Biphasic Immune Responses of Cats under Controlled Infection with a Feline Enteric Coronavirus-79–1683 Strain

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ABSTRACT. Kittens inoculated orally with 10^2 PFU of feline enteric coronavirus developed no antibody to the virus despite the repeated challenges. However, they developed antibody for a long period with $5 \times 10^3 - 1.6 \times 10^5$ (mean 3×10^4) and with $2.5 \times 10^3 - 2 \times 10^4$ (mean 6×10^3) immunoperoxidase antibody titer when they were challenged with 10^5 and 10^3 PFU of virus following previous challenges, respectively. Viremia was found when kittens were inoculated with 10^5 PFU of virus, but not with 10^3 PFU of virus. The dose of 10^3 PFU of virus seemed to be a lower limit to establish infection. These results indicate that local infection induces a low antibody response and systemic infection induces a high antibody response.—KEY WORDS: dose-dependent reaction, FECV.

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Feline coronaviruses are composed of two etiologic agents, feline infectious peritonitis virus (FIPV) and feline enteric coronavirus (FECV). These two viruses are indistinguishable from each other, in immunofluorescent antibody test, virus neutralization, and size of viral component tested by immunoblotting, except for pathogenicity [1, 4]. These facts may indicate that FECV is a FIPV which is not capable of inducing FIP. In this report, we studied immune response induced by FECV to know the biological differences between FECV and FIPV.

FECV-79–1683 [6] was provided by H. Koyama (School of Veterinary Medicine, Kitasato University), propagated in fcwf-4 cells [5], and stored at −80°C until used. FIPV-HR strain [3] was used for antibody titration. Viruses were titrated on fcwf-4 cells preseeded on 24-well multidishes as described previously [3].

Three-month-old SPF cats were purchased from Liberty Lab. (Waverly, NY, U.S.A.), fed in separate cages in a feeding room. Every two cats formed an experimental group. Virus was diluted to 1×10^2, 1×10^3, and 1×10^5 PFU/m*l* with culture medium, and every 1 m*l* of virus suspension was inoculated orally with a plastic syringe. Sera were obtained for antibody titration by the immunoperoxidase antibody (IPA) method [2] at 1- or 2-week intervals after infection. The summary of the IPA method is as following. FIPV-HR strain was plated on fcwf-4 cells to make clear plaques on 96-well multiplates, to allow easy distinction between the specific antibody-binding to plaques and nonspecific antibody binding to uninfected-cells. Then the plates were fixed with methanol, washed with water, and dried. The antigen plates were treated once with phosphate buffered saline containing 0.1% tween 20 (PBST), then reacted with test sera appropriately diluted with PBST containing 10% calf serum. The bound antibody was visualized with peroxidase-conjugated goat anti-cat IgG, dianisidine and hydrogen peroxide. The antibody titer was expressed by the reciprocal of the endpoint dilution. To know viremia, plasmas were harvested at 2-day intervals for first seven times after infection and they were serially diluted 1:3 with medium, and aliquots of 50 μl were overlaid on fcwf-4 cells seeded in a 96-well multidish. Then the virus was titrated in the same manner as described previously [3]. All sera and plasmas were frozen once at −80°C until titrated.

Since the minimal viral dose to produce antibody to the virus is not known, we started the infection with a low dose of virus. Four cats were orally inoculated with 10^2 PFU of FECV and observed for 35 days. However, no detectable antibody was found (Fig. 1). Then the cats were divided into two groups, and group A received 10^5 PFU of virus (Fig. 1A), and group B was left uninoculated and further observed (Fig. 1B). One week after the second challenge, group A cats raised antibody to 1×10^4 IPA-titer, then maintained high antibody levels of 5–160 $\times 10^3$ (mean 3×10^4) (Fig. 1A). One of these cats were further challenged with 10^5 PFU of virus, but no effect was observed on the antibody titer. On the other hand, the group B cats were challenged further twice with 10^2 PFU of virus on 196th and 252nd day after the first challenge, but no antibody production was observed. So, further challenge with 10^3 PFU was carried out on 315th day. Two weeks after the last challenge, the group B cats raised antibody to 5–10 $\times 10^3$ IPA titer. Then the antibody level ranged within 2.5–20 $\times 10^3$ IPA-titer (mean 6×10^3), and the mean value was about 5-fold less than that of group A.

