Leukemia Specific Antigens: FOCMA and Immune Surveillance*

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A. Introduction

The domestic cat is one of few species where most cases of naturally occurring leukemia and lymphoma are known to be caused by viruses [14]. The RNA retroviruses that cause these diseases are well characterized, and they are related to the viruses that cause similar tumors in laboratory mice [13,15]. The malignancies associated with feline leukemia virus (FeLV) infection include T and B cell lymphomas, lymphoblastic leukemias, and myeloid leukemias [7,44,48,56,63]. Feline sarcoma viruses (FeSV), which are defective for replication, induce multicentric fibrosarcomas and melanomas in vivo [36,62,82], and transform fibroblasts in vitro [5,73].

Many studies on the biology and natural history of feline leukemia have been directed to issues that seem appropriate for a further understanding of leukemia of man. Among these, we have addressed the following questions: a) is leukemia transmitted in a horizontal (infectious) manner or in a vertical (genetic) manner? b) does a specific immunosurveillance response to the tumor cells serve to protect infected cats from leukemia development? c) do tumor cells have tumor specific antigen markers that are expressed in the absence of virus structural proteins? and finally, d) is it possible to establish whether the feline leukemia virus (FeLV) causes lymphoid tumors that neither make virus particles nor express virus structural proteins, nor contain full copies of the viral genome. The latter question appears important to our understanding of any possible role that retroviruses may play in human malignancies since they have generally not been found to be associated with these tumors in man. Recent information gathered in the feline model which relates to these questions will be discussed below.

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B. Virus-Induced Tumors of Cats

I. Etiologic Agents

The genetic map of the feline retroviruses is apparently the same as the map for the murine agents [59,83]. From the 5′ end of the mRNA, the first gene (gag) codes for a polyprotein of approximately 75000 daltons which is subsequently cleaved after translation to form 4 smaller peptides which all become located in the core of the mature virion. The peptides, designated p15, p12, p30, and p10, occur in the latter order starting from the 5′ end of the genome. The major capsid protein is p30, a protein which is immunogenic in cats [6,84]. The second gene from the 5′ end is pol, which codes for the reverse transcriptase-RNase H complex.

The third gene, env, codes for a polyprotein which is cleaved to form two components situated at the surface of the virion. The first, usually designated gp70, becomes the virion envelope knobs or spikes. It is also immunogenic, and the target for virus neutralizing antibody. The feline viruses have been divided into 3 subgroups (A–C) based on type specific antigenic differences manifested by the gp70 molecule [74]. The subgroups which are designated on the basis of virus neutralization serology show a comparable distinction in interference concerning host-cell attachment [74]. Differences in sensitivity of infection of cells from heterologous species are also partially related to the virus subgroup and some evidence suggests a partial correlation between subgroup designation and pathogenicity [55]. The second peptide associated with the env gene polyprotein is designated p15e. It is highly conserved among viruses that infect various species and it appears to be associated with immune suppression by the virus [3,65].

II. Pathobiology

FeLV is associated with lymphoma as well as lymphoid and myeloid leukemia. Although one highly selected strain of virus has been regularly associated with the induction of the thymic form of lymphoma [50], most isolates produce various forms of hematopoietic tumors when inoculated into neonatal kittens [64]. The reported relative ratio of lymphoma incidence to leukemia incidence in the natural population has varied widely according to geographical location and/or institution. In the British Isles, for example, cases of true leukemia appeared to be very rare [18,56]. Conversely, in Boston the relative incidence of true leukemia was about the same as the incidence of lymphoma [18]. Similarly, geographical variations occurred concerning the form of lymphoma observed. In Glasgow, the alimentary form was the most common type of lymphoma reported [18]. In Boston, the alimentary form accounted for less than 10% of all lymphoma cases while the thymic form was most common [18]. A pathologic breakdown of the cases observed at the Angell Memorial Animal Hospital from 1972 to 1976 is presented in Table 1.

