Sero-Diagnostic Aspects of Feline Herpesvirus Infection

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The serum-neutralization (SN) test has been only one serologic tool most frequently employed in the study in vitro of feline viral rhinotracheitis (FVR) and its causal virus. As the initial immune response of cats to feline herpesvirus (FHV) infection is of low magnitude when measured by the SN test [1, 9], serological diagnosis by this test is not always practical. Moreover, some clinical cases suggesting partial immunity have been described [1, 6, 9]. In them, cats survived severe infection without developing any demonstrable antibody titer, or convalescent cats without detectable antibody titers developed a mild disease following exposure to the virus. To solve these questions, the present experiment was performed. In it, the SN test was carried out in tubes with or without addition of complement, as well as the hemagglutination-inhibition (HI) test with ether-treated hemagglutinin of FHV, employing rabbit immune serum and cat serum samples.

Seven strains of FHV described previously [5] were employed. A lined feline lung cell culture growing at the authors' laboratory was used for virus titration and SN test. The growth and maintenance media and the virus titration procedure used were the same as previously described [5].

Rabbit immune serum was prepared as follows. One rabbit was inoculated intravenously (i.v.) with 5 ml (10^4.0 TCID₅₀/0.2 ml) of the C7301 strain of FHV at weekly intervals for eight weeks. Serum samples were collected before every inoculation and three weeks after the last inoculation. Three rabbits were inoculated i.v. with 5 ml of the C7301 strain. Serum samples were collected from two of them on the 1st, 3rd, 5th, 7th, 10th, 14th and 21st day after inoculation, and from the other in the 4th, 5th, 6th, 7th, 8th and 11th week after inoculation.

Feline serum samples were collected from 33 cats. Of these cats, twenty-nine were apparently healthy, two manifested upper respiratory signs, and the other two presented nervous signs. Serum samples were also collected from cat No. 0576 [5] on the 32nd, 41st and 60th days after inoculation.

The SN test was performed by the authors’ routine method previously described [5]. Complement supplementation to rabbit immune serum and cat serum samples in this test was also investigated by the
method of Yoshino and Taniguchi [10].

Phosphate-buffered saline solution (PBSS; 0.01 M, pH 7.5) was used as a diluent for serum and hemagglutinin obtained by treatment with peroxide-free ethyl ether [5] in the HI test. Cat serum was subjected only to inactivation by heating at 56°C for 30 minutes. Rabbit serum was inactivated in the same manner and absorbed with an equal volume of 10% feline erythrocyte suspension at 37°C for three hours prior to test. Then 0.25 ml of twofold serum dilution was mixed with an equal volume of hemagglutinin suspension containing 4 HA units in each tube, and two volumes of 0.5% feline erythrocyte suspension in PBSS were added to the tube. Reading was made after incubation at 37°C for three hours. The HI titer was expressed as a reciprocal of the highest serum dilution that inhibited hemagglutination completely.

Fig. 1 illustrates the antibody response of rabbits inoculated with a single dose (5 ml; 10^4.0 TCID_{50}/0.2 ml) or multiple doses of the FHV C7801 strain. In the rabbits inoculated with a single dose, neither SN nor complement-requiring SN (CRSN) antibody was detected during the observation period. An HI antibody, however, was detected from them on the 5th day after inoculation, and an HI titer of 1:8 to 1:16 was maintained for 11 weeks during the observation period. In the rabbits inoculated with multiple doses, no CRSN antibody was detected, but HI antibody titer slightly higher than SN antibody titer was maintained with a close correlation throughout the observation period.

As shown in Table 1, crude and ether-treated hemagglutinin were used. It was impossible, however, to distinguish the
seven strains from one another even by the cross HI test.

As shown in Table 2, SN antibody could be detected in two samples obtained from cats presenting nervous signs, but did not rise by addition of complement. An HI antibody could be detected in a cat with upper respiratory signs and the two samples mentioned above at the same level of titer as SN antibody.

In a cat (No. 0576) which recovered clinically 25 days after inoculation, neither SN nor CRSN antibody was observed in convalescent serum on the 32nd day after inoculation, but an HI antibody was detected in a titer of 1:8, which was maintained for an observation period of 60 days.

Yoshino and Taniguchi [10] first reported CRSN antibody against herpes simplex viruses, which was an early antibody detected in human and rabbit sera. This antibody has also been detected in other viral diseases, e.g., equine arteritis [4, 8], in which it is known as a late antibody. In the present study, neither clinical signs nor SN antibodies were observed in rabbits inoculated i.v. with FHV suspension. These rabbits differed from rabbits inoculated with herpes simplex virus in antibody responses [10]. Furthermore, no significant difference was observed in SN titer between cat serum samples with complement supplemented and such samples without complement, as reported previously [7].
mentioned above, FHV may be unable to produce CRSN antibody, but it will be necessary to investigate it in detail using the early sera of infected cats. Therefore, it is considered that supplementation with complement is not necessary for the routine SN test at present.

There has been only one report on the HI test for FHV [3], which was developed to use only rabbit and goat immune sera. No report has been found as yet on the detection of the HI antibody in cat serum and on serotyping of the virus by the HI test. The HI antibody could be detected specifically in cats in the present study.

It is known by the SN test that there have been two serotypes of the virus (FVR virus and FHV 2) [2]. The seven strains used in the present study are considered to belong to the FVR type viruses [5]. They were proved to be of one serotype by the HI test.

Consequently, the HI test with viral hemagglutinin obtained by ether-treatment is much more available for the sero-diagnosis of FHV infection than the SN test.

References