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Authors: Roelke, M. E., Pecon-Slattery, J., Taylor, S., Citino, S., Brown, E., et al.

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T-LYMPHOCYTE PROFILES IN FIV-INFECTED WILD LIONS AND PUMAS REVEAL CD4 DEPLETION

M. E. Roelke,[1] J. Pecon-Slattery,[2,8] S. Taylor,[3] S. Citino,[4] E. Brown,[2,7] C. Packer,[5] S. VandeWoude,[6] and S. J. O'Brien[2]

[1] Laboratory of Genomic Diversity, Basic Research Program, SAIC Frederick, National Cancer Institute, Frederick, Maryland 21702, USA
[2] National Cancer Institute–Frederick, Frederick, Maryland 21702, USA
[3] Environmental Protection Agency, Research Triangle Park, North Carolina 27711, USA
[4] White Oak Conservation Center, Yulee, Florida 32097, USA
[5] Department of Ecology, Evolution and Behavior, University of Minnesota, St. Paul, Minnesota 55108, USA
[6] Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, Colorado 80523, USA
[7] Present address: U.S. Food and Drug Administration, College Park, Maryland 20740, USA
[8] Corresponding author (email: slattery@mail.ncifcrf.gov)

ABSTRACT: Feline immunodeficiency virus (FIV) is a lentivirus related to human immunodeficiency virus (HIV) that causes feline AIDS in the domestic cat (*Felis catus*). Serological surveys indicate that at least 25 other species of cat possess antibodies that cross-react with domestic cat FIV. Most infected nondomestic cat species are without major symptoms of disease. Long-term studies of FIV genome variation and pathogenesis reveal patterns consistent with coadaptation of virus and host in free-ranging FIV-Ple–infected African lions (*Panthera leo*) and FIV-Pco–infected pumas (*Puma concolor*) populations. This report examined correlates of immunodeficiency in wild and captive lions and pumas by quantifying CD5+, CD4+, and CD8+ T-cell subsets. Free-ranging FIV-Ple–infected lions had immunofluorescence flow cytometry (IFC) profiles marked by a dramatic decline in CD4+ subsets, a reduction of the CD4+/CD8+ ratio, reduction of $CD8+\beta^{high}$ cells, and expansion of the $CD8+\beta^{low}$ subset relative to uninfected lions. An overall significant depletion in CD5+ T-cells in seropositive lions was linked with a compensatory increase in total CD5− lymphocytes. The IFC profiles were altered significantly in 50% of the seropositive individuals examined. The FIV-Pco–infected pumas had a more generalized response of lymphopenia expressed as a significant decline in total lymphocytes, CD5+ T-cells, and CD5− lymphocytes as well as a significant reduction in CD4+ T-cells. Like lions, seropositive pumas had a significant decline in $CD8+\beta^{high}$ cells but differed by not having compensatory expansion of $CD8+\beta^{low}$ cells relative to controls. Results from FIV-infected lions and pumas parallel human and Asian monkey CD4+ diminution in HIV and SIV infection, respectively, and suggest there may be unrecognized immunological consequences of FIV infection in these two species of large cats.

Key words: CD4 T-cells, Felidae, FIV, flow cytometry, immune depletion, lion, lymphocytes, puma.

INTRODUCTION

Feline immunodeficiency virus (FIV) is a pathogenic lentivirus related to human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV). Initially isolated in 1986, FIV was subsequently identified as the etiologic agent of acquired immune deficiency syndrome or feline AIDS in the domestic cat (*Felis catus*) (Pedersen et al., 1987; Brunner and Pedersen, 1989; Pedersen et al., 1989; Gardner, 1991). Feline immunodeficiency virus–infected domestic cats exhibit disease symptoms, immune suppression, and mortality markedly similar to HIV infection in humans (Yamamoto et al., 1988; Pedersen et al., 1989; Yamamoto et al., 1989; Willett et al., 1993; English et al., 1994; Bendinelli et al., 1995; Liang et al., 2000). Feline immunodeficiency virus strains are species-specific as indicated by comparative genomic analyses of strains sequenced from domestic cats (FIV-Fca), Pallas cats (*Otocolobus manul*; FIV-Oma), cheetahs (*Acinonyx jubatus*; FIV-Aju), leopards (*Panthera pardus*; FIV-Ppa), pumas (*Puma concolor*; FIV-Pco), and African lions (*Panthera leo*; FIV-Ple) (Brown et al., 1994; Carpenter et al., 1996, 1998; Troyer et al., 2005). Additional serological surveys indicate at least 25

other species of cats possess antibodies that cross-react with FIV (Olmsted et al., 1992; Brown et al., 1994; Carpenter and O'Brien, 1995; Carpenter et al., 1996, 1998; Troyer et al., 2004, 2005). The observed worldwide prevalence of FIV in multiple cat species is made more intriguing by the apparent lack of discernable disease in nondomestic cat species (Lutz et al., 1992; Hofmann-Lehmann et al., 1996; Packer et al., 1999), although an FIV-positive captive lion with end-stage disease reminiscent of domestic cats with feline AIDS has been reported (Poli et al., 1995; Bull et al., 2003).

Phylogenetic studies of the *gag* and *pol* genes from FIV in natural populations of pumas in North and South America and African lions in eastern and southern Africa and *env* regions from FIV-Fca sampled from domestic cats worldwide depict unique patterns of virus-host coevolution. In pumas, FIV-Pco forms distinct divergent evolutionary lineages that are distributed throughout this species range (Carpenter et al., 1996; Biek et al., 2003). In lions, FIV-Ple is endemic to populations within east and south Africa and forms at least three different evolutionary clades with high levels of genetic differences (Brown et al., 1994; Troyer et al., 2004, 2005). Genetic analyses of FIV-Ple within a large out-bred population of lions in the Serengeti region of Tanzania revealed >90% prevalence with 43% of the individuals multiply infected with at least two subtypes (Brown et al., 1994; Troyer et al., 2004). In domestic cats, FIV-Fca subtype classification is made using the more variable *env* region rather than the *pol* or *gag* analyzed in FIV from other species. Even using the *env* region, FIV-Fca has lower levels of intersubtype divergence than FIV-Pco and FIV-Ple (Carpenter et al., 1998). Further, three of the five recognized subtypes or clades of FIV-Fca are composed of closely related strains from cats dwelling on different continents (Carpenter et al., 1998). Thus, the low genetic diversity and demonstrable pathogenicity of FIV in domestic cats suggest that this species acquired FIV relatively recently, whereas FIV-Pco and FIV-Ple descend from an earlier species experience with endemic virus and became attenuated as a natural outcome of a longer period of virus-host coevolution (Carpenter and O'Brien, 1995; Carpenter et al., 1996, 1998).

