Differences in Acute and Convalescent-Phase Antibodies of Cats Infected with Feline Picornaviruses

RICHARD G. OLSEN, DONALD E. KAHN, EDWARD A. HOOVER, NANCY J. SAXE, AND DAVID S. YOHN

Department of Veterinary Pathobiology, Ohio State University, Columbus, Ohio 43210

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Complement fixation inhibiting and complement-fixing antibodies were demonstrated in sera from cats during the acute and convalescent stages, respectively, of feline picornavirus infections. Complement-fixing antibody activity was present in 19S and 7S globulins, whereas complement fixation inhibiting antibody activity was confined to the 7S globulins. Sera with complement-fixing antibody and sera with complement fixation inhibiting antibody were also shown to have neutralizing and precipitating antibodies. In a study of antibody responses to homotypic and heterotypic strains of feline picornavirus, less serological cross-reactions were observed between strains of feline picornavirus in sera from cats in the acute phase of feline picornavirus infection than in the convalescent phase. The temporal relationship of complement fixation inhibiting and complement-fixing antibody responses of cats after acute viral respiratory infections is contrasted with previously observed antibody response in cats to feline leukemia virus.

It is generally recognized that most animal species produce various classes and subclasses of immunoglobulins (Ig) that differ in functional properties. Benacerraf et al. (2) demonstrated distinguishing biological and serological properties between guinea pig IgG1 and IgG2 subclasses; the latter fixed complement, whereas the former mediated local anaphylaxis. Recently, numerous investigators have demonstrated nonprecipitating antibodies in dogs (16), guinea pigs (19), and non-complement-fixing (non-CF) antibody in cats (15), horses (12), man (20), and monkeys (6). Few studies (12) have examined, however, the sequential and potential roles of functionally different antibodies in animals during viral infection.

Previous studies in our laboratory (13) have shown that cats respond to C-type ribonucleic acid viruses by producing a non-CF 7S antibody specific for feline leukemia virus group-specific antigen, but a CF antibody species with similar affinity has not been detected. The significance of the appearance of only non-CF immunoglobulin in the pathogenesis of an infectious neoplastic disease has yet to be established, but could have potential bearing on the evolution of the host-virus relationship.

To extend our observations on the development of cats’ immunological response and evaluate the biological significance of the different types of antibodies that arise after stimulation with various antigens, the serological responses of cats infected with feline picornaviruses (FPV) were examined. FPV are cytoplastic viruses that cause acute respiratory tract infections. The severity of the resultant clinical illness has been shown to vary with the virulence of the FPV strain involved (E. A. Hoover and D. E. Kahn, manuscript in preparation). Neutralizing antibodies occur in postexposure sera (11), but identification of other functional antibody species has not been reported. In this study the humoral antibody responses were determined sequentially in cats undergoing FPV infections.

MATERIALS AND METHODS

Animals. Cats used in this investigation were obtained from a specific pathogen-free (SPF) breeding colony developed in the Department of Veterinary Pathobiology of Ohio State University. The cats are of caesarian-derived, gnotobiotic ancestry (18) and have been maintained in isolation as a closed colony free of spontaneous infectious diseases since 1966. Cats for this study were between 3 and 4 months of age and were housed in separate cages in a room isolated from other cats. Neither FPV nor antibody to picornavirus could be demonstrated prior to inoculation.

Viruses. The viruses used in this study belong to the genus Calicivirus, according to the recent proposal of the Internation Commission for the Nomenclature of Viruses which would create three separate subgroups of the agents belonging to the picornavirus family (23).

Two virus strains of FPV were tested in this study, isolate no. 255 (FPV-255) (7, 10) and strain F-9
Animal inoculation. For group 1 six cats were exposed to aerosol of FPV-F9 and observed for 35 days. On postinoculation day 35, the cats were exposed by aerosol to FPV-255. For group 2 twelve cats were exposed to aerosol to FPV-255 only.

Viral exposures were accomplished by enclosing the head of the cat within a 7-inch (17.78-cm) diameter plastic chamber (11) into which an aerosol of the appropriate viral stock was generated by forcing air through a hand-operated DeVilbis no. 40 nebulizer for 2 min. The FPV-F9 and FPV-255 inocula contained 10^4.8 and 10^4.4 of mean tissue culture infective doses per 0.1 ml, respectively. The amount of inoculum aerosolized was 0.2 ml per cat. Control cats were exposed to an equivalent volume of aerosolized supernatant fluid from a Crandell feline embryonic kidney (CRFK) cell culture that had been subjected to three successive cycles of freezing and thawing. The CRFK cell line was used for production of the viral stocks.

Serum samples were collected from each cat in group 1 prior to FPV-F9 exposure and on postinfection days (PID) 7, 14, 21, 28, 35, and 42. Three cats were also sampled on PID 49. Serum was collected from cats in group 2 prior to FPV-255 exposure and at intervals varying between 2 and 21 days after inoculation. All sera were heat-inactivated for 30 min at 56°C.

