Natural Feline Leukemia Virus Infection and the Immune Response of Cats of Different Ages

Chris K. Grant, M. E. Essex, M. B. Gardner, and W. D. Hardy, Jr.

ABSTRACT

Forty-two kittens and 28 adult cats were placed as tracers in leukemia cluster environments in contact with resident cats, 30% of which were persistently infected with feline leukemia virus (FeLV). After 7 months exposure, FeLV viremia had been detected in 71% of the tracer kittens, although only 55% of these remained persistently infected; in the same period, 11% of tracer adults became infected, but by 2 years the figure reached 43%. Mean latent periods before detection of viremia were 3.4 ± 1.8 (S.D.) and 13.0 ± 5.9 months for kittens and adults, respectively. First detection of FeLV infection was accompanied by a sharp although transient drop in peripheral white blood cell numbers, and infection onset triggered the humoral immune response which was comprised of separate antibodies with virus-neutralizing and tumor lysis activities. High titers of virus-neutralizing antibody appeared in transiently viremic cats immediately following elimination of viremia; this antibody was rarely detected in cats that remained persistently viremic. Lytic complement-dependent antibody to feline oncornavirus-associated cell membrane antigen appeared in most cats 1 to 2 weeks after FeLV infection was first detected, and subsequently high titers of this antibody remained in both transiently and persistently infected cats. If the rate of FeLV infection was summarized by using viremia and/or antibody appearance, then 95% of the kittens became infected within 1 year and 61% of the adults within 2 years. Adult cats are, therefore, susceptible to FeLV infection following natural exposure, and their apparent resistance cannot be attributed to a protective humoral immune response that developed immediately after exposure commenced.

INTRODUCTION

FeLV4 is infectious among cats, thus exemplifying a horizontally transmitted oncornavirus of an outbred species (5, 22, 25, 29, 33). Infection results after contact between individuals, and saliva is the most likely vehicle for virus transmission (13). In MCH's there is a high incidence of FeLV infection and within such environments leukemia clusters frequently arise (4, 9, 14, 22). Cats which remain healthy in MCH environments commonly have detectable antibody to FeLV and also to virus-induced, transformation-specific FOCMA (4, 8, 9, 16, 19, 23, 37). Prospective studies have shown that FOCMA antibody is not detected in cats which subsequently develop tumors and that high titers of antibody protect cats from tumor development in most cases (4, 9, 10, 16). The humoral effector mechanism involved appears to be CDA, since this FOCMA-CDA reactivity is not directed at FeLV, but it does lyse FOCMA-bearing cat tumor cells in the presence of cat complement (1, 8, 16, 17, 39). In this system, we have been unable to detect antibody-dependent cellular cytotoxicity with FOCMA immune sera and cat effector cells.5

Following virus inoculation, young kittens are most susceptible to the pathogenic effects of FeLV and the closely related feline sarcoma virus (8, 16, 26). Results reported here show that young cats are more rapidly infected following natural contact exposure, but that older cats become infected after a protracted exposure period. In most cats, the onset of detectable viremia was found to be directly associated with a drop in circulating WBC numbers and, after a short delay, with the independent appearances of FOCMA-CDA and/or VNA. A minority of cats reverted to the leukemia virus-negative state after a transient viremia which was terminated by the appearance of VNA. While the latent period before infection became established was longer in adults than in kittens, no evidence was found to suggest that this resulted from the ability of older cats to mount a protective humoral immune response shortly after exposure commenced.

MATERIALS AND METHODS

Cats. The private MCH's studied were located in California, Connecticut, and Massachusetts. Resident cats were predominantly random bred and of domestic short or long hair varieties; most were spayed or castrated, and >20% were related. Persistent FeLV infection was confirmed in 25, 27, and 32% of the resident cats in the separate MCH's used in this study. As tracers, 42 kittens (3 to 4 months old) and 28 adults (≥6 months old) were placed in the MCH's and were allowed to intermix freely. The kittens were raised in specific-pathogen-free breeding colonies and screened for preexisting FeLV infection or immunity prior to, or immediately after, MCH entry. Only tracers remaining in the MCH's continuously for ≥12 months are reported. At regular intervals, all cats were examined clinically, and jugulovenous blood samples were removed

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2 Scholar of the American Cancer Society (Massachusetts Division). To whom requests for reprints should be addressed.
3 Scholar of the Leukemia Society of America.
4 The abbreviations used are: FeLV, feline leukemia virus; MCH, multiple cat household; FOCMA, feline oncornavirus-associated cell membrane antigen; CDA, complement-dependent antibody; FOCMA-CDA, complement-dependent antibody to feline oncornavirus-associated cell membrane antigen; VNA, virus-neutralizing antibody; GSA, group-specific antigens; FIP, feline infectious peritonitis.
5 J. McCartney and C. K. Grant, Manuscript in preparation.

