FIV as a Model for HIV: An Overview

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1. INTRODUCTION

Animal models for human immunodeficiency virus (HIV) infection play a key role in understanding the pathogenesis of AIDS and the development of therapeutic agents and vaccines. As the only lentivirus that causes an immunodeficiency resembling that of HIV infection, in its natural host, feline immunodeficiency virus (FIV) has been a unique and powerful model for AIDS research. FIV was first described in 1987 by Niels Pedersen and co-workers as the causative agent for a fatal immunodeficiency syndrome observed in cats housed in a cattery in Petaluma, California. Since this landmark observation, multiple studies have shown that natural and experimental infection of cats with biological isolates of FIV produces an AIDS syndrome very similar in pathogenesis to that observed for human AIDS. FIV infection induces an acute viremia associated with T-cell alterations including depressed CD4:CD8 T-cell ratios and CD4 T-cell depletion, peripheral lymphadenopathy, and neutropenia. In later stages of FIV infection, the host suffers from chronic persistent infections that are typically self-limiting in an immunocompetent host, as well as opportunistic infections, chronic diarrhea and wasting, blood dyscracias, significant CD4 T-cell depletion, neurologic disorders, and B-cell lymphomas. Importantly, chronic FIV infection induces a progressive lymphoid and CD4 T-cell depletion in the infected cat. The primary mode of natural FIV transmission appears to be blood-borne facilitated by fighting and biting. However, experimental infection through transmucosal routes (rectal and vaginal mucosa and perinatal) have been well documented for specific FIV isolates. Accordingly, FIV disease pathogenesis exhibits striking similarities to that described for HIV-1 infection. Recent observations regarding functions of FIV accessory and structural genes, FIV...
tropism, and immunopathogenesis have further corroborated similarities shared by FIV and HIV-1. This chapter will serve as an overview of the FIV animal model for HIV AIDS and as such, will focus on FIV molecular biology and virology and address recent developments in FIV viral vector development as well as nondomestic FIV biology. FIV pathogenesis, vaccine development, and antiviral therapies are critical topics for discussion regarding the value of the FIV animal model and will also be described briefly in this overview, but will be examined in more significant detail in subsequent chapters.

2. FIV GENOME, STRUCTURE, AND GENE FUNCTION

Virion Structure

The morphology of the FIV virus particle is similar to that of other lentiviruses.\textsuperscript{1,14,25} The mature extracellular virion is spherical to ellipsoid, 100 to 125 nm in diameter, and bordered by an outer envelope with poorly defined short projections or knobs. An elongated conical shell surrounds an eccentrically positioned electron-dense viral nucleoid. A polygonal electron-lucent halo is often visible between the core and a granular layer located just inside the envelope. Similar to other lentiviruses, the density of FIV was shown to be 1.15–1.17 g/cm\textsuperscript{3} by continuous sucrose gradient centrifugation.\textsuperscript{26}

Typical of other retroviruses, the FIV genome consists of an identical pair of single-stranded RNA molecules that are approximately 9,200 bases in length and densely packed within the virion by their association with nucleocapsid protein (NC, p7).\textsuperscript{27,28} A t-RNA\textsubscript{lys} is hydrogen-bonded to each RNA molecule at the primer binding site (PBS) located within the 5\textsuperscript{¢} terminal 180 bases of the genome and serves as the primer for negative strand reverse transcription. The ribonucleoprotein complex is contained within a protein core largely composed of the viral capsid protein (CA, p24) that is associated with and surrounded by a roughly spherical shell consisting of myristylated matrix protein (MA, p14). Also contained in the viral capsid are viral enzymes involved in particle maturation and replication of the viral RNA genome including protease (PR), reverse transcriptase (RT), integrase (IN), and dUTPase (DU).\textsuperscript{29–31} NC, CA, and MA are expressed from the \textit{gag} gene, while PR, RT, DU, and IN are products of the \textit{pol} gene. Outside the matrix coat is a lipid bilayer of the virion envelope, which confers the characteristic icosahedral morphology to the enveloped retrovirus. Embedded within the lipid bilayer are viral envelope glycoproteins, with the transmembrane subunit (TM, gp40) present as a single-pass transmembrane protein anchor, and the surface unit (SU, gp95) as an entirely extravirion protein bound to TM.\textsuperscript{27} Both TM and SU are the products of
the \textit{env} gene. \textit{Env} gene products mediate binding of the virus to cell surface receptors and fusion with the target cell membrane\textsuperscript{32–36} and are critical targets for host humoral and cellular immune responses.

\textbf{Genome Organization and Expression}

Sequence organization of the FIV genome is similar to that of HIV-1 and other lentiviruses\textsuperscript{37,38}. Flanked by two long terminal repeats (LTR) at both ends, the FIV proviral DNA genome contains three large open reading frames (ORFs), \textit{gag}, \textit{pol}, and \textit{env}, encoding internal structural proteins, RT and other viral enzymes, and envelope proteins, respectively, as well as various small ORFs encoding regulatory and accessory proteins (Figure 1). The FIV genome also contains nonencoding regulatory sequences important for virus replication. These sequences include transcriptional elements within the LTRs, a posttranscriptional regulatory sequence located in 3′ half of the genome\textsuperscript{39–43} and encapsidation determinants within the U5 domain and the first 90–300 nucleotides of \textit{gag}.\textsuperscript{44,45} Other critical noncoding sequences include the central polyuridine tract (cPPT) involved in priming plus-strand DNA synthesis and the central termination sequence (CTS) important for formation of a central DNA flap during reverse transcription.\textsuperscript{46}

The approximately 355 bp long FIV LTR accommodates multiple regulatory sequences and is composed of three domains designated U3, R, and U5. Located at each terminus of the proviral DNA genome, the LTRs are bordered by two bp-inverted repeats. Critical transcriptional regulatory sequences located in the FIV U3 domain consist of enhancer elements including AP-1, ATF (also known as the cAMP response element or CRE), and cEBP sites. These \textit{cis}-acting elements have been shown to be important for FIV LTR promoter activity in vitro and for virus replication in vitro.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{FIV_HIV.png}
\caption{Comparison of FIV and HIV-1 genomic organization.}
\end{figure}
and in vivo. Previous studies also demonstrated binding of these specific LTR sequences by cellular proteins using DNase I footprinting and gel shift assays. Although cis-acting transcriptional elements within the FIV U3 domain differ from those described as critical for the HIV LTR (NFκB and SP1), FIV-encoded AP-1 and ATF sequences are positive regulatory elements that respond to host cell activation states, a property also shared by the NFκB site within the HIV-1 LTR. Other retroviruses encoding LTRs regulated by ATF/CREB family of transcription factors include human T-lymphotropic virus type (HTLV-1) and bovine leukemia virus (BLV). Similarly, an AP-1 site encoded by the visna virus LTR was found to be critical for basal activity and for transactivation of the viral LTR resulting from interactions of the visna virus putative transcriptional transactivator (Tat) protein with cellular transcription factors Fos and Jun. Interestingly the structure of Orf-A, an FIV accessory gene product previously regarded to be a Tat protein, is very similar to that of visna virus Tat. Collectively, these observations suggest that FIV LTR promoter activity may be regulated by multiple cell activation pathways involving possible interactions between a viral accessory protein (Orf-A) with cellular proteins that bind either AP-1, ATF, or cEBP elements. However, a definitive characterization of these potential interactions has not yet been reported. Knowledge of potential complex interactions between the FIV LTR and viral and cellular proteins that are most likely involved in regulation of FIV expression is still rudimentary at best.

The FIV LTR is also distinguished from the HIV-1 LTR by its strong basal promoter activity that does not require activation by a viral transactivator. Similarly, other animal lentiviruses including caprine arthritis encephalitis virus (CAEV) and visna virus (VV) also encode LTR promoters capable of high basal levels of transcription in the absence of a viral transactivator. In contrast, LTRs encoded by primate lentiviruses including HIV and simian immunodeficiency virus (SIV), include a transcriptional element designated TAR (Tat responsive element) that possesses a stem-loop structure for binding of the virus encoded transactivator Tat and other cellular proteins. Tat transactivation of the HIV LTR is required for elongation of initiated RNA transcripts and for efficient transcription of viral genes. The FIV LTR does not contain a stem-loop structure of similar complexity nor does FIV encode a transcriptional transactivator with structure or activity similar to that described for HIV Tat. Previous reports have proposed that the gene product of FIV accessory gene orf-A may encode a viral transactivator or FIV Tat. However, these studies have generated conflicting data regarding the ability of FIV-encoded Orf-A to transactivate the FIV LTR and revealed either no effect, a small effect, or a moderate effect imposed by Orf-A on FIV LTR-directed gene expression in transient expression assays. Taken together, these data suggest that the FIV LTR may be regulated in part by accessory protein Orf-
A although by mechanisms unlike those described for the HIV LTR and HIV Tat.

Examination of FIV mRNA species from infected cells revealed the presence of at least five short multiply spliced transcripts in addition to unspliced genomic RNA and env-containing singly spliced transcripts. Nuclear export of unspliced and singly spliced FIV mRNA transcripts involves binding of a postranscriptional regulatory sequence, designated the rev response element (RRE), by the FIV regulatory protein Rev. The FIV RRE is a 243 nucleotide sequence that forms a stem-loop structure within viral mRNA species and serves as a binding site for FIV regulatory protein Rev and is structurally and functionally similar to the HIV-1 RRE. However, the FIV RRE is located at the 3’ terminus of the env gene and partially overlaps the 3’ LTR, whereas the HIV-1 RRE is positioned between the junction of the SU and TM open reading frames within env. Binding of the FIV RRE by the viral protein Rev is critical for cytoplasmic accumulation of unspliced and singly spliced FIV mRNA transcripts and for FIV structural (Gag and Env) and enzymatic (Pol) protein expression.

FIV Structural and Enzymatic Proteins

FIV Gag proteins are necessary and sufficient for the formation of the noninfectious virus-like particles. Similar to other lentivirus systems, expression of FIV Gag polyprotein precursor (p50) from the gag gene within the unspliced genome RNA is dependent on viral Rev. Nevertheless, the FIV Gag polyprotein, when expressed in the absence of other viral structural proteins such as env gene products, is capable of self-assembly into virus particles that are released from the plasma membrane of Gag-expressing cells. Formation of mature virus particles, however, requires cleavage of the FIV Gag polyprotein by virus-encoded PR during or shortly after budding from the cell to generate three mature Gag proteins: MA, CA, and NC (Figure 2). Examination of the proteolytic sites within the FIV Gag polyprotein revealed processing of Gag proteins similar to that for HIV-1 Gag proteins, and in particular to HIV-1 CA protein. Within the mature virion, MA is attached to the viral lipid membrane while CA forms the virus core, and NC is present in the virus core in a ribonucleoprotein complex with the viral RNA genome.

Reports describing studies focused on FIV Gag protein processing and characterization of functional domains are still limited. Similar to HIV MA, myristylation of FIV MA is required for targeting of MA to the plasma membrane during late events of viral assembly. In contrast to observations for HIV-1 MA, targeting of FIV MA to the cellular plasma membrane is not dependent on a N-terminal polybasic domain that is present in both FIV and primate lentivirus MA proteins. Instead, this conserved polybasic domain (lysine-rich) in FIV MA appears necessary for
either particle assembly or release. The role of FIV MA in other steps of virus replication, where HIV-1 MA is thought to be important, such as early postentry events, has not yet been determined.

The principal function of FIV NC involves encapsidation of full-length, unspliced viral genomic RNA into virions. Similar to other retroviruses, FIV NC protein contains two copies of a zinc finger motif, which has been characterized as a zinc-binding moiety for HIV-1 RNA. Although studies describing FIV NC interactions with viral RNA are few, recent observations suggest that FIV NC binds to viral RNA at, or upstream of, the PBS and may thereby initiate RNA dimerization and promote initiation of minus strand DNA. However, the role of NC in viral RNA encapsidation or determinants responsible for FIV NC binding of RNA have not yet been reported. Interestingly, examination of proteolytic cleavage of NC revealed a secondary cleavage site within the C-terminus of NC that produces a mature NC 7.1kD protein and a C-terminus 1.9 kD protein. Although further characterization of this C-terminus 1.9 kD protein has not yet been described, amino acid sequence of this cleavage product contains a PSAP motif similar to the PTAP motif also characterized as a “late” or L domain encoded by the HIV-1 p6 Gag protein. This motif within HIV-1 p6 functions in virus particle budding and release and is a binding motif for proteins encoding WW domains. Interactions of the L domain of HIV-1 p6 with cellular protein TSG101 are reported to be critical for HIV-1
budding and are under investigation as potential targets for antiviral therapeutics. Examination of the significance and potential function of this C-terminal cleavage product of FIV NC as a L domain for FIV Gag may be warranted and provide another lentiviral model for characterizing cellular proteins important for lentivirus assembly and release.

In addition to MA, CA, and NC proteins encoded by FIV gag, the FIV Gag polyprotein contains a CA-NC spacer region shown to regulate temporal Gag processing for several retroviruses including HIV-1, bovine immunodeficiency virus (BIV), and Rous sarcoma virus (RSV). The FIV CA-NC spacer region consists of nine residues and contains a LAEAL motif also found in the HIV-1 CA-NC spacer region and reported to be indispensable for HIV-1 Gag assembly. Although a recent study showed that the FIV CA-NC spacer region was capable of functionally replacing the BIV CA-NC spacer region for BIV Gag assembly, studies examining the FIV CA-NC spacer region in FIV Gag assembly have not been reported. This observation, however, suggests that FIV Gag may provide another model for examination of this Gag motif in HIV-1 assembly.

The FIV pol gene positioned downstream of gag, encodes four enzymes: protease (PR), reverse transcriptase (RT), dUTPase (DU), and integrase (IN) (Figure 2). FIV pol overlaps the gag gene by 109 nucleotides and is in a −1 reading frame with respect to that of gag. Similar to other retroviruses, pol is translated as a Gag-Pol fusion polyprotein produced by ribosomal frameshifting facilitated by a consensus frameshift signal sequence of GGGAAAC within the gag-pol overlap region, together with a sequence displaying potential for a pseudoknot tertiary structure immediately downstream of the signal sequence. The Pol polypeptide of the Gag-Pol fusion precursor protein is cleaved by viral PR into functional enzymes during virus assembly.

FIV PR is a 14.3 kD protein that facilitates processing of Gag and Gag-Pol polyproteins into individual structural and enzymatic proteins during assembly and maturation of the virus particle. Focus on antiviral therapies targeted to HIV-1 PR have promoted interest in FIV PR as a model for design of protease inhibitors as well as structural studies characterizing FIV PR. Based on three-dimensional crystal structure analysis, FIV PR is a homodimeric aspartyl proteinase with quaternary structures very similar to those of HIV-1 PR despite a conservation of only 27 amino acids between the two enzymes. However, each monomer of FIV protease is composed of 116 amino acids compared to 99 amino acids for HIV-1 PR. Regardless of similarities observed between FIV PR and HIV-1 PR, FIV PR exhibits a substrate specificity that is restricted to FIV Gag cleavage sites and excludes sites within HIV-1 Gag. Multiple residue substitutions are required within FIV PR to modify this specificity to include HIV-1 Gag cleavage sites. Furthermore, residues peripheral to the active site of PR, as well as those within the active site, influence binding of substrate by stabilizing
crucial residues within the active site that directly contact substrate and may account for differences in substrate specificities observed between FIV and HIV-1 PR activities.95 Regardless of disparate substrate specificity displayed by FIV PR and HIV-1 PR, similarities in their structure have been utilized in the development of broad-based inhibitors that will bind both HIV PR and FIV PR.94,96,97 These comparative analyses should significantly increase the understanding of the molecular basis for lentivirus PR substrate specificity and may possibly facilitate the development of PR inhibitors less susceptible to resistance development.

