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Replication of feline coronaviruses in peripheral blood monocytes

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Summary. Feline infectious peritonitis virus (FIPV) (*Coronaviridae*) causes the most lethal viral infection in cats: FIP. The related feline enteric coronavirus (FECV) causes mild enteritis. Why these feline coronaviruses manifest so differently *in vivo* is not known. In this study, infection kinetics (titres and antigen expression) of FIPV 79-1146, and FECV 79-1683, were determined in peripheral blood monocytes from 3 donor cats and compared to those in Crandell feline kidney (CrFK) cells. The infection kinetics in monocytes were host dependent. Monocytes from 1 cat were resistant to both FIPV- and FECV-infection. Monocytes from the other 2 cats could initially be infected by both FIPV and FECV but FIPV infection was sustained in monocytes of only one cat. FECV-infection was never sustained and viral production was up to 100 times lower than in FIPV-infected monocytes. In CrFK cells, FIPV and FECV infection kinetics did not differ. In monocytes of a larger cat population ($n = 19$) the 3 infection patterns were also found. Considering all 22 investigated cats, 3/22 were not susceptible for FIPV and FECV. The rest could be infected with FECV and FIPV but 10/22 cats had monocytes that only sustained FIPV infection and 9/22 sustained neither FIPV nor FECV infection.

Introduction

Two coronaviruses are described in cats: feline infectious peritonitis virus (FIPV) and feline enteric coronavirus (FECV). These feline coronaviruses are spread world-wide and infect cats and other members of the family *Felidae*. An infection with FECV is usually subclinical, except in young kittens where it may cause mild to severe diarrhoea [23]. In contrast, FIPV infection causes a chronic and

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very often fatal pleuritis/peritonitis. It is the most important cause of death of infectious origin in cats. Two forms of FIP exist: the effusive or wet form with the typical effusions in body cavities and the less common non-effusive or dry form [232]. Characteristic lesions of both forms are granulomas on the surface of target tissues. Despite the large biological differences, more than 98% of the genome is identical in FIPV and FECV isolates from the same environment [33]. Therefore, it has been proposed that FIPV arises from FECV by mutation but the exact mutation and the inducing factors have not yet been clarified [24, 34].

The main difference between FECV and FIPV is the invasive nature of FIPV. FECV replicates mainly locally, in enterocytes of the intestine, whereas FIPV also infects blood monocytes and spreads systemically [37, 38]. The reason for this pathogenic difference is not understood. After infiltration of infected monocytes in the perivascular tissue, the infected monocytes and surrounding cells release numerous chemotactic and vasoactive factors [8, 9, 39]. This leads to vasodilatation and increased vascular permeability and attraction of new monocytes to the area, which can be infected in turn. The outcome of the inflammatory reaction is a characteristic vasculitis which causes the venules to leak large amounts of protein rich plasma into the body cavity. The release of progeny virus also leads to the formation of virus-antibody-complement complexes which are concentrated around the small venules in the target organs [15]. These complexes further activate inflammation.

Although the difference between FIPV and FECV is very clear *in vivo*, it is not *in vitro*. The first *in vitro* characterisation of FIPV strain 79-1146 and FECV strain 79-1683 was done by McKeirnan et al. [18] in Crandell feline kidney (CrFK) cells. They found similar growth curves for FIPV and FECV. The replication of FIPV and FECV was also studied in peritoneal macrophages [29]. It was reported that FECV infected fewer macrophages and reached lower production titres than FIPV. The *in vivo* relevance of these infection studies is most likely higher than those performed in a continuous cell line. But, until now, the FIPV and FECV replication cycles have never been studied in the *in vivo* target/carrier cell of FIPV: the feline blood monocyte.

In the present study, we present the *in vitro* replication kinetics of FIPV and FECV in the target cell of FIPV, the blood monocyte. It was found that the replication kinetics were dependent on the origin of the cells. No differences between FIPV and FECV were found in CrFK cells.

Materials and methods

Viruses

A third passage of FIPV strain 79-1146 and FECV strain 79-1683 on CrFK cells was used [17]. FECV strain 79-1683 was obtained from the American Type Culture Collection (ATCC) and FIPV strain 79-1146 was kindly provided by Dr. Egberink (Utrecht University, the Netherlands).

Antibodies

Polyclonal antibodies originating from cats infected with FIPV 79-1146 were kindly provided by Dr. Egberink (Utrecht University, the Netherlands). These antibodies were purified and biotinylated according to manufacturer's instructions (Amersham Bioscience, Buckinghamshire, UK). The monoclonal antibodies (mAb) 7-4-1, F19-1, E22-2, recognising respectively the S-, M- and N-protein, were kindly provided by Dr. Hohdatsu (Kitasato University, Japan). A monocyte marker, DH59B, recognising CD 172a was purchased from Veterinary Medical Research and Development (Pullman, Washington, USA).

Cats

Three cats of a non-specific breed from a FCoV free closed household were used as blood donors for the extensive infection kinetics study. Seventeen stray cats brought to the clinic of small animals in the Faculty of Veterinary Medicine (Ghent University) and 2 SPF cats were used for a study on the distribution of the infection kinetics patterns. The sex and FeLV, FIV and FCoV status of the cats are listed in Table 1.

Isolation of blood monocytes

Six ml blood was collected on heparin (15 U/ml) (Leo, Zaventem, Belgium) from the *vena jugularis* and blood mononuclear cells were separated on Ficoll-Paque (Pharmacia Biotech AB, Uppsala, Sweden) following manufacturer's instructions. Mononuclear cells were resuspended in RPMI-1640 (Gibco BRL, Merelbeke, Belgium) medium containing 10% fetal bovine serum (FBS), 0.3 mg/ml glutamine, 100 U/ml penicillin, 0.1 mg/ml streptomycin, 0.1 mg/ml kanamycin, 10 U/ml heparin, 1 mM sodium pyruvate, and 1% non-essential amino-acids 100× (Gibco BRL). Afterwards, cells were seeded in a 24-well dish with cell culture coating (Nunc A/S, Roskilde, Denmark) at a concentration of 2×10^6 cells/ml and cultivated at 37 °C with 5% CO_2. Non-adherent cells were removed by washing the dishes two times with RPMI-1640 at 2 and 24 h after seeding. The adherent cells consisted for $86 \pm 7\%$ of monocytes (as assessed by fluorescent staining with the monocyte marker DH59B).

