Antibody-Dependent Enhancement Occurs Upon Re-Infection with the Identical Serotype Virus in Feline Infectious Peritonitis Virus Infection

Tomomi TAKANO1), Chisako KAWAKAMI1), Shinji YAMADA1), Ryoichi SATOH1) and Tsutomu HOHDATSU1)*

1)Laboratory of Veterinary Infectious Disease, School of Veterinary Medicine, Kitasato University, Towada, Aomori 034–8628, Japan

(Received 9 June 2008/Accepted 22 August 2008)

ABSTRACT. Feline infectious peritonitis virus (FIPV) is classified into serotype I and serotype II according to the amino acid sequence of its spike(S) protein. Antibody dependent enhancement (ADE) of macrophage infection occurs in the presence of antibodies to FIPV S protein, and a close relationship between ADE and neutralizing epitopes has been reported. The importance of differences in FIPV serotype on the induction of ADE remains unclear. In this study, we investigated whether the same or different serotype of FIPV induces ADE in cats. Specific pathogen-free cats were passively immunized with anti-type I or II FIPV antibodies, and we investigated the induction of ADE following subcutaneous inoculation with type I FIPV. Inoculation using FIPV serotype I enhanced the onset of FIP in cats passively immunized with FIPV serotype I-specific antibodies but not in those passively immunized with antibodies to FIPV serotype II. These data suggest that re-infection with the same serotype induces ADE in cats infected with FIPV.

KEY WORDS: ADE, coronavirus, FIPV, serotype.

Feline infectious peritonitis virus (FIPV), a feline coronavirus (FCoV) in the family Coronaviridae, causes fatal disease in wild and domestic cat species. FCoV is an enveloped virus with a single-stranded positive-sense RNA genome. The FCoV virions consist of three main structural proteins, peplomer spike (S) protein, transmembrane (M) protein and nucleocapsid (N) protein [16]. FCoV is classified into types I and II according to the amino acid sequence of its S protein and the reactivity to anti-FIPV S monoclonal antibodies (MAb) [9, 14, 15]. FCoV is also classified into 2 biotypes based on differences in pathogenicity: feline enteric coronavirus (FECV) and FIPV. Cats infected with FECV do not develop disease, but FIPV infection induces feline infectious peritonitis (FIP). FIP is a fatal disease, mainly leading to the development of immune complex vasculitis accompanied by necrosis and pyogenic granulomatous inflammation. It has been proposed that FIPV arises from FECV by mutation but the exact mutation and the inducing factors have not yet been clarified [21, 27].

Type II FCoV is known to utilize feline aminopeptidase N (fAPN) as its virus receptor [7]. FIPV targets macrophages, and macrophage infection is enhanced in the presence of antibodies (antibody-dependent enhancement, ADE). ADE activity in FIPV infection is enhanced when anti-FIPV-S antibody-bound viruses infect macrophages by binding of the Fc region of the antibody to the Fc receptor on the macrophage surface [2, 10, 18]. In addition to FIPV, there have been several reports concerning ADE of other virus infections (dengue virus, human immunodeficiency virus and respiratory syncytial virus, etc.) [4, 5, 20, 22, 23, 25].

We previously reported that when feline macrophages or human cells of monocyte lineage were inoculated with type II FIPV that had been reacted with the serum of type I or II FIPV-infected cats, ADE was observed only when type II FIPV was reacted with the serum of type II FIPV-infected cats [12]. However, despite the fact that more than 70% of FIP cats have been infected with type I FIPV, it is not clear whether type I FIPV, like type II, induces ADE by re-infection with the same serotype FIPV. Moreover, it is unclear whether ADE is induced by infection with the same serotype virus alone in cats as in the in-vitro study.

In this study, we examined whether ADE was induced in cats that had been passively immunized with anti-type I or II FIPV antibodies. Subsequently inoculated with type I FIPV, that is, whether type I FIPV, like type II, induced ADE by re-infection with the same serotype FIPV.

