[Frontiers in Bioscience 12, 3668-3682, May 1, 2007]

Immunopathogenesis of feline immunodeficiency virus infection in the fetal and neonatal cat

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1. ABSTRACT

The global incidence of pediatric HIV infection is estimated at 2.3 million children, most acquiring the infection from their mothers *in utero*, peripartum, or postpartum. Pediatric HIV infection typically causes a rapidly progressive disease when compared with adult infection, due in part to the profound susceptibility of the neonatal thymus to productive infection or degenerative changes. Failed production of naïve T-lymphocytes further limits the success of antiviral therapy to restore immunologic function. In this review, we explore the use of feline immunodeficiency virus (FIV) infection of domestic cats as an animal model for pediatric HIV infection. Cats infected with FIV represent the smallest host of a naturally occurring lentivirus, and the immunodeficiency syndrome elicited by FIV infection is similar to that of HIV-AIDS. The feline-FIV model uniquely reproduces several key aspects of immunosuppressive lentivirus infection of the thymus, allowing investigators to define viral determinants of pathogenicity, influence of host age on disease outcome, and therapeutic strategies to restore thymus function.

2. INTRODUCTION

As of December 2006, approximately 39.5 million people worldwide were infected with human immunodeficiency virus (HIV), including 2.3 million children (1). Of the childhood infections, there were approximately 530,000 new infections and 380,000 deaths due to AIDS and related diseases. The vast majority of pediatric HIV cases are a result of vertical transmission from infected women, which can occur *in utero*, during birth, or following the ingestion of infected milk (2). Infected newborns often experience a shorter incubation time and more rapid disease progression than do infected adults. Newborns may develop clinical symptoms during the first year of life and up to 16% succumb to AIDS before four years of age (3, 4). Those infected *in utero* have a two-fold risk of progression to AIDS or death by 12 months of age as compared with those infected during childbirth (5). Whether this is the result of profound susceptibility of the fetal thymus to infection or to the selective transmission of pathogenic viral variants needs to be defined in an animal model. Feline immunodeficiency virus (FIV) is a naturally occurring, immunosuppressive lentivirus of cats

that provides a useful model in which to define the role of age-related factors on disease pathogenesis because the inoculum can be precisely controlled and delivered at defined stages of fetal development (6, 7).

FIV and HIV are both members of the family *Retroviridae*, genus *Lentivirus*. FIV was first isolated in 1986 from a colony of group-housed cats with a high prevalence of opportunistic infections and degenerative conditions (8). Many of these cats had originated from stray or feral populations and all tested negative for feline leukemia virus (FeLV), the only recognized immunosuppressive feline retrovirus at the time. Since this initial recognition two decades ago, FIV is now recognized as an endemic pathogen in domestic cat populations worldwide (9). As an animal model for HIV/AIDS, the FIV-infected cat has three major strengths (6): (1) Close phylogenetic relationship of the viruses, which translates into similar virus morphology, biochemical properties, and replication; (2) similar virus-host interaction, which is characterized by the infection and progressive depletion of circulating T-helper ($CD4^+$) lymphocytes; and (3) progressive deterioration of immune status leading to an AIDS-like syndrome of degenerative and infectious disease. Furthermore, because of its long-standing presence in the cat population, FIV provides a unique perspective from which to study viral evolution and adaptation in the host. Both viruses exhibit tropism for many cells of the immune system, including the $CD4^+$ subset of T lymphocytes, which orchestrate the acquired immune response (6). As the number of $CD4^+$ cells declines over the course of infection, the host becomes susceptible to opportunistic infections and degenerative disorders (10).

3.1. General pathogenesis of FIV infection

Once FIV is transmitted to a susceptible host, the virus persists within the blood and lymphoid tissues even in the presence of a strong antiviral immune response. The course of FIV infection can be characterized by a gradual transition through three general phases defined by changes in clinical signs, level of viremia, strength of antiviral immunity, and the ratio of $CD4^+$ lymphocytes to T-cytotoxic/ suppressor ($CD8^+$) lymphocytes (CD4:CD8) in peripheral blood.

The acute stage of infection typically occurs within the first three months of virus exposure. Clinical signs during this period include transient fever, neutropenia, and generalized lymphadenopathy (11). During this relatively short time interval, FIV replicates and disseminates throughout the body and antiviral immunity is initiated. After inoculation, a two-week quiescent period ensues, in which circulating virus and antiviral antibodies are generally undetectable. Shortly after this period, virus can be identified in the plasma using reverse transcription-polymerase chain reaction (RT-PCR) to amplify viral nucleic acids or by incubating plasma with susceptible cells *in vitro* and subsequently isolating the virus (12). This initial burst of viremia is closely associated with a high level of circulating $CD4^+$ lymphocyte infection (13, 14) and a precipitous decline in $CD4^+$ lymphocytes from the peripheral blood (12). By the third week of infection, the virus has disseminated throughout the lymphoid tissues of the body, replicating within the thymus, regional lymph nodes, and mucosa associated lymphoid tissues (13, 15, 16). Virus is also detected at this time in many body secretions, including saliva (17), milk/colostrum (18, 19), and vaginal secretions (17). The immunologic response to viremia first occurs with the production of neutralizing antibodies by 3 to 4 weeks p.i. (20) and cell-mediated immunity between 2 weeks (21) and 7 weeks (22). The emergence of an effective antiviral immune response correlates with a sharp decline in viremia that defines the end of the acute stage of infection.

The dynamic events of the acute stage precede a relatively stable, clinically asymptomatic stage of infection that characterizes most lentivirus infections of animals. In nature, the median age of asymptomatic cats (4 years) is much less than the median age for FIV-infected cats with AIDS-like disease (10 years), suggesting that the this asymptomatic stage lasts for several years (23). In an experimental setting, the duration of this stage is highly variable, being influenced by the virus strain (12) and by the degree of immunologic challenge to the host (24). Plasma viremia is maintained at extremely low levels (12) and cell-associated infection is maintained primarily in $CD8^+$ T lymphocytes and B lymphocytes (13, 14). A persistent increase in IgG concentration emerges, partly attributable to the production of FIV-specific antibodies (25). Within a year of infection, memory-effector $CD8^+$ lymphocytes localize within the spleen, lymph nodes, and thymus where their presence is associated with reduced virus replication within these tissues (21, 26, 27). The compromised immune status of these animals is characterized by a sustained reduction in the number of $CD4^+$ lymphocytes (reduced CD4:CD8), and lower proliferative responses by T lymphocytes *in vitro* (10, 25, 28-32). Clinical findings are often minimal during this period but may include generalized lymphadenopathy, uveitis, gingivitis/stomatitis, and neutropenia. While these cats may not become overtly ill when housed under conditions that limit exposure to infectious agents, they are more susceptible than FIV-negative cats to fulminant clinical disease when challenged with normally subpathogenic doses of infectious agents such as *Toxoplasma gondii* (24).

