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Advances in Understanding Molecular Determinants in FeLV Pathology

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Abstract

Feline leukemia virus (FeLV) occurs in nature not as a single genomic species but as a family of closely related viruses. The disease outcome of natural FeLV infection is variable and likely reflects genetic variation both in the virus and the naturally outbreeding host population. A series of studies have been undertaken with the objectives of examining natural FeLV genetic variation, the selective pressures operative in FeLV infection that lead to predominance of natural variants, and the consequences for infection and disease progression. Genetic variation among FeLV isolates was examined in a cohort of naturally infected cats with thymic lymphoma of T-cell origin, non-T-cell multicentric lymphoma, myeloproliferative disorder or anemia. The predominant isolate in the cohort, designated FeLV-945, was identified exclusively in disorders of non-T-cell origin. The FeLV-945 LTR was shown to contain a unique 21-bp repeat element, triplicated in tandem downstream of enhancer. The 21-bp triplication was shown to act as a transcriptional enhancer and to confer a replicative advantage through the assembly of a distinctive transcription factor complex. Oncogene utilization during tumor induction by FeLV-945 was studied using a recombinant Moloney murine leukemia virus containing the FeLV-945 LTR. This approach identified novel loci of common proviral integration in tumors, including the regulatory subunit of PI-3Kgamma. Mutational changes identified in FeLV-945 SU were shown not to alter receptor usage as measured by host range and superinfection interference, but to significantly increase the efficiency of receptor binding. To determine whether the unique sequence elements of FeLV-945 influence the course of infection and disease in vivo, recombinant viruses were constructed in which the FeLV-945 LTR alone, or the FeLV-945 SU gene and LTR were substituted into the prototype isolate FeLV-A/61E. Longitudinal studies of infected animals showed that substitution of the FeLV-945 LTR into FeLV-A/61E resulted in a significantly more rapid disease onset, but did not alter the tumorigenic spectrum. In contrast, substitution of both the FeLV-945 LTR and SU gene changed the disease outcome entirely. Together, these observations indicate that the distinctive LTR and SU gene of FeLV-945 mediate a rapid pathogenesis with distinctive clinical features and oncogenic mechanisms.

Keywords

feline leukemia virus; lymphoma; pathogenesis; oncogenesis

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The gammaretroviruses represent a group of mammalian oncogenic retroviruses typically associated with the long-latency induction of leukemia and lymphoma in the natural host. Moloney murine leukemia virus (M-MuLV), a prototype laboratory gammaretrovirus, induces a T-lymphoblastic lymphoma of the thymus in virtually 100% of susceptible neonatal mice with a latency of 3 to 4 months (Fan, 1997). Feline leukemia virus (FeLV) is a naturally occurring gammaretrovirus endemic in an outbreeding mammalian species, the domestic cat. Unlike M-MuLV, FeLV infection in the natural host is associated with a variable and rather unpredictable disease outcome. Natural FeLV infections in the domestic cat are associated with malignant and proliferative diseases including lymphomas and leukemias of lymphoid, myeloid or erythroid origin as well as degenerative diseases including anemia (Rezanka et al., 1992). Analysis of FeLV-mediated disease induction offers a rare opportunity to study mechanisms of pathogenesis in an outbred mammalian host during infection with a natural pathogen.

