BASIC SCIENCE

Mario Stevenson, PhD

Presentations in the basic science categories underscored the steady progress being made in understanding the functions of viral accessory genes. The identification of cellular intermediaries that interact with viral accessory gene products has revealed cellular pathways utilized by these proteins. This is beginning to provide insight into how these proteins may be important for virus replication. New findings on the interactions of primate lentiviruses with their coreceptors are promoting our understanding of how these interactions affect viral tropism, how the virus impacts on host cell physiology, and how levels of expression of coreceptor molecules impact on disease progression. Reservoirs of primate lentivirus infection, which may explain long-term latency/viral persistence in the face of antiretroviral therapy, were described by a number of investigators. The actual cells involved in maintaining long-term viral latency/persistence still remain an important but unresolved issue.

ACCESSORY GENES

The accessory proteins arguably represent the most enigmatic of the primate lentivirus gene products. Several are unique to the primate lentiviruses and are conserved across primate lentivirus lineages. The accessory genes appear necessary for the pathogenicity of the virus, both in monkeys and in humans. Although a number of properties have been described for each of the accessory gene products, the actual role served by these proteins is not known for any of them. Ultimately, a greater understanding of these roles can be gained through the identification of cellular intermediates with which these accessory gene products interact and manifest their effect and it is in the identification of such cellular factors that the greatest progress is being made.

A well-recognized feature of virus infected CD4 cells in vitro is the rapid downregulation of the CD4 receptor (and also of coreceptor molecules). Three proteins, namely envelope, Nef, and Vpu, working at mechanistically different levels, have been shown to reduce the expression of CD4 on the cell surface. Vpu interacts with CD4 in the endoplasmic reticulum and targets CD4 for degradation likely in the proteasomes. A cellular protein that likely interacts with Vpu and directs Vpu/CD4 complexes for degradation was described **(Abstract 25).** A yeast genetic screen was used to identify cellular proteins that interact with Vpu. Using this approach, a protein has been identified (β-TRCP) that may be involved in Vpu-mediated targeting of CD4 to the degradation pathway. β-TRCP and Vpu were shown to interact in a yeast two-hybrid assay. The recombinant proteins could interact in vitro and could be co-immunoprecipitated when expressed in mammalian cells. Two serine residues shown previously to be necessary for downregulation of CD4 by Vpu were also important for interaction of Vpu with β-TRCP. Thus, it is likely that Vpu, CD4, and β-TRCP form a trimolecular complex where Vpu links CD4 to β-TRCP. Truncated mutants of β-TRCP prevented CD4 downregulation by Vpu. Further support for the role of β-TRCP in Vpu-mediated CD4 downregulation was provided by the demonstration that β-TRCP interacts with a second cellular factor (Ski P1), a protein that targets the ubiquitin pathway. Therefore, following interaction of Vpu with CD4 in the endoplasmic reticulum, β-TRCP likely mediates interaction of the Vpu/CD4 complex with Ski P1 and, as a result, the complex is targeted for degradation in the proteasome. This elegant study provides a mechanism through which Vpu downregulates CD4. However, it is still not clear as to why downregulating its primary receptor is advantageous to the virus.

Research findings presented at the conference attempted to shed light on why CD4 downregulation is important for efficient virus replication. In addition to Vpu, CD4 receptor expression on the cell surface is also downregulated by the Nef accessory gene product. Nef protein appears to induce accelerated endocytosis of CD4. Thus, cells that express only HIV or SIV Nef proteins exhibit less CD4 on the cell surface. Studies by Trono **(Abstract S40)** indicate that Nef and Vpu counteract CD4-mediated inhibition of HIV infectivity. During virus exit from the producer cell, nascent envelope proteins need to be transported to the cell membrane for incorporation into assembling viral complexes. However, the interaction of nascent envelope glycoproteins with CD4 molecules poses a problem in that some envelope complexed to CD4 may be incorporated into viral particles. As a consequence, these virion-incorporated CD4 molecules may compete for available receptor binding sites on the target cell. In support of this notion, evidence was provided that overexpression of CD4 in producer cells markedly impaired HIV-1 infectivity. Virions released from such cells were shown to contain CD4 molecules. Nef and Vpu, by reducing surface CD4 expression in the producer cell, prevented virion

