MINIREVIEW

Human Immunodeficiency Virus and AIDS: Insights from Animal Lentiviruses

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A well-recognized and frustrating hurdle in human immunodeficiency virus (HIV) and AIDS research is the lack of an authentic, reproducible animal model system that recapitulates the entire infectious process of HIV from virus entry and replication to the pathogenic manifestations of the disease and eventual AIDS-like outcome. Several animal models currently in use capture one or a number of events typical of HIV infection, but none encompasses the full spectrum of the disease.

A small animal model that is extensively used is based on the severe combined immunodeficient (SCID) mouse, engrafted with either human peripheral blood mononuclear cells (hu-SCID-PBMC) or with human fetal thymus and liver tissue (hu-SCID thy/liv). Because these models are based on human cells or tissue (albeit in a surrogate host), intervention and cellular pathogenesis studies specific to HIV type 1 (HIV-1) can be conducted. Nonetheless, fundamental species differences, lack of inducible immune responses, limited life span of the engrafted human cells, and other basic differences vis-à-vis infected humans, preclude studies bearing on virus compartmentalization, reservoirs, immune responses, and disease progression—areas that are essential for the design of therapeutic strategies and for containment of the disease.

In contrast to the hu-SCID models, simian immunodeficiency virus (SIV) infection of macaques has many of the pathogenic and disease hallmarks of HIV infection in humans and is therefore widely used for pathogenic, therapeutic, vertical transmission, and prevention (vaccine) studies. A large body of knowledge related to the human disease has accumulated, and significant advances in the areas mentioned have been made with this nonhuman primate (NHP) model. The system, however, is far from ideal. Several drugs (e.g., nonnucleoside reverse transcriptase inhibitors or NNRTIs) effective against HIV-1 are ineffective against SIV and cannot be evaluated in vivo for efficacy and other pharmacologic parameters (e.g., short- and long-term drug effects, synergistic or antagonistic effects with other drugs, and optimal dosing). Moreover, because of cost, logistics, limited availability, and the special expertise required for NHP work, the system does not easily lend itself to large and diverse studies; most studies are done in primate centers and are restricted to small experimental groups (four to six animals per experimental arm), often lacking sufficient statistical power for definitive outcomes.

Other natural hosts infected by their cognate lentiviruses exist. Examples of lentiviruses for which information on the genomic organization is available and for which aspects of host-virus interaction have been characterized include feline immunodeficiency virus (FIV) infection of domestic cats, canine arthritis-encephalitis virus (CAEV) infection of goats, equine infectious anemia virus (EIAV) infection of horses, bovine immunodeficiency virus (BIV) infection of cattle, and visna virus infection of sheep. Among these, the FIV/cat system is the most extensively studied, owing to the small size and relative ease of working with cats vis-à-vis the other hosts mentioned. FIV infection in cats closely resembles that of HIV in humans (see below), and a number of anti-HIV drugs are also effective against FIV and manifest similar toxicity profiles. Furthermore, cats are easier to breed and have a shorter breeding cycle than macaques. These and additional attributes of the model provide opportunities to explore new therapeutic strategies as well as prevention strategies against maternal and other modes of transmission. Nonetheless, the FIV/cat and other nonprimate lentivirus models have not engendered a level of enthusiasm equal to the macaque model.

Nonhuman lentiviral systems are critical for exploring questions that cannot be addressed in humans. An example may be to assess in vivo the requirement of genes shared by all lentiviruses (e.g., those for RNase H and integrase) for virus viability and survival and thereby provide the proof of principle to justify an aggressive search for antiviral agents targeting these gene products. A number of genes have also been identified in some, but not all, members of the lentivirus class. It is important to define the role of these gene products in the replication cycle of the viruses where they are found and to explore how lentiviruses lacking such genes provide for, or acquire, the function. Is the function provided by a viral gene that has dual activities? Is the function provided by a surrogate host factor? Or, is the absence of the function indicative of an evolutionary adaptation that can be exploited to counteract viral survival?

In addition to viral elements (structural, regulatory, and auxiliary), various host-encoded factors have been identified as having essential roles in lentivirus replication and others are likely to be identified. Insight into what these factors are, how they enhance (or repress) viral functions, how they modulate the disease process, and how they resemble and/or differ from those provided by the human cell may lead to additional intervention strategies.

A prototypic retrovirus consists of the gag, pol, and env genes that encode the structural proteins necessary for virion assembly as well as the enzymes needed for genome replication (RNA-dependent DNA polymerase and RNase H), provirus integration (integrate [IN]), and polyprotein processing (protease [PR]). Lentiviruses demonstrate a genomic complexity far greater than that of a prototypic retrovirus, possessing as
many as six additional genes, which are involved in regulatory processes (Table 1). All known lentiviruses encode an apparent transactivating protein (herein referred to as Tat) and a Rev protein that are both essential for virus replication (9, 12). In addition to tat and rev, HIV-1 contains four additional regulatory genes: nef, vif, vpr, and vpu, encoding the so-called accessory proteins. It is noted that whereas HIV-2 and SIV genomes lack the vpu gene, they contain another gene, vpx. Some of the accessory proteins are not absolutely required for viral replication in all in vitro systems but represent critical virulence factors in vivo. Nef is expressed from a multiply spliced mRNA and is therefore Rev independent (see below). In contrast, Vif, Vpr, and Vpu are the products of incompletely spliced mRNA and thus are expressed only during the late, Rev-dependent phase of infection from singly spliced mRNAs. Most of the small accessory proteins of HIV have multiple functions manifested at different stages of virus replication.

