Zeist) and kept in a laminar flow hood throughout the experiments. Litters containing 7 to 14 animals not older than 24 hours were infected by the intracerebral route, inoculating 5 μl/animal of a 10 % (w/v) cat liver suspension (starting material) or of a 40 % mouse brain homogenate (passage material), using a Hamilton syringe. A control passage series was done using a normal cat liver homogenate as starting material. The animals were examined daily for clinical signs. After 7 days the mice were decapitated and the brains removed and divided into two portions each, one serving for demonstrating viral antigen using the direct IFT, the other (after pooling and homogenizing) for further passaging.

Cats

A group of three kittens nine weeks of age was obtained as a gift from the aforementioned SPF cat breeding colony (courtesy of J. C. J. van Vliet, CPB, Zeist) which had been shown seronegative for TGE virus (6). The animals were housed together in a pressurized sterile stainless steel glove box; it formed part of an isolator unit belonging to an institute (Gezondheidsdienst voor Pluimvee, Doorn, The Netherlands) where cats had no access. Two of the animals were inoculated with passage material by injection of 1.0 ml.-quantities of a clarified (2.5 min at 10.000 $\times$ g.) 40 % (w/v) mouse brain homogenate via the intraperitoneal route. The third kitten served as a contact control. The animals were checked daily for rise in body temperature and overt clinical signs.

Immunofluorescence tests

From the ascitic fluid of an FIP-field case γ-globulin was prepared by repeated precipitation (3 times) with ammonium sulphate at 50 % saturation. The preparation was labelled with FITC using standard techniques (9). Cryostat sections from mouse brains and cat organs were acetone-fixed (10′ at − 20 °C), dried, washed with PBS and with distilled water and dried again. A working dilution of the conjugate in PBS was applied to the sections and the preparations were incubated for 30′ at 37 °C in a moist chamber. After three rinses in PBS and one in distilled water the slides were dried and mounted in Uvak (Searle, High Wycombe Bucks. Eng.). The specificity of the con-

jugate was demonstrated in a blocking test using TGE virus (strain Purdue) infected porcine thyroid cells; significant quenching of fluorescence was observed when the antigen preparations had been preincubated with unlabelled anti-FIP serum prior to the application of the FITC conjugated γ-globulin.

Indirect immunofluorescence tests were performed essentially as described previously (6), using infected porcine kidney cells as TGE antigen source and an FITC labelled rabbit anti cat γ-globulin as second antibody.

Results

Demonstration of virus multiplication in the brains of suckling mice

Three litters of one day old mice were inoculated intracerebrally with infectious cat liver material and one litter with normal cat liver material; passages were performed at weekly intervals as described above. No clinical signs were observed in the experimental animals during the short observation period. The results of direct IFT applied to brain sections at different passage levels are shown in Table 1. Using the labelled anti FIP γ-globulin, clearcut fluorescence was observed in one of the three FIP virus-infected mouse series (A), from the second passage onward; the two other FIP-series and the control series remained negative through four subsequent passages. To avoid any personal bias, coded samples were examined by one of the authors (R. M. S. W.) in a different institute. Predominantly cytoplasmic fluorescence was observed in neurons and glia cells (Fig. 1) in focal accumulations throughout the brain. Their number and size increased during the first positive passages.

In order to confirm the results, reisolation was attempted from the same cat liver material. Virus multiplication was detected in two of the four passage series (Table 1, E and F) from the third passage on.

Table 1
Passage history of FIP virus (strain Dahlberg) in suckling mouse brain

Passage number	1	2	3	4	5	6	7	8	9
Isolation									
A	–[1]	+[2]	+	+	10/10[3]	10/10	8/8	10/10	10/10
B	–	–	–	–					
C	–	–	–	–					
D (control)	–	–	–	–					
Reisolation									
E	–	–	4/10	10/10	10/10				
F	–	–	9/9	8/8	10/10				
G	–	–	–	–	–				
H	–	–	–	–	–				
I (control)	–	–	–	–	–				

1) no fluorescence observed
2) fluorescence observed in $\geqslant$ 1 brain section
3) numerator: number of IFT-positive mice; denominator: number of inoculated mice

Specificity criteria: serology

In order to determine the specificity of the observed fluorescence for FIP virus, indirect IFT were carried out in parallel on positive mouse brain sections (homologous reaction) and on porcine kidney cells infected with TGE virus (heterologous reaction). The preinoculation sera of experimentally FIP virus-infected cats (sera Utrecht and Davis) showed no fluorescence whereas

the postinoculation sera were unequivocally positive. Twenty sera from the Dutch open cat population (6) with varying titers in the heterologous reaction were assayed on mouse-brain sections; complete agreement was observed between the results of both tests: the 13 sera positive in the heterologous reaction