Inoculation of CrFK cells and monocytes

CrFK cells and monocytes were inoculated with FIPV strain 79-1146 or FECV strain 79-1683 at a multiplicity of infection (m.o.i.) of 5. After 1 h incubation at 37 °C with 5% CO_2, cells were washed 3 times with RPMI-1640 and further incubated in medium.

Growth curves of FCoV

At different time points post inoculation, culture medium was harvested and centrifuged at $400 \times g$ for 10 min. The supernatants were used for determination of extracellular virus titres. The cells were removed from the well by scraping and added to the pellet for determination of intracellular virus titre. Virus was released from the cells by 2 freeze-thaw cycles. The samples were stored at −70 °C until titration. Both intra- and extracellular virus titres were assessed by a 50% tissue culture infective dose assay using CrFK cells. The fifty percent end-point was calculated according to the method of Reed and Muench [25]. A virus inactivation curve was determined by keeping cell free virus in medium at 37 °C with 5% CO_2. Samples were taken at different time points and stored at −70 °C until titration.

Table 1. Sex and FeLV, FIV and FCoV status of the cats

Cat no.	Sex[a]	FeLV antigen[b]	FIV antibody[b]	FCoV titer[c]
Closed household				
1	M	−	−	<20
2	M	−	−	<20
3	M	−	−	<20
Population of stray cat				
1	F	−	−	<20
2	F	−	−	<20
3	F	+	−	<20
4	F	−	+	<20
5	F	−	+	<20
6	M	−	−	<20
7	M	+	−	<20
8	F	+	−	<20
9	F	+	−	<20
10	F	−	−	80
11	M	−	−	<20
12	M	−	−	<20
13	F	−	−	<20
14	F	−	−	<20
15	F	−	−	<20
16	M	−	−	<20
17	F	−	−	<20
SPF cats				
1	F	−	−	<20
2	F	−	−	<20

[a]M: male, F: female

[b]Tested on plasma samples with SNAP®FIV Antibody/FeLV Antigen Combo Test (IDEXX)

[c]IPMA antibody titer

Three independent assays were carried out and the inactivation curve was calculated by linear regression.

Visualisation of viral antigens in FCoV infected cells

At different time points post inoculation, cells seeded on glass coverslips, were fixed with 1% formaldehyde. Surface-expressed viral proteins were labelled with biotinylated anti-FIPV polyclonal cat antibodies and streptavidin-FITC (Molecular Probes, Eugene, Oregon, USA). After permeabilisation with 0.1% Triton X-100 (Sigma-Aldrich GmbH, Steinheim, Germany), cytoplasmic viral proteins were stained with a mixture of monoclonal antibodies (7-4-1, F19-1 and E22-2) and with goat anti-mouse-Texas Red (Molecular Probes). Finally, the glass coverslips were mounted on microscope slides using glycerin-PBS solution (0.9:0.1, vol/vol) with 2.5% 1,4-diazabicyclo(2,2,2)octane (Janssen Chimica, Beerse, Belgium) and analysed

by fluorescence microscopy. For the stray cats and SPF cats, only cytoplasmic viral proteins were stained with FITC labelled anti-FIPV antibodies (VMRD Inc, Pullman, Washington, USA).

Confocal laser scanning microscopy

The samples were stained to visualise the cytoplasmic and the surface-expressed viral proteins as described above and examined with a Leica TCS SP2 laser scanning spectral confocal system (Leica Microsystems GmbH, Wetzlar, Germany) linked to a DM IRB inverted microscope (Leica Microsystems). Argon and Helium/Neon laser lights were used to excite FITC (488 nm line) and Texas-Red (543 nm line) fluorochromes. The images were obtained and processed with Leica confocal software.

Statistical analysis

All experiments were repeated 2 or more times. The "area under the curve" was calculated for each experiment. Triplicate assays were compared using a Mann-Withney U test. Statistical analysis were performed with SPSS 11.0 (SPSS Inc. Chicago, Illinois, USA).

Results

Growth curves of feline coronaviruses in CrFK cells

The growth curves of FIPV and FECV in CrFK cells are given in Fig. 1. Production of progeny virus started between 3 and 6 hpi and increased strongly until 12 hpi. Between 12 and 24 hpi there was only a slight increase of virus titres to reach a maximum of 6.8 $\log_{10}$TCID$_{50}$/10^{6}cells at 24 hpi. There was no significant difference between the growth curves of FIPV and FECV.

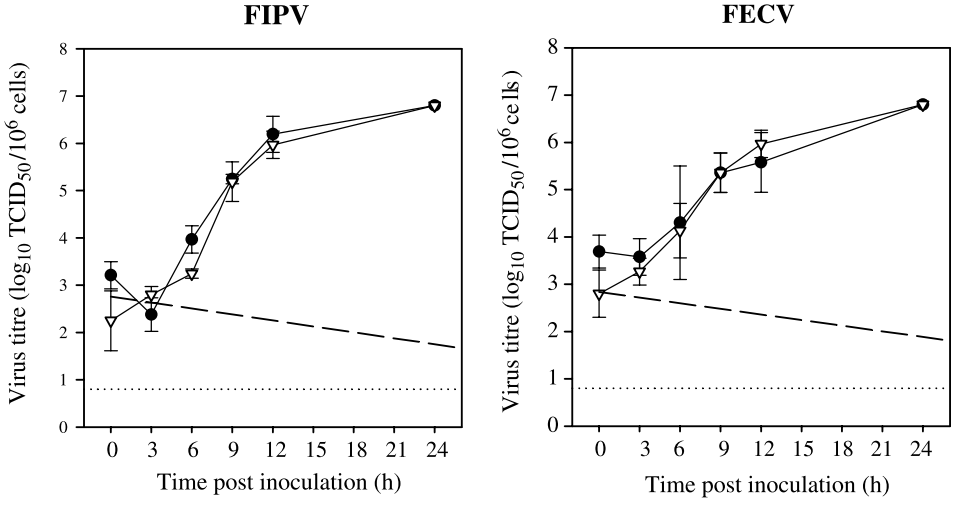


Fig. 1. Kinetics of FCoV replication in CrFK cells. The cells were inoculated with FIPV 79-1146 or FECV 79-1683 at a m.o.i. = 5. At designated time points post inoculation, the intracellular (●) and extracellular (▽) virus titres were determined. The dashed line represents the inactivation curve and the dotted line is the detection limit. The data represent means ± SD of triplicate assays