FeLV occurs in nature not as a single genomic species but as a genetically complex family of closely related viruses. Genetic variation in FeLV is generated during virus replication through error-prone reverse transcription and by recombination with endogenous FeLV-related sequences. The consequence of this variation is a genetically diverse virus population that is continuously shaped by selective pressures *in vivo* and from which variants arise as predominant species (Overbaugh and Bangham, 2001). Our research objective over many years has been to examine the selective pressures operative in natural FeLV infection that lead to the predominance of viral variants, many of which have significant consequences for infection and disease progression. Natural isolates of FeLV most commonly exhibit sequence variation within the viral long terminal repeat (LTR) or the surface glycoprotein (SU) gene (Neil et al., 1991; Overbaugh and Bangham, 2001). The LTR of FeLV, like that of other retroviruses, is a modular structure in which the U3 region contains the transcriptional promoter and enhancer elements required to direct gene expression. The LTR encodes the major determinant of tumorigenic potential and disease specificity of the gammaretroviruses, particularly within the repeat elements characteristic of the central enhancer motif (Fan, 1997; Chandhasin et al., 2004). The LTR of M-MuLV or FeLV, like other gammaretroviruses, is implicated in the malignant process in two ways: (1) by directing high levels of virus expression in relevant target tissues, and (2) by insertionally activating oncogenes at or near the sites of proviral integration. Indeed, the FeLV LTR is a region of remarkable genetic variation among natural isolates, and LTR variants have been linked to particular disease outcome. For example, FeLV proviruses cloned directly from T-cell lymphomas typically contain two or three tandemly repeated enhancers in the LTR (Fulton et al., 1990; Matsumoto et al., 1992). In contrast, FeLV LTRs derived from nonneoplastic diseases or from non-T-cell malignancies typically contain only a single copy of the enhancer (Jackson et al., 1996) but may contain repeated elements elsewhere in the LTR (Athas et al., 1995; Nishigaki et al., 2002; Nagashima et al., 2005).

The FeLV surface glycoprotein gene (SU) represents another source of genetic variation among natural isolates. It has been shown that subtle mutational changes accumulate in FeLV SU during infection *in vivo*, and may alter biological properties of the virus such as receptor utilization or affinity, replication kinetics or pathogenic potential (Neil et al., 1991; Brojatsch et al., 1992; Kristal et al., 1993; Rohn et al., 1998; Gwynn et al., 2000; Lauring et al., 2001; Overbaugh and Bangham, 2001). As is typical of gammaretroviruses, the FeLV SU protein comprises an aminoterminal receptor-binding domain (RBD) followed by a proline-rich region (PRR) that mediates conformational changes required for entry. Two variable regions within RBD, designated VRA and VRB, define the specificity for receptor binding (Battini et al., 1995; Boomer et al., 1997; Tailor and Kabat, 1997; Sugai et al., 2001). FeLV occurs in nature in four subgroups, designated A, B, C and T, that are distinguished genetically by sequence differences in SU and functionally by interaction with distinct host cell receptors for entry. FeLV-A is a weakly pathogenic, ecotropic virus thought to represent the predominant agent

spread horizontally cat-to-cat in nature. The known isolates of FeLV-A share ~97% amino acid sequence identity in SU, particularly remarkable since they were isolated over more than a decade from distant geographic locations across the world (Donahue et al., 1988; Neil et al., 1991; Overbaugh and Bangham, 2001). FeLV-B, -C and -T subgroups are thought to arise from FeLV-A *de novo* through point mutation, insertion and/or recombination with endogenous FeLV-related sequences during virus replication in the infected animal. While FeLV-A infection is typically associated with the induction of thymic lymphoma of T-cell origin (Neil et al., 1991; Rezanka et al., 1992), the FeLV-B, -C and –T subgroups are specifically associated with lymphoma, anemia or immunodeficiency disease, respectively (Donahue et al., 1991; Neil et al., 1991; Rohn et al., 1998).

We previously described a natural isolate of FeLV, termed **FeLV-945**, as the predominant species in a geographic and temporal cohort of naturally infected cats (Athas et al., 1995; Athas et al., 1995; Chandhasin et al., 2004). The U3 region of the FeLV-945 LTR was shown to contain a unique repeat sequence motif, specifically, a single copy of the canonical transcriptional enhancer followed 25-bp downstream by the tandem triplication of a 21-bp repeat element. It was striking that the sequence and position of the 21-bp triplication within the LTR was precisely conserved among independent isolates from the cohort (Prabhu et. al., 1999; Chandhasin et al., 2004). The SU protein of FeLV-945 was shown to be most closely related to natural horizontally-transmissible FeLV-A, but was observed to differ from an FeLV-A prototype to a greater extent than the known FeLV-A isolates differ among themselves (Athas et al., 1995; Prabhu et al., 1999). The following review describes studies designed to understand the selective advantages that may have led to the predominance of FeLV-945 in a naturally infected population, and the consequences of its predominance on infection and disease progression.

