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Disease outcome and cytokine responses in cats immunized with an avirulent feline infectious peritonitis virus (FIPV)-UCD1 and challenge-exposed with virulent FIPV-UCD8

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Summary Eight cats were immunized with an avirulent strain of feline infectious peritonitis virus (FIPV)-UCD1, then challenge-exposed to a highly virulent cat passaged strain (FIPV-UCD8). Th1 and Th2 cytokine profiles in the peripheral blood mononuclear cells (PBMCs) were measured throughout in the experiment. No clinical signs of FIP were evident in the experimental cats after immunization. After challenge, the immunized cats demonstrated one of four clinical outcomes: (1) classical effusive FIP; (2) accelerated FIP; (3) non-effusive FIP, or (4) resistance to challenge. Only minor cytokine changes were observed following immunization, however, several cytokine changes occurred following challenge-exposure. The most noteworthy changes were in tumor necrosis factor-alpha (TNF-α) and interferon gamma (IFN-γ) levels. Our preliminary findings suggest that immunity against FIP is associated with TNF-α and IFN-γ response imbalance, with high TNF-α/low IFN-γ mRNA responses favouring disease and low TNF-α/high IFN-γ mRNA responses being indicative of immunity.
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Introduction

Feline infectious peritonitis (FIP) is a highly fatal disease in *Felidae* caused by a coronavirus and usually affects cats between 6 months and 3 years of age (reviewed by Pedersen, 1995). The FIPV is a common mutant of the far more ubiquitous and largely non-pathogenic feline enteric coronavirus (FECV) (Pedersen, 1987a; Vennema et al., 1998). The enteric virus is highly tropic to the mature epithelium of the small intestine, whereas the mutant FIPV gains a tropism for macrophages. This macrophage tropism allows the virus to disseminate throughout the body and is responsible for pathogenicity (Stoddart and Scott, 1989). The disease presents in two major forms: (1) an effusive form associated with peritonitis and/or pleuritis and vasculitis, and (2) a non-effusive form characterized by more classical granulomatous disease of major abdominal organs, uveal tract, and meninges (reviewed by Pedersen, 1995). Both forms of the disease are uniformly lethal, with affected cats dying over several days to many months. Immunity to FIPV is presumed to be cellular (Pedersen, 1987b), as is typical of highly cell-associated pathogens (Tizard, 2000). Antibody responses, by themselves, are actually harmful by facilitating virus uptake by macrophages and participating in

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Arthus like reactions (Pedersen and Boyle, 1980). Therefore, whether a cat develops wet or dry FIP is thought to depend on the strength of the cat's cell-mediated immune response. Cats producing a negligible cell-mediated response and a strong antibody response rapidly develop acute wet FIP. Cats producing a partial cell-mediated response develop the chronic form of the disease. There is strong circumstantial evidence that susceptibility to FIP has a significant genetic component (Foley et al., 1997).

The goal of developing effective FIPV vaccines has been elusive. Most vaccines either fail to protect or enhance the infection (Pedersen, 1989). Partial success has been obtained by using temperature sensitive mutants of a type II strain of FIPV (Gerber et al., 1990; Hoskins et al., 1995; Reeves et al., 1992). We were fortunate to create another avirulent FIPV, but to a preferred type I rather than type II strain (FIPV-UCD1). Type II strains are recombinants with canine coronavirus, while type I strains are uniquely cat. Type I strains cause 70–95% or more of disease (Hohdatsu et al., 1992).

We decided to test our avirulent strain of FIPV-UCD-1 as a vaccine. After primary vaccination cats were then challenge-exposed to a highly virulent type I strain of FIPV (FIPV-UCD8). In order to see whether there was a relationship between vaccine immunity and challenge-exposure outcome, we also analysed Th1 and Th2 cytokine profiles during immunization and following challenge-exposure.

Materials and method

Experimental animals

Ten 5-month-old male cats were obtained from the specific pathogen free breeding colony of the Feline Nutrition Laboratory, School of Veterinary Medicine, UC Davis, USA. Cats were housed in the facilities of the Center for Companion Animal Health, School of Veterinary Medicine, UC Davis, USA under the supervision of the Center for Laboratory Animal Sciences.

