

Advances in Basic Science

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Basic science continues to occupy a substantial portion of the program at the Conference on Retroviruses and Opportunistic Infections. At the 17th conference this year, presentations focused on advances in our understanding of cellular factors that regulate the interplay between the virus and the host cell and, in particular, cellular defenses such as tetherin (or BST-2) that antagonize viral replication. Research into basic mechanisms of primate lentiviral pathogenicity was also an area of great interest at the conference. An analysis of the evolution of lentiviral and primate genomes highlights the conflict between the virus and its host and illustrates the considerable gyrations that lentiviruses have undergone to acquire the ability to replicate within their primate hosts. It is now apparent that all 4 accessory proteins of lentiviruses are in some way involved in overcoming natural antiviral restrictions in primate cells. Therefore, because lentiviruses such as HIV-1 are hard pressed to avoid the intrinsic antiviral defenses of the cell, there is strong rationale for the development of strategies that harness the antiviral capacity of these natural cellular restrictions.

Cellular Factors Influencing the Interplay Between Virus and Host Cell

All viruses must commandeer cellular factors to replicate within the host. In the case of the immunodeficiency viruses, research over the past 2 decades has revealed the existence of cellular factors that are required for various steps in the viral replication cycle. This progress is best illustrated by the receptor and coreceptor molecules that primate lentiviruses use to gain access to the interior of the cell. Research into the interaction between the viral envelope glycoprotein and the receptor and coreceptor molecules on the cell surface has led to the development of novel small-molecule inhibitors like maraviroc that target the interaction between viral envelope glycoprotein and the CC chemokine receptor 5 (CCR5) coreceptor.

Research featured at the 17th conference revealed that it may be possible to therapeutically target not only the viral function but also the cellular

cofactors upon which the viral function is dependent. The viral integrase is an enzymatic protein against which small-molecule inhibitors have most recently entered the clinic. Integrase processes viral and cellular DNA for ligation that leads to the establishment of the integrated provirus. Detailed biochemical information on the end processing and ligation reactions promoted the discovery of integrase inhibitors like raltegravir that are now widening treatment options for people living with HIV-1 infection (eg, Abstracts 263, 514, 515).

Furthermore, a high-resolution crystal structure for integrase has recently been determined,¹ which will further aid in the development of an expanded list of integrase antagonists. Although integrase is necessary for the integration of viral with host cell DNA, it is not sufficient. Research from Debyser's group in 2003 identified lens epithelium-derived growth factor p75 (LEDGF/p75) as a cellular protein that strongly binds to HIV-1 integrase.² Since that time, research from several groups has revealed that the ability of integrase to catalyze the integrase reaction depends upon its interaction with LEDGF/p75. When LEDGF/p75 expression is reduced by RNA interference, viral integration and replication is inhibited.

The crystal structure of the integrase core domain in complex with the integrase binding domain of LEDGF/p75 has been resolved; it reveals an interface to which small-molecule inhibitors might be directed.³ Abstract 49 described the structures of first-in-class inhibitors of the LEDGF/p75 interaction. These 2-(quinolin-3-yl)acetic acid derivatives are micromolar inhibitors of the integration step that compete with LEDGF/p75 for integrase interaction. These agents were found to inhibit HIV-1 replication at micromolar concentrations and importantly, were active against raltegravir- and elvitegravir-resistant HIV-1 variants. This research points the way to the development of a new class of antiretroviral drugs that targets a cellular cofactor of integrase and raises the attractive possibility of targeting viral replication simultaneously on the viral and on the cellular interfaces of the integration reaction.

It was originally thought that primate lentiviruses like HIV-1 integrate randomly within chromatin. However, advances in the technology used to identify integration sites have revealed that HIV-1 preferentially integrates within actively transcribed genes. At present, it is not clear how the selective integration of HIV-1 within actively transcribed genes might impact or be advantageous to viral replication. In vivo studies have shown that depletion of LEDGF/p75 reduces the frequency of integration events into active genes. Therefore, LEDGF/p75 may function as a molecular tether to link integrase to sequences within transcriptionally active genes.

