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Acute virulent infection with feline immunodeficiency virus (FIV) results in lymphomagenesis via an indirect mechanism

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ABSTRACT

Four cats (24%) experimentally infected with FIV unexpectedly developed neoplastic changes within four months of inoculation. While FIV has previously been associated with neoplasia, the rapidity and high attack rate seen here is highly unusual. PCR for antigen receptor rearrangements (PARR) detected clonally rearranged T cells in two animals diagnosed with B cell follicular lymphoma by classical means. All cats were negative for feline leukemia virus; gamma-herpesvirus DNA was not amplified using degenerate primers. FIV proviral load in neoplastic tissue was two orders of magnitude lower than in the periphery, lower in neoplastic vs non-neoplastic lymph node, and clonal integration was not detected. We hypothesize that neoplasia was secondary to FIV immune dysregulation, and show that PARR can augment our capacity to phenotype these tumors and distinguish follicular hyperplasia from lymphoma. Age of exposure and relative virulence of the inoculum likely contributed to this unusual presentation of FIV infection.

Introduction

Feline immunodeficiency virus (FIV) is a lentivirus in the family *Retroviridae* that causes immune dysfunctions in cats similar to those observed in people infected with human immunodeficiency virus (HIV) (Gabor et al., 2001; Ishida et al., 1989; Pedersen et al., 1989; Pedersen, 1994; Zenger, 1990). FIV was first described in 1987 in a large multiple cat household experiencing immunodeficiency related diseases (Pedersen et al., 1987). Clinical symptoms during the initial few weeks of infection include fever, leucopenia, gingivitis, and generalized lymphadenopathy. Clinical signs usually regress by 1–4 months post experimental infection, concurrent with seroconversion, and a latent stage of variable duration occurs wherein viral load attains a steady state. End stage disease typically does not occur for several years post-infection, and is marked by loss of CD4 immunocytes and high circulating viral load. Clinical signs reported during this phase of disease include neurologic manifestations, enhanced susceptibility to opportunistic infections, and neoplasia (Ravi et al., 2010).

The association between FIV and an increased incidence of neoplasia has been frequently reported in the years since FIV was first described (Alexander et al., 1989; Barr et al., 1993; Callanan et al., 1992; Gabor et al., 2001; Gruffydd-Jones et al., 1988; Hopper et al., 1989; Hutson et al., 1991; Magden et al., 2011; Pedersen et al., 1989; Pedersen, 1994; Poli et al., 1994; Shelton et al., 1989a, 1989b; Terry et al., 1995; Yamamoto et al., 1989; Zenger, 1990). The incidence of FIV-associated neoplasms ranges from 1 to 21% of FIV-positive cats, consistent with the reported incidence in HIV-positive individuals, and neoplasia typically rises sporadically several years post-infection (Feder and Hurvitz, 1990; Sabine et al., 1988; Zenger, 1990). The most frequently reported type of neoplasia associated with FIV infection is lymphoma, typically a high grade B cell tumor, also consistent with reports of HIV-associated neoplasia (Callanan et al., 1996; Gabor et al., 2001; Poli et al., 1994; Rabkin et al., 1991; Terry et al., 1995; Wang et al., 2001).

Infectious etiologies other than FIV and HIV have been associated with increased incidence of neoplasia. Recent publications have implicated infectious diseases in up to 17% of reported new cancers (Fontham, 2009). In cats, feline leukemia virus (FeLV) is known to result in the development of lymphoma (Ahmad and

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Levy, 2010). Human and primate herpesviruses have been associated with a variety of cancers, often in association with lentiviral co-infection (Bruce et al, 2012; Carbone et al., 2008; Delecluse et al., 2007; Wen and Damania, 2010). Some other examples of agents that are associated with neoplastic transformation include human papilloma virus, hepatitis B and C viruses, and *Helicobacter pylori* (Fontham, 2009).

The ability of viruses to induce neoplasia typically relates to specific features of the viral replication cycle (Maeda et al., 2008). A potential direct role of FIV-induced lymphomagenesis, i.e. FIV clonal integration disruption of oncogene function, has been reported in two select cases (Beatty et al., 1998b, 2002; Wang et al., 2001). In one study, FIV sequences were detected in DNA isolated from a cat experimentally infected with FIV that developed lymphoma of the lymph nodes, liver, and omentum. The tumor was of high grade B-cell origin and demonstrated monoclonal integration of FIV (Beatty et al., 1998b). A second study demonstrated clonal FIV proviral integration in two of fourteen cases of feline lymphosarcoma by PCR and confirmed with Southern blot analysis (Wang et al., 2001). These two studies suggest that in a subset of cases, a direct oncogenic role of FIV in proto-oncogene stimulation may lead to malignant transformation (Rosenberg et al., 1991).