In this review, we highlight certain aspects of lentivirus-host systems that can be utilized to gain additional insight into the interaction of HIV with its human host in terms of the viral and host factors involved in viral replication and those involved in disease manifestation and possibly its containment. The examples provided are arbitrary and are designed to be illustrative rather than comprehensive.

**Tat**

The Tat protein is produced early in the replication cycle and plays a primary role in the expression of viral transcripts from a promoter within the lentiviral long terminal repeat (LTR). In the primate (i.e., HIV-1, HIV-2, and SIV), bovine (i.e., BIV), and equine (i.e., EIAV) lentiviruses, but not in visna virus, CAEV, or FIV, the Tat protein interacts with a Tat activation region (TAR) that consists of a stem-loop structure located 3' of the transcription initiation site within the LTR. Findings indicate that HIV-1 Tat primarily enhances transcription elongation rather than initiation (21, 22). At least two cellular cofactors are involved in this process. The prevailing model is that cellular cyclin T binds to the activation domain of Tat, thus increasing the affinity and specificity of the resulting complex for the TAR element (51). Next, a host cell-encoded Tat-associated kinase is recruited to the Tat-cyclin T-TAR complex and phosphorylates the carboxyl-terminal domain of the host cell RNA polymerase II. Hyperphosphorylation of RNA polymerase II, in turn, enhances transcript elongation by a mechanism yet to be resolved.

Experiments on cyclin T have shed light on the species restriction of HIV-1 replication related to the efficient functioning of the Tat protein. Analysis of murine cyclin T1 demonstrated that this protein could bind to the activation domain of HIV-1 Tat but was not able to bind to the TAR element (4). The inability was linked to a single amino acid difference between the human and murine cyclin T proteins. The inability of the Tat-murine cyclin T1 complex to bind to HIV-1 TAR accounts for the species restriction of HIV-1 gene expression in murine cells. The fact that murine cyclin T functions normally in the cell, yet is incapable of binding to HIV TAR, further suggests that these two cyclin T functions are distinct and nonoverlapping. It may therefore be possible to target the TAR binding function to human cyclin T without compromising the latter's role in cellular functions. However, since many cellular activities are redundant, it is conceivable that HIV may recruit or adapt to a surrogate host factor in lieu of cyclin T and thereby circumvent cyclin T-based viral inhibition. Thus, while targeting a cellular factor required for HIV replication eliminates the possibility of drug resistance due to a genetic mutation in the target gene, it does not necessarily eliminate the possibility of the emergence of viral variants capable of using an alternative cellular function to replace the one that is abrogated.

While the Tat protein serves a common function in all of the animal lentiviruses studied to date, there are differences in the mechanism of action of this essential regulatory protein. In transcription activation of visna virus and CAEV genes, the Tat protein acts via cellular transcription factors and not through binding to a TAR element or to any other nucleic acid sequence. In the case of visna virus, Tat binds to the cellular transcription factors Fos and Jun which target the resulting transcription elongation rather than initiation (21, 22). At least two cellular cofactors are involved in this process. The prevailing model is that cellular cyclin T binds to the activation domain of Tat, thus increasing the affinity and specificity of the resulting complex for the TAR element (51). Next, a host cell-encoded Tat-associated kinase is recruited to the Tat-cyclin T-TAR complex and phosphorylates the carboxyl-terminal domain of the host cell RNA polymerase II. Hyperphosphorylation of RNA polymerase II, in turn, enhances transcript elongation by a mechanism yet to be resolved.
visna virus model in determining whether the level of a cellular factor (those mentioned above or others) affects Tat function and infectivity in vivo.

Like visna virus and CAEV, FIV does not possess a stem-loop structure within the LTR analogous to the TAR of HIV-1. Until recently, it was not known whether FIV encoded a tat gene, although a short open reading frame (ORF) was identified that was comparable in size and genomic position to the tat gene of the ungulate lentiviruses. Recently, the orf2 gene was shown to encode a protein of 79 amino acids able to stimulate in vitro gene expression from the FIV LTR, strongly suggesting a Tat-like activity for this protein (10). The target of FIV Tat appears to be AP-1, cEBP, and ATF sites within the viral LTR. Thus, the mechanism of action of FIV Tat is most similar to that of visna virus and CAEV, both of which interact with an AP-1/AP-4 motif.