MATERIALS AND METHODS

Cell cultures and viruses: Felis catus whole fetus-4 (fcwf-4) cells were cultured in a 1:1 mixture (v/v) of Eagle’s minimum essential medium and L-15 medium, supplemented with 5% fetal calf serum (FCS) and antibiotics. Type I FIPV strain KU-2 (type I FIPV KU-2) and type II FIPV strain 79–1146 (type II FIPV 79–1146) were grown in fcwf-4 cells at 37°C. The type I FIPV KU-2 was isolated in our laboratory, and type II FIPV 79–1146 was supplied by Dr. M. C. Horzinek of State University Utrecht, The Netherlands.

Experimental animals: Anti-FCoV antibody-negative twenty-one specific pathogen-free (SPF) cats aged 4–6 months were used. The cats were maintained in a temperature-controlled isolated facility. All experiments were performed in accordance with the Guidelines for Animal Experiments of Kitasato University.

Passive immunization with anti-FIPV antibodies and
virus inoculation in SPF cats: Serum from FIPV-seropositive healthy cats and ascites derived from FIP cats were used in the experiment. The sera and ascites were heated at 56°C for 30 min before administration into cats.

Four cats were subcutaneously administrated with serum (3 ml or 30 ml) from type I FIPV KU-2-infected cats, and two cats were administrated with IgG (3 ml or 30 ml) purified from type I FIPV KU-2-infected cats-derived ascites by ammonium sulfate precipitation. The cats were subcutaneously inoculated with type I FIPV KU-2 (10^{4.3} TCID_{50}/head) 3 days after passive immunization. Five SPF cats inoculated with the virus without passive immunization were used as the control group.

Similarly, six cats were subcutaneously administrated with serum from type II FIPV 79–1146-infected cats. The cats were subcutaneously inoculated with type I FIPV KU-2 (10^{4.3} TCID_{50}/head) 3 days after passive immunization. As control, 4 cats without passive immunization were inoculated with only the virus.

These cats were checked daily for their clinical signs, and measured their body temperature and weight every 4–6 days. Serum was collected every 6–7 days for the detection of antibodies against FIPV. FIP diagnosis was confirmed upon postmortem examination, revealing peritoneal or pleural effusion.

**Experimental conditions: Serum from FIPV-seropositive cats or ascites were heated at 56°C for 30 min, each well of the plates was added 100 µl of the serum sample. Horseradish peroxidase-conjugated goat anti-cat IgG (ICN Pharmaceuticals, Inc., U.S.A.) were diluted to the optimal concentration with PBS containing 10% FCS and 0.05% Tween-20. 100 µl of the dilution was pipetted into each well of 96-well flat-bottomed plates. After washing with PBS containing 0.02% Tween-20, each well of the plates was added 100 µl of the test serum sample. The test sera were serially 2-fold diluted in medium and mixed with an equal volume of a virus suspension containing approximately 200 TCID_{50}/100 µl and the mixtures were incubated at 37°C for 60 min. Each mixture was then inoculated into the fcrf-4 cell cultures in 96-well flat-bottomed plates, and incubation was made in an atmosphere of 5% CO_{2} in air at 37°C for 3 days. For each serum dilution tests were duplicated. The antibody titer was expressed as a reciprocal of the highest dilution of the test sera that inhibiting cytopathic effect completely.

**Statistical analysis:** Data were analyzed by the Mann-Whitney test. Differences between comparisons were considered significant if p<0.01.

**RESULTS**

**ADE after type I FIPV infection in cats passively immunized with antisera against type I FIPV and purified ascitic IgG from type I FIPV:** SPF cats were passively immunized with serum or purified ascitic IgG from type I FIPV KU-2-infected cats and inoculated with type I FIPV KU-2. The neutralizing antibody titer against type I FIPV KU-2 was 1:320, the ELISA antibody titer against purified soluble type II FIPV 79–1146 antigen was 1:12600 in both the serum- and purified ascitic IgG-administered cats.