Under this scenario, FIV-Fca, FIV-Ple, and FIV-Pco would be expected to elicit different clinicopathologic abnormalities in their respective host species. However, evaluation of such parameters using a systematic approach in lions and pumas has been impeded by logistical difficulties and infrequent opportunities associated with sample collection in the wild, adequate preservation of samples in field conditions, and lack of reagents or reagent validation to measure blood cell responses for exotic felids accurately. Consequently, only one previously published study has reported lymphocyte subset alterations in five captive FIV-Ple seropositive lions (Bull et al., 2003) Additionally, the high seroprevalence rate of some free-ranging populations (up to 100%) (Brown et al., 1994; Carpenter and O'Brien, 1995; Biek et al., 2003; Troyer et al., 2004) may affect statistical analyses of seropositive versus seronegative groups.

Analysis of domestic cats infected with FIV-Fca, on the other hand, has been more intensively studied. Domestic cats infected with FIV-Fca experience profound changes in T-cell subsets concurrent with clinical immune deficiency. Like HIV in humans, the continued deterioration of the host immune system in FIV-positive domestic cats is correlated with the reduction in circulating CD4+ lymphocytes (Ackley et al., 1990; Novotney et al., 1990). In the initial acute phase, the CD4+/CD8+ ratio is lowered as a consequence by both the reduction of CD4+ T-cells and the marked expansion of CD8+ T-cells (Willett et al., 1993). However, subtype and/or strain-specific effects on the host circulating lymphocyte kinetics,

which parallels the degree of immunosuppression or virulence, are observed in domestic cats infected with a less virulent strain of FIV characterized by low viral load (Hosie et al., 2002). Experimental infection of domestic cats with FIV-Pco or FIV-Ple results in apathogenic but productive infection (VandeWoude et al., 1997a, b, 2003; Terwee et al., 2005), which strengthens the hypothesis that nondomestic cat FIVs are host-adapted or perhaps less virulent lentiviruses.

We examined changes in T-cell lymphocytes in response to FIV infection in a cohort of both captive and free-ranging populations of lions and pumas and found significant changes in circulating lymphocyte T-cell subsets associated with FIV infection. These findings may have significant implications for management of FIV-positive endangered feline species and for the health of individual animals.

METHODS

Study animals: pumas and lions

The FIV-positive and -negative individuals were sampled from pumas in North America and African lions from the Serengeti National Park in Tanzania and Kruger National Park in South Africa (Table 1). All pumas and free-ranging African lions were captured, anesthetized, examined, and bled as part of ongoing field studies (Roelke et al., 1993; Roelke-Parker et al., 1996). The puma sample consisted of 10 uninfected individuals (1–9 yr-old) and six FIV-Pco–infected animals (5–12 yr-old); five were captive, and 11 were free-ranging, all originating from the same population in southern Florida, USA. Twelve FIV-Ple–infected lions (2–12 yr-old) and five zoo-bred captive naive lions of unknown ages with no record of ancestral geographic origin were sampled. All seropositive lions were free-ranging in either Kruger National Park, South Africa, or Serengeti National Park, Tanzania. Captive lions were bled during routine yearly physical examinations.

Sample collection and processing

Peripheral whole blood collected from both captive and free-ranging animals was processed within 48 hr. Whole blood collected with EDTA was used for an absolute white blood cell count (WBC), determined by the Unopipette® counting system and hemocytometer, and an absolute lymphocyte count, determined by staining blood films with a modified Wright-Giemsa stain. To process samples for lymphocyte analyses, peripheral blood mononuclear cells (PBMCs) were separated from whole anticoagulated blood collected by using Histopaque-1077 (Sigma, St. Louis, Missouri, USA) and viably frozen. Five milliliters of whole blood was overlaid on 5 ml histopaque and spun at 400 × G for 30 min. The PBMC layer was removed, washed in phosphate buffered saline (PBS), and spun at 250 × G for 10 min at least twice. Cell pellets were gently suspended in 90% fetal calf serum with 10% dimethyl sulfoxide (DMSO) and viably frozen, in aliquots of 10^7 cells/ml/cryotube, at a rate of 1 C/min and stored in liquid nitrogen.

Serum samples were simultaneously collected and screened for antibodies to both FIV-Pco and FIV-Ple antigens. Western blot analysis for FIV-reacting antibodies was performed as previously described (Diehl et al., 1995; VandeWoude et al., 1997a, b; Troyer et al., 2005). Tissue culture supernatant containing either virus strain grown on 3201 T-cells was concentrated by ultracentrifugation. Protein content was determined, and viral antigen was subjected to polyacrylamide electrophoresis and transferred to nylon membranes. Diluted serum was reacted with membrane strips, and bound antibody detected by horse radish peroxidase-labeled secondary antibodies. To ensure FIV antibody detection, FIV-Fca antigen was used in separate tests in addition to FIV-Pco for pumas and FIV-Ple for lions. Positive and negative sera served as controls for comparison to unknown samples.

TABLE 1. Species, identity, health status, origin and contact for samples used in this study.