The unique FeLV-945 LTR predominates in non-T-cell diseases in a cohort of naturally infected animals

FeLV-945 was originally identified from diseased tissues in a cohort of 21 naturally infected cats collected from essentially a single veterinary practice in Pasadena, California over a period of six years (the gift of Dr. Murray Gardner). Thus, the cohort represents a geographic and temporal cluster presumably exposed to a similar spectrum of horizontally transmissible FeLV. The cohort included four cases of thymic lymphoma, twelve cases of multicentric lymphoma, one case of mast cell leukemia, two cases of myeloproliferative disease and two cases of anemia. The cases of thymic lymphoma were shown by Southern blot analysis to represent clonal tumors of T-cell origin. The multicentric lymphomas were shown by similar analysis to represent clonal tumors of unknown cell origin, but which did not contain clonally expanded T-cells or B-cells (Athas et al., 1995). FeLV LTR sequence variation in the cohort was examined using PCR amplification and nucleotide sequence analysis. Specifically, genomic DNA from diseased tissues was amplified by PCR using primers specific for the U3 region of exogenous FeLV LTR (Prabhu et al., 1999; Chandhasin et al., 2004). Amplification products of multiple sizes were detected from most animals, indicating the presence of FeLV LTR sequence variation within the tissues of individuals. In thymic lymphomas, LTRs uniformly contained enhancer duplications that varied in length from 39 – 77 bp. The termini of the enhancer repeat unit varied among these LTRs, but the LVb/Ets and CORE binding sites were conserved within the repeat unit regardless of its length. This observation underscores the apparent requirement for repetition of the LVb/Ets and CORE binding sites in the induction of thymic lymphoma. It was noteworthy that LTRs containing the 21-bp tandem triplication characteristic of FeLV-945 were not detected in any of the thymic tumors in the cohort. In contrast, LTRs amplified from non-T-cell diseases contained 1, 2, 3 or 4 tandem copies of the 21-bp sequence element characteristic of FeLV-945 with the tandem triplication predominating. Southern blot analysis confirmed the occurrence of all four LTR structures in

tumor DNA, as well as the predominance of the triplicated form. LTRs containing 21-bp repeats were identified in seven of twelve multicentric lymphomas and in all cases of myeloproliferative disease and anemia. Unlike the enhancer duplications, the termini of the 21-bp repeat units were precisely conserved (Chandhasin et al., 2004). Functional studies were then initiated to establish the selective advantage responsible for the precise conservation of the 21-bp triplication in non-T-cell diseases in the cohort.

Predominance of FeLV-945 LTR reflects replicative fitness

A series of studies of the 21-bp triplication showed that it contributes transcriptional enhancer function to the FeLV-945 LTR, that it functions preferentially in a cell type-specific manner and that it confers a replicative advantage to the virus in those cells (Levesque et al., 1990; Athas et al., 1995; Prabhu et al., 1999; Chandhasin et al., 2004). In one such study (Chandhasin et al., 2004), recombinant FeLV LTRs were constructed and substituted into infectious FeLV-A/61E in order to compare the replication of virus with one, two, three or four copies of the 21-bp repeat element. Replication kinetics of recombinant FeLVs were then compared in three feline cell lines: FEA embryonic fibroblasts, FC6.BM adherent bone marrow-derived cells and 3201 T-lymphoid cells. Plasmid DNA encoding each infectious recombinant provirus was introduced into the cells, and culture supernatants were collected at regular intervals thereafter for measurement of reverse transcriptase activity. As a confirmatory approach, cells were infected with equivalent amounts of virus particles representing each recombinant FeLV, and were similarly treated for measurement of reverse transcriptase activity in culture supernatants. The results showed that virus containing the 21-bp triplication (i.e., the FeLV-945 LTR) replicated significantly faster in feline fibroblasts, bone marrow cells and T-lymphocytes than virus containing 1, 2 or 4 copies of the repeat element. Thus, a replicative advantage conferred by the FeLV-945 LTR is readily detectable in relevant feline target cells. This observation is consistent with the predominance of the triplicated form in animals from the cohort, and suggests that the predominance of the FeLV-945 LTR in the cohort reflects replicative fitness.

