Relationship between Feline Leukemia Virus Antigen Expression and Viral Infectivity in Blood, Bone Marrow, and Saliva of Cats

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SUMMARY

Correlation was greater than 90% between feline leukemia virus (FeLV), group-specific antigen (GSA) in leukocytes, and viral infectivity (VI) in serum or plasma from 132 cats infected with either the Rickard strain of FeLV, the Snyder-Theilen strain of feline sarcoma virus, or field strains of FeLV. Detection of GSA in blood cells was at least as sensitive as detection of VI in serum. In 45% of FeLV GSA-positive cats inoculated with FeLV-Rickard strain, VI was detected in saliva. No saliva samples from GSA-negative cats had VI. Sequential bone marrow biopsies from 34 cats inoculated with Snyder-Theilen feline sarcoma virus indicated that the correlation between FeLV GSA in bone marrow cells and blood cells was virtually 100%. FeLV GSA appeared in bone marrow leukocyte precursors 1 week before its appearance in peripheral blood leukocytes in 50% of the cats. The FeLV GSA-positive state was transient (3 to 6 weeks) in 34% of the Snyder-Theilen feline sarcoma virus-inoculated cats.

INTRODUCTION

After infection with FeLV, either of 2 host-virus relationships develops in most cats: (a) the FeLV GSA-positive state in which FeLV GSA is continuously demonstrable in circulating leukocytes (7–12), no virus-neutralizing antibody develops, and variable titers of antibody develop against the feline oncornavirus-associated cell membrane antigen (2–4, 9–12); (b) the FeLV GSA-negative state is maintained, and usually both virus-neutralizing and feline oncornavirus-associated cell-membrane antigen antibodies are produced (3, 4, 9–12, 19). Usually, the GSA-positive state is irreversible, results in development of FeLV-related disease, and is associated with capacity for contagious transmission of FeLV to other cats (2, 8, 10, 12). A likely mode of virus transmission by GSA-positive cats is via saliva. FeLV has been found in salivary gland (6, 7), and upper respiratory epithelium (12) of cats with lymphosarcoma and horizontal transmission of FeLV appears to involve the opportunity for relatively close contact (2, 11). Data reported here deal with relationships between FeLV GSA in blood and bone marrow cells and infectious FeLV in oral secretions, serum, and tissues of cats infected experimentally with FeLV or with FeSV.

MATERIALS AND METHODS

Cats and Viruses. The SPF cats used were from a hysterecmy-derived (17) breeding colony maintained by the Department of Veterinary Pathobiology, The Ohio State University. Cats infected with feline oncornaviruses were of 3 classes. The 1st group consisted of SPF cats inoculated with the FeLV-R (16) [serotype A (18)]. The inoculum was 20% (w/v) tissue homogenate that represented the 4th in vivo passage of FeLV-R in SPF cats and contained 10⁸ FFU/ml of infectious FeLV, as assayed in clone 81 feline cell cultures (5). This inoculum was oncogenic in 85 to 100% of cats less than 8 weeks old (10). The cats were inoculated i.p. with 1 ml and were between 2 and 12 weeks old when inoculated.

The 2nd group consisted of SPF cats inoculated with the Snyder-Theilen (20) isolate of FeSV. The inoculum was a 20% (w/v) fibrosarcoma tissue homogenate that produced a 100% incidence of fibrosarcomas at the site of s.c. inoculation. Seventy-five % of the tumors developed progressive (malignant) growth in cats 12 weeks old at time of inoculation (13). FeSV-ST stocks contain a mixture of defective FeSV and FeLV [type AB (18)]. All cats that develop progressive fibrosarcomas and some cats in which fibrosarcomas regress become persistently positive for FeLV GSA in circulating leukocytes (13).

The 3rd group was composed of cats that were naturally infected with field strains of FeLV. These cats were patients presented to the Veterinary Teaching Hospital of The Ohio State University with a variety of symptoms. The group included clinically healthy cats as well as cats with lymphosarcoma, aplastic anemia, myeloproliferative disorders, miscellaneous intercurrent infections, and digestive disturbances. The biological activity of the various field isolates of FeLV was not evaluated under conditions of experimental inoculation.