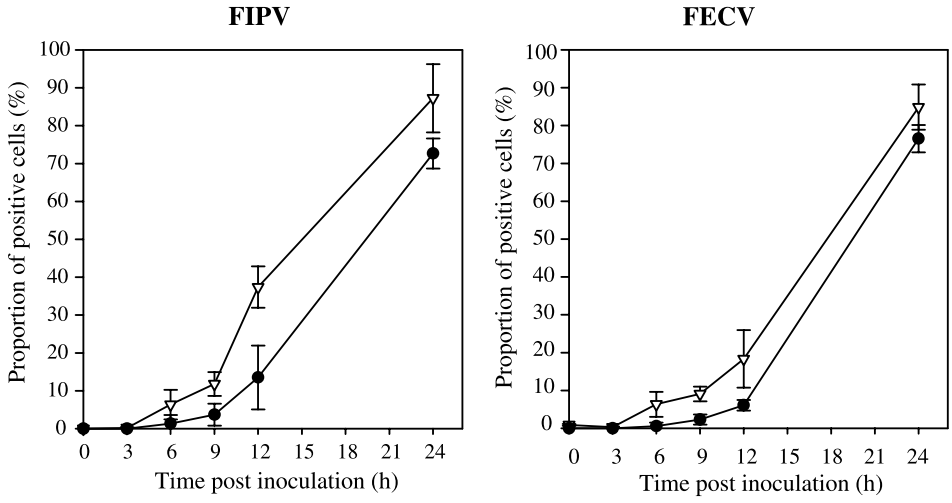


Fig. 2. Kinetics of expression of viral antigens in FCoV infected CrFK cells. Cells were inoculated with FIPV 79-1146 or FECV 79-1683 at a m.o.i. = 5. At designated time points post inoculation the cells were fixed and cytoplasmic (▽) and surface-expressed (●) viral proteins were visualised with an immunofluorescence staining. The data represent means ± SD of triplicate assays

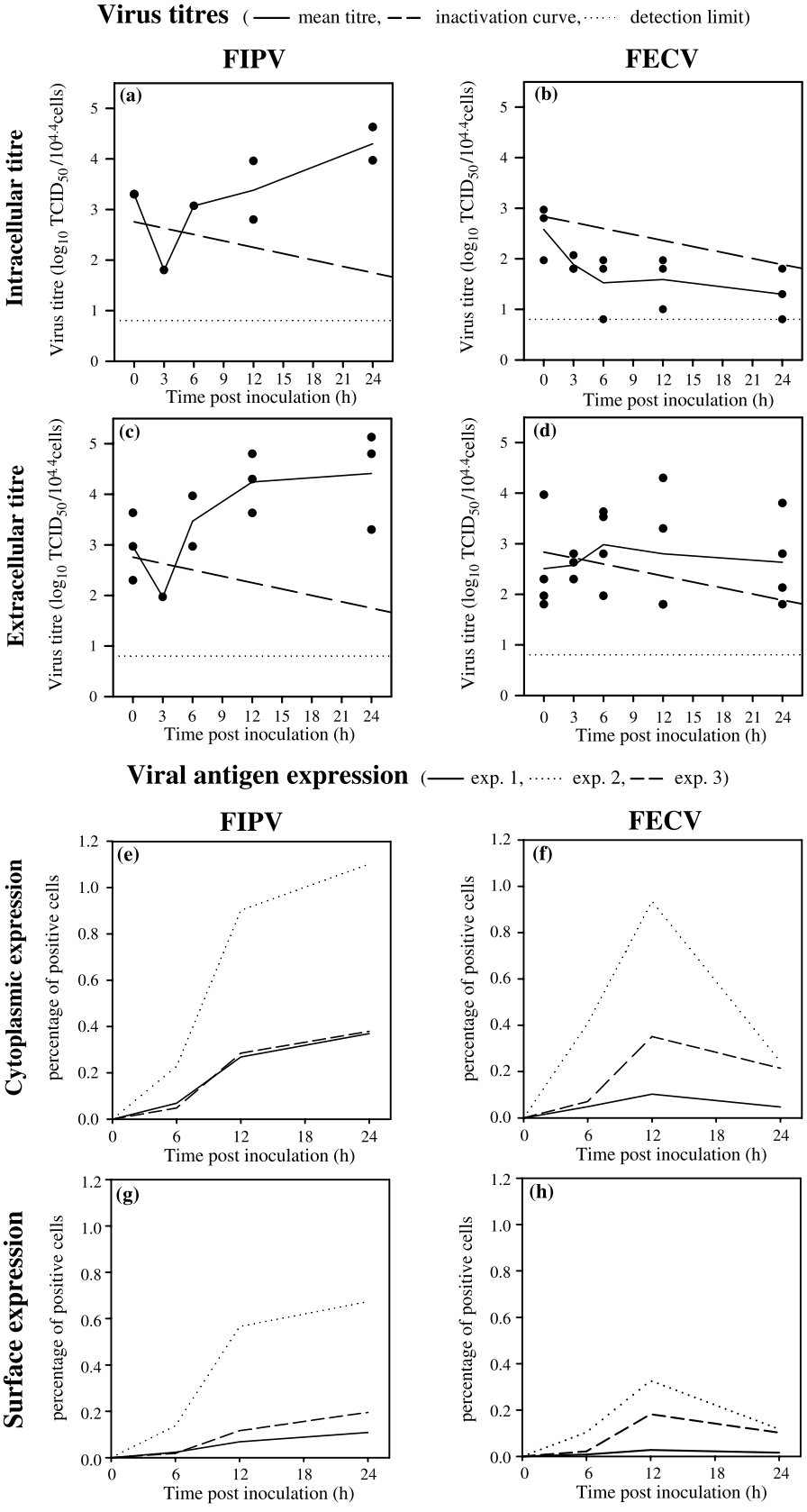
Expression kinetics of cytoplasmic and surface-expressed viral antigens in feline coronavirus-infected CrFK cells