incorporation of CD4 and restored HIV infectivity. Overexpression of a CD4 mutant that did not bind envelope also did not impair viral infectivity. Similarly, overexpression of CD4 did not block infectivity of virus particles that contained a VSV-G envelope protein in place of HIV-1 envelope. Thus, by downregulating CD4, primate lentiviruses have evolved a mechanism that prevents receptor molecules in the producer cell from being incorporated into virions as complexes with envelope glycoproteins. Since such a process would be predicted to impair viral infectivity, the study by Trono may explain the functional significance of receptor downregulation by primate lentiviruses. It remains to be determined whether a similar problem is posed by interaction of envelope glycoproteins with coreceptor molecules and whether the virus has evolved a mechanism to prevent incorporation of coreceptor molecules into virions.

Several presentations addressed functions of the HIV-1 accessory gene product, Vpr. A number of properties have been described for this accessory gene product. Vpr facilitates nuclear translocation of the viral reverse transcription complex in nondividing cells. Vpr, when expressed in cells, causes a delay in cell cycle progression. Vpr has also been shown to associate with the DNA repair enzyme, uracil DNA glycosylase, and, in addition, has been shown to function as a weak transcriptional activator of HIV-1 gene expression. A number of presenters focused on the nuclear import functions of Vpr. While Vpr has been demonstrated to localize to the nucleus and facilitate nuclear translocation of viral reverse transcription complexes, the protein contains no prototypic nuclear targeting signal that would support such a role. However, recent studies **(Abstract 29)** demonstrate that Vpr interacts with karyopherin-α which is involved in shuttling nuclear proteins to the nuclear pore complex. Vpr appears to act in a novel fashion. Normally, proteins containing a nuclear targeting signal interact with karyopherin and are subsequently targeted to the nuclear pore complex. Vpr appears to increase the affinity of proteins containing nuclear targeting signals for karyopherin, thus increasing the rate and extent of their nuclear transport. A second protein implicated in nuclear targeting of the viral reverse transcription complex is the structural gag MA protein. However, there have been several conflicting studies with regards to whether gag MA is a nuclear protein which promotes nuclear translocation of the viral reverse transcription complex. Vpr was shown to promote the interaction of gag MA with karyopherin. Vpr appears to act in a novel fashion in promoting nuclear targeting of viral proteins. Thus, it appears that Vpr strengthens the interaction of both weakly and strongly nucleophilic proteins for karyopherin. This study also provides a mechanistic explanation for why mutations in gag MA and Vpr result in an additive impairment in nuclear translocation of viral reverse transcription complexes in nondividing cells.

Studies presented at the conference provided evidence that HIV-1 Vpr enters the cell nucleus by a novel nuclear transport pathway **(Abstract 26).** Two classical transport pathways have been implicated in directing nuclear proteins to the nuclear pore complex. These include the importin-α/importin-β dependent and transportin-dependent pathways. The nuclear translocation of Vpr in this study was independent of either importin or transportin receptor pathways. In addition, through mutagenesis, two nuclear targeting motifs in Vpr were identified. Each of these nuclear localization signals function in a non-nuclear localization signal-dependent manner. Studies outlined by another group **(Abstract 27)** further addressed the role of Vpr nuclear localization in nuclear translocation of viral nucleic acids. The nuclear translocation of Vpr was dependent upon an intact α-helical domain and Vpr alleles with mutations in this domain (Q65E) localized to the cytoplasm. Surprisingly, different phenotypes were observed depending on the types of mutations introduced into Vpr. For example, some mutations in the α-helical domain prevented nuclear localization of Vpr and prevented provirus establishment in nondividing macrophages. Some mutations in Vpr did not affect nuclear translocation of the protein, yet markedly impaired virus replication in macrophages. Mutations that completely abrogated Vpr expression resulted in viruses that were able to integrate within macrophages, but which were unable to produce infectious virions. This phenotype has not been described previously for Vpr mutants and it will be important to confirm these results in order to identify how Vpr is influencing infectious virus production.