Species ID	Date collected	FIV[a]	Sex	Age (yr)	Captivity (status)	Health (status)	Location	Source
Puma (*Puma concolor*)								
PCO-166	8 Aug 1991	−	M	5	Captive	Healthy	Everglades Holiday Park, Florida	M. Roelke
PCO-387	6 Aug 1991	−	M	9	Captive	Healthy	Everglades Holiday Park, Florida	M. Roelke
PCO-390	6 Aug 1991	−	F	8	Captive	Healthy	Everglades Holiday Park, Florida	M. Roelke
PCO-392	7 Aug 1991	−	M	7	Captive	Healthy	Everglades Holiday Park, Florida	M. Roelke
PCO-533	16 Dec 1996	−	M	5	Wild	Healthy	Big Cypress Swamp, Florida	S. Taylor/D. Land
PCO-539	15 Jan 1997	−	M	2	Wild	Dermatophytosis	Big Cypress Swamp, Florida	S. Taylor/D. Land
PCO-717	8 Mar 1997	−	F	5	Wild	Dermatophytosis	Big Cypress Swamp, Florida	S. Taylor/D. Land
PCO-718	18 Mar 1997	−	M	1	Wild	Healthy	Big Cypress Swamp, Florida	S. Taylor/D. Land
PCO-719	15 Apr 1997	−	M	2	Wild	Healthy	Big Cypress Swamp, Florida	S. Taylor/D. Land
PCO-898	5 Mar 1997	−	F	1	Wild	Healthy	Big Cypress Swamp, Florida	S. Taylor/D. Land
PCO-075	14 Jan 1997	+	F	12	Captive	Anemia, neurologic symptoms	White Oak Plantation, Florida[b]	S. Citino
PCO-154	25 Jan 1992	+	M	5	Wild	Healthy	Big Cypress Swamp, Florida	M. Roelke
PCO-160	21 Feb 1997	+	F	10	Wild	Mange infection	Big Cypress Swamp, Florida	S. Taylor/D. Land
PCO-730	18 Dec 1996	+	F	6	Wild	Healthy	Big Cypress Swamp, Florida	S. Taylor/D. Land
PCO-733	19 Dec 1996	+	F	6	Wild	Healthy	Big Cypress Swamp, Florida	S. Taylor/D. Land
PCO-736	10 Jan 1997	+	F	5	Wild	Healthy	Big Cypress Swamp, Florida	S. Taylor/D. Land
African lion (*Panthera leo*)								
PLE-030	5 Feb 1997	−	M	3	Captive	Healthy	Wildlife Waystation, California	R. Yates
PLE-031	28 Feb 1997	−	M	n/a	Captive	Healthy	Wildlife Waystation, California	R. Yates
PLE-032	28 Feb 1997	−	M	n/a	Captive	Healthy	Wildlife Waystation, California	R. Yates
PLE-033	7 Mar 1997	−	F	9	Captive	Asthma	Druid Park Zoo, Baltimore Maryland	M. Cranfield
PLE-034	1 Apr 1997	−	M	2	Captive	Healthy	Wildlife Waystation, California	R. Yates
PLE-133	26 July 1992	+	F	Adult	Wild	Healthy	Kruger National Park, South Africa	M. Bush
PLE-134	27 July 1992	+	F	Adult	Wild	Healthy	Kruger National Park, South Africa	M. Bush
PLE-135	26 July 1992	+	F	Adult	Wild	Healthy	Kruger National Park, South Africa	M. Bush
PLE-138	27 July 1992	+	M	Adult	Wild	Healthy	Kruger National Park, South Africa	M. Bush

TABLE 1. Continued.

Species ID	Date collected	FIV[a]	Sex	Age (yr)	Captivity (status)	Health (status)	Location	Source
PLE-569	28 June 1994	+	F	3	Wild	Chronic CDV infection, myoclonas, lymphadenopathy	Serengeti National Park, Tanzania	M. Roelke/ C. Packer
PLE-586	1 Apr 1994	+	F	2	Wild	Acute CDV infection, emaciated, lymphadenopathy	Serengeti National Park, Tanzania	M. Roelke/ C. Packer
PLE-599	20 July 1994	+	F	13	Wild	Healthy, seropositive CDV	Serengeti National Park, Tanzania	M. Roelke/ C. Packer
PLE-605	19 July 1995	+	F	6	Wild	Healthy	Serengeti National Park, Tanzania	M. Roelke/ C. Packer
PLE-618	7 July 1994	+	F	4	Wild	Prior CDV infection, lymphadenopathy	Serengeti National Park, Tanzania	M. Roelke/ C. Packer
PLE-645	18 Mar 1995	+	F	8 to 10	Wild	Infected snare wound, lymphadenopathy	Serengeti National Park, Tanzania	M. Roelke
PLE-647	10 July 1995	+	M	6 to 8	Wild	Infected snare wound, emaciated, lymphadenopathy	Serengeti National Park, Tanzania	M. Roelke
PLE-649	17 Jan 1996	+	M	6 to 8	Wild	Emaciated, lymphadenopathy	Serengeti National Park, Tanzania	M. Roelke

[a] FIV status determined by Western blot (see Methods).

[b] Removed from the wild in 1987.

Immunofluorescence flow cytometry

Viably frozen PBMCs were quickly thawed at 37 C, washed in PBS, counted, and resuspended in a buffer solution of PBS. The PBMCs (1×10^6) were combined in separate tubes with monoclonal antibodies that recognize the following: feline CD4+ (Fel7 clone CD4; Klotz and Cooper, 1986; Ackley et al., 1990); feline CD8+ (FT2 clone fCD8-Beta; Klotz and Cooper, 1986); and feline Pan-T+ cell CD5 equivalent (clone f43; Ackley and Cooper, 1992). The T-cell profiles were generated by double labeling with CD5+/CD4+, CD5+/CD8+, or CD4+/CD8+ and quantified by two-color immunofluorescence flow cytometry (IFC) using Becten-Dickenson FACscan and Cell Quest software. A dot-plot of side and forward scatter was used to construct a live lymphocyte gate of 5,000–10,000 cells per assay. The absolute number of T-cells was determined by multiplying the fraction of respective CD4+, CD8+, and CD5+ subpopulations times the number of lymphocytes/ml within the original sample. The CD5− cells, within the gated lymphocyte fraction, were considered primarily B cells, though it is possible that this fraction could also contain small proportions of monocytic cells. No conversion values were available for lions sampled from Kruger Park (Ple 133, 134, 135, 138). The potential bias introduced by using viably frozen PBMC versus those from fresh blood for IFC was addressed by setting the gates to exclude nonspecific binding and thus compensate for background staining due to cell death. Moreover, IFC analysis provided comparable results whether performed on frozen or fresh PBMC in our experience (Vande-Woude, unpublished data) as well as that of others (Bull et al., 2003).