Both myeloid and lymphoid leukemias appear to be caused by FeLV [8,44]. The clinicopathologic parameters observed in feline lymphoid leukemia

Table 1. Classification by pathologic form of cases of lymphoid malignancies observed at the Angell Memorial Animal Hospital in Boston for 1972 through 1976

Pathologic form	Number of cases	Proportion of total	Number virus positive	Proportion virus positive
Lymphoid leukemia	77	42%	52	68%
Lymphoma (total)	107	58%	71	66%
Thymic	49	27%	37	76%
Multicentric	20	11%	11	55%
Alimentary	14	8%	7	50%
Other	24	13%	16	67%

have been compared to the parameters observed in acute lymphoblastic leukemia of children [8].

Most cases of spontaneous feline lymphoid malignancies appears to be T cell tumors [7,48]. Essentially all cases that have a primary location in the thymus are T cell tumors, although many that arise at a different site are also of T cell origin. Some B cell tumors occur, primarily originating in the gut wall as the alimentary form of lymphoma [63]. Null cell tumors have also been reported [48].

When inoculated into newborn kittens, the Rickard strain of FeLV causes thymic involution as the first apparent pathologic alteration [57]. Following this involution, the first cells observed in the thymic site are malignant T cells which contain the tumor specific antigen designated the feline oncornavirus associated cell membrane antigen (FOCMA) [25]. See Table 2.

Table 2. Expression of FOCMA and FeLV proteins on freshly biopsied lymphoid cells from cats inoculated with FeLV[a]

		Nonthymic lymphoid cells			*Thymic lymphoid cells*		
Time after inoculation (weeks)	Disease status	FeLV (infectious centers)	Presence of FOCMA	Membrane FeLV gp70 and p30	FeLV (infectious centers)	Presence of FOCMA	Membrane FeLV gp70 and p30
4	normal	4/4[b]	0/4	4/4	4/4	0/4	4/4
8	normal	4/4	0/3	3/3	3/3	0/3	3/3
12	normal	2/3	0/3	3/3	2/3	1/3	3/3
16	normal	1/1	0/1	1/1	1/1	1/1	1/1
	leukemic	2/2	0/2	2/2	2/2	2/2	2/2
20–24	leukemic	6/6	2/6	6/6	6/6	6/6	6/6

[a] Cells from bone marrow, spleen, mesentric lymph nodes, and buffy coat were tested from each cat. All preps from different organs of the same animal (other than thymus) gave the same result. The only exception was the presence of a significant population of FOCMA positive cells in the spleens of 2 of 6 leukemic animals even though less than 10% of the cells in the bone marrow, buffy coat, or mesentric lymph nodes of the same animals were FOCMA positive.

[b] Number positive over total number tested

The proportion of spontaneous cases of feline lymphoma and leukemia which contain detectable FeLV varies with both geographical location and pathologic form [18]. Both virus isolation procedures and serologic tests have established that from 50% to 90% of the spontaneous cases of leukemia and lymphoma contain readily detectable FeLV. Although the proportion of virus negative cases appears to be highest for the B cell alimentary form of the disease, all pathologic forms include a significant minority that appear to be "virus-negative" by all criteria [32].

FeLV is regularly excreted in saliva from essentially all healthy and leukemic cats that are viremic [34]. Infectious virus is not ordinarily present in significant levels in feces, urine, or fleas taken from infected cats but the levels of infectious virus in saliva are as high or higher than the levels found in blood plasma [34]. FeLV is inactivated rapidly within a few minutes at 56°C, but stable for several days at 25–35°C [33].

C. Epidemiology of Feline Leukemia and Lymphoma

That FeLV is transmitted as a contagious agent in cats is now well established [19,46,49,58]. Initially this conclusion was doubted, largely because it had been previous established that many related retroviruses were genetically transmitted in inbred strains of mice [52]. The first suggestion that FeLV and leukemia might be horizontally transmitted in the cat came with reports that leukemia [9,10] and lymphoma [22,46,56,76] occasionally occurred in clusters. Since these reports were sometimes anecdotal and difficult to evaluate due to a lack of data on the base cat population, their significance was questioned [75].