The third phase of FIV infection is the classical form of feline AIDS often marked by the emergence of multiple degenerative, neoplastic, and secondary infectious diseases, including chronic periodontal disease, weight loss, tracheobronchitis, abscesses, neurologic disease, and lymphoma. While there is considerable overlap in clinical findings between this and the previous category, manifestations may become multiple or more severe. This stage is best characterized in naturally infected animals, but has also been reproduced in an experimental setting (10, 12). AIDS coincides with a re-emergence of circulating virus, a marked decline in the number of circulating $CD8^+$ T lymphocytes (10), a persistent deficiency of $CD4^+$ lymphocytes (33) and a further reduction in proliferative response to mitogen stimulation. A rapid progression to

AIDS has been attributed to elevated levels of plasma viremia due to ineffective immunologic control of virus replication (34).

3.2. Impact of FIV infection on the neonate

FIV infection causes a more severe immunologic dysfunction in the neonatal cat than in the adult cat. In experiments with the Petaluma strain of FIV, severe disease in kittens was characterized by persistent generalized lymphadenopathy, profound neutropenia, persistent decreases in CD4:CD8 T cell ratio, and variably decreased $CD4^+$ T cell counts (35-37). A number of other FIV isolates are associated with impaired weight gain, increased numbers of circulating $CD8^+$ T cells, and decreases in $CD4^+$ T cell counts within 12 weeks of neonatal inoculation. Some of these isolates include FIV-NCSU-1 (7, 26), FIV-Ch (36, 38, 39) and V1CSF, a primary isolate from a cat with neurological disease (36, 37). The pathogenic molecular clone JSY3 (40) reduces $CD4^+$ T cell numbers and CD4:CD8 T cell ratios in neonates, and these effects are partially abrogated with an inactivating mutation of the ORF-A gene (41, 42). Similar changes in $CD4^+$ T cells and $CD4^+/CD8^+$ T cell ratios have been observed with pFIV-PPR (43).

Considerable work has focused on the role of $CD8^+$ T cells in control of, and resistance to, HIV infection. A subset of chronically HIV-infected people progress slowly to AIDS and certain repeatedly exposed but uninfected individuals possess strong cytotoxic T lymphocyte responses (44). Children with non-cytotoxic $CD8^+$ T cell viral suppression have lower plasma HIV concentration, higher $CD4^+$ T cell percentages at one year of age, and slower progression to AIDS (45). Neonatal cats infected with FIV-JSY3 were examined for generation of $CD8^+$ T cell responses and their effects on viral burden (26). Development of $CD8^+$ T cell-mediated FIV suppressive activity and increased numbers of effector $CD8^+$ T cells within blood, thymus and lymph node samples over the 16-week study period were associated with a reduction in viral load. However, viral suppression was not strongly associated with improved $CD4^+$ cell counts or $CD4^+/CD8^+$ T cell ratios.

Neutrophil counts are reported to both increase and decrease in FIV-infected cats, suggesting that stage of disease and viral properties may influence neutrophil production (46). Neutropenia during acute infection of cats with the Petaluma strain of FIV is associated with a decline in marrow precursors belonging to the granulocyte-macrophage lineage between 4 and 12 weeks post-inoculation (47). Kubes et al. infected neonatal kittens with FIV-Ch and followed their neutrophil counts for 8 or 12 weeks, at which time neither quantitative differences nor neutrophil infection were observed (39). Using *in vivo* microscopy, it was determined that the 12-week-old cats in this study exhibited a profound impairment in neutrophil rolling, adhesion, and emigration in postcapillary venules in response to endotoxin treatment.

3.3. Vertical transmission

Transmission of FIV from pregnant cats (queens) to their kittens provides a unique system in which to develop strategies for the prevention and treatment of lentivirus-induced disease in neonates (48, 49). Vertical transmission of FIV is known to occur during pregnancy (17, 49, 50), parturition (17), or postpartum through the ingestion of infected colostrum or milk (17, 19, 51), resembling the diverse timing of vertical transmission of HIV (52-55). Transmission in naturally infected cats occurs much less frequently than in experimentally induced infections. In fact, early investigations of FIV often failed to detect any serologic evidence of vertical transmission (11, 56), and immunologic surveys of natural populations suggest that vertical transmission of native virus is relatively uncommon (57, 58). However, vertical FIV transmission in experimental settings occurs frequently, ranging from 47-95%, depending on viral strain, size of inoculum, and study design (49, 50). Cats that were experimentally inoculated with FIV strains that were generated by serial *in vivo* passage to produce an accelerated disease (59) have increased vertical transmission rates when compared with wild type virus. Likewise, HIV vertical transmission rates are variable, ranging from 15-90% depending on geographic location, maternal viral load, severity of maternal disease, and mode of delivery (52, 60). Vertical transmission is most efficient when pregnant cats are acutely infected during pregnancy. Pregnant women with high virus loads are also more likely to transfer infection to the infant, but other factors facilitate transmission when maternal viremia is low (61). Chronic infection (i.e., maternal infection that precedes the onset of pregnancy) is the norm in the case of natural FIV infection, and transmission rates are typically low in this situation due to low levels of viremia (6, 19).

Transmission *in utero* within the first and second trimesters is considered rare in FIV (49) and HIV (53, 55, 62) infections, as fetal blood and tissues, including thymus, tested early in pregnancy are usually negative for virus. One study of FIV vertical transmission demonstrated increasing rates of infection with advancement of gestation, as no fetal kittens were found to be infected at 3 weeks of gestation, but up to 60% were infected by the last trimester as determined by positive DNA PCR from at least one fetal tissue (49). Most transmission of HIV occurs during late gestation or within the peripartum interval (63). However, many studies of HIV transmission are based on the evaluation of the blood of near-term fetuses. Such approaches are not effective in identifying covert infections where the virus is sequestered in the thymus or lymph node in the absence of detectable virus in the blood (48-50). Misidentification of these infected kittens, and a failure to recognize fetal resorption, may result in an underestimation of the frequency of vertical transmission (64).