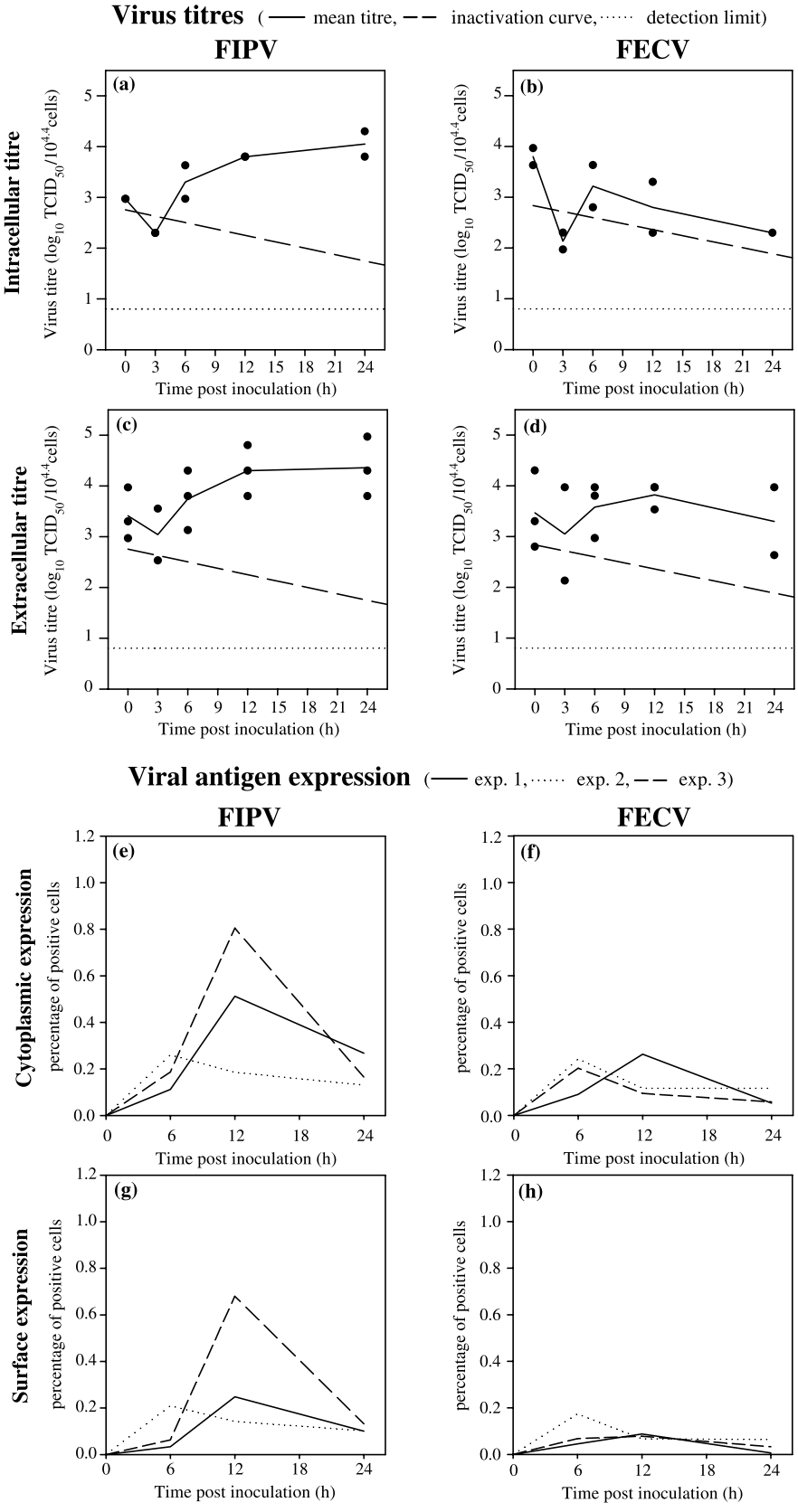
Figure 2 shows that the first viral antigen positive cells appeared between 3 and 6 hours post inoculation. Between 12 and 24 hpi, there was a vast increase of infected cells. At 24 hpi, 86% of the cells showed cytoplasmic expression of viral proteins and 75% surface expression. There is no significant difference (area under the curve) between the FIPV curve and the FECV curve. The amount of infectious virus produced per cell can, theoretically, be calculated from the virus titres and the percentage of infected cells. For both FIPV- and FECV-infected CrFK cells, productivity was less than 10 infectious viruses per infected cell.

