

Enhancement and Neutralization of Feline Infectious Peritonitis Virus Infection in Feline Macrophages by Neutralizing Monoclonal Antibodies Recognizing Different Epitopes

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Abstract: The interaction between the enhancing and neutralizing activities of three monoclonal antibodies (MAbs) (5-6-2, 6-4-2 and 7-4-1) to the spike protein of feline infectious peritonitis virus (FIPV) strain 79-1146 was determined using feline macrophages. At a high MAb concentration, all of the three MAbs completely inhibited the FIPV infection at 37 C. However, two of them (6-4-2 and 7-4-1) enhanced FIPV infection when either the MAb concentration or reaction temperature was lowered. These MAbs also exerted an immediate infectivity-enhancing activity for up to 10 min of reaction and by 20 min, neutralizing activities were observed. Only MAb 5-6-2 consistently showed neutralizing activity regardless of the reaction conditions. Competition with sera from cats experimentally infected with FIPV strain 79-1146 or feline enteric coronavirus strain 79-1683 showed that the two epitopes recognized by MAb 5-6-2 and MAb 6-4-2, respectively, are also recognized by the natural host.

Key words: Antibody-dependent enhancement, Monoclonal antibody, Feline infectious peritonitis virus

Feline infectious peritonitis virus (FIPV) is a member of the coronavirus group, and causes a chronic progressive immune-mediated disease in their natural host. Antibodies to FIPV have been shown to accelerate both the rate of FIPV infection and the disease onset in cats (15, 16, 21). Cats immunized with closely related coronaviruses, such as feline enteric coronavirus (FECV), are also sensitive to exposure to virulent FIPV (15). These phenomena are thought to be caused by a mechanism similar to that of dengue shock syndrome (21). Macrophages are known to be one of the target cells for FIPV, as in dengue virus infection (8, 14, 22). We previously reported that FIPV infection of feline alveolar macrophages is enhanced by monoclonal antibodies (MAbs) to the peplomer spike (S) protein and the transmembrane (M) protein (10). This antibody-dependent enhancement (ADE) (1, 4-7, 11, 17-19) was completely offset or reduced by pretreatment of the

MAb with protein A or when F(ab')₂ fragments of MAb were used. Olsen et al (13) and Corapi et al (2) have recently supported our findings by showing enhancement of FIPV infection in primary feline peritoneal macrophages with MAbs to the viral S protein. In many instances, neutralizing MAbs were able to induce ADE, demonstrating a direct relationship between neutralization and enhancement.

We describe in this paper the relationship between neutralizing and enhancing activities of MAbs (5-6-2, 6-4-2 and 7-4-1) that recognize three distinct epitopes on the S protein and have the

Abbreviations: ADE, antibody-dependent enhancement; CrFK, Crandell feline kidney; ELISA, enzyme-linked immunosorbent assay; fcwf-4, feline whole fetus cells; FECV, feline enteric coronavirus; FIPV, feline infectious peritonitis virus; HBSS, Hanks' balanced salt solution; IFA, indirect fluorescent antibody assay; MAb, monoclonal antibody; MAbs, monoclonal antibodies; M protein, transmembrane protein; NT test, neutralization test; PBS, phosphate-buffered saline solution; RS virus, respiratory syncytial virus; S protein, peplomer spike protein; TCID₅₀, 50% tissue culture infectious dose.

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ability to neutralize FIPV strain 79-1146 in feline macrophages. We further investigate whether or not the epitopes recognized by our MAbs are also immunogenic in cats experimentally infected with FIPV.

FIPV 79-1146 strain and FECV 79-1683 strain were used in this study. These strains were kindly provided by Drs. H. C. Horzinek (The State University, Utrecht, The Netherlands) and A. J. Mckeirnan (The Washington State University, Pullman, Wa., U.S.A.), respectively. The MAbs to FIPV strain 79-1146 were produced as previously described (9).

The MAbs 5-6-2, 6-4-2, and 7-4-1 used in the present study recognize S protein of the virus by immunoblot. These MAbs have the ability to neutralize FIPV strain 79-1146 and FECV strain 79-1683, and recognize different epitopes of the viral S protein. A stock culture fluid from the three hybridoma lines was used as the MAb source for all the experiments. Peroxidase-labeled MAb was prepared by the method of Nakane and Kawaoi (12). Macrophages were collected from coronavirus antibody-negative adult cats. Alveolar macrophages were obtained by washing alveoli with Hanks' balanced salt solution (HBSS).

