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The molecular biology and evolution of feline immunodeficiency viruses of cougars

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Abstract

Feline immunodeficiency virus (FIV) is a lentivirus that has been identified in many members of the family Felidae but domestic cats are the only FIV host in which infection results in disease. We studied FIVpco infection of cougars (*Puma concolor*) as a model for asymptomatic lentivirus infections to understand the mechanisms of host-virus coexistence. Several natural cougar populations were evaluated to determine if there are any consequences of FIVpco infection on cougar fecundity, survival, or susceptibility to other infections. We have sequenced full length viral genomes and conducted a detailed analysis of viral molecular evolution on these sequences and on genome fragments of serially sampled animals to determine the evolutionary forces experienced by this virus in cougars. In addition, we have evaluated the molecular genetics of FIVpco in a new host, domestic cats, to determine the evolutionary consequences to a host-adapted virus associated with cross-species infection. Our results indicate that there are no significant differences in survival, fecundity or susceptibility to other infections between FIVpco-infected and uninfected cougars. The molecular evolution of FIVpco is characterized by a slower evolutionary rate and an absence of positive selection, but also by proviral and plasma viral loads comparable to those of epidemic lentiviruses such as HIV-1 or FIVfca. Evolutionary and recombination rates and selection profiles change significantly when FIVpco replicates in a new host.

Keywords

Feline immunodeficiency virus; Puma; Ecology; Evolution; Lentivirus; Recombination; Co-adaptation; Cross-species infection

Why do some hosts sustain infection without disease while the outcome of infection in other species is fatal? A potential reason for differential affects of infection on hosts is that long-

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None of the authors has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the paper entitled “The molecular biology and evolution of feline immunodeficiency viruses of cougars”.

term virus-host coevolution results in infections that minimally impact host fitness. Coadaptation of host and viral pathogen has been used to explain the presumed asymptomatic nature of lentivirus infections in their natural hosts (Carpenter and O'Brien, 1995). In fact, among all primate and feline lentivirus infections, only the human and feline immunodeficiency viruses (HIV and FIV) are associated with overt clinical signs. Thus, asymptomatic infections by lentiviruses may be the rule and not the exception. However, to date, there have been no detailed studies of the consequences of lentivirus infections in native, free-ranging, host populations. To this end, we evaluated both virus genetics and population demographics of cougars naturally infected with feline immunodeficiency virus (FIVpco) to determine the effect of infection on both virus and host.

Epidemiology of FIVpco in free-ranging cougars

Cougars are a solitary species and contact among individuals is limited to mating or territorial defense. Thus it is remarkable that the prevalence of FIVpco in some cougar populations is as high as 58%. (Biek et al., 2003). Animals are infected both vertically and horizontally. The rate of vertical transmission from infected females to offspring is 53%. The risk of being infected increases with age, and in most populations there is a 50% risk of being infected with FIVpco by the age of 2.5 years.

To determine whether, FIVpco infection affected cougar survival we estimated the monthly survival of 160 infected and uninfected animals derived from two populations (Biek et al., 2006c). Radio telemetry was used to determine if animals were alive or dead on a weekly basis, which resulted in a total of 2672 cougar-months observations. Monthly survival was determined using program MARK (White and Burnham, 1999) with known-fate models. In addition to the factors ‘population’, ‘sex’, and ‘FIV infection’, we considered ‘age’ by distinguishing subadults (<2yr) from adults (≥2yr). Data were entered as 16 groups according to all possible combinations of these factors. The Akaike Information Criterion with small sample size correction (AIC_C) was used to rank models and unconditional survival estimates and their variances were calculated for each group using model averaging (Burnham and Anderson, 2002). There was no difference in survival between infected and uninfected cougars regardless of age, gender or population (Biek et al., 2006c). It is also noteworthy that similar analyses demonstrated that there was no affect of FIVple infection in African lions (Packer et al., 1999). Thus in two free-ranging feline species harboring FIV, mortality is not measurably increased by infection.

We also assessed the affect of FIVpco infection on fecundity by estimating the annual probability that a female would produce a litter, the average litter size, and the average number of offspring per litter surviving to independence (Biek et al., 2006c). Here the models considered FIVpco status and population affiliation and model selection criteria were similar to those used for survival analysis. The fecundity of infected cougars was consistently lower than for uninfected animals but this difference was not significant. Further, our simulations indicated that the small effect observed would not have a measurable impact on cougar population growth.

Because the principle clinical manifestation of FIV infection in domestic cats relates to immune dysfunction, we reasoned that if FIVpco infection in cougars had any clinical consequence, it would be manifest as an increased susceptibility to other common feline viral infections. We collected serological data on 207 individual cougars from four populations in the Northern Rockies Ecosystem (Biek et al., 2006b). Samples were evaluated for feline parvo, corona, herpes, and caliciviruses, canine distemper virus and Yersinia pestis. Our statistical analysis evaluated whether FIVpco infection was a risk factor for concurrent infection with one of these pathogens. Although age and group explained much of the observed serological data, there

was no support for models that included FIVpco infection (Biek et al., 2006b). We conclude that FIVpco has no significant affect on the susceptibility of cougars to these other infections.