**Rev**

The Rev protein plays an essential role in the replication cycle of all lentiviruses studied by activating the export of partially spliced and unspliced viral mRNAs. This function of Rev is crucial for the expression of genes encoded by the partially spliced and unspliced viral mRNAs, since it overrides the cellular machinery that processes transcripts expressed by eukaryotic genes. Eukaryotic genes consist of multiple exons separated by noncoding (intron) regions. After transcription of intron-containing genes, splicing must take place in the cell nucleus before the mRNA is exported to the cytoplasm for translation of the gene product. Incompletely spliced mRNAs are retained in the nucleus to allow splicing to occur and thereby to ensure export of these transcripts and subsequent translation of functional proteins. Although lentiviruses encode a number of genes with extensive splicing requirements that fare well in such an environment, the virus replication cycle also depends on successful export of full-length transcripts. Specifically, while Nef, Rev, and Tat are encoded by fully spliced transcripts, Gag, Env, Pol, Vif, Vpr, and Vpu are encoded by unspliced or singly spliced transcripts. Rev is encoded by a fully spliced transcript and is expressed early in virus infection. Once produced in the cytoplasm, a nuclear localization signal on the protein facilitates its entry into the nucleus. Multimeric Rev then binds to the cis-acting Rev response element (RRE) found on unspliced viral RNAs and targets them for export to the cytoplasm by utilizing a nuclear export signal sequence found on the carboxyl-terminal domain of Rev. Nuclear export is accomplished via the host cell export pathway. Thus, the early expression of Rev facilitates export of unspliced RNAs whose translated products appear later in the virus replication cycle. The nuclear receptor involved in export was recently shown to be CRM1 (exportin 1). In addition to serving as the translational template for expression of the Gag, Env, and Pol proteins, the full-length transcript also serves as the genomic molecule and is incorporated during the assembly of newly formed virions.

The study of Rev has been particularly valuable in understanding the pathogenesis of EIAV. Acute EIAV infections are highlighted by rapid virus replication, sometimes leading to the death of the host within 1 to 4 weeks. In chronic EIAV infection there are cycles of fever, anemia, thrombocytopenia, and viremia. The cycles occur as frequently as biweekly early in infection but usually abate over time, leading to an apparent disease state with low viral load. EIAV Rev is unique in several ways. First, it is produced from a bicistronic four-exon mRNA that also encodes the Tat protein. Second, it downregulates its own production by fostering exon 3 skipping of the bicistronic mRNA, permitting continuous production of Tat at the expense of Rev. Recently, it has been suggested that the variability of EIAV Rev may contribute to changes in clinical disease (3, 29). Data demonstrated that highly competent rev genes are present during rapid virus replication, whereas less competent rev genes predominate during periods of long-term persistence in the presence of a host immune response (3). Analysis indicates a significant sequence variation in the second exon of rev in the latter instances, and such changes evoke biological attenuation in EIAV. The suggested hypothesis is that a limited amount of competent Rev restricts expression of the genes encoding structural proteins which could permit the virus to evade the host immune response during periods of clinical quiescence.

Identification of safe and effective inhibitors of Rev is of particular importance because of the central role that this lentivirus protein plays in the virus replication cycle. The fact that it is encoded by all known lentiviruses highlights Rev as a critical viral protein and an attractive target for drug therapies that can be tested in several animal model systems. In this regard, leptomycin B (LMB) has been shown to inhibit the function of the EIAV and FIV Rev proteins (36). LMB is an antibiotic that was identified as an inhibitor of the nuclear export function of HIV-1 Rev. LMB specifically blocks the export of mRNA by using the nuclear export signal (NES) pathway, but it does not block the export of other mRNAs. The NES usually contains hydrophobic amino acids. In the case of HIV-1, four conserved leucines are present in the NES as well as a moeity termed the core tetramer that contains two of these residues. However, the NESs of EIAV and FIV are atypical in that other hydrophobic amino acids are utilized and a core tetramer is not present. Nonetheless, LMB is able to inhibit Rev-mediated export of mRNA of these two viruses, suggesting that these animal lentiviruses, like HIV-1, use the exportin 1 receptor for binding the Rev NES. Thus, the equine and feline animal model systems may serve in the in vivo identification and testing of Rev inhibitors and may lead to compounds that are likely to also interfere with HIV replication.

**Vif**

Another well-conserved regulatory protein found in all lentiviruses except EIAV is the virion infectivity factor (Vif) protein. Current thought is that Vif influences the late stages of virion assembly, since virus particles produced in the absence of Vif are incapable of incorporating the proviruses into host cell chromosomes. The finding that this effect is cell-type specific led to the discovery that Vif counteracts the activity of a naturally occurring antiviral activity present in human cells (32, 44). Vif is predominantly cytoplasmic but also exists as a membrane-associated form. Phosphorylation appears to be important for the interaction of Vif with cellular and viral proteins and may be involved in targeting Vif to specific cellular compartments. Vif appears to be phosphorylated by p44/42 mitogen-activated protein kinase (MAPK) at sites conserved among the primate lentiviruses (52). The HIV-1 Gag p17, Nef, Rev, and Tat proteins were also shown to be directly phosphorylated in vitro by MAPK, suggesting that other HIV proteins are potential substrates for MAPK or related kinases (53). In this case, the SIV model may be useful in exploring in vivo the role of MAPK in lentivirus replication.