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for testing. The mean point between 2 consecutive tests, in
which the second revealed infection or immunity, was taken as
the conversion time from commencement of exposure.

**FeLV Infection.** The presence of FeLV GSA in fixed blood
smears was determined by immunofluorescence (20). Positive
results from this test correlate ≥95% with other tests for
presence of FeLV, e.g., infectious virus isolation and electron
microscopy (14, 20, 27). The existence of transient viremia
was confirmed by virus isolation using the focus-forming assay
with CCC81 cells (11).

VNA. This procedure was modified slightly from that de-
scribed by Schaller and Olsen (38). Serum dilutions (1 :5, 1 :1:
25, 1:125, and 1:625) were tested for their ability to neutralize
subgroup A, virus, harvested from the feline lymphoblastoid
line F442 (35), prior to infection of CCC81 cells (11). Serum
dilutions causing neutralization of 50% of the foci num-
ers detected in control plates were considered positive.

**Antibody to FOCMA.** Serum antibody activity was titrated (at
doubling dilutions of 1:2 to 1:256) using immunofluorescence
(8) and 51Cr release (15, 17) as previously described, except
that the 51Cr release assay was performed in microcytotoxicity
plates using the Flow semiautomated Titertek harvesting sys-
tem (Flow Labs, Inc., McLean, Va.). Target cells for both assays
were the feline FL74 lymphoblastoid cell line F442 (35), prior to infection of CCC81 cells (11). Serum
dilutions causing neutralization of 50% of the foci num-
ers detected in control plates were considered positive.

**Rate of FeLV Infection in Tracer Cats.** Tracers (42 kittens
and 28 adult cats), which were negative for FeLV GSA and
FOCMA-CDA, were placed in MCH's where 25 to 32% of the
resident cats were FeLV GSA positive. As controls, 20 cats
were isolated in laboratory environments free of FeLV infection;
throughout the study period, these controls remained FeLV
GSA negative and did not develop FOCMA-CDA or VNA.

The rates of FeLV infection of the tracers are shown in Chart
1. By 7 months of continuous contact with MCH residents, 30
of the 42 tracer kittens (71%) became FeLV GSA positive,
although only 23 (55%) remained persistently viremic until
death or experimental termination. The 7 kittens which became
FeLV GSA positive for a short period [mean, 3.2 ± 1 (S.D.)
weeks] thereafter returned permanently to the FeLV GSA-neg-
ative state. In future discussion, these cats are classed sepa-
ately as transient viremics, although in Chart 1 and Table 2
they are included among the FeLV GSA-positive cats.

Infection of adult tracers occurred more slowly (Chart 1).
After 7 months, only 11% of these cats were FeLV GSA
positive, but after 2 years of continuous exposure 43% of the
tracers had become infected. In this group of adult tracers,
evidence was also found for 3 transiently viremic cats, but,
insomuch as intervals between removing samples were longer
than those used for kittens, we were unable to establish the
duration of transient viremia in the cases.

Mean intervals between commencement of exposure and
appearance of FeLV viremia in the groups of tracers are sum-
marized in Table 2.

**Antibody Responses of Tracer Cats.** Several classes of
response to exposure could be distinguished within the tracer
kittens according to (a) the susceptibility to FeLV infection and
(b) the subsequent appearance of humoral immunity in the form
of VNA and/or FOCMA-CDA. The number of kittens exhibiting
each type of response is shown in Table 3, and from these data
it is apparent that detectable VNA and FOCMA-CDA immune
responses occurred independently. While most kittens (17 of
19) that resisted persistent FeLV infection (i.e., FeLV GSA
detected transiently or not at all) developed VNA, this antibody
was rarely detected in cats which succumbed to persistent
viremia. On the other hand, FOCMA-CDA was detected in 69%
of all exposed kittens irrespective of their FeLV GSA status.

