Post-exposure Treatment of Cats with Mouse-Cat Chimeric Antibodies against Feline Herpesvirus Type 1 and Feline Calicivirus

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(Received 29 March 2002/Accepted 5 August 2002)

ABSTRACT. In order to confirm the in vivo effectiveness of anti- feline herpesvirus type 1 (FHV-1) mouse-cat chimeric antibody (FJH2), and anti-feline calicivirus (FCV) mouse-cat chimeric antibody (F1D7), cats that had been experimentally infected with FHV-1 or FCV were administered intravenously with the chimeric antibodies, and observed for clinical manifestations. The symptoms due to FHV-1 or FCV infection in the cats administered FJH2 or F1D7 were obviously decreased when compared with those of the non-administered control cats. From these results, it was confirmed that both FJH2 and F1D7 were effective at reducing the appearance of symptoms due to FHV-1 and FCV infection, respectively.

KEY WORDS: feline calicivirus, feline herpesvirus-1, mouse-cat chimeric antibody, post-exposure treatment.

FULL PAPER Immunology

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Feline viral rhinotracheitis (FVR) and feline calicivirus infection (FCI) of which the respective causal agents are feline herpesvirus-1 (FHV-1) and feline calicivirus (FCV), are known as major feline viral infections. Although vaccines have been used for the control of these infections, they are of no use for purposes such as urgent prophylaxis and therapy. In accordance with such general limitations of vaccines, the use of other strategies such as passive immunizations and interferons have been proposed for the control or therapy of these viral infections. We reported previously that repeated administration to cats of anti-FCV mouse-cat chimeric antibody (F1D7) did not induce any anaphylactic reactions, whereas repeated administration of cats with mouse monoclonal antibody did [10]. In the present study, we mainly discuss the treatment effects of F1D7 and FJH2 in cats experimentally infected with FHV-1 or FCV.

MATERIALS AND METHODS

Test materials: FJH2 was developed according to the modified method for the development of F1D7 by Umehashi et al. [12]. Briefly, the variable region genes, JH2-VH and JH2-VL of JH2 were isolated from JH2-producing hybridoma cells that had been established against the FHV-1, K1 strain [11]. It was confirmed that JH2-recognizing epitope is didected to gp60 (Hemagglutinin) of the FHV-1, K1 strain (unpublished data).

The heavy chain gene (CB25) [12] and the light chain gene (CE κ) [12] of the feline immunoglobulin constant region were connected with JH2-VH and JH2-VL, respectively. Each of the chimeric heavy and light chain genes were introduced into mouse myeloma cells P3X63Ag8.653(ATCC CRL1580), then screened for stable transformant cells and adaptation cultures of them in a serum free medium (Gibco BRL). F1D7-producing hybrida cells which had been established by the authors [12] were also used for the present study. The culture supernatants of F1D7-producing cells and FJH2-producing cells were harvested, concentrated and applied to protein A column chromatography for purification of F1D7 and FJH2.

Virus neutralization tests: Chimeric antibodies, FJH2 and F1D7 and their parental mouse antibodies, JH2 and 1D7 were used as test and control materials, respectively. The K1 strain of FHV-1 and C-14 strain (ATCC VR-653) of FCV were used as the challenge viruses for the virus neutralization tests. Serial two-fold dilutions were made for the test and control materials in which the protein concentrations were previously adjusted to 100 µg/ml with phosphate-buffered saline (PBS).

Challenge virus solutions were prepared to contain 1014 50% tissue culture infectious dose (TCID50)/ml (100 TCID50/25 µl) with the same buffer used for the dilution of test and control materials. To each the serial two-fold dilution of test or control material was added with the same volume of challenge virus solution, then the mixtures were incubated at 37°C in 5% CO2 atmosphere for 60 min. Each incubated mixture solution (50 µl) was inoculated on to Crandell-Rees Feline Kidney (CRFK) cell monolayer wells of 96-well microplates, incubated at 37°C in 5% CO2 atmosphere for 7 days, and then observed for CPE. The virus-neutralizing antibody titer was expressed as the reciprocal of the dilution of the test or control materials that showed 50% CPE inhibition (ED50/25 µl).

Post-exposure treatment of cats with FJH2 and F1D7: Sixteen week old specific pathogen free (SPF) cats introduced from Liberty Laboratory were monitored for their general condition and weight during the 6-day period of quarantined taming. After confirming that their health was satisfactory, we employed a total number of 21 cats in the tests. Their weights at the time of the experiments were

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KEY WORDS: feline calicivirus, feline herpesvirus-1, mouse-cat chimeric antibody, post-exposure treatment.