Another function of Vif may be to block premature processing of Gag precursor protein by PR in the cytoplasm. This temporal control of Gag processing by PR ensures the availability of Gag-derived peptides (CA, MA, and NC) at the plasma membrane for assembly with other viral components.
However, the Vif proteins from SIV AGM and SIV SYK were not sufficient viruses to restore full genetic competency to Vif capacity of SIV. This would be important in confirming (or refuting) the proposed hypothesis with an appropriate animal model (e.g., the FIV/cat system). Vif is still controversial and awaits definitive proof. Studies of lentiviruses, with their limited genetic information, may lead to the development of a new class of PR inhibitors, distinct from current anti-PR drugs, for treatment of HIV infection. Of note, the PR-inhibitory activity of Vif proteins from SIV AGM and SIV SYK was decreased 10- to 100-fold (EIAV), and tissue distribution of dUTP enzyme was altered (visna virus and FIV). Furthermore, G to A substitutions accumulated in the genome of both CAEV and FIV dUTPase mutants during in vivo growth (28, 49). This finding is consistent with misincorporation of dUTP into viral DNA during reverse transcription. In the FIV model a similar effect was seen in virus obtained from macrophages but not in virus from lymphocytes (28). Thus, dUTPase appears to be involved in reducing the occurrence of genome mutations. While these data suggest that dUTPase is not essential for lentivirus replication, this conclusion is not as simple as suggested at face value. Viruses lacking dUTPase may be impaired to a point where they are unable to compete with wild-type viruses and are consequently selected against in a mixed pool of dUTPase-mutant and wild-type viruses.

UDG

Uracil DNA glycosylase (UDG) belongs to a specific class of ubiquitous DNA repair enzymes encoded by eukaryotic and prokaryotic organisms. The biological activity of UDG is to specifically remove a misincorporated uracil (RNA base) from DNA, thereby initiating the excision-repair process. Another ubiquitous enzyme that minimizes uracil incorporation into DNA, albeit by a slightly different mechanism, is deoxyuridine triphosphatase (dUTPase). dUTPase hydrolyzes dUTP to dUMP, which is subsequently used for the synthesis of thymidine triphosphate (TTP) by thymidylate synthetase. dUTPase thus maintains a low dUTP/TTP ratio, which in turn reduces the likelihood of uracil incorporation into DNA.

The levels of these and other DNA excision-repair enzymes are generally high during cell division and low in resting or terminally differentiated cells. Consequently, nonreplicating cells have a relatively high dUTP/TTP ratio and a greater probability of uracil misincorporation, which may ultimately result in genetic mutations. Large viruses (i.e., herpesviruses and poxviruses) that replicate in the cytoplasm independently of the cell cycle encode their own ribonucleotide reductase and other DNA synthesis/repair enzymes. This genetic independence enables them to replicate within resting cells where these endogenous cellular enzymes are deficient or inactive. But how do lentiviruses, with their limited genetic information, maintain the capacity to replicate with fidelity in nondividing cells? One clue to this dilemma is provided by the nonprimate lentiviruses. These viruses (i.e., visna virus, CAEV, EIAV, and FIV) encode dUTPase (see above), which hydrolyzes dUTP to dUMP. The reaction has the dual benefit of providing a source of dUMP, an essential precursor of TTP, and maintaining a low dUTP/TTP ratio to minimize dUTP incorporation into chromosomal DNA.

Studies on the role of virally encoded dUTPase in virus replication have focused on visna virus, CAEV, and EIAV, viruses that replicate predominantly in macrophages, and on FIV, a lentivirus with a broader tropism that includes lymphocytes (28, 30, 38, 49). Viruses with in-frame nucleotide insertions or deletions in the dUTPase gene have been studied both in vitro and in vivo. Generally, replication of dUTPase mutants in vitro is severely affected in nondividing host cells (e.g., primary macrophages), while replication in actively dividing cells (e.g., mitogen-stimulated T cells and continuous T-cell lines) is only minimally decreased, if at all. In animal models, there are indications that replication of the dUTPase-mutant virus was less efficient. Nonetheless, the dUTPase-mutant virus was infectious in each case, suggesting that virally encoded dUTPase is not essential for replication in vivo. However, viral loads were decreased 10- to 100-fold (EIAV), and tissue distribution of dUTPase mutants was somewhat altered (visna virus and FIV). Furthermore, G to A substitutions accumulated in the genome of both CAEV and FIV dUTPase mutants during in vivo growth (28, 49). This finding is consistent with misincorporation of dUTP into viral DNA during reverse transcription. In the FIV model a similar effect was seen in virus obtained from macrophages but not in virus from lymphocytes (28). Thus, dUTPase appears to be involved in reducing the occurrence of genome mutations. While these data suggest that dUTPase is not essential for lentivirus replication, this conclusion is not as simple as suggested at face value. Viruses lacking dUTPase may be impaired to a point where they are unable to compete with wild-type viruses and are consequently selected against in a mixed pool of dUTPase-mutant and wild-type viruses.