Studies from the laboratory of Emerman **(Abstract S42)** provided an update on studies into both nuclear import and cell cycle arrest properties of HIV-1 Vpr. Utilizing site specific mutagenesis, residues in Vpr that are important for its nuclear localization were identified. A Vpr F34I mutation caused a redistribution of this protein from the nucleus to the cytoplasm. This redistribution did not affect the ability of Vpr to delay cell cycle progression. However, this mutation introduced back into an infectious molecular clone markedly impaired the ability of the virus to infect nondividing macrophages without influencing its infection of T-cells. These data further substantiate the notion that the nuclear translocation of Vpr is important for the ability of the virus to infect nondividing cells. On the other hand, the role served by cell cycle

delay properties of Vpr in the virus lifecycle are less well-understood. Recent studies presented at the meeting **(Abstract S42)** may shed light on this process. Vpr causes a delay in cell cycle progression such that the G_2 phase of the cell cycle is greatly extended. The extent of virus production was increased three- to fourfold when cells were in the G_2 stage of the cell cycle. This was found to correlate with a concomitant increase in the level of transcriptional activity of the viral LTR in the G_2 phase of the cell cycle. The increased transcriptional activity of the virus was not directly mediated by Vpr. Cells that were arrested in the G_2 phase but that did not express Vpr also exhibited elevated LTR activity. This suggests that cellular factors necessary for efficient transcriptional activity of the LTR are more abundant in the G_2 phase of the cell cycle, and by extending the G_2 phase, Vpr maximizes the level of virus production during the life of the cell. These studies may also explain the weak transcriptional activating effect previously reported for Vpr. Thus, while Vpr may itself not be a transcriptional activator, it promotes cellular conditions in which viral gene expression is more active.

There is also selective pressure to maintain a functional Vpr open reading frame by HIV-1 in vivo. The study documented sequence evolution in a laboratory worker who was infected with a laboratory strain of HIV-1 that contained an inactivating mutation in *vpr* **(Abstract S42).** Over a course of several years, *vpr* mutant alleles were gradually replaced with variants containing functional *vpr* open reading frames. A similar phenomenon was observed in HIV-1 infected chimpanzees where the original Vpr-minus strain was gradually replaced by a population of viruses that had a functional Vpr. Since both infection of a laboratory worker with a laboratory-adapted strain of HIV-1 and infection of chimpanzees with HIV-1 represent non-pathogenic infections, this study suggests that viruses containing functional Vpr have a selective advantage even in such non-pathogenic infections. At present, however, it is unclear which of the Vpr functions (nuclear import, cell cycle arrest, uracil DNA glycosylac [UDG] association) was being selected for.

Additional effects of Vpr on host cell physiology were reported. Vpr was shown to induce T-cell receptor (TCR)-triggered apoptosis **(Abstract 564).** Thus, in the absence of TCR-mediated activation Vpr induced apoptosis, while in the presence of TCR-mediated activation Vpr interfered with the apoptotic pathway. This effect of Vpr on regulation of apoptosis was shown to be dependent on the suppression of NF-κβ activity by Vpr. Independent studies **(Abstract 565)** demonstrated that HIV-1 Vpr induces gross morphologic changes within bone marrow cells cultured in vitro. Treatment of human marrow cells resulted in the promotion of adhesion of mononuclear phagocytes to culture plates with a concomitant increase in the association with nucleated and nonnucleated cells. This pattern is reminiscent of accelerated phagocytosis, which is itself indicative of monocyte/macrophage activation. These studies suggest a role for Vpr in mononuclear phagocyte activation, which may promote productive viral infection or, through monocyte/macrophage activation, may provide a basis for the induction of cytopenias which are typical of HIV-1 infected individuals.