Experimental design

Eight cats were inoculated intraperitoneally, first with a non-pathogenic strain of FIPV-UCD1, then 32 days later with pathogenic FIPV-UCD8. Attenuated FIPV-UCD1 was passaged on Fcwf-4 cells and animals inoculated with 1 ml of infectious tissue culture fluid. FIPV-UCD8 was serially passaged in laboratory cats and infectious material was in the form of 1 ml of pooled ascitic fluid. This fluid had been harvested from cats dying of experimentally induced effusive FIP. Two control animals were mock vaccinated and challenge-exposed with PBS. The clinical status of the cats was monitored throughout the experiment. Three millilitres of heparinized blood were collected at the following time points: day −3, day 0 (time of experimental infection with UCD-1), day 4, day 7, day 11, day 14, day 18, day 21, day 28, day 32 (time of experimental infection with UCD-8), day 35, day 39, day 42, day 46, and day 49. All animals were euthanased on day 49. A complete necropsy was performed on each animal, including histologic examination of a range of potential target tissues. Necropsies were done to confirm the form of disease and to rule out the presence of subclinical infections in animals that appeared outwardly normal (Hoskins et al., 1995).

Assay procedures

Blood samples were immediately centrifuged in order to separate the buffy coat and plasma for cytokine analysis and serology, respectively. All fractions were stored at −80 °C until further processing. Three time points (day 21, day 42, and day 49) were selected for the serological investigations of the plasma samples by IFA technique using serum dilutions of 1:25 and 1:100 (Pedersen, 1976). IFA substrate slides were made from Fcwf-4 cells infected with FIPV-UCD1. Cytokine mRNA measurements from unstimulated peripheral blood mononuclear cells (PBMC) were performed for the following cytokines: IL4, IL6, IL10, IL12 p40, IL18, IFN-γ, and TNF-α as described previously (Kipar et al., 2001; Leutenegger et al., 2000). Relative cytokine mRNA levels were determined at the time points indicated above and calibrated against cytokine mRNA levels measured 3 days prior to immunization and from the two control animals. Experimentally infected cats were euthanased and necropsied when it became obvious that their disease was terminal or at the completion of the study (day 49). The form of FIP was determined grossly and histologically.

Results

Effect of immunization with avirulent FIPV-UCD1

No clinical signs of illness were observed in the eight experimental cats after immunization with FIPV strain UCD1, except for slight rise in rectal temperatures lasting 1 to 3 days (data

Table 1 Disease outcome, and FIPV antibody titers 21 days following immunization with avirulent FIPV-UCD1, and 10 and 17 days (days 42 and 49) after challenge-exposure with virulent FIPV-UCD8

Cat ID	Disease outcome	Day 21	Day 42	Day 49
00522	Classical wet FIP	1:25	1:25	1:100
00616	Classical wet FIP	1:25	0	1:100
00622	Classical wet FIP	0	1:25	1:100
00527	Accelerated FIP	1:100	NS[a]	NS
00623	Non-effusive FIP	1:25	0	NS
00624	Non-effusive FIP	1:25	1:25	NS
00524	Immune	1:25	0	0
00625	Immune	1:100	1:25	0
00626	Control	0	0	0
00627	Control	0	0	0

[a]NS=not sampled (animal dead).

not shown). All but one (cat 622) vaccinated cats seroconverted, but to low titer (Table 1).

Disease outcome of challenge-exposure to FIPV-UCD8

Three of eight vaccinated cats (nos 522, 616, 622) developed effusive FIP within 2 weeks of challenge-exposure to FIPV-UCD8, typical of classical non-enhanced disease (Pedersen and Boyle, 1980) (Table 1). One of the eight cats (no. 527) developed FIP within 4 days, characteristic of enhanced disease (Pedersen and Boyle, 1980). Two cats (nos 623, 624) developed non-effusive FIP. Two of eight cats (nos 524, 625) showed no signs of illness, as did the two control animals.

Relationship of antibody responses to disease outcome

Secondary antibody responses appeared to reflect disease outcome (Table 1). The three cats (nos 522, 616, 622) dying of classical effusive FIP had a rise in antibody titer post-challenge-exposure. Cats nos 623 and 624, which developed non-effusive FIP, had low primary antibody responses at day 21 post-immunization and with the same or decreased antibody titers 10 days post-challenge-exposure (day 42). The antibody titers of the two cats (nos 524 and 625) that resisted challenge-exposure declined to negligible levels even after challenge-exposure with FIPV-UCD8.

Cytokine mRNA changes in PBMCs post-immunization and following challenge-exposure

No major changes in cytokine mRNA levels were detected following the initial non-pathogenic FIPV-UCD1 immunization (Figs. 1–4) when compared to individual pre-infection values and to parallel cytokine responses in the two control cats (data not shown). A moderate elevation of TNF-α mRNA occurred between the second and third week after vaccination in cat 527. A small post-immunization increase of the IFN-γ mRNA level was seen in cat 524, which was one of the ultimate survivors.