Reverse transcriptases are encoded by all retroviruses and are RNA-dependent DNA polymerases that reverse transcribe viral genomic RNA into a double-stranded proviral DNA copy that is subsequently integrated into the host cellular genomic DNA.98 FIV RT is comparable to HIV-1 RT in amino acid sequence, structure and physical properties, catalytic activities, and susceptibility to multiple nucleoside analogs.99–101 Amino acid sequence analysis reveals a 48% identity and 67% similarity between HIV-1 RT and FIV RT.101 Like HIV-1 RT, FIV RT exists as a heterodimer consisting of a 66 kD subunit (p66) and a 51 kD subunit (p51), each of which contains a common N-terminus and are present in equimolar concentrations.100 The p51 subunit is generated by cleavage of the RNase H domain from C-terminus of p66. The RNase H domains in FIV RT and HIV-1 RT function in reverse transcription to degrade RNA from the DNA-RNA hybrid.102 Importantly, FIV RT and HIV-1 RT exhibit a similar susceptibility to multiple nucleoside analogs.100,103–105 However, FIV RT resistance to specific nucleoside analogs such as 3′-azido-3′-deoxythymidine (AZT), 2′,3′-dideoxyinosine (ddI), 2′,3′-didehydro-3′-deoxythymidine (d4T), and 2′,3′-dideoxycytidine (ddC) does not map to homologous residues within similarly drug-resistant HIV-1 RT mutants. In contrast, FIV RT and HIV-1 RT susceptibilities to nucleoside analog (3TC) map to corresponding codon, M184 in the YMDD active site of the RT palm subdomain.106,107 Other studies also revealed that a unique 3TC-resistant FIV mutant encoded a novel proline to serine change at position 156, analogous to proline residue 157 residing within the template grip of HIV-1 RT.108 This observation suggested that mutations within a region (template grip) close to, but distinct from the RT active site could influence substrate recognition, a conclusion further substantiated by examination of homologous HIV-1 RT mutant P157S.103 Furthermore, functional studies assessing chimeric RT molecules composed of FIV and HIV-1 p51 and p66 subunits demonstrated the importance of p51 in maintaining optimal structural integrity of RT and shed some light on significance of the p51 subunit.109,110 These studies also produced observations showing the lack of FIV RT sensitivity to non-nucleoside RT inhibitors (NNRTI) found to block HIV-1 replication, although amino acids lining the NNRTI-specific pocket of HIV-1 RT exhibit a higher similarity to the correspond-
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ing FIV RT residues than to HIV-2 RT.\textsuperscript{109,110} Catalytic activity of HIV/FIV chimeric RTs was also found to be significantly decreased compared to wild type HIV and FIV RTs, despite similarities observed between the two molecules.\textsuperscript{109} In summary, similarities and differences observed between HIV-1 and FIV RT have generated somewhat limited support for use of FIV RT as a model for HIV-1 RT-targeted drug design and studies of drug resistance both in cell culture and in vivo.\textsuperscript{111}

A DU gene product is expressed from the \textit{pol} gene in genomes from nonprimate lentiviruses including FIV and the type-D retroviruses, but not from primate lentiviruses.\textsuperscript{30} FIV DU resides immediately downstream of RT in the Pol polyprotein and is packaged in active form in FIV virions. DU catalyzes the hydrolysis of dUTP to dUMP and inorganic pyrophosphate (PP\textsubscript{i}), and is believed to minimize misincorporation of dUTP into DNA, which can be mutagenic.\textsuperscript{112} For those viruses encoding a DU, enzymatic activity is required for productive viral replication in cells such as primary macrophages that express low dUTPase activity.\textsuperscript{113–115} Infection of cats with a DU mutant of FIV resulted in fivefold increase in the number of mutations observed in the viral genome.\textsuperscript{114,115} Although HIV-1 does not encode a dUTPase activity, recruitment of a cellular DNA repair enzyme, uracil DNA glycosylase (UNG), into HIV-1 virions by accessory protein Vpr, also acts to modulate viral mutation rate.\textsuperscript{116–119} Accordingly, HIV Vpr activity imparts a similar effect to that of FIV DU on virus replication but through a different mechanism.

Integration of double stranded proviral DNA into the host genome is a function of all retroviral integrase proteins and is a distinguishing feature of retrovirus replication.\textsuperscript{120} FIV IN is a 32 kD protein that is approximately 37% identical to HIV-1 IN by amino acid sequence.\textsuperscript{121} Similar to other retroviral IN proteins, FIV IN contains three domains including an N-terminal domain, a central catalytic core domain, and a C-terminal domain.\textsuperscript{122} Studies testing activity of a recombinant FIV IN expressed in \textit{E. coli} revealed that FIV IN exhibits a relaxed sequence requirement for site-specific cleavage and integration of viral DNA termini and is active on FIV, HIV, and Moloney murine leukemia virus (MoMLV) DNA termini.\textsuperscript{121} A difference noted between FIV IN and HIV-1 IN was their choice of nucleophiles in vitro with FIV IN preferentially using the 3' OH viral DNA ends and HIV-1 IN using H\textsubscript{2}O and glycerol. In vitro analyses of recombinant FIV IN also demonstrated that the central catalytic core domain determined target site selection and the importance of a central aspartic acid (D118) in 3' terminus processing and joining activities.\textsuperscript{123,124} Virus replication studies testing FIV IN mutants in the context of FIV vectors showed that mutation of either D66 and or both D66 and D118 within the catalytic core domain blocked transduction of dividing fibroblast and integrations, as would be predicted for type I IN mutants.\textsuperscript{125} These observations for mutants involving FIV IN residues D66 and D118 that correspond to D64 and D116 in
the catalytic triad of HIV-1 IN, characterized properties of catalytic core IN mutants for a non-HIV-1 lentivirus, and verified similarities between FIV and HIV-1 IN.

In addition to a direct role in integration of proviral DNA into host cell genomic DNA, HIV-1 IN is a component of the PIC that also contains newly synthesized proviral DNA, viral MA and Vpr, and the viral central DNA flap. Each of these viral components are thought to contribute to nuclear import of the PIC and IN may play the primary role. Similar to HIV-1 IN, FIV IN exhibits karyophilic properties. Determinants encoded by FIV IN for nuclear import map to a N-terminal zinc-binding domain and to a region rich with basic residues near the C-terminal domain, rather than to a canonical nuclear localization signal (NLS). The NLS for HIV-1 IN is also thought to involve a bipartite signal that instead includes a 13 residue peptide within the central core domain of IN, but does not include the N-terminal zinc-binding domain, suggesting that mechanisms for nuclear import of HIV-1 and FIV integrase molecules are different.

The env gene of FIV and other lentiviruses is the most diverse viral gene in size and sequence. Lentivirus envelope proteins play a major role in the virus life cycle by encoding determinants that interact with cell surface receptor and mediate fusion between the lipid bilayer of the viral envelope and host cell plasma membrane. Accordingly, variation in viral envelope proteins, particularly the surface glycoprotein, affects virus host cell tropism and fusogenicity, as well as virus replication. In addition, the Env glycoproteins contain epitopes that elicit immune responses important for both diagnosis and protective immunity.

FIV env expression from a singly spliced mRNA is Rev-dependent, similar to other structural proteins. In contrast to primate lentivirus Env proteins, FIV Env and other nonprimate lentiviruses Env proteins encode a lengthy N-terminal presequence upstream of the hydrophobic region of the Env signal peptide (Figure 3). This N-terminal presequence of FIV Env, containing 149 amino acids, represents a 20 kD polypeptide and includes the L region of env that encodes the N-terminal exon of FIV Rev. The early gene product of FIV env is a full-length uncleaved precursor 145–150 kD glycoprotein that is subsequently processed to a 130 kD precursor (gp130) by cleavage of the N-terminal 20 kD polypeptide and hydrophobic signal sequence. Precursor gp130 is then transported to the Golgi and proteolytically cleaved to produce mature FIV surface glycoprotein (SU) gp95 and characteristic hydrophobic membrane-spanning glycoprotein (TM) gp40. SU forms a noncovalent association with TM, which anchors the envelope complex to the lipid bilayer. Studies using glycosylation inhibitors have confirmed extensive glycosylation of FIV envelope proteins similar to that observed for HIV Env and demonstrated cell type-specific glycosylation of Env. These studies also verified the role of envelope protein glycosylation in virus infectivity.
nificance, structure, or function of this 20 kD N-terminal polypeptide cleavage product, apart from proper Env processing, is not well understood, although one study revealed that partial deletion of this peptide produced a virus unable to infect primary feline astrocytes while still infectious for feline lymphocytes and macrophages. Structural models based on x-ray crystallography or NMR spectroscopy indicate that HIV-1 envelope proteins form trimers on the viral surface. Furthermore, SU binding of the primary receptor, CD4, results in conformational changes facilitating formation of a ternary complex composed of CD4, SU, and coreceptor molecules (β-chemokine receptors CCR-5 and CXCR-4). This ternary complex triggers additional conformational changes in TM that mediate fusion of envelope proteins with the cellular plasma membrane. Models for spatial folding of FIV Env SU and TM proteins based on predictive algorithms using Env amino acid sequence from multiple isolates, reveal structural similarities to HIV-1 envelope proteins, including conserved and hypervariable domains for both FIV SU and TM. A principal immunodominant domain (PID) within the extracellular region of TM that is conserved among lentivirus transmembrane glycoproteins, including HIV-1 TM, has also been described for FIV TM (Figure 3). Despite conservation of the PID, mutation of this TM...
domain was not found to alter FIV infectivity. In contrast, disruption of a tryptophan-rich domain also conserved among lentivirus TM proteins and located in the extracellular region immediately upstream of the membrane-spanning domain abrogated virus entry. Findings from a second study demonstrated a role for the FIV TM tryptophan-rich domain in Env-mediated fusion between viral and cellular membranes, thus providing a probable mechanism by which this domain affects virus infectivity. Finally, synthetic peptides modeled on this tryptophan-rich domain were found to inhibit FIV replication. These findings parallel other data demonstrating the importance of a similar tryptophan-rich domain encoded by HIV TM for HIV-1 entry and HIV-1 fusogenic effects and suggest that FIV and HIV-1 TM may share one mechanism necessary for fusion of viral and cellular membranes. However, a more detailed structural analysis of FIV envelope proteins and their interactions with cell surface receptors based on x-ray crystallography will be needed for a thorough comparison of FIV and HIV-1 envelope proteins.

As stated earlier in this chapter, envelope proteins exhibit the greatest diversity in amino acids among all proteins encoded by lentiviruses. By comparison of different HIV isolates, five interspersed conserved (C1 to C5) and five variable domains (V1–V5) were identified for HIV-1 env gene. Similarly, analysis of amino acid sequences derived from the env gene of different isolates of FIV resulted in identification of nine variable regions (V1–V9), separated by regions that are more conserved (Figure 3). Furthermore, examination of V3, V4, and V5 domains within SU-coding sequence revealed up to 26% genetic diversity for different isolates and allowed separation of FIV isolates into five clades, or subtypes, designated A to E and possibly a sixth subtype arising from a subtype B cluster found in Texas. Diversity between subtypes varies from 17 to 26% whereas variation within a subtype ranges from 2.5 to 15%, findings that are similar to those for HIV-1 isolates. Although geographic separation is most likely a major factor for emerging genetic diversity of FIV, individual subtypes have been identified in geographically isolated regions of the world (Table I). FIV subtype A and B isolates have been detected in United States, Europe, Japan, and Australia, whereas subtype C variants have been found in North America, Europe, and Taiwan. Subtype A isolates have also been detected in Central and South America and all three FIV subtypes A, B, and C have been isolated from a single location (Munich, Germany). Subtype D and E isolates, characterized so far, are few and restricted to Japan and Argentina respectively. Multiple genetic analyses indicate that the vast majority of FIV isolates are either subtype A or B. Interestingly, findings from one report revealed twice as many synonymous site changes within FIV subtype B envelope V3 to V4 domains compared to those detected for subtype A envelope, although both subtype A and B envelopes encoded similar numbers of nonsynonymous site changes.
The greater diversity observed for subtype B suggests that this FIV subtype may represent an older virus. Significant diversity of FIV subtype B variants was also shown for viruses isolated from cats in Italy where this subtype is highly prevalent.\textsuperscript{144} Findings from this study were generated by phylogenetic analysis focused on sequences from a Gag fragment from 32 isolates and on SU sequences from four isolates. Finally, higher diversity for this subtype was further supported by two recent studies. Examination of both Gag and envelope sequences derived from FIV subtype B isolates in Austria, where subtype B predominates, revealed subtype B clusters with sufficient genetic diversity to support their designation as subclades.\textsuperscript{146} A second study characterized a cluster of FIV isolates from Texas more closely related to FIV subtype B viruses than to other subtypes, yet clearly distinct from this subtype.\textsuperscript{151} Findings from this study suggested that this virus cluster was either a separate subtype that recently emerged from subtype B or represented a subgroup within subtype B. Collectively, these findings strengthen the hypothesis of FIV subtype B as an older virus existing within the domestic cat population for a longer time period than other FIV subtypes. Host adaptation and lower virulence has been suggested to be associated with FIV B subtype isolates but not confirmed by rigorous examination.

Several reports have also presented evidence of both naturally occurring\textsuperscript{149,150} and experimentally induced\textsuperscript{152} intersubtype recombinant viruses derived from recombination within the \textit{env} gene. Observation of intersubtype recombinants involving a common break-point within \textit{gag} was also recently reported.\textsuperscript{153} These findings for FIV agree with reports of recombinant HIV-1 isolates generated by divergent HIV subtypes in geographic regions where multiple HIV subtypes are found.\textsuperscript{154–156} Identification of multiple subtypes, significant divergence within the major subtype B viruses, and confirmation of naturally occurring FIV recombinants are relevant issues for FIV vaccine design and suggest that vaccines specific to geographic area and subtype prevalence may be required. Similar issues exist for HIV-1 vaccine design and provide an opportunity for the FIV

| Subtype | Location |
|---------|----------|
| A       | United States, Europe, Japan, Australia, Central and South America |
| B       | United States, Europe, Japan, and Australia |
| C       | North America, Europe, and Taiwan |
| D       | Japan |
| E       | Argentina |

\textbf{TABLE I}

Geographic Distribution of FIV Subtypes
vaccine model to address the importance of multisubtype vaccine approaches.

Regulatory and Accessory Genes

Only one regulatory gene (\textit{rev}) and two accessory genes (\textit{vif} and \textit{orf-A}) have been characterized for the FIV genome. Although several research groups have shown that FIV Rev and FIV Vif provide functions in virus replication similar to those described for analogous HIV-1 proteins, exhaustive examination of these FIV gene products has not been reported. FIV accessory genes, that are clearly analogous to primate lentivirus regulatory and accessory genes, including \textit{tat}, \textit{vpr}, \textit{vpu}, \textit{vpx}, or \textit{nef}, have not been identified. Early reports proposed that FIV Orf-A encoded a HIV-1 Tat gene product. However, studies testing Orf-A transactivation of the FIV LTR did not demonstrate Orf-A activity comparable to HIV-1 Tat.\textsuperscript{39,40,43,61} Recent findings suggest that FIV Orf-A may express functions more similar to those of HIV-1 Vpr or perhaps Vpu, although data supporting this premise are very limited.\textsuperscript{157,158} However, the possibility that viral functions and activities expressed by multiple unique HIV-1 accessory proteins may be encompassed by a single FIV accessory protein along with specific domains within FIV structural proteins warrants further examination.