We became convinced that the clusters were not due to chance alone when the high incidence of leukemia/lymphoma continued to occur, in a prospective sense, in the same cluster households [9,22]. Subsequently, extensive supporting seroepidemiologic evidence for FeLV transmission was obtained in some of the same households [9,12,19,28,31,37,38,42,43,45,46,84].

Another clue which suggested that FeLV-associated malignancies were horizontally transmitted was the finding that healthy uninoculated control cats developed leukemia/lymphoma more often than would be expected due to chance alone following exposure to leukemic cats [45,58,71]. This occurred under both laboratory and field conditions.

Using serologic techniques, healthy cats known to be exposed to either leukemic cats and/or cats viremic with FeLV were examined for the presence of antibodies to FOCMA [9,19,22,26,28,37,38], complement dependent cytotoxic antibodies [37,38,66], FeLV neutralizing antibodies [19,42,45,72], radioimmunoprecipitating antibodies to FeLV p30 [19,31,84], radioimmunoprecipitating antibodies to FeLV gp70 [19,31,84], and antibodies which neutralized the activity of FeLV reverse transcriptase [53]. The healthy cats examined included animals exposed to FeLV under both experimental and natural conditions. In each case populations of cats known to be exposed to FeLV had much higher frequencies of detectable levels of antibody than cats

of comparable genetic backgrounds with no history of FeLV exposure. Similarly, the geometric mean antibody titers were regularly higher for cats that were known to have been exposed to FeLV when compared to cats that were not known to be exposed to FeLV. The latter category included both "specific pathogen free" laboratory cats, and conventional cats from laboratory and household pet backgrounds.

Along with the examination of such FeLV-exposed cat populations for various antibody activities, numerous individuals from the same populations were checked for the presence of circulating infectious virus [19,42,46,55], the presence of major viral antigens by fixed cell immunofluorescence [6,22, 42,46,47,84], and the presence of FeLV p30 and gp70 antigens by radioimmunoprecipitation [31,84]. Again, healthy cats from populations known to be contact exposed to either known FeLV-infected animals and/or known cases of leukemia/lymphoma were examined. Up to 50% of the healthy cats in such populations were found to be actively infected with FeLV [9,22,41, 42,46]. Conversely, no more than 1–2% of the healthy cats with a history of no known exposure to FeLV were found to be actively infected [17,41,46].

Along with the serologic examination of existing cat populations for the presence or absence of FeLV-related antigens and antibodies, tracer specific-pathogen-free cats were placed in natural environments where FeLV was known to be present (leukemia cluster households) and subsequently examined for prospective seroconversion [19]. The cats placed in this new environment were previously confirmed to be negative by all of the serologic tests. Essentially all developed evidence of either transient or persistent FeLV infections within a few months after being introduced to the new environment [19].

As a final measure, a study was undertaken to determine if subsequent infections could be prevented in known high-risk FeLV exposure environments by the elimination of FeLV-excretor cats [43,45]. This approach was also successful, demonstrating that the numbers of subsequent FeLV infections and cases of FeLV-associated diseases could be either drastically reduced or completely eliminated by these procedures [43,45].