Infants are considered infected *in utero* if there is a positive culture or DNA PCR test within 48 hrs of birth. Intrapartum infection is identified by a positive test prior to 3 months of age in the absence of breast-feeding and with negative HIV tests within the first week of life (65). Using these definitions, 43% of infants infected *in utero* died of AIDS prior to one year of age, as opposed to 29% of those infected intrapartum (54), illustrating the clinical and epidemiological significance of accurately determining the

timing of transmission. If intrapartum infection occurs at an appreciable rate, then cesarean delivery might be justified over vaginal delivery; however, data in support of this presumption are conflicting. In the case of HIV, delivery by elective cesarean section before rupture of the amniotic membranes or the onset of labor, and the administration of antiretroviral agents during the peripartum period, significantly reduce vertical transmission of HIV (54). However, in a 1996 study of FIV infected queens by O'Neil et al., 25% of kittens born to chronically FIV-infected queens, delivered vaginally and denied maternal colostrum and milk, were FIV positive at birth (18). Some kittens, which had tested FIV negative at birth, became positive by 6 months of age, resulting in an overall infection rate of 50%. In the same study, 75% of kittens delivered by cesarean section and foster nursed by FIV-naïve queens were FIV-positive at birth, and an additional 20% of kittens later tested positive, resulting in a total infection rate of 95%. Both groups had a 20-25% increase in detectable infection rate by 6 months of age, which is similar to infection rates attributed to intrapartum exposure of HIV (54). These findings suggested that the kittens were infected near parturition, but the benefit of cesarean over vaginal delivery was not established. Although the queens in this study were chronically infected, the majority developed clinical signs related to FIV infection during pregnancy. There was a significant positive association between lowered maternal $CD4^+$ cell count (specifically when less than 200 cells per microliter), increased duration of maternal infection, and increased vertical transmission rate (18).

3.4. Impact of FIV infection on the fetus

Cats infected *in utero* or at birth tend to exhibit accelerated progression of disease and decreased postnatal viability (17, 49, 50). Arrested fetal development, abortion, stillbirth, and lowered birth weights are relatively common when compared with the incidence in non-infected kittens (17). Advanced maternal disease during pregnancy is also associated with faster lentiviral disease progression in children (66) and in kittens (18). Furthermore, queens with high viral loads tend to have a higher rate of stillbirth or death in their kittens within 48 hours of birth (17). This is particularly true when infection occurs early in pregnancy, as the number of nonviable kittens is higher from acutely infected queens (53%) than from chronically infected queens (30%) (49-51). Data concerning fetal viability are somewhat variable, however, as Weaver et al. reported slight differences in litter size, and significantly increased numbers of non-viable kittens, either due to arrested development or fetal resorption, in queens infected with FIV-B-2542 (64), while other studies utilizing this same viral strain reported no differences in litter size and no evidence of an increase in fetal gross morphologic abnormalities (49). In a separate study, 25 of 83 kittens (30%) born to queens chronically infected with either FIV-B-2542 or FIV-AB-2771 were nonviable, compared to an 8% rate of nonviable kittens born to non-infected queens (18). Nine of these 25 nonviable kittens (36%) were born prematurely, 7 (78%) of which were also FIV infected, indicating that prematurely born, nonviable kittens are more likely to be infected than are full-term, nonviable kittens (18). Other FIV strains have also shown deleterious effects on fetal development as well, as 53% of kittens born to FIV-CSU-2771-infected queens were either stillborn or died within 48 hours (17). Mean birth weights were often low for the nonviable kittens (avg. <90g) when compared with normal controls (avg. 101g). The average birth weight of FIV-infected kittens was significantly lower than that of uninfected littermates; moreover, the birth weights of both of these groups were lower than the average weights of kittens born to uninfected queens (18). Postnatal weight gains were also below normal for virus isolation-negative, but antibody-positive kittens born from FIV-infected queens. Thus, FIV-infected queens are less supportive of fetal and neonatal growth even in the absence of vertical transmission.

Some seropositive and PCR-positive kittens born to FIV-infected queens are reported to develop a transient infection, with progressive decline–often to undetectable levels–of antiviral antibody, virus, and provirus within peripheral blood (48, 67). However, FIV was detectable within certain tissues greater than one year post-infection, indicating that conventional indicators of infection may be negative, even in the face of undetectable latent infection (48, 67). Thus, small quantities of virus may remain within tissues, yet be insufficient to induce antibody production. In some cases, proviral DNA was detected, but there were no measurable RNA transcripts via *in situ* hybridization, suggesting that these were not active or productive infections at that time, even though *ex vivo* cultures confirmed viral viability (49).

HIV-infected fetuses exhibit a reduced rate of intrauterine growth (63). Infants born prematurely and with low body weight (when corrected for gestational age) have significantly higher risks of intrapartum infection (68). Additionally, in HIV-infected pregnant women with clinical symptoms of disease, there is a significantly increased risk of low birth weight, premature delivery, intrauterine fetal death, and miscarriage (69), and an approximately three-fold greater incidence of fetal loss than the general population (63). Embryopathy and multiple malformations, including thymic hypoplasia (70) occurs occasionally in spontaneously or electively aborted human fetuses born to HIV-infected mothers, even when the fetus does not have demonstrable infection (55, 71). Interestingly, as noted in both FIV and HIV infection, not all infected fetuses harbor virus within the placenta (50), yet fetal and placental tissues with the highest viral expression are often associated with earlier gestational loss (55).

HIV may induce changes that contribute to immune rejection of the fetus, possibly through an imbalance of maternal and fetal T-helper (Th)-1 and Th-2 cytokines, leading to early spontaneous abortion and an increased incidence of reproductive failure (72). In comparable studies of kittens from FIV-infected pregnant queens, there are trends for increased expression of Th-1 cytokines, which are usually suppressed during normal pregnancy, and a decrease in Th-2 cytokine levels in the placentas of infected versus noninfected cats (64).