Various cytokine mRNA changes were observed following challenge-exposure with FIPV-UCD8 (Figs. 1–4). IL-4 and IL-6 mRNA levels did not change from pre-infection or control cat levels in any of the eight infected animals. Slight to low increases in IL-10 mRNA were seen following FIPV-UCD8 infection in all cats, while IL-18 mRNA levels increases were negligible to low following challenge-exposure and bore no relationship to disease outcome (data not shown).

Changes, or lack thereof, were deemed significant for three cytokines mRNAs, IFN-γ, TNF-α and IL-12p40. The level of IFN-γ mRNA was strongly elevated in one of the surviving two cats; in the other one it remained unaltered. Cats that developed FIP had negligible or below normal IFN-γ responses, save cat 527 that showed slightly elevated levels of this cytokine on the day of challenge-exposure. All of the cats that developed FIP, regardless of form, had elevated post-challenge-exposure levels of TNF-α mRNA. IL-12 mRNA responses were increased following infection with FIPV-UCD8, but did not appear to relate to disease outcome or IFN-γ/TNF-α mRNA responses.

Discussion

Efficacy of vaccination

FIPV-UCD1 immunization induced only partial protection at best, as gauged against historical data. Animal-passaged FIPV-UCD8 usually kills from 90–100% of inoculated cats, almost always from effusive FIP (NC Pedersen, UC Davis, unpublished information). In this study, two of eight vaccinated cats (nos 524 and 625) appeared immune to challenge-exposure with virulent FIPV-UCD8 and two (nos 623 and 624) developed non-effusive FIP (indicative of partial immunity; Pedersen, 1995). Three immunized cats (nos 522, 616, and 622) died of the classical form of effusive FIP, indicating a predominance of non-protective humoral immunity. One cat (no. 527) developed a highly accelerated form of effusive FIP, indicating that immunization had elicited enhancing type antibodies (Pedersen and Boyle, 1980). Therefore, immunization induced a spectrum of immune

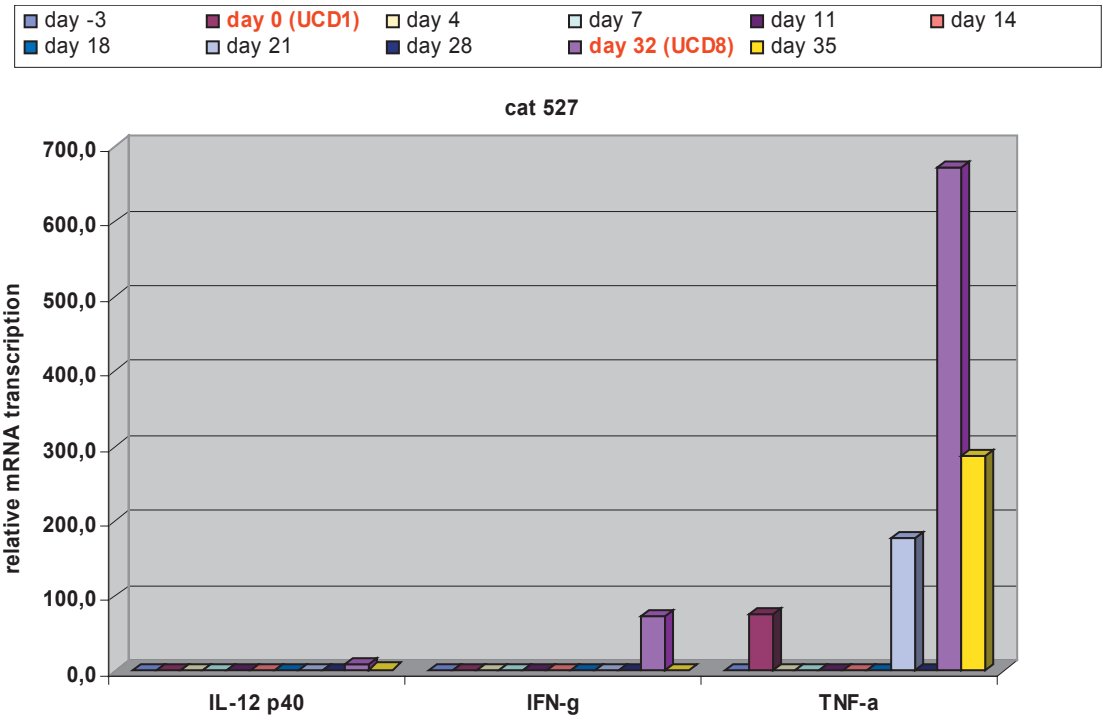

Figure 1 Cytokine mRNA levels in PBMCs of a cat with accelerated effusive FIP.

effects, ranging from protection (two cats), partial immunity (two cats), typical humoral immunity (three cats), to antibody enhancement (one cat). This reflects past experiences with attenuated live FIPV vaccines (McArdle et al., 1995; Pedersen and Black, 1983; Scott et al., 1995).