Statistical analysis

The total T-cell (CD5+) profile generated by IFC displayed the relative proportions of CD4+, CD8+, and CD4−CD8− subpopulations within the PBMC sample. These ratios were not independent because they were derived from the total CD5+ T-cells; therefore, we performed heterogeneity G-tests (Sokol and Rohlf, 1991). Each profile was tested against the expected profile from control naive animals to determine if the individual T-cell subset profile changed with FIV infection. Significance was determined by computation of a G-statistic that approximates a chi squared distribution.

For each IFC profile, the relative proportions of CD4+, CD8+, and CD4−CD8−CD5+ cells were converted into absolute numbers of cells based on the absolute cell count of lymphocytes. We evaluated the following absolute cell counts for each animal: WBC/ml, total lymphocytes/ml, total CD5− cells/ml, CD5+ T-cells/ml, CD4+/ml, CD8+/ml, and CD4−CD8−CD5+/ml. In addition, we examined two subsets, CD8+β^{low} and CD8+β^{high}, identified by the antibody FT2 as it binds specifically to the beta chain of the CD8+ heterodimer molecule. Estimates of cell counts for each were computed by multiplying the relative proportion times the estimated CD8+ cells/ml count for each individual.

The distribution of each cell count variable was tested for normality using the Shapiro-Wilks' W suitable for small sample sizes. The relative impact of FIV status on the cell count variables for each species was tested using analysis of variance (ANOVA) and paired *t*-tests of means (SAS, 2001).

RESULTS

The majority of lymphocytes of 17 lion and 16 puma blood samples were CD5+ T-cells by IFC. The relative proportion of CD4+, CD8+, and CD4−CD8− subsets among CD5+ T-lymphocytes were determined and presented as a CD5+ T-cell profile per animal (Table 2). Further, cell count data were used to test for changes in WBC/ml, total lymphocytes/ml, total CD5− cells/ml, CD5+ T-cells/ml, CD4+ cells/ml, CD8+ T-cells/ml, CD8β^{high}/ml, CD8β^{low}/ml, and CD4−CD8−CD5+ cells/ml. Shapiro-Wilks' W test indicated normal distributions for each of these cell count variables. Two of these variables, WBC/ml and CD8+ T-cells/ml, did not change with FIV status in either lions or pumas (Table 3; Figs. 1a, b).

T-cell alterations with FIV infection in pumas

The IFC profiles of the relative proportions of CD4+, CD8+, and CD4−CD8−CD5+ cells within FIV-negative pumas were uniform among individuals (G-test, NS). The mean T-cell profile for pumas infected with FIV-Pco was 42:27:29 (percentage of CD4+, CD8+, and CD4−CD8−CD5+, respectively). Three of the six FIV-Pco–infected pumas (Pco-075, Pco-733, and Pco-736) had significant differences in IFC profiles (Table 2), but the overall mean profile for positive animals was not significantly altered relative to negative controls (G-test, $P=0.069$).

Infection with FIV-Pco was correlated with an overall reduction in cell counts of total lymphocytes (*t*-test, $P=0.03$) that involved both CD5+ T-cells and CD5− lymphocyte subsets and was accompanied by a 50% reduction in CD4+ cells/ml relative to uninfected pumas (*t*-test, $P=0.008$) (Table 3 and Fig. 1a). No significant changes were observed in the CD8+ and CD4−CD8−CD5+ T-cell subsets relative to the negative controls (Tables 2 and 3). Further examination of the CD8+ T-cell subset revealed no change in CD8+β^{low} cells, but a significant decline in mean CD8+β^{high} cells was detected with FIV-Pco infection (*t*-test, $P= 0.008$) (Table 3 and Fig. 2a, b).

TABLE 2. Immunofluorescent profiles of lymphocytes in FIV infected pumas and lions and uninfected control animals.

		Proportion of CD5+			Proportion of CD8β+ (%)		Ratio
Species ID	FIV	CD4+	CD8+	CD4−CD8−	CD8+β^{high}	CD8+β^{low}	CD4+:CD8+
Puma (*Puma concolor*)							
PCO-166	−	48.99	35.81	15.20	70.08	29.92	1.37
PCO-387	−	55.18	25.39	19.43	63.83	36.17	2.17
PCO-390	−	46.39	27.66	25.96	47.93	52.07	1.68
PCO-392	−	44.67	30.34	24.99	52.45	47.55	1.47
PCO-533	−	48.16	23.60	28.24	55.72	44.28	2.04
PCO-539	−	42.70	30.67	26.63	56.54	43.46	1.39
PCO-717	−	47.78	28.62	23.59	50.18	49.82	1.67
PCO-718	−	61.81	21.14	17.06	67.49	32.51	2.92
PCO-719	−	58.89	23.41	17.70	39.89	60.11	2.52
PCO-898	−	57.77	27.53	14.70	66.11	33.89	2.10
Mean		**51.23**	**27.42**	**21.35**	**57.02**	**42.98**	**1.93**
PCO-075[a]	+	40.54	15.76	43.70	25.84	74.16	2.57
PCO-154	+	44.68	22.25	33.07	46.27	53.73	2.01
PCO-160	+	43.54	32.23	24.23	31.68	68.32	1.35
PCO-730	+	53.26	22.35	24.38	32.35	67.65	2.38
PCO-733[a]	+	35.33	32.14	32.54	38.69	61.31	1.10
PCO-736[a]	+	38.87	41.25	19.88	49.70	50.30	0.94
Mean		**42.70**	**27.66**	**29.63**	**37.42**	**62.58**	**1.73**
African lion (*Panthera leo*)							
PLE-030	−	73.34	14.54	12.12	52.67	47.33	5.04
PLE-031	−	58.94	20.78	20.28	79.54	20.46	2.84
PLE-032	−	60.84	25.42	13.74	85.68	14.32	2.39
PLE-033	−	55.06	24.54	20.40	66.71	33.29	2.24
PLE-034	−	52.29	23.42	24.30	85.78	14.22	2.23
Mean		**60.09**	**21.74**	**18.17**	**74.07**	**25.93**	**2.95**
PLE-133[a]	+	24.52	28.23	47.25	22.48	77.52	0.87
PLE-134[a]	+	19.99	21.20	58.81	34.55	65.45	0.94
PLE-135[a]	+	28.10	18.76	53.14	38.18	61.82	1.50
PLE-138[a]	+	44.80	17.43	37.77	48.98	51.02	2.57
PLE-569[a]	+	27.43	18.33	54.24	28.30	71.70	1.50
PLE-586[a]	+	16.79	38.46	44.75	31.84	68.16	0.44
PLE-599[a]	+	25.73	22.53	51.74	39.81	60.19	1.14
PLE-605[a]	+	24.85	18.67	56.48	45.77	54.23	1.33
PLE-618[a]	+	6.21	30.20	63.59	36.10	63.90	0.21
PLE-645[a]	+	32.00	39.45	28.55	51.84	48.16	0.81
PLE-647[a]	+	27.17	16.45	56.38	27.20	72.80	1.65
PLE-649[a]	+	21.69	20.34	57.97	46.22	53.78	1.07
Mean		**24.94**	**24.17**	**50.89**	**37.61**	**62.39**	**1.17**