Sampling. For cats inoculated with FeLV-R or FeSV-ST, blood samples were collected at weekly or biweekly intervals. Serum samples were stored at −70°C for 2 weeks to 6 months prior to testing. Samples of oropharyngeal secretions (saliva) were collected by swabbing the oropharynx.
with Dacron swabs and expressing the salivary secretions in 1 ml of Hanks' balanced salt solution containing penicillin and streptomycin. Bone marrow biopsies were obtained from the ilium with 19-gauge Osgood biopsy needles previously rinsed with EDTA solution. Marrow smears were prepared on glass slides rather than coverslips to facilitate handling. Marrow samples for VI assay were diluted 1:10 in Hanks' balanced salt solution immediately after collection.

**FeLV GSA Test.** The indirect immunofluorescence procedure of Hardy et al. (9) was used to determine the presence of FeLV GSA in blood and bone marrow cells. The primary reagent was goat anti-FeLV serum that had been absorbed in vivo to remove nonspecific reactivity to feline cells (9).

**VI Assay.** Tenfold dilutions of plasma, serum, marrow, and saliva were assayed for FeLV by focus formation in clone 81 cells, a murine sarcoma virus genome-positive-leukemia virus-negative feline cell culture derived by Fischinger et al. (5). Cultures were seeded at 4 x 10^4 cells/50-mm Falcon Petri dish. After 18 hr, the cultures were treated with DEAE-dextran prior to inoculation of 0.2 ml of test sample per well and incubation at 37° for 1 hr. Growth medium (McCoy's + 15% fetal calf serum) was then added, and the cultures were incubated at 37° in 5% CO2 for 3 to 4 days. At this time, the medium was changed to McCoy's + 5% serum until the cultures were fixed in formalin and stained by the Giemsa method at 8 to 10 days postinoculation. The number of discernible foci of hyperchromatic retracted cells per well were counted, and the dilution factor was used to calculate the number of FFU of FeLV in the sample (FFU/ml = no. foci x dilution x 1/inoculum volume).

**RESULTS**

**Relationship between FeLV GSA in Blood Cells and Infectious FeLV in Serum.** Correlation between GSA and VI was high. In each of 61 VI-positive blood samples from cats inoculated with FeLV-R 1 to 85 weeks earlier, FeLV GSA was detected in leukocytes (Table 1). Of 68 FeLV GSA-positive blood samples, VI was demonstrated in 61 (90%). The correlation between negative GSA and negative VI tests was 100%, and the overall concordance of the 2 tests was 94.7% (Table 1). The mean titer of infectious FeLV in sera was 3.3 ± 0.8 FFU/ml (log_{10} ± S.D.).

Similar data were obtained for cats inoculated with FeSV-ST (1 to 7 weeks earlier) and for cats infected with FeLV in nature (Table 1). In both instances, the overall correlation of GSA and VI was 100%. Mean VI titer in serum samples from cats infected with FeSV-ST was 2.9 ± 1.2 FFU/ml, and for cats infected with field strains of FeLV it was 3.6 ± 0.5 FFU/ml.

Sequential comparison of GSA and VI assays in weekly or biweekly samples collected from 10 cats between 0 and 29 weeks postinoculation with FeLV-R is in Table 2. Overall correlation was 92%. In 3 of 10 cats, the GSA test became positive earlier than VI during the initial conversion to the viremic state. In instances of discordance (8%), the GSA test was positive and VI negative, but in each case both tests were positive at the next sample interval.

**Relationship between FeLV GSA in Blood Cells and Infectious FeLV in Saliva.** In 56% of GSA-positive cats (15/27), infectious FeLV was detected in saliva obtained by oropharyngeal swabbing. None of the swab samples from GSA-negative cats yielded virus (Table 3). All of the cats had been inoculated with FeLV-R between 2 and 29 weeks earlier. The mean FeLV infectivity titer in the diluted saliva samples was 2.4 (log_{10}) as compared with a mean of 3.4 in the serum of the same cats. The dilution factor involved in expressing saliva from swabs into 1 ml of balanced salt solution before these samples were inoculated into cell culture was approximately 1:10. The mean postinoculation interval for GSA-positive cats with positive salivary samples was 35.5 weeks (range, 18 to 65 weeks) as compared to 21.7 weeks (range, 8 to 30 weeks) for GSA-positive cats in which p.o. swabs were negative.