Cytokine mRNA responses in relation to disease outcome

Although this pilot study of a potential FIP vaccine was deemed largely a failure as far as protection was concerned, there were interesting findings in regards to Th1 and Th2 type cytokine responses and post-challenge exposure disease course. We were fortunate in this study to have cats representing each of the four possible disease outcomes: (1) classical effusive FIP occurring about 2 weeks following infection; (2) enhanced FIP occurring almost immediately after challenge, (3) non-effusive FIP, and (4) resistance. Based on the cytokine profile analysis of the different groups, we suggest that disease, regardless of form, is associated with a strong TNF-α mRNA response in PBMC and a failure to induce IFN-γ mRNA. In contrast, immune cats failed to upregulate TNF-α mRNA and one manifested strong IFN-γ mRNA responses. The former profile tends to favor Th2 (humoral) immunity, while the latter favors Th1 (cell-mediated) immunity. FIPV is an intracellular pathogen of macrophages, and as such, cell mediated immunity would be most important.

It was interesting to note the relationship between responses to avirulent or virulent virus and the magnitude and even duration of cytokine mRNA responses in PBMCs. Cats infected with the avirulent FIPV only showed transient fevers (data not shown) and low to negligible changes in cytokine mRNAs in PBMCs. The cat that had the most pronounced primary cytokine responses was also the animal that developed the most severe febrile reaction to the vaccine. This indicated that changes in cytokine mRNAs within PBMCs were only noticeable when reactions within internal lymphoid organs reached a certain threshold, thus allowing the responses to spill over into the blood. This was also observed after challenge-exposure. Cats that became very sick with FIP tended to have marked upregulation of cytokine mRNAs in their PBMCs. A dichotomy in responses was seen between the two cats that were immune to challenge exposure with virulent FIPV-UCD8. Cat no. 524 developed weak cytokine reactions during immunization and strong cytokine responses following challenge-exposure, while cat 625 showed very little changes during either immunization or challenge-exposure. This suggested that cat no. 524, while being immune, was none the less infected by the challenge virus