Growth curves of feline coronaviruses in monocytes

The growth curves of FIPV and FECV in monocytes varied between the different donor cats. Figures 3a and 4a show that the production of FIPV started between 3 and 6 h post inoculation for both cat 1 and 2. Between 12 and 24 h post inoculation there was a slight increase in virus titre for cat 1 whereas the curve from cat 2

Fig. 3. Kinetics of FCoV replication in blood monocytes from cat 1. The monocytes were inoculated with FIPV 79-1146 or FECV 79-1683 at a m.o.i. = 5. At designated time points post inoculation, the intra- and extracellular virus titres were determined and the viral protein expression was visualised


Virus titres (mean titre, inactivation curve, detection limit)
FIPV
FECV
Intracellular titre
Extracellular titre
(a)
(b)
(c)
(d)
Virus titre (log10 TCID50/10^4.4cells)
Time post inoculation (h)
Viral antigen expression (exp. 1, exp. 2, exp. 3)
Cytoplasmic expression
Surface expression
(e)
(f)
(g)
(h)
percentage of positive cells


Virus titres (mean titre, inactivation curve, detection limit)
FIPV
FECV
Intracellular titre
Extracellular titre
(a)
(b)
(c)
(d)
Virus titre (log10 TCID50/10^4.4cells)
Time post inoculation (h)
Viral antigen expression (exp. 1, exp. 2, exp. 3)
Cytoplasmic expression
Surface expression
(e)
(f)
(g)
(h)
percentage of positive cells

reached a plateau at 12 h post inoculation. The virus release curves were similar (Figs. 3c and 4c). The growth curves of cat 1 for FECV showed a low-level production (Fig. 3b and d). The growth curves of cat 2 for FECV began with a slight titre increase, similar to the FIPV growth curve, but then the virus titre decreased with a slope comparable to the inactivation curve (Fig. 4b and d). These findings suggest that monocytes could be infected by FECV but that the cells did not sustain a productive infection. Figure 5 shows that the growth curves for cat 3 followed the inactivation curve, suggesting that there was no progeny virus produced.

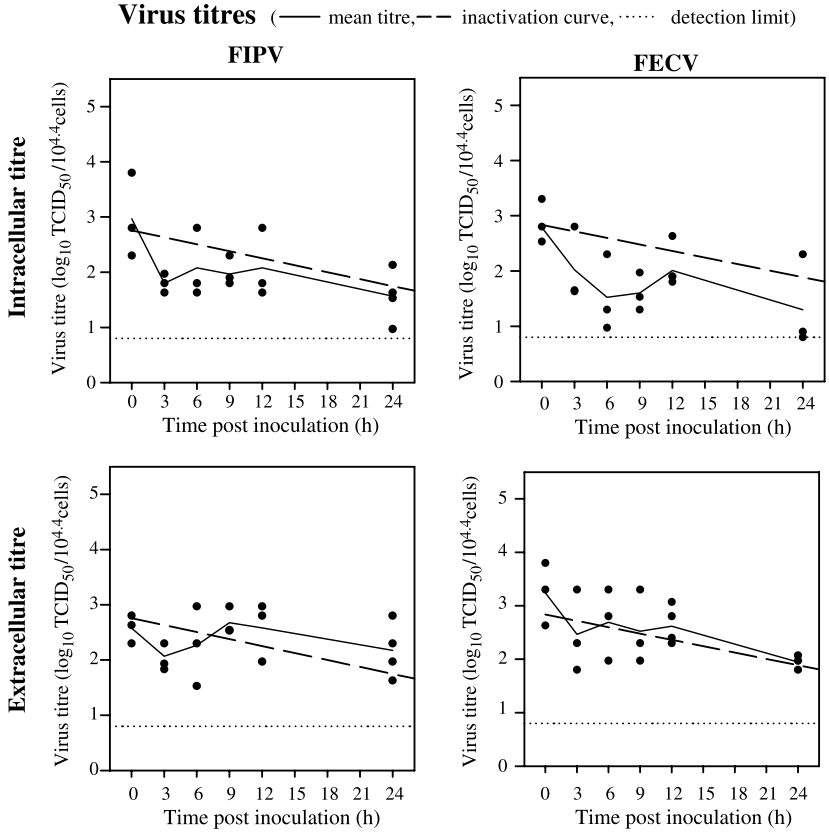


Fig. 5. Kinetics of FCoV replication in blood monocytes from cat 3. The monocytes were inoculated with FIPV 79-1146 or FECV 79-1683 at a m.o.i. = 5. At designated time points post inoculation, the intra- and extracellular virus titres were determined

Fig. 4. Kinetics of FCoV replication in blood monocytes from cat 2. The monocytes were inoculated with FIPV 79-1146 or FECV 79-1683 at a m.o.i. = 5. At designated time points post inoculation, the intra- and extracellular virus titres were determined and the viral protein expression was visualised