Increased placental expression of interferon (IFN)-gamma and IL-1-beta, both Th-1 cytokines, were significantly associated with increased fetal resorption in infected animals (64). Increased Th-1 cytokines and decreased interleukin (IL)-10, a Th-2 cytokine, has also been linked to spontaneous abortion and underweight infants born to HIV-infected women (73). In acutely FIV infected cats, elevations of IFN-gamma, IL-12p40, IL-4, and IL-10, among others, have been measured in various tissues, including thymus, lymph nodes, and spleen (74, 75). Infection may additionally produce placentitis, thereby facilitating the transfer of virus from mother to fetus (76) via altered local cytokine levels and disruption in membrane and vascular integrity.

In two related studies, Rogers and Hoover reported on the *in utero* transmission of FIV viruses from differing clades, FIV-A-Pet, FIV-B-2542, and FIV-C-Pgmr, which exhibit variable virulence to the infected fetus (49, 50). FIV-A-Pet virus was most frequently isolated in the placenta and mesenteric lymph nodes, less commonly in the fetal thymus, PBMC, spleen, and bone marrow, and never in the brain or liver. Interestingly, 63% of FIV-A-Pet positive fetuses had FIV-negative placentas, suggesting that demonstrable placental infection is neither sufficient nor necessary for fetal infection (50). The FIV-B-2542 isolate, a known macrophage-tropic virus (77), was most often localized to tissues rich in mononuclear cells, including the brain, blood, and thymus, and less commonly to the bone marrow, mesenteric lymph nodes, spleen, and liver, indicating a distinctly different cellular tropism than FIV-A-Pet. The FIV-C-Pgmr virus, reported to be immunopathogenic, was most commonly isolated in the placenta, PBMC, and bone marrow, and less commonly in the brain, thymus, liver, mesenteric lymph nodes, and spleen, further illustrating alterations in cellular tropisms of different viral subtypes with variable pathogenicity (50).

3.5. Thymus pathogenesis

The thymus is the major site of production for T lymphocytes, and replacement of these cells during the course of immunosuppressive lentivirus infection is by cell division in the periphery and *de novo* synthesis in the thymus. Therefore, a major contributor to the progression of pediatric HIV infection is the loss of thymus function (78-83). Thymocytes are highly susceptible to lytic HIV infection, resulting in cortical atrophy and varying degrees of inflammatory cell infiltration from the periphery (84). Treatment of HIV infection with highly active antiretroviral therapy (HAART) appears to partially restore thymic function; however, the pathogenesis of the thymic infection appears to include mechanisms beyond those of productive and lytic viral infection (80). Compared to uninfected children, vertically infected HIV-positive children have lower levels of T cell receptor rearrangement excision circles (TREC) in blood lymphocytes, which are markers for recent thymic emigrants and serve as an indicator of thymic output (85); this decrease was not directly associated with viral load in the blood. The complex relationship between viral load, disease progression, and thymus dysfunction is difficult to assess in humans due to the relatively inaccessible location of the thymus, difficulties in defining the timing of transmission, and the variable properties of the transmitted virus.

The domestic cat infected with FIV offers specific advantages in the modeling of HIV infection in the thymus. Tissue can be collected for examination at defined intervals after inoculation, litter-matched controls can be used to assess maternal and environmental influences, and a cloned inoculum of defined infectious potential can be precisely administered (7). Furthermore, FIV exerts similar effects on the thymus as does HIV, providing a suitable physiologic model (7). Neonatal thymus infection with FIV results in a reduction of thymus-body weight ratio, selective depletion of $CD4^+CD8^+$ thymocytes, cortical atrophy, infiltration of B cells, formation of lymphoid follicles and deformation of the thymic architecture (7, 41, 42, 75, 86). Interestingly, these changes persist despite treatment with antiretroviral therapy (87) and are produced in cats harboring FIV with an inactivating mutation of the ORF-A gene that yields lower viral replication and a lower thymic proviral load (41). This suggests that host factors and inflammatory processes may be significant contributors to the pathogenesis of thymus infection without extensive productive infection. Infection of the thymus was associated with the emergence of $CD8^+$ T cells expressing CD8alpha+beta^{low} and CD8alpha+beta^{neg} phenotypes (26, 75), and the activity of these cells was found to correlate with a reduction in viral load in the thymus and blood (26). Immunohistochemistry of thymic samples showed significant staining for IgG outside of lymphoid follicles that did not correlate with positive staining for a B cell marker, suggesting that thymocytes were coated with antibody (42). Infection was associated with a 10-fold increase in thymic expression of interferon (IFN)-gamma messenger RNA (mRNA) by cells located in perivascular areas along the corticomedullary junction and adjacent to lymphoid follicles (75). As with HIV, the overall number of FIV expressing cells within the thymus was low and the lowest frequency of productively infected cells correlated with the most severe histologic lesions (7).

Fetal kittens inoculated *in utero* develop acute, but transient, thymic atrophy, which partially regenerates after birth (7, 88). Thymus-body weight ratios were initially decreased, with severe thymic cortical depletion, reduction of thymocyte numbers, and decreased corticomedullary distinction in those infected fetally, but this later began to rebound or approach normal. In contrast, neonatally infected kittens exhibited a progressive decline in thymus-body weight ratio, a moderate decline in cortical thymocyte density, a loss of corticomedullary distinction, and the formation of lymphoid follicles (7). Similar thymic responses have been noted in SIV-infected rhesus macaque models of HIV, in which infected animals initially had dramatically increased levels of thymocyte depletion and apoptosis, with a rebound in thymocyte progenitor numbers and increased levels of cell proliferation that occurred in the following weeks (89). Viral loads also varied between the two groups, as *in situ* hybridization and immunohistochemistry revealed an increased propensity to support viral mRNA and protein expression in the fetal