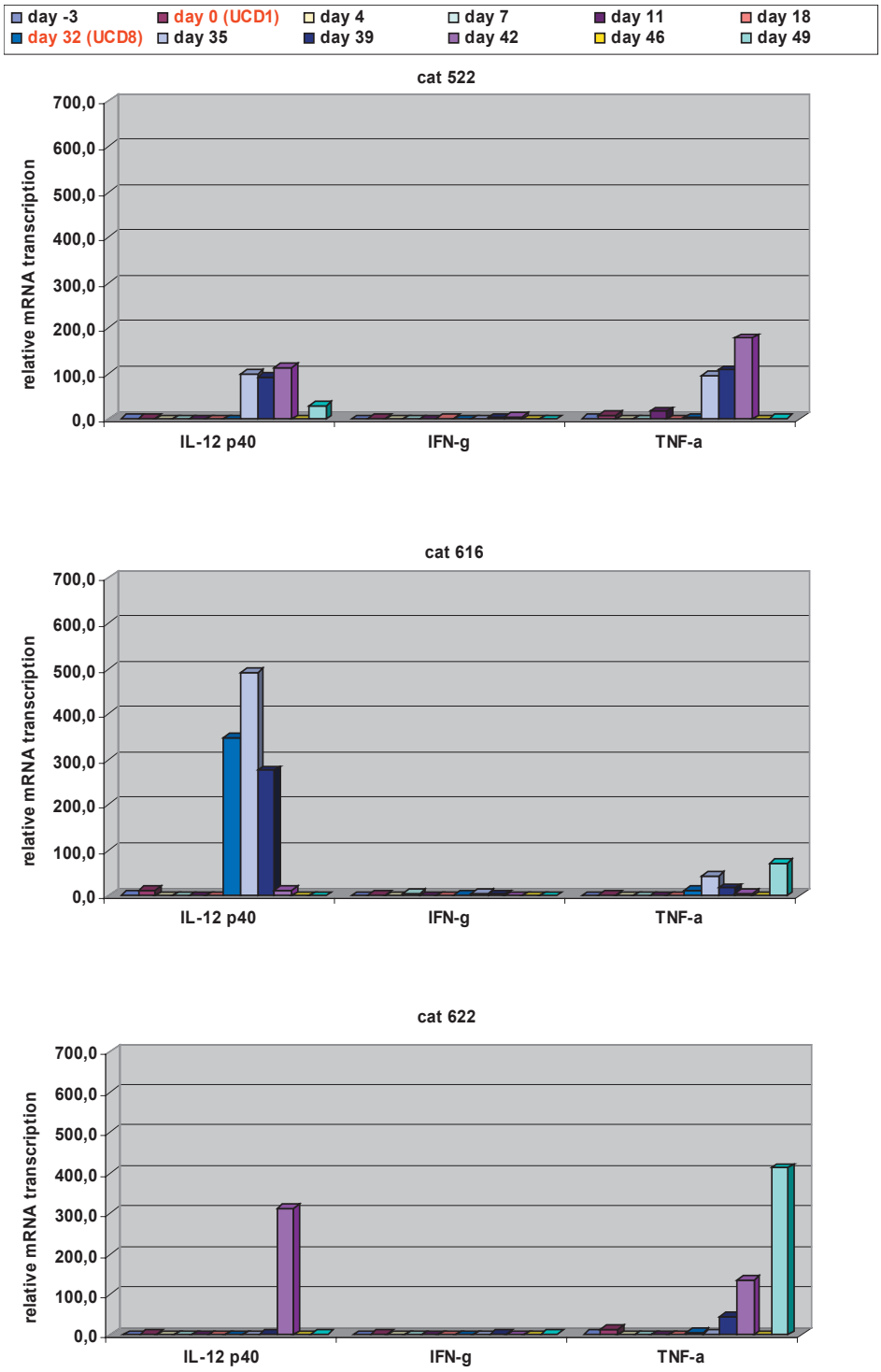

Figure 2 Cytokine mRNA levels in PBMCs of three cats with classical effusive FIP.

and did mount a vigorous secondary immune response (at the cytokine, but not antibody level). In contrast, cat 625 appeared to develop exceptionally strong protective immunity from the onset, precluding the need for a systemic response. Therefore, the magnitude and duration of cytokine mRNA responses in PBMCs does not always correlate with strength of immunity.