To know whether viremia caused by virus propagation affects the antibody titer, we tested four new kittens by orally inoculating with either 10^5 or 10^3 PFU of virus. In cats of group C (Fig. 2C) challenged with 10^5 PFU of virus, viremia was observed early after infection and the recovered virus titers were 105 and 30 PFU/m*l*, 2 days after challenge, and 180 and 10 PFU/m*l*, 4 days after challenge from the plasmas of cat Nos. 5 and 6, respectively. Hereafter, no virus was recovered up to 14th day. Two weeks after infection, the antibody rose up to 2–4 $\times 10^4$ IPA-titer. Cats of group D (Fig. 2D) received 10^3 PFU of virus. However, no virus was found in the plasmas of No. 7 and No. 8 cats, during the first 14 days. Only No. 7 cat produced antibody of 4×10^4 IPA-titer 2 weeks after infection. No. 8 cat was further challenged with 10^5 PFU of virus, and produced antibody to 4×10^4 IPA-titer 2 weeks after the second challenge.

According to Pedersen *et al.* [6], FECV is recovered not only from the enteric organs but also from the tonsil, thymus, lung, spleen etc. This spread of infection may be

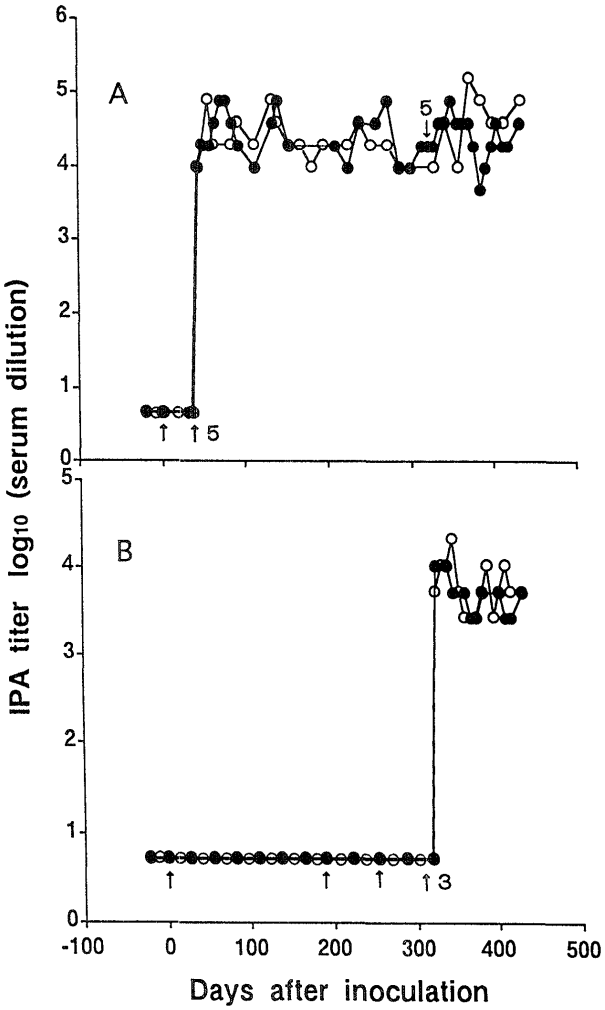

Fig. 1. Sequential infections of SPF cats with FECV 79–1683. All cats received orally 10^2 PFU of FECV (↑), then group A cats, No. 1 (○) and No. 2 (◍), received 10^5 PFU (↑ 5 or 5 ↓ : A); and group B cats, No. 3 (○) and No. 4 (●), received 10^3 PFU (↑ 3; B), on the indicated days by arrows. The IPA-titers are expressed by $\log_{10}$ (reciprocal of serum dilution). The lowest IPA-titers of 5 plotted in the figure mean 'below 5'.