Genomes of all members of lentivirus subfamily encode for a HIV-1 Rev-like posttranscriptional regulatory protein that is expressed early in the viral life cycle from a multiply spliced mRNA species containing either \textit{orf-A} and the L region of \textit{env} and \textit{orf-H}, or the L region of \textit{env} and \textit{orf-H} only.\textsuperscript{42,66} FIV Rev is a 23 kD protein that is expressed from two exons derived from the L region of \textit{env} and from \textit{orf-H} (Figure 2). The first \textit{rev} exon extends through the 5’ terminal L region of the \textit{env} gene and is in the same open reading frame as \textit{env}. The second exon (\textit{orf-H}) is located at the 3’ terminus of the \textit{env} gene and overlaps the 5’ terminus of the 3’ LTR. Similar to HIV-1 Rev, FIV Rev is a nucleolar protein that binds as multimers to the \textit{cis}-acting regulatory RRE sequence contained within unspliced and singly spliced viral mRNAs to promote nuclear export of these mRNA species to the cytoplasm and to also increase mRNA stability and protein translation.\textsuperscript{41,68,69,159,160} FIV Rev contains a highly basic domain similar to that described for other lentivirus Rev proteins. This basic domain located in the second exon, most likely serves as an RRE binding domain, although studies confirming the function of this FIV Rev element have not been reported. Another viral mRNA containing only the second Rev exon (\textit{orf-H}) has also been identified and encodes for a truncated Rev protein designated p15\textsuperscript{rev}.\textsuperscript{66} P15\textsuperscript{rev} contains the highly basic domain and effector domain of Rev but lacks the N terminus derived from the first exon. This truncated Rev protein exhibited Rev activity by in vitro assays but only 20% of the activity observed for wild type full-length FIV Rev. The significance
of p15

v in virus replication has not been characterized. Expression of FIV structural and enzymatic proteins, including Vif, is dependent on Rev expression and activity. Accordingly, the Rev regulatory system is essential for productive virus replication.

Although FIV Rev shares similar activities and functions with HIV-1 Rev, the FIV Rev effector (activation) domain located immediately downstream of the basic domain, contains a nuclear export signal (NES) that differs significantly in amino acid sequence from those described for Rev effector domains of primate and ungulate lentiviruses. The FIV Rev effector domain/NES lacks organized hydrophobic residues (leucine-rich clusters) and a core tetramer, which are properties that define the HIV-1 Rev NES. Instead, the FIV Rev effector domain/NES is characterized by basic residues and dispersed leucine residues similar to that observed for equine infectious anemia virus (EIAV) Rev. However, both FIV and EIAV Rev effector domains can functionally replace the effector domain of HIV-1 Rev, a finding that suggests FIV and HIV-1 Rev proteins utilize similar cellular pathways for their activity. Similarity between FIV Rev and HIV-1 Rev is further supported by studies demonstrating that nuclear export of FIV and EIAV Rev proteins, as well as Rev function, is inhibited by leptomycin B, a drug previously shown to block HIV-1 Rev NES interactions with CRM1 (exportin 1). This observation suggests that binding of CRM1 for nuclear export may be a property that FIV Rev shares with HIV-1 Rev, regardless of their dissimilar activation domains. Another functional property shared by the FIV and HIV-1 Rev regulatory systems involves an interaction with cellular eukaryotic initiation factor 5A (eIF-5A), which is also associated with Rev’s nuclear export of unspliced and singly spliced viral mRNA. Biologically active eIF-5A was previously shown to be required for HIV-1 Rev function and to specifically facilitate binding of Rev/RRE complex to CRM1. Activation of eIF-5A requires synthesis of the unique amino acid hypusine, which is sustained by deoxyhypusine synthetase. A recent report revealed that 1,8-diaminooctane, an inhibitor of deoxyhypusine synthetase, significantly inhibited FIV replication and specifically restricted FIV Rev function. Taken together, these observations demonstrate similarities shared between HIV-1 and FIV Rev proteins and validate FIV Rev as a model for potential antiviral therapies that target HIV-1 Rev.

An accessory gene designated as \( vif \) is conserved among all the lentiviruses with the exception of EIAV and has been determined to encode a factor necessary for virion cell-free infectivity, although not required for virion production. FIV \( \textit{vif} \) resides at the 3’ terminus of the \( \textit{pol} \) gene in the viral genome and is translated from a singly spliced mRNA to express a 23-kD basic protein (Figure 2). The role of FIV Vif in viral infectivity for primary feline lymphocytes and macrophages has been clearly established. However, biochemical characterization of FIV Vif has been limited and restricted to one report describing nuclear
localization for this FIV accessory protein. Studies regarding mechanisms of Vif function have so far focused instead on primate lentivirus Vif proteins. Early studies demonstrated that the replication phenotype of vif-deleted HIV mutants was cell type-dependent and specifically dependent of the cell producing the virion. Cell types, including primary lymphocytes and macrophages as well as specific T-cell lines, required the presence of HIV-1 Vif expression for production of infectious HIV and were designated as “nonpermissive” for replication of vif-deleted mutants of HIV-1. However, specific human T-cell lines designated as “permissive cells,” including SupT-1 cells and Jurkat cells, supported productive virus replication for HIV-1 vif-deleted variants. Cell fusion experiments showed that heterokaryons formed between permissive and nonpermissive cells displayed the nonpermissive phenotype, suggesting that nonpermissive cells naturally express an antiviral activity that inhibits the replication of vif-deficient virus. Virions produced in nonpermissive cells in the absence of the vif gene are impaired for reverse transcription of genomic RNA and therefore fail to establish full-length proviruses after entry into a target cell. The small number of reports focused on FIV Vif to date have not revealed a feline cell line “permissive” for vif deletion mutants of FIV. This observation may be due in part to the very few established feline T-cell lines that are available and permissive for primary wild type isolates of FIV.

Recent reports have revealed major breakthroughs in the understanding of HIV-1 Vif functions and interactions with host cell proteins. Sheehy and colleagues first identified a cellular protein designated CEM15 as the “nonpermissive” cell factor responsible for production of noninfectious virus particles in the absence of HIV-1 Vif. CEM-15 was later identified as APOBEC3G, a cell protein closely related to APOBEC1 and a member of the cytidine deaminase family of nucleic acid-editing enzymes. Numerous studies have now established APOBEC3G as a cellular factor that exerts an antiviral effect by deamination of cytosines to uracils in single-stranded minus-strand DNA during reverse transcription, resulting in either degradation of newly synthesized minus-strand DNA or guanidine to adenine hypermutations in the final double-stranded proviral DNA product. In the absence of HIV-1 Vif, APOBEC3G is packaged into virions, allowing this cellular protein to exert its antiviral effect during reverse transcription after virion entry into a target cell. However, if present during virion assembly, HIV-1 Vif forms a complex with human APOBEC3G that targets the cellular factor for proteosomal degradation and thereby prevents virion encapsidation of APOBEC3G to facilitate particle infectivity. These advances in the elucidation of HIV-1 Vif function have generated new enthusiasm for designing antiviral therapeutics targeting Vif and Vif-APOBEC3G interactions. Recent studies have also confirmed that other primate lentivirus (SIV) Vif proteins also target APOBEC3G in simian cells, although these interactions appear to be species-specific for some of
the simian species. Whether nonprimate lentivirus Vif proteins, including FIV Vif, function similarly by interacting with a species-specific APOBEC3G analog has yet to be determined. Evidence that supports this possibility is derived from reports demonstrating that APOBEC3G degradation is mediated by the HIV-1 Vif SLQ(Y/F)LA domain, an amino acid motif that is conserved among all lentivirus Vif proteins including FIV Vif.\textsuperscript{175,182,183}

FIV gene orf-A (also referred to as orf-2) encodes a 77 amino acid accessory protein previously implicated to encode a Tat-like protein and is critical for efficient viral replication in peripheral blood mononuclear cells (PBMC) in vitro and in vivo.\textsuperscript{61,157,185–188} Although the orf-A gene product contains a cysteine-rich domain within its 3' terminus, similar to Tat proteins encoded by ungulate lentiviruses, this gene product does not include core and basic domains comparable to those found in Tat proteins encoded by primate lentiviruses, EIAV, or BIV. Amino acid sequence alignments of FIV Orf-A with visna virus and CAEV Tat proteins reveal a similar organization of conserved putative domains, including N-terminal hydrophobic, central leucine-rich, and C-terminal cysteine-rich regions.\textsuperscript{60} In addition, FIV Orf-A encodes previously unrecognized conserved tryptophans at positions 43 and 66 positioned similarly to conserved tryptophan residues 63 and 85 of visna virus Tat. Two recent studies demonstrated a moderate upregulation of the FIV LTR promoter activity by coexpression of Orf-A in transient reporter gene expression assays.\textsuperscript{66,67} In contrast, earlier reports revealed either a small effect or no effect imposed by Orf-A on FIV LTR-directed gene expression.\textsuperscript{39,40,43,61} These findings suggest that Orf-A very likely drives FIV-LTR-directed transcription, but by indirect mechanisms involving interactions with cellular transcription factors.

A recent report revealed Orf-A to be important in the late steps of the FIV life cycle involved in virion formation and in early steps involved in virus infectivity and mapped critical Orf-A domains needed for these steps in replication.\textsuperscript{157} Central leucine-rich and C-terminal cysteine-rich regions, along with a conserved central tryptophan (residue 43) within Orf-A, were shown to be critical determinants for efficient virus replication and infectivity. The leucine-rich domain was important for infectivity, whereas tryptophan 43 and the cysteine-rich domain were important for both infectivity and virion formation. Importantly, deletions and point mutations in orf-A imposed a small or no effect on FIV LTR-driven viral gene expression and no effect on viral protein expression. These findings suggested that orf-A represents a FIV-encoded analog more similar to accessory genes vpr, vpu, or nef rather than the regulatory tat gene encoded by the primate lentiviruses. This concept was further supported by another recent study using mammalian expression plasmids encoding wild type or deletion mutant Orf-A proteins fused to the C'-terminus of green fluorescent protein (GFP) to evaluate Orf-A subcellular localization and effects on cell function.\textsuperscript{158} Findings from this study demonstrated nuclear localization
for the GFP-Orf-A fusion protein and allowed mapping of a NLS (residues 44–54) critical for nuclear import of FIV Orf-A. Furthermore, assessment of cell cycle profiles of cells transiently expressing GFP-Orf-A revealed that Orf-A causes an arrest at G2 of the cell cycle. These novel findings suggested that Orf-A is a nuclear protein that expresses some properties similar to those reported for HIV-1-encoded Vpr. Collective data generated from both reports suggest the possibility that Orf-A may encode specific functions attributed to several different HIV-1 encoded accessory protein but not all functions characterized for a single HIV-1 accessory protein. Additional support for this possibility is provided by a recent report showing that CAEV Tat, which shares structural homology with FIV Orf-A, also localizes to the nucleus and arrest cells in G2. Verification of these findings for FIV Orf-A from other research groups and for Orf-A proteins encoded by multiple FIV isolates will be needed to confirm the similarities between FIV Orf-A and HIV-1 Vpr and other HIV-1 accessory proteins.

3. FIV TROPISM

FIV Receptor Usage

Natural and experimental infection of cats with biological and molecularly cloned isolates of FIV consistently induce an acute viremia associated with T-cell alterations including depressed CD4:CD8 T-cell ratios and CD4 T-cell depletion. Early studies revealed that targets for FIV in vitro and in vivo included CD4 T-cells, macrophages, dendritic cells, microglia, and astrocytes similar to those for HIV infection in humans, but also included CD8 T-cells, and B-cells (Table II). Importantly, experimental inoculation studies in cats revealed that cell culture-adapted viruses represented a particular subset of viral variants that exhibited reduced replication and virulence in vivo. FIV infection of feline CD4-negative adherent cell lines provided indirect evidence that FIV differs with HIV-1 and does not utilize CD4 as a primary receptor. In addition, direct evidence refuting FIV usage of CD4 was provided by studies revealing an absence of virus infectivity for non-lymphoid cells expressing feline CD4. Subsequent reports described blocking of FIV infectivity by an antibody specific for feline CD9, a cell surface antigen belonging to the four-transmembrane-spanning domain superfamily (TM4SF). However, anti-CD9 antibody was later shown to
block virus infection by inhibition of virus release and not by interference with virus-receptor binding. 

A major breakthrough in characterization of FIV cell surface receptors resulted from observations showing that cell culture-adapted FIV isolates primarily utilize the β-chemokine receptor CXCR4 for infection in a similar fashion to T-cell line-adapted isolates of HIV-1 (Table II). 

Induced cell surface expression of CXCR4 was shown to mediate a susceptibility to FIV infection that could be abrogated by treatment with natural ligands for CXCR4 such as stromal cell-derived factor (SDF-1) and with CXCR4 antagonists including AMD3100. Furthermore, critical determinants for FIV infection were mapped to the extracellular loop of CXCR4. Multiple reports have now confirmed that cell culture-adapted FIV isolates are capable of using CXCR4 exclusively for virus entry and infection.

Results of subsequent reports suggesting FIV usage of chemokine receptors CCR5 and CCR3 have conflicted with observations of other investigators and may have resulted from enhanced expression of CXCR4 associated with ectopic expression of CCR5. Use of other chemokine receptors by particular FIV isolates is not clear at this time and is under investigation. Similar to HIV-1 envelope proteins, binding of FIV Env to

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**TABLE II**  
Comparison of FIV and HIV-1 Replication Properties In Vitro

| Property                     | FIV                                         | HIV-1                                                 |
|------------------------------|---------------------------------------------|-------------------------------------------------------|
| Permissive primary cells     | PBMC, CD4 T-cells, CD8 T-cells, macrophages, various other cell types including microglia and astrocytes | PBMC, CD4 T-cells, macrophages various other cell types including microglia and astrocytes |
| Permissive cell lines        | IL-2-dependent T-cell lines IL-2-independent T-cell lines fibroblastic adherent cell lines (GrFK and G355-5) | Various CD4 T-cell lines macrophage cell lines |
| Cytopathic effects           | Syncytium, giant cell formation, cell lysis, apoptosis | Same as FIV |
| Receptors                    |                                             |                                                       |
| Primary isolates             | CD134 and CXCR4                             | CD4 and CCR5 or dual-tropic (R5X4)                   |
| Adapted isolates             | CD134 and CXCR4 or CXCR4 only               | CD4 and CXCR4 or other “orphan” chemokine receptors or CXCR4 only |
| Host range                   | Feline cells only (?)                       | Human, some nonhuman primate cells                   |
| Viral tropism determinants   | Env: V3 region and TM Orf-A, U3 domain of LTR | Env: V3, V1/V2                                       |

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216. A major breakthrough in characterization of FIV cell surface receptors resulted from observations showing that cell culture-adapted FIV isolates primarily utilize the β-chemokine receptor CXCR4 for infection in a similar fashion to T-cell line-adapted isolates of HIV-1 (Table II). 33,217 Induced cell surface expression of CXCR4 was shown to mediate a susceptibility to FIV infection that could be abrogated by treatment with natural ligands for CXCR4 such as stromal cell-derived factor (SDF-1) and with CXCR4 antagonists including AMD3100. Furthermore, critical determinants for FIV infection were mapped to the extracellular loop of CXCR4. Multiple reports have now confirmed that cell culture-adapted FIV isolates are capable of using CXCR4 exclusively for virus entry and infection.