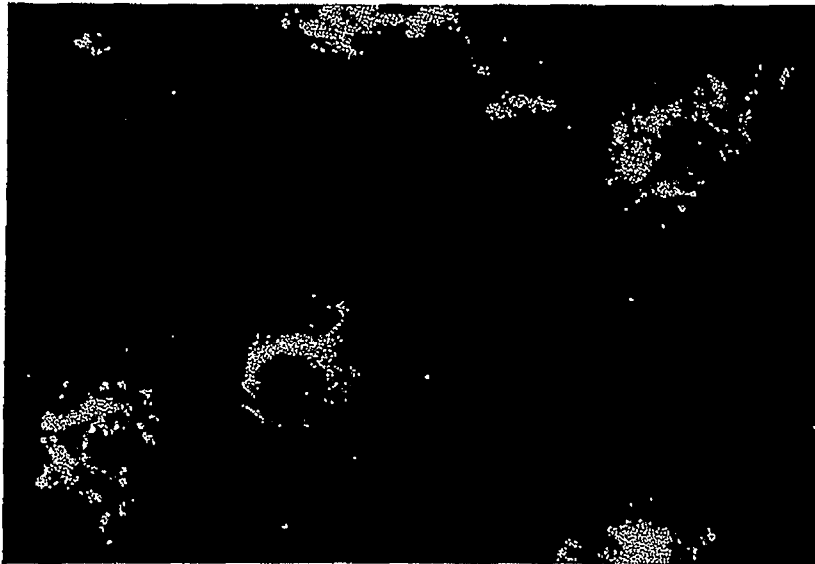

Fig. 1. Immunofluorescence in a positive mouse brain section (Table I A, passage 8) using the FITC labelled anti FIP γ-globulin preparation; note cytoplasmic fluorescence of single cells, shown at high magnification (objective 40 ×)

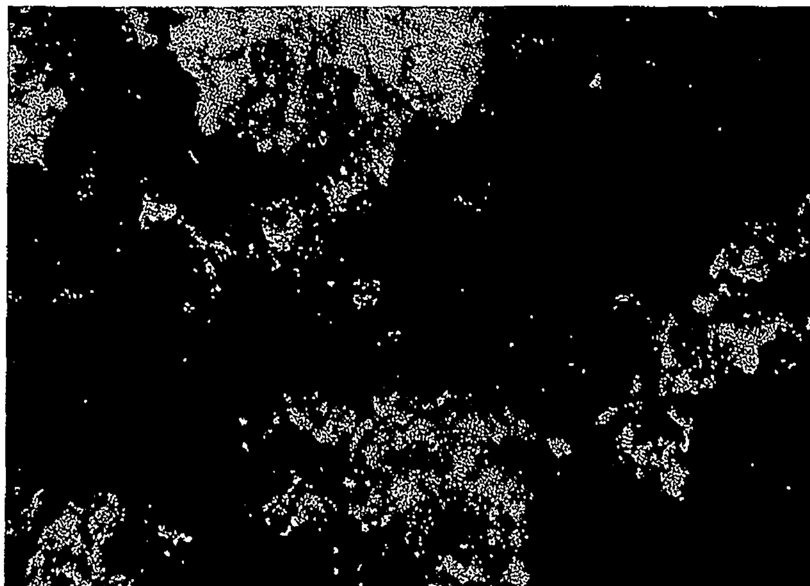

Fig. 2. Indirect immunofluorescence using a positive field serum; sections of the same material as in Fig. 1 were examined. Note foci of fluorescent cells shown at lower magnification. (objective 16 ×)

were also positive in the homologous reaction on infected mouse brain sections. Fluorescence using the indirect test was localized in foci throughout the brain (Fig. 2) as was the case with the direct method. None of the 20 sera from the SPF cat colony showed a reaction with FIP mouse brain sections, as they were also negative in the heterologous test (6).

Specificity criteria: cat inoculation experiments

The conclusive experiment for establishing the FIP virus specificity of the immunofluorescence in mouse brain was performed by inoculating SPF kittens with fluorescence-positive material of the 6th mouse passage (isolation series A, Table 1). Employing the heterologous indirect IFT, no antibodies reacting with TGE virus were found in the preinoculation sera of the animals. The temperature curves of two inoculated kittens and one control animal are given in Figure 3. One inoculated cat showed a distinct rise in body temperature on day 13 p. i. and gradually developed classical FIP symptoms: anorexia, depression, enlarged abdomen; death occurred on day 20 p. i. Upon post mortem examination the clinical diagnosis was confirmed. Granulomatous foci were present in the liver, spleen, lung, mediastinal lymph nodes, kidneys, bladder wall and the small intestine; all these tissues showed specific fluorescence.