In the absence of a dUTPase-like encoded activity, primate lentiviruses may have acquired an alternative mechanism to minimize uracil incorporation into proviral DNA. Several studies suggest that this indeed may be the case. The Vpr proteins of HIV-1, HIV-2, and SIVsm (Fig. 1) have been shown to interact with cellular UDG (5, 43). That the interaction is conserved across several primate lentivirus lineages suggests that the Vpr-UDG interaction plays an important role in the replication of these lentiviruses. This argument is supported by the observation that the enzymatic activity of UDG is retained while in complex with Vpr. Conversely, the two activities ascribed to HIV-1 Vpr, T-cell arrest at the G2 phase in the cell cycle and facilitating transport of the preintegration complex to the nucleus, are not dependent on the binding of Vpr to UDG. This implies that Vpr may have yet a third function related to its capacity to bind UDG. In single-round replication assays, the mutation rate of HIV-1 is reduced fourfold in the presence of Vpr (33). Related to this finding is the observation that uracil incorporation into viral DNA correlates with reduced replication of EIAV in macrophages (48). From these studies it may be surmised that in binding UDG, Vpr facilitates the import and concentration of the enzyme into the preintegration complex, thereby reducing the mutation rate of the newly transcribed viral DNA. Further testing and validation of this model should determine whether UDG and perhaps other cellular enzymes involved in DNA synthesis/repair constitute bona fide inhibition targets that can specifically be blocked to affect viral replication without compromising cellular function and viability.

The role of a host-encoded dUTPase in the replication of primate lentiviruses, if any, is not known at present. In addition to the human cellular dUTPase gene, a dUTPase-encoded sequence has been identified in the human endogenous retrovirus HERV-K, a defective multicopy virus that is transmitted...
host factors

As exemplified above with the involvement of MAPK in Vif activity, various factors encoded by the host cell have been shown to be required for replication of lentiviruses in addition to the structural, regulatory, and accessory gene products encoded by these viruses. The extent to which these factors overlap or diverge from those used by HIV in human cells can provide important insight into the understanding of viral replication, the disease process, and importantly, possible intervention strategies.

protease

Retroviruses encode a protease (PR) that is essential for virus maturation and infectivity. Comparative studies are beginning to yield information on similarities and differences among lentivirus proteases which may eventually be exploited to yield inhibitors broadly active against members of the lentivirus family. Crystal structures have been determined for the PR of several lentiviruses, including HIV-1, HIV-2, SIV, EAIV, and FIV (25). The active site structures of the FIV and HIV-1 PR are, for example, superimposable yet the two enzymes have distinct substrate and inhibitor specificities (26). Of note, six amino acid residues of HIV-1 PR that are associated with resistance to PR inhibitors are found in the corresponding residues of HIV-2 PR (27). Current strategies to counteract the resistance of HIV to protease inhibitor drugs are being developed in the FIV/cat model (27).

nuCLEOcAspid zInC Finger Miot

Eukaryotic cells possess an abundant class of proteins (zinc fingers) that coordinate Zn and possess sequence-specific DNA-binding activity. While the function of some of these proteins is unknown, many appear to function as transcription factors (e.g., Sp1, GATA-1, and PARP). Retroviral proteins have one or more highly conserved structural motifs similar to the DNA-binding site of eukaryotic zinc finger proteins (42). This motif in HIV consists of a peptide segment Cys-X2-Cys-X2-His-X2-Cys (CCHC), where X is any amino acid, that forms a three-dimensional Zn-coordinating structure. Importantly, the retroviral zinc finger motif varies from the classical motif found in transcriptional factors and other zinc finger proteins which typically use CCCC or CCHH motifs and have loops of variable size. These differences should facilitate the development of selective antiviral agents.

HIV-1 has two retrovirus-type zinc finger motifs localized to the p7 nucleocapsid (NC). Both copies are required for normal RNA packaging and for infectivity. The importance of the zinc finger motif in multiple steps of the virus life cycle has been demonstrated in experiments where mutation (1, 16) or chemical modification (41) was used to disrupt the structure of this domain. Such studies have shown that this viral motif can be selectively targeted by chemical agents (20), culminating in the production of noninfectious particles. Importantly, the zinc finger structures in intact virions are accessible to chemical modification by several agents studied thus far. Recent work has shown that SIV virions inactivated by one such agent retained the structural integrity and the native conformation of the envelope proteins (2). These observations suggest that the highly conserved retroviral zinc finger motif can serve as a valuable target both for the treatment and prevention (as vaccines and topical microbicides) of lentivirus infection.
Among the host cell factors that appear to be conserved between HIV and FIV is the chemokine entry receptor CXCR4. The recent discovery that FIV Env can also use human CXCR4 as a primary fusion receptor (B. J. Willett, M. J. Hosie, J. C. Neil, J. D. Turner, and J. A. Hoxie, Letter, Nature 385:587, 1997) highlights the similarities in entry mechanisms that are likely to exist between members of this family of viruses. As with HIV, the isolates of FIV that use CXCR4 as a receptor have been derived from animals with advanced disease and have been propagated in laboratory cell lines. The finding of shared entry receptors points to the potential utility of the FIV model in the development of novel AIDS therapies. Similar to the potential role of the FIV model in the development of reverse transcriptase (RT), PR, and IN inhibitors (11), the use of shared mechanisms of entry may well represent a circumstance that can be exploited in the development of broad-based therapies that target this very first step in the viral replication cycle. Indeed, recent data suggest that, similar to HIV, infection of feline cells with FIV can either be inhibited or enhanced in vitro by SDF-1, the ligand for CXCR4, depending on the assay conditions employed (19). The FIV/cat model may, therefore, provide a complementary system for coreceptor inhibitor development to the SIV/ma-caque model, where in vivo coreceptor usage appears to predominantly favor CCR5.

