8

FIV as a Model for AIDS Pathogenesis Studies

THOMAS W. VAHLENKAMP, MARY B. TOMPKINS, and WAYNE A. F. TOMPKINS

1. INTRODUCTION

The domestic cats (*Catus domesticus*) from which feline immunodeficiency virus (FIV) was first isolated showed clinical signs of immunodeficiency, and the incidence of disease was associated with the prevalence of FIV. The first publication by Niels Pedersen and his collegues[1] at the University of California describes a number of important genomic, structural, and biochemical characteristics of the virus that were found to be remarkably similar to human immunodeficiency virus (HIV). During the past two decades, FIV infection in the domestic cat has become an excellent comparative model for studying several aspects of HIV biology, vaccine development, and particularly immunopathogenesis. Although the lentiviruses genetically most closely related to FIV are those of the small ruminants, maedi-visna virus of sheep, and caprine arthritis encephalitis virus,[2,3] the type of disease produced by FIV is remarkably similar to acquired immunodeficiency syndrome (AIDS) in humans.

HIV infection results in a transient lymphopenia at the time of seroconversion followed by a partial rebound in CD4+ T-cell numbers and the development of a $CD8^+$ lymphocytosis after several weeks. The characteristic decrease in the $CD4^+/CD8^+$ T-cell ratio progresses throughout the asymptomatic stage of infection as $CD4^+$ T-cells continue to decline. By the

THOMAS W. VAHLENKAMP, MARY B. TOMPKINS and WAYNE A. F. TOMPKINS • Immunology Program, College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough St., Raleigh, NC 27606

time HIV+ patients develop AIDS, the $CD4^+$ T-cell numbers are markedly reduced.[4] Parallel with a loss in $CD4^+$ T-cell numbers is a progressive decline in $CD4^+$ T-cell proliferative response to mitogenic and antigenic stimulation that is unrelated to $CD4^+$ cell numbers.[5] The number of $CD8^+$ T-cells also decreases as AIDS develops, but unlike $CD4^+$ T-cell counts, is not a good predictor of disease progression.[6,7] The loss of $CD4^+$ cell numbers and function is highly predictive of the development of AIDS, suggesting that the immunopathogenesis of HIV and the development of AIDS are centered around this selective loss. Despite intensive research in the 20 years after the first isolation of the virus, the mechanisms responsible for the changes in circulating lymphocyte subsets in HIV+ patients are not fully understood.

The development of the FIV animal model with virus-immune system interactions similar to those seen with HIV has greatly facilitated the study of the immunopathogenesis of AIDS, which will be focus of this chapter.

2. COURSE OF THE DISEASE

As in HIV+ patients, progressive immune dysfunctions in naturally or experimentally FIV+ domestic cats usually result in four clinical stages of disease (Figure 1). The acute phase of the infection is characterized

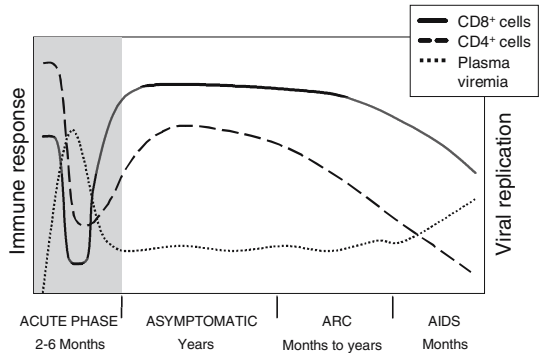

FIGURE 1. Natural history of FIV infection. The acute stage infection is characterized by an initial plasma viremia and a decrease in $CD4^+$ and $CD8^+$ cells in the peripheral blood that is followed by a rebound of $CD4^+$ and $CD8^+$ cells and a marked decline in plasma viremia. The initial phase of the immune response (gray underlayed area) appears to be normal and similar to other viral infections.[198] With transition to the asymptomatic stage of infection a low level plasma viremia persists and $CD4^+$ cell counts in the peripheral blood remain lower than physiologic numbers before the infection. With time of infection $CD4^+$ cells and in later stages also $CD8^+$ cells progressively decrease. Clinical symptoms appear as the infection progresses to the AIDS-related-complex (ARC) and AIDS stages that are characterized by a marked immunodeficiency and an increase in plasma viremia.

by an early peak in plasma viremia generally three to four weeks post-infection. Transient clinical signs may accompany this early period of virus replication, commonly manifested as mild pyrexia, anorexia, depression, and neutropenia. Acute diarrhea, conjunctivitis, and upper respiratory tract symptoms may develop in more severely affected cats.[8] During this period, productive infection is seen within the thymus, mucosal and systemic lymphoid tissues, and bone marrow, and the virus can be detected in plasma, saliva, vaginal secretions, semen, and milk/colostrums.[9–13] Virus also enters the central nervous system early in the course of infection and can be isolated from cerebrospinal fluid.[10] Some cats develop a persistent generalized lymph-adenopathy without obvious secondary or opportunistic infections.[8] Concurrent with the humoral and cellular immune response the level of viral load in plasma decreases and the animals become clinically asymptomatic. Usually the infection remains clinically inapparent for prolonged periods of time, lasting for several years. Despite the lack of clinical disease symptoms, there is progressive immune dysfunction characterized by loss of $CD4^+$ cells, altered cytokine profiles, inability of $CD4^+$ cells to produce interleukin (IL)-2, and loss of T-cell proliferative response to mitogen and major histocompatibility complex (MHC) II restricted recall antigens. Antibody responses in FIV+ cats have been reported to be normal or enhanced,[14] but usually infected cats mount reduced antibody responses to a variety of antigens and microorganisms.[15–17] As immune dysfunctions progress, infected cats eventually move into the AIDS-related complex (ARC) stage of infection. This stage is characterized by recurrent episodes of infection and inflammation, and cats suffer from infections of the oral cavity, weight loss, anemia, leukopenia, upper respiratory tract infections, chronic skin disorders, diarrhea, and lymphoid neoplastic disorders. Neurologic symptoms associated with the ARC stage of FIV infection may include sleep disorders, behavioral changes, and altered visual and auditory evoked potentials.[18–20] As in HIV+ patients,[21] the great majority of lymphoid tumors are of B-cell origin.[8,22,23] Clinical symptoms worsen over a period of months to years, and animals may develop a disease picture comparable to AIDS in man. Cytopenia with $CD4^+$ T-cell numbers less than 200–300/μl of blood is usually seen with this stage of disease that is also characterized by increased plasma viral load, weight loss, pancytopenia, severe emaciation, chronic disease symptoms, and opportunistic infections. In the individual cat the degree of $CD4^+$ cell depletion, however, does not solely predict the clinical outcome, although for the larger population of FIV^+ cats, this correlate can be made.[24,25] Cats usually die within months of developing AIDS-associated clinical disease.[22,23,26,27]

Whatever the mechanisms leading to the loss of $CD4^+$ cell numbers and function, data suggest that FIV and HIV affect the immune system in a similar manner and share a common immunopathogenesis.

3. FELINE IMMUNODEFICIENCY VIRUS (FIV)

A major feature of retrovirus infection is virus persistence in the host. Lentiviruses achieve this through the integration of a reverse transcribed DNA copy of the viral RNA into the host cell chromosomal DNA, which is maintained as provirus for the life of the cell. Lentivirus persistence is also promoted by the establishment of effective mechanisms to evade the host's specific immune responses to viral antigens. The latter can be achieved by constant mutational changes in viral antigens presented to the immune system or by interference with immune regulatory mechanisms essential for the induction of an effective immune response.

Cell Tropism

HIV and FIV are both lymphotropic lentiviruses. The majority of HIV strains utilize the host cell CD4 molecule as the primary receptor. It was originally thought that FIV has a somewhat broader lymphocyte tropism than HIV as it does not utilize the CD4 receptor and has the ability to infect $CD8^+$ cells and B-cells.[28] However, HIV and SIV infection of $CD8^+$ cells and B-cells has been reported, and some strains of HIV and SIV have been demonstrated to enter target cells independent of their CD4 expression (Table I).[29]

In vivo, FIV infects $CD4^+$, $CD8^+$ T-cells, B-cells, cells of the monocyte/macrophage lineage, astrocytes, and megacaryocytes.[1,10,28,30,31] The vast majority of bone marrow cells apparently remain virus-free throughout the course of FIV infection. By in situ hybridization, FIV RNA was detected only in limited numbers of megacaryocytes and mononuclear cells.[32,33] For both FIV and HIV, only low numbers of circulating monocytes have been found

TABLE I
Main Cell Tropism and Chemokine Receptor Usage

Cell Tropism	HIV	SIV	FIV
$CD4^+$ T-cells	+	+	+
$CD8^+$ T-cells	(+)	(+)	+
B-cells	−	−	+
Monocytes/macrophages	+	+	+
Astrocytes/Glia cells	+	+	+
Chemokine receptor[a]			
CCR5	+	+	−
CXCR4	+	−	+

[a] Belong to the seven-transmembrane domain chemokine receptor family

infected.[28,34] Both viruses, however, are prevalent in tissue macrophages and follicular dendritic cells.[33,35]

The principal in vivo target of FIV early after infection is $CD4^+$ T-cells, which carry the highest proviral burden.[28] As the infection progresses to the asymptomatic phase the provirus burden shifts from the $CD4^+$ cells to the $CD21^+$ B-cells and $CD8^+$ cells.[28,32,36] Several reasons may account for the shift in proviral burden within weeks after acute infection. As with HIV infection, FIV can be lytic for $CD4^+$ cells in vitro, raising the possibility that a highly susceptible population of $CD4^+$ cells are infected and rapidly lysed. Immune-mediated destruction of infected $CD4^+$ cells as a means of reducing virus burden may also occur. In support of this the decrease in $CD4^+$ cell proviral burden in FIV^+ cats correlates with the increase in activated $CD8^+$ cells.[37] $CD8^+$ cells may control virus levels by CTL-mediated FIV-infected $CD4^+$ cell lysis or by suppression of virus replication in a noncytotoxic, contact-dependent non-MHC restricted mechanism.[38–41] Whatever the reason, continued viral replication in the $CD4^+$ T-cell population may then depend on a slow renewal of these cells or on a smaller $CD4^+$ T-cell subset supportive of a nonlytic virus infection.

Differential susceptibility of $CD4^+$ cell subsets has been reported for both HIV and SIV.[42,43] Subsets of activated $CD4^+$ cells are preferentially infected by HIV-1 and SIV in vitro and the same subsets of highly differentiated, activated CD45RO expressing $CD4^+$ cells contain the majority of provirus in vivo during the entire course of the infection.[44,45] In vitro studies demonstrated that productive HIV infection is associated with a $CD4^+$ T-cell subset characterized by a partial activation phenotype, as indicated by cell surface expression of the CD25 receptor (IL-2 receptor α chain). Peripheral blood mononuclear cells (PBMC) depleted of $CD25^+$ cells are markedly diminished in their ability to replicate HIV when infected in vitro.[42,43] $CD4^+CD25^+$ cells were also shown to be susceptible to productive HIV infection in vitro, whereas highly purified resting $CD4^+CD25^-$ cells were resistant to infection.[46] Interestingly, a naturally occurring $CD25^+$ subset of $CD4^+$ T-cells (T regulatory cells) with unique immune regulating properties has been described in humans and rodents.[47,48] While the $CD4^+CD25^+$ cells reported to support HIV replication were not identified as T regulatory cells, these observations are of interest, as the partial activated phenotype of $CD4^+CD25^+$ T regulatory cells could make it a particularly favorable target for sustained HIV infection. Studies in our laboratory demonstrated that both $CD4^+CD25^+$ and $CD4^+CD25^-$ cells are susceptible to FIV infection in vitro and in vivo, but only $CD4^+CD25^+$ cells replicate the virus in the absence of mitogenic stimulation. In contrast to $CD4^+CD25^-$ cells, $CD4^+CD25^+$ cells, whether or not infected with FIV, do not proliferate in response to Concanavalin A (Con A) stimulation and are relatively resistant to activation-induced programmed cell death. These observations plus the fact that $CD4^+CD25^+$ cells are partially activated and yet are anergic

and incapable of progressing through the G1 cell cycle suggest that they could represent a long-lived reservoir of productive FIV infection.[49]

Receptor Usage

Despite the similar cell tropism to HIV, there is no evidence that FIV uses the CD4 molecule as a cellular receptor.[50,51] FIV infects a range of $CD4^-$ cell lines, including monocyte/macrophages,[30,52,53] which in cats do not express CD4 and cells of neuronal lineage.[10] CD4 expression on cells also does not correlate with susceptibility to in vitro infection with FIV.[54] It is intriguing that FIV infection induces $CD4^+$ T-cell associated AIDS-like disease in cats and yet does not share a common primary cellular receptor with HIV.

Binding of HIV to the primary receptor induces a conformational change in the envelope glycoprotein that enables interaction with a chemokine coreceptor. Following a second conformational change, the viral envelope glycoprotein fuses with the cellular membrane of the target cell allowing virus entry into cells.[55,56] HIV-1 coreceptors belong to the seven-transmembrane domain chemokine receptor family, with CCR5 and CXCR4 being the main receptors for macrophage and T-cell tropic isolates, respectively (Table I).[57–59] The expression of CCR5 on human peripheral blood T-cells is largely restricted to $CD4^+CD45RO^+$ (memory) cells, while CXCR4 is expressed on B-cells and on both $CD4^+CD45RA^+$ (naïve) and memory T-cells.[60] CCR5-dependent viruses are detected early in HIV infection. In about 50% of infected individuals progression of the disease coincides with the emergence of CXCR4-dependent viruses. The differential expression of CXCR4 and CCR5 on cells of the immune system may provide a basis for the specific targeting of memory T-cells early in infection and the additional cell types targeted with the emergence of CXCR4-dependent strains in the later stages of infection.