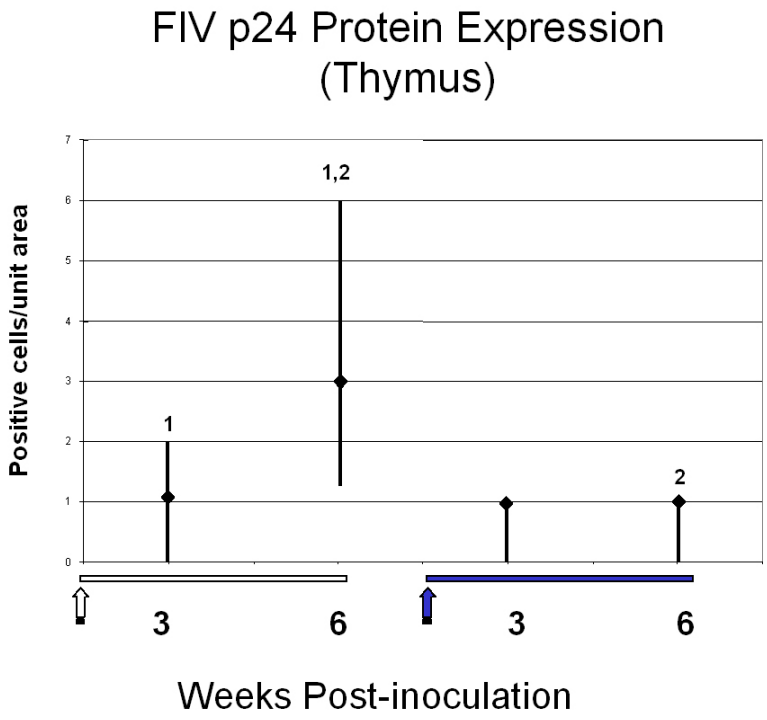

Figure 1. Frequency of productively infected thymic cells in two groups of cats: those inoculated with FIV between gestational days 40-45 (white bar with arrow indicating the time of inoculation), and those inoculated with FIV within one day after birth (blue bar with arrow indicating time of inoculation). Thymic cells were evaluated in both groups at 3 and 6 weeks after birth. Data are expressed as the median number of cells expressing FIV *gag* per unit area (diamonds) and corresponding interquartile ranges (lines). Note that the frequency of positive cells at 3 weeks after fetal inoculation is significantly lower ($P<0.05$, Mann-Whitney *U* test) than the frequency at 6 weeks after fetal inoculation (1). Furthermore, the frequency of positive cells at 6 weeks after fetal inoculation is significantly greater than at 6 weeks after neonatal inoculation (2).

inoculates, while those infected neonatally had reduced virus levels, supporting the theory that kittens infected *in utero* are more likely to harbor productive infection (Figure 1) (7). Thymocytes and stromal cells with dendritic morphology appear capable of harboring productive infection (88, 89). Viral effects on the developing fetal thymus, which is exquisitely vulnerable to productive infection, may result in enhanced pathogenicity and progression of disease, acute and profound thymic atrophy, and increased levels of viremia near birth (Figure 2). The unique changes noted within the thymus after fetal versus neonatal inoculation may reflect differences in productively infected cell types or distinctly different regenerative capacities of progenitor cells at different stages of development. The impact of fetal infection on peripheral blood T cell subpopulations became most apparent after birth, where there was a significant depletion of $CD4^+$ and $CD8^+$ lymphocytes from the peripheral blood (Figures 3 and 4) resulting in a failure to maintain T-cell homeostasis in cats with fetal infection (Figure 5). Recently, a mAb (755) was reported to recognize the feline homologue to CD45RA, allowing the enumeration of naïve CD4 and CD8 lymphocytes in cats (90). FIV-infected cats had reduced percentages of $CD4^+$ lymphocytes, which were deficient in $CD4^+CD45RA^+$ (naïve $CD4^+$) cells (Figure 6). Likewise, percentages of naïve $CD8^+$ lymphocytes were also reduced with FIV infection, resulting in a predominance of $CD8^+CD45RA^{neg}$ (memory $CD8^+$) cells, as reported for FIV-infected adult cats (91). Depletion of naïve cells may have occurred through lytic infection, activation-induced apoptosis, activation of immunosuppressive regulatory T cells, transition from a naïve to a memory phenotype, or thymus insufficiency (14, 92-96).

Thymopoiesis requires a controlled microenvironment and is regulated by direct cellular interactions and paracrine influences by cytokines. Retroviral diseases and some autoimmune disorders induce thymic inflammation, which could influence the cytokine milieu and potentially reduce thymopoiesis. Ongoing studies in our laboratory have focused on the role of aberrantly expressed cytokines in FIV pathogenesis. Aside from the upregulation of IFN-gamma associated with peripheral CD8 cell influx (75), there appears to be a marked reduction in the endogenous production of IFN-alpha mRNA by infected animals at 16 weeks of age, which correlates with a greater depletion of developing thymocytes and higher proviral loads (97). These changes prompted an investigation of the resident plasmacytoid dendritic cells (PDC), the major producers of Type I interferons such as IFN-alpha. In infected cats, these cells appear to be increased in number and stain positively with anti-FIV gag antibody along the corticomedullary junction and within germinal centers (97). Ongoing development of

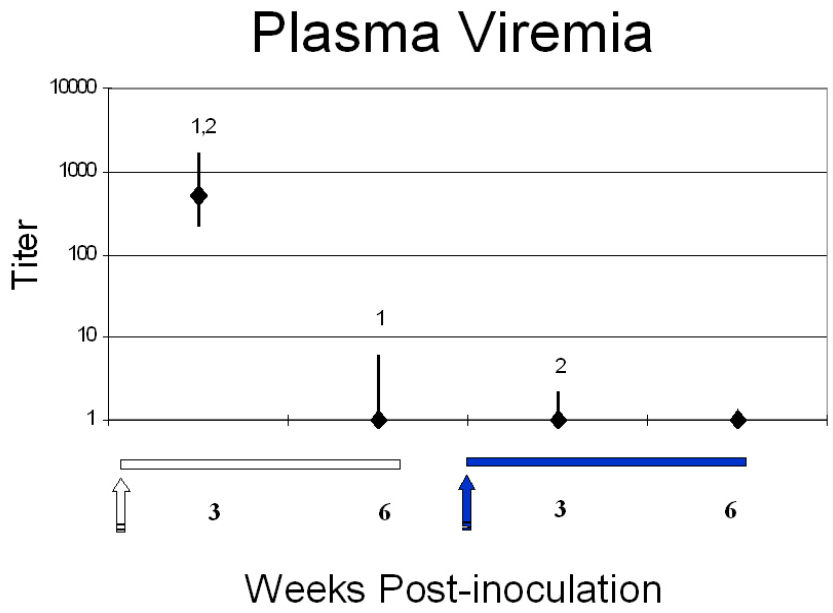

Figure 2. Titer of infectious virus within the plasma of infected cats belonging to two treatment groups: those inoculated with FIV between gestational days 40-45 (white bar with arrow indicating time of inoculation), and those inoculated with FIV within one day after birth (blue bar with arrow indicating time of inoculation). Thymic cells were evaluated in both groups at 3 and 6 weeks after birth. Data are expressed as medians (diamonds) and interquartile ranges (lines). Titers are expressed as the reciprocal of the greatest dilution of plasma that was positive by virus isolation. Note that the titer of infectious virus in plasma at 3 weeks after fetal inoculation is significantly greater than the titer at 6 weeks after fetal inoculation (1). Furthermore, the titer of virus in plasma at 3 weeks after fetal inoculation is significantly greater than at 3 weeks after neonatal inoculation (2).