Regarding the mechanism by which the FeLV-945 LTR confers a replicative advantage, our studies identified binding sites for the transcription factor, c-Myb, that cross the repeat junctions of the 21-bp triplication (Prabhu et al., 1999; Finstad et al., 2004). It was intriguing to identify binding sites that cross the repeat junctions, because such sites would not occur in the absence of the repeat; thus, a requirement for c-Myb binding to the repeat junctions of the triplication might represent a selective pressure to conserve its sequence precisely. Electrophoretic mobility shift assays were used to verify the binding of c-Myb to the 21-bp triplication, and using reporter gene assays, the FeLV-945 LTR was shown to respond to c-Myb in a manner requiring both c-Myb binding sites in the triplication. Results further indicated that c-Myb in complex with the 21-bp triplication recruits the transcriptional co-activator, CBP, a regulator of normal hematopoiesis. Indeed, FeLV-945 replication was shown to be positively regulated by CBP in a manner dependent on the presence of the 21-bp triplication. Thus, the FeLV-945 LTR binds and is regulated by two major transcriptional control proteins in the bone marrow, c-Myb and CBP (Finstad et al., 2004). These studies demonstrate the assembly of a distinctive transcription factor complex on the FeLV-945 LTR that might account for its replicative advantage in hematopoietic cells in which both c-Myb and CBP are present.

The FeLV-945 LTR activates a distinctive set of oncogenes in lymphomas

The gammaretroviral LTR functions in the malignant process not only by directing high levels of virus expression in relevant target tissues but also by insertionally activating oncogenes at or near the sites of proviral integration. When the same genetic locus is found to be interrupted by proviral integration in multiple independent tumors, it is inferred that the commonly interrupted locus encodes an oncogene whose activation is relevant to tumor induction (Hansen

et al., 2000; Mikkers and Berns, 2003). Such a locus is then referred to as a common insertion site (CIS). Based on several preliminary observations, we hypothesized that the FeLV-945 LTR would insertionally activate a distinctive set of oncogenes in tumors. First, the hypothesis was consistent with our observations from lymphomas in the natural cohort from which FeLV-945 was identified. While the c-*myc*, *pim*-1, *bmi-1* and *fit-1* loci, among others, are most commonly involved in thymic lymphomas induced by FeLV (Levy and Lobelle-Rich, 1992; Levy et al., 1993; Tsatsanis et al., 1994), none of these loci was targeted as a CIS in the non-T-cell multicentric lymphomas from which FeLV-945 was originally identified. Rather, a locus in feline DNA of unknown function, termed *flvi*-1, is a common target of FeLV-945 proviral integration in those tumors. Presumably, the unique structure of the FeLV-945 LTR is relevant to activation of a gene sequence within or near *flvi*-1 (Levesque et al., 1990; Athas et al., 1995; Levy et al., 1997). Second, we examined CISs in lymphomas induced by a novel synthetic retrovirus termed MoFe2. MoFe2 is a murine-feline recombinant retrovirus in which the triplication-containing FeLV-945 LTR was substituted into Moloney murine leukemia virus (M-MuLV). This recombinant virus was developed to permit analysis of FeLV-945 LTR integrations in a larger number of lymphomas than could reasonably be developed in cats. We showed that MoFe2 is infectious in mice, and that it induces thymic lymphomas with kinetics similar to M-MuLV. However, the oncogenes commonly targeted for proviral insertion in M-MuLV-induced lymphomas were not involved in MoFe2-induced tumors. Indeed, fewer than 10% of the lymphomas contained proviral integrations at previously identified CISs (Starkey et al., 1998). These observations suggested that a novel set of oncogenes might be activated by the FeLV-945 LTR in MoFe2-induced lymphomas.