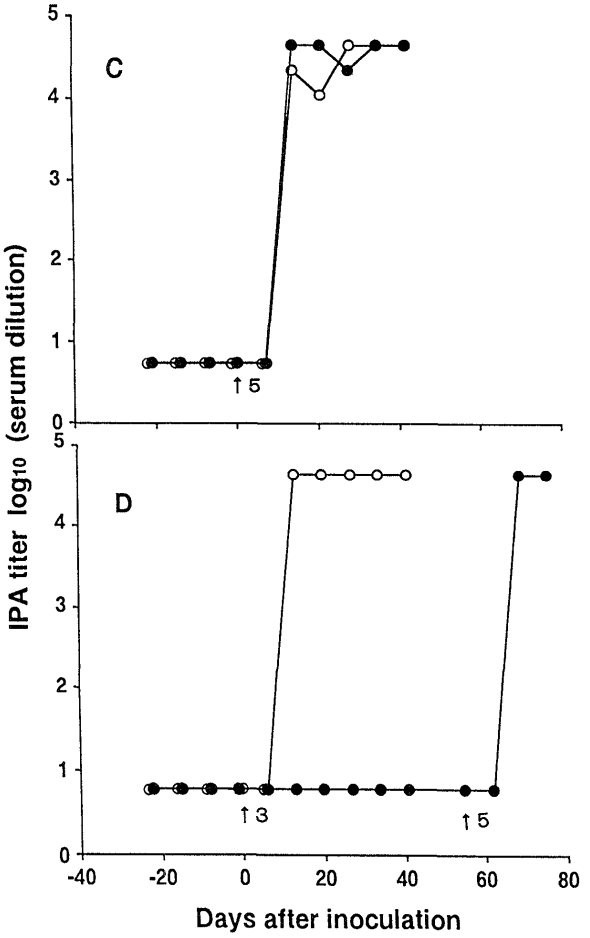

Fig. 2. Antibody induction in cats by a single infection with FECV 79–1683. Group C cats, No. 5 (○) and No. 6 (●), received 10^5 PFU of FECV (C); and group D cats, No. 7 (○) and No. 8 (●), received 10^3 PFU (D). The No. 8 cat further received 10^5 PFU on the day indicated by arrow (↑ 5).

associated with viremia. In the present study, viremia was observed only in group C cats inoculated with 10^5 PFU of virus. If FECV always induces viremia regardless of viral dose, viremia should occur even in group D cats receiving a smaller amount of virus (10^3 PFU). Thus, it seems probable that the mode of FECV infection differs by inoculated viral dose, and a higher dose may induce systemic infection through viremia, while a lower dose may induce limited or local infection. However, this probability of limited or local infection (may be at enteric organ) under low inoculum of 10^3 PFU, should be proven in the future. It should also be proven whether the dose below 10^3 PFU induces the local infection, though the antibody was not evoked in our case. If the antibody production means "establishment" of infection, 1×10^3 PFU of FECV seems to be the lowest dose to establish infection, because Nos. 3 and 4 (Fig. 1B), and 7 (Fig. 2D) but not 8 (Fig. 2D) cats produced antibody.

The mode (systemic or local) of infection may affect the antibody production. In fact, as shown in Fig. 1, we observed a biphasic immune response. The maintenance of the antibody level for a long period indicates the persistent virus infection. The minimum dose, 10^3 PFU of FECV, induced antibody levels ranging from 5×10^3 to 4×10^4 IPA-titer during a long period of observation, and never descended under 5×10^3. When these results are compared with our previous results on FIP [2], we can find some differences. Cats infected FIPV were calssified into three groups by titrating antibody to FIPV. One group represents a major part of infected cats living healthily and having antibody under 1×10^4 IPA-titer (designated as type I). The second group represents cats having wet type diseases and ocular disorders, with antibody increasing acutely upto over 1×10^5 IPA-titer (Type II). The third group represents cats having disorders with hind leg paralysis, with antibody ranging 8–16 $\times 10^4$ for a long time, then descending to 2–4 $\times 10^4$ IPA-titer (Type III).

among type I cats, there are cats that have low-titer antibody under 10^3 during infected period. This may indicate that cats having low-titer antibody under 10^3 are infected with FIPV but not FECV. Also, type III cats may be comparable with those shown in Fig. 1A. However, the variation in antibody titer is wide in the case of FECV infection but not FIPV infection. And the mean values of antibody-titers were around 3×10^4 and about 10^5 in the cases of FECV infected cats and type III cat, respectively. Thus, group A cats seems to be distinguishable from type III cats, if observation on a cat for some period is possible. For confirmation, the IPA- titrations were carried out not only with FIPV-HR strain but also with FECV in the same manner, but the obtained titers were same in both cases (data not shown).

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