[a] Denotes individuals with significantly different IFC profile from mean profile of control animals.

T-cell alterations with FIV infection in lions

Homogeneous IFC profiles of uninfected lion controls had an average ratio of 60 : 22 : 18 for relative CD4+, CD8+, and CD4−CD8−CD5+ percentages. In contrast, IFC profiles for infected lions were more highly heterogeneous (G-test, $P=9.0\times10^{-9}$), and each had significant deviation from the mean profile (G-test, $P=2.5\times10^{-5}$) (Table 2).

Measured cell counts indicated total lymphocytes did not vary with FIV-Ple infection as observed with FIV-Pco in pumas (Table 3 and Fig. 1b). Instead, infected lions had both a significant reduction in CD5+ T-cells (*t*-test,

TABLE 3. Absolute cell counts standardized by total T-cell counts (see Methods, Table 2).

		Absolute cell counts (10^3/ml)		Estimated Cell Counts (10^3/ml)						
Sample ID	FIV	WBC	Lymphocytes	Total T-cells	CD4+	CD8+	CD4–8−	CD8β^{High}	CD8β^{Low}	Other Lymphocytes (CD5−)
Puma (*Puma concolor*)										
PCO-166	−	5800	3074	2165	1061	775	329	543	232	909
PCO-387	−	9700	2037	1593	879	405	310	258	146	444
PCO-390	−	10900	2507	1656	768	458	430	219	238	851
PCO-392	−	11600	6264	4349	1942	1320	1087	692	627	1915
PCO-533	−	7800	2340	1840	886	434	520	242	192	500
PCO-539	−	13900	5143	2799	1195	859	746	485	373	2344
PCO-717	−	8600	4730	3634	1736	1040	857	522	518	1096
PCO-718	−	14000	4760	3434	2123	726	586	490	236	1326
PCO-719	−	16100	4347	3045	1793	713	539	284	429	1302
PCO-898	−	7400	3256	2307	1333	635	339	420	215	949
Mean±s.e.		**10580±1052**	**3845±443**	**2682±293**	**1371±155**	**736±90**	**574±80**	**415±50**	**320±50**	**1163±187**
PCO-075	+	11800	427	301	122	47	132	12	35	126
PCO-154	+	11100	1110	766	342	170	253	79	92	344
PCO-160	+	8900	3204	2492	1085	803	604	254	549	712
PCO-730	+	7700	2233	1556	829	348	379	113	235	677
PCO-733	+	11100	3774	2799	989	900	911	348	551	975
PCO-736	+	10300	1854	1307	508	539	260	268	271	547
Mean±s.e.		**10150±635**	**2100±512**	**1537±395**	**646±156**	**468±139**	**423±117**	**179±53**	**289±52**	**563±122**
LION (*Panthera leo*)										
PLE-030	−	9800	1960	1692	1241	246	205	130	116	268
PLE-031	−	10890	3594	2723	1605	566	552	450	116	871
PLE-032	−	13900	2780	2549	1551	648	350	555	93	231
PLE-033	−	15800	1580	1156	637	284	236	189	94	424
PLE-034	−	11900	2261	1802	942	422	438	362	60	459
Mean±s.e.		**12458±1074**	**2435±350**	**1984±288**	**1195±183**	**433±78**	**356±64**	**337±79**	**96±10**	**451±114**
PLE-569	+	25905	2591	907	249	166	492	47	119	1684
PLE-586	+	11380	2845	1160	195	446	519	142	304	1685
PLE-599	+	12320	1725	926	238	209	479	83	126	799
PLE-605	+	11715	2460	1426	354	266	806	122	144	1034
PLE-618	+	11275	1917	882	55	266	561	96	170	1035
PLE-645	+	19415	2912	1206	386	476	344	247	229	1706
PLE-647	+	17400	2262	1123	305	185	633	50	135	1139
PLE-649	+	17985	1619	716	155	146	415	67	78	903
Mean±s.e.		**15924±1848**	**2291±175**	**1043±80**	**242±38**	**270±44**	**531±50**	**107±23**	**163±25**	**1248±134**

$P=0.0025$) and a dramatic 80% decline in CD4+ cells (*t*-test, $P=0.0001$) relative to controls (Fig. 1b). This CD4+ cell decline was observed without a significant increase in overall CD8+ levels. However, within the CD8+ subset, β^{high} cells declined precipitously with FIV-Ple infection to one-third the levels observed in controls (*t*-test, $P=0.006$) and was accompanied by an expansion of β^{low} cells (*t*-test, $P=0.03$) (Fig. 2a, b). Further unique changes were observed including an increase in the cell counts of the CD4−CD8−CD5+ subset (*t*-test, $P=0.05$) and an increase in the CD5− lymphocytes (*t*-test, $P=0.001$), presumed to be mostly B cells, in infected lions relative to negative controls (Table 3 and Fig. 1b).