218. Use of other chemokine receptors by particular FIV isolates is not clear at this time and is under investigation. Similar to HIV-1 envelope proteins, binding of FIV Env to
other cell surface makers, including heparan sulfate proteoglycans (HSPGs) and DC-SIGN, has been shown and is specific for particular isolates. Binding of DC-SIGN was observed with recombinant Env proteins derived from primary and cell culture-passaged FIV isolates, whereas binding of HSPGs was observed only with a cell culture-passaged FIV strain. Although virus entry was shown to be mediated exclusively by CXCR4, virus infectivity was enhanced by factors including temperature or HSPGs, which either increase concentration of or binding for CXCR4, respectively. The roles of binding of DC-SIGN and HSPGs by FIV Env in FIV infection and pathogenesis in vivo are currently not well understood and warrant further examination.

Previous studies confirming CXCR4 as a receptor also revealed that CXCR4 expression was not sufficient for infectivity of primary FIV isolates and suggested the likelihood that primary isolates required a second receptor for binding and entry. One report describing coimmunoprecipitation of a recombinant FIV envelope protein with a 40 kD cellular protein provided further evidence of a non-CXCR4 receptor for FIV. A second major advance in FIV receptor biology was the recent identification of this cellular protein as CD134, a 43 kD cell surface marker, and the receptor utilized by primary FIV isolates in conjunction with CXCR4. CD134 is a member of the tumor necrosis factor-receptor superfamily and is expressed primarily on activated CD4 T-cells (mouse and human) after T-cell receptor (TCR) engagement. A recent report confirmed a similar phenotype for feline CD134 by showing up-regulation of CD134 expression on mitogen-activated feline CD4 T-cells. Binding of cell surface CD134 with cellular CD134 ligands provides a costimulatory signal that results in pro-inflammatory effects, as well as proliferation, migration, and cytokine production by memory T-cells. Low level CD134 expression has also been reported for activated CD8 T-cells, activated B-cells, and macrophages in mice and humans but has not been confirmed for the same feline cell lineages.

So far, preliminary reports indicate that infection by primary isolates requires binding of both CD134 and CXCR4. Assessment of receptor usage by larger panels of primary FIV isolates will be needed to determine if additional chemokine receptors may also be used for FIV infection, as previously observed for HIV-1, and may be associated with specific FIV replication/pathogenic phenotypes. Whether infection of CD8 T-cells, B-cells, and macrophages previously observed in later stages of FIV infection is due to CD134-independent usage of CXCR4 expressed on these cell populations, or results from usage of both CD134 and CXCR4, has not yet been clarified. Clearly, usage of CD134 as a receptor by primary FIV isolates explains the specific targeting of the CD4 T-cell population for depletion observed with FIV infection and the similarities in the acquired immunodeficiencies induced by both HIV-1 and FIV. Furthermore, these obser-
vations elucidate a mechanism by which FIV and HIV-1 utilize unique receptors (CD4 and CD134) to target a similar subset of T-cells (i.e., activated and resting memory CD4 T-cells) to impart disease pathogenesis.232–237 Accordingly, these findings further validate the importance of FIV infection as a model for examination of HIV-1-induced AIDS. Examination of pathogenic effects and cellular dysfunction imposed by virus-binding of CD134 expressed on CD4 T-cells in vitro will also be important for defining potential viral mechanisms for induction of feline acquired immunodeficiency in vivo.

Viral Determinants for Cell Tropism

Replication phenotypes in vitro and in vivo clearly distinguish cell culture-adapted FIV isolates from primary isolates. Most primary FIV isolates examined to date require both CD134 and CXCR4 for infection and exhibit a cell tropism restricted to primary feline PBMC, selected IL-2-dependent T-cell lines, and possibly primary macrophages and astrocytes,2,42,61,196–199,238 although testing for tropism to the latter two cell types has been infrequently reported (Table II). In contrast, FIV variants passaged in vitro utilize CXCR4 solely for efficient infection and replication in feline adherent cell lines including CrFK cells,33,36,218–221,223,224 as well as feline PBMC, IL-2-dependent and independent feline T-cell lines and macrophages.2,42,61,187,190,197,211,239–242 These highly passaged isolates, however, exhibit severely restricted virus replication and pathogenicity in vivo as discussed above.111,192,197,212 Multiple studies using FIV molecular clones have mapped Env as a major determinant that expands cell tropism to include feline adherent cell lines including CrFK cells and G355–5 cells (feline glial cell line), as well as feline astrocytes (Table II).33,212,223,243–246 Similar to the V3 domain of HIV-1 Env, the V3 domain of the FIV SU encodes specific tropism determinants212,223,243,244,249–251 in addition to important neutralizing antibody epitopes (Figure 3).252–254 Critical amino acid residues mapped within the FIV Env V3 domain appear to determine adherent cell tropism by modifying the overall charge of the V3 loop.212,243,244 A recent report describing the evolution of a nonpathogenic FIV isolate to a more pathogenic virus in vivo over time suggested that mutation E409K within the V3 loop not only imposed CrFK cell tropism but also contributed to virus attenuation.212 This hypothesis is supported by the consistent observation of severely restricted replication in vivo observed for viruses exhibiting a tropism for CrFK cells in vitro.111,192,197,212 Other Env determinants affecting FIV cell tropism for adherent cells have been mapped to the second constant domain within SU, the extracellular domain of TM,247 and the cytoplasmic domain of TM.245 Although V3 and V4 domains of FIV SU are also reported to influence FIV macrophage and brain microglia tropism,209,255 viral determinants critical for macrophage tropism as well as the frequency
of macrophage tropism across many primary isolates have not been well examined for FIV. Mechanisms by which these important Env residues or domains contribute to virus infectivity (for all cell types) either by effects on Env conformation or binding affinity to either CXCR4 or CD134 are also not well understood at this time and warrant investigation to further characterize the FIV animal model.

Multiple reports confirmed that the gene product of accessory gene orf-A is a critical determinant for infection of feline PBMC but is not required for infection of adherent cell lines including CrFK cells. However, the role of Orf-A in FIV tropism for feline macrophages has received only limited study and is unclear based on conflicting data reported from different studies. Mechanisms by which Orf-A impacts virus infectivity of PBMC are also undetermined at this time.

Interestingly, early studies suggested that restriction of productive FIV infection in human cells resulted primarily from a block in FIV transcription rather than a block in virus entry. The capability of FIV isolates to establish latent infections in human cells was later explained by demonstration of cell culture adapted-FIV usage of human CXCR4 for fusion and infection of human cells. Subsequent studies focused on FIV vector development revealed that the FIV U3 domain was the determinant responsible for restricted virus replication in human cells and showed that replacement of U3 with the cytomegalovirus immediate early promoter produced FIV vectors capable of viral gene expression in human cells. Studies reported by one research group showing that FIV is capable of productive infection of primate cells both in vitro and in vivo have not been confirmed by other independent researchers but suggest FIV infection of nonfeline cells may warrant further examination.

4. EPIDEMIOLOGY AND TRANSMISSION

Similar to other lentiviruses, the presence of FIV-specific antibodies signifies an established virus infection that will persist throughout the remaining lifetime of the host. Antibody detection ELISAs and other immunochromatographic methods (excluding western blot) have served as the primary screening diagnostic assays for FIV infection in clinical veterinary practice and epidemiologic studies. Although most FIV-infected cats produce antibodies to both Gag and Env structural proteins, a small proportion of cats will test positive for antibodies specific to only one of these two structural proteins. Therefore, currently available commercial FIV antibody assays include both FIV Gag and Env recombinant antigens for optimal sensitivity as well as specificity. Current and earlier serologic studies have shown that FIV is enzootic worldwide. Evaluation of mutations within virus subtypes suggests that FIV has been
present in domestic cats for a significant period of time, especially when compared to the relative short evolution of HIV. FIV prevalence varies greatly depending on geographic location and other variables of the tested cat populations. Among clinically healthy cats, the prevalence of FIV may be as low as 1% or less as observed in central European countries and the United States, or as high as 30% as reported in Japan and Australia. The seroprevalence rates in sick cats appear to be several times higher than those in their healthy counterparts and reflect the disease-inducing potential of FIV. Age and gender also markedly affect FIV prevalence. Most infections are acquired after one year of age, and prevalence increases up to approximately 10 years of age and then remains stable or tends to decline as the mean lifespan of a domestic cat is about 15 years. Viruses similar to FIV have been documented in several nondomestic felids such as lions, panthers, and bobcats. The large genetic diversity observed for lentiviruses among different nondomestic felids and between nondomestic feline lentiviruses and FIV, however, do not support the likelihood that nondomestic feline lentiviruses contribute significantly to the circulation of FIV in domestic cats. There is little evidence that FIV is transmissible to any other species including humans with the exception of a recent single report describing experimental FIV infection of non-human primates (cynomolgus macaques).

Precise modes of natural FIV transmission among domestic cats are not yet clear. Nevertheless, strong epidemiologic evidence implies that biting and fighting may be the predominant route of transmission (Table III). The importance of this route of transmission is corroborated by observations of the highest prevalence of infection in rural feral cat populations and urban areas containing a high density of freely roaming cats, as in Japan, and a higher prevalence of infection in adult tomcats. In fact, one epidemiologic study revealed that a pattern of increased FIV infection in orange cats compared to nonorange cats correlated with the pattern of more aggressive behavior also exhibited by orange cats. In addition, evidence of virus in saliva harvested from infected cats and an infection experiment involving virus inoculation by simulating biting further substantiate that transmission by this route is highly effective.

Early epidemiologic and experimental infection studies refuted the possibility of naturally occurring vertical transmission of FIV. However, later experimental inoculation studies confirmed virus transmission to newborn kittens from queens either acutely or chronically infected with different FIV isolates. Such transmission appeared to occur via in utero and postnatal routes, although intrapartum transmission has also been implicated. Interestingly, recent studies provided evidence of vertical transmission resulting in occult infection of kittens characterized by the presence of viral DNA in tissues in the absence of replicating
virus in peripheral blood, as well as absence of antiviral antibody apart from maternal antibody.\textsuperscript{206,296,297} Although these occult infections resulting from vertical transmission were considered regressive or transient as reported by one study,\textsuperscript{296} the duration of persistence of this type of infection has not been thoroughly examined. These covert or occult infections resemble previously described restricted FIV infections that were detectable only by PCR amplification of viral DNA from blood or tissues and that resulted either from inoculation with extremely attenuated viruses\textsuperscript{192} or from persistent but nontraumatic contact between naive and infected cats.\textsuperscript{298} Furthermore, similar sequestered virus infections in the absence of antibody have been reported for SIV infection\textsuperscript{299,300} and implicated in particular human populations at high risk for HIV infection.\textsuperscript{301,302} Collectively, these findings suggest the possibility that the incidence of natural FIV infection by vertical transmission may be underestimated, since diagnostic assays focus on antibody or virus in peripheral blood only.

Detection of virus in semen of infected cats has been observed\textsuperscript{303,304} and experimental infection by artificial insemination has also been reported.\textsuperscript{305,306} Numerous experimental studies have shown the feasibility of infection by the vaginal route\textsuperscript{16–18,21–23,307–311} and demonstrated the utility

| Property                  | FIV                                                                 | HIV-1                                                                 |
|---------------------------|----------------------------------------------------------------------|----------------------------------------------------------------------|
| **Transmission (natural)**| Blood (bite wounds), vertical (?)                                   | Blood, vertical, sexual                                               |
| **Transmission (experimental)**| Blood (bite wounds), vertical, vaginal and rectal mucosa             | Not applicable                                                        |
| **Cell tropism**          | CD4 T-cells, CD8 T-cells, B cells, macrophages, dendritic cells, microglia follicular dendritic cells CD4+CD25+ T-cells, thymocytes, megakaryocytes | CD4 T-cells, macrophages dendritic cells, microglia follicular dendritic cells thymocytes, CD8 T-cells and others (?), CD4+CD25+ T-cells (?) |
| **Tissue tropism**        | Blood, lymphoid tissues, gastrointestinal tract, CNS genital tract, liver, kidney | Blood, lymphoid tissues, gastrointestinal tract, CNS genital tract |
| **Host range**            | Restricted to felids                                                | Restricted to humans and some nonhuman primates                      |
| **Immunopathology**       | Lymphoid follicular hyperplasia (early) and CD8αβ T-cell subset expansion, followed by lymphoid depletion, CD4 T-cell depletion, cytokine dysregulation, and AIDS | Same as FIV                                                           |
of this feline animal model for examination of mucosal transmission and viral pathogenesis. Although these observations and experimental studies suggest the possibility of sexual transmission of FIV, definitive observations of natural transmission by this route so far have not been reported.

5. VIRUS INFECTION AND HOST RESPONSES

Virus Infection in Cell Culture

Primary feline PBMC activated by concanavalin A (ConA) and specific feline IL-2-dependent T-cell lines, including FET-1 cells,242 MYA-1 cells,312 FCD4E cells,201 104C-1 cells,227 and MCH5–4 cells,211 have proven to be highly permissive for propagation of biological and specific molecularly cloned isolates of FIV. As described in the section discussing FIV receptor usage, productive virus replication in these cell types results from expression of feline CXCR4 and CD134 cell surface molecules that has been confirmed, at least for activated feline PBMC and 104C-1 cells (Table II).32,227 Established feline adherent cell lines, including CrFK cells and G355-5 cells, as well as feline IL-2-independent lymphoid cell lines such as 3201 cells, MCH5-4DL, and 104-C1DL, have been used for propagation of CD134-independent isolates.13,25,42,211 Feline IL-2-independent lymphoid cell lines (FL-4 cells) chronically infected with FIV isolate FIV-Petaluma were also generated for production of virus for use in whole killed virus vaccines and diagnostics.242

Assays used to confirm virus replication and production in vitro have included FIV p24Gag antigen capture ELISAs, RT assays, indirect immunofluorescence or immunocytochemical assays for viral structural proteins, and PCR assays for viral nucleic acids. Quantitative real-time PCR assays for both FIV RNA and DNA have been developed for quantitation of viral nucleic acids of different isolates in either cell culture supernatants, plasma, or cells.11,157,190,212,309,313–316 The appearance of virus-induced cytopathic effects (CPE) consisting of syncytium, giant cell formation, and cell lysis (Table II) in feline PBMC and T-cell lines may be PBMC donor and FIV isolate-dependent and has proven less dependable for use as a marker of virus infection in these cell types.1,212,317,318 In contrast, infection of adherent cell lines CrFK and G355-5 and IL-2-independent lymphoid cells lines (3201, MCH5-4DL, 104-C1DL) with CD134-independent FIV isolates may result in prolific CPE that is most consistently observed in CrFK cells and which may serve as a indicator of infection for these isolates.13,32,211,238,241,242,248 CD134-independent FIV strains have been selected by extensive cell culture passage of biological isolates and mimic CD4-independent HIV-1 isolates that are also extremely cytopathic and efficient for replication in established T-cell lines in vitro.73
Virus Localization in the Host

Most knowledge regarding acute FIV infection and virus localization and dissemination has been derived from experimental inoculation studies using both biological and molecularly cloned FIV isolates. Factors that may affect virus localization and distribution during the acute phase of infection are virus pathogenicity, virus tropism, titer of virus inocula, presence of virus-infected cells in the inocula, route used for virus infection, and age of host. Experimental studies testing highly pathogenic and attenuated FIV isolates, viruses inoculated by parenteral or mucosal routes, and cell-free or cell-associated virus inocula have been reported. However, careful examination of effects imposed by each of these variables on viral distribution and ultimate disease, as well as mechanisms for these effects, has yet to be described. Regardless, reports so far describe a virus distribution pattern for pathogenic FIV isolates in vivo that is similar to that reported for HIV-1 (Table III).319–321