FIV strains of clade A and tissue culture adapted strains of clade A and B have been shown to utilize CXCR4 as a receptor for envelope-mediated fusion.[61–63] The second extracellular loop of CXCR4 acts as the primary determinant of usage by the FIV envelope V3 to V5 region,[64,65] and two CXCR4 ligands, stromal cell derived factor 1 alpha (SDF1∀) and the bicyclam AMD3100 are able to inhibit FIV infection.[62,63,66] In addition, the feline CXCR4 displays 94.9% amino acid homology with the human counterpart and FIV is able to utilize both species of CXCR4 for cell infection and syncytia formation. It is unknown whether CXCR4 acts as a primary receptor for some strains of FIV. Certain primate lentivirus strains can utilize CXCR4 independently of the primary receptor CD4.[29] In contrast to the chemokine distribution in the human immune system, the expression of feline CXCR4 in vivo is mainly restricted to monocytes and B-cells and found to be unaltered after FIV infection.[67] CXCR4 expression has also

been reported on freshly isolated thymocytes and on a proportion of T-cells in the lymph nodes. In vitro, expression of CXCR4 was shown to be upregulated on mitogen-activated feline T-cells.[62] If CXCR4 is also upregulated upon T-cell activation in vivo, FIV might selectively target activated $CD4^+$ cells in early stages of the infection, as has been reported.[28,67] In support of this, FIV has been shown to productively infect partially activated CD25 expressing $CD4^+$ T-cells in vitro.[49]

It is, however, likely that FIV also uses alternate cellular receptors, in agreement with the differential receptor usage by HIV strains.[57] Consistent with this, a number of CXCR4+ cell lines are resistant to infection by primary isolates of FIV, and viral infection of T-cells is not consistently inhibited by natural ligands for CXCR4.[68,69] In addition, RANTES, the natural ligand for CCR5, inhibits FIV infection of feline PBMC and antibodies against CCR5 or CCR3 reduce FIV-dependent fusion of human PBMC.[68,70] Feline cell surface molecules can also function as coreceptors for HIV and SIV infection, as HIV-2 and SIV can infect feline cells expressing the human CD4 molecule.[71] These results suggest a shared chemokine receptor usage between primate and feline lentiviruses and a close evolutionary link of entry mechanisms between FIV and HIV. In addition to chemokine receptors, cell surface heparans have been shown to contribute to the envelope glycoprotein binding of cell culture adapted strains of FIV,[69] similar to that described for HIV.[72] FIV envelope binding to primary T-cells that cannot be inhibited by RANTES, SDF-1α, macrophage inflammatory protein 1∃, or heparan also suggests the usage of additional cellular receptor(s) for some FIV strains.[69]

Antibody and complement binding has been shown to enhance susceptibility of B-cells for HIV infection.[73] An indirect antibody and complement-dependent mechanism of viral entry into B-cells might also occur in FIV+ cats. This is supported by the fact that proviral burden in B-cells increases dramatically as the anti-FIV antibody response develops and would explain why B-cells are not susceptible to FIV infection in vitro.[28,74]

Viral Clades

Phylogenetic analysis of the nucleotide sequences encompassing the gag and envelope variable (V) 3 to 5 regions revealed that the degree of divergence among FIV isolates from different places is a function of the geographical distance and segregation.[75–79] Five distinct clades (A-E) of FIV have been identified based on greater than 15 to 20% variability in envelope amino acid sequences. The majority of viruses identified to date belong to clade A or clade B.[75–77,80] While both virus groups are distributed worldwide, clade A viruses have been found to predominate in the western United States, northern Japan, Germany, and South Africa, whereas clade B viruses predominate in eastern Japan, Italy, Portugal, and the central and

eastern United States.[75,77] Clade B viruses are significantly more diverse than clade A and can be distinguished by three evolutionary subgroups.[77] Clade C viruses are common in northern Taiwan, Japan, and Vietnam.[77,81–83] Several clade D viruses have been characterized from Japan and Vietnam. Two Argentine strains represent subtype E viruses.[84]

It is currently not known whether infections with viruses from different clades or superinfections with viruses from different clades are associated with an accelerated disease progression. Experimental studies, however, suggest that FIV isolates from different clades differ in their pathogenicity, tissue cell tropism, and clinical disease.[85–87] In one study, peak plasma viremia and tissue provirus burden were significantly greater in cats infected with clade B virus (FIV-2542) compared to those infected with a clade A virus (FIV-PPR).[13] In another study comparing clade C (FIV-CPGammar) and clade A (FIV-Petaluma) viruses, greater plasma viremia, PBMC proviral burden, tissue viral burden, and histologic lesions were detected in the clade C virus infected cats.[88] Further studies are necessary to determine whether differences in the pathogenicity described for these clade B and clade C viruses can be extrapolated to other members of the clades.

4. IMMUNE RESPONSE TO FIV

The hallmarks of FIV infection, as with HIV infection, is the progressive loss of $CD4^+$ T-cell numbers and immune function. The immune defects in FIV+ cats are characterized by decreased responses to antigen and mitogen stimulation, reduced antibody responses to a variety of antigens, and increased susceptibility to secondary pathogen. As FIV induces a similar disease state as HIV, despite using an alternative cellular receptor and having a broader cell tropism, understanding the immunopathogenesis of FIV is of prime importance to elucidate the mechanism(s) of $CD4^+$ T-cell depletion and the pathogenesis of lentivirus-induced immunodeficiency.

Humoral Immune Response

B-cell numbers do not differ significantly from normal in either naturally or experimentally FIV+ cats.[17,27,89,90] Most naturally and experimentally infected cats develop a polyclonal gammopathy (mainly IgG) directed against nonviral proteins that can be detected as early as 6 weeks post-infection.[89,91,92] The hypergammaglobulinemia correlates histologically with the activation of the B-cell compartment in lymphoid organs.[25]

Antibodies directed against FIV can be detected in serum of experimentally infected animals by ELISA or western blot as early as 2 weeks

postinfection and typically persist throughout the course of the disease.[12,93] Antibody responses against Gag and Env usually develop at approximately the same time and tend to remain high throughout the lifespan of the infected animal.[94,95] Using recombinant, partially overlapping peptides, immunodominant domains have been localized within the surface and transmembrane glycoproteins of FIV. Within the surface glycoprotein, an immunodominant region has been identified within the V3 region, which shares both structural and functional homology with the V3 domain of HIV.[96–99] Other immunodominant domains have been localized to the carboxy terminus of the surface and transmembrane glycoprotein, as well as to at least four different epitopes on the Gag protein.[100–102] In addition, the ectodomain of the FIV transmembrane glycoprotein contains an immunodominant domain, also termed principal immunodominant domain, analogous to that found in other lentiviruses.[98] A pentapeptide comprised between two cysteine residues represents a linear B-cell epitope, which is recognized by 100% of sera from FIV+ cats.[100] Characterization of this domain revealed that chimeric FIV envelope protein containing the principal immunodominant domain of HIV is correctly processed and is capable of inducing syncytia in feline cells, whereas chimeric FIV envelope proteins containing the similar domain of maedi-visna virus (MVV) or equine infectious anaemia virus (EIAV) are nonfunctional. Computer modeling of this domain in FIV, HIV, MVV, and EIAV suggested a conservation of structure between FIV and HIV, whereas there is little similarity in structure between HIV and MVV or EIAV.[98]

Neutralizing Antibodies

The V3 region is an important target for virus-neutralizing antibodies. Synthetic peptides consistent with this region have been found to inhibit FIV-neutralizing activity of pooled immune cat sera, and cat sera raised against these peptides effectively neutralized FIV infectivity for Crandell feline kidney (CrFK) cells.[96] Residual neutralizing activity in the absorbed sera suggests that other nonlinear determinants in the viral envelope are also involved in neutralization. The involvement of the V4 and V5 regions of the surface glycoprotein in virus neutralization has been shown as single amino acid changes in these regions conferred resistance to virus neutralization.[103,104] These data are supported by findings that the V3 to V5 region of the FIV envelope interacts with the second extracellular loop of CXCR4 to mediate fusion.[64,65] It is of interest to note that neutralizing epitopes and also at least in part the immunodominant regions on the viral envelope coincide with genetically highly variable regions.

Although FIV infection induces a strong neutralizing antibody response, the role of these antibodies in controlling virus infection is controversial. The presence of neutralizing antibodies does not correlate with

virus clearance or disease progression in FIV$^+$ cats, as virus neutralizing titers are similar in asymptomatic and symptomatic FIV$^+$ cats.[105,106] Also, sera that exhibited elevated titers of highly efficient, cross-reactive neutralizing activity when assayed on CrFK cells exerted a much lower neutralizing effect when assayed in PBMC, thymocytes or T-cell lines.[103,106,107] In contrast, the activity of neutralizing antibodies in vivo has been shown in adoptive transfer experiments, as sera from vaccinated cats effectively protect cats against subsequent challenge with the homologous strain of FIV.[108,109] Cats without detectable antibody production are also reported to progress more rapidly to terminal immunodeficiency.[110,111]

Cellular Immunity to FIV

CD4$^+$ T-Cells

In healthy domestic cats, CD4$^+$ cells represent approximately 66% of the T-cells in circulation, whereas CD8$^+$ cells represent a much smaller proportion, yielding normal CD4:CD8 ratios between 1.5 and 3 (Figure 2A).[17,27,90] In cats, similar to the situation in humans, CD4$^+$ cells are the main source of IL-2,[112] which explains the "help" that CD8$^+$ cells must receive from CD4$^+$ cells in mounting an immune response. CD4$^+$ T-helper function is manifested by IL-2-promoted expansion and differentiation of natural killer (NK) cells and antigen-specific CD8$^+$ cells into effector cells. As such, antigen-specific CD4$^+$ T-cell responses are essential for controlling viral infection, including HIV and FIV infections.

The rapid expansion of activated CD8$^+$ cells during the acute phase of HIV and FIV infection suggests that in the initial immune response, CD4$^+$ cells are able to provide the necessary T-cell help. Studies suggest, however, that even at the acute stage of infection HIV-1 specific CD4$^+$ immune responses are not sustained as there is a relative absence of CD4$^+$ cell proliferative responses to HIV antigens despite normal CD4$^+$ cell responses to other antigens.[113–115] During the chronic phase of infection, HIV-specific CD4$^+$ T-cell responses in the peripheral blood of HIV$^+$ individuals, in particular those to the envelope, are also often difficult to detect.[116] In patients undergoing continuous highly active antiretroviral therapy (HAART) reconstitution of functional antigen-specific CD4$^+$ T-cell responses to HIV and several other antigens has been observed.[117–119] The restoration of antigen-specific CD4$^+$ T-cell responses in patients undergoing HAART, however, does not always correlate with a significant decrease in plasma viremia.[118]

Activation of CD4$^+$ T-cells has been documented in both FIV and HIV infection as CD4$^+$ cells show an upregulated expression of B7.1 and B7.2 costimulatory molecules.[120–123] Additional evidence of chronic and pro-

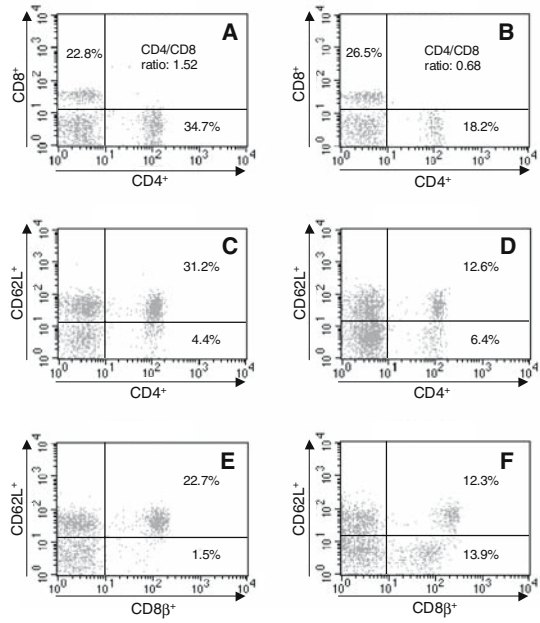

FIGURE 2. FIV infection results in decreased $CD4^+/CD8^+$ cell ratios and activation of T-cell subsets. Flow cytometric analysis of feline $CD4^+$ and $CD8^+$ cells in the peripheral blood derived from control (A, C, E) and FIV^+ cats (B, D, F) are shown. In uninfected animals, the number of $CD4^+$ cells exceeds the number of $CD8^+$ cells (A), whereas in FIV^+ cats the decreased $CD4^+$ cell numbers result in an inverted CD4/CD8 cell ratio (B). Numbers in the upper and lower quadrants represent percentage of positive cells of the total $CD45^+$ lymphocyte population of representative blood samples. Two-color flow cytometric analysis reveals a loss of CD62L expression on $CD4^+$ and $CD8^+$ cells in FIV^+ cats (D, F) as compared to control animals (C, E). Note that the loss of CD62L expression on $CD8^+$ cells is restricted to the $CD8\alpha^+\beta^{low}$ cell population. Numbers in the upper and lower right quadrants represent percentage of positive cells of representative blood samples. The increase in the percentage of $CD4^+CD62L^-$ as well as $CD8^+CD62L^-$ cells progresses throughout the course of FIV infection.

gressive T-cell activation in FIV^+ cats and HIV^+ patients comes from studies investigating the expression of L-selectin (CD62L), a surface marker that is lost on activated T-cells. Analysis of CD62L expression on $CD4^+$ cells in FIV^+ cats demonstrated that with time after infection the percentage of $CD4^+CD62L^-$ cells increase such that the $CD4^+CD62L^-$ cell population represents about 80% of the total $CD4^+$ cells in long-term (>7 years) infected cats (Figure 2C and D).[41] In other words, 80% of the circulating $CD4^+$ as well as $CD8^+$ T-cells (Figure 2E and F) in the blood of chronic, long-term FIV^+ cats have an activation phenotype. A similar phenotype change has been reported for $CD4^+$ cells of HIV^+ patients.[124,125]

$CD8^+$ T-Cells

FIV and HIV infection results in increased total CD8 cell counts in the peripheral blood, which can usually be detected early after infection, remain high throughout the long asymptomatic phase of infection, and decrease only late in the infection prior to the development of AIDS-like disease symptoms.[27,89,126] In both infections, the CD8 lymphocytosis correlates with the emergence of $CD8^+$ cells that provide antiviral immunity through cytotoxic and virus-suppressive mechanisms.[38,40,41,127–129]

Cytotoxic T Lymphocytes (CTL). CTL responses in the peripheral blood of FIV^+ cats can be detected as early as 2 weeks postinfection,[130] which is similar to the very early detection of virus-specific CTL activity in SIV^+ macaques.[131] Early detection of CTL activities corresponds with a reduction in viremia and transition to the asymptomatic phase.[128,130] In chronically FIV^+ cats, CTL responses against Gag, Pol, and Env epitopes are also detectable in the lymph nodes and spleen.[128,129,132] As the lymph node is a known site of virus sequestration and replication following HIV infection, and CTL responses in HIV^+ patients are associated with the maintenance of a low viral burden in the asymptomatic phase of long-term progressors,[133] the demonstration of CTL at these sites in FIV^+ cats may reflect the attempts of the host immune system to control virus replication.[134] In support of this, vaccine-induced protection against FIV challenge has been associated with CTL responses directed against the Env protein.[135]

Noncytolytic Antiviral Activity. In addition to the virus-specific CTL responses, FIV and HIV infection induces a population of nonantigen specific $CD8^+$ effector cells that suppress virus replication in $CD4^+$ cells by a noncytotoxic, contact-dependent, non-MHC restricted mechanism.[38–41] $CD8^+$ effector cells that mediate immunity through cytolytic or noncytolytic suppressor mechanisms can be divided by differential expression of surface markers. Early detection of noncytolytic $CD8^+$ effector cells in FIV^+ cats is associated with reduced expression of the CD8 β chain and increased expression of MHC class II molecules.[14,37,40,41] As reported for HIV^+ patients,[125] FIV-induced $CD8\alpha^+\beta^{lo}$ cells are also characterized by a progressive downregulation of CD62L (Figure 2E, F) resulting in more than 70% CD62L negative cells of the total $CD8\beta^+$ population in the peripheral blood of long-term (>7 years) infected cats.[41] The appearance of these $CD8\alpha^+\beta^{lo}$ cells correlates with the acute stage of infection and the rapid decline in viremia.[38,41,136] In the case of FIV infection, activated $CD8\alpha^+\beta^{lo}CD62L^-$ cells possess strong anti-FIV activity[40,41] and are capable of controlling FIV expression in vivo and in ex vivo cultured PBMC, suggesting that they may play a major role in maintaining the low plasma viremia in cats during the asymptomatic stage of infection.[40,137,138] Further phenotypic characterization of the $CD8^+$ cell population in FIV^+ cats revealed a profound and sustained loss of the naïve $CD8\alpha^+\beta^{hi}CD62L^+CD44^{lo}CD49d^{lo}CD18^{lo}$ phenotype

with a concurrent expansion of an $CD8\alpha^{+}\beta^{lo}CD62L^{-}CD44^{hi}CD49d^{hi}CD18^{hi}$ activation phenotype in the circulation.[41] Similar to FIV infection, in patients with long-term HIV infection, activated $CD8^{+}CD11a^{hi}$ cells, including a major subpopulation of $CD8^{+}CD62L^{-}CD11a^{hi}$ cells, represent approximately 80% of the total $CD8^{+}$ T-cells in the circulation.[124,125] The loss of CD62L and increase in the adhesion molecule (CD44) and integrin (CD49d, CD18) on the surface of $CD8^{+}$ cells is indicative of T-cell activation and therefore suggests that naïve CD8 cells are largely replaced by activated $CD8^{+}$ cells during the course of FIV and HIV infection.