Studies provided further insight into the mechanism of action of Vif (viral infectivity factor) **(Abstract S39).** The *vif* gene is essential for virus replication within primary T-cells, macrophages, and certain cell lines, whereas some cell lines are permissive for productive infection by *vif*-minus viruses. Permissiveness cannot be restored when Vif is expressed in the target cell, suggesting that Vif modulates infectivity at a step late in the virus lifecycle, for example assembly, budding, or maturation. Although Vif has been shown to be contained within virus particles, reducing the amount of virion associated Vif by eightfold did not lead to a concomitant reduction in infectivity. To determine how Vif influences viral infectivity, Malim et al examined the abilities of *vif* genes from divergent lentiviruses to complement each other. It was demonstrated that the Vif proteins of HIV-1 enhanced infectivity of SIV_{SM} only in human cells. However, Vif proteins of SIVs from African green monkeys or Sykes monkeys were unable to influence infectivity of either HIV or SIV when expressed in human T-cells. Vif proteins of HIV also augmented the infectivity of the oncoretrovirus, murine leukemia virus (MLV) in human cells, even though MLV does not itself contain a recognized *vif* gene. Thus, Vif interacts with infected cells in a species-specific manner. The restricted nature of primate lentivirus/host cell interactions may be governed by the interaction between Vif and its cellular protein and this interaction itself may be species-specific.

Studies documenting the effects of the Nef (negative factor) accessory gene product on lymphocyte activation were presented **(Abstract S41).** Infection of interleukin-2 (IL-2)-dependent T-cell lines by $SIV_{MAC}221$ confers IL-2 independence. These infected cells release IL-2, suggesting that SIV augments IL-2 production in the infected host cell. The induction of IL-2 independence was found to be Nef-dependent. Intriguingly, HIV-1 Nef was found to functionally substitute for SIV-Nef in inducing IL-2 independence. These results raise the possibility that primate lentivirus Nef genes may influence lymphocyte acti-

vation via the IL-2/IL-2 receptor axis. Thus, virus-infected cells that are suboptimally activated may be brought to a higher level of activation through action of Nef on IL-2/IL-2 receptor-mediated activation, which would lead to a more permissive environment for virus replication in the host cell.

New data providing a mechanism for Nef-mediated CD4 downregulation were presented **(Abstract S25).** Nef-mediated CD4 downregulation was found to be dependent on CDC42/RAC1 and P21-activated kinase (PAK). A new Nef-binding protein (MBP1) was identified as the catalytic subunit of V-ATPase. Nef was shown to bind both MBP1 and CD4. The interaction of Nef with MBP1 resulted in the recruitment of Nef with bound CD4 to clathrin-coated pits, leading to rapid endocytosis of CD4 and subsequent degradation.

Regulatory Proteins

Exciting new data providing further insight into the mechanism of action of regulatory proteins were presented at the conference. The Rev protein is essential for retrovirus replication. The interaction of Rev with a Rev-response element (RRE) in intron-containing RNAs results in their export from the nucleus. Studies of Weis **(Abstract S24)** have identified a new cellular protein that mediates Rev-dependent RNA export. Rev contains a nuclear export signal (NES) and a yeast protein known as exportin 1 was shown to interact with the NES of Rev and mediate its nuclear export. This finding opens the way for the development of new strategies to block the action of this essential viral gene product.

The Tat protein represents another essential viral regulatory gene product that regulates viral gene expression. Utilizing a biochemical screen, compounds that inhibit Tat-dependent transcriptional elongation have been identified **(Abstract S27).** These compounds were shown to inhibit PTEFb, a novel cellular kinase that influences transcriptional elongation of cellular genes. Mutant forms of PTEFb kinase were shown to block, in a transdominant fashion, Tat-dependent LTR transcription without influencing host cell physiology. Thus, these inhibitors are able to influence the action of PTEFb kinase on viral gene expression at concentrations, which leaves transcription of cellular genes unaffected. These agents represent potential novel therapeutics for the inhibition of HIV-1 replication.