The recovered cells were centrifuged, washed three times with HBSS, and resuspended in growth medium (Eagle's minimum essential medium containing 50% Leibovitz L-15 medium, 10% fetal calf serum, 100 units per ml of penicillin, and 100 μ g per ml of streptomycin) at a final concentration of 2×10^6 cells/ml. One ml of cell suspension was added to a well (in 24-well multi-plates) containing sterile glass cover slip and incubated at 37 C for 2 hr. The cells on the cover slips were washed with HBSS, incubated in growth medium for additional 2 hr at 37 C, and washed with HBSS. The adherent cells thus obtained were used as macrophages in the experiments. Indirect fluorescent antibody assay (IFA) was carried out as described previously (10). Neutralization (NT) test was also carried out as described previously (9). Antibody-dependent enhancement (ADE) assay of FIPV infection was carried out as follows. Equal amounts of MAbs and viral suspension were allowed to react for 1 hr at 37 C, 25 C or 4 C. The mixtures were inoculated in 0.1 ml volumes into wells of multiplate cultures of macrophages that had been incubated for 3 days. After adsorption at 37 C for 1 hr, followed by washing with HBSS, 1 ml of growth medium was added, and the plates were incubated at 37 C for 36 hr in a CO₂ incuba-

tor. The cover slips were removed and examined by IFA. The percentage of ADE was obtained by the following formula: percent of ADE = $(A - B/B) \times 100$, where A is the rate of positivity of IFA in the presence of the antibodies and B is the rate of positivity of IFA in the absence of the antibodies. For competitive enzyme-linked immunosorbent assay (ELISA), the antigen was prepared with the FIPV strain 79-1146 grown in fcfw-4 cell cultures. The antigens were purified from the infectious culture fluid by the method previously described (9). Cat serum (100 μ l of a 1 : 100 dilution in PBS containing 10% calf serum and 0.05% Tween-20) was added to 96-well Microelisa plates coated with viral antigens, and the plates were incubated at 37 C for 1 hr. After the plates were washed with PBS containing 0.02% Tween-20 three times, peroxidase-conjugated MAb (optimally diluted in PBS containing 10% calf serum and 0.05% Tween-20) was added. After incubation at 37 C for 30 min, each well received 100 μ l of substrate solution and the plates were incubated at 25 C for 20 min in a dark room. The substrate solution was prepared by dissolving *O*-phenylenediamine dihydrochloride at a concentration of 0.4 mg/ml in 0.1 M citric acid-0.2 M Na₂HPO₄ buffer (pH 4.8) and adding 0.2 μ l/ml of 30% H₂O₂. After incubation, the reaction was stopped with 3 N H₂SO₄ solution and the optical density (OD) at 492 nm was measured. The percent inhibition was calculated by the formula $100(A - B/A)$, where A is the OD in the dilution buffer and B is the OD in the test serum.

The interaction between the neutralizing and enhancing activities of MAbs 5-6-2 [neutralization (NT) titer; 1 : 128], 6-4-2 (NT titer; 1 : 256) and 7-4-1 (NT titer; 1 : 64) to the S protein of FIPV 79-1146 strain was studied in feline alveolar macrophages. All the three undiluted MAbs completely neutralized the infectivity of FIPV (2,000 TCID₅₀) upon preincubation of the MAbs with virus for 1 hr at 37 C (Fig. 1). However, under the same reaction conditions, MAbs 6-4-2 and 7-4-1 diluted to 1 : 10 enhanced the FIPV infection. MAb 6-4-2 had a higher enhancing activity. Enhancing activity of MAb 6-4-2 was observed even in undiluted samples when the reaction temperature of the MAb-virus mixture was lowered to 25 C or 4 C. At 25 C and 4 C, even undiluted MAb 7-4-1 had no neutralizing activity and instead, exhibited weak enhancing activity. In contrast, MAb 5-6-2 had neutralizing activity under any reaction conditions employed, except that the neutralizing activity