Abstract 145 presented evidence that it may be possible to reroute the HIV-1 genome to heterochromatic, gene-poor regions of chromatin. The authors fused the integrase-binding domain of LEDGF/p75 to the heterochromatin-binding protein CBX1. The authors then reduced expression of endogenous LEDGF/p75 and infected

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cells with HIV-1 and equine infectious anemia virus (EIAV). This CBX1 fusion protein was able to rescue HIV-1 and EIAV integration in LEDGF/p75-depleted cells. Active genes were disfavored by integration sites in cells expressing CBX1 fusion protein. Therefore, the normal integration site preferences exhibited by HIV-1 were reversed in the presence of this CBX1–LEDGF/p75 fusion protein.

These results underscore a model in which LEDGF/p75 supports integration by tethering integrase to chromatin and LEDGF/p75's tethering activity selectively targets active genes. One possible practical application of this research is that it might be used to retarget lentiviral vectors away from active genes, thereby reducing the probability of insertionally activating oncogenes in gene therapy trials. It would also be interesting to know whether retargeting of HIV-1 to heterochromatin might increase the probability of latent infection (see also Abstracts 251 and 252 for host factors that influence HIV integration site selection).

The capsid protein (CA) forms a cone-shaped core that protects genomic viral RNA in the extracellular viral particle. Mutations in capsid can affect capsid assembly and morphology of the core as well as core stability. In addition, the cellular factor TRIM5 α from rhesus macaques can affect stability of the incoming core and greatly impact virus infectivity. Therefore, assembly, morphology, and stability of the capsid core are all essential for HIV-1 infectivity.

Abstracts 50 and 495 described the identification of compounds that can bind to capsid and affect viral core morphology and that exhibit antiviral activity. Both research groups used cell-free capsid assembly assays to screen small-molecule libraries. Compounds emerging from the inhibitor screens were found to affect viral core morphology or to prevent the formation of virions. In some cases, virions produced in the presence of inhibitor were non-infectious. Inhibitor-resistant variants (Abstract 50) could be generated by serial passage of virus in the presence of the inhibitors. Resistance was correlated with the presence of mutations

in highly conserved residues at the N-terminal domain of capsid and within the C-terminal domain of capsid. Cell-free capsid assembly assays offer a strong opportunity for high-throughput screening and identification of novel inhibitors of capsid assembly and core morphology.

Intrinsic Cellular Defenses

Some cellular factors such as LEDGF/p75 and CCR5 are co-opted by the virus in its replication cycle, whereas other cellular factors antagonize viral replication. These antiviral cellular proteins, commonly referred to as cellular restrictions, are able to potentially antagonize viral replication. Cellular restrictions acting at various points in the viral life cycle have been identified. Because of the existence of these cellular restrictions, primate lentiviruses have been forced to evolve defense strategies that counteract the antiviral action of cellular restrictions.

It is now apparent that the viral accessory proteins (Vif, Vpu, Nef, Vpr/Vpx) are all, in some way, involved in the antiviral defense against cellular restrictions. The Vif protein, which is encoded by all primate lentiviruses, counteracts the antiviral activity of APOBEC 3 cellular cytidine deaminases. The cytidine deaminase APOBEC 3G causes G-to-A substitutions in nascent viral complementary DNA (cDNA) and inhibits reverse transcription of viral cDNA. The viral Vif protein overcomes this defense by targeting APOBEC 3 for proteasomal degradation.