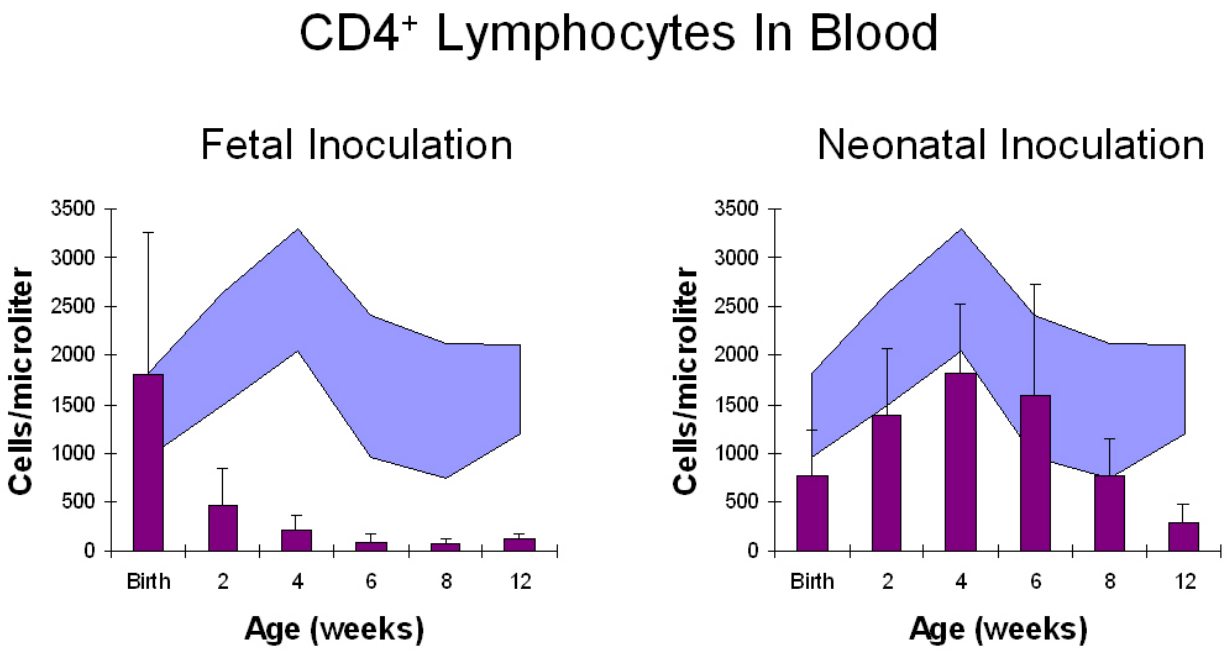

Figure 3. $CD4^+$ lymphocytes in the peripheral blood of cats inoculated between days 40-45 of fetal development (left graph) or within 1 day of birth (right graph). Bars depict median values from 7 (fetal inoculation) and 10 (neonatal inoculation) cats, and lines represent one standard deviation. The shaded region in each graph represents the 95% confidence interval for age-matched uninfected cats. Note the early and persistent decline in $CD4^+$ lymphocytes by two weeks of age following fetal infection, and the late decline (12 weeks) following neonatal infection.

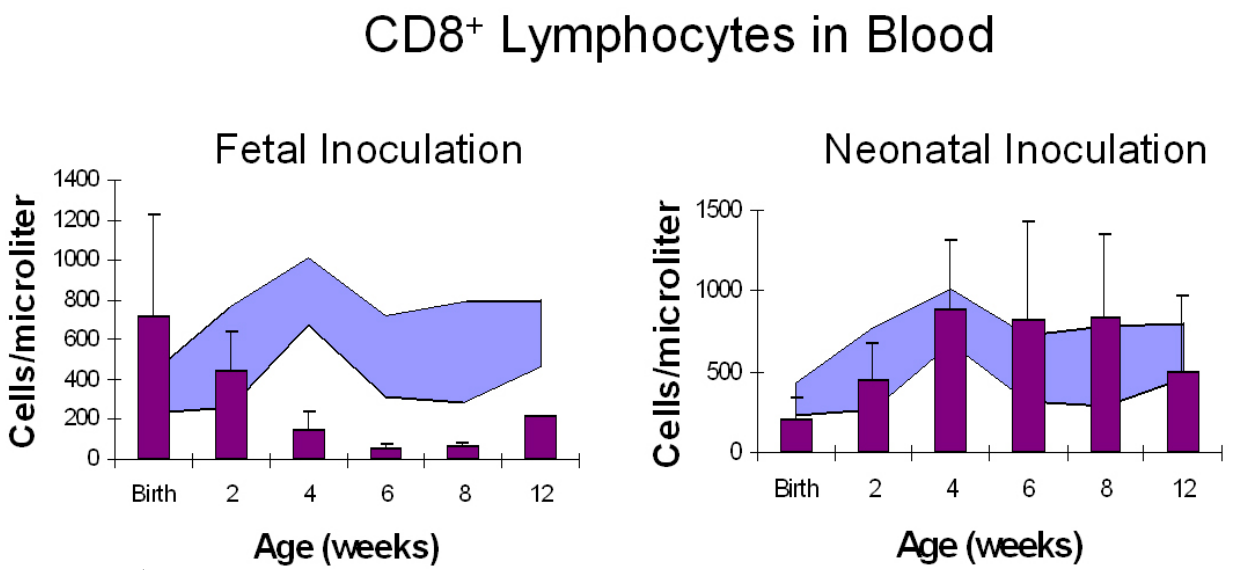

Figure 4. $CD8^+$ lymphocytes in the peripheral blood of cats inoculated between days 40-45 of fetal development (left graph) or within 1 day of birth (right graph). Bars depict median values from 7 (fetal inoculation) and 10 (neonatal inoculation) cats, and lines represent one standard deviation. The shaded region in each graph represents the 95% confidence interval for age-matched uninfected cats. $CD8^+$ cells remain beneath control values after 4 weeks of age in fetal inoculates. In contrast, $CD8^+$ lymphocytes remain within the 95% confidence interval throughout the 12-week study interval for kittens inoculated at birth.