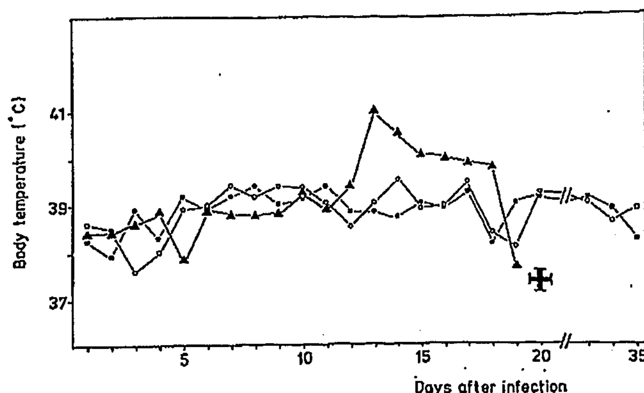

Fig. 3. Cat infection experiment using FIP virus infected mouse brain material (Table I A, pass. 6). Rectal temperature curves are given of three SPF animals kept in sterile glove boxes. Of the two infected cats, one animal (triangles) succumbed on day 20, the other (empty circles) survived > 35 days, as well as the contact cat (full circles)

Discussion

The experiments described in this paper have established that FIP virus multiplies in brain tissue of one-day-old laboratory mice. The presence in the material from several subsequent mouse passages of an antigen reacting with FIP antibodies of naturally and experimentally infected cats is one line of evidence; the reproduction of the clinical and anatomo-pathological picture of the feline disease by inoculation of brain material of the 6th mouse passage into the natural host constitutes the final proof. It must be emphasized that all experiments were done under conditions of careful isolation using SPF animals.

One reason for selecting the mouse as an experimental animal has been given in the Introduction: the multiplication of coronaviruses of several species in this host. Ecologic reasons supported this choice. As pointed out in an earlier

publication (5) the sporadic occurrence of clinical FIP could reflect virus spillover from a non-feline reservoir. Although serology has shown a rather high percentage of healthy cats to possess antibodies (indicating cat-to-cat-transmission of the virus), the mouse as predominant prey mammal could still play a role in FIP epidemiology. Since clinical symptoms have never been observed even in older FIP virus infected mice (Osterhaus and Horzinek, unpublished observations), they might be inapparent carriers; this possibility is being investigated.

The authors feel that their findings are significant in the virology of FIP. Although in vitro cell systems will certainly follow, important questions of virus characterization, demonstration of (cross-)neutralizing antibodies, virus attenuation etc. can be answered now, using an easily available small laboratory animal.

Summary

Feline infectious peritonitis (FIP) virus multiplication was demonstrated in the brains of one-day-old laboratory mice using direct immunofluorescence tests. Specificity was assessed by virus reisolation, indirect immunofluorescence and reproduction of FIP after inoculation of SPF kittens using brain material from the 6th mouse passage.

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Zusammenfassung

Virus der felinen infektiösen Peritonitis

II. Vermehrung im Gehirn von Säuglingsmäusen

Mit Hilfe der direkten Immunofluoreszenz wurde die Vermehrung des Virus der Felinen Infektiösen Peritonitis (FIP) im Gehirn eintägiger Laboratoriumsmäuse nachgewiesen. Die Spezifität wurde durch Reisolierung des Virus und indirekte Immunofluoreszenz belegt, sowie durch die Auslösung der FIP nach Inokulation von Gehirnmaterial der sechsten Mäusepassage in SPF Katzenwelpen.

Résumé

Virus de la péritonite infectieuse du chat

II. Multiplication dans le cerveau de la souris nouveau-née

A l'aide de l'immunofluorescence directe la multiplication du virus de la péritonite infectieuze féline a été démontrée dans le cerveau de la souris nouveau-née. Epreuves de spécificité étaient le réisolement du virus, l'immunofluorescence indirecte et la reproduction de la maladie par inoculation de chats SPF utilisant du material cerveau provenant du 6ième passage en souris.

Resumen

Virus de la peritonitis infecciosa del gato
II. Multiplicación en cerebro de ratoncitos recién-nacidos

Utilizando la inmunofluorescencia directa se mostró la multiplicación en cerebro de ratoncitos recién-nacidos del virus de la peritonitis infecciosa del gato. Se pudo evidenciar la especificidad por medio de re-aislamiento del virus y de la inmunofluorescencia indirecta; además, se logró reproducir la enfermedad en gatos SPF, inoculándoles material del 6° pasaje en cerebros de ratoncitos.

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