To test the hypothesis that the FeLV-945 LTR directs the activation of a novel set of oncogenes, CISs in MoFe2-induced tumors were identified by three strategies, including cloning of size-selected restriction fragments, construction and screening of genomic DNA libraries, and PCR-based high throughput screening. Several CISs were identified that encode potential oncogenes, including *Rasgrp1*, *Ahi-1*, and *Jundm2*. Perhaps most intriguing was the identification of a CIS on mouse chromosome 11:B3, immediately upstream or within the gene encoding the p101 regulatory subunit of phosphoinositide-3-kinase-gamma (PI3K□). PI3K□. is known to be activated by G protein coupled receptors, is an activator of MAPK signaling and is expressed preferentially in cells of hematopoietic lineage (Lopez-Ilasaca et al., 1997; Baier et al., 1999; Brock et al., 2003). Thus, PI3K□ function may be relevant to growth regulation in the induction of lymphoma by MoFe2. None of the CISs identified in MoFe2-induced tumors had previously been reported in tumors induced by either parent virus in wild type animals, and one had not previously been reported in any model (Johnson et al., 2005). These findings support the hypothesis that the unique FeLV-945 LTR activates a novel set of oncogenes in the induction of lymphoma, possibly via the distinctive set of transcription factors shown to assemble on the 21-bp triplication. Finally, it was observed that one of the newly identified CISs contained two predicted matrix attachment regions (MARs). Further analysis of integration patterns for three retrovirus groups was performed to assess whether MARs may be a factor for integration site selection. Results indicated that all three groups integrate preferentially near MARs and that integration near MARs may have a role in the insertional activation of oncogenes by gammaretroviruses (Johnson and Levy, 2005).

The FeLV-945 SU gene encodes distinctive mutational differences as compared to FeLV-A

By sequence analysis, the SU protein of FeLV-945 is most closely related to natural horizontally-transmissible FeLV-A, but was observed to differ from an FeLV-A prototype to a greater extent than the known FeLV-A isolates differ among themselves (Athas et al., 1995; Prabhu et al., 1999). The sequence differences in FeLV-945 SU included point mutations restricted largely to domains that play essential roles in receptor recognition and entry, i.e.,

VRA, VRB and PRR (Battini et al., 1992; Boomer et al., 1997; Tailor and Kabat, 1997; Sugai et al., 2001). These sequences differences were considered remarkable because FeLV SU is strongly conserved (~97%) among natural FeLV-A isolates (Donahue et al., 1988; Neil et al., 1991; Overbaugh and Bangham, 2001). Experiments were performed to test the possibility that the mutational changes in FeLV-945 SU may alter receptor utilization, increase growth kinetics, or change disease spectrum relative to a prototype FeLV-A isolate. For this purpose, a recombinant FeLV was constructed in which the SU gene of FeLV-945 was substituted for that of FeLV-A/61E. Measurements of receptor utilization, including host range and superinfection interference confirmed the assignment of FeLV-945 to subgroup A by demonstrating utilization of the FeLV-A receptor for entry of the recombinant virus (Chandhasin et al., 2005). Despite the ecotropic host range and common assignment to FeLV-A, the mutational changes in FeLV-945 SU were observed to confer distinctive biologic properties. First, preliminary studies using flow cytometric virus binding assays indicated that FeLV-945 binds to the FeLV-A receptor with significantly greater efficiency than does FeLV-A/61E (C. Chandhasin and L.S. Levy, unpublished observations). Second, as might be predicted from a more efficient interaction with receptor, FeLV-945 SU was shown to confer a small but statistically significant replicative advantage in some feline cell lines (Chandhasin et al., 2005). The observations of increased receptor-binding affinity predict that FeLV-945 SU may influence the outcome of infection by at least two mechanisms: (1) by increasing kinetics of virus spread *in vivo*, and/or (2) by permitting efficient entry into a novel population of target cells, perhaps one with low receptor density. In addition, as described below, FeLV-945 SU acts together with the unique FeLV-945 LTR to determine an unusual disease spectrum.