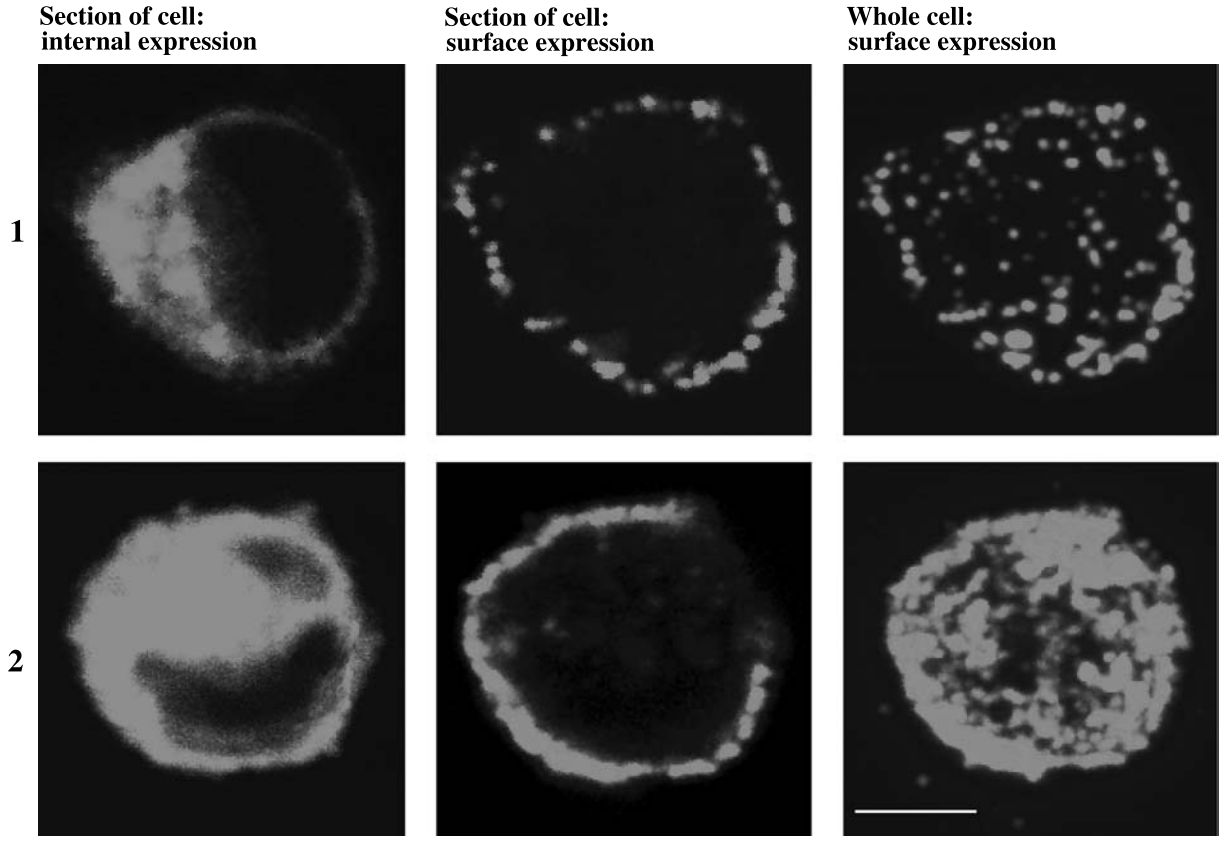


Fig. 6. Cytoplasmic expression (left) and surface expression (right) of viral proteins in a monocyte infected with FIPV 79-1146, visualised by confocal microscopy. Bar = 5μm

Expression kinetics of cytoplasmic and surface-expressed viral antigens in FCoV-infected monocytes

Figure 6 shows confocal images of cytoplasmic and surface-expressed viral antigens in monocytes infected with FIPV 79-1146. Surface expression was only detected in an average of 49% of the infected monocytes (24 hpi). No differences in the amount of infected cells with surface expression were seen between the cats or between FIPV and FECV infection. Depending on the cell, the amount of viral antigens expressed on the surface varied. The majority of the infected monocytes showed a small amount of surface-expressed viral proteins (Fig. 6, lane 1). Some showed a larger amount of surface-expressed viral proteins (Fig. 6, lane 2).

The antigen expression kinetics varied between the donor cats. Figure 3e and f show the FIPV and FECV cytoplasmic expression kinetics for cat 1. The percentage of FIPV infected cells with cytoplasmic expression increased till 24 hpi. The infection of monocytes with FECV initiated in the same manner but at 12 hpi the curve started to decline. The cytoplasmic expression in monocytes of cat 2 is shown in Fig. 4e and f. Infection with FIPV or FECV led to the same expression kinetics. After an increase till 6 or 12 h post inoculation the percentage

of cells with viral expression decreased rapidly. The number of FECV infected monocytes was lower than the FIPV-infected monocytes. The FIPV and FECV surface expression, for both cat 1 and 2, followed the same curve as the cytoplasmic