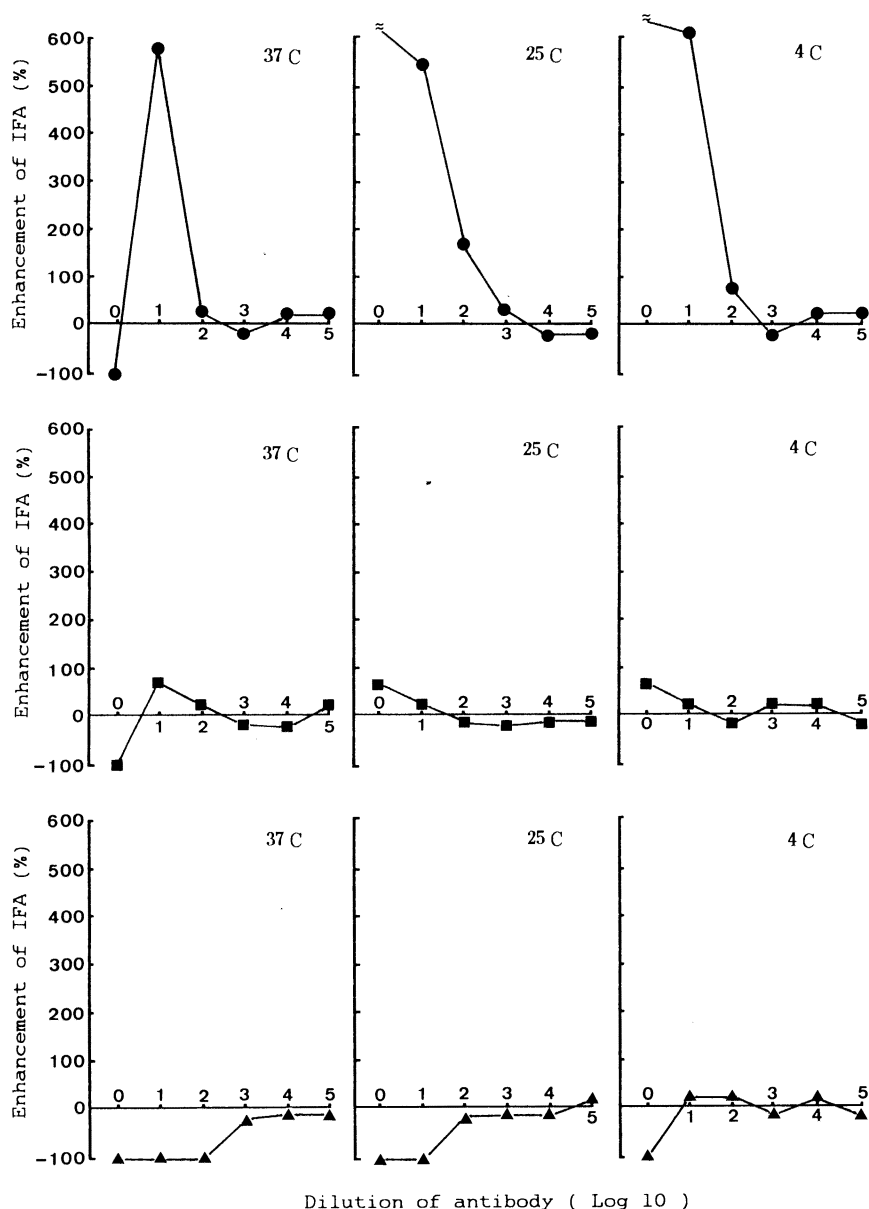


Fig. 1. Neutralization and enhancement of FIPV infection in feline macrophages by neutralizing MABs at different temperatures. (●), MAB 6-4-2; (■), MAB 7-4-1; (▲), MAB 5-6-2.

decreased by lowering the reaction temperature.

When undiluted MAB and the virus (2,000 TCID₅₀) were added to the macrophage culture immediately (0 min) or 5 min after reaction at 37 C, strong enhancing activity was observed with MAB 6-4-2 (Fig. 2b). No enhancement but strong neutralization was observed when the MAB and the virus were allowed to react for at least 20 min. Weak enhancing activity was observed with MAB 7-4-1 after 0-5 min of reaction (Fig. 2c); thereafter, such activity was gradually lost and neutralizing activity became detectable. MAB 5-6-2 remained able to neutralize the virus infectivity throughout the reaction period (0-60 min) tested

(Fig. 2a).