Molecular genetics of FIVpco

The molecular genetics of pathogenic lentivirus infections such as with HIV-1 are characterized by rapid evolution, high intrahost diversity, and evidence for positive selection on several virus genes (Coffin, 1995; Crandall et al., 1999; Rambaut et al., 2004). Further, high viral loads in HIV-1 and FIV infected individuals are associated with a poor prognosis (Diehl et al., 1996; Mellors et al., 1996). We reasoned that in a co-evolved host/virus system there would be little positive selection on the virus because the virus would have achieved high fitness in the host environment. To test this hypothesis, we evaluated the molecular evolution of FIVpco *gag* and *env* from 52 infected cougars.

Intrahost diversity of FIVpco in cougars based on either gene is 1% or less. There was no evidence of hypervariable regions. The majority of substitutions within a host were synonymous and purifying selection was the dominant force shaping these genes (Biek et al., 2003). Evolutionary rates for FIVpco *pol* and *env* based on maximum likelihood and MCMC methods (Rambaut, 2000; Drummond, 2002) yielded similar estimates of 0.5–1.0% per site per decade. Estimates of intrahost evolution based on serially sampled animals were similar to the population averages. Because lower evolutionary rates could be due to low levels of virus replication, we determined viral production by real time PCR. There was an average of 1 in 100 PBMC carrying an integrated provirus based on samples from 38 cougars. Circulating plasma virus ranged from 10^4 to 10^6 copies per ml (Blake et al., 2006). Thus molecular evolution of FIVpco is characterized by a slower evolutionary rate relative to an epidemic lentivirus such as HIV-1 but comparable proviral and plasma viral loads, indicating that this virus is actively replicating in the host. In contrast to positive selection described on HIV-1, the FIVpco genome is under purifying selection.

Despite the lower evolutionary rates and intrahost diversity, the genetic diversity of FIVpco across the northern Rocky Mountain region is high, ranging from 5–34%. The virus can be resolved into 2 major clades and 8 distinct viral lineages (Biek et al., 2006a). Because of the high levels of diversity within the population, we proposed that FIVpco could be used as a surrogate, rapidly evolving, host gene that could be used to detect recent changed in cougar demography. Indeed, we demonstrated that whereas there was no evidence of population structure in the cougar population based on host genes, there was distinct regional segregation of viruses obtained from these cougars. The population subdivision suggested by viral genetics is consistent with the recovery of cougars in the western states subsequent to years of persecution from predator elimination programs. Estimates of the time to most recent ancestor for viruses obtained in contemporary cougar populations were approximately 80 years, which coincides with imposing controls on bounty hunting. The unique evolutionary history of lentiviruses and their hosts thus provides a tool for understanding host population biology that will be useful to epidemiological and conservation research alike.

FIVpco in a new host

The ecological and evolutionary data on FIVpco-infected cougars discussed above strongly supports the hypothesis that this feline lentivirus is well adapted to its cougar host and has evolved a strategy for successful maintenance in the population that is minimal pathogenic. However, it is clear that the outcome of lentivirus infections is not always benign; lentiviruses recently emerged in a host population such as FIV and HIV-1 and HIV-2, are virulent in their new host. To evaluate the process by which a well-adapted lentivirus establishes infection in an individual of another species, domestic cats were infected with a FIVpco isolate derived from a cougar from Vancouver Island, BC, called PLV.

Cats can be persistently infected with PLV by either the oral nasal (ON) or intravenously (IV) routes but there are no clinical consequences of the infection (VandeWoude et al., 1997; Terwee et al., 2005). Despite the fact that in the majority of ON-inoculated cats the virus levels dropped below the level of detection at time points when viremia was still apparent in IV- inoculated cats, there were no differences in immunological profiles of cats. Because any response of the host that affects virus fitness is likely to yield a genetic change in the virus genome, we conducted a thorough molecular evolutionary analysis of sequences derived from ON and IV inoculated cats.