D. Role of the Immune Response

All of the FeLV structural proteins appear to be immunogenic in adult cats that become exposed to virus. These include each of the gag gene peptides p15, p12, p30, and p10 [20,84,85], reverse transcriptase [53], and the env gene proteins gp70 [20,84,85], and p15e [86]. At least in the case of the major core protein (p30) and the major envelope protein (gp70), several antigenic determinants are present on the same molecules, including those with group, subgroup, or type, and interspecies specific determinants. In cats the major immunogenic determinants of the p30 is group specific while the major immunogen on the gp70 is subgroup specific [6,55]. Conversely, different primary antigenic determinants on the same molecules may be recognized as significant immunogens when non-feline species such as goats or rabbits are inocu-

lated with FeLV [20]. This might appear to be inconsequential, except for the possibility that different antigenic determinants might be expressed on the surface of FeLV-infected cells when compared to reactivities seen with either intact virus or purified immunoprecipitating virion components. For example, it is conceivable that anti-gp70 serum from the homologous species (cat) might effectively neutralize circulating cell-free FeLV but not attack FeLV-infected cells while goat or rabbit antiserum to the same molecule might cause cytolysis of infected cells. A major consequence of this spectrum of reactivity might be a more beneficial anti-tumor therapeutic effect in the presence of antiserum from a heterologous species which would otherwise react similarly to cat antiserum when tested by a radioimmunoassay with the purified molecule.

An effective antibody response by cats to the gp70 is associated with the reduction and/or elimination of circulating FeLV [19,42,84]. The clinical significance of the antibody responses to the other virion structural antigens has not been determined. Antibodies to other FeLV antigens are frequently present, but they are not always concordant with the presence of antibodies to gp70 [31,84]. Cats with persistent viremia appear to lack free antibodies to all of the FeLV structural proteins [20,84,85].

Aside from the virus structural antigens, an important antigen in both FeLV and FeSV induced tumors is FOCMA [23,24,30]. As opposed to the situation with the viral structural antigens, the antibody response to FOCMA is closely correlated with protection from development of leukemia, lymphoma, and fibrosarcoma, whether laboratory induced or naturally occurring [12,14,20, 23,24,26,28,30,31,37,38]. Cats that become naturally infected with FeLV that remain healthy and viremic for long periods, for example, usually also maintain readily detectable levels of antibody to FOCMA [14,26]. Cats with either naturally occurring leukemia or lymphoma as well as those cats bearing tumors induced by FeLV and FeSV contain either no detectable antibody to FOCMA or very low levels of such antibodies [14,18,26,28]. Additionally, a poor antibody response to FOCMA serves, in a prognostic sense, as a risk factor for subsequent tumor development [14,26]. FOCMA antibody titers determined for cats in several populations are summarized in Table 3.

Table 3. FOCMA antibody titers for healthy and neoplastic cats from laboratory and field environments

	Number tested	Number and percent of cats with antibody titers of:		
		<4	4–16	>16
[illegible][b]	131	116 (89)	15 (11)	0
[illegible]	76	6 (8)	48 (63)	22 (29)
[illegible]exposed to virus[c]	221	218 (99)	3 (1)	0

[illegible]kemia, lymphoma, and fibrosarcoma
[illegible]nd/or FeSV under either laboratory or field conditions
[illegible]nd minimal disease free laboratory cats

Feline serum samples which contain detectable levels of antibodies to FOCMA usually also contain antibodies which are lytic for cultured feline lymphoma cells using the ^{51}Cr release test [37,38]. The complement-dependent antibodies (CDA) function best in the presence of cat complement, but the lysis requires up to 20 hours to reach maximum effect. A 90–95% overall correlation was found between CDA as detected with cat complement and FOCMA antibodies as detected by indirect membrane immunosurveillance [37,38]. When guinea pig complement or rabbit complement rather than cat complement was used in the CDA assay, the correlation was reduced to 79% and 65%, respectively [37,38].

Cats that become infected with FeLV manifest a generalized syndrome of immunosuppression [3,21,42,57,70]. As a result, viremic healthy cats have a significantly increased risk for development of various infectious diseases [11,21,42]. Many possible explanations can be considered to explain the observed immunosuppression. FeLV causes thymic atrophy and wasting disease, following either laboratory injections or natural infections [1,46,57]. Persistent viremia with FeLV has been associated with depressed numbers of peripheral blood lymphoid cells [21], depressed counts of peripheral T cells [16], variations in complement levels [39], and increased suppressor cell activity [3,4]. One of the viral structural proteins, p15e, has been demonstrated to have a profound effect on various lymphocyte functions [3,65]. Persistently viremic cats also have a high risk for development of immune complex glomerulonephritis [54].