Preparation of viral antigen. Viral antigen for CF, complement fixation inhibition (CFI), and precipitin tests was produced in CRFK cell monolayers grown in glass roller bottles. The cells were grown in Eagle minimal essential medium plus 1% amino acids, 1% glutamine, 0.5% lactalbumin hydrolysate, and 10% fetal bovine serum. The serum concentration was reduced to 2% on preconfluent monolayers infected with virus.

When cytopathic changes typical of picornavirus infection involved 75% of the cell monolayer (36 to 48 h after infection), the cultures were lysed by three consecutive cycles of freezing and thawing and clarified by low-speed centrifugation for 15 min. Both FPV-F9 and FPV-255 antigen preparations contained 10^4.3 mean tissue culture infective doses per 0.1 ml.

Virus isolations from nasal, ocular, and pharyngeal swabs collected during the postexposure period confirmed that cats became infected after exposure to FPV-F9 and FPV-255.

Sero logical tests. The CF test utilized 25 aliters containing 6 CF units of viral antigen, 25 aliters of heat-inactivated cat serum, and 3 complement hemolytic dose 50 (CHs0) units of guinea pig complement (50 aliters). Antiserum, antigen, and complement were incubated at 4°C for 16 h and subsequently mixed with 25 aliters of optimally sensitized sheep red blood cells. The CF antibody titer was expressed as the highest dilution of serum that gave a 3+ or greater fixation of complement.

The CFI tests consisted of incubating 2 CF units of viral antigen with varying dilutions of heat-inactivated cat (test) serum for 16 h at 4°C. Two CF units of reference goat anti-FPV serum and 5 CHs0 of guinea pig complement were added to the test mixture, and incubation was continued for an additional 16 h at 4°C. Sensitized sheep red blood cells were subsequently added, and the tests were incubated for 30 min at 37°C. After centrifuging the plates at 800 x g for 1 min, the CFI antibody titers were scored. The antibody titer was expressed as the highest serum dilution that inhibited the CF reaction between reference antibody and reference antigen by 50% or greater.

The procedure for preparing the CF and CFI tests simultaneously on the same microtiter plate with microtiter needles and loops (Cooke Engineering Co., Alexandria, Va.) was previously described (13). Controls for the CF and CFI test included anticomplementary control for reference and test serum and viral antigen. Control antigens included 25% homogenate of packed CRFK cells and fetal calf serum. CFI reference antigens were titrated against homologous reference serum by the block "checker board" method. The reference sera were goat anti-FPV-255 (10) and cat anti-FPV-F9. Both reference sera were adsorbed with fetal calf serum and were shown to be negative in the CF test against fetal calf serum and control cellular antigen.

Micro-immunodiffusion tests and immunoelectrophoresis tests were performed by previously described methods (14).

For the serum neutralization (SN) test, sera were serially diluted in fourfold increments with Eagle minimum essential medium. Equal volumes of each serum dilution and a preparation of virus (FPV-F9 or FPV-255) calculated to contain 10^6 mean tissue culture infective doses were mixed and incubated for 1 h at room temperature. Each serum-virus mixture (0.2 ml) was distributed into each of four monolayer cultures of CRFK cells. The cultures were grown in Limbro Disposo Plates (FB-16-24TC) (Linbro Chemical Co., New Haven, Conn.) and maintained in an atmosphere supplemented with 5% CO2. Final readings of the tests were made on the fifth day and the median neutralizing titers were calculated by the method of Spearman and Karber (5).

Separation of 19S and 7S antibodies by gel filtration. Whole cat serum (2 ml) was applied to a G-200 Sephadex (Uppsala) column (2.5 by 45 cm) and chromatographed at a constant flow rate of 5.0 ml/hr. Eluates were monitored at 280 nm with an ultraviolet analyzer and collected in 2-ml portions. All protein peaks were concentrated back to the original volume by dialysis against an aqueous solution of Aquacide III (Calbiochem, Los Angeles, Calif.).

RESULTS

Disease induced. The 12 cats exposed to FPV-255 developed clinical disease characterized by fever, malaise, anorexia, oral ulcerations, and pneumonia (10). Cats exposed to FPV-F9 developed small ulcerations of the mucosa of the tongue or nostril (Kahn and Hoover, unpublished data). Improvement in clinical condition and resolution of the oral and pulmonary lesions were apparent by postinoculation day (PID) 10. The convalescent phase of FPV infection, therefore, was considered to be PID 10 and beyond.