Time-course changes in the serum anti-FIPV antibody level were investigated by ELISA using purified soluble FIPV antigen. In cats passively immunized with 3 ml of antisera against type I FIPV KU-2 and cat passively immunized with 3 ml of IgG purified from type I FIPV KU-2-infected cat-derived ascites, the ELISA OD value was increased to 0.1–0.2 on the day of virus inoculation (day 3 post passive immunization). In cats without passive immunization (control group), the ELISA OD value continuously increased from day 12 after inoculation with type I FIPV KU-2 (Fig. 1a). In cats immunized with 30 ml of the antisera and immunized with 30 ml of the purified ascitic IgG, the ELISA OD value was increased to 0.4–0.8, respectively, on the day of virus inoculation (Fig. 1b). A continuous increase in the ELISA OD value from day 12 after inoculation was also noted in cats immunized with antisera against type I FIPV KU-2 (Fig. 1b). However, the ELISA OD value started to decrease on day 6 after inoculation in Cat Nos. 2F and 0F passively immunized with 30 ml of antisera against type I FIPV KU-2, and then re-increased from day 18 or 24.

In cats passively immunized with 3 ml of antisera against type I FIPV KU-2, the neutralizing antibody titer was increased to four fold on the day of virus inoculation (day 3 post passive immunization). In cats immunized with 30 ml of the antisera, the neutralizing antibody titer was increased to 8 and 16 fold, respectively, on the day of virus inoculation. In cats without passive immunization, the neutralizing antibody titer started to increase on day 6 after inoculation with type I FIPV KU-2, and reached 16–64 fold on day 24 (Fig. 2a). In cats passively immunized with 3 ml of IgG purified from ascites of cats infected with type I FIPV KU-2, the neutralizing antibody titer was increased to four fold on the day of virus inoculation. In cat immunized with 30 ml of the purified ascitic IgG, the titer was increased to eight fold (Fig. 2b). The neutralizing antibody titer also increased after inoculation with type I FIPV KU-2 in cats passively immunized with antisera against type I FIPV KU-2 (Fig. 2b).

In the control group, FIP developed 69 days after type I FIPV KU-2 inoculation only in Cat No. 9E out of 5 animals. In cats immunized with antisera against type I FIPV KU-2, Cat Nos. 0F, D3, and 8E developed FIP on day 24, Cat Nos. Q8 and C3 developed FIP on day 30, and Cat No. 2F developed FIP on day 36 after type I FIPV KU-2 inoculation. Figure 3 shows the survival rate of cats immunized
ADE OF FIPV INFECTION WITH SAME SEROTYPE VIRUS

with antiserum against type I FIPV KU-2 and inoculated with type I FIPV KU-2. The survival rate of cats immunized with antiserum against type I FIPV KU-2 was significantly lower than that of the control group, and the survival time after infection with type I FIPV KU-2 was also shorter.

ADE after type I FIPV infection in cats passively immunized with antiserum against type II FIPV: SPF cats were passively immunized with serum from anti-type II FIPV 79–1146-infected cats and inoculated with type I FIPV KU-2. The neutralizing antibody titer against type II FIPV 79–1146 was 1:6400, the ELISA antibody titer against purified soluble type II FIPV 79–1146 antigen was 1:12600 in the serum administered cats.

Time-course changes in the serum anti-FIPV antibody level were investigated by ELISA using purified soluble FIPV antigen. In cats immunized with 3 ml of antiserum against type II FIPV 79–1146, the ELISA OD value was increased to 0.1 on the day of virus inoculation (day 3 post passive immunization). In cats without passive immunization (control group), the ELISA OD value continuously increased from day 15 after inoculation with type I FIPV KU-2 (Fig. 4a). In cats immunized with 30 ml of the antiserum against type II FIPV 79–1146, the ELISA OD value was increased to 0.4–0.5 (Fig. 4b). A continuous increase in the ELISA OD value was also noted in cats immunized with antiserum against type II FIPV 79–1146 from day 22 or 29 after inoculation (Fig. 4b). However, the ELISA OD value started to decrease on day 7 after inoculation in cats passively immunized with 30 ml of antiserum against type II FIPV 79–1146 from day 22 or 29 after inoculation (Fig. 4b). However, the ELISA OD value was maintained at about 0.3 from day 29 to 85 after inoculation in Cat No. SV13 immunized with 3 ml of antiserum against type II FIPV 79–1146.