Structural Proteins

Previous studies documented the existence of tyrosine-based sorting signal on SIV-1 envelope glycoprotein that promoted rapid endocytosis of envelope via clathrin-coated pits. Thus, in infected cells, envelope glycoproteins have two fates: they are either incorporated into virus particles or they are rapidly endocytosed by clathrin-coated pits. The gag MA protein diverts HIV-1 envelope away from the endocytosis pathway. It has been suggested that this mechanism ensures that only those envelope glycoproteins that are competent for virion incorporation (which are MA associated) are targeted to the membrane. New studies presented at the conference **(Abstract 520)** investigated the in vivo significance of tyrosine-based sorting signals on lentiviral envelope glycoproteins. A highly conserved tyrosine (amino acid 721) in gp41 of SIV-MAC239 that forms part of a sorting signal, was mutated, and in vitro and in vivo properties of viruses containing substitutions at this residue were examined. Replication of a gp41 Y721 mutant in macaque peripheral blood mononuclear cells (PBMCs) or in CEM X174 T-cells was unaffected, and there was no reversion of the mutation during multiple passages in culture. In contrast, the gp41 Y721 mutant exhibited an impaired phenotype in rhesus macaques. In one infected animal, reversion of the mutation back to wild-type sequence within three months of infection was observed. In one animal that showed no reversion, there was an approximately 2 log reduction in peak virus load. These studies provide evidence that tyrosine-based sorting signals in the gp41 portion of the envelope glycoprotein are important for in vivo virus replication and pathogenicity.

Virus-Host Cell Interactions and Viral Coreceptors

Previously published studies have suggested that polymorphisms (V64I) in the CCR2 gene delay disease progression. These results were subsequently challenged by a report demonstrating no effect of CCR2 polymorphisms on disease progression. Studies presented by Doms **(Abstract S6)** addressed this issue. In vitro experiments demonstrated that CCR2b containing a V64I mutation supported viral fusion and infection as efficiently as did wild-type virus. Expression of CCR2b did not affect expression of other coreceptor molecules *in trans.* However, both wild-type and V64I versions of CCR2b were able to desensitize the major HIV-1 coreceptors. Collectively, however, the studies failed to show a direct effect of the CCR2b V64I polymorphism on receptor function and virus infection.

In contrast, data on CCR2b genotypes in 954 DNA samples from infected and uninfected patients in the Chicago Multicenter AIDS Cohort Study (MACS) cohort were presented by Moore **(Abstract S3),** which may clarify the debate on the role of CCR2b polymorphisms on disease progression. Data were presented that the CCR2 64I allele was associated

with increased survival and reduced rate of CD4+ cell depletion, but only in a sero-incident and not in a sero-converter cohort. The V64I mutation in the CCR2b allele was found to have a genetic association with polymorphisms in the CCR5 regulatory region. These polymorphisms in the CCR5 promoter likely reduce the level of CCR5 expression and subsequently impair virus replication and disease progression. In further studies, 8 different substitutions in the CCR5 promoter were identified (for example, 353C, 402A). These promoter polymorphisms had a protective effect on disease progression in the context of individuals with a heterozygous CCR5Δ32 genotype or a CCR2b 64I genotype. In some cases, these polymorphisms were correlated with a reduced binding of MIP1-β by CCR5. Thus, the combination of CCR5 promoter polymorphisms with either CCR2b 64I or heterozygous Δ32 CCR5 mutations results in delayed disease progression. Why this is apparent in sero-incident and not in sero-converter cohorts is unknown.

Studies examining the correlation between the syncytium-inducing (SI) phenotype of primary isolates and their tropism were presented **(Abstract S4).** Several studies demonstrated previously that SI HIV-1 strains usually emerge late in disease and are associated with a more rapid decline in CD4+ T-cell counts. The SI phenotype is the result of adaptation to the use of CXCR4 coreceptor usage in vitro. A panel of primary isolates was examined for the ability to infect macrophages from normal donors and from individuals with a homozygous Δ32 CCR5 deletion. Some of these viruses (for example, HIV-1_{2044}) were able to infect macrophages from normal and from Δ32 CCR5 homozygous individuals with equal efficiency. The infection of macrophages by HIV-1_{2044} was not inhibited by AOP RANTES (which blocks CCR5 coreceptor usage) but was inhibited by SDF-1, suggesting that some viruses are able to use CXCR4 for entry into primary macrophages. Variations on this theme were also noticed in that some viruses could use either CCR5 or CXCR4 for macrophage infection.

