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The frequency of occurrence and nature of recombinant feline leukemia viruses in the induction of multicentric lymphoma by infection of the domestic cat with FeLV-945

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ABSTRACT

During feline leukemia virus (FeLV) infection in the domestic cat, viruses with a novel envelope gene arise by recombination between endogenous FeLV-related elements and the exogenous infecting species. These recombinant viruses (FeLV-B) are of uncertain disease association, but have been linked to the induction of thymic lymphoma. To assess the role of FeLV-B in the induction of multicentric lymphoma and other non-T-cell disease, the frequency of occurrence and nature of FeLV-B were examined in diseased tissues from a large collection of FeLV-infected animals. Diseased tissues were examined by Southern blot and PCR amplification to detect the presence of FeLV-B. Further analysis was performed to establish the recombination junctions and infectivity of FeLV-B in diseased tissues. The results confirmed the frequent association of FeLV-B with thymic lymphoma but showed infrequent generation, low levels and lack of infectivity of FeLV-B in non-T-cell diseases including multicentric lymphoma.

Introduction

Feline leukemia virus (FeLV) is a naturally occurring, horizontally transmitted gammaretrovirus associated with malignant, proliferative and degenerative diseases in the domestic cat. 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 in the domestic cat genome. Such variation has led to four naturally occurring FeLV subgroups, designated A, B, C, and T, that are distinguished genetically by sequence differences in the surface glycoprotein gene (SU) and functionally by interaction with distinct host cell receptors for entry. The weakly pathogenic FeLV subtype A (FeLV-A) is thought to represent the predominant agent spread horizontally cat-to-cat in nature, from which FeLV-B, -C and -T arise *de novo* in the infected animal by envelope (*env*) gene recombination, mutation or insertion events (Levy, 2008; Overbaugh and Bangham, 2001). While FeLV-A is associated with prolonged asymptomatic infection in the cat that may lead to malignant disease, typically a T-cell lymphoma of the thymus (Neil et al., 1991; Rezanka et al., 1992), the FeLV-B, -C and -T subgroups facilitate and/or redirect disease outcome to lymphoma, anemia or immunodeficiency disease, respectively (Levy, 2008; Overbaugh and Bangham, 2001).

Of particular interest in the present study is the generation of FeLV-B, a polytropic virus that arises by recombination *in vivo* between the infecting exogenous species and endogenous FeLV-related sequences in the cat genome. As a consequence of recombination, FeLV-B viruses contain a novel *env* gene with SU sequences containing variable representation of endogenous FeLV-derived DNA (Neil et al., 1991; Roy-Burman, 1995; Stewart et al., 1986). The endogenous, FeLV-related elements in the domestic cat occur at 9–16 copies per haploid genome (Pontius et al., 2007; Roca et al., 2005), differ in load among domestic cats of different breeds (Tandon et al., 2007) and represent a genetic set of fluid, polymorphic composition (Roca et al., 2004). The endogenous FeLV-related elements are defective and do not encode infectious particles, although some elements remain transcriptionally active and may be expressed in leukemic cells and in lymphoid tissues from healthy animals (McDougall et al., 1994; Roy-Burman, 1995). Recombination between endogenous and exogenous FeLV *env* sequences is thought to occur in a manner analogous to the generation of MCF recombinant viruses during MuLV infection (Fan, 1997). Unlike MCF viruses, which are known to represent the proximal leukemogen in MuLV infection (Fan, 1997), the disease association of FeLV-B infection remains unclear. While FeLV-B can be identified in 30%–50% of natural infections, always in conjunction with FeLV-A (Coelho et al., 2008; Neil et al., 1991; Roy-Burman, 1995), FeLV-B is overrepresented in the diseased tissues of animals with lymphoma as compared to asymptomatic,

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FeLV-infected cats, and has been identified in the majority of thymic lymphomas in particular (Jarrett, 1980, 1992; Roy-Burman, 1995; Sheets et al., 1993; Tsatsanis et al., 1994). In a recent study, however, FeLV-B recombinants were identified only infrequently by PCR or Southern blot analysis in a set of unusual multicentric lymphomas of B-cell origin induced experimentally by viruses containing the unique sequence elements of a natural variant designated FeLV-945 (Chandhasin et al., 2005a). FeLV-945 had been identified originally in a geographic and temporal cohort of naturally infected animals in which non-T-cell disease, including multicentric lymphoma, was the condition most commonly identified. As the predominant isolate in non-T-cell diseases in the cohort, FeLV-945 was shown to contain unique sequence elements in the LTR and SU gene. The substitution of these elements into prototype FeLV-A significantly reduced the latency to disease and shifted the disease spectrum entirely to recapitulate the multicentric lymphoma from which FeLV-945 was originally identified (Chandhasin et al., 2005b). Considering the relatively rapid disease induction in animals infected with chimeric viruses containing elements of FeLV-945, the infrequent detection of FeLV-B in preliminary analysis (Chandhasin et al., 2005a,b) suggested that the generation of FeLV-B recombinants may not be involved in the induction of multicentric lymphoma, or alternatively, may occur infrequently or inefficiently when FeLV-945 is the exogenously infecting species. To address this question further, the frequency of occurrence and nature of FeLV-B recombinants was examined in a large collection of diseased tissues from naturally and experimentally infected cats. Twenty-two naturally infected animals were examined, representing the geographic and temporal cohort from which FeLV-945 was originally identified (Chandhasin et al., 2004; Levesque et al., 1990). Twenty experimentally infected animals were examined from several previous studies (Chandhasin et al., 2005a,b; Levy et al., 1988) in which neonatal cats were inoculated with prototype FeLV-A/61E, with chimeric viruses in which the unique LTR and/or SU gene of FeLV-945 were substituted into FeLV-A/61E, or with the *myc* oncogene-containing LC-FeLV isolate. Diseased tissues were examined by Southern blot and PCR amplification to detect the presence of FeLV-B. Further analysis was performed to establish the recombination junctions and infectivity of FeLV-B in diseased tissues.