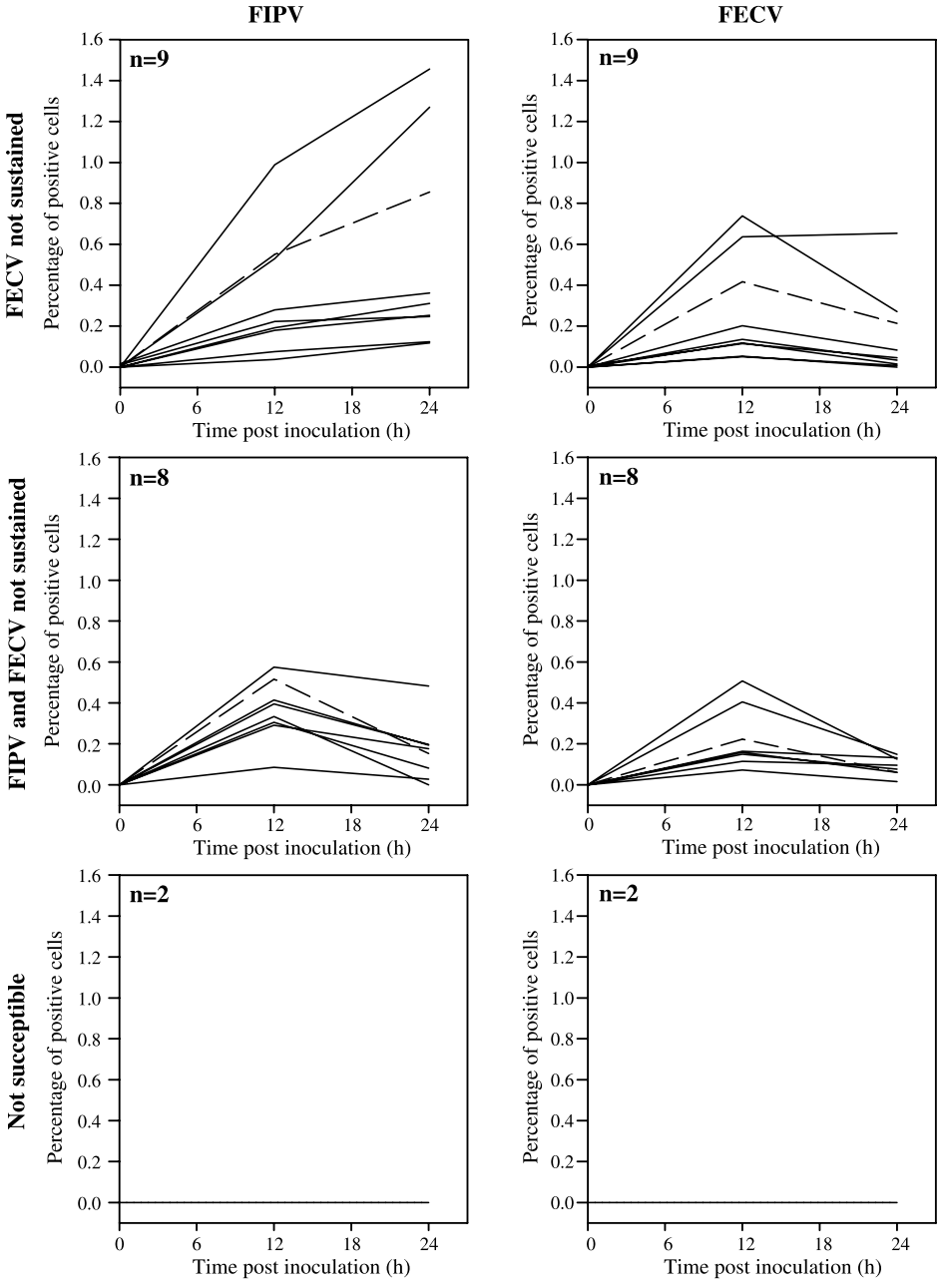


Fig. 7. Kinetics of FCoV replication in blood monocytes from 17 stray cats (solid line) and 2 SPF cats (dashed line). The monocytes were inoculated with FIPV 79-1146 or FECV 79-1683 at a m.o.i. = 5. At designated time points post inoculation, the cytoplasmic viral protein expression was visualised. Each curve represents the FIPV and FECV infection kinetics from 1 cat

expression but at a lower percentage (Fig. 3g and h; Fig. 4g and h). The results of cat 3 were quite different from cat 1 and 2. Here, viral antigen positive monocytes were not found.

Knowing the total production of infectious progeny virus and the number of infected cells, it can be calculated that FIPV-infected monocytes from both cat 1 and 2 have produced approximately 200 infectious viruses per infected cell at 12 h post inoculation. FECV-infected monocytes from cat 1 produced 10 times less progeny virus at 12 h post inoculation whereas the FECV-infected monocytes from cat 2 produced the same amount of progeny virus as the FIP-infected monocytes.

Infection kinetics in a larger population of cats

In order to clarify the prevalence of the patterns of viral replication observed in this study in a bigger cat population, the antigen expression kinetics were studied in 17 stray cats and 2 SPF cats for both FIPV and FECV. The antigen expression was visualised at 0, 12 and 24 hours post inoculation. The results are presented in Fig. 7. The 3 different expression kinetics that were found in monocytes from the closed household cats were also seen in monocytes from the stray cats and the SPF cats. Within this population of 19 cats, the monocytes isolated from 9 cats showed a continuous increase in viral antigen positive cells during a 24 hour time span after inoculation with FIPV. When these monocytes were inoculated with FECV, the number of viral antigen positive cells increased until 12 hours post inoculation and then diminished. In monocytes from 8 cats, the percentages of both FIPV- and FECV-infected cells increased until 12 hpi and then decreased. The monocytes from 2 cats were resistant to infection.

Discussion

In this study, *in vitro* infection kinetics of FIPV (strain 79-1146) and FECV (strain 79-1683) were established in peripheral blood monocytes from 22 cats (3 cats of a closed household, 17 stray cats and 2 SPF cats). It is the first time that infection studies were performed in peripheral blood monocytes, the host/carrier cell of FIPV. Three distinct patterns were found in the infection studies.

Monocytes from 3 cats were not infected by either strain (first pattern). The reason for the insusceptibility of these cells is not yet clear. Virus particles were detected in the cells shortly after inoculation of the cells but no production of viral antigens was observed using polyclonal antibodies (data not shown). Thus, it seems that new viral proteins were not formed. This suggests that the block of infection is located after entry of the virus but before (or at) the translation step. *In vivo*, resistance to FIPV infection has been observed in experimental inoculations. After inoculation with a lethal dose of FIPV, a varying part of the cats (depending on experiment 8–50%) showed no clinical signs and some of them remained seronegative [24, 36]. This was also seen in control groups of vaccination trials (no vaccination, only FIPV challenged) [16, 27]. Resistance to FCoV infection has also been suggested to occur in natural infections in the field

[1]. A small percentage of cats in FCoV endemic households had no shedding, remained seronegative or had a low antibody titre over a time period of 5 years. It would be most interesting to investigate the correlation between *in vitro* and *in vivo* resistance to FCoV. This might give perspectives for selection of cats insusceptible for FIP.

Monocytes from 10 cats showed an increase of FIPV antigen positive cells till 24 hpi whereas the amount of FECV antigen positive cells dropped after 12 hpi. This shows that the FIPV infection was sustained whereas the FECV infection was not sustained (second pattern). Monocytes from 9 cats did not sustain both FIPV and FECV infection since the number of viral antigen positive cells dropped after 6 or 12 hpi (third pattern). The drop in antigen positive cells after 6 or 12 hours post inoculation may be explained by the fact that the infected cells died due to infection and were washed away during the staining. However, the same kinetics were found with staining in suspension, a technique which prevents cell loss (data not shown). Another explanation is that monocytes stopped producing viral proteins and assembling new virions. The extracellular virus titres showed indeed that (almost) no new progeny virus was produced between 12 and 24 hours post inoculation. Some graphs show differences in virus titres between 2 experiments (with the same virus and with monocytes from the same donor cat) of up to 2 $\log_{10}$units. These differences are intrinsic to working with primary cells and are reported in viral infection studies with porcine and equine monocytes as well [21, 30].

Although FECV initially infects monocytes, the infection is never sustained. This implicates that FECV might reach the blood circulation *in vivo*. In several studies, healthy cats from FCoV endemic households were investigated [5, 10, 13, 14, 19, 28]. In such households, where the FCoV was most likely FECV, a part of these healthy cats were viraemic for FCoV. FCoV was detected both in plasma and in monocytes. Therefore, it may be hypothesised that when FECV reaches the blood circulation, the lack of sustainability and long-term production of progeny virus (the total virus production was up to 100 times lower in FECV-infected monocytes) may be the reason for the lack of disease progress. This might form the basis for the difference with FIPV since FIPV infection is sustained and reaches higher titres. However, the non-sustained FECV infection, might also be attributed to the virus strain that was used. Although FECV 79-1683 is a reference strain, it may act differently from other FECV strains due to its deletion in the 7b ORF [35]. It has been described that loss of the 7ab ORFs results in loss in virulence [11]. It could be that this loss in virulence is translated in loss of the ability to replicate efficiently in monocytes. Thus, whether the hampered replication of strain 79-1683 in monocytes is a universal property of FECV strains or only of 7b deleted/mutated strains, remains to be determined.