The in vivo pattern of coreceptor expression was presented **(Abstract 276).** In both lymphoid and non-lymphoid tissues, CD4+ T-cells and macrophages were found to be the major cell populations expressing CXCR4, CCR3, and CCR5. High-level CXCR4, CCR3 and CCR5 expression were observed in T-cells and macrophages at various anatomic sites. However, the expression is limited to a small fraction of these cells. Surprisingly, S100 dendritic cells did not appear to express significant levels of coreceptor molecules. Macrophages in the colon were found to express high levels of CXCR4 and CCR3. Significant levels of coreceptor expression were observed in the cells in the cervix but not in the vagina. Brain neurons were found to be highly positive for CXCR4 and CCR3 molecules, but surprisingly, no CCR5 expression was observed in these cells. Studies examining the expression of coreceptor molecules on CD34+ stem cells were examined **(Abstract 275).** CD34+ stem cells obtained from umbilical cord blood were found to express low to undetectable levels of CD4 and CCR5. Upon differentiation in culture, CD34 expression decreased while there was a concomitant increase in CD4 and CCR5 expression. As expected, these cells were permissive to HIV infection only after differentiation and upregulation of primary and coreceptor molecules. Upon differentiation in culture, these cells were only infectible by macrophage tropic HIV and not by T-cell tropic viruses, suggesting that CXCR4 is either present at suboptimal levels or cannot be utilized for infection of these cells.

Primary peripheral blood monocytes are refractory to HIV-1 infection. Upon differentiation to macrophages in vitro, cells become fully permissive to virus infection. This increased permissiveness to virus infection upon differentiation was shown to correlate with levels of CCR5 expression **(Abstract 40).** Surface expression of CCR5 and levels of CCR5 mRNA were increased upon monocyte differentiation. Furthermore, studies from another group **(Abstract 39)** presented evidence that HIV-1 infection of macrophages promotes CCR5 expression. FACS analysis demonstrated that CCR5 was expressed on a subpopulation of uninfected peripheral blood monocyte-derived macrophages. Following HIV-1 infection, however, a significant expansion of CCR5+ cells was observed and this was directly reflected by increases in the level of CCR5 mRNA expression. Thus, HIV-1 is able to augment CCR5 expression and thus, promote conditions for further virus spread and replication.

Several groups have turned their attention to establishing small animal models of primate lentivirus infection. In addition to receptor restrictions, the poor activity of regulatory genes in mouse cells has precluded the use of the mouse as a small animal model for primate lentivirus infection. However, expression of CCR5 and human CD4 in rabbit epithelial cells was shown to increase their permissiveness to HIV-1 infection **(Abstract 43).** These cells were infectible by CCR5-dependent HIV-1 strains but were not infectible by CXCR4-dependent strains. These studies raise the possibility that transgenic rabbits expressing human CCR5 and CD4 may provide a permissive small animal model for primate lentivirus infection. Studies presented at the conference suggested that CXCR4 usage is a fundamental feature of lentivirus biology **(Abstract 44).** Thus, infectivity and

syncytium-inducing capacity of FIV in human cells was found to be CXCR4 dependent and CD4 independent. Thus, CXCR4 usage appears to be exhibited by distantly related lentiviruses that cause an AIDS-like disease.

Infection of cells by HIV and SIV in vitro leads to the onset of viral cytopathic effects. The mechanisms through which primate lentiviruses induce host cell cytolysis are poorly understood, but have previously been shown to be envelope-dependent. Studies from the Baltimore laboratory **(Abstract 285)** attempted to examine the role of apoptosis in virus-mediated cytopathicity. HIV-1 was shown to direct cell killing of infected T-cells rather than indirect killing of uninfected cells and that this killing appeared to involve apoptosis. In addition, viruses with T-tropic envelopes exhibited greater cytopathic properties than viruses containing macrophage tropic envelopes. HIV-1 mediated cell killing was also observed in primary lymphocytes from patients who had a genetically defective Fas pathway, suggesting HIV-1 is killing T-cells by Fas-independent mechanism. These studies challenge earlier studies implicating Fas in HIV-1 mediated cytolysis.