E. Tumor Specific Antigens

FOCMA was first described on cultured feline lymphoma cells taken from a cat inoculated with FeLV [23,24]. The antisera which reacted with the lymphoma cell surface were taken from cats previously inoculated with FeSV [23,24,30]. Subsequent studies indicated antisera taken from cats either inoculated with FeLV or horizontally exposed to FeLV gave the same reaction [12,17,19,22,26,49]. Thus, it was clear that FeLV was capable of inducing the antigen. Whether or not FeSV alone could also induce the antigen was unclear because the FeSV preparations inoculated into cats always contained an excess of FeLV helper virus.

To determine if FeSV alone could induce FOCMA it was necessary to study FeSV transformed nonproducer cells, which were not superinfected with FeLV [27,80,81]. The initial approaches involved the examination of mink cells transformed by either FeSV or murine sarcoma viruses. FOCMA was regularly expressed on cells transformed by FeSV, but not expressed on cells that were morphologically transformed by other viruses. The antigen was not expressed on nontransformed cells infected by FeLV, and no change in the level of expression was seen on nonproducer transformed cells following superinfection with FeLV. No relationship was found between expression of FOCMA and either activation of the endogenous mink virus or expression of the mink virus structural proteins [27,80,81,85]. Additionally,

FOCMA was found to be specifically induced by FeSV in cells from various other species including subhuman primates, dogs, and rats [81]. Conversely, FOCMA was not found in feline cells carrying the murine sarcoma virus genome, even when these cells were superinfected with FeLV [27,80]. The observation that FOCMA induction could be transmitted across species barriers with FeSV strongly suggested that this transformation-specific antigen was encoded for by FeSV. The correlation between FOCMA expression and FeSV transformation is summarized in Table 4.

FeSV-transformed nonproducer mink cells were examined by radio-immunoprecipitation for all of the major FeLV-related virion proteins [77,85]. The cells were found to be free of the major env gene product gp70 and free of the major gag gene product p30. The cells were also free of p10, but significant levels of the gag 5′ end peptides p15 and p12 were present in the FeSV-transformed nonproducer cells. Using antisera to FOCMA and/or p15 and p12, several classes of molecules were detected in the transformed cells. The first was an 85 000 dalton molecule which could be precipitated with antisera to each of the three antigens. The second species, which could be precipitated with antisera to FOCMA but not with antisera to p15 or p12, was about 65 000 daltons. The 65 000 dalton species was found in largest amounts after apparent cleavage from the 85 000 dalton species. Along with the 65 000 species, the cells also contained free p15 and p12, and a precursor of approximately 25 000 daltons which had both p15 and p12 activity [85].

The 85 000 dalton species was also identified in pseudotyped feline sarcoma virions rescued from the mink cells using various serologically distinguishable helper viruses such as the endogenous baboon retrovirus and the xeno-

Table 4. FOCMA expression in malignant and non-malignant cells of feline and mink origin

Species	Description of cells	Malignant phenotype	Antigens present on cell surface		
			FOCMA	FeLV gp70	FeLV p30
cat	cultured FeLV producer lymphoma cells	+	+	+	+
cat	freshly biopsied FeLV producer lymphoma or leukemia cells	+	+	+	+
cat	freshly biopsied nonproducer lymphoma or leukemia cells	+	+	–	–
cat	freshly biopsied or cultured normal cells infected with FeLV	–	–	+	+
cat	fibroblasts transformed with FeSV, superinfected with FeLV	+	+	+	+
cat	fibroblasts transformed with murine sarcoma virus, superinfected with FeLV	+	–	+	+
mink	fibroblasts infected with FeLV	–	–	+	+
mink	fibroblasts transformed with FeSV, nonproducer	+	+	–	–
mink	fibroblasts transformed with FeSV, superinfected with FeLV	+	+	+	+
mink	fibroblasts transformed with murine sarcoma virus	+	–	–	–