DC isolation and culture techniques will assist in the clarification of the infectious status of these cells.

3.6. Potential modes of therapy for thymus dysfunction

The unique lymphoid and stromal microenvironment of the thymus can be manipulated therapeutically to enhance T-cell production (98). While antiviral pharmaceuticals are currently used to a limited extent in clinical treatment of FIV-infected cats, the FIV model holds significant promise as a system to explore gene therapy, cytokine therapy, or *ex vivo* methods designed to reconstitute depleted T cells through thymic regeneration (87, 99).

The antiretroviral drug 3'-azido-2', 3'-dideoxythymidine (AZT, zidovudine, Retrovir) is a thymidine analogue and reverse transcriptase inhibitor and is currently the most extensively studied anti-retroviral drug used in FIV treatment (100). The benefits of AZT include attenuated clinical symptoms, less severe FIV related stomatitis, and an increased CD4:CD8 ratio (101). However, these clinical improvements are quite variable in their duration and effect and AZT does not prevent the spread of the virus to other tissues or prolong the life of FIV infected cats (102, 103). AZT use also requires concurrent monitoring of erythrocytes, as anemia is a common side effect of its use (101). When compared with AZT, the antiviral drug 9-(2-phosphonylmethoxyethyl) adenine (PMEA) appears more potent at reducing clinical signs of FIV infection, including stomatitis, and in increasing the CD4-CD8 ratio in peripheral blood (101). The antiviral complex carbohydrate acemannan has been administered to FIV infected cats, where it was associated with increased lymphocyte counts, decreased neutrophil counts, and decreased frequency of sepsis (104). Human recombinant IFN-alpha is an antiviral cytokine that may hold promise in FIV treatment to decrease viral replication (105). Although relatively limited research has addressed the use of IFN-α in clinical FIV treatment, it is thought to have a synergistic effect when used with AZT against other retroviruses (106). A report suggests that IFN-alpha may have little to no beneficial effect in reversing changes in peripheral blood lymphocyte subsets of FIV-infected cats (107).

Specific cytokines are potentially promising in inducing thymic T cell production in HIV-infected patients (108). IL-7, IL-2, IL-12 and IL-15 are considered possible factors in promoting T cell reconstitution (109). Of these interleukins, IL-7 is the most extensively studied. Endogenous IL-7 is a natural growth factor for T and B cells produced by thymic stromal cells (110). Lymphopenic HIV-1 patients are shown to have an inverse correlation between total CD4 cells and IL-7, alluding to its function in reconstituting the T cell population (111). The primary value for use of IL-7 in antiretroviral therapy lies within its anti-apoptotic and proliferative effects on T cells. IL-7 transgenic mice over-expressing IL-7 have shown increased numbers of $CD4^+$ and $CD8^+$ cells during and after lymphopenia-induced proliferation (112). IL-7 has also been shown to enhance reconstitution of T cells in hematopoietic stem cell (HSC) transplant recipient mice and to have profound effects on peripheral T cell proliferation in non-human primates (113). Given its initial promising results, IL-7 may prove to be an effective tool in stimulating T cell reconstitution. However, despite its suggested proliferative effects, some research suggests a possible detrimental effect of IL-7 in HIV patients. CXCR4 is a co-receptor for both HIV and FIV (114), and IL-7 has been shown to positively regulate CXCR4 mRNA

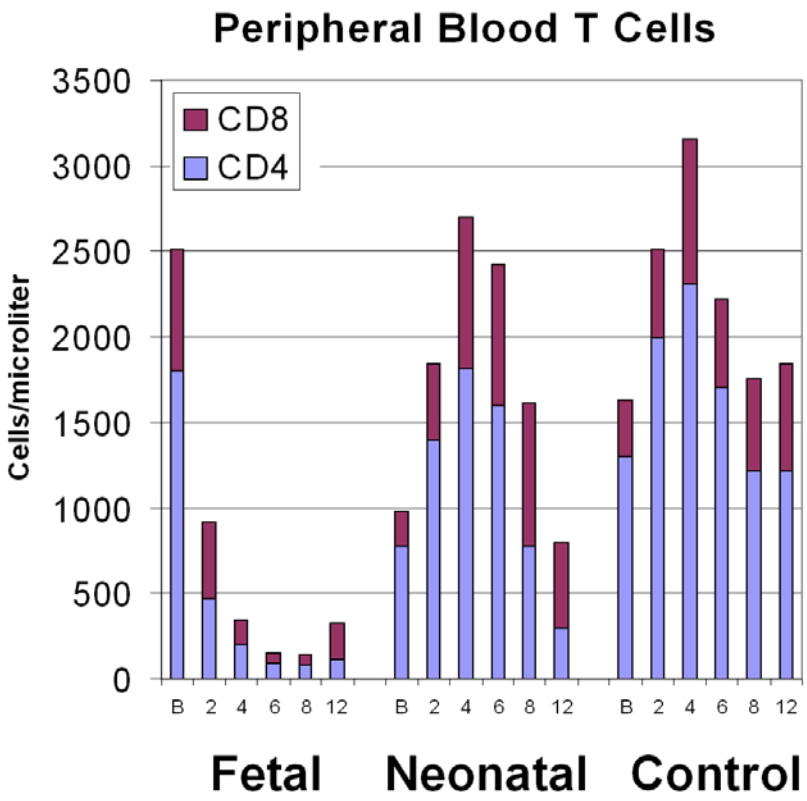

Figure 5. Total peripheral blood T cells are depicted as the sum of $CD4^+$ and $CD8^+$ lymphocytes at birth (B) and biweekly intervals after birth for kittens inoculated between gestational days 40-45 (Fetal), during the first day after birth (Neonatal), or without infection (Control). T cells from kittens inoculated on days 40-45 of fetal development are severely deficient beginning at 2 weeks of age as compared to controls. In comparison, T cells from kittens inoculated on day 1 of age are slightly reduced when compared with controls.

expression in mature lymphocytes (115). Therefore, IL-7 may indirectly promote infection in $CD4^+$ lymphocytes.