Results and discussion

Demonstration of integrated FeLV-B proviral DNA in diseased tissues by Southern blot

To evaluate the frequency with which integrated FeLV-B proviral DNA could be detected in diseased tissues, large molecular weight genomic DNA (8 μg) was digested with KpnI and examined by Southern blot analysis. The blot was hybridized to probe B/S, a *Sau3A* fragment from the *env* gene of FeLV-B/Gardner-Arnstein specific for the major classes of endogenous FeLV that serve as substrates for recombination (Chandhasin et al., 2005b; Tsatsanis et al., 1994). Analysis revealed the 3.6-kb fragment distinctive of FeLV-B proviral DNA in thymic lymphoma samples, but not in samples of multicentric lymphoma or in uninvolved tissues of the same animals (Fig. 1A). By comparison, hybridization of KpnI-digested DNA with a probe for the LTR of exogenous FeLV clearly demonstrated the pattern of clonally integrated proviruses distinctive of the monoclonal expansion in all tumor samples examined, but not in uninvolved tissues of the same animals (Fig. 1B). By applying this approach to the entire collection, FeLV-B provirus was detected in the DNA of diseased tissues from 5 of 22 naturally infected animals, including 2 of 4 cases of thymic lymphoma and 3 of 12 cases of multicentric lymphoma. FeLV-B was not detected in myeloproliferative disease, mast cell leukemia, feline infectious peritonitis, chronic glomerulonephritis, or in healthy, FeLV-infected animals (Table 1). In experimentally infected animals, FeLV-B was detected in 9 of 13 cases of thymic lymphoma and in two additional animals for which FeLV-B was included in the original inoculum. FeLV-B was not detected by this measure in multicentric lymphoma, in an animal inoculated with *myc*-containing FeLV in the presence of FeLV-B in the inoculum, or in mock-infected animals (Table 2). Considering all cases together, the incidence of FeLV-B in thymic lymphoma (11 of 17) was significantly higher than in multicentric lymphoma (3 of 15) as measured by Fisher Exact Test ($p = 0.015$).

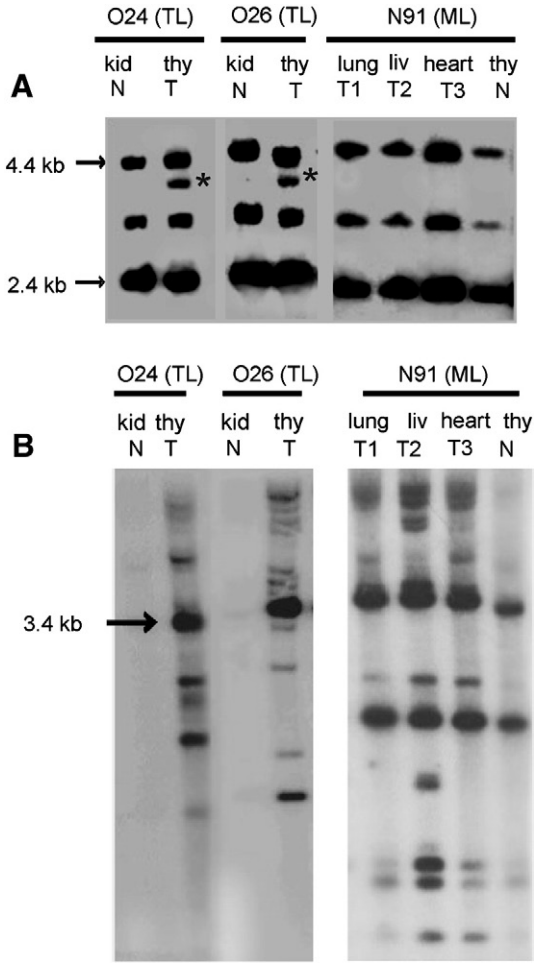

Fig. 1. Southern blot analysis of genomic DNA from tumor (T) or paired normal (N) tissue from FeLV-infected cats O24, O26 and N91 bearing thymic lymphoma (TL) or multicentric lymphoma (ML) (Chandhasin et al., 2005b). A. DNA samples (8 μg) were digested with KpnI and hybridized to probe B/S, a *Sau3A* fragment from the *env* gene of FeLV-B/Gardner-Arnstein specific for the major classes of endogenous FeLV that serve as substrates for recombination. The distinctive hybridizing fragment of ~3.6-kb (*) indicates recombinant FeLV-B provirus in genomic DNA (Chandhasin et al., 2005b; Tsatsanis et al., 1994). B. KpnI-digested DNA samples were also hybridized to a probe representing the U3 region of the LTR of exogenous FeLV. By this analysis, clonally integrated proviruses are visualized as host-virus junction fragments in tumor DNA (Levy et al., 1984).

PCR amplification of FeLV-B proviral DNA from diseased tissues

PCR amplification was then used to validate the results and to extend the findings to cases in which FeLV-B integrations may have occurred at a frequency in the tumor mass below the level of detection by Southern blot analysis. Genomic DNA samples were amplified using forward and reverse primers specific for the endogenous FeLV-related CFE-6 element and exogenous FeLV-945, respectively. CFE-6 was selected for this purpose because it represents the major class of nearly full-length proviruses in the cat genome and contains a complete *env* gene with open reading frame (Kumar et al., 1989).