tropic murine retroviruses [77,78]. As in the case of cell extracts, the pseudotyped FeSV's contain FOCMA, FeLV p15, and FeLV p12. All the p30, p10, and gp70 found in the pseudotyped virions was specific for the helper virus [77]. FOCMA is not found in either FeLV or the helper viruses used to rescue FeSV. A radioimmunoassay has now been developed for the 85000 dalton species of FOCMA [78].

Since FOCMA activity was originally detected using a cultured FeLV-producer feline lymphoma line, studies were undertaken to determine the association between FOCMA, FeLV production, and the malignant phenotype in various biopsied lymphoid cell preparations [25,27,29,47,48]. FOCMA was found to be present on biopsied malignant feline lymphoid cells, irregardless of whether they were taken from T or B cell tumors, or whether or not detectable FeLV was also present (see following section), or whether the cells were from cases of lymphoblastic leukemia or lymphoma, or whether the malignant cells were from natural cases of tumors or laboratory induced cases following inoculation of the animals with FeLV [25,27,29,47,48]. FeLV-producer lymphoid cells that were morphologically normal were negative for FOCMA, even when the normal cells were taken from cats with lymphoma.

Taken together, these results indicate that FOCMA is encoded for by FeSV, but they do not reveal whether or not the same antigen (or a closely related cross-reactive antigen) is also encoded for by FeLV. Several possible explanations can be considered. For example, FOCMA might represent the src gene of FeSV, which was originally acquired from normal fibroblasts by non-transforming FeLV. In this case, the gene would presumably be somewhat related to those expressed during normal differentiation processes. FeLV might then act by derepressing the gene when the virion genome interacts with the cell in such a way as to cause leukemia. If FeLV was uniquely qualified to do this, because of some evolutionary process of co-development between the virus and that specific region of the cell genome, we might expect that tumors of mesodermal cell origin caused by factors other than FeLV would not express FOCMA. Alternatively, if FOCMA was a common requirement to the process of de-differentiation associated with malignancy, we might expect that tumors induced in lymphoid and/or fibroblastoid cells with chemicals, irradiation, or other viruses might also express FOCMA. Since "FeLV-negative" lymphoid tumors normally express FOCMA, we would obviously have to postulate that the later tumors were initiated by FeLV if FOCMA is either FeLV encoded or induced in the cell only by FeLV.

The possibility that FOCMA is also encoded for by FeLV can also be considered. Although it does not appear to be gag, pol, or env, it is possible for example that feline retroviruses which actually cause leukemia might possess a "leuk" or "onc" gene analogous to "src" which codes for FOCMA. An FeLV carrying such a gene has not been detected as yet, but classical nucleic acid hybridization experiments would not detect such an agent anyway if it existed as a small minority in an excess of non-leukemogenic helper virus. The latter situation is of course present for FeSV, as well as for the defective acute leukemia viruses of chickens [2]. Still another possibility is that FOCMA

might be encoded for by sequences normally found in replication competent FeLV. In the case of Rauscher virus a protein "by-product" of unknown function has been identified which arises from the gag-pol precursor polyprotein. This protein does not become part of the subsequent gag or pol virion proteins [78]. Although perhaps even more unlikely, information to make FOCMA might be generated from standard FeLV gag, pol, or env nucleotides in the form of a "spliced" mRNA made from integrated DNA provirus.