The unique LTR and SU gene sequences of FeLV-945 act as determinants of disease outcome in experimentally infected cats

Serological survey of free-roaming urban domestic cats indicates that at least 50% of adult animals have been infected with FeLV (Rogerson et al., 1975). While most animals successfully clear the infection without disease consequences, the majority of persistently infected animals succumb to degenerative diseases including anemia or immunodeficiency, neoplastic or proliferative diseases including lymphoma, leukemia or myeloproliferative disorder (Mullins and Hoover, 1990; Rezanka et al., 1992). The determinants of disease outcome in natural FeLV infection are complex and probably involve a combination of host and viral factors. To study the influence of the distinctive FeLV-945 LTR and SU gene on pathogenesis, recombinant infectious FeLV proviruses were constructed in which the SU gene and/or LTR of FeLV-945 were substituted for homologous sequences in FeLV-A/61E, a prototype member of FeLV subtype A. The recombinant FeLVs were designated 61E/945L and 61E/945SL (Table 1). It is important to note that the substitutions into recombinant viruses actually contained the entire *env* gene of FeLV-945; however, the TM genes of FeLV-945 and FeLV-A/61E are nearly identical and sequence differences are limited largely to SU. Thus, substitution of the FeLV-945 *env* gene into FeLV-A/61E is essentially a substitution of SU and is designated as such. Neonatal kittens were inoculated intraperitoneally with FeLV-A/61E, 61E/945L or 61E/945SL and viremia was measured at regular intervals thereafter by antigen capture ELISA for the major capsid protein p27Gag in peripheral blood. By this measure, persistent viremia was detected in all animals beginning at 2 – 4 weeks post-inoculation. As the kittens were housed together with the dam until weaning, the possibility of horizontal transmission to the dam was also examined by ELISA. Interestingly, persistent viremia was detected only in the dam of animals inoculated with 61E/945SL. FeLV p27Gag ELISA showed that mean serum antigen levels for all challenge groups were highest during the first four weeks of infection and then declined slowly thereafter. Complete blood count performed at regular intervals during the course of disease revealed significant premalignant

changes. A significant depression in the number of circulating red blood cells and segmented neutrophils was observed during the first 4 weeks post-inoculation, particularly in animals infected with FeLV-A/61E or 61E/945L, but had recovered to normal levels by 8 weeks post-inoculation. In the case of animals infected with 61E/945SL, a significant peripheral blood lymphocytosis was observed beginning at 8 weeks post-inoculation and persisting for at least one month (Chandhasin et al., 2005).

The results of neonatal inoculations showed that the FeLV-945 LTR determined the kinetics of disease. Specifically, Kaplan-Meier survival estimate and analysis of the data by the log-rank test showed that virus containing the FeLV-945 LTR (i.e., either 61E-945L or 61E-945SL) caused disease significantly more rapidly than did FeLV-A/61E (avg. 47 weeks vs. avg. 78 weeks). Substitution of the FeLV-945 LTR did not alter the tumorigenic spectrum, in that both FeLV-A/61E and 61E-945L induced a thymic lymphoma of T-cell origin. Flow cytometric analysis of tumor cells from 61E-945L-infected animals demonstrated surface phenotypes characteristic of immature thymocytes, e.g., CD4+CD8+ or CD4−CD8−. One case was of mixed phenotype including CD4+CD8− (65%), CD4−CD8− (23%), and CD4+CD8+ (12%). These findings are consistent with other studies showing that FeLV-A typically induces thymic lymphoma of T-cell origin, and that tumor cells exhibit the surface phenotype of immature thymocytes (Rohn et al., 1994; Phipps et al., 2000). In contrast, substitution of both the FeLV-945 LTR and SU gene into FeLV-A/61E changed the disease outcome entirely. Specifically, 61E/945SL-infected animals developed disease relatively rapidly (average = 50 weeks), but in no case developed thymic lymphoma of T-cell origin. Rather, animals developed a multicentric lymphoma that involved multiple organs including liver, kidney and lungs but excluded the thymus. Flow cytometric analysis of tumor cells demonstrated the absence of cell surface CD4 or CD8 expression, but demonstrated high levels of expression of CD45R/B220, indicative of B-cell origin. Immunohistochemical analysis confirmed the B-cell origin of tumor cells by demonstrating abundant expression of CD79a. In addition, immunohistochemical analysis demonstrated surface IgM expression on a fraction of tumor cells and FeLV SU protein on the majority of tumor cells. Taken together, these findings clearly implicate the FeLV-945 LTR and SU gene as determinants of disease outcome indicating that the LTR determines the kinetics of disease induction and SU determines the tumorigenic spectrum (Chandhasin et al., 2005; Chandhasin et al., 2005).