Alteration of the CD4+/CD8+ ratio with FIV infection

Both lions and pumas were examined for reduction of the CD4+/CD8+ ratio commonly observed in FIV-Fca infection of domestic cats. In lions, the CD4+/CD8+ ratio sharply declined from mean=2.95 (range of 2.23–5.04) in controls to mean=1.17 (range of 0.21–2.57) in FIV-Ple–infected individuals (Table 2). In contrast, the puma CD4+/CD8+ T-cell ratio was not significantly altered (Table 2) with values slightly less in pumas with FIV-Pco mean=1.73 (range of 0.91–2.57) compared with mean=1.93 (range of 1.37–2.92) in negative animals. The drop in CD4+ cells in positive pumas was accompanied by a proportional, albeit not significant, reduction in CD8+ cells (Fig. 1b) sufficient to maintain the CD4+/CD8+ ratio as unchanging with FIV-Pco status.

DISCUSSION

To understand the interaction between FIV and target cells in the host immune system, we examined T-lymphocyte profiles in free-ranging and captive lions and pumas naturally infected with species-specific strains FIV-Ple and FIV-Pco, respectively. The uninfected controls of the two species had only minor differences in the relative mean proportions of CD4+:CD8+:CD4−CD8−CD5+ T-cell subsets (51:27:22 in pumas; 60:22:18 in lions). However, FIV infection appeared to affect lions and pumas differently, causing marked changes in lymphocytes unique to each species. The T-cell subset profile perturbations were more pronounced in lions than pumas with FIV infection. Specifically, all seropositive lions, but only 50% of FIV-infected pumas, had significantly altered IFC profiles relative to negative controls.

The major alteration in the relative proportion and absolute cell count of CD4+, CD8+, and CD4−CD8− subsets in both seropositive lions and pumas was a reduction in CD4+ T-cells. In domestic cats infected with FIV-Fca, the CD4+/CD8+ ratio declines during the asymptomatic phase from two or more to less than one (English et al., 1994; Bendinelli et al., 1995;) because of CD4+ depletion concurrent with relative or absolute CD8+ increase. The magnitude of CD4+ depletion was profound in FIV-Ple lions and resulted in a sharp decline in the CD4+/CD8+ ratio. By contrast, the CD4+/CD8+ ratio was not significantly altered in pumas even though FIV-Pco infection was correlated with a 50% decline in CD4+ T-cells.

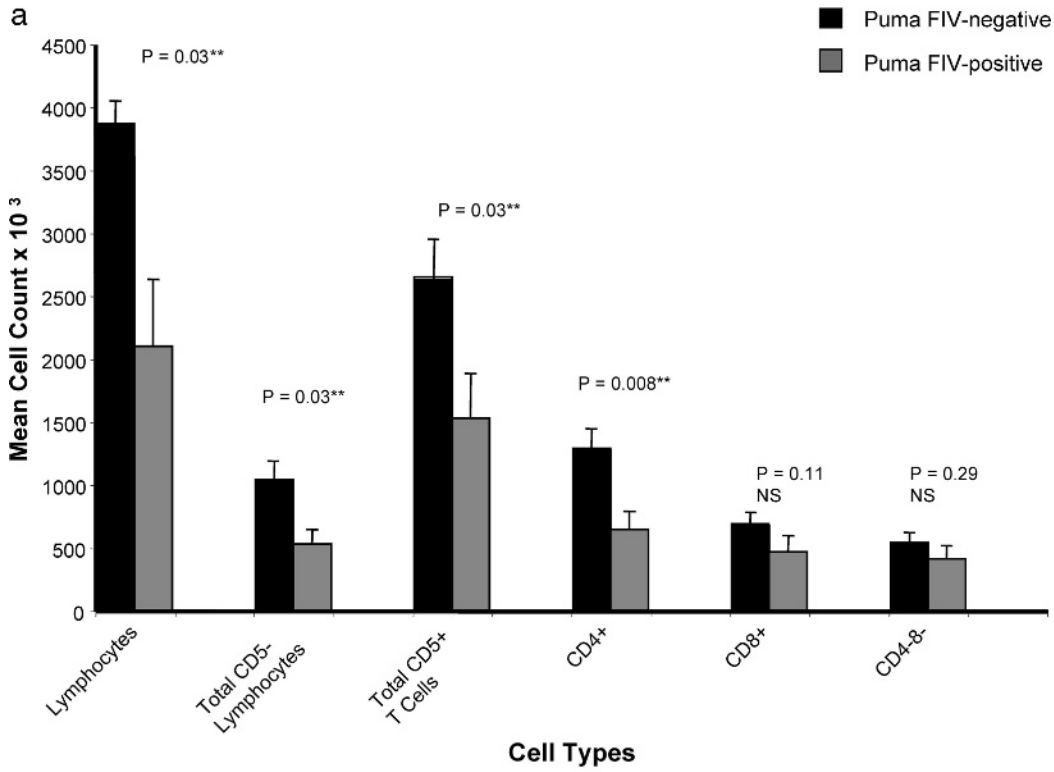
Additional differences between lions and pumas included alterations of total lymphocytes and CD5+ T-cells; neither of these changes paralleled the typical findings in domestic cats. For example, pumas infected with FIV-Pco had relative lymphopenia, indicated by the 41% lower total lymphocyte count, which was distributed equally between CD5+ and CD5− cell subsets. Although lymphopenia was not noted in seropositive lions (Fig. 1b), a significant decrease (48%) in CD5+ T-cells was accompanied by a compensatory increase in CD5− lymphocytes that masked this loss. Most studies have not recorded lymphopenia or pan-T-cell loss as a striking finding in domestic cat FIV infection. Therefore, assuming CD5−