Abstract 90 presented evidence that deamination of viral cDNA by APOBEC 3 may contribute to the acquisition of drug resistance mutations. The authors hypothesized that some Vif alleles may harbor mutations that compromise their ability to neutralize the antiviral effects of APOBEC 3 proteins. The authors identified a Vif mutant that appeared to be associated with virologic failure. This Vif mutant (K22H) exhibited impaired viral replication in the presence of APOBEC 3. Patients harboring K22H viruses exhibited a greater propensity to harbor drug-resistant

mutations resulting from G-to-A substitutions. The authors propose that polymorphisms that impact the ability of Vif to neutralize APOBEC 3G can lead to an increase in the G-to-A mutations that promote the emergence of drug resistance mutations.

Tetherin (also known as BST-2 or CD317) is an interferon-inducible cell protein that inhibits the detachment of nascent virions from the surface of infected cells.^{4,5} The Vpu protein that is encoded by HIV-1 and some simian immunodeficiency virus (SIV) strains opposes the action of tetherin/BST-2. Abstract 144 presented evidence that the ability of Vpu to counteract tetherin/BST-2 correlated with its ability to down-regulate tetherin/BST-2 from the cell surface. The authors observed that Vpu transmembrane domain mutants that down-regulated tetherin/BST-2 from the cell surface were able to enhance virion release, whereas a Vpu mutant that did not down-regulate cell-surface tetherin/BST-2 lacked the ability to enhance virion release. The ability to enhance virion release by Vpu was found to be independent of ion channel activity, which has previously been hypothesized to underscore Vpu's biologic activity. The authors propose that Vpu and tetherin/BST-2 interact within post-endoplasmic reticulum endosomal membranes and that this interaction mislocalizes tetherin/BST-2 away from sites of virus budding. Similar results underscoring a role for tetherin/BST-2 internalization by Vpu were presented in Abstract 213.

Although the Vpu protein specifically counteracts the antiviral activity of tetherin/BST-2, Vpu is not encoded by HIV-2 and most SIV strains. Therefore, these viruses have evolved different mechanisms to counteract the antiviral activity of tetherin/BST-2. Research groups led by Evans and Bieniasz have recently demonstrated that SIV Nef can counteract restriction by rhesus macaques' tetherin/BST-2.^{6,7}

In Abstract 142, the authors presented a fascinating story of the evolutionary changes in tetherin/BST-2 that drove the evolution of Vpu and Nef functions. The authors examined tetherin/BST-2 evolution in primates using

a maximum-likelihood approach. They observed strong signals of positive selection within the cytoplasmic tail of tetherin/BST-2, which is also the site of Nef interaction. However, the selection predated modern Vpu-encoding primate lentiviruses, suggesting that the evolution in tetherin/BST-2 was driven by selection from ancient viruses. In HIV-1 that encodes both Vpu and Nef, Vpu antagonizes tetherin/BST-2 but Nef is inactive against tetherin/BST-2. Conversely, in the ancestors to HIV-1 (chimpanzee SIV, SIVcpz), Nef antagonizes the host's tetherin/BST-2 but Vpu in these viruses is inactive against tetherin/BST-2. Therefore, as HIV-1 adapted to humans, the ability to counteract tetherin/BST-2 was lost in Nef and acquired in Vpu. However, the *nef* gene has been functionally retained by HIV-1, indicating that *nef* harbors other functions required for virus replication.

Data in Abstract 220 support the model in which tetherin/BST-2 is physically incorporated into HIV-1 virions. The authors visualized the location of tetherin/BST-2 using correlative fluorescence and electron microscopy. Virion incorporation of tetherin/BST-2 was evaluated by a bead-based immunocapture assay and by immunoblotting of tetherin/BST-2 in partially purified virions. By immuno-electromicroscopy, the authors visualized tetherin/BST-2 among and between virions as well as between virions and the plasma membrane. Virions tethered to the cell surface by tetherin/BST-2 could be released by proteolysis with subtilisin. The authors propose a model in which cell-associated and virion-associated tetherin/BST-2 molecules interact with each other to restrict virion release.