Table 1. Standard of observation

<table>
<thead>
<tr>
<th>Item</th>
<th>Score</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activity</td>
<td></td>
<td>normal</td>
<td>disappear a little</td>
<td>disappear</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Appetite&lt;sup&gt;al&lt;/sup&gt;</td>
<td></td>
<td>≥90 g</td>
<td>50 g–90 g</td>
<td>&lt;50 g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drinking&lt;sup&gt;bl&lt;/sup&gt;</td>
<td></td>
<td>≥125 ml</td>
<td>50 ml–125 ml</td>
<td>&lt;50 ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feces</td>
<td></td>
<td>normal</td>
<td>soft</td>
<td>diarrhea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vomiting</td>
<td></td>
<td>–</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>General condition</td>
<td></td>
<td>normal</td>
<td>sensitive/piloerection</td>
<td>trembling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dehydration&lt;sup&gt;cl&lt;/sup&gt;</td>
<td></td>
<td>promptly</td>
<td>2–5 sec</td>
<td>6–20 sec</td>
<td>≥ 21 sec</td>
<td></td>
</tr>
<tr>
<td>Sneezing&lt;sup&gt;dl&lt;/sup&gt;</td>
<td></td>
<td>0</td>
<td>1–4 times</td>
<td>5–9 times</td>
<td>≥ 10 times</td>
<td></td>
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<tr>
<td>Stalorrhea</td>
<td></td>
<td>–</td>
<td>a little</td>
<td>much</td>
<td>purulent</td>
<td></td>
</tr>
<tr>
<td>Ophthalmia</td>
<td></td>
<td>–</td>
<td>Conjunctivitis</td>
<td>purulent Conjunctivitis</td>
<td>corneal ulcer</td>
<td></td>
</tr>
<tr>
<td>Rhinorrhea</td>
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<td>watery (a little)</td>
<td>watery (much)</td>
<td>purulent</td>
<td></td>
</tr>
<tr>
<td>Muzzle ulcer</td>
<td></td>
<td>–</td>
<td>bulla/reddening</td>
<td>skin deficit</td>
<td>ulcer</td>
<td>erosive ulcer</td>
</tr>
<tr>
<td>Stomatitis&lt;sup&gt;el&lt;/sup&gt;</td>
<td></td>
<td>–</td>
<td>bulla/reddening</td>
<td>ulcer (≥ 2 places)</td>
<td>ulcer (≤3 places)</td>
<td>erosive ulcer</td>
</tr>
<tr>
<td>Respiration</td>
<td></td>
<td>normal</td>
<td>nasal alar breathing</td>
<td>dyspnea</td>
<td>two-phase</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Food: 100 g/day.  
<sup>b</sup> Water: 250 ml/day.  
<sup>c</sup> Time for recovering to the normal condition in the wither-picking up test.  
<sup>d</sup> Observed for 5 min.  
<sup>e</sup> Gum, tongue, lips and palate observed.

1.80–2.25 kg for the FHV-1 infection tests, and 1.35–2.70 kg for the FCV infection tests, respectively. For easing pain of the cats, administration volumes of the chimeric antibodies were considered.

In the FHV-1 infection test, 9 cats were infected intranasally with 10<sup>4.0</sup> TCID<sub>50</sub> of K1 strain of FHV-1. On the 2nd day following the infection, 3 cats in each group were administered intravenously 10 mg/kg or 30 mg/kg of FJH2. The remaining 3 control group cats were administered intravenously 2.2 ml/kg of physiological saline.

In the FCV infection test, 12 cats were infected intranasally with 10<sup>7.0</sup> TCID<sub>50</sub> of C-14 strain (ATCC VR-653) of FCV. On the 2nd day following the infection, 4 cats in each group were administered intravenously 5 mg/kg or 10 mg/kg of F1D7. The remaining 4 control group cats were administered intravenously 1.9 ml/kg of physiological saline.

Various items including the clinical symptoms listed in Table 1 were monitored, and scored prior to infection and until the 21st day after infection. All of the scores with regard to each subject were summed, and the average value for each group was calculated. Monitoring of the weight and body temperature was also conducted daily from prior to infection until the 21st day after infection. Throughout the present study, all the cats were kept in a well-controlled atmosphere of 55 ± 5% moisture and 24 ± 1°C for easing their stress, and after the present study, all the cats used were euthanatized by exsanguination under general anesthesia.

Table 2. The virus neutralizing activities of FJH2 and F1D7

<table>
<thead>
<tr>
<th>Virus</th>
<th>Antibodies</th>
<th>Neutralizing activity titers</th>
</tr>
</thead>
<tbody>
<tr>
<td>FHV-1</td>
<td>JH2</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>FJH2</td>
<td>20.8</td>
</tr>
<tr>
<td>FCV</td>
<td>I1D7</td>
<td>16.0</td>
</tr>
<tr>
<td></td>
<td>F1D7</td>
<td>29.3</td>
</tr>
</tbody>
</table>

Antibody concentration of each test material was previously adjusted to contain 100 µg/ml. Titer was expressed as the reciprocal of the dilution showing 50% CPE inhibition (ED<sub>50</sub>/25 µl) of the test materials.