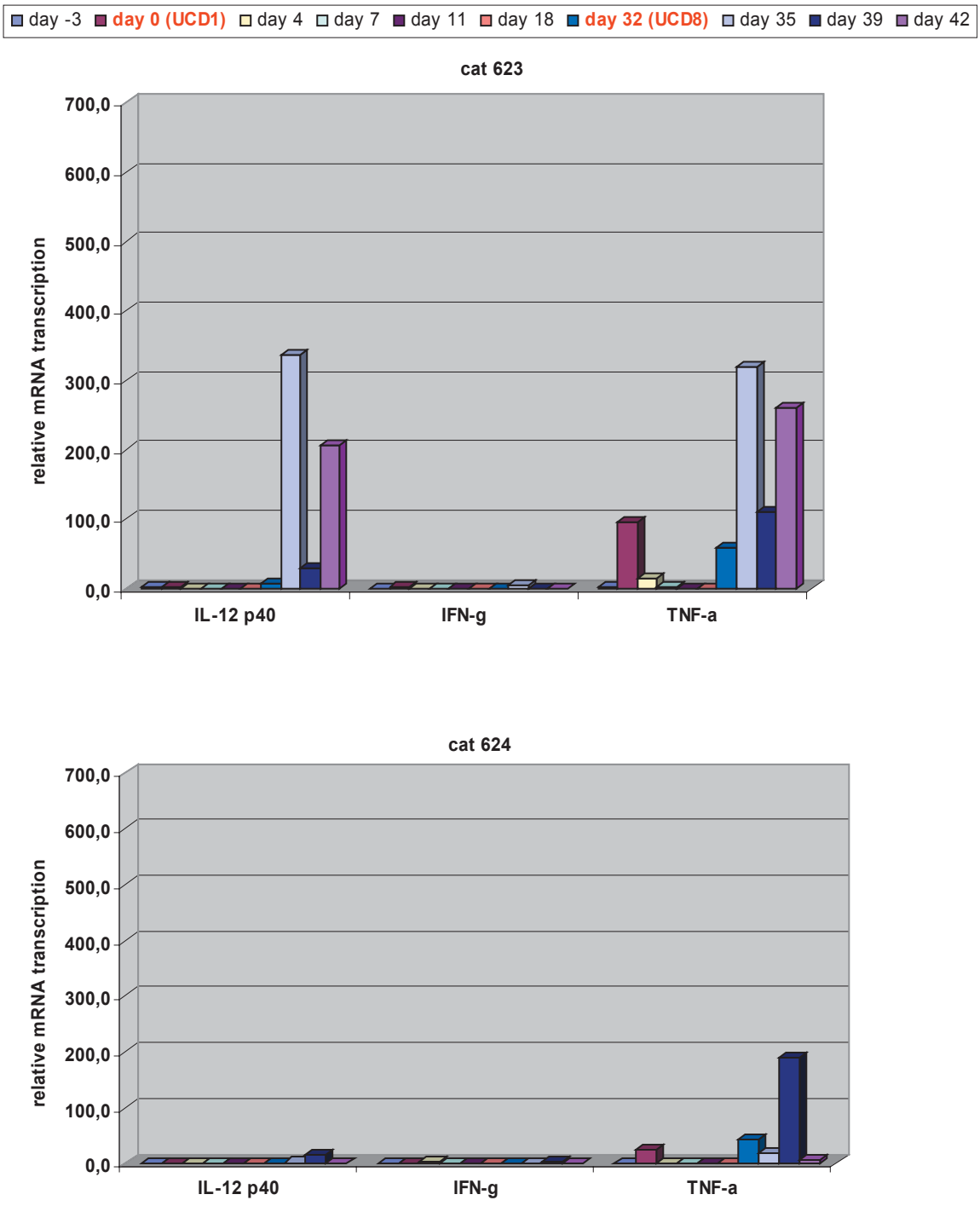

Figure 3 Cytokine mRNA changes in PBMCs for two cats with non-effusive FIP.

Similarities between FIP and MHV disease in IFN-γ knock-out mice

The importance of IFN-γ responses in FIPV immunity is strongly supported by what has been described recently for both experimental (Kyuwa et al., 1998a,b) and natural (France et al., 1999) mouse hepatitis virus (MHV) infections. MHV, like the feline coronaviruses (Horzinek et al., 1995), exists in two biotypes, a naturally occurring and largely enterotropic biotype and a more laboratory-associated polytropic biotype (Homberger et al., 1998). The enterotropic biotype of MHV is analogous to FECV, while polytropic biotypes have many parallels with FIPV. The similarities between feline and murine coronaviruses and their