Humoral antibody response to FPV. Fig-

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ures 1A and B summarize the mean CF and CFI antibody responses of six SPF cats infected with FPV-F9 at PID 0 and challenged with FPV-255 at PID 35. In all six cats infected with FPV-F9 (Fig. 1A), CFI antibodies to homotypic viral antigens were detected on PID 7. The peak mean CFI titer (approximately 1:30) occurred between PID 7 and PID 14. The range was 1:8 to 1:64. All cats had a CFI antibody titer of ≥1:2 through PID 42.

Figure 1B summarizes the CF responses in the six cats infected with FPV-F9. Three of the six cats developed CF antibodies of ≥1:32 by PID 21. The remaining three animals produced humoral CF antibody to FPV-F9 by PID 28, 35 and 42, respectively. Although neutralizing antibodies (Fig. 2A) appeared in the sera of FPV-F9-infected animals at the same time as CFI antibody, they persisted throughout the observation period.

The CFI and CF responses of 12 FPV-255-infected cats was similar to the response of cats exposed to FPV-F9. CFI and SN antibodies appeared during the acute phase of the infection followed by the replacement of CFI antibody by CF antibody in the convalescent phase of FPV-255 infection.

The six FPV-F9-infected cats were challenged (10^5.5 mean tissue culture infective doses) with FPV-255 at PID 35. These animals were clinically protected after exposure to FPV-255; however, the serological response (Fig. 1A and B) did not indicate a relative change in the CFI/CF antibody, i.e., CF antibody titers continued to rise and CFI antibody continued to diminish.

Although FPV-F9 appears to elicit immuno-
protection against the more virulent FPV-255 strain, serological studies with CF/CFI and SN tests indicate differences among the two strains. Figure 2A represents the homotypic response of a single cat to FPV-F9 antigen, and Fig. 2B represents the serological results after testing the same sera against heterotypic (FPV-255) virus antigen. CFI and SN cross-reacting antibodies to FPV-255 appeared in sera collected on PID 21 and PID 35, respectively (Fig. 2B). By contrast, CFI and SN to homologous virus antigen was evident by PID 7. However, the time of appearance of CF antibody to homotypic and heterotypic virus was nearly the same (Fig. 2A and B). In the five other cats infected with FPV-F9, the antibody response to FPV-255 antigen was similar.

Serological specificity of CF and CFI tests. The CFI reference sera, goat anti-FPV-255 and cat anti-FPV-F9, contained no CF activity when tested with 25% homogenate of CRFK cells and fetal calf serum. In addition, selected cat test sera from both FPV-255- and FPV-F9-infected cats were tested for CF antibody to the same control antigens with negative reactions. To confirm the specificity of the CFI test, the order of addition of CFI-positive test (cat) serum and reference antibody was reversed, i.e., reference antibody was incubated with reference antigen before the addition of test serum. The CF titers of three of the test cat sera were reduced by ≥3 units, one serum was reduced by 2 units, and one serum was reduced by 1 unit, therefore demonstrating the inhibition effect of cat antibody as being viral antigen dependent.

Comparison of SN test with CF and CFI tests. Thirty-nine cat sera (immune and nonimmune) were tested for SN and CF or CFI antibodies to determine the relative degree of correlation between the two types of tests (Table 1). Of the 16 preimmune sera tested, no antibody titers were detected by the SN test (≥1:4) or by CF and CFI tests (≥1:2). The 23 cat sera from FPV-infected cats (≥7 PID) all had SN antibodies and all but one contained CF or CFI antibodies.

Characterization of CF and CFI antibodies by gel filtration. Table 2 summarizes the serological tests from fractions of the PID 42 serum sample from one cat. This serum was chosen because it contained both CF and CFI antibody activity to FPV-F9 and FPV-255 viruses. CF antibody to homologous antigen was detected in the 19S (1:32) and the 7S (1:128) protein peak. The CFI antibody, however, was detected only in the 7S fraction. Similar fractionation of a cat serum (PID 7) that contained only CFI antibody demonstrated only CFI antibody to FPV-F9 antigen in the second (7S) peak. Immunoellectrophoresis of the Sephadex fractions revealed that the first protein peak was a beta-globulin with trace amounts of IgG. The 7S (second peak) fraction was composed of at least two heterodispersed precipitin arcs characteristic of IgG.

Precipitin activity in CF and CFI antibody. In the immunodiffusion test 13 cat sera, 6 with CF antibody of ≥1:4 and 7 with CFI antibody of ≥1:16, were tested for precipitin activity to homologous antigen (Table 3). All 13 cat sera

<table>
<thead>
<tr>
<th>Material tested</th>
<th>PDI 7</th>
<th>PDI 42</th>
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<tbody>
<tr>
<td></td>
<td>CF</td>
<td>CFI</td>
</tr>
<tr>
<td>Whole serum</td>
<td>&lt;1:2</td>
<td>1:32</td>
</tr>
<tr>
<td>G-200 Sephadex gel filtration</td>
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</tr>
<tr>
<td>Peak I</td>
<td>&lt;1:2</td>
<td>1:2</td>
</tr>
<tr>
<td>Peak II</td>
<td>&lt;1:2</td>
<td>1:8</td>
</tr>
<tr>
<td>Peak III</td>
<td>&lt;1:2</td>
<td>&lt;1:2</td>
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</tbody>
</table>

*Whole serum was obtained from a FPV-F9-infected SPF cat.