Early published experimental infection studies designed to examine FIV localization in vivo used either intraperitoneal, intravenous, intramuscular,4,7,180,200,201,322–324 intrathecal, or bone marrow inoculation198 of specific pathogen-free cats with various FIV isolates. Blood and tissues harvested from infected cats were assayed for infection by virus isolation from PBMC, viral nucleic acid detection by PCR or in situ hybridization, or viral antigen detection by immunocytochemical analyses. Later reports described experimental studies testing virus localization after virus mucosal delivery by either vaginal, rectal, or oral/nasal routes,16,18,21,23,307,309,325 in addition to either intraperitoneal or intravenous routes193,197,202,207,326–328 or intracranial injection.329,330 Virus localization in systemic lymphoid and central nervous system (CNS) tissues after FIV proviral DNA inoculation has also been examined.331,332 Collectively, these studies revealed localization of virus in PBMC and plasma, peripheral, and systemic lymphoid tissues, small and large intestinal tracts, and CNS tissues within 10 to 21 days after inoculation of the host, regardless of route of infection tested. In fact, one report described virus detection in gastrointestinal mucosa and associated lymphoid tissue by 1 to 3 days after oral/nasal infection and rapid dissemination of virus to systemic lymphoid tissues, bone marrow, and PBMC within 7 to 10 days after infection.23 Similarly, virus was detected in vaginal mucosa and spleen within 3 days after vaginal delivery of virus.23 However, virus infection of vaginal mucosa has been examined in very few studies and was observed only in those testing vaginal delivery of specific virus isolates (FIV-B-2542 and FIV-PPR)16,23,309 and was not observed after vaginal infection with other FIV isolates,18,23 or after intravenous virus infection.207 Similar to observations for the SIV animal model,299,333 experimental FIV infection by mucosal routes generally required higher titered virus inocula than
required by parenteral injection,\textsuperscript{16,309} and one study reported a higher efficiency for infection with cell-free virus inocula compared to cell-associated virus.\textsuperscript{334} Virus has also been detected in other nonlymphoid organs including liver and kidney.\textsuperscript{4,200,207} Importantly, FIV infection of the CNS has been well documented\textsuperscript{4,198,200,202,255,294,324,335,336} and utilized as an animal model for HIV-1-induced neurologic deficits.\textsuperscript{210,324,329,330,332,337–344}

Kinetics of virus emergence in blood and individual tissues has varied depending on the route of inoculation, virus strain, and infectious titer of virus inocula. Virus load in peripheral blood based on either virus isolation from PBMC or plasma viral RNA quantitation, may peak anywhere from 14 to 56 days after experimental infection.\textsuperscript{4,11,21,191,200} Significant virus loads during the acute stage of infection may also be observed in both peripheral and systemic lymph nodes, gastrointestinal tissues (predominantly submucosa and lamina propria), spleen, thymus, and bone marrow\textsuperscript{4,23,185,193,200,207} and will precede the appearance of peak viremia in peripheral blood. These findings generally mimic virus distribution during acute infection for both HIV-1 and pathogenic SIV isolates.\textsuperscript{319,321,345} It is important to note the similar robust virus replication in gastrointestinal mucosal lymphoid tissue (GALT) observed during early time points of FIV,\textsuperscript{4,200,207} SIV,\textsuperscript{346,347} and HIV-1\textsuperscript{348–350} infection. Although virus has been detected in the CNS during both acute and chronic phases of infection, quantitative data describing virus load in specific CNS tissues has been scarce. Virus loads in the PBMC and plasma generally decrease to lower set points during chronic asymptomatic infection, although this finding may be variable and dependent on virus strain pathogenicity\textsuperscript{17,212} and has not been as well defined for experimental FIV infection as reported for experimental SIV infection of rhesus macaques. FIV loads in individual tissues at sequential time points spanning acute, chronic asymptomatic, and terminal AIDS stages of disease also have not been well examined.

Similar to findings for HIV-1 and SIV infection,\textsuperscript{319–321,345,351} cellular subsets targeted during the acute stage of FIV infection have included CD4 T-cells, monocytes, macrophages, mucosal dendritic cells, mature and immature thymocytes, brain microglia and lymph node follicular dendritic cells (FDC) (Table III).\textsuperscript{4,195,194,197,199,201–204,207,208,307,327,338,352,353} As discussed in a previous section of this chapter, FIV differs from HIV-1 by exhibiting a broader tropism in vivo that also includes megakaryocytes,\textsuperscript{323} CD8 T-cells and B-cells,\textsuperscript{186,193,197,201,307} although a few reports have described either HIV-1 or SIV infection of CD8 T-cells.\textsuperscript{354,355} The variation in observations from different reports regarding the frequency and stage of infection for which virus or viral nucleic acid are detected in macrophages, CD8 T-cells, and B-cells may relate to differences in virus isolates, routes used for virus infection, age of the host, and virus detection assays. However, several studies report that either T-cells, or specifically CD4 T-cells, in both blood and
tissues, are the predominant cell type harboring virus during the acute stage of infection. Virus load in CD4 T-cell populations decrease over time as the host progresses into chronic infection, while virus loads in macrophages and B-cells appear to increase. The relationship of stage of infection to virus load in other specific cell populations, including CD8 T-cells, mucosal dendritic cells, lymph node follicular dendritic cells, thymocytes, cells within the CNS, and other nonlymphoid tissues, has received little examination so far, although one report revealed a decrease in FIV-infected CD8 T-cells in lymphoid tissues by 10 weeks after infection. A recent report described FIV infection in vivo of CD4+CD25+ T-cells, a cell population reminiscent of immunosuppressive CD4 T regulatory (Treg) cells. This CD4+CD25+ Treg cell population isolated from FIV-infected cats was shown in another recent report to coexpress costimulatory molecules B7.1, B7.2, and CTLA4 and to be anergic and resistant to clonal deletion. These findings warrant further investigation and suggest that CD4+CD25+ Treg cells may serve as an important long-lived reservoir for latent FIV in lymphoid tissues and currently are under examination as potential reservoirs for HIV-1 infection in vivo.

Clinical Disease

FIV infection results in progressive impairment of the immune system, including loss of CD4 T-cells, inverted CD4:CD8 ratios, heightened susceptibility to infectious agents, disruption of immune cell function, and deterioration of major lymphoid tissues and organs of the host. Observations from studies involving either experimental or natural infections show that FIV disease course is very similar to that induced by HIV-1 infection and can be similarly divided into four to five stages based on type and severity of the clinical signs of infection. These stages of infection have been described as acute or primary, chronic asymptomatic, persistent generalized lymphadenopathy (PGL), AIDS-related complex (ARC), and feline AIDS (FAIDS). Notably, FIV and HIV-1 infection are usually associated with a prolonged asymptomatic phase that can last 10 years or more for infected humans and cats, and which constitutes most of the lifetime of the infected cat. In contrast, pathogenic SIV isolates induce an accelerated progression of immunodeficiency, resulting in death within 2 years of infection of rhesus macaques with a relatively short or absent asymptomatic phase of infection.

The acute phase begins 1 to 4 weeks after FIV infection and may span a time period of 2 to 6 months. This stage of infection is characterized by a transient peak in peripheral blood (plasma and PBMC) virus load that is accompanied by a precipitous decline in CD4 T-cell counts and CD4:CD8 T-cell ratios. Depending on pathogenicity of virus isolate and age of the host, clinical and hematological abnormalities may include generalized
lymphadenopathy, mild pyrexia, dullness, depression, anorexia, and neutropenia. Similar to findings for HIV-1 infection, FIV pathogenicity and clinical prognosis correlate to virus replication and load and to severity of clinical signs and hematologic deficits exhibited during the acute phase of infection.11,21,185,191,192,212,313,367,368 Another property shared by FIV and HIV-1 acute infection369,370 relates to the effect of age of the host on severity of clinical and hematologic disease at the time of infection. Multiple studies have shown that severity of primary phase FIV-induced disease was increased for neonatal kittens experimentally infected at birth.190,318,371–374

Primary FIV infection recedes as the host generates virus-specific immune responses and as virus loads decrease. Concordant with emergence of antiviral immune responses and reduced peripheral blood virus loads is an increase in CD4 T-cell count and CD4:CD8 T-cell ratio, although peripheral blood CD4 T-cells counts do not usually return to preinfection concentrations. The infected cat enters a relatively asymptomatic phase of FIV infection where control of virus load by host immune responses is presumed and may last for 5 to 6 years or for a significant proportion of the remaining life span of the cat. However, long-term observation of both experimentally and naturally infected cats has shown that peripheral blood CD4 T-cell counts slowly but progressively decrease during the asymptomatic phase and that clinical disease eventually becomes apparent.8,9

Careful observation of infected cats over time may reveal the reappearance of PGL as an early manifestation of clinical disease that is associated with vague signs of disease including recurrent fevers and weight loss.9,13–15 After the appearance of PGL and other vague clinical signs, infected cats generally progress into ARC, a phase characterized by the development of chronic persistent infections with pathogens that are usually self-limiting and involve the oral cavity, upper respiratory tract, ocular tissues, skin, and other body sites. Progression from ARC to FAIDS may be distinguished by infections with opportunistic pathogens, severe wasting, neoplastic disorders including non-T-cell lymphomas, neurologic disease, leukopenia, and anemia. Virus load increases and severe depletion of peripheral blood CD4 T-cells are observed. Survival time is usually less than a year after the onset of FAIDS.

**FIV Immunodeficiency and Pathology**

A gradual but progressive CD4 T-cell depletion that mimics primary immune deficiency observed in HIV-1 AIDS is the hallmark of immunodeficiency associated with both experimental and natural FIV infection in cats.5–8,10,11,21,185,190,193,200,212,361,375 CD4 T-cell subset depletion has been observed in peripheral blood and lymphoid tissues including thymus22,193,374 during both early and chronic stages of infection (Table III). An increase in CD8 T-cell concentration is frequently associated with reduc-
tion in CD4 T-cell counts and either contributes to, or largely accounts for, lower CD4/CD8 T-cell ratios observed during acute and chronic infection with either FIV or HIV-1. This elevation in the CD8 T-cell count is due to an increase in a CD8αβ\textsuperscript{low} subset that is concurrent with a reduction in CD8αβ\textsuperscript{high} of CD8 T-cells and involves lymphocyte populations from peripheral blood, lymph nodes, and thymus. A similar expansion of an CD8αβ\textsuperscript{low} T-cell subset has also been observed in HIV-1 infection. Furthermore, this cell population has been shown to express markers associated with lymphocyte activation and adhesion and to exhibit antiviral activity comparable to a noncytolytic CD8 antiviral activity previously described for HIV-1 infection.

FIV-induced immunodeficiency is characterized by other defects that are similar to those described for HIV-1 infection and include reduced proliferative T-cell responses to mitogens, dysregulation of cytokine networks, and humoral immune response deficits. Several studies have revealed significantly reduced mitogen-induced blastogenic responses from T-cells isolated from cats during acute and chronic FIV infection, a finding that may result from virus-associated defects in T-cell growth and proliferation, as well as defects in cell surface expression of receptors required for transmission of antigen/mitogen signals. Although cytokine responses in different lymphoid tissues harvested from cats acutely infected with FIV have been shown to be heterogenous, findings from a number of reports suggest that dysregulation of cytokines such as IL-10, tumor necrosis factor (TNF)-alpha, IL-6, IL-1, and interferon gamma may play a role in FIV-induced immune deficiency. Deficits in generation of antibody responses to multiple antigens and specifically to T-cell-dependent immunogens have also been reported for FIV-infected cats. Bone marrow abnormalities that may result in neutropenia, leukopenias, and pancytopenias, as well as deficits in neutrophil and monocyte/macrophage function, may further impair immunologic function in FIV-infected cats.

Histologic lesions associated with FIV infection are predominantly localized to lymphoid tissues including GALT for both early and later stages of FIV infection, although severity of acute stage lesions is dependent on virulence and titer of the infecting FIV isolate. Lymph node abnormalities during the acute stage of infection include a mixture of follicular hyperplasia and lymphoid depletion resulting in loss of lymph node architecture and medullary plasmacytosis. Lymphoid hyperplasia has also been observed in other lymphoid tissues including spleen, bone marrow, and mucosal-associated lymphoid tissue. Several studies have shown the thymus to be a primary target during early FIV infection, with pathologic changes that include thymic atrophy, thymitis, medullary B-cell hyperplasia, and cortical involution. Lesions in the gastrointestinal tract have frequently been observed and include severe inflam-
mation, necrosis, and villous atrophy. Brain and lung are non-lymphoid tissues that also frequently exhibit pathologic changes. CNS lesions have consistently included gliosis, glial nodules, and perivascular cellular infiltrates and less frequently included inflammatory changes associated with encephalitis and meningitis and neuronal abnormalities such as neuronal stress and neuronal satellitosis. Giant cell formation is a frequent lesion in SIV and HIV-1-associated neuropathology but has only rarely been observed by histologic examination of CNS tissues from FIV-infected cats. Pulmonary lesions associated with FIV infection have consisted of inflammatory infiltrates suggestive of pneumonia.

Lymphoid depletion and involution within multiple lymphoid tissues have been dominant and consistent findings in tissues harvested from cats during the later stages of FIV infection. Neoplastic lesions, including B-cell lymphomas and other sarcomas, have also been frequently observed in later stages of both experimental and natural FIV infection. All together, these histologic lesions of lymphoid hyperplasia frequently characterized as B-cell hyperplasia mixed with severe lymphoid depletion, as well as thymic atrophy and the severe inflammatory lesions observed in the gastrointestinal mucosa, mimic pathological changes reported for both HIV-1 and pathogenic SIV and further support the use of the FIV animal for examination of HIV immunopathogenesis.

Mechanisms for either FIV or HIV-1-induced CD4 T-cell depletion and dysfunction in vivo most likely involve multiple processes such as direct cytolysis from virus infection or from virus-specific immune responses, and indirect strategies including chronic immune activation, cellular dysregulation, and inappropriate killing of uninfected bystander cells. In vitro syncytia formation of FIV-infected cells has been well documented and is due to viral envelope fusion with cellular membranes and associated with specific cell types and FIV strains. Programmed cell death or apoptosis has been hypothesized as one of several important mechanisms involved in FIV/HIV-1 immunopathogenesis based on observations of apoptotic cells in virus-infected cell cultures and in PBMC and lymphoid and thymic tissues isolated from FIV-infected cats. Recent reports have shown that binding of cell surface chemokine and virus receptor CXCR4 by FIV TM may trigger apoptosis. Although some recent reports suggest that the Fas-TNF-α receptor pathway may be important for FIV-induced apoptosis, mechanisms by which FIV uses this pathway for apoptosis are not well understood and are currently under investigation.