First detection of serum antibodies in individual tracers varied
with the onset of FeLV infection, and data from tracer kittens
are exemplified in Table 4. Neither FOCMA-CDA nor VNA

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**Table 1**

<table>
<thead>
<tr>
<th>Household</th>
<th>Total no. of cats</th>
<th>FeLV GSA only</th>
<th>FOCMA-CDA only</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>22</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>15</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>C</td>
<td>22</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>D</td>
<td>18</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>E</td>
<td>50</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>F</td>
<td>56</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td>G</td>
<td>12</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>173</td>
<td>21</td>
<td>32</td>
</tr>
<tr>
<td>Total (Households B to G combined)</td>
<td>12.1</td>
<td>18.5</td>
<td>43.4</td>
</tr>
</tbody>
</table>

* The remaining 26% of cats in Households B to G were negative for FeLV GSA and FOCMA-CDA.
Immunity following FeLV Infection

Table 2

<table>
<thead>
<tr>
<th>Age at initial exposure</th>
<th>3-4 mos.</th>
<th>6-60 mos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>FeLV GSA detected(\text{a})</td>
<td>71</td>
<td>43</td>
</tr>
<tr>
<td>Mean conversion time (mos.)</td>
<td>3.4 ± 1.8</td>
<td>13.0 ± 5.9</td>
</tr>
<tr>
<td>FOCMA-CDA detected</td>
<td>69</td>
<td>50</td>
</tr>
<tr>
<td>Mean conversion time (mos.)</td>
<td>3.9 ± 2.4</td>
<td>13.4 ± 6.2</td>
</tr>
</tbody>
</table>

\(\text{a}\) Mean ± S.D., 18.6 ± 14.8 months.

appeared before FeLV GSA was detected in persistently or transiently infected cats, although these antibodies appeared in a minority of exposed cats that were never viremic (FeLV GSA negative) (see "Discussion"). The mean interval separating the first appearance of FeLV GSA and FOCMA-CDA was 2.4 ± 1.9 weeks. After appearance, FOCMA-CDA titers increased to peak values by 5.0 ± 2.1 weeks later, and in most of these cats (19 of 29) antibody then persisted at peak titer for at least 6 months before any decline was evident. In the remainder, antibody titers dropped after peaking to about one-half the maximum level detected, and then the titer persisted at the lower level.

VNA appeared in serum at the same time as FOCMA-CDA in cats which were never detectably viremic. In all transiently viremic cats, VNA was first detected approximately 1 week after the disappearance of FeLV GSA, although FOCMA-CDA appeared and increased in titer during the transient episode of viremia. In individual cats, no correlations were apparent between FOCMA-CDA and VNA in regard to detection or to the quantitative levels of the antibodies.

The immune responses of adult cats followed patterns similar to those seen in kittens, except that the interval between commencement of exposure and development of immunity was relatively long. As in kittens, FOCMA-CDA appeared in adults soon after first detection of FeLV GSA (Table 2), and subsequently the magnitude of the antibody responses was of the same order as that detected in young cats.

Ages of adult tracers and the time elapsed to infection and/or development of immunity are summarized in Table 5. In each age group, the numbers of cats are small, but the data suggest a trend in which cats of 12 to 17 months of age require the longest exposure period before becoming infected.

Some cats developed humoral immunity without detectable FeLV infection. If all cats which became FeLV GSA positive and/or immune are considered together, then after 1 year of exposure 95% of kittens had exhibited some form of infection.
or resistance, whereas after 2 years of exposure 61% of the adult tracers had responded in a similar manner.

Geometric mean titers for all antibody-positive cats are compared in Table 6. Mean FOCMA-CDA titers were similar in the FeLV-infected cat groups (residents and tracers) and in the corresponding groups of FeLV-free but immune housemates. In contrast, almost all persistently infected cats lacked detectable VNA, whereas almost all exposed but immune cats had high VNA titers. The mean VNA titer, in the minority of persistently infected cats that had detectable neutralizing antibody, was 1 log lower than the mean titer found in transiently infected individuals after they had eliminated their infection.

Changes in Peripheral WBC Numbers Associated with FeLV Infection. Peripheral WBC counts varied in the individual tracer kittens, but when FeLV GSA was first detected there was a coincident decrease in hemopoietic cell numbers to approximately one-half of the preinfection levels (Chart 2). In transiently viremic cats, the duration of leukopenia was short, and cell numbers returned to normal levels by 4 to 6 weeks. In most cats with persistent FeLV infection, the mild leukopenia lasted for at least 3 months.