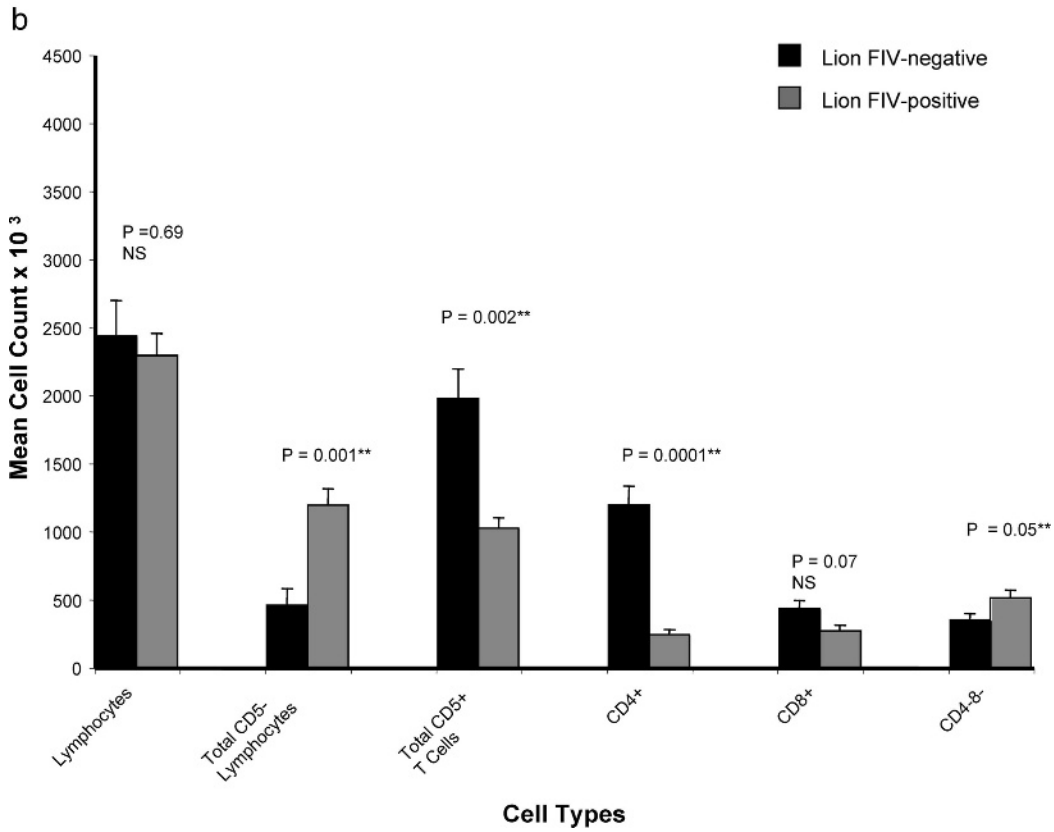
cells are predominantly B-cells, this increase would be a unique feature to FIV-Ple infection as B-cell lymphocyte kinetics in FIV-Fca infected cats have not been reported to be altered relative to naive controls (Ackley et al., 1990; Novotney et al., 1990).

Reduction in CD4+ cells in lions and pumas was not accompanied by an absolute increase of CD8+ cells as observed in domestic cats. During the acute and asymptomatic phases of FIV infection, CD8+ T-cells are both cytotoxic and virus-suppressive in domestic cats (Prince et al., 1991; English et al., 1994). In particular, the heterodimer CD8+ molecule exhibits changes in the composite α and β chains correlated with time course of viral infection, antiviral activity, and pathogenesis of disease (Shimojima et al., 1998). The CD8+ subset changes in lions and pumas in this study were based on the FT-2 antibody that binds exclusively to the β chain of the heterodimer. Previous studies of the CD8+ β chain in the asymptomatic phase of FIV infection in domestic cats showed depletion of CD8+β^{high} cells and the appearance and expansion of CD8+β^{low} T cells, which secrete a soluble factor inhibitory for in vitro FIV infection (Bucci et al., 1998a, b; Shimojima et al., 1998; Gebhard et al., 1999). This simultaneous expansion of CD8+β^{low} T-cell subsets offsets a decrease in total CD5+ T-cells with FIV-Fca infection (Willett et al., 1993). In this study, lions and pumas resembled domestic cats by having a significant depletion of CD8+β^{high} cells and a reduction of CD5+ cells, but only lions had a concomitant expansion of CD8+β^{low} cells. A similar result in a study of five seropositive captive lions with an expanded CD8+β^{low} subset relative to naive animals (Bull et al., 2003) suggests this is a signature of FIV-Ple infection. Results from FIV-Pco–infected pumas demonstrated a decline in lymphocytes in general, and CD4+ cells in particular, which was not offset by expansion of any subset presented here. Rather, a substantial number of CD8+β^{low} cells are present in pumas irrespective of FIV status. Perhaps these cells are part of an immune response to another unspecified pathogen, or this may reflect the outcome of coevolution of virus and host that has resulted in a "standing army" within the species as a whole.

The changes in CD4+ subpopulations in lions and to a lesser extent in pumas in response to FIV infection provide strong support for T-cell dyscrasia as observed with domestic cats. Yet other lymphocyte changes specific to lions and pumas suggest that additional immune responses occur in both species, and perhaps clues reside within an uncharacterized T-cell population present within the CD4− CD8− subset. Although pumas showed no change in CD4−CD8− cell counts with FIV-Pco infection, FIV positive lions had a significant increase relative to controls. Thus, the more muted immune response of pumas consisting of general lymphopenia and reduction of CD4+ cells not accompanied by a change in the CD4+/CD8+ ratio suggests FIV-Pco causes less T-cell perturbation in pumas than FIV-Ple in lions. The FIV-Ple elicits a dramatic decline in CD4+ cells, yet lions may be in the process of coadapting to the virus, as suggested by the unique combination of increased CD5− cells, expansion of the CD8+β^{low} subset, and increased numbers of CD4−CD8− cells.