Competitive ELISA was performed to determine whether epitopes recognized by MABs 5-6-2 and 6-4-2 can be recognized by cats experimentally infected with FIPV or FECV. Both MABs competed with the sera from both FIPV- and FECV-infected cats (Fig. 3). Such competition increased with increasing the NT titer of the serum. In general, sera from FIPV-infected cats competed more effectively against the MABs than those from FECV-infected cats. Both sera competed more effectively against the epitope recognized by MAB 6-4-2 than the one recognized by MAB 5-6-2.

Both enhancing and neutralizing antibodies

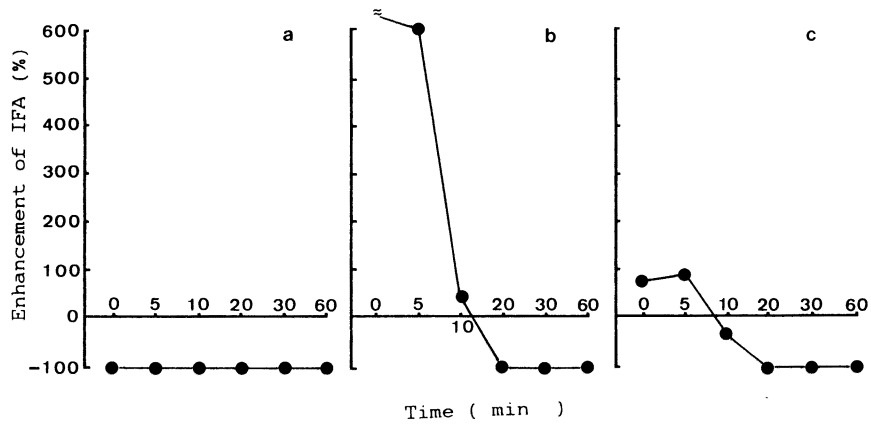


Fig. 2. Effect of reaction time at 37 C on the enhancement of FIPV infection in feline macrophages by neutralizing MAb. a, 5-6-2; b, 6-4-2; c, 7-4-1.