While animals in our studies were inoculated through the intraperitoneal route, the most frequent route of exposure to FeLV in nature is thought to be oronasal contact with infectious saliva. Parenteral routes of inoculation used experimentally (e.g., intraperitoneal, intravenous) have been speculated to mimic the natural introduction of FeLV in the course of cat bites during fights or copulation (Rojko and Kociba, 1991). Indeed, a seminal study in which young cats were inoculated through either the oronasal or intraperitoneal route demonstrated a generally similar pattern of spread in vivo (Rojko et al., 1979). It is noteworthy that, in the natural cohort, FeLV-945 was not identified in T-cell tumors. The natural cohort did include thymic lymphomas, but such tumors did not contain FeLV-945. Rather, FeLV-945 was identified in non-T-cell multicentric lymphomas like those induced experimentally by intraperitoneal inoculation of the recombinant virus, 61E/945SL (Athas et al., 1995; Chandhasin et al., 2004). Thus intraperitoneal inoculation recapitulated the disease spectrum of FeLV-945 in nature.

Conclusions

Genetic variability is a hallmark of natural FeLV infection. Indeed, FeLV occurs in nature not as a single genomic species but as a family of closely related viruses. The disease outcome of natural FeLV infection is variable and rather unpredictable, including degenerative, proliferative and neoplastic diseases. While the molecular determinants of disease outcome are

not fully understood, the variability likely reflects genetic variation in both the host and the virus. FeLV-945 was identified as the predominant isolate in a geographic and temporal cohort of naturally infected cats and was shown to contain unique genetic features in the LTR and in the SU gene. FeLV-945 did not occur in T-cell lymphomas in the cohort, but was identified in non-T-cell disorders including multicentric lymphoma, myeloproliferative disorder and anemia. Thus, analysis of FeLV-945 afforded the opportunity to explore the selective advantages responsible for its predominance in a natural population as well as the genetic determinants of its distinctive disease spectrum. Results of a series of studies showed that the unique FeLV-945 LTR confers a replicative advantage to the virus in a cell type-specific manner and activates a novel set of oncogenes in tumor induction. Mutational changes in the FeLV-945 SU gene do not alter the ecotropic host range or entry through the FeLV-A receptor, but affect biological properties of the virus related to receptor interaction. The substitution of both the FeLV-945 LTR and SU gene into prototype FeLV-A was shown to increase the kinetics of tumor induction *in vivo*, cause distinctive premalignant features during disease induction and alter the tumorigenic spectrum. Taken together, the studies define mechanisms that may account for the predominance of FeLV-945 in a natural population and its association with a distinctive disease spectrum.

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Table 1

Recombinant FeLVs used in pathogenesis studies (Chandhasin et al., 2005).

Recombinant Virus	Parent Virus	Source of LTR	Source of SU gene	Kinetics of disease induction	Disease outcome
FeLV-A/61E (prototype FeLV-A)	FeLV-A/61E	FeLV-A/61E	FeLV-A/61E	slow	Thymic lymphoma; T-cell origin
61E/945L	FeLV-A/61E	FeLV-945	FeLV-A/61E	rapid	Thymic lymphoma; T-cell origin
61E/945SL	FeLV-A/61E	FeLV-945	FeLV-945	rapid	Multicentric lymphoma; B-cell origin

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