ANTIVIRAL IMMUNE RESPONSES

In addition to providing essential functions for lentivirus replication, host factors, specifically the components of the host immune system, play a pivotal role in control of viral replication. As noted above, the clinical course of EIAV infection is marked by periods of high viral replication interspersed with periods of quiescence. Usually, the cycling period of quiescence and recrudescence resolves to an asymptomatic carrier state marked by low or undetectable levels of viral expression. The factors contributing to pathogenesis during the symptomatic phases of the disease are yet to be defined but do not appear to be immune mediated, since SCID horses also experience anemia and thrombocytopenia with EIAV infection. However, control of the virus during acute infection, during the cycling phase of the disease, and during the carrier state appears to be mediated by antiviral immune responses. Several lines of evidence support this conclusion. Immunodeficient animals are incapable of controlling viral replication and experience a rapid disease course culminating in death (37). Further, animals that are immunologically suppressed during the asymptomatic phase of the recurrent cycling period experience a recrudescence of disease and viremia. Viral phenotype also varies during each successive course of viral re-emergence (29), consistent with the notion that viral recrudescence may result from the generation of immune escape variants. However, immune-competent animals are ultimately able to control the infection and develop a persistent carrier state. At this stage, it also appears that viral control is immune mediated since it is known that EIAV carrier horses experience viral recrudescence following the administration of immunosuppressive agents. These findings may provide important therapeuic directions for the control of HIV infection. If EIAV control and recrudescence can be mimicked in HIV infection by intermittent highly active antiretroviral therapy (HAART), the EIAV model would suggest that multiple rounds of inter-mittent HAART followed by treatment withdrawal may be necessary before stable immune control of the infection can be achieved. In this regard, several structured treatment interruption (STI) studies were recently initiated in HIV-infected pa-
tients at either the acute or the chronic disease stage. Preliminary data from patients with early HIV disease suggest a pronounced anti-HIV immune response (both T-helper and cytotoxic T-cell response) subsequent to STI (Bruce Walker, personal communication). While it is premature to draw definitive conclusions at this point, these results strongly suggest that the immune system of an HIV-infected individual is sufficiently resilient to respond to HIV antigens during the rise and fall in viral load which occurs during STI. Clearly, this prediction and optimization of this strategy for maximal immune response and viral containment can be easily modeled using a number of natural lentivirus infection models.

Although the specific mechanism(s) of immune control of EIAV is yet to be delineated, several findings suggest that mechanisms other than neutralizing antibody responses can control viral replication. Consistent with this notion, neutralization escape variants have been isolated from EIAV carrier horses as early as 5 days after corticosteroid treatment, when antibody levels have not significantly changed. Similar to the control of HIV viremia following acute infection, control of corticosteroid-induced viremia in carrier horses occurs before the appearance of neutralizing antibody to the endogenous viral strain (23). The finding of cytotoxic T lymphocytes (CTL) specific for several epitopes on EIAV Gag in EIAV carrier horses (54) leaves open the possibility that this cell type may be involved in viral control. It is of interest to note that in HIV infection, CD8 CTL against Gag are found in individuals with slow disease progression and, conversely, are lacking (or present at marginal levels) in individuals with a rapid disease course.

OTHER CLUES TO VIRAL PATHOGENESIS

Another example of a lentivirus that causes minimal pathol-ogy in its natural host is SIVsm infection of sooty mangabeys. In this situation, the virus replicates to very high levels (up to 30 million RNA copies per ml of plasma) in the infected host (40). Despite these high viral loads, which can be sustained for many years, infection results in no evident pathology or clinical manifestations. The lack of gross pathology in these animals is mirrored by the lack of evident pathology at the cell and tissue level: lymph node and spleen architectures are essentially intact despite the high levels of viral replication occurring within these organs, and CD4 levels are maintained as is the CD4 to CD8 ratio. These findings might be explained if the virus was not cytopathic for infected cells. However, when infected animals are treated with antiretroviral agents, the bimodal kinetics of disappearance of the virus are similar to those seen with HIV-1 infection in humans and in SIV infection of macaques. Since high viral loads would be expected to remain if virus producer cells were not quickly killed, these results suggest that the virus is cytopathic for infected host cells. Thus, no gross differences in the behavior of the virus in this host have been identified to explain the nonpathogenic nature of SIV in sooty mangabeys. Since identical SIVsm isolates are asymptomatic in sooty mangabeys and pathogenic in rhesus macaques, current thinking implicates the host response to the virus as responsible for the different disease courses in these animals. Indeed, a CD8+ cell-derived soluble factor has been implicated in the protective effect seen in the sooty mangabeys (50). Interestingly, a soluble CD8 factor has also been implicated in rendering an anti-HIV effect in humans, and an intense search is in progress to identify it (14, 31). Subtle differences in viral tropism in the two species might also play a role in the pathogenic response in one host and lack of it in the other.
SIV infection of macaques has already been demonstrated as a useful model in studying the role of macrophages in the generation of established infection. The role of HIV Vpr is subsumed in SIV by two proteins, Vpr and Vpx. Vpx mediates macrophage infection by virtue of its role in nuclear entry (15, 18). SIV Vpr mediates all other functions ascribed to HIV Vpr. Challenge experiments with Vpx variants of SIV have therefore provided insight into the importance of macrophages in the disease process. Investigators have recently reported (18) that SIV Vpx mutants are out-competed by wild-type virus when the two are coadministered by either mucosal or systemic routes. In single infection experiments, the Vpx variants fail to induce local lesions at the site of infection that are typically seen with wild-type virus and show replication rates that are much delayed in comparison to those of the wild type.