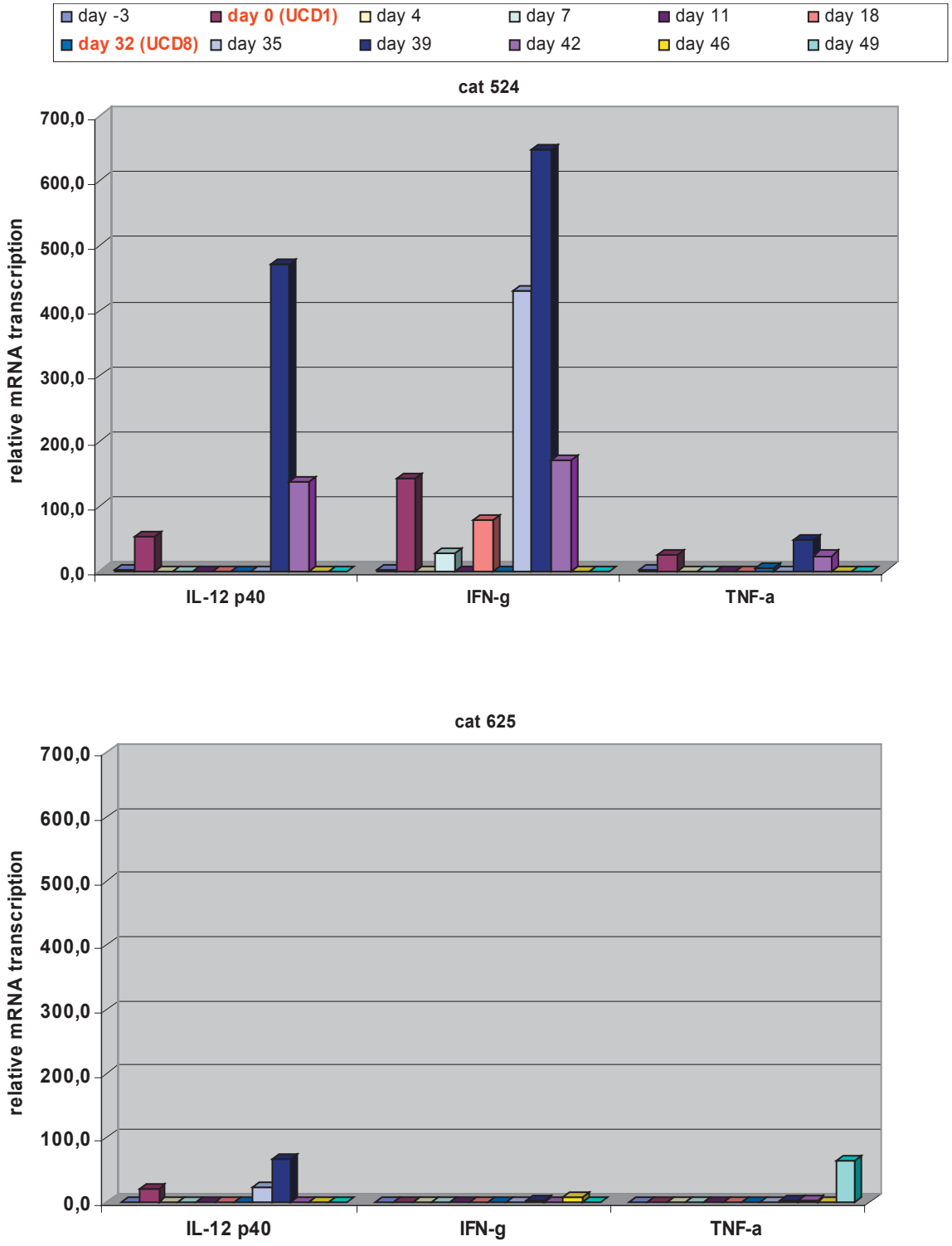

Figure 4 Cytokine mRNA levels in PBMCs from two FIP-immune cats.

biotype-associated diseases were quickly noted following the creation of IFN-γ deficient mice. IFN-γ knockout mice developed severe peritonitis, identical to FIP, upon experimental challenge with a polytropic MHV (Kyuwa et al., 1998a, 2002) and this disease could be partially inhibited with exogenously administered IFN-γ (Kyuwa et al., 2002). This supports our findings on the importance of IFN-γ mRNA responses in cats exposed to FIPV. A granulomatous peritonitis and pleuritis has also

been described in a colony of IFN-γ knockout mice infected naturally with enterotropic MHV (France et al., 1999). This latter observation parallels what is seen in a group of retrovirus immunocompromised cats exposed to FECV (Poland et al., 1996). Other similarities exist between murine and feline coronavirus infections. Age and genetic factors have been shown to play a role in naturally occurring MHV infection, with 1 week olds being more susceptible than 3 and 12 weeks, and Balb and ICR mice more susceptible than SJL mice (Barthold, 1987). Age and genetic susceptibility have been shown to be important risk factors for FIP in purebred cats (Foley et al., 1997; Foley and Pedersen, 1996). The central question coming out of this preliminary experiment and previous MHV-related studies concerns the role of IFN-γ in FIPV immunity.

Conclusions

In the presented preliminary experiment, vaccination of cats with an attenuated live strain of FIPV-UCD1 appeared to induce a degree of protection, in that two of eight cats were immune and two more developed non-effusive FIP post challenge. A more significant finding was the possible relationship between certain cytokine mRNA responses and disease outcome. Cats developing FIP after challenge-exposure failed to demonstrate IFN-γ mRNA responses in PBMCs, but did make high levels of TNF-α mRNA. Conversely, immune cats did not make detectable levels of TNF-α mRNA, and one made markedly high level of IFN-γ. Although only a pilot study, the findings are supported by parallel observations in MHV disease in IFN-γ knockout mice, and suggest the need for more in depth studies of the role of IFN-γ in FIPV disease and immunity.

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References

Barthold, S.W., 1987. Host age and genotype effects on enterotropic mouse hepatitis virus infection. Laboratory Animal Science 37, 36–40.

Foley, J.E., Pedersen, N.C., 1996. Inheritance of susceptibility to feline infectious peritonitis in purebred catteries. Feline Practice 24(1), 14–22.

Foley, J.F., Poland, A., Carlson, J., Pedersen, N.C., 1997. Risk factors for feline infectious peritonitis among cats in multiple-cat environments with endemic feline enteric coronavirus. Journal of the American Veterinary Medical Association 210, 1313–1318.

France, M.P., Smith, A.L., Stevenson, R., Barthold, S.W., 1999. Granulomatous peritonitis and pleuritis in interferon-gamma gene knockout mice naturally infected with mouse hepatitis virus. Australian Veterinary Journal 77, 600–604.

Gerber, J.D., Ingersoll, J.D., Gast, A.M., Christianson, K.K., Selzer, N.L., Landon, R.M., Pfeiffer, N.E., Sharpee, R.L., Beckenhauer, W.H., 1990. Protection against feline infectious peritonitis by intranasal inoculation of a temperature-sensitive FIPV vaccine. Vaccine 8, 536–542.

Hohdatsu, T., Okada, S., Ishizuka, Y., Yamada, H., Koyama, H., 1992. The prevalence of types I and II feline coronavirus infections in cats. Journal of Veterinary Medical Science 54, 557–562.

Homberger, F.R., Zhang, L., Barthold, S.W., 1998. Prevalence of enterotropic and polytropic mouse hepatitis virus in enzootically infected mouse colonies. Laboratory Animal Science 48, 50–54.