F. "Virus Negative" Tumors

About 20–30% of the spontaneous lymphoid tumors found in cats from the northeastern USA lack detectable FeLV [18]. In Glasgow, up to 50% of the feline lymphomas are negative for FeLV [18,57]. Although the proportion of B cell tumors that are "virus negative" is highest (up to 65%) a significant minority of the T cell tumors also lack detectable FeLV [48]. Additionally, all of the major gross pathologic forms of lymphoid malignancies may occur in the "virus-negative" state. The examination of tumor cell homogenates using a sensitive radioimmunoprecipitation technique failed to yield detectable levels of any of the virus proteins [84,85]. As in the case of FeSV transformed nonproducer mink cells, the "virus-negative" tumors lacked detectable p30, p10. or gp70. Unlike the FeSV transformed nonproducer cells, the "virus-negative" tumors also lacked detectable p15 or p12. Thus, the question obviously arises concerning the etiology of the "virus-negative" tumors. If, for example, it could be established that the "virus-negative" tumors were originally caused by FeLV, as were the morphologically indistinguishable "virus-positive" tumors, one might expect that the "virus-negative" feline tumors would be an excellent model for attempting to understand "virus-negative" leukemia and lymphomas of man.

One obvious approach to study the possible role of exogenously acquired FeLV in the etiology of "virus-negative" tumors is the approach of nucleic acid hybridization. Several studies have been reported. These include the use of DNA probes, made from FeLV with reverse transcriptase, to test for hybridization with lymphoma cell RNA [35,61]. The DNA probes used represented all 3 of the subgroups of FeLV, as well as two independently distinguishable FeSV's. The results failed to reveal hybridization with the "virus-negative" lymphoma cells which was above background levels detected in normal feline tissue. A second approach employed iodinated virion RNA as a probe to hybridize with lymphoma cell DNA [60]. Again, "virus-negative" lymphoid cells lacked evidence of FeLV-related provirus sequences above the background levels found in normal cells. Levels of hybridization obtained with virus negative tissues varied from about 15% to more than 60%, but tumor tissues did not necessarily have values that were any higher than normal tissues.

Other approaches can be used to study the possible association between FeLV and the "virus-negative" tumors. Feline lymphoid tumors often occur

in clusters within households where cats are exposed to large doses of FeLV [10,22,46,76]. We recently sought to determine if "virus-negative" leukemia occurred in a large FeLV exposure leukemia cluster household at a higher rate that it would be expected to occur in the population at large. This was found to be the case.

Under experimental conditions, serum immunotherapy directed to circulating FeLV and/or FOCMA has been employed [40,67,69]. Both classes of antiserum appear to have a beneficial effect under appropriate conditions. Antiserum to virion antigens could presumably function by eliminating FeLV-producer cells, as well as by eliminating free virus. We recently postulated that immunoselection might be one possible mechanism by which "virus-negative" tumors might be caused by FeLV and/or FeSV [20]. If fo example, an anti-FeLV response was to eliminate virus producer cells in vivo, allowing nonproducer transformed cells to "sneak-through", the result might be a "virus-negative" tumor. We have observed this apparent course of events in a few fibrosarcoma-bearing cats treated with anti-FeLV [68]. The absence of a parallel anti-FOCMA response apparently allowed the development of "virus-negative" tumors following the initial regression of "virus-positive" tumors. Whether or not the "virus-negative" secondary tumors were also negative for the provirus genome by hybridization remains to be determined. Additionally, a few cats have been observed to develop "virus-negative" leukemia, under natural conditions after previous infections with FeLV [40]. Direct evidence that naturally occurring "virus-negative" feline lymphomas are caused by FeLV is lacking. However, the regular presence of FOCMA, an antigen known to be encoded by FeSV, on "virus-negative" tumor cells, as well as the curious epidemiologic association between exposure to FeLV and the existence of "virus-negative" tumors cannot be ignored. Further investigations on the identity of FeLV-related partial provirus sequences in the "virus-negative" tumor cells seems warranted. If such tumors can be clearly linked to FeLV, the rationale for an intensified search for analogous defective viruses in human tumor tissues becomes obvious.