A second probable pathway for FIV-induced apoptosis involves the B7-CTLA4 pathway. Data from recent reports indicate that PBMC and lymph node lymphocytes isolated from FIV-infected cell cultures in vitro or freshly isolated from FIV-infected cats are down-regulated for expression of
costimulatory molecule CD28. These cells are instead up-regulated for expression of T-cell costimulatory molecule CTLA4, a cell surface molecule expressed on activated T-cells and reported to induce anergy in activated T-cell subsets.\(^{432-435}\) Furthermore, these reports presented flow cytometric analyses that verify induction of apoptosis in FIV-infected cultures by T-cells that coexpress CTLA4 and B7 cell surface molecules and suggested that CD4 T-cell depletion and lymph node apoptosis in vivo may partially result from chronic B7-CTLA4-mediated T-cell interactions.\(^{357,432,434}\) Similarly, reduced CD28 expression and increased CTLA-4 expression have been observed in HIV-1 infection\(^{436-440}\) and hypothesized to promote immune hyporesponsiveness and apoptosis through binding of CTLA4 with B7 costimulatory molecules. Although mechanisms by which FIV infection induces an increase in B7+CTLA4+ T-cell populations in vivo are not well understood, chronic immune activation in the virus-infected host has been hypothesized as one possible etiology for this effect. These observations further illustrate similarities in FIV and HIV-1-associated disease and potential value of the FIV animal model for in vivo studies focused on mechanisms of immunopathogenesis of lentivirus-induced acquired immunodeficiency.

**Immune Responses**

Emergence of cellular and humoral host immune responses during primary infection with either FIV or HIV-1 coincides with reduction in peak peripheral blood virus loads.\(^{14,17,24}\) These responses are thought to be important for controlling virus replication during acute and chronic stages of infection based on CD8 T-cell and B-cell depletion studies involving SIV-infected rhesus macaques.\(^{441-443}\) Regardless of robust virus-specific immune responses, most infected hosts fail to eliminate the virus, leading to prolonged clinical latency, eventual immunologic exhaustion with subsequent increasing virus loads, and AIDS. Currently, factors responsible for this inability of host responses to effectively clear virus during primary infection are not well understood. Identification of such factors will be necessary for understanding immunopathogenesis of lentiviral infections and design of vaccines capable of inducing sterilizing immunity.

Based on experimental inoculation studies, antibodies to viral proteins SU gp95, CA p24, and MA p14 are the first to appear in serum, usually within 2 to 4 weeks after infection, and are quickly followed by the appearance of antibodies to TM gp40, Gag precursor p50, and reverse transcriptase.\(^{14,28,444,445}\) Lentiviral Gag proteins are highly expressed immunogenic proteins and FIV-infected cats typically exhibit high titers of antibody specific for viral CA p24. Four B-cell epitopes have been mapped for FIV CA using mouse monoclonal antibodies.\(^{446}\) However, evidence that CA-specific antibodies function in either FIV or HIV-1 clearance has been scarce. Inter-
Interestingly, a recent examination of serological responses in HIV-1-infected patients undergoing prolonged antiretroviral therapy with structured treatment interruptions (STIs) showed that kinetics of CA-specific antibody responses revealed clear differences in patients’ immune functions. Patients exhibiting rapid and large increases in CA antibody responses also experienced significantly decreased viral set points. Antibodies specific to CA have not been shown to express significant antiviral activity, but may instead reflect enhanced virus-specific CD4 helper activity that is predictive of the capabilities of the host’s antiviral immune responses. Similar careful analyses of FIV Gag antibody responses have not been reported but may be useful for characterizing the value of these responses as markers that aid in distinguishing protective from nonprotective vaccine-induced immune responses.

Only antibodies that bind to surface domains of the envelope are thought to exhibit virus neutralizing activity, and accordingly, identification of envelope B-cell epitopes is critical to the characterization of potentially protective host immune responses. Multiple reports have identified the V3 region of SU, carboxy terminal of SU, and a highly conserved PID located within the ectodomain of TM as major immunodominant domains of FIV envelope (Figure 3). Four or more linear B-cell epitopes have been mapped within the V3 domain of SU, and other SU epitopes are localized within V4 and V5 domains. B-cell epitopes within extracellular TM include the PID previously discussed, a second domain within the extracellular membrane-proximal domain of TM that is downstream of the PID, and a third domain within the intracytoplasmic region of TM. In contrast, the tryptophan-rich motif within the extracellular domain of TM that functions in virus fusion and infectivity was not found to be immunogenic.

Although the V3 immunodominant domain is the only FIV Env determinant consistently shown to induce antibodies capable of neutralizing virus in vitro, determinants within domains V4 and V5, including residues 481 and 551, were also shown to confer broad neutralization resistance (BNR) in primary isolates passaged in vivo (Figure 3). The remaining FIV envelope linear epitopes mapped by binding assays are most likely inaccessible for neutralization due to the complex oligomeric structure and extensive glycosylation of native FIV SU, a property shared with HIV-1 SU. Expression of both neutralizing epitopes and tropism determinants by the FIV hypervariable V3 domain is an important function also described for the V3 domain of HIV-1 surface glycoprotein. It is also significant that FIV SU linear epitopes, capable of inducing neutralizing antibodies, are located within hypervariable regions of envelope that may change in response to selective pressures in vivo.

Importantly, an epitope encoded within the FIV extracellular membrane-proximal domain of TM (designated as the TM3 epitope) is
similarly positioned to that of a HIV-1 epitope (2F5), which is recognized by an extremely potent broadly neutralizing HIV monoclonal antibody (Figure 3). Although feline antisera raised against this FIV TM epitope was not shown to exhibit virus neutralizing activity in vitro, a peptide vaccine based on this epitope was shown to be capable of inducing partial immunity to FIV challenge. These observations suggest that this extracellular membrane-proximal TM3 domain may encode a neutralizing epitope conserved across multiple lentiviruses and may support further investigation of this TM determinant in FIV vaccine design.

Neutralizing antibodies emerge in the host as peak peripheral blood virus loads decline during primary infection and are thought to contribute to control of virus load and replication in lentivirus infections including HIV-1, SIV, and FIV. Control of infection by neutralizing antibody is also based on CD8 T-cell and B-cell depletion studies of rhesus macaques infected with either SIV or chimeric SIV/HIV-1 (SHIV) isolates and on reports describing successful passive immunization of either neonatal kittens or neonatal macaques with hyperimmune serum. However, despite apparent successes observed recently with therapeutic administration of exogenous highly potent neutralizing antibodies in nonhuman primate animal models, antibodies induced in the host by virus infection have been consistently ineffective in virus clearance, particularly within lymphoid tissue reservoirs. Furthermore, FIV and HIV-1 assay systems using established cell lines, rather than primary lymphocytes for virus infectivity or highly passaged viruses compared to primary strains directly isolated from infected cats, generate significantly different data regarding neutralization activity for identical serum samples. Notably, previous studies have shown that both HIV-1 and FIV primary isolates are resistant to neutralization by autologous sera, especially when primary PBMC are used to assay infectivity. However, more recent findings regarding HIV-1 neutralizing antibody biology and use of more sophisticated approaches for analysis of antibody activity have triggered renewed interest and support for investigation of humoral immune responses in virus control during acute and chronic HIV-1 infection and for HIV-1 vaccine design. Similar investigations seem warranted in the FIV animal model, especially for examination of antibody kinetics and specificity associated with mucosal versus parenteral routes of virus exposure and for investigation of epitopes conserved across different virus clades as vaccine immunogens.

Strong supporting data exist for CD8 T-cell-mediated suppression of virus load in both HIV-1-infected patients and SIV-infected rhesus macaques. Investigation of FIV-specific cellular immune responses has previously been restricted by a deficiency of key feline-specific reagents, including antibodies specific for feline cell surface markers and cytokines, as well as a lack of knowledge of FIV T-cell epitopes. However, recent peptide mapping of FIV-specific T-cell epitopes and
established assays for FIV-specific CD4, CD8, and cytotoxic T-cell (CTL) activity have now permitted some characterization of cellular immune responses induced by either virus infection or vaccination. Furthermore, a report showing that adoptive transfer of blood cells isolated from FIV-vaccinated cats induced resistance in MHC-matched recipient cats to FIV challenge infection, provides some evidence of the importance of virus-specific cellular responses in virus clearance.

Longitudinal examinations of cats experimentally infected with FIV reveal virus-specific CTL activity in PBMC within 2 to 7 weeks after infection, time points in primary infection that coincide with rising virus loads and are similar to those reported for CTL emergence in HIV-1 infection. Virus-specific CTL activity has also been detected in lymphocytes isolated from lymphoid tissues including systemic and peripheral lymph nodes and spleen during the primary phase of infection. Assay for CTL activity during chronic stages of infection at 47 and 127 weeks after virus exposure demonstrated persistence of cellular responses, although detection of antiviral CTLs were more consistent from lymphoid tissues compared to peripheral blood. Activity specific to FIV Gag rather than Env was more consistently observed in FIV-infected cats described in these reports. However, currently described protocols testing for FIV-specific CTL by chromium release assay are restricted by use of only two immunogens provided by vaccinia recombinant viruses, one of which expresses a Gag protein derived from isolate FIV-GL14 (subtype A) and the other expressing an Env protein encoded by FIV-Petaluma (also subtype A). Therefore, lower Env-specific CTL activity may be partially attributed to deficient assay detection due to variability within recently mapped Env-encoded T-cell epitopes, since cats described in these reports were experimentally infected with FIV isolates distinct from FIV-Petaluma. Measurement of CTL responses to variable proteins such as Env will require virus-specific reagents for optimal assay sensitivity. Characterization of FIV-specific CTL responses will also call for assay of activity against other viral proteins including RT, Rev, and Orf-A. Although limited mapping of Env T-cell epitopes was previously reported, a recent report described peptide mapping of FIV T-cell epitopes across all FIV genes using a feline interferon gamma ELISpot assay. These newly identified epitopes will facilitate use of peptides for other FIV CD8 assays and further characterize FIV CD8 responses to different FIV isolates, as well as examine the occurrence of FIV CTL epitope escape variants, a well-described trend in SIV and HIV infection. Another unique test for FIV-specific CTL activity involved assay of perforin expression and may also be useful for future examination of FIV-specific CD8 immune responses.

Interestingly, a CD8α+ βlow T-cell subset associated with antiviral activity in FIV-infected cats has actually been more carefully examined than the topic of virus-specific CTL activity. This cell population has also been
described for HIV infection and was initially distinguished for its expansion in peripheral blood during the primary phase of FIV infection and for a capacity to suppress virus replication in cultured PBMC by release of a soluble factor in vitro. Significantly, appearance of this cell population correlated with reduction of peak PBMC-associated virus, suggesting possible antiviral activity in vivo as well as in vitro. Other FIV infection studies have confirmed an expansion of this CD8αβlow T-cell subset and also reveal expression of lymphocyte activation and adhesion markers by this subset. However, these studies also show conflicting data on the issue of a restriction of antiviral activity to the CD8αβlow subset with some data suggesting that both CD8αβlow and CD8αβ+ subsets are capable of noncytolytic antiviral activity.

A lack of agreement is also apparent among studies regarding correlations between CD8 T-cell noncytolytic antiviral activity and either CD4 T-cell counts, clinical disease in vivo, distribution patterns of this cell subset in blood and lymphoid tissues, or persistent expansion of the CD8αβlow subset throughout the course of virus infection. More recent reports indicate that CD8 antiviral activity production could be enhanced or induced in vitro by exposure to either virus-infected cells or to cells expressing an irrelevant antigen, but is not induced by mitogen activation. These conflicting reports indicate that additional studies using standardized methodologies will be needed for a more precise definition of this CD8 T-cell antiviral activity. However, data generated from almost all studies reported so far suggest that this noncytolytic CD8 antiviral activity is reminiscent of a still undefined secreted CD8 antiviral factor (CAF) previously described for HIV-1 infection. Noncytolytic CD8 antiviral factors associated with both HIV-1 and FIV infections, although not clearly defined, have been hypothesized to be associated with the innate immune system. Multiple factors proposed in previous reports to represent human CAF include human β-chemokines RANTES, MIP-1α, MIP-1β, MCP-1, SDF-1, and alpha defensins. These proteins, however, do not meet the criteria of CAF definition, either due to their biochemical nature or because they are not exclusively expressed by CD8 T-cells. Observation of CD8 T-cell antiviral activity in both HIV-1 and FIV infection confirms the importance of this host immune response to lentivirus infection and provides another opportunity for use of the FIV animal model for assessment of HIV-1 immunopathogenesis.

6. FIV VACCINE DEVELOPMENT

A safe efficacious vaccine that prevents the spread of HIV will be essential to arresting the spread of the AIDS epidemic. Studies with nonhuman primates and SIV and chimeric SHIV isolates have demonstrated that
live-attenuated viruses are highly effective; \textsuperscript{505–507} however, such vaccines maintain a low level of pathogenicity.\textsuperscript{508–510} Other vaccine trials in the rhesus macaque animal model have described noninfectious DNA vaccines that control viremia and suppress clinical disease but do not induce sterilizing immunity against SIV/SHIV infection.\textsuperscript{511–513} FIV vaccine research and development have been fairly well supported due to the value of this animal model for HIV vaccine development and to the significance of FIV as a natural pathogen in cats. A wide variety of vaccine approaches have been examined, although efforts have concentrated on particular vaccine methods including whole killed virus (WKV)-based vaccines, DNA vaccines, and viral protein subunit vaccines.\textsuperscript{514} A commercial FIV vaccine (Fel-O-Vax FIV, Fort Dodge Animal Health), containing whole killed viruses representing two distinct FIV subtypes, was approved by the USDA in 2002 for use in the domestic pet cat population and is one of only two commercial lentiviral vaccines currently in use, including a live attenuated EIAV vaccine widely used in China.\textsuperscript{515,516} However, use of this FIV WKV vaccine is still not widely accepted due to significant issues including interference of vaccine-induced antiviral antibodies with commercial FIV diagnostic assays\textsuperscript{517} and the potential of vaccine-induced enhancement of virus infection.\textsuperscript{485,518–520}

**FIV WKV and Fixed-Cell Vaccines**

FIV immunization studies based on conventional WKV or fixed virus-infected cell (FC) vaccines have produced a diverse array of experimental findings. Vaccine efficacy for different and identical FIV WKV-based vaccines has varied greatly, most likely due to modifications of vaccine inactivation procedure, producer cell types used, vaccination schedule, vaccine adjuvants, vaccine doses, routes of challenge, and variability in virulence or subtypes of challenge FIV isolates.\textsuperscript{514} WKV-based vaccine studies have reported both complete or partial protection against challenge with homologous and sometimes heterologous FIV isolates and have also described vaccine-induced enhancement of FIV infection. Interestingly, enhancement of challenge virus infection has been more frequently associated with FC vaccines prepared with autologous feline lymphocytes.\textsuperscript{485,518,519} In contrast, FC and WKV vaccines based on virus-infected cell lines have proven efficacious, although protection may be considerably reduced against challenge with either heterologous or more virulent FIV isolates.\textsuperscript{487,521–526} The current commercial FIV WKV vaccine is composed of two isolates including FIV-Petaluma subtype A and FIV-Shizuoka subtype D, specifically to broaden virus-specific immune responses, and has demonstrated improved protection against multiple subtypes when compared to single subtype WKV vaccines.\textsuperscript{487,527} Use of a FIV WKV vaccine as a booster to a priming
immunization with a recombinant canarypoxvirus (ALVAC)-based FIV vaccine also improved protective responses against challenge with isolates distinct from the vaccinating strains. Duration of WKV and FC vaccine-induced protection has been another concern, with some vaccine studies revealing a breakthrough in vaccine-induced protection a year after vaccination despite boosting the primary immunization. Another concern relates to differences in WKV or FC vaccine-induced protection observed against similar challenge viruses but delivered by different routes of exposure, including parenteral and mucosal delivery. Collectively these findings suggest that WKV vaccines demonstrate significant potential for development of lentivirus vaccines, but that multiple issues including WKV vaccine-induced enhancement still require attention for achievement of optimal protection.