PENDSPECTIVE

In addition to the lentivirus animal models cited above, a number of transgenic (TG) animals (predominantly mice, rabbits, and recently, rats) are being established containing either specific HIV genes or the entire proviral DNA genome. Still, these models cannot address the most basic questions related to the HIV replication cycle, such as entry, uncoating, reverse transcription, integration, assembly, budding, and disease processes. A lingering uncertainty associated with TG models is the extent to which an isolated viral function reflects on the same function when it occurs during the continuum process of virus replication in a susceptible host. Current TG models focus on engineering the human receptors (CD4 and the chemokine coreceptors CCR5 and/or CXCR4) to allow for HIV entry. To date, mice transgenic for the human receptors have failed to generate a host that is fully susceptible to HIV-1, most likely due to a deficiency in a host-specific factor(s) required subsequent to viral entry. Nonetheless, such failures are instrumental in their own right in identifying host-specific factors essential for virus replication. How much engineering will be needed before (and if) a mouse or other species is tailored to recapitulate the complete HIV replication cycle? How well will such a system mimic natural events in HIV replication? Answers to these questions are conjectural at best. What is clear, however, is the great divide that exists between the many remaining questions bearing on the human disease and the in vivo animal models available to tackle them.

Are we then at an impasse for lack of effective animal systems to model HIV and AIDS? Not quite. It should be recognized that animals infected with comparable lentiviruses are models for the human disease and not replicas of it. They should complement rather than replace and are valuable as adjuncts to in vitro and human clinical studies rather than as stand-alone models. The model selected should be compatible with, and fit, the questions asked. This approach is exemplified in the type of questions pursued in the simian/human immunodeficiency virus (SHIV)/macaque model currently in use. SHIVs are chimeric viruses comprised of HIV-1 and SIV genes. A SHIV derivative, consisting of HIV-1 envelope, tat, rev, and vpu genes, with the remaining genes derived from SIV, is of particular value owing to its ability to initiate a complete, pathogenic infectious cycle in the macaque host. This SHIV construct is thus particularly suited for in vivo studies specific to the HIV-1 envelope, such as the mechanism of HIV fusion and entry, the role of envelope in HIV pathogenesis, the role of envelope-specific neutralizing antibodies in controlling the onset of infection or ameliorating a chronic infection, and importantly, for design and efficacy studies of vaccines that target HIV envelope fusion and entry. Moreover, since SHIV contains the HIV tat, rev, and vpu genes, specific therapeutic interventions that target these HIV genes and their protein products may be evaluated in a fully pathogenic model. Indeed, the success achieved in developing a pathogenic SIV/HIV-1 chimera consisting of structural and regulatory HIV-1 genes suggests that other SIV/HIV-1 chimeras may be designed and used in therapeutic intervention studies that target a specific gene or a combination of genes in vivo. Moreover, the success in constructing a pathogenic SIV/HIV-1 hybrid suggests that other chimeric viruses (for example, FIV/HIV-1) may be designed for testing novel therapeutic and vaccine concepts in the cat.

The SIV/macaque model illustrates that a considerable body of useful information can be obtained when the questions addressed are compatible with the model used even if the model does not reproduce every aspect of HIV infection in humans. Indeed, dissimilarities may be as valuable as similarities in yielding information of importance bearing on HIV-human interaction and the human disease (see below). Unfortunately, this logic has not transcended the use of other natural lentivirus models in delineating specific questions that can be addressed in the specific model. The following illustrates several scenarios whereby a number of natural lentivirus-host models can be utilized to gain insight into HIV molecular biology and pathogenicity.

NATURAL LENTIVIRUS-HOST MODELS

Virus-host interaction and disease processes. As described above, SIV infection of sooty mangabeys is an intriguing infectious model in which the nonpathogenic outcome is attributable to host factors rather than unique viral features. Host factors that prevent the development of disease in the sooty mangabey host are either lacking or are inhibited in the rhesus monkey host. Clearly, understanding the host factors or genetic disposition that renders protection against a pathogenic disease may identify therapeutic and vaccine venues for HIV disease in humans.

Natural host systems may also broaden the understanding of events leading to an asymptomatic phase (BIV and EIAV), natural control of viremia by the host (EIAV), innate factors operating in the emergence of viral variants during the course of the disease (EIAV), host factors involved in preventing immune deficiency and other clinical symptoms in spite of significant viremia (SIV in sooty mangabeys and other African monkeys), neurologic complications (FIV), and means to reduce proinflammatory symptoms subsequent to lentivirus infection (CAEV).