In cats immunized with 3 ml of antiserum against type II
FIPV 79–1146, the neutralizing antibody titer against type II FIPV 79–1146 was increased to 8 or 16 fold on the day of virus inoculation (day 3 post passive immunization). In cats immunized with 30 ml of the antiserum against type II FIPV 79–1146, the neutralizing antibody titer against type II FIPV 79–1146 was increased to 128 fold (Fig. 5d). However, the neutralizing antibody titer against type I FIPV KU-2 did not increase in response to type I FIPV KU-2 inoculation in any cat immunized with antiserum against type II FIPV 79–1146 (Fig. 5c). In contrast, no increase in the neutralizing antibody titer against type II FIPV 79–1146 in response to type I FIPV KU-2 inoculation was noted in the control group (Fig. 5b). In the group passively immunized with antiserum against type II FIPV 79–1146, the neutralizing antibody titer against type II FIPV 79–1146 decreased to a level lower than the detection limit (lower than two) on days 28 and 77 after type I FIPV KU-2 inoculation, respectively, corresponding to the initial antibody titer passively administered (Fig. 5d).

In cats immunized with antiserum against type II FIPV 79–1146, FIP developed on day 56 after type I FIPV KU-2 inoculation only in Cat No. SV63 out of 6 cats, but no cat

---

F. T. TAKANO ET AL.
ADE OF FIPV INFECTION WITH SAME SEROTYPE VIRUS

developed FIP in the control group. Figure 6 shows the survival rate of cats passively immunized with serum from type II FIPV 79–1146-infected cats and inoculated with type I FIPV KU-2. No significant difference was noted in the survival rate between cats immunized with antiserum against type II FIPV 79–1146 and control groups.

DISCUSSION

The antibody titer, including the neutralizing antibody...
titer, increases in FIPV infection, as in most virus infections. However, anti-FCoV antibodies are not effective for elimination of the virus, and, inversely, they enhance FIP development in the laboratory experiment [19]. Analysis using anti-FIPV MAb revealed that epitopes involved in ADE exist mainly on the S protein. Antibodies which neutralized virus propagated in Fc receptor-negative cells, such as the feline kidney cell line CrFK, induced strong ADE activity in Fc receptor-positive feline macrophages infected with FIPV, suggesting a close association between the neutralizing and infection-enhancing epitopes [2, 11, 17, 18].

There are two serotypes of FIPV, types I and II [9, 14, 15]. Four different serotypes of dengue virus exist, to which antibodies induce ADE, as with FIPV. The host infected with other serotypes of dengue virus develops high-mortality dengue hemorrhagic fever associated with ADE [3, 6]. In FIPV infection, unlike in dengue virus infection, ADE was observed in cats that had been passively immunized with anti-type I FIPV antibodies and subsequently infected with the same serotype (I) of FIPV, but not with the different type (II) of FIPV. These results support the in vitro experimental results that ADE was observed when cat macrophages or human cells of monocyte lineage were inoculated with type II FIPV that had been reacted with the serum of type II FIPV-infected cats [12].

We investigated ADE of infection of feline alveolar macrophages with FIPV KU-2, FIPV UCD-1 and FIPV Black strain, which are type I FIPV [12]. These viruses did not multiply in cultured feline alveolar macrophages, and enhancement of the infection by antibodies was not observed. However, in this study, in cats passively immunized with the serum of type I FIPV-infected cats, ADE was observed after inoculation with type I FIPV. Since Van Hamme et al. [26] have suggested that monocyes may be the target of type I FIPV, we need to investigate whether the occurrence of ADE of type I FIPV infection can be reproduced in vitro using monocyes instead of feline alveolar macrophages. We intend to prepare monoclonal antibodies reacting with type I FIPV S protein to evaluate the development of ADE of type I FIPV infection in detail.