Although the metabolic effects of growth hormone (GH) and insulin-like growth factor-1 (IGF-1) are well established, they also hold promise as thymotrophic agents. Clinical studies involving administration of GH and IGF-1 to HIV-infected patients have resulted in increased thymic volume and modest effects on T cell function (108, 113, 116). Infusion of human recombinant IGF-1 has been shown to increase thymic mass in FIV-infected cats (99). In this study, FIV-inoculated cats treated for 12 and 20 weeks with IGF-1 resulted in structural evidence of cortical regeneration, reduced thymic inflammation, and reduced thymic viral load. This effect corresponded with a rise in peripheral blood $CD4^+$ lymphocyte counts after 14 weeks of therapy. In humans, significant side effects are linked to GH and IGF-1 therapy, including myalgia, edema, and diabetes, which could hinder their clinical application (116).

Keratinocyte growth factor (KGF) is a cytokine with thymus-promoting effects produced by mesenchymal cells. Its receptor, FgfR2-IIIb, is located on a variety of cells including thymic epithelial cells (117). Mice deficient in FgfR2-IIIb are shown to have deficits in thymopoiesis and thymic epithelial cell development (118). When given pre-insult, KGF has been shown to be protective against thymic injury due to radiation, chemotherapy, and oxidative damage. Furthermore, KGF given pre-bone marrow transplant has been shown to subsequently facilitate thymic function normally impaired by pre-transfer cytotoxic treatment (119). This was represented by increases in $CD4^+$ T cells and improved humoral responses to T-lymphocyte dependent antigens. Therefore, much like IL-7, KGF seems to have an effect on immune reconstitution that may be useful in thymus reconstitution following lentivirus infection. Sex steroids contribute to age-related thymic involution—Research in both mice and humans has shown that both surgical and chemical castration after the administration of luteinizing hormone-releasing hormone reverses age-related thymic atrophy, resulting in an increase in thymic tissue and thymopoiesis (120, 121). Further research may show that anti-androgen therapy could provide a relatively safe and simple way to increase thymic tissue and T cells in immunosuppressive lentiviral infections.

The ability to deliver and express genes in the thymus could represent a significant advancement in thymus reconstitution during HIV infection. Methods for delivering genes to the thymus, particularly within the thymic stroma, are currently in development. Lentivirus vectors injected directly into the thymus transduce a large

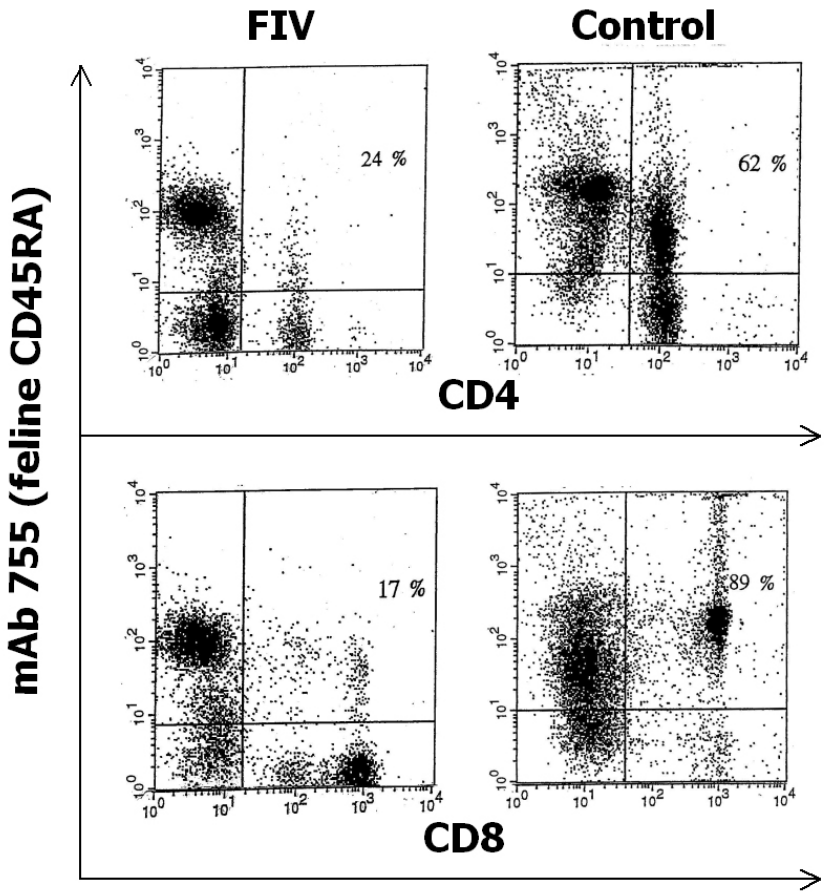

Figure 6. Two-color flow cytometry dot plot of T cell phenotypes. Peripheral blood lymphocytes from adult cats inoculated with FIV within 1 day of birth (left plots) or control cats (right plots). Lymphocytes were labeled with mAb 755 (feline CD45RA equivalent) in conjunction with either CD4 (upper plots) or CD8 (lower plots). FIV infection is associated with a reduction in the percentage of CD4 and CD8 lymphocytes that co-label with mAb 755 (located in the upper right quadrant of each plot), indicating a relative loss of both naïve T cell populations.

proportion of thymic stromal cells and a relatively low number of thymocytes (122). Significant dissemination of the virus from the site of injection, primarily into the liver, suggests that a more direct and efficient means of gene delivery may be necessary. *Ex vivo* approaches are also being developed, including a three-dimensional porous growth substrate that provides a framework in which hematopoietic progenitor cells and thymic stromal cells may be added along with thymotrophic cytokines to promote the production of T cells and reconstitute positive and negative selection (123). The feline-FIV animal model will provide a unique system in which to test novel therapeutic approaches that are designed to reverse thymic disease caused by immunosuppressive lentiviruses.

4. ACKNOWLEDGEMENTS

This work was supported by NIH R29HD33983 (CMJ), NIH R01AI42563 (AM), the Auburn University Biogrant Program, and the Grace Kemper Research Fund. The authors thank Sandra Bortnick, Abigail Carreño, Cynda Crawford, Robert Norway, Janelle Novak, Marlene Orandle, George Papadi, Mary Tompkins, Wayne Tompkins, and Tina Yanchis for their contributions.

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Key Words: AIDS, FIV, Animal Model, Vertical Transmission, HIV, Feline, Thymus, Review

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