Diseases Apparent in FeLV-exposed Tracer Cats. Twelve of the tracer kittens died after entry into FeLV-infected MCH's, and all but one of these were persistently infected with FeLV. Causes of death were nonregenerative anemia (5 cats), FIP (5 cats), and leukemia-lymphoma (2 cats). Adult tracer cats remained essentially healthy. High FOCMA-CDA titers (1:16 to 1:64) were apparent in 3 cats with fatal FIP and one cat which died from anemia, and a low titer of FOCMA-CDA (1:4) was detected in sera removed both before and at death of one tracer with leukemia. No FOCMA-CDA was detected in the cat which developed lymphoma.

DISCUSSION

Laboratory studies have shown that cats inoculated with FeLV at a young age are most susceptible to the pathological effects of the virus (8, 16, 26). Results reported here extend these observations and show that young cats are more susceptible to naturally transmitted FeLV infection, but that many...
older cats eventually become persistently infected after a protracted exposure period. We cannot be sure that protracted and continuous natural exposure is necessary to produce persistent viremia, because we did not remove tracers after only short-term exposure. Nevertheless, it seems likely that long-term exposure plays some role in causing persistent infection, because most pet cats that have been exposed to FeLV are found to be immune but not viremic, and usually these pets are housed singly and exposure occurs through short-term chance contact (10, 16, 19).

The transient FeLV-associated leukopenia which was observed confirms findings by others (7, 33) that infection causes acute disturbances within hemopoietic tissue. These results correlate with the finding that both FOCMA-CDA and VNA appeared in response to an episode of viremia, probably because FeLV must infect and/or transform host cells before it will present an adequate stimulus to induce humoral immunity. It seems likely that those cats which became immune while apparently remaining FeLV GSA negative were actually transiently infected for a very short interval between tests for FeLV. In the case of FOCMA-CDA, which is directed at a tumor or transformation-specific antigen (4, 8, 9, 16, 17), prior virus-induced transformation of host cells would appear to be a prerequisite for stimulation of an antibody response. Conceivably, infection might sometimes occur at a localized site (e.g., the oropharyngeal region) in which case virus would not be detected by the FeLV GSA test performed on peripheral blood smears.

The differing susceptibilities to FeLV infection of young and old cats may be explained by the possibility that young cats are less immunocompetent. If so, the difference involves mechanisms other than humoral immunity, since there was no evidence that most older tracers resisted primary FeLV infection by a rapid production of antibody after placement in FeLV-infected environments. In a separate study, we have seen 2 cats become FOCMA-CDA immune and one cat become FeLV GSA positive after residence in an infected MCH for 3 to 4 years. Inasmuch as we have studied most of the adult tracer cats for approximately 2 years only, it is possible that our figure of 61% total FeLV-infected and/or immune adults (Table 5) is conservative at this time, since in MCH's where FeLV infection has been fully established, 74% of all cats show some sign of infection or immunity (Table 1). Possibly the mode of virus transmission plays some role in governing the latent period between commencement of exposure and onset of infection. Virus is shed in saliva (13, 27), and it has been shown that for infection to occur cats must be caged together rather than singly in juxtaposed cages (25). Apparently, social grooming or sharing of feeding utensils is more important than aerosol transmission, and subjectively we have noticed that the most asocial adult cats seem to be the least readily infected. There seems to be no evidence for a significantly large population of cats which are genetically refractory to FeLV, because almost all (40 of 42) of the exposed kittens developed viremia and/or immunity, and others have shown that all cats inoculated or otherwise exposed to virus become infected or immune as a result (5, 10, 25, 33, 37).

The function of VNA seems to be to terminate FeLV infection, for the antibody was commonly detected in the absence of virus, and in transiently viremic cats it appeared rapidly at or after elimination of viremia. Infection of cats occurs with FeLV A subgroup only or with a mixture of FeLV A and B subgroups (19, 28). Theoretically, cats recently infected with virus A or AB might make VNA to the subgroups concerned and thereby eradicate their viremia (most cats), or they may respond with VNA to the B subgroup thereby remaining viremic with FeLV A (cf. Ref. 30). Serologically, A and B virus subgroups sometimes cross-react in vitro (36), so that VNA directed to B subgroups might be detected in a neutralizing test using A subgroup indicator virus. We believe this explains the apparent paradox that a minority of viremic cats (3 of 21) had low but persistently detectable levels of VNA.