Our analyses were based on complete genome sequences, which were amplified and cloned in two overlapping fragments (Poss et al., 2006b). The evolutionary rate for the 5' and 3' half-genome sequences derived from IV and ON cats was similar (0.26 and 0.37% per site per year, respectively). The number of stop codons and single nucleotide insertions and deletions was notable in sequences from all cats. The accumulation of errors was particularly high in the RNAase H domain of *pol*. There was no phylogenetic signal to distinguish sequences as they evolved over time or in individuals. However, the substitution matrix derived for the best model of evolution for each gene demonstrated that the frequency of substitutions to A was high in all genes but was most pronounced in *pol*. Retrovirus genomes normally have a high base frequency of A. Thus, to determine if the frequency of substitutions resulting in A was higher than expected, we generated a frequency distribution from 1000 simulation replicates across a tree containing the ancestral PLV sequence and sequences obtained from cats at the last time point. The number of codons degenerate for G or A in the third position was compared to this distribution. From this analysis, we determined that 6 of the 13 codons contained A in the third position at a frequency exceeding expectation. Four of these codons were located in *pol*, which was consistent with the substitution bias observed for this gene. We conducted a similar analysis on a fragment of *pol* from naturally infected cougars and demonstrated that there was no bias toward increased substations with A in this region. To provide further support for a nucleotide bias towards A in the PLV sequences derived from domestic cats, we determined the state change of each nucleotide along the branches of the phylogenetic tree generated for each gene. This analysis demonstrated that 32–51% of all substitutions resulted from a G to A substitution, again supporting the observation that there are excessive substitutions towards A in this sequence data set. In contrast, only 13.6% of substitutions arising during evolution in the natural cougar host were G to A. Finally, we evaluated the distribution of observed G to A substitutions against a distribution generated by random assignment of substitutions across the genome while maintaining the correct substitution frequency. Using this method, we demonstrated that G to A substitutions were not randomly distributed and tended to cluster (hotspots) in *pol*.

Our data demonstrate that fatal mutations accumulate in the PLV genome during this cross-species infection and that the majority of substitutions that occur are G to A. These findings are consistent with the action of cytidine deaminases on the viral genome. Cytidine deaminases in the APOBEC family, specifically APOBEC3G, have recently been shown to edit HIV-1 genomes resulting in a G to A hypermutation (Sheehy et al., 2003; Zhang et al., 2003; Harris and Liddament, 2004). The lentiviral vif protein confers protection against some APOBEC enzymes in a species-specific fashion by targeting them for degradation. APOBEC3G acts on single stranded DNA, which is produced as a result of first strand synthesis by reverse transcriptase (Yu et al., 2004). As single stranded DNA is generated, the RNA genome is degraded by the RNAse H activity of RT but RNA primers are retained at specific sites to initiate second strand synthesis. These short RNA primers protect that region of the DNA from modification resulting in patches devoid of G to A substitutions. In our analyses, the central polypurine tract (cPPT) is such a region; it contains no G to A changes despite the fact that there are target G throughout the region. Our findings that *pol* is particularly sensitive to editing by cytidine deaminases can also be explained by considering the process of reverse

transcription. If second strand synthesis is initiated at the cPPT, which is located at the 3' end of *pol*, then the last section of the viral genome to be converted into double stranded DNA will be *pol*. Because *pol* is present as single-stranded DNA longer than other regions of the viral genome, it is subject to more extensive editing by these cytidine deaminases.

The innate ability to recognize and modify foreign DNA is a powerful means for a host to prevent infection by novel viruses. Based on the viral genomes that we evaluated, which sustained extensive deleterious mutations, this primary protection should have been effective. However, the majority of cats in this experiment sustained a persistent viremia over the 37 week period. We evaluated the viral genomes to determine if there was any evidence of positive selection that would indicate the virus was increasing fitness in this new environment (Poss et al., 2006b). We used two methods to assess the selective pressures on each codon in each gene and demonstrated that there were five sites under positive selection that all fell in RT. These sites were located at positions important for RT-template contacts. We hypothesized that changes in stability of RT on the nucleotide template could decrease processivity and result in increased recombination rates (Poss et al., in press). At seventeen days post-infection with PLV, there were between 0.02 to 0.06 recombination events per genome per day in the 5' portion of the genome in three of four cats and in the 3' genome in two of the cats. We used the Shimodaira-Hasegawa test to evaluate topological congruence of phylogenetic trees generated for each viral gene and genome fragment. There was significant incongruence among the genome fragment containing the 3' portion of *pol* and the OrfA-*env* fragment in sequences obtained from the two cats with measurable recombination rates in the 3' half genome. The approximate breakpoint was near the splice acceptor site for *vif*. Of interest, we were also able to detect positive selection at one of the previously identified sites in RT from sequences from these two cats. Thus, despite an intensive innate response of cats to this novel feline lentivirus, infection can be sustained. Persistence is possibly due to enhanced recombination rates resulting from substitutions that accrue in RT.

We have demonstrated that FIVpco bears all the predicted signatures of a well-adapted virus to its natural host. It is efficiently transmitted in this population of solitary hosts and, despite high viral titers, infection has no measurable effect on the cougar host. However, in a new host environment, FIVpco is capable of rapidly responding by increasing the rate of recombination and evolution. These detailed analyses of the molecular evolution of a lentivirus in a native and new host will be valuable in understanding mechanisms of establishment and adaptation in emerging viral infections.

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