The neutralizing antibody titer of the type II FIPV-infected cat serum used in this study was approximately 10 times higher than that of type I FIPV-infected cat serum or IgG purified from ascitic fluid. It is known that the neutralizing antibody titer in the serum of type I FIPV-infected cats against type I FIPV is lower than that of type II FIPV-infected cats against type II FIPV. The ELISA antibody titers of serum and IgG purified from ascitic fluid against purified soluble FIPV antigen were similar. Probably, a similar level of anti-FIPV antibodies was contained, although the neutralizing antibody titers against the specific viruses were different. The cats passively immunized with type II FIPV-infected cat serum had a high neutralizing antibody titer to the homologous virus, whereas those passively immunized with type I FIPV-infected cat serum had a high ELISA OD value. Based on these facts, groups of cats were passively immunized with 3 or 30 ml of FIPV-infected cat serum for the intergroup comparison of responses after inoculation with type I FIPV. Cats immunized with 30 ml of type I FIPV-infected cat serum or ascitic IgG and those immunized with 3 ml of type II FIPV-infected cat serum had a similar neutralizing antibody titer on day 3 after passive immunization (on the day of virus inoculation). All 3 cats immunized with 30 ml of type I FIPV-infected cat serum or ascitic IgG developed FIP after type I FIPV inoculation, whereas the cats immunized with 3 ml of type II FIPV-infected cat serum did not. These results indicate that, when cat serum contained antibodies with a similar neutralizing titer, ADE of type I FIPV infection occurred in the anti-type I FIPV-immunized cats alone.

The possibility cannot be excluded that the FIPV antigen and heat-stable substances other than anti-type I FIPV antibodies were present in FIPV-infected cat serum and FIP cat-derived ascitic IgG, thereby being involved in ADE induction. However, if substances in the serum other than anti-type I FIPV antibodies had been involved in ADE induction, ADE would also have occurred in cats passively immunized with type II FIPV-infected cat serum, which was not the case in this study. In addition, in cats passively immunized with type II FIPV-infected cat serum, the neutralizing antibody titer rose temporarily after immunization, and then gradually decreased. In other words, even if the type II FIPV antigen was present in the serum, it was not at a level sufficient to induce antibody production, probably not affecting the present experimental system. Thus, we consider that mainly anti-type I FIPV antibodies induced ADE in this study.

It was also confirmed that the probability of developing FIP increased when anti-type I FIPV antibody-positive cats were infected with type I FIPV. Many domestic cats possess anti-type I FCoV antibodies, and numerous field cases of FIP are caused by type I FIPV infection [8, 13], suggesting that FIPV re-infection induces ADE and advances FIP development in domestic cats. However, Addie et al. [1] reported that FCoV re-infection of anti-FCoV antibody-positive domestic cats might not result in the development of ADE. Although the details are unclear, differences in the immunological condition of FCoV-infected cats may be the reason why the phenomenon noted in this experiment does not occur in domestic cats. It is assumed that ADE does not occur in cats which have acquired strong cellular immunity, even if they possess anti-FCoV antibodies, escaping from FIP development. It may also be possible that anti-FCoV antibodies prevent FIPV infection, avoiding ADE, when the anti-FCoV neutralizing antibody titer is high. We previously reported that FIPV infection of macrophages was completely neutralized at a high MAb concentration, but dilution of the antibodies inversely induced ADE in an in vitro study [10].

We demonstrated that ADE was induced by re-infection with the identical serotype virus in FIPV infection, unlike dengue virus infection. We have recently reported that TNF-alpha released from macrophages showing ADE of FIPV infection is closely involved in the progression of the
pathological state of FIP [24]. The ADE of FIPV infection is a barrier to the development of FIPV vaccines. Moreover, it is possible to study ADE of FIPV infection using cultured cells (in vitro) or experimental infection of cats (in vivo), thereby comparing responses in cultured cells and the host. Thus, the study of ADE of FIPV infection provides an appropriate animal model for the understanding of human infection with deadly viruses associated with ADE, such as dengue virus and ebola virus infection. It seems very important to conduct studies on the occurrence of ADE of FIPV infection.

ACKNOWLEDGEMENT. This work was supported in part by Ministry of Health, Labor, and Welfare (grant H16-Shinkoh-9) and The Waksman foundation of Japan Inc. (grant 07–9).

REFERENCES