RESULTS

**Virus neutralization tests:** The neutralizing antibody titers of FJH2 and F1D7 were comparable to or rather higher than those of the parental mouse monoclonal antibodies JH2 and I1D7, and no declines in the neutralizing activities were observed (Table 2).

**FHV-1 infection tests:** The cats of the control group to which was physiological saline administered began to show clinical symptoms on the 3rd day post-infection, and the onset reached a peak on the 9th day. In contrast to this, the cats of the groups that were administered 10 mg/kg or 30 mg/kg of FJH2 were almost entirely free of the onset of symptoms (Fig. 1). Furthermore, the control group cats
showed a clear weight loss until the 10th day post-infection, but the cats of the FJH2 groups did not show such weight loss and steadily gained weight (Fig. 2). Neither the control group cats nor FJH2 administered group cats showed any obvious increase in body temperature (data not shown).

**FCV infection tests:** In the cats administered physiological saline, the onset of clinical symptoms reached a peak on the 5th day post-infection. In contrast to this, the cats receiving 5 mg/kg or 10 mg/kg of F1D7 were almost entirely free of the onset of symptoms (Fig. 3). However, the average scores of FCV infections were fewer than those of FHV-1 infections. The only common symptom observed in all the cats infected with FCV was stomatitis. Onset of stomatitis in the control group began to emerge on the 2nd day post-infection, and peaked around the 8th to 10th day. In contrast to this, the F1D7-administered groups were almost entirely free of the onset of stomatitis (Fig. 4). In addition body weight loss was observed in the control group until the 6th day post-infection, whereas the F1D7-administered groups demonstrated a steady weight gain (Fig. 5). Neither the control group nor the F1D7-administered groups showed any obvious increase in body temperature (data not shown).
DISCUSSION

In order to develop therapeutic antibodies which are safe for cats and easy to supply, we tried to develop FJH2 and F1D7, because FVR and FCI are major infectious diseases among feline viral infections. We previously reported on F1D7, because FVR and FCI are major infectious diseases for cats and easy to supply, we tried to develop FJH2 and F1D7, because FVR and FCI are major infectious diseases among feline viral infections. We previously reported on F1D7 for SPF cats in repeated administration tests, and found that the substitution of the constant regions of heterologous mouse immunoglobulin could decrease anaphylactic reactions [12].

In the present study, we confirmed the efficacy of the mouse-cat chimeric antibodies F1D7 and FJH2 using cats experimentally infected with FHV-1 or FCV. Declines in virus neutralizing activity of the chimeric antibodies due to felinization of the parental mouse-monoclonal antibody were suspected, but both chimeric antibodies maintained rather higher virus neutralizing activity than that of the parental mouse monoclonal antibody (Table 2). From these facts, we conclude that substitution of the constant region gene of the parental mouse monoclonal antibody into the feline antibody constant region gene had no influence at all on the virus neutralizing activities.

Following the early stage of the manifestations of illness such as anorexia, enervated condition, fever and sneezing, cats with FVR in the field showed salivation, conjunctival edema, spasmodic cough, secretion/excretion from eyes/nose, and opening breathing due to rhinostenosis [6]. In serious cases dehydration and weight decrease are also shown. Ulcers on the tongue and oral mucous membranes are also recognized in FVR, but the frequency and degree of these manifestations seem to be lower and not as serious as those observed in FCI [7]. In the present studies, SPF cats infected with FHV-1 did not show obvious fever, but all observations agreed well with those observed in cats with weak pathogenic strain infections of FCV in the field. There was no difference in the suppression of stomatitis between administration doses of F1D7 of 5 mg/kg and 10 mg/kg.

These results indicate that both the experimental infections of FHV-1 and FCV in SPF cats reflected the field states, and the clinical symptoms of these infections were reduced by the post-exposure treatment with FJH2 and F1D7.

In conclusion, it seems difficult to distinguish between FVR and FCI by clinical diagnosis [7, 8], and it is also known that mixed infections of FHV-1 and FCV are present in the field. Furthermore, the risk of the outbreak of these infections seems to be high in cat breeding firms where a large number of cats are bred with intensive conditions of entrance and exit [1, 2, 9]. Taking into consideration the prevalence of FVR/FCI in the field, a mixture of FJH2 and F1D7 would be extremely useful for the treatment of these infectious diseases. There have been no reports on intravenous treatment with antibodies against local viral infections in animals. Although there have been some reports regarding intravenous antibody-treatment against viral infections in humans [3], those targets were systemic infections and not local ones. It is very interesting that intravenous treatment of cats with virus-specific chimeric antibodies in the present study resulted in obvious suppression of the clinical symptoms after experimental infections of FCV and FHV.

ACKNOWLEDGEMENTS. The authors thank Dr. Yukinobu Tohya (Department of Veterinary Microbiology, Faculty of Agriculture, Tokyo University) for providing 1D7 hybridoma cells, and Mr. Kiyoto Nishiyama and Miss Yoko Tomita (The Chemo-Sero-Therapeutic Research Institute) for their support in the present study.

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