Conclusions regarding neutralizing antibodies as immune correlates of vaccine protection frequently varied in early reports describing WKV and FC FIV vaccines. Nonetheless, other early studies, as well as more recent reports, present findings that suggest a correlation between appearance of virus neutralizing antibodies and WKV and FC vaccine-induced protection. WKV and FC vaccines have also induced potent cellular immune responses that are thought to be important correlates of protection. The current commercial FIV WKV vaccine containing inactivated whole viruses of subtypes A and D, elicited strong cellular responses against both vaccine strain viruses and moderate neutralizing antibody activity, particularly when commercial Fort Dodge vaccine adjuvant FD-1 was supplemented with human IL-12. In fact, the commercial dual subtype FIV WKV vaccine administered with human IL-12 provided broad protection against homologous and heterologous virus challenges containing in vivo-derived FIV inoculum. However, the actual contribution provided by cellular and humoral immune responses to protection induced by this vaccine approach is still not clear and may ultimately require immune cell depletion studies for determination. Despite reports of FIV WKV and FC vaccine efficacy, acceptance of this vaccine approach in the veterinary community is restricted by concern of vaccine antibody interference with FIV antibody-based diagnostics. Furthermore, exploration of WKV vaccines in the SIV and SHIV animal model has been extremely limited and was discouraged by early studies showing SIV WKV vaccine-induced protection resulted from immune responses to cellular antigens in vaccine preparations, rather than to viral antigens. However, renewed enthusiasm for vaccines that induce strong neutralizing antibody responses has lent increased support for multiple modality vaccine approaches that include WKV as a component. Inclusion of a WKV vaccine as a component is expected to enhance and broaden virus-specific humoral immune responses induced by other vaccine components such as
live viral vectors and DNA. The sizable body of data already generated for FIV WKV and FC FIV lends strong support for use of the feline animal model for further investigation of WKV and FC vaccine approaches for HIV-1.

FIV Subunit and Peptide Vaccines

Various FIV subunit vaccine approaches have been tested including SU (primarily V3 and C2 epitopes), TM and Gag peptides, recombinant SU proteins generated from bacterial expression plasmids administered as single immunogens, or in combination with recombinant SU proteins expressed from vaccinia or baculovirus vector systems and immunoaffinity-purified SU from FIV-infected cell lysates. Absence of protection against FIV challenge, observed with many of these frequently highly immunogenic subunit approaches, recapitulates findings reported for subunit vaccine approaches in the nonhuman primate model. However, partial protection against challenge infection was revealed in a few reports describing immunization with either immunoaffinity-purified SU or SU proteins conjugated to autologous erythrocytes via biotin-avidin-biotin bridges, although immune correlates of protection were not determined. As described in the section on immune responses, a FIV peptide vaccine based on an extracellular TM3 epitope, similarly positioned to that of the highly neutralizing HIV-1 2F5 epitope, was also shown to be capable of inducing partial immunity to FIV challenge. Vaccine-induced enhancement of FIV challenge virus infection has also been observed, with a subunit approach consisting of coimmunization of a bacterially expressed full-length envelope protein with either SU or SU-TM vaccinia recombinant proteins. However, coinoculation of recombinant SU proteins, with a plasmid expression vector encoding the FIV NC protein, abrogated vaccine-induced enhancement of challenge infection that was observed for vaccination with recombinant SU alone and actually improved vaccine efficacy in another study. Loss of vaccine-induced enhancement and increased vaccine efficacy associated with coinoculation of NC expression plasmid with recombinant SU were hypothesized to result from a plasmid DNA adjuvant effect based on altered cytokine expression profiles and the absence of FIV NC-specific immune responses associated with this approach. All together, these data do not support use of vaccines consisting of single viral protein subunits. Instead, these findings suggest that FIV may provide another vaccine model for testing peptides encoding broadly reactive neutralizing epitopes as components of a multiple modality vaccine that also includes immunogens for induction of cellular responses, a balanced vaccine approach currently of interest in HIV-1 vaccine design.
FIV DNA Vaccines

Assessment of other vaccine approaches, including DNA vaccines, attenuated virus vaccines, and live attenuated viral or bacterial vectors for expression of FIV antigens, is still somewhat limited at this time. FIV DNA vaccine approaches, based on deletion mutants of FIV provirus plasmids, have shown considerable efficacy when challenged with less virulent FIV isolates. DNA vaccines, including either defective proviruses or SU and TM expression cassettes along with feline cytokine expression vectors, have shown particular promise in limited studies. Extremely low or absent humoral immune responses observed with FIV DNA vaccines may partially result from failure to incorporate codon optimization of viral structural genes encoded by these vaccine plasmids, a process found critical for improved immunogenicity of HIV-1 DNA vaccines that do not coexpress the viral Rev protein. DNA vaccine efficacy has been observed despite low antibody responses and may be attributed in some measure to strong virus-specific CTL responses, despite the lack of a clear correlation between measured CTL activity and DNA vaccine-induced protection. Unfortunately, DNA vaccine-induced protection has proven to be reduced or negligible against more pathogenic isolates. Moreover, DNA vaccines encoding envelope genes used without cytokine adjuvants have been associated with enhancement of challenge virus infection.

FIV Vaccines Using Live Viral and Bacterial Vectors

Mixed results regarding efficacy have been reported for the few FIV vaccines utilizing either live viral or bacterial vectors. Immunization of cats with a replication-defective adenovirus type 5 expressing FIV Env was poorly immunogenic and failed to induce protection against FIV challenge. A vaccine composed of Venezuelan equine encephalitis (VEE) virus replicon particles engineered to express the FIV matrix/capsid region of Gag and full length Env also failed to induce protection against FIV challenge, although the vaccine elicited both humoral and cellular responses. A combination vaccine using priming inoculations with a feline herpes virus (FHV) vector expressing the FIV gag gene and a FHV vector expressing FIV env and booster inoculations with a FIV FC vaccine also failed to protect immunized cats from FIV challenge infection. In contrast, partial protection against FIV challenge was observed for cats immunized with live attenuated Salmonella typhimurium aroA strains expressing FIV CA and truncated SU encoding hypervariable regions V3–V5, although correlates of protection were not defined. Partial protection against challenge was also observed by vaccination of cats with a recombinant canarypoxvirus (ALVAC)-based FIV vaccine used alone or in combination with a FIV FC vaccine. Furthermore, a single oral immu-
nization of cats with a live recombinant *Listeria monocytogenes* strain, which both expresses FIV Gag and delivers an FIV truncated Env-expressing plasmid, was found to confer protection by reduction of virus load and virus-induced disease after FIV challenge. The limited number of FIV vaccine studies utilizing live viral vectors is surprising, given the interest and success of multiple modality vaccines consisting of live recombinant viral vectors and DNA expression plasmids recently observed for the SIV/SHIV animal models.

**FIV Attenuated Virus Vaccines**

Attenuated live virus vaccines have been extensively examined in the SIV/SHIV animal models due to consistent efficacy shown by these viruses for inducing protection against challenge with highly pathogenic SIV and SHIV isolates. A widely used vaccine based on an attenuated strain of equine lentivirus EIAV has protected 75 million horses and donkeys over the past 30 years in China. Studies describing efficacious molecularly cloned attenuated EIAV and CAEV vaccine viruses have also been reported. Examination of attenuated FIV vaccines has been limited but shown that this approach is also effective for the feline AIDS animal model. The FIV-Petaluma virus variant used in the commercial FIV WKV vaccine was recently shown to establish a low-level infection of cats without induction of apparent FIV-associated disease. Furthermore, infection with the attenuated FIV-Petaluma variant protected against infection with a different pathogenic FIV isolate (FIV GL8), although of the same subtype. Complete protection was conferred by this attenuated FIV against FIV GL8 delivered by the intraperitoneal route and partial protection was demonstrated against challenge by a mucosal route. Complete protection was also imparted against wild-type FIV challenge by inoculation of cats with infectious molecularly cloned FIV deletion mutants including a LTR mutant encoding a deletion of the AP-1 site and a *vif*-deleted provirus (FIV-Dvif) administered as a DNA vaccine. Lastly, superinfection of domestic cats with either nonpathogenic lion or puma nondomestic feline lentiviruses has been shown to confer resistance to infection with pathogenic domestic FIV. Immune correlates were not apparent from any of these attenuated FIV vaccine studies. In fact, the substantial genetic variation observed between domestic and nondomestic feline lentiviruses argues against initial control of challenge virus infection driven by epitope-specific immune responses and instead suggests currently undetermined resistance factors or mechanisms possibly associated the innate immune system. An important limitation of attenuated lentiviral vaccines is the concern for long-term safety as demonstrated by experiments performed by Ruprecht and coworkers, which showed that a SIV *nef* deletion mutant may cause fatal AIDS-like disease in newborn macaques and may become pathogenic after
Globally, there is a rich history of long-term infection of adult macaques. Regardless of whether attenuated lentivirus vaccines will ever be safe enough for general use, examination of these viruses in animal model systems, including FIV, offers an important opportunity to identify and fully characterize immune responses and undefined host resistance factors that confer protection against pathogenic virus infection or disease.

Critical Issues for FIV Vaccine Development

FIV vaccine development faces critical issues that are very similar or identical to those facing HIV-1 vaccines. Characterization of immune correlates of protective immunity for any of the FIV vaccine approaches examined so far has been elusive, a situation that is common to all lentivirus vaccines including those for SIV and SHIV, and must be addressed for successful HIV-1 vaccine design in the future. Vaccine-induced enhancement of pathogenic virus challenge has been consistently observed with FIV and EIAV vaccine approaches, involving either WKV, FC, or protein subunits/peptides and for FIV, was thought to result from enhancing antibodies that might be specific to epitopes within Env hypervariable regions V3–V5 or to the PID epitope. However, vaccines using recombinant Env proteins with regions V3–V5 deleted were shown to remain capable of inducing enhancement of challenge infection. Occurrence of FIV DNA vaccine-induced enhancement of challenge virus infection despite negligible antibody responses, also suggested mechanisms other than enhancing antibodies, such as lymphoid activation. A very recent report described enhancement of challenge virus infection after vaccination of rhesus macaques with an attenuated recombinant varicella-zoster virus vaccine expressing SIV Env. Vaccine-associated enhancement in this study correlated with appearance of robust anamnestic virus-specific CD4 proliferative responses in the absence of strong CD8 responses and again suggested that immune activation may play a role in vaccine-induced enhancement along with cellular responses skewed against CD8 T-cells. These findings suggest major concerns for clinical testing of HIV-1 vaccines for which less than optimal CD8 responses are already predicted and validate use of the FIV animal model for characterization of this potentially devastating complication of HIV vaccine use. A third issue of importance for both future and the current commercial FIV vaccine design as well as future commercial HIV-1 vaccines, concerns interference of vaccine-induced antibodies with FIV diagnostics. All lentivirus diagnostic assay systems utilize seropositivity as a marker for virus infection. PBMC virus isolation and plasma viral RNA detection systems using PCR, although specific, are not sufficiently sensitive to detect all virus infections or currently feasible as routine diagnostic assays. Future HIV-1 vaccine design will even-
tually need to address this issue for which the FIV animal model may prove most useful.

7. FIV AND ANTIVIRAL THERAPIES

Relatively soon after the initial isolation and characterization of FIV, considerable efforts were focused on characterizing this lentivirus as an animal model for antiviral therapies for HIV-1. These studies were encouraged by similarities between FIV and HIV-1 RT in amino acid sequence, structure and physical properties, catalytic activities, and susceptibility to multiple nucleoside analogs, including AZT, 3TC, 9-(2-phosphonomethoxyethyl)adenine (PMEA) and (R)-9-(2-phosphonylmethoxypropyl)-2,6-diaminopurine {((R)-PMPDAP)}, as discussed earlier in this chapter. Conversely, FIV has proven relatively resistant to therapies including nonnucleoside RT inhibitors and protease inhibitors used in highly active antiretroviral therapy (HAART) protocols for HIV-1-infected patients. Nevertheless, ongoing studies are evaluating protease inhibitors in vivo that show activity against proteases of multiple lentiviruses in vitro, as well as drugs that target lentiviral TM fusion domains conserved across different lentiviruses. Identification of such compounds will be important for design of efficacious HAART protocols for FIV infection and continued development of FIV as a model for HIV-1 antiviral drug therapy.

Examination of FIV susceptibility to AZT in vitro resulted in the first description of emergence of an AZT-resistant lentivirus through virus passage in cell culture and led to a rigorous examination of multiple drug-resistant FIV RT mutants that arise in vitro. Although FIV resistance to AZT did not map to homologous residues within AZT-resistant HIV-1 RT mutants, both FIV and HIV-1 do share a similar determinant (M184 in the YMDD active site of the RT palm subdomain) for susceptibility to nucleoside analog 3TC. Accordingly, a Met-to-Thr mutation in the YMDD motif of RT has been observed in 3TC-resistant mutants for both FIV and HIV-1. Studies assessing drug-resistant FIV mutants in vivo have been very few but have revealed attenuation of an AZT-resistant FIV when inoculated into cats. These findings recommended the potential use of this model for characterizing pathogenesis of other FIV variants resistant to drugs, targeting domains conserved between lentiviral enzymatic proteins.

Examination of FIV susceptibility to nucleoside analogs AZT, PMEA, and (R)-PMPDAP in vitro has been complemented by a variety of in vivo studies that illustrate the value of this animal model for HIV-1 antiviral drug development and assessment of drug efficacy. Studies testing the merit of
AZT as monotherapy for either reducing virus load, improving CD4:CD8 T-cell ratios, or clinical status in both experimental primary infection and chronic natural infection with FIV have produced somewhat mixed results. Altogether, however, investigations have revealed fairly limited efficacy for AZT used as a single therapeutic regimen for FIV infection, as previously found in HIV-1 infection, and frequently reported side effects such as anemia that are also observed in human patients.580–587 Furthermore, a single report examining combination therapy of AZT and 3TC for cats either acutely or chronically infected with FIV described only slightly improved efficacy compared to AZT treatment alone.588 In contrast, nucleoside analog PMEA and its derivative (R)-PMPDAP have proven very efficacious as antiviral therapies for FIV infection both in vitro and in vivo by significantly reducing virus load and improving CD4:CD8 T-cell ratios.572,573,575,581,580 Combination therapy consisting of AZT, 3TC, and a third nucleoside analog abacavir also blocked FIV replication in a synergistic manner in vitro.589 These observations suggest that this drug combination or combinations including PMEA may warrant future assessment as HAART protocols using the FIV animal model.

A shortage of HIV-1 protease inhibitors that effect FIV replication due to differences in substrate specificity displayed by FIV PR and HIV-1 PR90–95 has hampered use of the FIV animal for analysis of combination drug protocols used in HAART protocols for HIV-1-infected patients. However, attempts to identify a protease inhibitor universally active against multiple lentivirus PRs revealed that a statine-based inhibitor LP-13096 and a C2-symmetric competitive inhibitor identified as TL-393,592 were both capable of inhibiting PR expressed by HIV-1, SIV, and FIV. Interestingly, comparison of crystal structures of FIV PR and HIV-1 PR in complex with TL-3 reveals differences in the position of the flaps in FIV PR and HIV-1 PR, whereas complexes of FIV PR and HIV-1 PR with inhibitor LP-130 are nearly identical in conformation.593 Recent reports have shown TL-3 to inhibit FIV and HIV-1 replication and to be active against protease inhibitor-resistant HIV-1 mutants in vitro.93,592 Furthermore, TL-3 treatment of FIV-infected cats reduced virus load and disease, including clinical neurologic dysfunction and severe acute phase immunodeficiency.343,594 These findings support further testing of TL-3 in combination drug protocols for FIV infection both in cell culture systems and in vivo. Assessment of combination protocols with TL-3 may be particularly warranted for testing a proposed hypothesis that therapy with compounds broadly reactive against proteases of multiple lentiviruses will less likely be associated with emergence of protease inhibitor-resistant virus mutants in vivo.