G. Summary

In cats, horizontally transmitted viruses cause leukemia and lymphoma under natural conditions. As with other retroviruses, feline leukemia virus (FeLV) contains products of 3 major genes; the virus core gag gene products, the polymerase, and the virus envelope glycoprotein. When cells are transformed in vitro by the related feline sarcoma virus (FeSV), an additional protein, FOCMA is expressed at the cell membrane. FOCMA, which is FeSV-coded, is transformation and/or tumor specific and expressed regardless of whether or not the cells make virus or contain virus structural antigens. Lymphoid leukemia cells also express FOCMA, both when FeLV is used to induce the disease in laboratory cats and when the tumors occur under natural conditions. FOCMA is expressed on both T and B lymphoid leukemia cells, but not expressed on non-malignant lymphoid cells, even when they are infected

with FeLV. About one-third of the naturally occurring lymphoid tumors of cats lack detectable FeLV proteins and varying portions of the FeLV provirus. Despite this, they regularly express FOCMA, which is the target of an immunosurveillance response that functions effectively under most conditions. FOCMA thus provides a useful model for antigens that might be expressed in "virus-negative" leukemias of man.

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in clusters within households where cats are exposed to large doses of FeLV [10,22,46,76]. We recently sought to determine if "virus-negative" leukemia occurred in a large FeLV exposure leukemia cluster household at a higher rate that it would be expected to occur in the population at large. This was found to be the case.

Under experimental conditions, serum immunotherapy directed to circulating FeLV and/or FOCMA has been employed [40,67,69]. Both classes of antiserum appear to have a beneficial effect under appropriate conditions. Antiserum to virion antigens could presumably function by eliminating FeLV-producer cells, as well as by eliminating free virus. We recently postulated that immunoselection might be one possible mechanism by which "virus-negative" tumors might be caused by FeLV and/or FeSV [20]. If fo example, an anti-FeLV response was to eliminate virus producer cells in vivo, allowing nonproducer transformed cells to "sneak-through", the result might be a "virus-negative" tumor. We have observed this apparent course of events in a few fibrosarcoma-bearing cats treated with anti-FeLV [68]. The absence of a parallel anti-FOCMA response apparently allowed the development of "virus-negative" tumors following the initial regression of "virus-positive" tumors. Whether or not the "virus-negative" secondary tumors were also negative for the provirus genome by hybridization remains to be determined. Additionally, a few cats have been observed to develop "virus-negative" leukemia, under natural conditions after previous infections with FeLV [40]. Direct evidence that naturally occurring "virus-negative" feline lymphomas are caused by FeLV is lacking. However, the regular presence of FOCMA, an antigen known to be encoded by FeSV, on "virus-negative" tumor cells, as well as the curious epidemiologic association between exposure to FeLV and the existence of "virus-negative" tumors cannot be ignored. Further investigations on the identity of FeLV-related partial provirus sequences in the "virus-negative" tumor cells seems warranted. If such tumors can be clearly linked to FeLV, the rationale for an intensified search for analogous defective viruses in human tumor tissues becomes obvious.

G. Summary

In cats, horizontally transmitted viruses cause leukemia and lymphoma under natural conditions. As with other retroviruses, feline leukemia virus (FeLV) contains products of 3 major genes; the virus core gag gene products, the polymerase, and the virus envelope glycoprotein. When cells are transformed in vitro by the related feline sarcoma virus (FeSV), an additional protein, FOCMA is expressed at the cell membrane. FOCMA, which is FeSV-coded, is transformation and/or tumor specific and expressed regardless of whether or not the cells make virus or contain virus structural antigens. Lymphoid leukemia cells also express FOCMA, both when FeLV is used to induce the disease in laboratory cats and when the tumors occur under natural conditions. FOCMA is expressed on both T and B lymphoid leukemia cells, but not expressed on non-malignant lymphoid cells, even when they are infected

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