Additional evidence of chronic and progressive $CD8^{+}$ T-cell activation in HIV^{+} patients and FIV^{+} cats comes from the analysis of B7 costimulatory molecules and CTLA4 expression on these cells. Similar to what is seen on $CD4^{+}$ cells, the $CD8^{+}B7^{+}$ phenotype represents approximately 80% of the total $CD8^{+}$ cells in long-term (>7 years) infected cats, and B7 expression has been shown to be largely confined to the $CD8\alpha^{+}\beta^{lo}$ cell population.[122] The role of these activated $CD8^{+}$ T-cells in controlling FIV or HIV infection in vivo is unknown, although Bucci et al.[136] has shown by $CD8^{+}$ cell depletion studies that they have a potent suppressive activity on FIV replication in $CD4^{+}$ cells cultured ex vivo.

5. IMMUNOPATHOGENESIS OF FIV INFECTION

The decline in the number of $CD4^{+}$ T-cells and the inversion of the $CD4^{+}/CD8^{+}$ cell ratio is seen in naturally and experimentally FIV^{+} cats regardless of the route of infection (Figure 2A and B).[12,27,36,110,136] The onset of $CD4^{+}$ T-cell depletion usually coincides with the peak plasma viremia during the acute stage of infection,[139] and after a partial rebound progresses throughout the asymptomatic and symptomatic phases of the infection. Several mechanisms of $CD4^{+}$ cell loss have been proposed. FIV is lytic for $CD4^{+}$ cells in vitro, suggesting that this may occur in vivo as well. Immune-mediated destruction of infected cells may also occur. $CD8^{+}$ CTL and NK cells capable of lysing virus-infected cells have been demonstrated in both FIV and HIV infection.[38,128,129] Due to the high viral load during the acute phase of infection, lytic infections of target cells might at this stage of infection contribute predominantly to the $CD4^{+}$ cell decline. However, as the infection progresses, the percentage of infected cells among the total lymphocyte population is relatively low, indicating that mechanisms other than lysis of infected cells may be involved in the continued $CD4^{+}$ T-cell loss. Whatever the mechanism(s) responsible for $CD4^{+}$ T-cell loss with disease progression, increased secondary infection is a manifestation of this immune dysfunction.

The types of secondary infections seen in HIV and FIV infections are those that are controlled by a functional cell-mediated immune (CMI)

response. While the development of these secondary infections can be attributed to the decline in $CD4^+$ cell numbers and the resulting decrease in cytokines (IL-2, IFN-γ) required for a successful CMI response, numerous studies indicate that the immune dysfunction develops before a significant decrease in $CD4^+$ cell numbers. Clerici et al.[5] described a sequential loss of immune functions in HIV infections beginning with an inability to respond to recall antigens and progressing to lack of response to human leukocyte antigen (HLA) alloantigens and finally to phytohemagglutinin (PHA) stimulation. These defects occurred in many HIV^+ individuals prior to $CD4^+$ cell loss. Similar immune dysfunctions have been described in FIV infection.[17,140] In our laboratory we developed a *Toxoplasma gondii*-FIV coinfection model that allowed us to examine FIV-induced dysfunctions in the CMI response. Normal cats challenged with *T. gondii* develop a transient chorioretinitis that resolves 3 to 4 weeks post-challenge.[141,142] However, as early as 16 weeks after infection with FIV, FIV^+ cats challenged with *T. gondii* develop severe interstitial pneumonia resulting in 50% to 75% mortality.[141,143] This suggests that severe immune dysfunction develops early after lentivirus infection. Several mechanisms responsible for this immune dysfunction have been proposed including cytokine deregulation,[144] defective or altered antigen presentation,[145,146] and inappropriate activation of immune regulatory cells.[147] The remainder of this chapter will address these proposed mechanisms of HIV/FIV immunopathogenesis.

Cytokine Alterations

There have been numerous studies evaluating cytokine levels in FIV infections, suggesting that the perturbations in cytokine production observed in the human immune system following HIV infection are paralleled in FIV^+ cats. Clerici et al.[5] reported that PBMC from HIV^+ individuals had decreased proliferation and IL-2 production in response to recall antigens as compared to seronegative controls. This nonresponsiveness has important consequences, as IL-2 has been shown to determine both the magnitude of a primary cellular immune response, as well as the development of memory cells. In contrast to many other cytokines, IL-2 is not redundant with regards to its role in the generation of a normal immune response and other cytokines or surface ligands cannot substitute for IL-2. In contrast to the lack of IL-2, serum levels of tumor necrosis factor-alpha (TNF-α) are found elevated in all AIDS patients, and approximately 50% of those with ARC, but none in the asymptomatic phase of HIV infection.[148] Lawrence et al.[140] reported similar results (decreased IL-2 and increased TNF-α levels) in FIV^+ cats early after infection. Elevated levels of IL-6 have been reported in the serum of both HIV^+ patients[149,150] and FIV^+ cats,[140] and

both infections have been shown to stimulate production of IL-6 by macrophages.[53,151] In FIV$^+$ cats, peak production of the proinflammatory cytokines IL-6, IL-1, and TNF-α by PBMC was found to coincide with periods of depressed immune responses as evidenced by a concurrent depressed responsiveness of PBMC to mitogen stimulation and the presence of clinical signs.[152] With the division of CD4$^+$ cells into two subsets based on cytokine production, Clerici and Shearer[144] speculated that HIV infection affects these subsets differently, causing a switch from a T-helper-1 (TH1; IL-2 and IFN-γ producers) to a T-helper-2 (TH2; IL-4, IL-5, IL-6, and IL-10 producers) type response to specific antigens. However, further studies did not support a clear TH1 to TH2 switch. Elevated levels of IL-10 and IFN-γ in PBMC and lymph nodes of HIV$^+$ patients have been described by Graziosi et al.[153] and Than et al.[154] The latter also reported increased expression of TNF-α and decreased IL-12 mRNA, whereas IL-2 and IL-4 mRNA were undetectable in both patients and controls. Others have also described decreased IL-12 p40 and p35 synthesis in HIV$^+$ patients.[155] However, decreases in IFN-γ production have also been reported in HIV$^+$ individuals,[156,157] while IL-4 has been reported to increase.[157,158] It is not surprising that the precise changes in cytokine expression leading to HIV-associated immune deficiency, and how such changes may impact immune responses to other antigens, remain controversial. The heterogeneity of the patient populations, including virus burden, disease progression, and exposure to other pathogens, makes meaningful comparisons difficult. Some of these issues have been addressed in FIV$^+$ cats.

FIV infection has provided a powerful animal model to examine lentivirus-induced cytokine dysfunctions, allowing for analysis of constitutive cytokine levels prior to and after FIV infection, as wells as cytokine responses following challenge with secondary pathogens. Using an FIV-*T. gondii* coinfection model, we were able to analyze constitutive cytokine production by lymph node cells from normal and FIV$^+$ cats before and after challenge with *T. gondii*.[159,160] FIV infection caused a marked increase in constitutive IFN-γ, TNF-α, and IL-10 mRNA expression in the lymph nodes during the acute stage infection. Challenge of control cats with *T. gondii* induced an increase in IL-2, IFN-γ, and IL-12 (p40) in lymph nodes, indicative of a normal CMI response, whereas IFN-γ and IL-10, but not IL-2 or IL-12, were increased in lymph nodes of FIV-*T. gondii* coinfected cats. Dean and colleagues[161,162] reported similar findings using an FIV-*Listeria monocytogenes* coinfection model. In both cases, prior to secondary challenge, the levels of IL-10 with respect to IL-12 in the FIV$^+$ cats were greatly elevated compared to negative cats. These data suggest that FIV-induced immunodeficiency may be derived from a failure to generate an IL-12 dependent response, in part due to elevated levels of IL-10, a cytokine known to suppress IL-12 production by dendritic cells.

Altered Antigen Presentation

A number of studies have examined the possibility that the $CD4^+$ T-cell immunodeficiency in HIV^+ patients is the result of inadequate or inappropriate antigen presentation. Defective antigen presentation can lead to T-cell anergy, which is characterized by decreased or no IL-2 production following T-cell receptor (TCR) stimulation. Studies have shown that anergy is more easily induced in IL-2/IFN-γ secreting $CD4^+$ T-cells than in IL-4 secreting $CD4^+$ T-cells.[163,164] The unresponsiveness of $CD4^+$ T-cells in HIV infections resembles anergy, and it has been suggested that it is the result of defective antigen presentation by antigen presenting cells (APC).[145,146] Mechanisms proposed for defective APC function include loss of dendritic cells due to direct virus infection,[165] decreased expression of accessory molecules such as MHC class II or B7 on APC,[145,165,166] and decreased cytokine or cytokine-dependent signaling.[145]

Inadequate APC costimulation is consistent with the observation that T-cells from asymptomatic HIV^+ patients and FIV^+ cats fail to produce IL-2 and proliferate after antigenic stimulation. The lack of IL-2 in the stimulated cells results in anergy and progression to activation-induced programmed cell death or apoptosis,[167,168] leading to the speculation that loss of T-cell function and subsequent immune deficiency in HIV^+ patients and FIV^+ cats is the result of anergy/apoptosis. This speculation is supported by the findings of abnormally high frequencies of apoptotic cells in the lymph nodes of HIV^+ individuals and FIV^+ cats.[169–172]

T-Cell-Mediated Immunopathology

Recent data suggest that the $CD4^+$ T-cell immune hyporesponsiveness in FIV^+ and HIV^+ patients may be unrelated to APC but result from unique immunosuppressive properties of virus activated T-cells. These potentially immunosuppressive T-cells fall into two categories: the activated $B7^+CTLA4^+$ T-cells that are part of the normal T-helper cell immunoregulatory response; and the $CD4^+CD25^+$ T-regulatory cells, a distinct population of $CD4^+$ T-cells with negative immunoregulatory properties.

$B7^+CTLA4^+$ T-Cell-Induced Anergy and Apoptosis

There is recent evidence that HIV and FIV infections may cause immunopathology by disrupting the normal process of activation-induced programmed cell death (apoptosis) that controls the magnitude and duration of the T-cell immune response. Apoptosis regulates the cellular repertoire throughout the life of an organism and is initiated by engagement of specific cell surface receptors with their ligands. The best described examples of receptor interactions that transduce intracellular signals mediating

cell death are between proteins that belong to the TNF family (e.g., FasL with Fas, TNF-α with TNF-α receptor, Apo2L with TRAIL), and interactions between the B7 costimulatory molecules and CTL antigen 4 (CTLA4). Apoptosis induced by proteins of the TNF family induce caspase-mediated cell death in many tissues, whereas the B7-CTLA4 apoptotic pathway induces T-lineage-specific cell death via the suppression of IL-2, resulting in antigen-specific anergy or clonal deletion of previously activated T-cells.[173,174] The inability of $CD4^+$ cells from HIV^+ individuals and FIV^+ cats to produce IL-2 and proliferate in response to recall antigens suggests that the increased apoptosis could be due to B7-CTLA4 interactions.

B7.1 and B7.2 costimulation receptors are normally found on professional APCs, such as dendritic cells, activated monocytes, and B-cells.[175] These molecules interact with CD28 and CTLA4 on T-cells, providing necessary second signals to either stimulate IL-2 synthesis (B7-CD28) thus promoting an immune response or suppression of IL-2 production (B7-CTLA4) and terminating the immune response. CD28 is constitutively expressed on T-cells, whereas CTLA4 is expressed only after T-cells have been activated, 2 to 3 days after peptide-TCR engagement. As the binding avidity of the B7 molecules for CTLA4 is 50–100 times greater than for CD28, negative signaling would dominate over activation on cells expressing both CD28 and CTLA4. While B7.1 and B7.2 are normally found on professional APCs, a number of studies have demonstrated that these molecules are upregulated on activated T-cells in vitro[176,177] and in vivo in situations of chronic antigenic stimulation such as autoimmune disease[178,179] and HIV infection.[121] Our laboratory has recently reported an increase in the percentage of $CD4^+$ and $CD8^+$ T-cells that express B7.1 and B7.2 molecules in FIV^+ cats compared to uninfected cats.[122] The numbers of B7 positive $CD4^+$ and $CD8^+$ T-cells increase with disease progression such that the majority of T-cells (≥75%) in the lymph nodes express B7 molecules in long-term (>7 years) infected cats. Interestingly, the majority of $B7^+CD4^+$ and $B7^+CD8^+$ T-cells in FIV^+ cats also express CTLA4, and we have been able to correlate the expression of these molecules to spontaneous T-cell apoptosis in vitro[180] and in LN and PBMC of FIV^+ cats in vivo.[122] Further, in a more recent study, we have found that T-cell apoptosis in the PBMC of HIV^+ individuals is associated with $CD4^+$ T-cells coexpressing B7 and CTLA4.[123] As ligation of CTLA4 by B7 transduces a signal for downregulation of IL-2 and induction of anergy and apoptosis, we speculated that persistent antigenic stimulation, as seen in HIV and FIV infection, chronically induces $B7.1/2^+CTLA4^+$ T-cells capable of T-T interactions that result in T-cell anergy and apoptosis. Such a mechanism could explain the progressive loss of T-cell immune function and increase in lymph node T-cell apoptosis, which is the hallmark of FIV and HIV infections.