**Relationship between FeLV GSA in Blood Cells and Bone Marrow Cells.** Weekly blood and bone marrow were collected from 34 cats inoculated with ST-FeSV. In 32 of 34 cats, GSA was detected in blood, bone marrow, or both at some point after inoculation (Chart 1). In 17 cats (50%), GSA was detected in marrow cells at least 1 week prior to their appearance in circulating leukocytes and platelets; in 1 cat (3%), GSA appeared in marrow only over a transient 2-week period; in 14 cats (41%), GSA in marrow and blood cells was detected on the same postinoculation week; 2 cats (6%) remained GSA-negative throughout the postinoculation period. Eleven of the 32 cats (34%) that became GSA positive, reverted to GSA negative in 3 to 6 weeks (mean, 4.2 weeks). In 7 of these 11 cats, GSA disappeared from blood cells 1 week prior to their disappearance from bone marrow. In the remainder, reversion to negative status was detected at the same week in blood and marrow cells.

In bone marrow smears, fluorescence specificity for FeLV GSA occurred principally in neutrophil precursors and was most intense in the more differentiated cells of the series (i.e., metamyelocytes, bands, segmented neutrophils). Eosinophilic granulocytes were recognized by red staining of their cytoplasmic granules with the counterstain Evans blue. Specific fluorescence was not detected in eosinophil precursors but conceivably could have been masked. In most samples, cells that appeared to be erythroid precursors also contained GSA, but differentiation of proerythroblasts and rubriblasts from progranulocytes and myelocytes often was not possible. In blood smears, specific fluorescence was most intense and most frequent in neutrophils, platelets, and monocytes.

**Comparison of FeLV Infectivity in Various Tissues of Cats.** GSA-positive cats inoculated with FeSV-ST had similar amounts of VI in serum (mean, 4.8 FFU/ml log_{10}) and in 20% homogenates of spleen (4.8), mesenteric lymph node...
Table 2
Sequential comparison of FeLV GSA in leukocytes and FeLV infectivity in serum of cats inoculated with FeLV-R

<table>
<thead>
<tr>
<th>Cat</th>
<th>Assay*</th>
<th>Wk postinoculation with FeLV-R</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0-1</td>
</tr>
<tr>
<td>88R-1</td>
<td>VI</td>
<td>&lt;1</td>
</tr>
<tr>
<td></td>
<td>GSA</td>
<td></td>
</tr>
<tr>
<td>88R-2</td>
<td>VI</td>
<td>&lt;1</td>
</tr>
<tr>
<td></td>
<td>GSA</td>
<td></td>
</tr>
<tr>
<td>88R-3</td>
<td>VI</td>
<td>&lt;0.7</td>
</tr>
<tr>
<td></td>
<td>GSA</td>
<td></td>
</tr>
<tr>
<td>88R-4</td>
<td>VI</td>
<td>&lt;0.7</td>
</tr>
<tr>
<td></td>
<td>GSA</td>
<td></td>
</tr>
<tr>
<td>921-1</td>
<td>VI</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>GSA</td>
<td></td>
</tr>
<tr>
<td>964-2</td>
<td>VI</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>GSA</td>
<td></td>
</tr>
<tr>
<td>964-3</td>
<td>VI</td>
<td>&lt;0.7</td>
</tr>
<tr>
<td></td>
<td>GSA</td>
<td></td>
</tr>
<tr>
<td>230R-1</td>
<td>VI</td>
<td>&lt;0.7</td>
</tr>
<tr>
<td></td>
<td>GSA</td>
<td></td>
</tr>
<tr>
<td>230R-2</td>
<td>VI</td>
<td>&lt;0.7</td>
</tr>
<tr>
<td></td>
<td>GSA</td>
<td></td>
</tr>
<tr>
<td>230R-3</td>
<td>VI</td>
<td>&lt;0.7</td>
</tr>
<tr>
<td></td>
<td>GSA</td>
<td></td>
</tr>
</tbody>
</table>

* Titer (log_{10}) in FFU/ml.