Viral Reservoirs

An examination of viral clearance rates following initiation of highly active antiretroviral therapy has demonstrated rapid and slow phases of viral clearance. A number of investigators have been characterizing viral reservoirs and the half-life of viruses within these reservoirs in order to determine the basis for these different clearance rates. To date, most of the information available on virus turnover have been gained from an examination of the decay curves of plasma virions following initiation of potent antiretroviral therapy. The initial decay phase induced by antiretroviral therapy occurs over the first two weeks and leads to a 1- to 2-log drop in plasma virion levels. It has been proposed that this initial rapid decay phase reflects the rapid clearance of productively infected T lymphocytes. Thus, an extremely important but unaddressed issue regards the nature of the viral reservoir that responds less effectively to antiretroviral therapy during the second decay phase. Ho **(Abstract S16)** examined changes in PBMC-associated viral DNA over time in 18 individuals who had no detectable plasma virions. Proviral DNA half-lives of between 83 and 124 days were observed. In agreement with these calculations, a half-life of between 104 and 108 days was observed for PBMC-associated proviral DNA in individuals undergoing potent antiretroviral therapy **(Abstract 515).** This study also demonstrated that there was an approximately 20-fold higher level of unintegrated viral DNA than integrated sequences in PBMCs of individuals who had barely detectable levels of plasma virions. In the retrovirus lifecycle, unintegrated viral DNA has a relatively short half-life (on the order of 1 to 7 days in vitro), whereas integrated or proviral DNA is maintained for the life of the cell. Thus, the presence of unintegrated viral DNA indicates that those cells were infected recently. This, therefore, documents continued replication in individuals undergoing potent antiretroviral therapy despite undetectable levels of plasma virions. It raises questions as to the possible existence of sanctuary sites in which cells (macrophages, dendritic cells, persistently infected T cells) can maintain virus production even in the face of potent antiretroviral therapy. An analysis of the frequency of virus-infected macrophages and T cells in early and late stages of infection **(Abstract S17)** also supported the notion that the major source of HIV in tissues following potent antiretroviral therapy are small numbers of productively infected cells rather than reactivation of latently infected cells. Studies by Bucy and colleagues demonstrated that following antiretroviral therapy, the relative viral RNA copy number in each infected cell did not change significantly. Only the frequency of infected cells showed a significant change. Furthermore, the frequency of infected cells did not correlate with amount of plasma virions. These data are more consistent with the maintenance of a small population of productively infected cells rather than reactivation of a pre-existing latent reservoir. It was also emphasized that due to rapid clearance of viral immune complexes by follicular dendritic cells, the amount of virus being produced in the host is not faithfully represented by the amount of virus that can be measured in plasma.

An examination of the viral clearance rates in monkeys provided indices that were very similar to those measured for HIV-1 clearance in infected humans. Studies outlined by Feinberg and colleagues **(Abstract 274)** documented viral clearance rates in naturally-infected sooty mangabeys. Such naturally-infected monkeys showed no evidence of disease despite high virus loads. Following inhibition of virus replication with PMPA, two viral decay phases were observed. The first was characterized by a half-decay rate of 0.66 to 1.15 days and the second, slower half-decay rate of 5.9 to 20 days. Remarkably, these decay rates are similar to what has been previously reported in pathogenic (HIV-1 infection of humans) infections. Therefore, viral turnover rates appear to be similar in pathogenic and non-pathogenic primate lentivirus infections. Ho and colleagues **(Abstract 272)** examined lymphocyte turnover dynamics in SIV infected rhesus monkeys. A debated issue is whether the increase in number of circulating CD4

lymphocytes following inhibition of virus replication with antiretrovirals represents protection of cells from virus-mediated killing or whether it is a consequence of lymphocytes redistributing between tissue compartments. Using in vivo labeling of cycling cells with bromodeoxyuridine, the Ho group provided solid evidence for T cell turnover in the face of SIV infection. ■

Dr Stevenson is Professor at University of Massachusetts Medical Center, Worcester, Massachusetts.