This speculation is supported by the observation that T-cells activated in vitro and shown to express B7 are capable of antigen presentation and

can engage the TCR on other activated T-cells in an antigen specific manner.[181] However, in contrast to professional APC, evidence suggests that T-T cell antigen presentation does not costimulate IL-2 production and proliferation, but primes T-cells for anergy and apoptosis.[182,183] Why T-T cell antigen presentation favors anergy over costimulation is not known. Greenfield et al.[183] demonstrated that while B7.1 expressed on T-cells was capable of binding both CD28 and CTLA4 on costimulated T-cells, B7.2 was capable of binding CTLA4 but not CD28, leading to T-cell anergy. As we have also documented increased expression of CTLA4 on T-cells from FIV$^+$ cats, one can envision activated B7 expressing CD4$^+$ cells being able to present antigen to other T-cells expressing CTLA4, resulting in anergy or apoptosis. The proposed mechanism for T-T antigen presentation leading to anergy and apoptosis is illustrated in Figure 3. As not all anergic cells progress to apoptosis, such a mechanism could explain the decreased

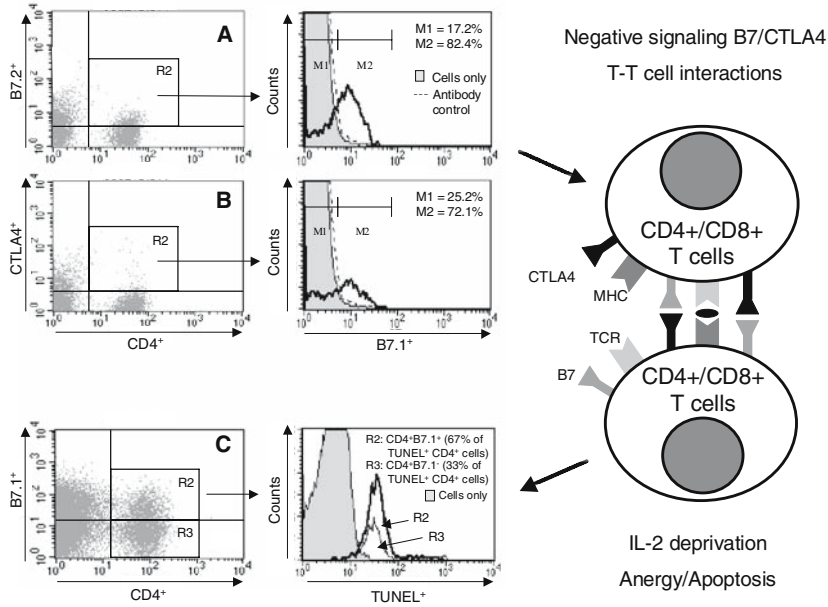

FIGURE 3. Coexpression of B7 and CTLA4 molecules on feline CD4$^+$ and CD8$^+$ cells correlates with the induction of apoptosis. Three-color flow cytometric analysis demonstrating colocalization of B7.1 costimulatory molecules on B7.2 and CTLA4 expressing CD4$^+$ cells. The majority of B7.2$^+$ (A) or CTLA4$^+$ (B) CD4$^+$ cells coexpress B7.1. The phenotype of B7.1$^+$B7.2$^+$CTLA4$^+$ CD4$^+$ cells enables T-T-cell interactions mediated by B7 receptors and CTLA4 capable of bidirectional signaling of anergy or apoptosis. Consistent with this, three-color flow cytometric analysis of apoptotic cells reveals that the majority of TUNEL$^+$ cells are among the B7.1$^+$ phenotype of CD4$^+$ cells (C). Similar up-regulation of costimulatory molecules, coexpression and correlation between B7$^+$CTLA4$^+$ cell phenotypes and apoptosis are also present on CD8$^+$ cells (not shown).

IL-2 production and immune unresponsiveness prior to $CD4^+$ cell loss. Moreover, the T-T cell interactions may not need to be antigen specific in their immunosuppressive function. Bienzle et al.[184] reported that HIV-specific CTL exerted a cytotoxic effect against uninfected, activated $CD4^+$ cells but not against nonactivated $CD4^+$ cells. This effector function was restricted to $CD8^+$ cells from HIV^+ individuals, not antigen specific or MHC-restricted, and killed uninfected $CD4^+$ cells via apoptosis as opposed to a normal CTL-mediated perforin mechanism. The studies of Bienzle et al.[184] are consistent with the reported loss of immunity to primary *T. gondii* infection in the FIV-*T. gondii* coinfection model. The large numbers of FIV-induced $B7^+CD8^+$ or $B7^+CD4^+$ effector cells in the lymph node could interact with activated $CD4^+$ cells responding to *T. gondii* antigens, resulting in anergy and eventual apoptosis and the inability to develop an immune response to *T. gondii*. While there is compelling evidence for a marked expansion of a unique population(s) of phenotypically and functionally activated $CD4^+$ and $CD8^+$ with disease progression in FIV/HIV infection, there is no direct evidence as yet that it plays a role in inhibiting protective T-cell responses to FIV/HIV antigen or to other unrelated pathogens. The most interesting data to date comes from reports by Vahlenkamp et al.[180] and Bull et al.[185] that anti-B7.1 or IL-2 treatment of cultured purified $CD4^+$ cells from FIV^+ cats significantly inhibits spontaneous apoptosis, as would be expected if apoptosis were the result of $B7^+$-$CTLA4^+$ mediated T-T interactions.

$CD4^+$ T Regulatory Cell-Mediated Anergy and Apoptosis

Studies in a number of experimental models have firmly established the existence of a "professional" $CD4^+$ regulatory T-cell population (Treg) that performs an important antiautoimmunity function by inhibiting the activation of autoreactive T-cells, thereby maintaining peripheral self-tolerance.[47,48] Evidence has accumulated that $CD4^+CD25^+$ Treg cells can also suppress immune responses against infectious agents, such as bacteria, fungi, viruses, and intracellular parasites,[186,187] and play a central role in regulating the T-helper-dependent immune response. The defining feature of $CD4^+CD25^+$ Treg cells in both rodents and humans is their ability, when activated through their TCR, to inhibit proliferation and induce apoptosis of other activated $CD4^+$ or $CD8^+$ T-cells in vitro. This in vitro suppression is mediated through a cell contact-dependent mechanism that transduces a signal for transcriptional down-regulation of IL-2, resulting in anergy and apoptosis.[188,189] Although activation of $CD4^+CD25^+$ cells is, with some exceptions (e.g., lipolysaccharide [LPS] and IL-2), antigen-specific, once activated, they suppress $CD4^+$ and $CD8^+$ T-cell responses in an antigen nonspecific manner.[188,190]

Phenotypic analysis of $CD4^+$ Treg cells reveal that, in addition to CD25, Treg cells express CTLA4, and when activated B7.1 and B7.2.[191,192] This

surface phenotype is reminiscent of the activated $CD4^+B7^+CTLA4^+$ cells that we have described in FIV^+ cats and HIV^+ patients that we propose may play a role in $CD4^+$ immune hyporesponsiveness in these lentivirus infections (Figure 3). $CD4^+CD25^+$ T-cells in normal and FIV^+ cats display the salient characteristics of $CD4^+$ Treg cells in humans and rodents,[47,48,187,193,194] as they constitute about 5 to 10% of the peripheral T-cell population, fail to proliferate in response to Con A stimulation (Figure 4B), and are relatively resistant to activation-induced programmed cell death (Figure 4C).[49,195] Phenotypic analysis of $CD4^+CD25^+$ T-cells from FIV^+ cats revealed that these cells also express B7.1, B7.2, and CTLA4,[195] suggesting that they were activated in vivo. Consistent with this interpretation, freshly isolated $CD4^+CD25^+$ T-cells from FIV^+ cats, but not normal cats, are able to suppress the proliferative response of Con A-stimulated autologous $CD4^+CD25^-$ T-cells (Figure 4E). This suppression was contact-dependent and correlates with the downregulation of IL-2 production,[195] providing further support that the $CD4^+CD25^+$ T-cells in FIV^+ cats are activated Treg cells capable of anergy induction.

It is possible that they are FIV antigen specific and activated by chronic antigenemia. It is also possible that they are nonspecifically activated by molecules such as LPS that has been shown to occur in vitro with murine as well as feline $CD4^+CD25^+$ Treg cells. Why Treg cells would be activated in FIV infection is not clear. However, it is easy to imagine their potential role in the immune dysfunctions associated with FIV/HIV infections. One can speculate that chronic FIV antigen presentation, and in particular aberrant antigen presentation by $CD4^+B7^+MCHII^+$ T-cells may activate Treg cells to dampen protective $CD4^+$ T-helper cell-dependent immune responses to FIV antigens. This negative immunoregulatory property of active $CD4^+$ Treg cells is well documented in a number of infectious diseases.[186,187,196,197] The activated $CD4^+CD25^+B7^+$ cells are found predominantly in the lymph node as opposed to the blood of FIV^+ animals, and it is the lymph node where we would expect immune responses to antigen to occur and where we would expect Treg cells to exert their suppressor function. It will be of much interest to further explore the possible role of FIV-activated $CD4^+CD25^+$ Treg cells on the $CD4^+$ T-helper-cell immunodeficiency in infected cats and determine if a similar mechanism contributes to human AIDS.

In addition to their potential role in immunosuppression in HIV and FIV infection, $CD4^+CD25^+$ Treg cells may contribute to the long-term virus persistence in these infections in vivo. In vitro studies have shown that activated $CD4^+$ cells expressing CD25 can be productively infected with HIV in vitro, whereas highly purified resting $CD4^+CD25^-$ cells were resistant to HIV infection.[46] The $CD4^+CD25^+$ cells reported to support HIV replication, however, were not identified as Treg cells. Recent studies in our laboratory using the FIV infection model demonstrated that both $CD4^+CD25^+$ and

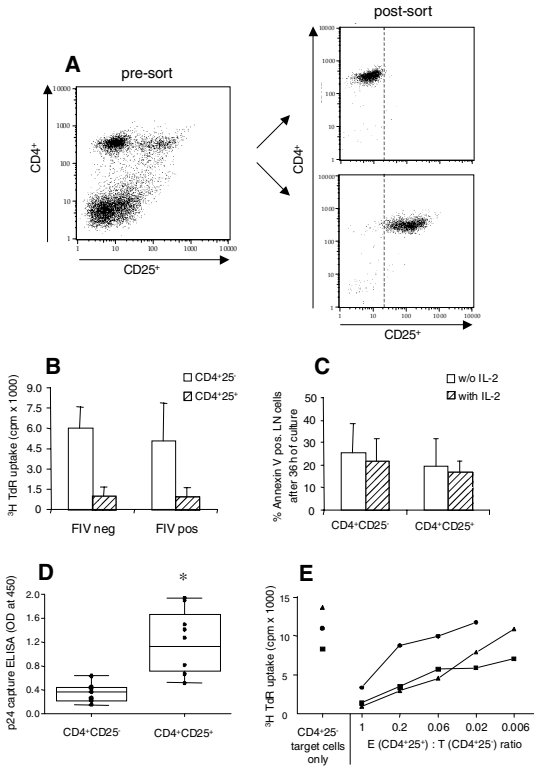

FIGURE 4. Feline $CD4^+CD25^+$ LN cells are anergic and productively infected with FIV. FIV infection results in activation of immunosuppressive function(s) of $CD4^+CD25^+$ T-regulatory cells in vivo. Lymph node (LN) cells of FIV^+ and control cats were sorted (MoFlo® cell sorter, Dako-Cytomation) into $CD4^+CD25^+$ and $CD4^+CD25^-$ cells (>97% purity) (A). Analysis of the purified cell subsets using ^{3}H TdR incorporation assays reveals that in contrast to $CD4^+CD25^-$ cells, $CD4^+CD25^+$ cells derived from FIV^+ and FIV^- cats are unresponsive to 2-day mitogen stimulation (Con A; 5 μg/ml) (B). As the percentage of Annexin V positive cells among the $CD4^+CD25^-$ and $CD4^+CD25^+$ cells cultured for 36h with or without IL-2 (100IU/ml) does not differ significantly (C), failure of $CD4^+CD25^+$ cells to proliferate upon mitogen stimulation indicates a state of nonresponsiveness/anergy. Coculture of FCD4E cells with in vitro unstimulated $CD4^+CD25^-$ and $CD4^+CD25^+$ cells from FIV^+ cats reveals that FIV can be rescued preferentially ($p < 0.05$) in cocultures with $CD4^+CD25^+$ cells (D), as determined by the presence of FIV-p24 in the culture supernatant. The results (mean ± standard deviation) of at least four independent experiments (B, C, D) are shown. Viral replication and the lack of increased apoptosis in these cells support the hypothesis that the partially activated, anergic $CD4^+CD25^+$ cells might serve as persistent reservoir of FIV replication in vivo. The phenotype of anergic $CD4^+CD25^+$ cells is reminiscent of immunosuppressive $CD4^+$ Treg cells. To test the ability of $CD4^+CD25^+$ cells in FIV^+ cats to suppress the proliferation of stimulated autologous $CD4^+CD25^-$ cells, we performed coculture experiments using Con A (5 μg/ml) stimulated $CD4^+CD25^-$ cells as targets and $CD4^+CD25^+$ cells as effector cells. The ^{3}H TdR incorporation assays reveal that the proliferation of $CD4^+CD25^-$ cells is markedly inhibited by coculture with unstimulated $CD4^+CD25^+$ cells in a dose-dependent manner as shown for three individual FIV^+ cats (E). As experiments using similar $CD4^+$ cell subpopulations derived from FIV^- cats reveal only a minor inhibition of target cell proliferation, the experiments suggest that FIV infection results in the activation of immunosuppressive $CD4^+CD25^+$ Treg cells in vivo.