Alternatively, extreme changes in CD4+ levels in lions also may be influenced by other determinants such as length of FIV infection or other microbial infections, as well as relative binding affinities of monoclonal antibodies originally developed for domestic cat T-cell antigens. The highly heterogeneous IFC profiles observed among individual wild seropositive lions relative to the more uniform profiles of control lions in zoos might reflect stress differences between captive and free-ranging populations. The fact that FIV status increases with age, and is seen with high seroprevalence in many free-





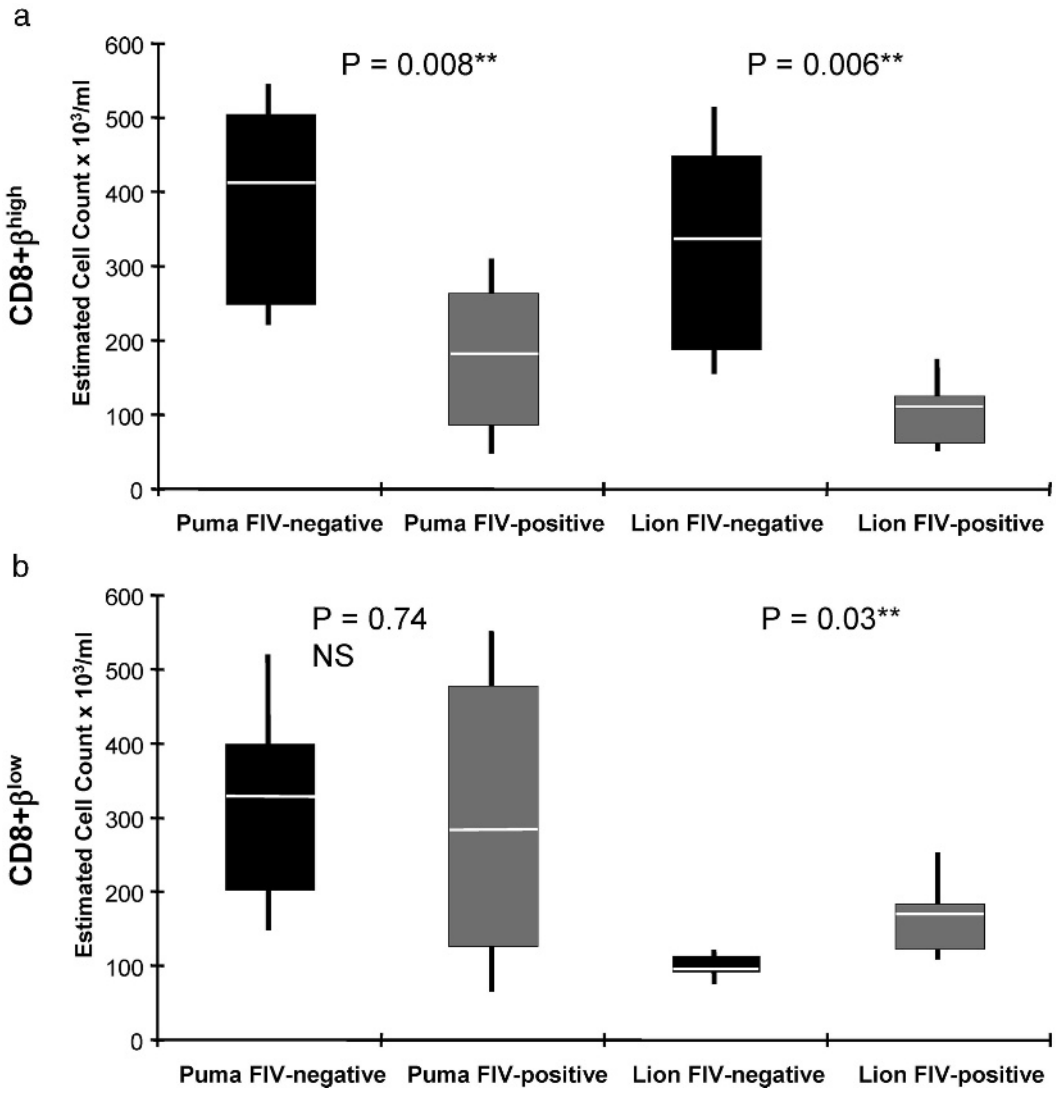


FIGURE 2. Comparison of CD8+ subsets β^{high} and β^{low} estimated cell counts in FIV-positive and -negative animals. Boxed regions reflect 25th–75th percentile range of individual values. Bars represent 10th–90th percentile range. Means are plotted as white line within boxes. *P*-values are *t*-test of mean between FIV positive (puma $n=6$; lion $n = 12$) and negative (puma $n=10$; lion $n=5$) animals within each species: (a) CD8+β^{high} in pumas and lion, (b) CD8+β^{low} in lions and pumas.

ranging populations of lions (Brown et al., 1994; Troyer et al., 2004), also makes it difficult to determine how age and environmental conditions may have contributed to our findings. We were unable to assess the effect of age in this study because of insufficient replicates per age class and unknown ages for some of both positive and

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FIGURE 1. Comparison of mean cell counts ($\times 10^3$/ml) of absolute lymphocytes and estimated total CD5− cells, total CD5+ T-cells, and T-cell subpopulations from whole blood between FIV-positive and FIV-negative individuals. (a) Puma positive $n=6$; puma negative $n=10$. (b) Lion positive $n=12$; lion negative $n=5$.

negative lions, leading to a prohibitively small sample for statistical analysis.

Overall, this immunological survey of naturally FIV-infected animals may serve as an indictor of the susceptibility of both captive and wild populations to emerging disease. Outbreaks of opportunistic infections, each with varying degrees of pathogenicity, have been documented in nondomestic species of cat. In lions, a canine distemper virus (CDV) outbreak caused significant mortality in free-ranging FIV-infected African lions of the Serengeti ecosystem in 1994 (Roelke-Parker et al., 1996) although other CDV outbreaks in the same seropositive populations had negligible mortality (Packer et al., 1999). In pumas, a recent outbreak of feline leukemia virus has been linked with, but no causality yet established for, the deaths of a small number of seropositive Florida panthers (M. Cunningham, unpubl. data). The precise role of FIV in regulating immune response to such opportunistic infection remains unknown, yet the T-cell changes observed in FIV-infected lions and pumas in this study suggest that further investigation is warranted and that it would be prudent to discourage the translocation of seropositive animals into naive populations.

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