Several groups have presented evidence that myeloid lineage cells harbor an antiviral restriction that is active against HIV-1, HIV-2, SIV, and murine leukemia virus (MLV).^{8,9} This restriction is specifically counteracted by the Vpx protein that is encoded by HIV-2 and most SIV strains. However, to date, there is no direct evidence that the Vpr protein of HIV-1 and SIV is able to counteract this restriction even though the restriction is active against HIV-1. Data presented in Ab-

stract 223 mapped the active domain in Vpx to the amino-terminus of Vpx. Collectively, the aforementioned data underscore the notion that cellular restrictions exert profound effects on the biology of primate lentiviruses and that accessory genes have evolved in these viruses primarily to counteract the antiviral activity of cellular restrictions.

Viral Replication and Pathogenicity

Infection of the cell is initiated when envelope glycoprotein on the surface of the virion binds to receptor and coreceptor molecules on the cell surface. This step is followed by a fusion event between viral and cellular membranes that deposits the viral core in the cytoplasm of the target cell. By comparison, many other viruses enter the cell by endocytosis and subsequently fuse with the membrane of the endosome in a process that requires low pH. For HIV-1, the fusion event at the plasma membrane does not require low pH.

Abstract 120 presented evidence that challenged the fundamental view that HIV-1 cannot enter cells by endocytosis (see also Miyauchi et al¹⁰). The authors employed time-resolved imaging of single virions as well as a beta-lactamase assay to follow virus–cell fusion. The authors froze the entry process at different stages with the use of specific fusion inhibitors at various intervals following virus–cell incubation. They observed that the majority of viral particles acquired resistance to membrane-impermeable inhibitors before acquiring resistance to low temperature that blocks fusion events. This evidence suggested that HIV-1 enters endosomal compartments before fusing with the cell membrane. Dynasore, a dynamin inhibitor that blocks endocytosis, was found to be active against HIV-1 infection. Collectively, these results indicate that HIV-1 can infect primary T cells and cell lines via receptor- and coreceptor-mediated endocytosis and subsequent pH-independent fusion with endosomes.

In the transmission of virus particles between cells, transmission can occur by cell-free virus particles or

during cell–cell contact in the form of a virologic synapse. Data presented in Abstract 121 examined the factors that impact the transmission of virus between cells during the formation of a virologic synapse. Specifically, the authors examined whether the antiviral restriction tetherin/BST-2 localized to virologic synapses. They compared the ability of wild-type and Vpu-deleted HIV-1 variants to be transmitted between cells in contact. The authors found that in virus-producing cells expressing tetherin/BST-2, wild-type virus was transmitted efficiently, whereas Vpu-deleted viruses did not transmit at the virologic synapse.

This evidence was consistent with the accumulation of viral Gag protein at the virologic synapse in tetherin/BST-2-expressing cells containing a Vpu-deleted virus. Tetherin/BST-2 did not directly inhibit the formation of virologic synapse; rather, it specifically localized to the membrane contacts at the virologic synapse. Curiously, when fluorescent viral particles were followed at the virologic synapse, wild-type viruses were transmitted as discreet, small dots that faded over time. In contrast, Vpu-deleted virus particles were localized in large patches of membrane and were transmitted as such. However, the fluorescent signal did not diminish over time, indicating that there was no fusion with the cell surface. These results indicate that tetherin/BST-2 has the potential to antagonize virus detachment and transmission, both in the cell-free state and during cell-to-cell transmission in the virologic synapse.

Xenotropic Murine Leukemia Virus-Related Virus

Xenotropic murine leukemia virus-related virus (XMRV) is a newly recognized human retrovirus first discovered in prostate tissues of cancer patients. XMRV is very similar to xenotropic viruses found in the mouse genome. Obviously, the presence of a new retrovirus recoverable from human tissue generates a lot of interest in the retrovirus research community, and a burning issue revolves around

whether XMRV is capable of infecting humans, whether it has a disease etiology, and whether action should be taken to control its replication. Goff presented a summary of the existing research on XMRV in a plenary lecture (Abstract 132). Recent studies have suggested a high degree of seroprevalence of XMRV in prostate cancer patients and in individuals with chronic fatigue (CF) syndrome. Much of the controversy surrounding the potential role of XMRV in human disease centers around the frequency with which XMRV can be detected in prostate cancer and in CF syndrome patients. The widely divergent prevalence rates reported in different studies may stem from several factors including methods used in the detection.