$CD4^+CD25^-$ cells are susceptible to FIV infection. In contrast to $CD4^+CD25^-$ cells, which become latently infected, $CD4^+CD25^+$ cells isolated from FIV^+ cats or infected in vitro harbor a productive FIV infection (Figure 4D), yet have functional characteristics of $CD4^+CD25^+$ Treg cells.[49] Thus, $CD4^+CD25^+$ Treg cells could potentially provide a long-term, stable reservoir for FIV, as well as HIV replication. Further experiments will address the role of cellular transcription factors known to regulate FIV replication in the activation state and function of $CD4^+CD25^-$ and anergic $CD4^+CD25^+$ cells. Figure 5 depicts the proposed mechanism of $CD4^+CD25^+$ Treg

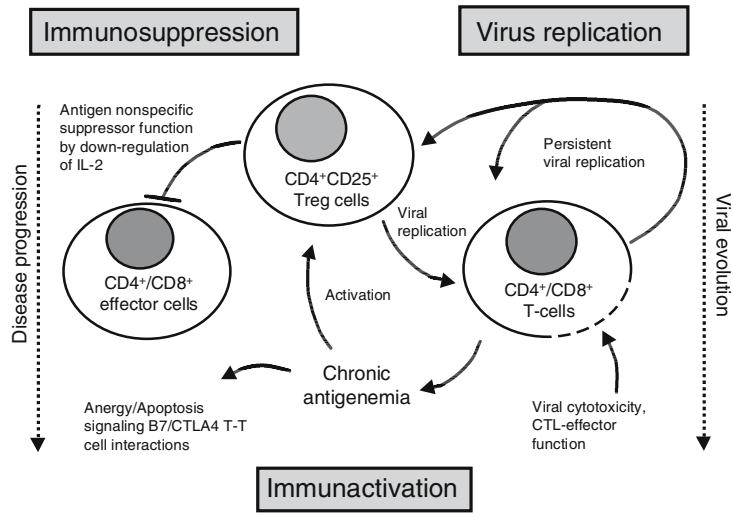

FIGURE 5. Potential role of immunosuppressive $CD4^+CD25^+$ Treg cells in the immunopathogenesis of FIV: A model for immunosuppression and persistent viral replication. FIV infection is characterized by the hallmarks of (i) immunosuppression, (ii) viral persistence and concurrent with disease progression, (iii) immunactivation. Studies in our laboratory show that $CD4^+CD25^+$ Treg cells harbor transcription factors required for lentiviral replication. Despite the fact that these cells are productively infected they are not apoptosis-prone in FIV+ cats, suggesting that these partially activated yet anergic (probably long-lived) regulatory cells might serve as a reservoir for persistent active viral replication in vivo. Infection of activated $CD4^+$ and $CD8^+$ cells supports viral replication throughout the course of the infection. Chronic viremia in infected animals could activate $CD4^+CD25^+$ Treg cells. Activated Treg cells suppress the proliferation of $CD4^+$ and $CD8^+$ effector cells in an antigen nonspecific manner by a transcriptional down-regulation of IL-2. Activated $CD4^+CD25^+$ Treg cells might therefore be responsible for the decreased responsiveness of T-cells to immune stimulation and the lack of IL-2 production detected early during the asymptomatic stage of FIV infection. With disease progression and increasing viral mutational diversity, chronic antigenic stimulation results in a continued activation of $CD4^+$ and $CD8^+$ cells as evidenced by the downregulated expression of adhesion (CD62L) molecules and by the abnormal expression of B7 costimulatory molecules, as well as their ligand CTLA4 leading to anergy/apoptosis, signaling T-T-cell interactions.

cell activation induced immunosuppression of T-cell-mediated immune responses and the possible role of $CD4^+CD25^+$ Treg cells in persistent viral replication.

6. CONCLUSION

FIV is a lentivirus of cats with a pathogenesis and clinical disease pattern that parallels HIV infection.[8,22,25,134] Cats develop an acute infection syndrome, including low-grade fever and transient generalized lymphadenopathy, followed by a long asymptomatic period in which the $CD4^+/CD8^+$ ratio declines due to an early increase in $CD8^+$ and a progressive decrease in $CD4^+$ cells.[28,37] This asymptomatic period is followed by the development of a variety of disorders, many of which mimic AIDS in humans.[8,22] Similar to HIV, the acute stage FIV infection is characterized by a high plasma viremia followed by a marked decline and persistent low-level viremia during the long asymptomatic stage of infection.[24]

The relevance of FIV as a model for HIV immunopathogenesis is underscored by the remarkable parallels in $CD4^+$ and $CD8^+$ phenotypic and functional changes during disease progression. Both infections are characterized by early and long-term changes in cytokine expression (e.g., elevation in IFN-γ and IL-10) and $CD4^+$ T-cell anergy and apoptosis. While the molecular mechanism(s) leading to immunodeficiency and AIDS is not known, recent new studies in the FIV animal model of AIDS suggest that anergy and/or clonal deletion of other antigen activated T-cells may be involved. Whatever, the mechanism(s) leading to $CD4^+$ immune dysfuntion in HIV^+ patients—and there have been many proposed—the FIV infection in cats has been and will continue to be an important animal model to study the immunopathogenesis of HIV infection. Based on data developed principally in the FIV model, one such scenario suggests that progressive deterioration of $CD4^+$ numbers and function characteristics of FIV/HIV infection could result from chronic anergic signaling mediated by virus activated T-cells expressing B7 molecules. More recent data developed in the FIV model support an argument that virus activated $CD4^+CD25^+$ Treg cells may abort protective T-helper-cell responses to antigens.

REFERENCES

1. N. C. Pedersen, E. Ho, M. L. Brown, and J. K. Yamamoto, Isolation of a T-lymphotropic virus from domestic cats with an immunodeficiency-like syndrome, *Science* 235, 790–793 (1987).
2. R. A. Olmsted, A. K. Barnes, J. K. Yamamoto, V. M. Hirsch, R. H. Purcell, and P. R. Johnson, Molecular cloning of feline immunodeficiency virus, *Proc. Natl. Acad. Sci.* 86, 2448–2452 (1989).

3. R. L. Talbott, E. E. Sparger, K. M. Lovelace, W. M. Fitch, N. C. Pedersen, P. A. Luciw, and J. H. Elder, Nucleotide sequence and genomic organization of feline immunodeficiency virus, *Proc. Natl. Acad. Sci.* 86, 5743–5747 (1989).
4. A. Fauci, A. Macher, D. Longo, H. Lane, A. Rook, H. Masur, and E. Gelmann, Acquired immunodeficiency syndrome: Epidemiologic, clinical, immunological, and therapuetic considerations, *Ann. Intern. Med.* 100, 92–106 (1984).
5. M. Clerici, N. Stocks, R. Zajac, R. Boswell, D. Lucey, C. Via, and G. Shearer, Detection of three distinct patterns of T helper cell dysfunction in asymptomatic, human immunodeficiency virus-seropositve patients, *J. Clin. Invest.* 84, 1892–1899 (1989).
6. J. L. Fahey, J. M. Taylor, R. Detels, B. Hofmann, R. Melmed, P. Nishanian, and J. V. Giorgi, The prognostic value of cellular and serologic markers in infection with human immunodeficiency virus type 1, *N. Engl. J. Med.* 322, 166–172 (1990).
7. B. F. Polk, R. Fox, R. Brookmeyer, S. Kanchanaraksa, R. Kaslow, B. Visscher, C. Rinaldo, and J. Phair, Predictors of the acquired immunodeficiency syndrome developing in a cohort of seropositive homosexual men, *N. Engl. J. Med.* 316, 61–66 (1987).
8. T. Ishida and I. Tomoda, Clinical staging of feline immunodeficiency virus infection, *Jpn. J. Vet. Sci.* 52(3), 645–648 (1990).
9. J. K. Yamamoto, H. Hansen, W. E. Ho, T. Y. Morishita, T. Okuda, T. R. Sawa, R. M. Nakamura, W. P. Kau, and N. C. Pedersen, Epidemiologic and clinical aspects of feline immunodeficiency virus infection in cats from the continental United States and Canada and possible mode of transmission, *J. Am. Vet. Med. Assoc.* 194, 213–220 (1989).
10. S. W. Dow, M. L. Poss, and E. A. Hoover, Feline immunodeficiency virus: A neurotropic lentivirus, *J. Acquir. Immune Defic. Syndr.* 3, 658–668 (1990).
11. H. Jordan, J. Howard, W. Tompkins, and S. Kennedy-Stoskopf, Detection of feline immunodeficiency virus in semen from seropositive domestic cats (Felis catus), *J. Virol.* 69(11), 7328–7333 (1995).
12. T. W. Vahlenkamp, H. F. Egberink, M. J. van Eijk, A. M. Slotboom-Kamphorst, E. J. Verschoor, M. C. Horzinek, and A. de Ronde, Competitive reverse transcription-polymerase chain reaction for quantitation of feline immunodeficiency virus, *J. Virol. Meth.* 52(3), 335–346 (1995).
13. M. J. Burkhard, C. K. Mathiason, K. O'Halloran, and E. A. Hoover, Kinetics of early FIV infection in cats exposed via the vaginal versus intravenous route, *AIDS Res. Hum. Retroviruses* 18(3), 217–226 (2002).
14. R. Lehmann, B. von Beust, E. Niederer, M. A. Condrau, W. Fierz, A. Aubert, C. D. Ackley, M. D. Cooper, M. B. Tompkins, and H. Lutz, Immunization-induced decrease of the CD4+:CD8+ ratio in cats experimentally infected with feline immunodeficiency virus, *Vet. Immunol. Immunopathol.* 35(1–2), 199–214 (1992).
15. S. Dawson, N. R. Smyth, M. Bennett, R. M. Gaskell, C. M. McCracken, A. Brown, and C. J. Gaskell, Effect of primary-stage feline immunodeficiency virus infection on subsequent feline calicivirus vaccination and challenge in cats, *AIDS* 5(6), 747–750 (1991).
16. A. Taniguchi, T. Ishida, T. Washizu, and I. Tomoda, Humoral immune response to T-cell dependent and independent antigens in cats infected with feline immunodeficiency virus, *J. Vet. Med. Sci.* 53(2), 333–335 (1991).
17. M. Torten, M. Franchini, J. E. Barlough, J. W. George, E. Mozes, H. Lutz, and N. C. Pedersen, Progressive immune dysfunction in cats experimentally infected with feline immunodeficiency virus, *J. Virol.* 65(5), 2225–2230 (1991).
18. O. Prospero-Garcia, N. Herold, T. R. Phillips, J. H. Elder, F. E. Bloom, and S. J. Henriksen, Sleep patterns are disturbed in cats infected with feline immunodeficiency virus, *Proc. Natl. Acad. Sci. USA* 91(26), 12947–12951 (1994).
19. T. R. Phillips, O. Prospero-Garcia, D. L. Puaoi, D. L. Lerner, H. S. Fox, R. A. Olmsted, F. E. Bloom, S. J. Henriksen, and J. H. Elder, Neurological abnormalities associated with feline immunodeficiency virus infection, *J. Gen. Virol.* 75(Pt 5), 979–987 (1994).

20. T. R. Phillips, O. Prospero-Garcia, D. W. Wheeler, P. C. Wagaman, D. L. Lerner, H. S. Fox, L. R. Whalen, F. E. Bloom, J. H. Elder, and S. J. Henriksen, Neurologic dysfunctions caused by a molecular clone of feline immunodeficiency virus, FIV-PPR, *J. Neurovirol.* 2(6), 388–396 (1996).
21. S. M. Astrin, E. Schattner, J. Laurence, R. I. Lebman, and C. Rodriguez-Alfageme, Does HIV infection of B lymphocytes initiate AIDS lymphoma? Detection by PCR of viral sequences in lymphoma tissue, *Curr. Top. Microbiol. Immunol.* 182, 399–407 (1992).
22. N. C. Pedersen and J. E. Barlough, Clinical overview of feline immunodeficiency virus, *J. Am. Vet. Med. Assoc.* 199(10), 1298–1305 (1991).
23. Y. Goto, Y. Nishimura, K. Baba, T. Mizuno, Y. Endo, K. Masuda, K. Ohno, and H. Tsujimoto, Association of plasma viral RNA load with prognosis in cats naturally infected with feline immunodeficiency virus, *J. Virol.* 76(19), 10079–10083 (2002).
24. N. C. Pedersen, Feline immunodeficiency virus, in: *The retroviruses, vol. 2* edited by J. A. Levy (Plenum Press, New York, 1993).
25. M. Bendinelli, M. Pistello, S. Lombardi, A. Poli, C. Garzelli, D. Matteucci, L. Ceccherini-Nelli, G. Malvaldi, and F. Tozzini, Feline immunodeficiency virus: an interesting model for AIDS studies and an important cat pathogen, *Clin. Microbiol. Rev.* 8(1), 87–112 (1995).
26. T. Ishida, A. Taniguchi, S. Matsumura, T. Washizu, and I. Tomoda, Long-term clinical observations on feline immunodeficiency virus infected asymptomatic carriers, *Vet. Immunol. Immunopathol.* 35(1–2), 15–22 (1992).
27. R. V. English, P. Nelson, C. M. Johnson, M. Nasisse, W. A. Tompkins, and M. B. Tompkins, Development of clinical disease in cats experimentally infected with feline immunodeficiency virus, *J. Infec. Dis.* 170, 543–552 (1994).
28. R. V. English, C. M. Johnson, D. H. Gebhard, and M. B. Tompkins, In vivo lymphocyte tropism of feline immunodeficiency virus, *J. Virol.* 67(9), 5175–5186 (1993).
29. M. J. Endres, P. R. Clapham, M. Marsh, M. Ahuja, J. D. Turner, A. McKnight, J. F. Thomas, B. Stoebenau-Haggarty, S. Choe, P. J. Vance, T. N. Wells, C. A. Power, S. S. Sutterwala, R. W. Doms, N. R. Landau, and J. A. Hoxie, CD4-independent infection by HIV-2 is mediated by fusin/CXCR4, *Cell* 87(4), 745–756 (1996).
30. D. Brunner and N. C. Pedersen, Infection of peritoneal macrophages in vitro and in vivo with feline immunodeficiency virus, *J. Virol.* 63(12), 5483–5488 (1989).
31. W. C. Brown, L. Bissey, D. S. Logan, N. C. Pedersen, J. H. Elder, and E. W. Collisson, Feline immunodeficiency virus infects both CD4+ and CD8+ T-lymphocytes, *J. Virol.* 65, 3359–3364 (1991).
32. A. Beebe, T. Gluckstern, J. George, N. Pedersen, and S. Dandekar, Detection of feline immunodeficiency virus infection in bone marrow of cats, *Vet. Immunol. Immunopathol.* 35, 37–49 (1992).
33. S. Lombardi, A. Poli, C. Massi, F. Abramo, L. Zaccaro, A. Bazzichi, G. Malvaldi, M. Bendinelli, and C. Garzelli, Detection of feline immunodeficiency virus p24 antigen and p24-specific antibodies by monoclonal antibody-based assays, *J. Virol. Methods* 46(3), 287–301 (1994).
34. A. Fauci, S. Schnittman, G. Poli, S. Koenig, and G. Pantaleo, Immunopathogenic mechanisms of human immunodeficiency virus (HIV) infection, *Ann. Intern. Med.* 114, 678–693 (1991).
35. H. Spiegel, H. Herbst, G. Niedobitek, H. D. Foss, and H. Stein, Follicular dendritic cells are a major reservoir for human immunodeficiency virus type 1 in lymphoid tissues facilitating infection of CD4+ T-helper cells, *Am. J. Pathol.* 140(1), 15–22 (1992).
36. G. A. Dean, G. H. Reubel, P. F. Boore, and N. C. Pedersen, Proviral burden and infection kinetics of feline immunodeficiency virus in lymphocyte subsets of blood and lymph node, *J. Virol.* 70, 5165–5169 (1996).