The different FIPV infection kinetics depending on the cat from which the monocytes were isolated suggests that cellular factors, influenced by genetic background and/or differentiation/activation status, are very important in determining the outcome of a FIPV infection. In an infection kinetics study where another cell type, feline peritoneal macrophages, was used, different results in the

antigen expression kinetics were obtained [29]. The number of FECV infected peritoneal macrophages was lower than the number of FIPV infected peritoneal macrophages throughout the infection kinetics. Since the viral antigen kinetics was only performed till 14 hours post inoculation, a possible drop in antigen expression, like reported here, could not be evaluated. In contrast, our results suggest that FIPV and FECV can initially infect the same amount of cells but at 24 h post inoculation, differences in sustainability of the infection are prominent. Since the same viruses were used as in our study, the different results are most probably due to cellular factors and/or a different differentiation status of the cells. Differences in susceptibility depending on the differentiation and/or activation status of the monocytes/macrophages has been reported for different viruses such as porcine reproductive and respiratory syndrome virus, caprine arthritis-encephalitis virus, suid herpes virus 1, herpes simplex virus, human immunodeficiency virus type 1 and maedi-visna virus [4, 20, 40]. The differences in activation status might explain the discrepancy between our results and those of Stoddart and Scott [29].

What this variation in susceptibility and sustainability means for the pathogenesis of FECV and FIPV *in vivo*, remains to be elucidated. In an inoculation study using FIPV 79-1146, different patterns of disease progression were detected, based upon survival time: progressors (rapid, intermediate and delayed) and survivors (prolonged and long-term) [3]. With natural *in vivo* FCoV infection, different clinical outcomes (besides resistance to FCoV) have been described: persistent carrier, transiently infection and development of FIP [2]. It is not clear what the viral and host factors are that determine the different clinical outcomes. Since in the inoculation study the same strain (FIPV-79-1146) was used and considering the fact that in the field cats are often infected with the same strain of FCoV, it is likely that genomic variation between cats contributes to a different clinical outcome. A genetic background was also suggested during a field study with pure-bred cats, in which it was shown that susceptibility to FIP is indeed inheritable [7]. A possible explanation for the different disease progression is the possibility of the cats to develop an efficient T-cell response [3]. However, it could also be that the susceptibility of the monocytes to FIPV plays a role, considering the results presented here. It would be interesting to investigate if cats that show a different outcome to an experimental or natural infection also show different infection kinetics *in vitro*. This might be important since a correlation between *in vitro* and *in vivo* infection kinetics would allow easy screening and selection.

In this study, it was shown that viral proteins can be expressed on the surface of FCoV infected cells. However, only a part of the infected cells showed surface-expressed viral antigens. On 24 hpi, 87% of the infected CrFK cells and 49% of the infected monocytes showed surface-expressed viral antigens. S- and M-proteins, but no N-proteins were found on the cell surface of both CrFK cells and monocytes using specific monoclonal antibodies (data not shown). This indicates that the observed surface expression does not represent virus particles. Possible explanations for the observed differences in amount of surface-expressed viral

antigens could be the retention of a part of the viral proteins or spontaneous internalisation of the surface-expressed viral antigens. Retention of viral proteins has been described for porcine coronavirus [26]. Spontaneous internalisation of viral proteins has been described for suid herpes virus 1 [32].

The presence of viral antigens on the cell surface can be of importance for the recognition and elimination of infected cells by the immune system. Binding of virus-specific antibodies to viral proteins present on the surface, makes infected cells recognisable for the classical complement pathway, phagocytes and natural killer cells, which will lead to lysis of the infected cell [12]. Interestingly, not all FIPV- and FECV-infected monocytes/macrophages showed surface expression. Absence of viral proteins on the cell surface has been described for other viruses, such as human cytomegalovirus and equine herpesvirus 1 as a strategy to avoid recognition by the antibody-dependent immune responses [6, 31]. Why only half of the infected cells showed surface expression and whether the cells without surface expression are indeed less susceptible towards antibody-dependent complement mediated lysis, remains to be elucidated.

In FIP research, the CrFK cell line is often used to perform *in vitro* experiments. The results of this study reveal that the CrFK cell line is not the best suitable *in vitro* model for the study of FIPV and FECV replication at a cellular level. Firstly, the course of infection of FIPV and FECV is similar in CrFK cells, whereas in monocytes there is a clear difference (as there is *in vivo*). Secondly, a high percentage of infected cells can be reached in CrFK cells (up to 90% of the inoculated cells) with each cell producing and releasing a relatively small amount of infectious virus (<10 viruses/cell). In monocytes on the other hand, less than 1% of the cells can be infected, but a single FIPV-infected monocyte releases up to 200 new infectious viruses. Thirdly, CrFK cells showed surface expression in almost all infected cells, in contrast to monocytes, which showed surface expression in only half of the infected cells.

In conclusion, it can be stated that FCoV infection kinetics *in vitro* are strongly dependent on cellular factors. Monocytes from some cats cannot be infected. If monocytes are susceptible to FCoV infection, then both FIPV and FECV can infect them. However, FECV infections are never sustained and production of viral antigens and progeny virus ceases at 24 h post inoculation. Sustainability of a FIPV infection depends on the origin of the host cells. FIPV production in susceptible monocytes was always 10 to 100 times higher than FECV production. What this variation in susceptibility and sustainability implicates for the development and pathogenesis of FIP and/or FECV *in vivo*, remains to be elucidated.

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