Recent preliminary studies have also described testing synthetic peptides targeted to determinants within the FIV TM ectodomain, such as the heptad repeat 2 (HR2) domain595 and the membrane-proximal tryptophan-rich region138,416 (Figure 3) as antiviral therapeutics. Previous studies
showed that HIV-1 peptides derived from the HR1 and HR2 regions are potent inhibitors of HIV-1 infection and function by blocking virus-mediated cell fusion. Similarly, the membrane-proximal tryptophan-rich region immediately downstream of HR2 within the TM ectodomain has also been shown to be important for virus-induced fusion and infectivity for both HIV-1 and FIV. Testing of combination FIV therapies that include nucleoside analogs, protease inhibitors such as TL3, and Env peptide inhibitors now proven active against FIV has not yet been reported but looks promising for further development of the FIV model for HAART therapies for HIV-1.

Various other antiviral approaches have shown activity against FIV replication either in vitro or in vivo but have been examined in a limited fashion. Strategies previously shown to successfully target the FIV Rev regulatory system in vitro include a ribozyme directed to the FIV RRE element and small molecule intervention using 1,8-diaminoctane that blocks the formation of hypusine required by eIF-5A, a cellular factor required for HIV-1 Rev function. Cytokines and cellular growth factors including recombinant human interferon-alpha2, human interferon-omega, human interferon-tau, recombinant human GM-CSF, IL-16, and recombinant human insulin growth factor-1, demonstrated limited success as treatment for FIV infection either in cell culture systems or in infected cats. However, cytokines as adjunctive therapy to HAART therapy are currently under evaluation in HIV-1-infected patients and may be worthy of similar examination in the FIV model, where mechanisms may be experimentally characterized. Cytokines may be useful both as immune reconstitution therapeutics and for activating expression of latent reservoir viruses for subsequent elimination by antiviral drugs. In conclusion, animal models such as FIV and SIV provide opportunities for examination of tissues and cell subset reservoirs for virus in hosts undergoing HAART therapy as reported for HIV-1-infected patients and, more importantly, characterization of viral mechanisms for persistence within these reservoirs.

8. FIV AS A VIRAL VECTOR

Advances in characterization of FIV molecular virology have facilitated development of FIV vectors as vehicles for gene transfer in both dividing and nondividing cells. FIV vector development has equally contributed significantly to the body of knowledge regarding viral determinants of FIV gene expression, virus packaging, and integration. Lentiviruses provide unique vector systems that allow reliable integration of foreign genes into chromosomal DNA of nondividing cells. Although cumulative data generated for HIV-based vectors suggest that safe and effective primate lentivirus
vectors will be possible, concern over the clinical use of gene vectors derived from a pathogenic human retrovirus (HIV-1) may restrict the use of such vectors. FIV has provided the first nonprimate lentivirus vector system capable of gene transfer efficiency comparable to that observed with HIV-based vectors. Development of lentivirus vectors based on FIV has received continued support due to the apparent lack of FIV replication competence in human cells, resulting from restriction of FIV LTR promoter activity. Although cell culture-adapted variants of FIV are capable of utilizing human chemokine receptor CXCR-4 for infection of human cells, FIV LTR-directed gene expression and virus production are abrogated in human cells. Lack of cross-reactivity of FIV proteins with those of HIV is another potential advantage for use of FIV-based vectors. As discussed above, FIV virion structure and genomic organization are typical of other lentiviral genomes except for the absence of specific accessory and regulatory genes such as vpr, vpu, nef, and tat and the presence of a pol-encoded dUTPase. The less complicated FIV genome provides another advantage for use of this vector system. FIV vectors systems infect human primary cell types from a broad array of tissues, including brain, eye, airway, hematopoietic system, liver, muscle, and pancreas. These and other factors have promoted enthusiasm for FIV vector systems for gene therapy in human disease.

The first FIV vector described was derived from FIV molecular clone 34TF10 and documented that FIV-based vectors were capable of transducing nondividing human cells. Subsequent studies reported by other research groups described biology and cell tropism for similar but modified FIV vector systems also based on FIV 34TF10. All FIV vector systems described to date use three plasmids: a transfer vector for encoding the gene of interest, a packaging vector for expression of structural and enzymatic genes, and a plasmid for expression of the vesicular stomatitis virus (VSV)-G envelope protein for pseudotyping of vector particles. A chimeric FIV LTR, composed of a human cytomegalovirus (hCMV) immediate early gene promoter replacing the FIV U3 element and fused to the R/U5 LTR domains, is found in all FIV transfer vectors and is necessary for FIV vector production from human cells. As stated earlier, studies focused on optimization of FIV vectors system have mapped specific replication domains that include encapsidation determinants, a central poly-purine tract, and a central termination sequence that generates a central DNA flap in the preintegration complex. Data from these studies revealed a divergence in sequence between the FIV cPTT and the FIV 3’ PTT, and also showed that the FIV cPTT and 3’ PTT are not entirely purine. These findings uncovered differences between FIV and HIV-1 reverse transcription and have been incorporated into FIV vector design. Vector optimization studies have also characterized class I FIV integrase mutants that prevent the integrase reaction but do not perturb other Gag/Pol
functions. These integrase mutants may be incorporated into preclinical gene therapy studies in animals for providing control vectors that identify tissues with a requirement for vector integration for gene expression. Finally, FIV vectors have been further modified by deleting noncoding and nonstructural FIV sequences as well as specific gag sequences for construction of minimal vectors with reduced cytotoxicity, yet efficient gene transfer. Continued FIV vector design studies will contribute to further understanding of the FIV life cycle and FIV molecular virology, as well as facilitate development of optimal lentiviral vector systems. Additional studies will also be required to fully characterize and compare relative efficiencies, as well as biosafety of FIV with primate lentivirus vectors.

9. FELINE LENTIVIRUSES OF NONDOMESTIC CATS

Incidence

The initial observation of infection of nondomestic cats with a feline lentivirus was subsequently confirmed by multiple reports revealing either virologic or serologic evidence of infection in both captive and free-ranging populations of several nondomestic feline species. Species-specific feline lentivirus isolates have since been characterized for lions (FIV-Ple), leopards (FIV-Ppa), pumas/cougars (FIV-Pco), and pallas cats (FIV-Oma). Nondomestic feline lentivirus infection of free-ranging nondomestic felids is worldwide and includes lion and leopard populations within Africa; cougar populations in southern and western United States, Canada, and South America; cheetahs in Africa; and Pallas cats in central Asia. Interestingly, the prevalence of feline lentivirus infection in specific nondomestic cat populations, including lions in the Serengeti National Park and Ngorongoro Crater of east Africa and Kruger National Park of South Africa and cougars within the Snowy Mountain Rage in Wyoming, is quite high ranging from 58% (cougars) to 90% (lions). Despite evidence of endemic infection of free-ranging lion and cougar populations, overt disease has not been associated with lentivirus infection in these populations. This apparent lack of virulence, along with the significant genetic diversity observed among feline lentiviruses, suggests that FIV-Pco and FIV-Ple are ancient viruses that have adapted to their hosts and may be comparable to African SIV isolates that are similarly nonpathogenic for their natural hosts.

Genomic Diversity

Characterization of a molecularly cloned FIV-Oma proviral genome revealed genomic organization similar to that of domestic cat FIV iso-
lates. However, current knowledge of genomic diversity of nondomestic FIV isolates is based primarily on phylogenetic analyses focused on viral sequences generated from endemic FIV-Ple- and FIV-Pco-infected cat populations. Although lentiviruses infecting nondomestic cats are clearly related to domestic cat FIV, comparison of amino acid and nucleotide sequences derived from the highly conserved \textit{pol} gene of nondomestic (lion, cougar, and pallas cat) and domestic feline FIV isolates reveals considerable variation, ranging from 21 to 30\% differences in nucleotides and 19 to 45\% in amino acids. These data indicate that divergence between domestic and nondomestic cat lentiviral genomes is similar to that observed between HIV-1 and HIV-2. Furthermore, significant genomic diversity reported for cougar FIV-Pco isolates gathered from North and South America defined two principal clades and 15 divergent subclades for this nondomestic FIV. Genetic divergence measured for the two FIV-Pco clades was similar to the diversity that distinguishes domestic cat FIV from FIV-Ple isolates. Similarly, analyses of FIV-Ple isolates from lion populations within the Serengeti Reserve and Ngorongoro Crater in southeastern Africa identified three phylogenetic clades exhibiting genetic diversity also similar to that which separates domestic cat FIV from FIV-Pco. Sequence diversity within the conserved \textit{pol} genes derived from both FIV-Pco and FIV-Ple isolates is greater than that reported for the few \textit{pol} sequences available for domestic cat FIV. In fact, this \textit{pol} gene variation is similar to diversity measured for the more variable \textit{env} genes used to define unique domestic cat FIV clades. These findings provide additional support to the theory that nondomestic feline lentiviruses are ancient viruses that have existed within cougar and lion species much longer than FIV in domestic cats.

Additional findings from phylogenetic analyses of endemic FIV-Pco- and FIV-Ple-infected cat populations include evidence of vertical transmission (Pco) and possible coinfection of different subtypes within a single host (Pco and Ple). A recent report examining a large free-ranging lion population that included 13 prides within the Serengeti National Park revealed the presence of all three FIV-Ple subtypes widely dispersed within the population. Infection with the three FIV-Ple subtypes within the same pride and possibly within the same host was also observed. Furthermore, a high incidence (43\%) of coinfection with two to three FIV-Ple subtypes within individual animals was reported, although the alternative possibility of infection with subtype-recombinant viruses was not ruled out. Significant genomic divergence between different FIV-Ple subtypes, most notable between subtype C and the two other subtypes (A and B), was also observed for this population of lions. Monophyletic clustering of FIV-Ple sequences was observed for only one of the prides under study.
Findings described above for lion populations within Serengeti National Park contrasted with those generated from a study of a smaller population of wild cougars located in a mountain range in southeast Wyoming. These animals supported an overall FIV-Pco prevalence rate of 58% and a remarkable 100% rate of infection for all adult animals. Although analysis of both pol and env sequences confirmed the presence of two distinct FIV-Pco lineages within this infected cougar population, sequence diversity between the lineages was low and evidence of coinfection of a single host with viruses of distinct lineages was not found. Importantly, investigation of the evolution of both FIV-Pco pol and env sequences within this cougar population over time revealed mean rates of 1 to 3% per 10 years, a rate considerably less than rates of 0.3 to 1% per year reported for HIV. Collective observations generated so far from phylogenetic analyses of nondomestic FIV isolates suggest that these lentivirus infections provide unique opportunities to evaluate virus dynamics and viral sequence evolution in a natural host for a nonpathogenic lentivirus.

Infection and Replication of Nondomestic Feline Lentiviruses

The extent of genetic divergence described so far between domestic and nondomestic feline FIV isolates would suggest that infection and replication of nondomestic isolates may not be possible in domestic cats or in primary lymphocytes isolated from domestic cats. Reports of in vitro replication properties of nondomestic feline lentiviruses are scarce and have been restricted by the inability of some nondomestic FIV isolates such as cougar FIV-Pco, to replicate in domestic cat primary lymphocytes. However, some nondomestic FIV isolates including FIV-Ple and FIV-Oma have been shown to replicate in domestic cat lymphocytes and some feline cell lines including CrFK cells (FIV-Oma) and a feline lymphoid cell line (3201 cells) (FIV-Ple). FIV-Pco was also shown to replicate in 3201 cells. Although characterization of receptor usage for nondomestic feline lentiviruses has not yet been reported, these current findings for in vitro growth properties suggest that nondomestic FIV isolates may also be capable of infecting and replicating in vivo in domestic cats. Rare, isolated cases of naturally occurring cross-species transmission of domestic cat FIV to nondomestic cats have been reported, which involved a cougar and a wild-caught Tsushima cat belonging to a subspecies of leopard cats located in Japan. Experimental transmission studies have also confirmed the infectivity of both FIV-Pco and FIV-Ple in domestic cats and revealed that establishment of persistent FIV-Ple viral infection is possible in the absence of FIV-associated disease. Moreover, prior infection of domestic cats with either FIV-Pco or FIV-Ple appears to impart some resistance to challenge with pathogenic domestic cat FIV based on lower challenge virus
loads and higher CD4 counts measured for FIV-Pco- and FIV-Ple-infected domestic cats compared to naive cats after exposure to domestic cat FIV. Additional studies with rigorous measurement of virus load and careful examination of viral immunopathogenesis over extended time periods will be needed to characterize nondomestic feline lentivirus infection of the domestic cat as a model for HIV infection.

10. OUTLOOK FOR THE FIV ANIMAL MODEL

Significant progress has been made in the development of antiviral drug regimens for HIV-1, although complete elimination of the virus from the host and full restoration of immunocompetency are still not possible. However, a commercial safe and efficacious vaccine for HIV-1 is still not available and is unlikely in the near future, despite exhaustive efforts in HIV-1 vaccine development that began 20 years ago immediately after the initial characterization of HIV-1. Several serious issues that must be resolved for future vaccine success include identification of immune correlates of protection against either lentivirus infection or virus-associated disease. The apparent difficulty of addressing this question was predictable since virus elimination has not been observed during natural infection with immunodeficiency-inducing lentiviruses, even when strong virus-specific cellular and humoral immune responses have been detected in the infected host. Vaccine-induced protection against pathogenic challenge has been difficult to achieve in both the SIV and FIV animal models, and when observed, has been inconsistent. An additional complication is presented by the specter of vaccine-induced immune responses that prove detrimental rather than protective as reported for FIV and more recently in the SIV animal model. The complication of virus strain diversity has barely been addressed by vaccine design in animal models and surely poses a major complication for HIV-1 vaccine success in the future. Successful resolution of these obstacles in vaccine development, and most particularly the lack of understanding of why some vaccines such as attenuated viruses are sometimes protective, absolutely requires continued examination of viral pathogenesis and host immune responses in animal models including both nonhuman primate and nonprimate lentivirus animal models. Elucidation of pathogenic mechanisms used by these immunodeficiency-inducing lentiviruses during the earliest stages of virus exposure and virus-host interactions will be critical for design of vaccines that will have any potential for sterilizing immunity, if such a goal is possible. Similarly, a far more precise understanding of viral mechanisms for immunodeficiency will also be necessary for design of therapies of immune reconstitution in both acutely and chronically HIV-1-infected hosts, which can only be gained through experimental animal model studies.
Significant progress in characterization of FIV infection has been accomplished in the past 10 years, especially regarding molecular characterization of viral proteins, FIV receptor usage, and virus tropism and localization in vivo. Progress in development of FIV vectors for gene therapy has also been accomplished and has contributed considerably to the molecular characterization of FIV. Continued efforts in FIV vaccine and broad-based antiviral therapeutic development has demonstrated the strong potential of the FIV model for investigation of novel vaccine approaches and antiviral drugs. Lastly, similarities observed for host cell targets for virus infection and immunodeficiencies associated with FIV and HIV infection in vivo offer strong support for use of this animal model for crucial studies focused on mechanisms of immunopathogenesis of lentivirus-induced acquired immunodeficiency.

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