37. B. J. Willett, M. J. Hosie, J. J. Callanan, J. C. Neil, and O. Jarrett, Infection with feline immunodeficiency virus is followed by the rapid expansion of a CD8+ lymphocyte subset, *Immunology* 78(1), 1–6 (1993).
38. C. E. Mackewicz, L. C. Yang, J. D. Lifson, and J. A. Levy, Non-cytolytic CD8 T-cell anti-HIV responses in primary HIV-1 infection, *Lancet* 344(8938), 1671–1673 (1994).
39. J. Levy, C. Mackewicz, and E. Barker, Controlling HIV pathogenesis: the role of the non-cytotoxic anti-HIV response of CD8+ cells, *Immunol. Today* 17, 217–224 (1996).
40. J. Bucci, D. Gebhard, T. Childers, R. English, M. Tompkins, and W. Tompkins, The CD8+ phenotype mediating antiviral activity in FIV-infected cats is characterized by reduced surface expression of the CD8 beta chain, *J. Inf. Dis.* 178, 968–977 (1998).
41. D. Gebhard, J. Dow, T. Childers, J. Alvelo, M. Tompkins, and W. Tompkins, Progressive expansion of an L-selectin-negative CD8 cell with anti-feline immunodeficiency virus (FIV) suppressor function in the circulation of FIV-infected cats, *J. Infect. Dis.* 180, 1503–1513 (1999).
42. O. Ramilo, K. D. Bell, J. W. Uhr, and E. S. Vitetta, Role of CD25+ and CD25-T-cells in acute HIV infection in vitro, *J. Immunol.* 150(11), 5202–5208 (1993).
43. J. Borvak, C. S. Chou, K. Bell, G. Van Dyke, H. Zola, O. Ramilo, and E. S. Vitetta, Expression of CD25 defines peripheral blood mononuclear cells with productive versus latent HIV infection, *J. Immunol.* 155(6), 3196–3204 (1995).
44. S. Schnittman, H. Lane, J. Greenhouse, J. Justement, M. Baseler, and A. Fauci, Preferential infection of CD4+ memory T-cells by human immunodeficiency virus type 1: Evidence for a role in the selective T-cell functional defects observed in infected individuals, *Proc. Natl. Acad. Sci. USA* 87, 6058–6062 (1990).
45. D. Willerford, M. J. Gale, R. Benveniste, E. Clark, and W. Gallatin, Simian immunodeficiency virus is restricted to a subset of blood CD4+ lymphocytes that includes memory cells, *J. Immunol.* 144, 3779–3783 (1990).
46. C. S. Chou, O. Ramilo, and E. S. Vitetta, Highly purified CD25- resting T-cells cannot be infected de novo with HIV-1, *Proc. Natl. Acad. Sci. USA* 94(4), 1361–1365 (1997).
47. K. J. Maloy and F. Powrie, Regulatory T-cells in the control of immune pathology, *Nat. Immunol.* 2(9), 816–822 (2001).
48. E. M. Shevach, CD4+ CD25+ suppressor T-cells: more questions than answers, *Nat. Rev. Immunol.* 2(6), 389–400 (2002).
49. A. Joshi, T. W. Vahlenkamp, H. Garg, W. A. Tompkins, and M. B. Tompkins, Preferential replication of FIV in activated CD4+CD25+ T-cells independent of cellular proliferation, *accepted for publication* (2004).
50. M. J. Hosie, B. J. Willett, T. H. Dunsford, O. Jarrett, and J. C. Neil, A monoclonal antibody which blocks infection with feline immunodeficiency virus identifies a possible non-CD4 receptor, *J. Virol.* 67(3), 1667–1671 (1993).
51. B. J. Willett and M. J. Hosie, The role of the chemokine receptor CXCR4 in infection with feline immunodeficiency virus, *Mol. Membr. Biol.* 16(1), 67–72 (1999).
52. T. W. Vahlenkamp, A. de Ronde, N. N. Schuurman, A. L. van Vliet, J. van Drunen, M. C. Horzinek, and H. F. Egberink, Envelope gene sequences encoding variable regions 3 and 4 are involved in macrophage tropism of feline immunodeficiency virus, *J. Gen. Virol.* 80(Pt 10), 2639–2646 (1999).
53. J. W. Ritchey, J. K. Levy, S. K. Bliss, W. A. Tompkins, and M. B. Tompkins, Constitutive expression of types 1 and 2 cytokines by alveolar macrophages from feline immunodeficiency virus-infected cats, *Vet. Immunol. Immunopathol.* 79(1–2), 83–100 (2001).
54. J. Norimine, T. Miyazawa, Y. Kawaguchi, K. Tomonaga, Y. S. Shin, T. Toyosaki, M. Kohmoto, M. Niikura, Y. Tohya, and T. Mikami, Feline CD4 molecules expressed on feline non-lymphoid cell lines are not enough for productive infection of highly lymphotropic feline immunodeficiency virus isolates, *Arch. Virol.* 130(1–2), 171–178 (1993).
55. S. Wain-Hobson, HIV. One on one meets two, *Nature* 384(6605), 117–118 (1996).

56. P. R. Clapham and A. McKnight, Cell surface receptors, virus entry and tropism of primate lentiviruses, *J. Gen. Virol.* 83(Pt 8), 1809–1829 (2002).
57. H. Deng, R. Liu, W. Ellmeier, S. Choe, D. Unutmaz, M. Burkhart, P. Di Marzio, S. Marmon, R. E. Sutton, C. M. Hill, C. B. Davis, S. C. Peiper, T. J. Schall, D. R. Littman, and N. R. Landau, Identification of a major co-receptor for primary isolates of HIV-1, *Nature* 381(6584), 661–666 (1996).
58. T. Dragic, V. Litwin, G. P. Allaway, S. R. Martin, Y. Huang, K. A. Nagashima, C. Cayanan, P. J. Maddon, R. A. Koup, J. P. Moore, and W. A. Paxton, HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC-CKR-5, *Nature* 381(6584), 667–673 (1996).
59. Y. Feng, C. C. Broder, P. E. Kennedy, and E. A. Berger, HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor, *Science* 272(5263), 872–877 (1996).
60. C. C. Bleul, L. Wu, J. A. Hoxie, T. A. Springer, and C. R. Mackay, The HIV coreceptors CXCR4 and CCR5 are differentially expressed and regulated on human T lymphocytes, *Proc. Natl. Acad. Sci. USA* 94(5), 1925–1930 (1997).
61. B. J. Willett, M. J. Hosie, J. C. Neil, J. D. Turner, and J. A. Hoxie, Common mechanism of infection by lentiviruses [letter], *Nature* 385(6617), 587 (1997).
62. J. Richardson, G. Pancino, R. Merat, T. Leste-Lasserre, A. Moraillon, J. Schneider-Mergener, M. Alizon, P. Sonigo, and N. Heveker, Shared usage of the chemokine receptor CXCR4 by primary and laboratory-adapted strains of feline immunodeficiency virus, *J. Virol.* 73(5), 3661–3671 (1999).
63. H. Garg, A. Joshi, and W. A. Tompkins, Membrane expressed feline immunodeficiency virus env glycoprotein mediates apoptosis in activated PBMC by a fusion dependent mechanism, *accepted for publication* (2004).
64. B. J. Willett, K. Adema, N. Heveker, A. Brelot, L. Picard, M. Alizon, J. D. Turner, J. A. Hoxie, S. Peiper, J. C. Neil, and M. J. Hosie, The second extracellular loop of CXCR4 determines its function as a receptor for feline immunodeficiency virus, *J. Virol.* 72(8), 6475–6481 (1998).
65. J. B. Johnston and C. Power, Feline immunodeficiency virus xenoinfection: the role of chemokine receptors and envelope diversity, *J. Virol.* 76(8), 3626–3636 (2002).
66. H. F. Egberink, E. De Clercq, A. L. Van Vliet, J. Balzarini, G. J. Bridger, G. Henson, M. C. Horzinek, and D. Schols, Bicyclams, selective antagonists of the human chemokine receptor CXCR4, potently inhibit feline immunodeficiency virus replication, *J. Virol.* 73(8), 6346–6352 (1999).
67. B. J. Willett, C. A. Cannon, and M. J. Hosie, Expression of CXCR4 on feline peripheral blood mononuclear cells: effect of feline immunodeficiency virus infection, *J. Virol.* 77(1), 709–712 (2003).
68. D. L. Lerner and J. H. Elder, Expanded host cell tropism and cytopathic properties of feline immunodeficiency virus strain PPR subsequent to passage through interleukin-2-independent T-cells, *J. Virol.* 74(4), 1854–1863 (2000).
69. A. de Parseval and J. H. Elder, Binding of recombinant feline immunodeficiency virus surface glycoprotein to feline cells: role of CXCR4, cell-surface heparans, and an unidentified non-CXCR4 receptor, *J. Virol.* 75(10), 4528–4539 (2001).
70. J. Johnston and C. Power, Productive infection of human peripheral blood mononuclear cells by feline immunodeficiency virus: implications for vector development, *J. Virol.* 73(3), 2491–2498 (1999).
71. A. McKnight, P. R. Clapham, and R. A. Weiss, HIV-2 and SIV infection of nonprimate cell lines expressing human CD4: restrictions to replication at distinct stages, *Virology* 201(1), 8–18 (1994).
72. Y. Ohshiro, T. Murakami, K. Matsuda, K. Nishioka, K. Yoshida, and N. Yamamoto, Role of cell surface glycosaminoglycans of human T-cells in human immunodeficiency virus type-1 (HIV-1) infection, *Microbiol. Immunol.* 40(11), 827–835 (1996).

73. G. S. Gras and D. Dormont, Antibody-dependent and antibody-independent complement-mediated enhancement of human immunodeficiency virus type 1 infection in a human, Epstein-Barr virus-transformed B-lymphocytic cell line, *J. Virol.* 65(1), 541–545 (1991).
74. A. M. Beebe, N. Dua, T. G. Faith, P. F. Moore, N. C. Pedersen, and S. Dandekar, Primary stage of feline immunodeficiency virus infection: viral dissemination and cellular targets, *J. Virol.* 68(5), 3080–3091 (1994).
75. D. L. Sodora, E. G. Shpaer, B. E. Kitchell, S. W. Dow, E. A. Hoover, and J. I. Mullins, Identification of three feline immunodeficiency virus (FIV) env gene subtypes and comparison of the FIV and human immunodeficiency virus type 1 evolutionary patterns, *J. Virol.* 68(4), 2230–2238 (1994).
76. S. Kakinuma, K. Motokawa, T. Hohdatsu, J. K. Yamamoto, H. Koyama, and H. Hashimoto, Nucleotide sequence of feline immunodeficiency virus: classification of Japanese isolates into two subtypes which are distinct from non-Japanese subtypes, *J. Virol.* 69(6), 3639–3646 (1995).
77. M. H. Bachmann, C. Mathiason-Dubard, G. H. Learn, A. G. Rodrigo, D. L. Sodora, P. Mazzetti, E. A. Hoover, and J. I. Mullins, Genetic diversity of feline immunodeficiency virus: dual infection, recombination, and distinct evolutionary rates among envelope sequence clades, *J. Virol.* 71(6), 4241–4253 (1997).
78. M. Pistello, G. Cammarota, E. Nicoletti, D. Matteucci, M. Curcio, D. Del Mauro, and M. Bendinelli, Analysis of the genetic diversity and phylogenetic relationship of Italian isolates of feline immunodeficiency virus indicates a high prevalence and heterogeneity of subtype B, *J. Gen. Virol.* 78 (Pt 9), 2247–2257 (1997).
79. T. Hohdatsu, K. Motokawa, M. Usami, M. Amioka, S. Okada, and H. Koyama, Genetic subtyping and epidemiological study of feline immunodeficiency virus by nested polymerase chain reaction-restriction fragment length polymorphism analysis of the gag gene, *J. Virol. Meth.* 70(1), 107–111 (1998).
80. A. Steinrigl and D. Klein, Phylogenetic analysis of feline immunodeficiency virus in Central Europe: a prerequisite for vaccination and molecular diagnostics, *J. Gen. Virol.* 84(Pt 5), 1301–1307 (2003).
81. G. Inada, T. Miyazawa, Y. Inoshima, M. Kohmoto, Y. Ikeda, C. H. Liu, J. A. Lin, T. F. Kuo, and T. Mikami, Phylogenetic analysis of feline immunodeficiency virus isolated from cats in Taiwan, *Arch. Virol.* 142(7), 1459–1467 (1997).
82. M. Uema, Y. Ikeda, T. Miyazawa, J. A. Lin, M. C. Chen, T. F. Kuo, C. Kai, T. Mikami, and E. Takahashi, Feline immunodeficiency virus subtype C is prevalent in northern part of Taiwan, *J. Vet. Med. Sci.* 61(2), 197–199 (1999).
83. K. Nakamura, Y. Suzuki, K. Ikeo, Y. Ikeda, E. Sato, N. T. Nguyen, T. Gojobori, T. Mikami, and T. Miyazawa, Phylogenetic analysis of Vietnamese isolates of feline immunodeficiency virus: genetic diversity of subtype C, *Arch. Virol.* 148(4), 783–791 (2003).
84. M. R. Pecoraro, K. Tomonaga, T. Miyazawa, Y. Kawaguchi, S. Sugita, Y. Tohya, C. Kai, M. E. Etcheverrigaray, and T. Mikami, Genetic diversity of Argentine isolates of feline immunodeficiency virus, *J. Gen. Virol.* 77(Pt 9), 2031–2035 (1996).
85. G. Pancino, S. Castelot, and P. Sonigo, Differences in feline immunodeficiency virus host cell range correlate with envelope fusogenic properties, *Virology* 206(2), 796–806 (1995).
86. T. Hohdatsu, H. Hirabayashi, K. Motokawa, and H. Koyama, Comparative study of the cell tropism of feline immunodeficiency virus isolates of subtypes A, B and D classified on the basis of the env gene V3-V5 sequence, *J. Gen. Virol.* 77(Pt 1), 93–100 (1996).
87. M. de Monte, H. Nonnenmacher, N. Brignon, M. Ullmann, and J. P. Martin, A multivariate statistical analysis to follow the course of disease after infection of cats with different strains of the feline immunodeficiency virus (FIV), *J. Virol. Meth.* 103(2), 157–170 (2002).