DISCUSSION

The data reported here demonstrated a high correlation between the immunofluorescence test for FeLV GSA in blood cells and direct viral isolation from serum. These observations agree with and extend those of several other investigators (2, 6, 8, 12). Moreover, examination of blood and bone marrow cells for GSA was at least as sensitive as viral isolation from serum in detecting viremia and the onset of the viremia. In no instance was VI detected in a GSA-negative blood sample. Occasional failure to detect infectious virus in serum samples from persistently GSA-positive cats could reflect loss of infectivity associated with handling or storage of the samples, since all were frozen (—70 °) for weeks to months prior to assay.

In over 50% of FeLV-R inoculated GSA-positive cats, relatively large amounts of infectious FeLV were detected in oropharyngeal secretions. In no instance was VI detected in saliva of an inoculated cat that was GSA negative. Since a single oropharyngeal swab was collected from each cat, the overall incidence of FeLV excretion probably underestimates what would be obtained with multiple samplings. Likewise, since the interval postinoculation with FeLV-R varied from 2 to 85 weeks, the relationship of the postinfection interval to the onset of salivary excretion could not be

(4.8), bone marrow (4.3), and liver (4.0) (Table 4). Infectivity in salivary gland, trachea, and bladder was at least 2 logs less than in serum (Table 4). Infectious FeLV was not detected in tissues from inoculated cats that remained GSA-negative.

Table 3
Relationship of FeLV GSA and VI in blood to excretion of infectious FeLV in saliva of cats inoculated with FeLV-R

<table>
<thead>
<tr>
<th>GSA (leukocytes)</th>
<th>VI (serum)</th>
<th>VI saliva</th>
<th>No./total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>15/27</td>
<td>56</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>—</td>
<td>12/27</td>
<td>44</td>
</tr>
<tr>
<td>—</td>
<td>—</td>
<td>—</td>
<td>21/21</td>
<td>100</td>
</tr>
</tbody>
</table>

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determined. However, the mean duration of viremia was greater in the group of cats with positive salivary samples. The data obtained support the hypothesis that contagious transmission of FeLV in p.o. secretions, probably involving relatively close contact (e.g., grooming, biting), is the dominant factor in the spread of FeLV infection and disease in the feral cat population (2, 4, 6, 8, 9, 12).

In 44% of cats inoculated with FeSV-ST, GSA was demonstrated in bone marrow granulocyte precursors 1 week prior to appearance in circulating neutrophils and platelets. More frequent marrow and blood sampling would be expected to increase this percentage since neutrophil transit time from the blast stage in the marrow to the peripheral blood is approximately 125 hr in cats (14). Neutrophil half-life in the blood would be expected to be 4 to 10 hr, assuming neutrophil kinetics in cats are similar to other species (1, 15, 21). The appearance of GSA in marrow cells in all but 1 cat was followed by viremia. We conclude, therefore, that the presence of GSA in circulating leukocytes and platelets reflects predominantly infection of hematopoietic precursors in bone marrow rather than phagocytosis of circulating virus or viral antigens (although we cannot exclude that the latter occurs).

The experiments reported here also indicate that transient viremia may occur under certain conditions. Eleven of 34 (34%) cats that became viremic after FeSV-ST inoculation reverted to GSA-negative after 3 to 6 weeks. In previous experiments, none of 42 cats recovered from the GSA-positive state after inoculation with FeLV-R (10). However, 3 of 5 cats that became viremic after contact exposure to FeLV-R-inoculated GSA-positive cagemates developed neutralizing antibody and reverted to GSA-negative after 4, 6, and 50 weeks, respectively (E. A. Hoover, unpublished data). In another study, Essex et al. (2) found that 6 of 6 cats that became GSA positive after introduction into a household with a high incidence of FeLV-positive cats remained viremic until death from FeLV-related diseases. The interplay between factors responsible for virus versus host dominance in the early phases of FeLV infection (virus strain and dose-versus-host immunological response) merits further study.

ACKNOWLEDGMENTS

We acknowledge the excellent work of Dagmar Imel and Kenneth Milliser.

REFERENCES

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