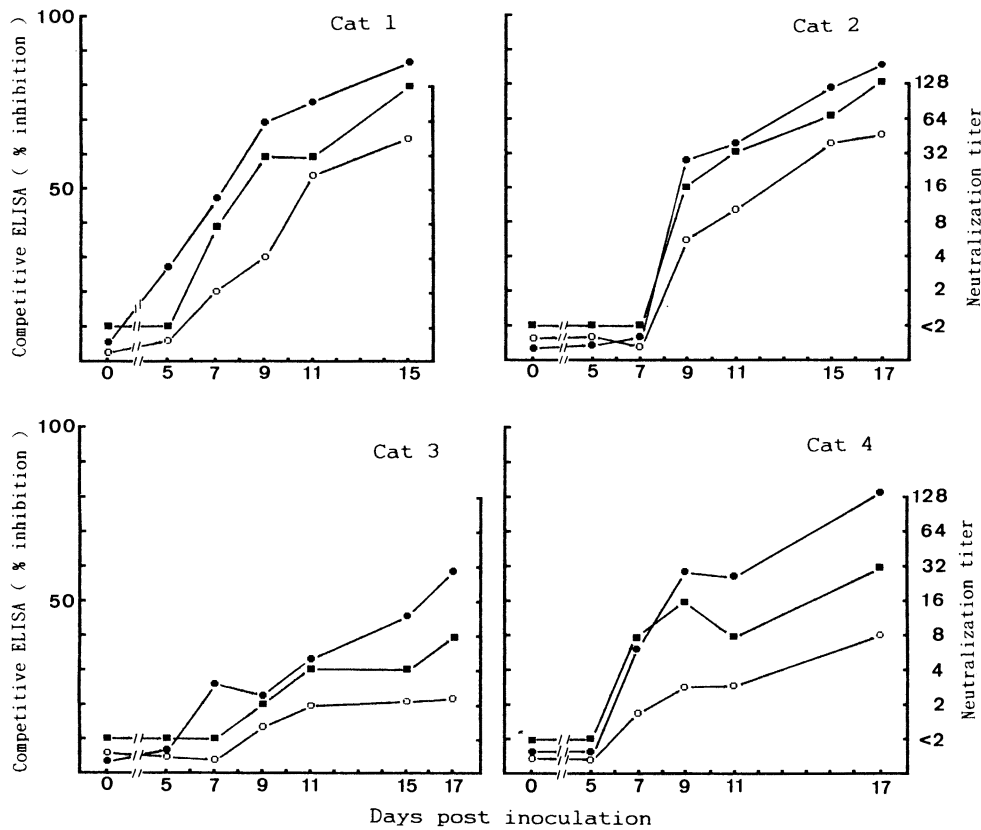


Fig. 3. Percent inhibition in competitive ELISA and neutralization titer of antisera from cats inoculated with FIPV strain 79-1146 (cats 1 and 2) or FECV strain 79-1683 (cats 3 and 4). (●), percent inhibition in competitive ELISA by MAb 6-4-2; (○), percent inhibition in competitive ELISA by MAb 5-6-2; (■), neutralization titer.

have been detected in cats infected with FIPV (13). Using MAbs we have attempted to identify the epitopes required for enhancing and neutralizing activities and evaluate the development of the antibodies to these epitopes in cats during FIPV infection. We have identified three distinct groups of MAbs to the FIPV S protein: those that have ADE activity only, neutralizing activity only and

both activities. In our previous studies, we have characterized two monoclonal antibodies that recognized viral S protein and possessed only ADE activity (10).

Our present study, characterized three MAbs (5-6-2, 6-4-2 and 7-4-1) that identified three distinct epitopes on viral S protein (9). MAbs 6-4-2 and 7-4-1 had both enhancing and neutralizing activities

depending upon the reaction conditions (concentration, reaction temperature and reaction time), suggesting that the enhancement is an event at an intermediate stage in the neutralization pathway. MAb 5-6-2 exerted neutralizing activity alone regardless of the reaction conditions. The inhibition of virus proliferation by the MAb may be exerted after adsorption of viruses to cells. However, when the MAb was allowed to react with viruses following adsorption of the viruses at 4°C for 2 hr after inoculation of the viruses into CrFK cell cultures, the MAb did not inhibit virus infection (data are not shown). Since MAb 5-6-2 recognizes the epitopes essential for neutralization, and since MAbs 6-4-2 and 7-4-1 recognize epitopes (enhancing epitopes) very closely related to the neutralizing epitopes, neutralization may be achieved by steric hindrance in the case of multi-bonds as a result of adequate reaction. Since the results obtained in this study were *in vitro* results with mouse MAbs, they cannot be applied directly to the *in vivo* situation.

It is clear, however, that the epitopes recognized by MAb 5-6-2 are closely related to the defence of the FIPV infection. This suggests that FIPV infection may be prevented by a neutralizing antibody.

The results of competitive ELISA revealed that the antibodies for epitopes recognized by MAbs 5-6-2 and 6-4-2 are raised in FIPV or FECV-infected cats. The percent inhibition by MAb 6-4-2 was always higher than by MAb 5-6-2. The difference in percent inhibition between MAb 6-4-2 and MAb 5-6-2 was more remarkable in the late stage than in the early stage of infection in FECV 79-1683 strain-infected cats. Preexisting heterologous FECV 79-1683 immunity often accelerated and enhanced the severity of disease caused by inoculation with FIPV (15). Gimenez et al (3) have reported that respiratory syncytial (RS) virus infection in U937 cells was enhanced by convalescent sera of patients with RS virus infection.

Vennema et al (20) combined the protein S gene of FIPV with vaccinia virus. Cats immunized with the recombinant virus showed no protection against challenge with virulent FIPV. The onset of disease was accelerated by the immunization. It is also clear from our results that enhancing epitopes are present on S protein. A vaccine without an enhancing epitope may be developed analysis of epitopes recognized by MAb 5-6-2. From this aspect as well, the results of this present study are meaningful.

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