88. N. C. Pedersen, C. M. Leutenegger, J. Woo, and J. Higgins, Virulence differences between two field isolates of feline immunodeficiency virus (FIV-APetaluma and FIV-CPGammar) in young adult specific pathogen free cats, *Vet. Immunol. Immunopathol.* 79(1–2), 53–67 (2001).
89. C. D. Ackley, J. K. Yamamoto, N. Levy, N. C. Pedersen, and M. D. Cooper, Immunologic abnormalities in pathogen-free cats experimentally infected with feline immunodeficiency virus, *J. Virol.* 64(11), 5652–5655 (1990).
90. C. Novotney, R. English, J. Housman, M. Davidson, M. Nasisse, C. R. Jeng, W. Davis, and M. Tompkins, Lymphocyte population changes in cats naturally infected with feline immunodeficiency virus, *AIDS* 4, 1213–1218 (1990).
91. A. Poli, C. Giannelli, M. Pistello, L. Zaccaro, D. Pieracci, M. Bendinelli, and G. Malvaldi, Detection of salivary antibodies in cats infected with feline immunodeficiency virus, *J. Clin. Microbiol.* 30(8), 2038–2041 (1992).
92. J. N. Flynn, C. A. Cannon, C. E. Lawrence, and O. Jarrett, Polyclonal B-cell activation in cats infected with feline immunodeficiency virus, *Immunology* 81(4), 626–630 (1994).
93. J. K. Yamamoto, E. Sparger, E. W. Ho, P. R. Andersen, T. P. O'Connor, C. P. Mandell, L. Lowenstine, R. Munn, and N. C. Pedersen, Pathogenesis of experimentally induced feline immunodeficiency virus infection in cats, *Am. J. Vet. Res.* 49(8), 1246–1258 (1988).
94. H. F. Egberink, C. E. Keldermans, M. J. Koolen, and M. C. Horzinek, Humoral immune response to feline immunodeficiency virus in cats with experimentally induced and naturally acquired infections, *Am. J. Vet. Res.* 53(7), 1133–1138 (1992).
95. S. Lombardi, M. Bendinelli, and C. Garzelli, Detection of B epitopes on the p24 gag protein of feline immunodeficiency virus by monoclonal antibodies, *AIDS Res. Hum. Retroviruses* 9(2), 141–146 (1993).
96. S. Lombardi, C. Garzelli, C. La Rosa, L. Zaccaro, S. Specter, G. Malvaldi, F. Tozzini, F. Esposito, and M. Bendinelli, Identification of a linear neutralization site within the third variable region of the feline immunodeficiency virus envelope, *J. Virol.* 67(8), 4742–4749 (1993).
97. G. Pancino, I. Fossati, C. Chappey, S. Castelot, B. Hurtrel, A. Moraillon, D. Klatzmann, and P. Sonigo, Structure and variations of feline immunodeficiency virus envelope glycoproteins, *Virology* 192(2), 659–662 (1993).
98. G. Pancino, L. Camoin, and P. Sonigo, Structural analysis of the principal immunodominant domain of the feline immunodeficiency virus transmembrane glycoprotein, *J. Virol.* 69(4), 2110–2118 (1995).
99. T. W. Vahlenkamp, E. J. Verschoor, N. N. Schuurman, A. L. van Vliet, M. C. Horzinek, H. F. Egberink, and A. de Ronde, A single amino acid substitution in the transmembrane envelope glycoprotein of feline immunodeficiency virus alters cellular tropism, *J. Virol.* 71(9), 7132–7135 (1997).
100. A. Avrameas, A. D. Strosberg, A. Moraillon, P. Sonigo, and G. Pancino, Serological diagnosis of feline immunodeficiency virus infection based on synthetic peptides from Env glycoproteins, *Res. Virol.* 144(3), 209–218 (1993).
101. G. Pancino, C. Chappey, W. Saurin, and P. Sonigo, B epitopes and selection pressures in feline immunodeficiency virus envelope glycoproteins, *J. Virol.* 67(2), 664–672 (1993).
102. A. de Ronde, J. G. Stam, P. Boers, H. Langedijk, R. Meloen, W. Hesselink, L. C. Keldermans, A. van Vliet, E. J. Verschoor, M. C. Horzinek, and et al., Antibody response in cats to the envelope proteins of feline immunodeficiency virus: identification of an immunodominant neutralization domain, *Virology* 198(1), 257–264 (1994).
103. K. H. Siebelink, G. F. Rimmelzwaan, M. L. Bosch, R. H. Meloen, and A. D. Osterhaus, A single amino acid substitution in hypervariable region 5 of the envelope protein of feline immunodeficiency virus allows escape from virus neutralization, *J. Virol.* 67(4), 2202–2208 (1993).

104. K. H. Siebelink, M. L. Bosch, G. F. Rimmelzwaan, R. H. Meloen, and A. D. Osterhaus, Two different mutations in the envelope protein of feline immunodeficiency virus allow the virus to escape from neutralization by feline serum antibodies, *Vet. Immunol. Immunopathol.* 46(1–2), 51–59 (1995).
105. J. K. Yamamoto, T. Okuda, C. D. Ackley, H. Louie, E. Pembroke, H. Zochlinski, R. J. Munn, and M. B. Gardner, Experimental vaccine protection against feline immunodeficiency virus, *AIDS Res. Hum. Retroviruses* 7(11), 911–922 (1991).
106. F. Tozzini, D. Matteucci, P. Bandecchi, F. Baldinotti, K. Siebelink, A. Osterhaus, and M. Bendinelli, Neutralizing antibodies in cats infected with feline immunodeficiency virus, *J. Clin. Microbiol.* 31(6), 1626–1629 (1993).
107. F. Baldinotti, D. Matteucci, P. Mazzetti, C. Giannelli, P. Bandecchi, F. Tozzini, and M. Bendinelli, Serum neutralization of feline immunodeficiency virus is markedly dependent on passage history of the virus and host system, *J. Virol.* 68(7), 4572–4579 (1994).
108. T. Hohdatsu, R. Pu, B. A. Torres, S. Trujillo, M. B. Gardner, and J. K. Yamamoto, Passive antibody protection of cats against feline immunodeficiency virus infection, *J. Virol.* 67(4), 2344–2348 (1993).
109. M. J. Hosie, R. Osborne, J. K. Yamamoto, J. C. Neil, and O. Jarrett, Protection against homologous but not heterologous challenge induced by inactivated feline immunodeficiency virus vaccines, *J. Virol.* 69(2), 1253–1255 (1995).
110. M. Burkhard, L. Obert, N. L. O, L. Diehl, and E. Hoover, Mucosal transmission of cell-associated and cell-free feline immunodeficiency virus, *AIDS Res. Hum. Retroviruses* 13, 347–355 (1997).
111. L. J. Diehl, C. K. Mathiason-Dubard, L. L. O'Neil, and E. A. Hoover, Plasma viral RNA load predicts disease progression in accelerated feline immunodeficiency virus infection, *J. Virol.* 70(4), 2503–2507 (1996).
112. Y. Liang, L. C. Hudson, J. K. Levy, J. W. Ritchey, W. A. F. Tompkins, and M. B. Tompkins, T-cells over expressing IFN-γ and IL10 are found in both the thymus and secondary lymphoid tissues of FIV-infected cats, *J. Infect. Dis.* 181, 564–575 (2000).
113. M. M. Reddy, A. Englard, D. Brown, E. Buimovici-Klien, and M. H. Grieco, Lymphoproliferative responses to human immunodeficiency virus antigen in asymptomatic intravenous drug abusers and in patients with lymphadenopathy or AIDS, *J. Infect. Dis.* 156(2), 374–376 (1987).
114. B. Wahren, L. Morfeldt-Mansson, G. Biberfeld, L. Moberg, A. Sonnerborg, P. Ljungman, A. Werner, R. Kurth, R. Gallo, and D. Bolognesi, Characteristics of the specific cell-mediated immune response in human immunodeficiency virus infection, *J. Virol.* 61(6), 2017–2023 (1987).
115. H. C. Kelker, M. Seidlin, M. Vogler, and F. T. Valentine, Lymphocytes from some long-term seronegative heterosexual partners of HIV-infected individuals proliferate in response to HIV antigens, *AIDS Res. Hum. Retroviruses* 8(8), 1355–1359 (1992).
116. S. Cohen, M. Tuen, and C. E. Hioe, Propagation of CD4+ T-cells specific for HIV type 1 envelope gp120 from chronically HIV type 1-infected subjects, *AIDS Res. Hum. Retroviruses* 19(9), 793–806 (2003).
117. T. Wendland, H. Furrer, P. L. Vernazza, K. Frutig, A. Christen, L. Matter, R. Malinverni, and W. J. Pichler, HAART in HIV-infected patients: restoration of antigen-specific CD4 T-cell responses in vitro is correlated with CD4 memory T-cell reconstitution, whereas improvement in delayed type hypersensitivity is related to a decrease in viraemia, *AIDS* 13(14), 1857–1862 (1999).
118. M. Peruzzi, C. Azzari, L. Galli, A. Vierucci, and M. De Martino, Highly active antiretroviral therapy restores in vitro mitogen and antigen-specific T-lymphocyte responses in HIV-1 perinatally infected children despite virological failure, *Clin. Exp. Immunol.* 128(2), 365–371 (2002).

119. G. A. Hardy, N. Imami, A. K. Sullivan, A. Pires, C. T. Burton, M. R. Nelson, B. G. Gazzard, and F. M. Gotch, Reconstitution of CD4+ T-cell responses in HIV-1 infected individuals initiating highly active antiretroviral therapy (HAART) is associated with renewed interleukin-2 production and responsiveness, *Clin. Exp. Immunol.* 134(1), 98–106 (2003).
120. K. Wolthers, S. Otto, S. Lens, D. Kolbach, R. van Lier, F. Miedema, and L. Meyaard, Increased expression of CD80, CD86 and CD70 on T-cells from HIV-infected individuals upon activation in vitro: regulation by CD4+ T-cells, *Eur. J. Immunol.* 26, 1700–1706 (1996).
121. C. Kochli, T. Wendland, K. Frutig, R. Grunow, S. Merlin, and W. Pichler, CD80 and CD86 costimulatory molecules on circulating T-cells of HIV infected individuals, *Immunol. Lett.* 65, 197–201 (1999).
122. M. B. Tompkins, M. E. Bull, J. L. Dow, J. M. Ball, E. W. Collisson, B. J. Winslow, A. P. Phadke, T. W. Vahlenkamp, and W. A. Tompkins, Feline immunodeficiency virus infection is characterized by B7+CTLA4+ T-cell apoptosis, *J. Infect. Dis.* 185(8), 1077–1093 (2002).
123. M. E. Bull, W. A. Tompkins, P. Menezes, J. J. Eron, C. D. Pilcher, J. L. Schmitz, J. A. Frelinger, and M. B. Tompkins, HIV infection is characterized by CD4+B7+CTLA4+MHCII+ T-cell apoptosis: A model for CD4+ T-cell loss and immunodeficiency, *Ph.D. thesis, North Carolina State University* (2003).
124. R. L. Rabin, M. Roederer, Y. Maldonado, A. Petru, L. A. Herzenberg, and L. A. Herzenberg, Altered representation of naive and memory CD8 T-cell subsets in HIV-infected children, *J. Clin. Invest.* 95(5), 2054–2060 (1995).
125. M. Roederer, J. G. Dubs, M. T. Anderson, P. A. Raju, L. A. Herzenberg, and L. A. Herzenberg, CD8 naive T-cell counts decrease progressively in HIV-infected adults, *J. Clin. Invest.* 95(5), 2061–2066 (1995).
126. H. E. Prince and E. R. Jensen, Three-color cytofluorometric analysis of CD8 cell subsets in HIV-1 infection, *J. Acquir. Immune Defic. Syndr.* 4(12), 1227–1232 (1991).
127. P. Borrow, H. Lewicki, B. H. Hahn, G. M. Shaw, and M. B. Oldstone, Virus-specific CD8+ cytotoxic T-lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type 1 infection, *J. Virol.* 68(9), 6103–6110 (1994).
128. J. Flynn, J. Beatty, C. Cannon, E. Stephens, M. Hosie, J. Neil, and O. Jarrett, Involvement of gag- and env-specific cytotoxic T lymphocytes in protective immunity to feline immunodeficiency virus., *AIDS Res. Hum. Retroviruses* 11, 1107–1113 (1995).
129. J. N. Flynn, P. Keating, M. J. Hosie, M. Mackett, E. B. Stephens, J. A. Beatty, J. C. Neil, and O. Jarrett, Env-specific CTL predominate in cats protected from feline immunodeficiency virus infection by vaccination, *J. Immunol.* 157(8), 3658–3665 (1996).
130. J. Beatty, B. Willett, E. Gault, and O. Jarrett, A longitudinal study of feline immunodeficiency virus-specific cytotoxic T lymphocytes in experimentally infected cats, using antigen-specific induction, *J. Virol.* 70(9), 6199–6206 (1996).
131. Y. Yasutomi, K. A. Reimann, C. I. Lord, M. D. Miller, and N. L. Letvin, Simian immunodeficiency virus-specific CD8+ lymphocyte response in acutely infected rhesus monkeys, *J. Virol.* 67, 1707–1711 (1993).
132. W. Song, E. Collisson, P. Billingsley, and W. Brown, Induction of feline immunodeficiency virus-specific cytolytic T-cell responses from experimentally infected cats, *J. Virol.* 66(9), 5409–5417 (1992).
133. C. R. Rinaldo, Jr., L. A. Beltz, X. L. Huang, P. Gupta, Z. Fan, and D. J. Torpey, III, Anti-HIV type 1 cytotoxic T lymphocyte effector activity and disease progression in the first 8 years of HIV type 1 infection of homosexual men, *AIDS Res. Hum. Retroviruses* 11(4), 481–489 (1995).
134. B. Willett, N. Flynn, and M. Hosie, FIV infection of the domestic cat: an animal model for AIDS, *Immunol. Today* 18, 182 (1997).