*Peaks were determined by adsorbance at 280 nm.

<table>
<thead>
<tr>
<th>Antibody activity in cat sera with CF or CFI antibodies to FPV</th>
<th>Reaction in the ID test</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF antibody</td>
<td>6/6</td>
</tr>
<tr>
<td>CFI Antibody</td>
<td>7/7</td>
</tr>
<tr>
<td>Neither</td>
<td>0/5</td>
</tr>
</tbody>
</table>

*Formed at least one precipitin line in the immunodiffusion (ID) test with homologous antigen.

*All sera tested had a CF titer to homologous antigen of ≥1:16.

*All sera tested had a CFI titer to homologous antigen of ≥1:4.

*These sera had neither CF or CFI antibody titers of ≥1:2.
formed one to three lines with appropriate antigen. Of the five sera tested with neither CF or CFI antibody none had precipitin antibodies to FPV-255 or FPV-F9 antigen.

Hyperimmune goat sera (anti-FPV-255) formed a single line of identity with antigens FPV-255 and FPV-F9. The line of precipitation formed by goat serum to FPV antigen was demonstrated to be continuous, with one of three precipitin lines developed by cat anti-FPV-F9 and cat anti-FPV-255.

**DISCUSSION**

The clinical disease associated with FPV infection in cats became evident by PID 2 as either oral ulcerations (both viruses) or pneumonia (FPV-255 only) (Kahn and Hoover, unpublished data). Transient viremia was demonstrated on PID 3 and 4 with FPV-255 (11). Resolution of the clinical signs and lesions of the disease were apparent by PID 10 (11), which we considered the onset of convalescence. Our results show that cats produce relatively high titers of humoral antibody to FPV by PID 7. This suggests that humoral immunity may be an important factor in recovery from FPV infection. The antibody detected in the early stage of FPV infection (PID 7) was non-CF, whereas sera from cats in the convalescent phase readily fixed guinea pig complement. Both the CF and non-CF antibody precipitated soluble viral antigen and neutralized virus.

The sequential appearance of CFI and CF antibody to FPV is similar to that reported by Perryman et al. (17) in cats immunized with various noninfectious antigens. By contrast, the humoral antibody response in cats to feline leukemia virus (FeLV) appears to be different. Olsen and Yohn (15) reported that FeLV-immunized cats and cats with FeLV-induced tumors produced only a 7S CFI antibody to viral core antigens.

The differences in the serological responses in the cat to an acute viral disease (FPV) as opposed to an oncogenic viral disease (FeLV) may be due to the differences in interreaction between virus and host lymphoid cells. Further research is needed to determine whether viral antigen behave similarly to more defined antigen. However, it is becoming recognized that the cellular events that culminate in the complete (primary and secondary) antibody response require the interreaction of T lymphocytes and B lymphocytes with immunogens (4). B cells respond to immunogenical determinants by producing IgM type antibody (primary response); however, the switch to IgG antibody (secondary response) requires a helper function from T lymphocytes (1). Additional studies are needed to determine whether the 7S CFI antibody to FPV contains gamma or Mu heavy-chain antigenic specificites and to determine whether T cell collaboration is needed to poteniate the switch from CFI to CF antibody.

The failure for cats horizontally infected with FeLV (9) to switch from CFI to CF antibodies (15) may be due in utero exposure (8) of the cat to homotypic C-type ribonucleic acid viruses, whereas endogenous nononcogenic (22) or oncogenic (9).

Other investigators (3) have reported considerable cross-reactivity between various FPV strains using hyperimmune sera in SN tests. However, the feline anti-FPV antibody elicited during the acute stage of infection by aerosol exposure (the probable route of natural infection) demonstrates little cross-reactivity with heterotypic FPV-255 antigen in the CFI and SN tests. Low titers of cross-reacting CFI antibody to heterotypic antigen were present by PID 21. In the immunodiffusion test hyperimmune antiserum capable sera formed only one precipitin line against homotypic and heterotypic antigen, whereas feline precipitins detected at least two FPV antigens. Similar findings that showed differences in the precipitin reactivity of hyperimmune and convalescent sera have been reported by Schmidt and Lennette (21) with Coxsackie viruses.

These data suggest, therefore, that sera taken from cats in the clinical phase of picornavirus infection may be more specific typing reagents, whereas convalescent sera or hyperimmune may be less type-specific.

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**LITERATURE CITED**