The majority of studies investigating mechanisms of FIV infection that underlie an increased rate of tumorigenesis have failed to demonstrate clonal integration of the FIV-provirus in lymphomas (Beatty et al., 1998a; Callanan et al., 1992, 1996; Terry et al., 1995). This implicates an *indirect* mechanism of tumorigenesis potentially involving impaired immune surveillance and removal of neoplastic cells secondary to FIV-induced immunodyscrasias. This theory is supported by observed increases in B cell proliferation following FIV infection, resulting in the production of large pools of circulating lymphocytes (Callanan et al., 1993). Acute FIV infection is consistently associated with lymphoid follicular hyperplasia and expansion of B cell regions resulting in a pre-neoplastic phenotype. Such vigorous lymphoid expansion may result in the enhancement of opportunities of malignant cell development and 'escape' (Callanan et al., 1992). While B cell lymphomas are the more frequently observed tumor type, T cell lymphomas can also occur with FIV infection (Endo et al., 1997; Gabor et al., 2001; Wang et al., 2001). Case reports of FIV-positive animals with multiple neoplasms, such as a spinal lymphosarcoma with concurrent disseminated mastocytoma (Barr et al., 1993), would also suggest a generalized mechanism of enhanced tumor susceptibility.

Cancer development during lentiviral infection has been associated with co-infection of a second viral agent. For example, co-infection with HIV and Kaposi's sarcoma-associated herpes virus [KSHV or human herpes virus 8 (HHV8)] results in epidemic Kaposi's sarcoma (KS), a cancer of lymphatic endothelium (Cesarman et al., 1995; Chang et al., 1994; Rabkin et al., 1991). AIDS patients are over 300 times more likely to develop KS than persons on immunosuppressive therapies (Beral et al., 1990). Non-human primates infected with both simian immunodeficiency virus (SIV) and simian gamma-herpes viruses (including the macaque equivalent of KSHV) have also been associated with increased incidence of gastrointestinal stromal tumor development and lymphomas (Bielefeldt-Ohmann et al., 2005, 2008; Bruce et al., 2012). These examples, and a recent report associating spontaneous B cell lymphoma in dogs to infection with an EBV-like gammaherpesvirus (Huang et al., 2012), suggests that an as-yet undescribed feline gamma-herpesvirus may be associated with lymphoma development in FIV-positive cats.

In this study we observed neoplastic changes in four of seventeen FIV-inoculated kittens as an unanticipated outcome in a protocol testing a novel anti-retroviral therapy. This

Table 1
PBMC proviral loads and histologic findings from one FIV-negative cat (1) and six cats infected with virulent FIV as quantitated by real time PCR. Cats 2 and 3 included as FIV-positive controls that did not develop neoplastic lesions. MLN = mesenteric lymph node, WNL = within normal limits, n.d. = not determined.

Cat ID	Status	Proviral load/10^6 cells		IHC	Neoplasia/clonality	Southern blot/ Herpesvirus	Histology descriptions
		PBMC	MLN				
1	Naïve	0	0	WNL	n.d.	Negative	No gross or histologic abnormalities observed.
2	FIV+ with no histologic evidence of neoplasia	1.68×10^5	1.50×10^3	WNL	n.d.	n.d.	Mesenteric lymph node; Mild expansion of paracortical zone, consistent with mild lymphoid hyperplasia.
3	FIV+ with no histologic evidence of neoplasia	6.35×10^4	8.69×10^3	WNL	n.d.	n.d.	Mesenteric lymph node: moderate lymphoid hyperplasia characterized by large numbers of secondary follicles that expand paracortical zone.
4	FIV+with rapid onset of pneumonia, lymphoid leukemia	3.71×10^5	3.37×10^3	inconclusive	n.d.	n.d.	Bone marrow: lymphoid leukemia with liver, lung, and spleen involvement; Lung: fibrinous interstitial pneumonia.
5	FIV+ with gross tumor (MLN)	5.89×10^4	3.18×10^2	Predominantly B Cell	Primarily B cell clonality with some T cell	negative	Ileum, mesenteric lymph node: *B cell follicular lymphoma* (CD79a positive).
6	FIV+ with histologic evidence of pre-neoplasic lesions (MLN)	3.29×10^4	3.70×10^2	Predominantly B Cell	n.d.	negative	Mesenteric lymph node: pronounced follicular hyperplasia with merging of germinal centers and many follicles with narrow or discontinuous marginal zones (*dysplasia*).
7	FIV+ with histologic evidence of neoplasia (MLN)	6.12×10^4	8.77×10^2	Predominantly B Cell with lower levels of T cell	T cellclonality	negative	Mesenteric lymph node: B cell follicular lymphoma (CD79a positive) with lower levels of T cell expression (CD3). *Mixed T/B lymphoma*