Viral reservoirs. Natural lentivirus infections may provide models to identify additional organ, tissue, and cellular viral reservoirs and the extent of viral gene expression in these sanctuaries. Strategies to reduce (and perhaps abrogate) replicating or latent virus may be devised and their impact on disease course can be assessed. Presently, strategies to purge viral reservoirs are in their formative stages and many entail an initial cellular activation step or an interruption in drug treatment—both entailing certain risks to the patient. Preliminary studies in nonhuman models would be of considerable value in assessing the risk-benefit in the design of human studies. Additionally, lentivirus models can be used to explore the driving force(s) operating in the establishment and maintenance of virus reservoirs and potential strategies to prevent these events from the outset.

Insights to therapeutic strategies. Fusion and entry of primate lentiviruses into cells involve two cellular receptors: the major CD4 receptor and a chemokine receptor (such as CCR5
and CXCR4). Based on this finding and the possibility of preventing the very first step in HIV infection, strategies to block or suppress CCRs have emerged. A lingering concern, however, is that in the presence of selective pressure the virus would adapt and find its way into cells by using an alternative CCR(s). Indeed, such a scenario was documented in experimental, in vivo testing using the hu-SCID-PBL mouse model (35) and in a natural setting (8), validating the concern: 86.6% of the African red-capped mangabeys (RCM) harbor a 24-base-pair deletion in the CCR5 receptor (the major CCR of RCM) which precludes infection by CCR5-tropic SIV. An RCM with the 24-base-pair deletion was found to be naturally infected with an SIV isolate that used CCR2b as its CCR. This observation suggests that CCR2b tropism may have been acquired as an adaptation in response to the CCR5 genetic defect appearing in the host (8).

The role of highly conserved viral or cellular genes for virus growth or induced disease can also be validated in natural virus-host systems. The critical nature of these genes in HIV infection or pathogenesis can then be confirmed and used as a platform for targeted therapeutic interventions. In contrast to highly conserved genes, certain genes may be unique to a specific virus-host model, such as the presence of the dUTPase gene in the FIV but not in the HIV genome (see above). Because of the general relatedness of lentiviruses, it is conceivable that additional lentivirus genes are also required for HIV replication but exist in a surrogate or alternative form: a cellular function serves in lieu of a lentivirus function (e.g., dUTPase-like activity); a viral gene in one lentivirus subsumes the function of a dedicated gene in another lentivirus (e.g., HIV-2); an Env protein which mediates viral entry and viral release vis-a-vis HIV-1 encodes two separate proteins, Env and Vpu, to accomplish these events (6); and a viral gene serves a dual instead of a single function (e.g., the combined activities of HIV-2 and SIV Vpr/Vpx proteins contained within the single HIV Vpr protein).

Evaluating genes discordant in different lentivirus-host systems may identify additional viral or cellular target elements for HIV therapeutic interventions.

Insights into immune deficiency and immune restoration. Current HIV therapeutic strategies emphasize immune restoration, in conjunction with conventional drug therapies. Lentivirus models with immunodeficiency manifestations can be used to assess and fine-tune a number of established and novel immune-based strategies, including immune suppression to reduce HIV replication and the optimal conditions to minimize the risk-benefit ratio, immune augmentation with therapeutic vaccines, and other mechanisms to enhance humoral and cellular immune responses in the infected individual. Lentivirus-induced immune deficiencies can also be used to identify specific cellular defects that contribute to the immune deficiency (e.g., antigen-presenting cells, CD8 CTL, and T-helper cells) and to develop strategies to specifically repair or circumvent these defects.

In conclusion, while not all lentivirus-host systems lend themselves to the comparative study of HIV interaction with its human host (due to phylogenetic distance from HIV, different mode of transmission, different target cells, dissimilar disease manifestations, and availability or size of the host), many have certain features that are relevant to the human disease. Moreover, several of these models have been studied for some time from economic (BIV and CAEV) or veterinary (EIAV and FIV) perspectives. Such information should be pooled and analyzed for trends common to the lentivirus class as a whole and for features specific to HIV in particular. Further, collaborative studies should be forged and studies designed in concert between investigators at ease with these lentivirus systems and HIV investigators to define suitable and relevant questions that can be addressed in vivo in nonprimate models to complement data generated in NHP and in human clinical studies.

ACKNOWLEDGMENTS

The authors thank C. Dieffenbach, E. Hoover, S. Plaeeger, and M. Stevenson for critical reading of the manuscript and insightful comments; R. Black for helpful discussions; and B. Foley, Department of Theoretical Biology and Biophysics, Los Alamos National Laboratory, for providing the phylogenetic tree in Fig. 1. The authors gratefully acknowledge the informative discussions of the following participants at the workshop “HIV/AIDS: Insights from Animal Lentiviruses,” held September 1998 in Bethesda, Md.: S. Carpenter, P. Cheever, J. Clements, J. Elder, M. Feinberg, B. Hahn, E. Hoover, J. Hoxie, P. Johnson, S. LeGrice, P. Marx, J. Mellors, R. Montelaro, B. Nikolic, S. O’Brien, R. Pearlman, W. Rice, M. Tompkins, W. Tompkins, A. Wlodawer, and C. Zink.

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