Horzinek, M., Herrewegh, A., de Groot, R.J., 1995. Perspectives on feline coronavirus evolution. Feline Practice 23(3), 34–39.

Hoskins, J.D., Taylor, H.W., Lomax, T.L., 1995. Independent evaluation of a modified live feline infectious peritonitis virus vaccine under experimental conditions (Louisiana experience). Feline Practice 23, 72–73.

Kipar, A., Leutenegger, C.M., Hertzel, U., Akens, M.K., Mislin, C.N., Reinacher, M., Lutz, H., 2001. Cytokine mRNA levels isolated feline monocytes. Veterinary Immunology and Immunopathology 78, 305–315.

Kyuwa, S., Shibata, S., Tagawa, Y., Iwakura, Y., Machii, K., Urano, T., 2002. Acute hepatic failure in IFN-gamma-deficient BALB/c mice after murine coronavirus infection. Virus Research 83, 169–177.

Kyuwa, S., Tagawa, Y., Machii, K., Shibata, S., Doi, K., Fujiwara, K., Iwakura, Y., 1998a. MHV-induced fatal peritonitis in mice lacking IFN-gamma. Advances in Experimental Medicine and Biology 440, 445–450.

Kyuwa, S., Tagawa, Y., Shibata, S., Doi, K., Machii, K., Iwakura, Y., 1998b. Murine coronavirus-induced subacute fatal peritonitis in C57BL/6 mice deficient in gamma interferon. Journal of Virology 72, 9286–9290.

Leutenegger, C.M., Boretti, F.S., Mislin, C.N. et al., 2000. Immunization of cats against feline immunodeficiency virus (FIV) infection by using minimalistic immunogenic defined gene expression vector vaccines expressing FIV gp140 alone or with feline interleukin 12 (IL-12), IL-16, or a CpG motif. Journal of Virology 74, 10447–10457.

McArdle, F., Tennant, B., Bennett, M., Kelly, D.F., Gaskell, C.J., Gaskell, R.M., 1995. Independent evaluation of a modified live FIPV vaccine under experimental conditions (University of Liverpool experience). Feline Practice 23(3), 67–71.

Pedersen, N.C., 1995. An overview of feline enteric coronavirus and infectious peritonitis virus infection. Feline Practice 23(3), 7–20.

Pedersen, N.C., 1989. Animal virus infections that defy vaccination. Equine infectious anemia, caprine arthritis encephalitis, maedi-visna, and feline infectious peritonitis. Advances in Veterinary Sciences and Comparative Medicine 33, 413–428.

Pedersen, N.C., 1987a. Virologic and immunologic aspects of feline infectious peritonitis. Advances in Experimental Biology and Medicine 218, 529–550.

Pedersen, N.C., 1987b. An overview of feline enteric coronavirus and infectious peritonitis virus infections. Feline Practice 23(3), 7–22.

Pedersen, N.C., 1976. Serologic studies of naturally occurring feline infectious peritonitis. American Journal of Veterinary Research 37, 1449–1453.

Pedersen, N.C., Black, J.W., 1983. Attempted immunization of cats against feline infectious peritonitis using either avirulent live virus or sublethal amounts of virulent virus. American Journal of Veterinary Research 44, 229–234.

Pedersen, N.C., Boyle, J.F., 1980. Immunologic phenomenon in the effusive form of feline infectious peritonitis. American Journal of Veterinary Research 173, 868–876.

Poland, A.M., Vennema, H., Foley, J.E., Pedersen, N.C., 1996. Two related strains of feline infectious peritonitis isolated from immunocompromised cats infected with feline enteric coronavirus. Journal of Clinical Microbiology 34, 180–184.

Reeves, N.C., Pollock, R.V., Thurber, E.T., 1992. Long-term follow-up study of cats vaccinated with a temperature-sensitive feline infectious peritonitis vaccine. The Cornell Veterinarian 82, 117–123.

Scott, F.W., Corapi, W.V., Olsen, C.W., 1995. Independent evaluation of a modified live FIPV vaccine under experimental conditions (Cornell experience). Feline Practice 23(3), 74–76.

Stoddart, C.A., Scott, F.W., 1989. Intrinsic resistance of feline peritoneal macrophages to coronaviruses correlates with virulence. Journal of Virology 63, 436–440.

Tizard, I.R., 2000. Resistance to viruses, in: Tizard, I.R. (Ed.), Veterinary Immunology: An Introduction, sixth ed. Philadelphia, PA, WB Saunders Company, pp. 276–277.

Vennema, H., Poland, A., Foley, J., Pedersen, N.C., 1998. Feline infectious peritonitis viruses arise by mutation from endemic feline enteric coronaviruses. Virology 243, 150–157.

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