The kittens which became ill following FeLV exposure were almost all viremic, and the diseases were limited to FIP, anemia, and lymphoid tumors. FIP is caused by a coronavirus which may be activated from a subclinical state due to FeLV-associated immunosuppression (4, 5, 16, 32–34). Anemia is also commonly associated with FeLV infection, and, when it is not fatal, the disease may represent a preleukemic state (4, 5, 16, 24, 33). Despite the relatively high FeLV infection rates detected, the appearance of only 2 cases of leukemia-lymphoma was not surprising for 2 reasons. First, the latent period between detection of FeLV GSA and appearance of overt neoplastic disease is protracted and may be as long as 40 months in natural circumstances (12, 16). Second, many of the persistently infected cats developed FOCMA-CDA, and prospective studies have shown that most cats which develop leukemia lack FOCMA antibody at all times prior to tumor appearance; moreover, cats immunized to elicit FOCMA antibody were subsequently resistant to progressive tumor development (4, 5, 8–10, 16, 19). The protective effect of FOCMA-CDA immunity is not absolute, however, since approximately 13% of immune cats had low levels of this antibody and yet developed lymphoid tumors (16–18). In some cases, tumor progression in the face of FOCMA-CDA can be explained by severe depletion of lytic complement activity (18, 31).

The possibility exists that immune mechanisms other than FOCMA-CDA play some role in feline leukemia resistance. Cytotoxicity mediated by antibody-dependent cells has been implicated in tumor resistance in nonfeline systems, but while feline peripheral lymphocytes have antibody-dependent cell-mediated cytotoxicity effector cell activity in systems containing heterologous target cells, they fail to mediate lysis to feline leukemia cells in the presence of FOCMA-CDA immune sera (data not shown). Two other mechanisms which may play roles in feline leukemia immunity are natural killer cells and cytotoxic macrophages. To date, natural killer cells have been detected in unexposed and FeLV-exposed field cats but they also exist in tumor-bearing animals; cytotoxic peritoneal macrophages have been detected only in cats immunized i.p. with allogeneic leukemia cells (16). Inasmuch as cats with FOCMA antibody resist tumor progression (4, 5, 8–10, 16, 19), as CDA is the only cytotoxic mechanism detected in FOCMA immune cats, and as CDA-mediated tumor cell lysis proceeds in culture mixtures containing only cat components, we believe that this complement-dependent lytic mechanism plays a primary role in the immunological defense of outbred cats against lymphoid tumors. One factor which may underlie this emphasis on a B-cell effector mechanism is that FeLV preferentially infects feline T-cells and that the majority of naturally occurring lymphoid
tumors are of T-cell origin (2, 3, 23).

The concept that FOCMA-CDA reactivity is directed at a virus-induced transformation-specific antigen and not to FeLV per se, (1, 6, 16–18, 39) was supported by the finding that geometric mean titers of FOCMA-CDA were the same in both FeLV-viremic and FeLV-free groups of immune cats (Table 6). This finding suggests that FOCMA-CDA is not absorbed in vivo by the large quantities of cell-free FeLV or virus-replicating cells that exist in peripherally viremic cats. Conversely, from data in Tables 3 and 4, it is apparent that cats can maintain persistently high levels of serum VNA without such sera containing any cytotoxic activity to virus-replicating tumor cells (FL74). Unlike FOCMA-CDA, VNA was rarely detected in viremic, and it is probable that in some circumstances FeLV is leukemic on a "hit and run" basis. During transient viremia, the opportunity exists for insertion of the onc or "leuk" gene into FOCMA-CDA. As such, FOCMA-CDA does lyse tumor cells which express only FOCMA and no viral antigens, but this point is relevant to only a minority of feline leukemias because most lymphoid tumors that arise replicate FeLV (12).

The demonstration here of transient viremia following natural FeLV infection may be relevant to that minority of feline leukemia virus-negative tumors (12). Recently, several lines of evidence have emerged to associate transient FeLV infection with a subsequent development of virus-negative tumors (16), and it is probable that in some circumstances FeLV is leukemogenic on a "hit and run" basis. During transient viremia, the opportunity exists for insertion of the onc or leuk gene into DNA of normal feline lymphoid cells, so that at a later date transformation may result in the absence of FeLV replication. The cats most susceptible to developing such tumors may be the minority of transient viremics that developed a protective titer of VNA but failed to produce detectable FOCMA-CDA.

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