135. M. J. Hosie and J. N. Flynn, Feline immunodeficiency virus vaccination: characterization of the immune correlates of protection, *J. Virol.* 70(11), 7561–7568 (1996).
136. J. Bucci, R. English, H. Jordan, T. Childers, M. Tompkins, and W. Tompkins, Mucosally transmitted feline immunodeficiency virus induces a CD8+ antiviral response that correlates with reduction of cell-associated virus, *J. Infect. Dis.* 177, 18–25 (1998).
137. P. C. Crawford, G. P. Papadi, J. K. Levy, N. A. Benson, A. Mergia, and C. M. Johnson, Tissue dynamics of CD8 lymphocytes that suppress viral replication in cats infected neonatally with feline immunodeficiency virus, *J. Infect. Dis.* 184(6), 671–681 (2001).
138. J. N. Flynn, S. Dunham, A. Mueller, C. Cannon, and O. Jarrett, Involvement of cytolytic and non-cytolytic T-cells in the control of feline immunodeficiency virus infection, *Vet. Immunol. Immunopathol.* 85(3–4), 159–170 (2002).
139. J. W. George, N. C. Pedersen, and J. Higgins, The effect of age on the course of experimental feline immunodeficiency virus infection in cats, *AIDS Res. Hum. Retroviruses* 9, 897–905 (1993).
140. C. Lawrence, J. Callanan, and O. Jarrett, Decreased mitogen responsiveness and elevated tumor necrosis factor production in cats shortly after feline immunodeficiency virus infection, *Vet. Immunol. Immunopathol.* 35, 51–59 (1992).
141. M. G. Davidson, J. Rottman, R. V. English, M. R. Lappin, and M. B. Tompkins, Feline immunodeficiency virus predisposes cats to acute generalized toxoplasmosis, *Am. J. Pathol.* 143, 1486–1497 (1993).
142. M. G. Davidson, M. R. Lappin, R. V. English, and M. B. Tompkins, A feline model of ocular toxoplasmosis, *Invest. Ophthalmol. Visual Sci.* 34(13), 3653–3660 (1993).
143. J. Yang, R. English, J. Ritchey, M. Davidson, T. Wasmoen, J. Levy, D. Gebhard, M. Tompkins, and W. Tompkins, Molecularly cloned feline immunodeficiency virus NCSU1 JSY3 induces immunodeficiency in specific-pathogen-free cats, *J. Virol.* 70(5), 3011–3017 (1996).
144. M. Clerici and G. M. Shearer, A T_H1 to T_H2 switch is a critical step in the etiology of HIV infection, *Immunol. Today* 14(3), 107–111 (1993).
145. L. Meyaard, H. Schuitemaker, and F. Miedema, T-cell dysfunction in HIV infection: anergy due to defective antigen-presenting cell function?, *Immunol. Today* 14(4), 161–164 (1993).
146. M. R. Helbert, J. L'Age-Stehr, and N. A. Mitchison, Antigen presentation, loss of immunological memory and AIDS, *Immunol. Today* 14(7), 340–344 (1993).
147. M. Ascher and H. Sheppard, AIDS as immune system activation. II. The panergic imnesia hypothesis, *J. Acquir. Immune Defic. Syndr.* 3, 177–191 (1990).
148. J. Lahdevirta, C. P. Maury, A. M. Teppo, and H. Repo, Elevated levels of circulating cachectin/tumor necrosis factor in patients with acquired immunodeficiency syndrome, *Am. J. Med.* 85(3), 289–291 (1988).
149. P. Gallo, K. Frei, C. Rordorf, J. Lazdins, B. Tavolato, and A. Fontana, Human immunodeficiency virus type 1 (HIV-1) infection of the central nervous system: an evaluation of cytokines in cerebrospinal fluid, *J. Neuroimmunol.* 23(2), 109–116 (1989).
150. E. C. Breen, A. R. Rezai, K. Nakajima, G. N. Beall, R. T. Mitsuyasu, T. Hirano, T. Kishimoto, and M. O. Martinez, Infection with HIV is associated with elevated IL-6 levels and production, *J. Immunol.* 144(2), 480–484 (1990).
151. L. Trentin, S. Garbisa, R. Zambello, C. Agostini, C. Caenazzo, C. Di Francesco, A. Cipriani, E. Francavilla, and G. Semenzato, Spontaneous production of interleukin-6 by alveolar macrophages from human immunodeficiency virus type 1-infected patients, *J. Infect. Dis.* 166(4), 731–737 (1992).
152. C. E. Lawrence, J. J. Callanan, B. J. Willett, and O. Jarrett, Cytokine production by cats infected with feline immunodeficiency virus: a longitudinal study, *Immunology* 85(4), 568–574 (1995).

153. C. Graziosi, G. Pantaleo, K. R. Gantt, J. P. Fortin, J. F. Demarest, O. J. Cohen, R. P. Sekaly, and A. S. Fauci, Lack of evidence for the dichotomy of TH1 and TH2 predominance in HIV-infected individuals, *Science* 265(5169), 248–252 (1994).
154. S. Than, R. Hu, N. Oyaizu, J. Romano, X. Wang, S. Sheikh, and S. Pahwa, Cytokine pattern in relation to disease progression in human immunodeficiency virus-infected children, *J. Infect. Dis.* 175(1), 47–56 (1997).
155. J. Chehimi, S. E. Starr, I. Frank, A. D'Andrea, X. Ma, R. R. MacGregor, J. Sennelier, and G. Trinchieri, Impaired interleukin 12 production in human immunodeficiency virus-infected patients, *J. Exp. Med.* 179(4), 1361–1366 (1994).
156. E. Maggi, D. Macchia, P. Parronchi, M. Mazzetti, A. Ravina, D. Milo, and S. Romagnani, Reduced production of interleukin 2 and interferon-gamma and enhanced helper activity for IgG synthesis by cloned CD4+ T-cells from patients with AIDS, *Eur. J. Immunol.* 17(12), 1685–1690 (1987).
157. L. Meroni, D. Trabattoni, C. Balotta, C. Riva, M. Clerici, and M. Galli, Evidence for type 2 cytokine production and lymphocyte activiation in early phases of HIV-1 infection, *AIDS* 10, 23–30 (1996).
158. M. Clerici, F. T. Hakim, D. J. Venzon, S. Blatt, C. W. Hendrix, T. A. Wynn, and G. M. Shearer, Changes in interleukin-2 and interleukin-4 production in asymptomatic, human immunodeficiency virus-seropositive individuals, *J. Clin. Invest.* 91(3), 759–765 (1993).
159. J. Levy, J. Ritchey, J. Rottman, M. Davidson, Y. Liang, H. Jordan, W. Tompkins, and M. Tompkins, Elevated interleukin-10-to-interleukin-12 ratio in feline immunodeficiency virus-infected cats predicts loss of type 1 immunity to Toxoplasma gondii, *J. Infect. Dis.* 178, 503–511 (1998).
160. J. K. Levy, Y. H. Liang, J. W. Ritchey, M. G. Davidson, W. A. Tompkins, and M. B. Tompkins, Failure of FIV-infected cats to control *T. gondii* challenge correlates with reduced IL2, IL6, and IL12 and elevated IL10 expression by lymph node T-cells, *Vet. Immunol. Immunopathol.* in press (2004).
161. G. A. Dean, J. A. Bernales, and N. C. Pedersen, Effect of feline immunodeficiency virus on cytokine response to Listeria monocytogenes in vivo, *Vet. Immunol. Immunopathol.* 65(2–4), 125–138 (1998).
162. N. C. Pedersen, G. A. Dean, J. Bernales, A. Sukura, and J. Higgins, Listeria monocytogenes and Serratia marcescens infections as models for Th1/Th2 immunity in laboratory cats, *Vet. Immunol. Immunopathol.* 63(1–2), 83–103 (1998).
163. T. M. Williams, K. R. Fox, and J. A. Kant, Interleukin-2: basic biology and therapeutic use, *Hematol. Pathol.* 5(2), 45–55 (1991).
164. D. De Wit, M. Van Mechelen, M. Ryelandt, A. C. Figueiredo, D. Abramowicz, M. Goldman, H. Bazin, J. Urbain, and O. Leo, The injection of deaggregated gamma globulins in adult mice induces antigen-specific unresponsiveness of T-helper type 1 but not type 2 lymphocytes, *J. Exp. Med.* 175(1), 9–14 (1992).
165. S. E. Macatonia, S. Patterson, and S. C. Knight, Primary proliferative and cytotoxic T-cell responses to HIV induced in vitro by human dendritic cells, *Immunology* 74(3), 399–406 (1991).
166. L. J. Eales, O. Moshtael, and A. J. Pinching, Microbicidal activity of monocyte derived macrophages in AIDS and related disorders, *Clin. Exp. Immunol.* 67(2), 227–235 (1987).
167. L. Meyaard, S. Otto, R. Jonker, M. Mijnster, R. Keet, and F. Miedema, Programmed cell death of T-cells in HIV-1 infection, *Science* 257, 217–219 (1992).
168. F. Miedema, Immunological abnormalities in the natural history of HIV infection: mechanisms and clinical relevance, *Immunodefic. Rev.* 3, 173–193 (1992).
169. T. Finkel, G. Tudor-Williams, N. Banda, M. Cotton, T. Curiel, C. Monks, T. Baba, R. Ruprecht, and A. Kupfer, Apoptosis occurs predominantly in bystander cells and not in productively infected cells of HIV- and SIV-infected lymph nodes, *Nat. Med.* 1, 129–134 (1995).

170. C. Muro-Cacho, G. Pantaleo, and A. Fauci, Analysis of apoptosis in lymph nodes of HIV-infected persons. Intensity of apoptosis correlates with the general state of activation of the lymphoid tissue and not with stage of disease or viral burden, *J. Immunol.* 154, 5555–5566 (1995).
171. A. Guiot, D. Rigal, and G. Chappuis, Spontaneous programmed cell death (PCD) process of lymphocytes of FIV-infected cats: cellular targets and modulation, *Vet. Immunol. Immunopathol.* 58, 93–106 (1997).
172. G. Sarli, L. Della Salda, L. Zaccaro, M. Bendinelli, G. Piedimonte, and P. Marcato, Apoptotic fraction in lymphoid tissue of FIV-infected SPF cats, *Vet. Immunol. Immunopathol.* 64, 33–44 (1998).
173. L. Boise, P. Noel, and C. Thompson, CD28 and apoptosis, *Curr. Opin. Immunol.* 7, 620–625 (1995).
174. E. Greenfield, K. Nguyen, and V. Kuchroo, CD28/B7 costimulation: a review, *Crit. Rev. Immunol.* 18, 389–418 (1998).
175. D. J. Lenschow, T. L. Walunas, and J. A. Bluestone, CD28/B7 system of T-cell costimulation, *Annu. Rev. Immunol.* 14, 233–258 (1996).
176. M. Azuma, H. Yssel, J. Phillips, H. Spits, and L. Lanier, Functional expression of B7/BB1 on activated T lymphocytes, *J. Exp. Med.* 177, 845–850 (1993).
177. T. Wyss-Coray, D. Mauri-Hellweg, K. Baumann, F. Bettens, R. Grunow, and W. Pichler, The B7 adhesion molecule is expressed on activated human T-cells: functional involvement in T-T-cell interactions, *Eur. J. Immunol.* 23, 2175–2180 (1993).
178. E. Ranheim and T. Kipps, Elevated expression of CD80 (B7/BB1) and other accessory molecules on synovial fluid mononuclear cell subsets in rheumatoid arthritis, *Arthritis Rheum.* 37, 1637–1646 (1994).
179. D. Folzenlogen, M. Hofer, D. Leung, J. Freed, and M. Newell, Analysis of CD80 and CD86 expression on peripheral blood B lymphocytes reveals increased expression of CD86 in lupus patients, *Clin. Immunol. Immunopathol.* 83, 199–204 (1997).
180. T. W. Vahlenkamp, M. E. Bull, J. L. Dow, E. W. Collisson, B. J. Winslow, A. P. Phadke, W. A. Tompkins, and M. B. Tompkins, B7+CTLA4+ T-cells engage in T-T-cell interactions that mediate apoptosis: A model for lentivirus-induced T-cell depletion, *Vet. Immunol. Immunopathol.* in press (2004).
181. V. Barnaba, C. Watts, B. M. de, P. Lane, and A. Lanzavecchia, Professional presentation of antigen by activated human T-cells, *Eur. J. Immunol.* 24, 71–75 (1994).
182. G. Lombardi, S. Sidhu, T. Dodi, R. Batchelor, and R. Lechler, Failure of correlation between B7 expression and activation of interleukin-2-secreting T-cells, *Eur. J. Immunol.* 24, 523–530 (1994).
183. E. Greenfield, E. Howard, T. Paradis, K. Nguyen, F. Benazzo, P. McLean, P. Hollsberg, G. Davis, D. Hafler, A. Sharpe, G. Freeman, and V. Kuchroo, B7.2 expressed by T-cells does not induce CD28-mediated costimulatory activity but retains CTLA4 binding: implications for induction of antitumor immunity to T-cell tumors, *J. Immunol.* 158, 2025–2034 (1997).
184. D. Bienzle, F. Smaill, and K. Rosenthal, Cytotoxic T-lymphocytes from HIV-infected individuals recognize an activation-dependent, non-polymorphic molecule on uninfected CD4+ lymphocytes, *AIDS* 10, 247–254 (1996).
185. M. E. Bull, T. W. Vahlenkamp, J. L. Dow, E. W. Collisson, B. J. Winslow, A. P. Phadke, M. B. Tompkins, and W. A. Tompkins, Spontaneous T-cell apoptosis in feline immunodeficiency virus (FIV)-infected cats is inhibited by IL-2 and anti-B7.1 antibodies, *accepted for publication* (2004).
186. Y. Belkaid, C. A. Piccirillo, S. Mendez, E. M. Shevach, and D. L. Sacks, CD4+CD25+ regulatory T-cells control Leishmania major persistence and immunity, *Nature* 420(6915), 502–507 (2002).
187. S. Sakaguchi, Regulatory T-cells: mediating compromises between host and parasite, *Nat. Immunol.* 4(1), 10–11 (2003).

188. T. Takahashi, Y. Kuniyasu, M. Toda, N. Sakaguchi, M. Itoh, M. Iwata, J. Shimizu, and S. Sakaguchi, Immunologic self-tolerance maintained by CD25+CD4+ naturally anergic and suppressive T-cells: induction of autoimmune disease by breaking their anergic/suppressive state, *Int. Immunol.* 10(12), 1969–1980 (1998).
189. A. M. Thornton and E. M. Shevach, CD4+CD25+ immunoregulatory T-cells suppress polyclonal T-cell activation in vitro by inhibiting interleukin 2 production, *J. Exp. Med.* 188(2), 287–296 (1998).
190. A. M. Thornton and E. M. Shevach, Suppressor effector function of CD4+CD25+ immunoregulatory T-cells is antigen nonspecific, *J. Immunol.* 164(1), 183–190 (2000).
191. K. Nakamura, A. Kitani, and W. Strober, Cell contact-dependent immunosuppression by CD4(+)CD25(+) regulatory T-cells is mediated by cell surface-bound transforming growth factor beta, *J. Exp. Med.* 194(5), 629–644 (2001).
192. I. Caramalho, T. Lopes-Carvalho, D. Ostler, S. Zelenay, M. Haury, and J. Demengeot, Regulatory T-cells selectively express toll-like receptors and are activated by lipopolysaccharide, *J. Exp. Med.* 197(4), 403–411 (2003).
193. B. Salomon, D. J. Lenschow, L. Rhee, N. Ashourian, B. Singh, A. Sharpe, and J. A. Bluestone, B7/CD28 costimulation is essential for the homeostasis of the CD4+CD25+ immunoregulatory T-cells that control autoimmune diabetes, *Immunity* 12(4), 431–440 (2000).
194. H. Jonuleit, E. Schmitt, M. Stassen, A. Tuettenberg, J. Knop, and A. H. Enk, Identification and functional characterization of human CD4(+)CD25(+) T-cells with regulatory properties isolated from peripheral blood, *J. Exp. Med.* 193(11), 1285–1294 (2001).
195. T. W. Vahlenkamp, M. B. Tompkins, and W. A. Tompkins, Feline immunodeficiency virus (FIV) infection phenotypically and functionally activates immunosuppressive CD4+CD25+ T-regulatory (Treg) cells, *accepted for publication* (2004).
196. B. Singh, S. Read, C. Asseman, V. Malmstrom, C. Mottet, L. A. Stephens, R. Stepankova, H. Tlaskalova, and F. Powrie, Control of intestinal inflammation by regulatory T-cells, *Immunol. Rev.* 182, 190–200 (2001).
197. S. Hori, T. L. Carvalho, and J. Demengeot, CD25+CD4+ regulatory T-cells suppress CD4+ T-cell-mediated pulmonary hyperinflammation driven by Pneumocystis carinii in immunodeficient mice, *Eur. J. Immunol.* 32(5), 1282–1291 (2002).
198. R. A. Seder and R. Ahmed, Similarities and differences in CD4+ and CD8+ effector and memory T-cell generation, *Nat. Immunol.* 4(9), 835–842 (2003).