Goff reviewed the literature for and against a role of XMRV in the etiology of CF syndrome and prostate cancer and presented evidence on the infectiousness of XMRV for human cells. All isolates of XMRV characterized to date appear to be exceedingly similar to each other (eg, 0.3% sequence diversions were found between the most distant pairs). Because sequence evolution is an inevitable consequence of error-borne reverse transcription, this extreme similarity would suggest that XMRV undergoes very few cycles of replication or, alternatively, that upon infection of a new host, the virus is fixed by host immune pressure. XMRV is antagonized by mouse APOBEC genes and by human tetherin/BST-2, and because XMRV has no obvious Vif- or Vpu-like functions, its ability to spread within the host may be limited by these cellular restrictions, thereby explaining its limited sequence diversion. Aside from this controversy surrounding its involvement in CF syndrome and prostate cancer, several lines of evidence indicate that XMRV is a bona fide infectious virus. It is able to replicate within peripheral blood mononuclear cells (PBMCs) and in fibroblasts and exhibits broad mammalian tropism.

As to the origin of XMRV, the limited divergence of XMRV isolates taken from different locations within the United States at different times sug-

gests a recent point of origin, and the relationship to xenotropic MLV further suggests recent cross-species transmission from mice. Because none of the xenotropic MLV variants has the exact sequence characteristic of XMRV, however, it is unlikely that xenotropic MLV is the immediate source. Rather, data are consistent with a relatively distant, signal cross-species transmission event followed by adaptation of the virus to humans and subsequent spread. Given that some MLV variants are transforming (via insertional activation), it is unclear as yet whether XMRV has transforming activity.

XMRV appears to be infectious for both B- and T-cell lines, and replication is associated with cytopathic effects. Abstracts 150LB and 151 described successful infection of rhesus macaques after intravenous inoculation with XMRV. Inoculation was followed by low-level transient plasma viremia and persistence of proviral DNA in circulating PMBCs for several weeks. XMRV-positive CD4+ T cells were detectable in most lymphoid organs including spleen, lymph nodes, and the gastrointestinal tract throughout the course of infection. Whereas complete XMRV dissemination had occurred by day 6 postinfection, prostate tissue was positive only during the acute phase of infection. Collectively, these studies indicate that lymphocytes are a primary target for replication in the absence of detectable plasma viremia. In addition, specific serologic markers were identified that can facilitate analysis of XMRV incidence in large-scale epidemiologic surveys.

Reservoirs and Mechanism of Viral Persistence

Antiretroviral therapy is able to sustain durable suppression of viremia. However, rapid recrudescence of viremia occurs if antiretroviral therapy is interrupted. Therefore, reservoirs exist that sustain HIV-1 persistence in the face of antiretroviral therapy. Some of these reservoirs are believed to harbor the virus in a latent state, one that is invisible to immune surveillance. How this state is established is a topic of great

interest. In particular, identification of mechanisms that permit interruption of latency is of interest because such understanding could lead to strategies to purge latently infected cells from the infected individual.

Abstract 253 examined HIV-1 integration sites in a primary CD4+ T-cell model of latency. In latently infected cells, the majority of integration sites were active genes, which were transcriptionally active according to serial analysis of gene expression. Furthermore, the orientation of the latent HIV-1 genome was the same as that of the upstream transcriptionally active gene. This orientation preference was not apparent in acutely or persistently infected cells. The authors hypothesized that transcriptional interference may play an important role in maintenance of HIV-1 latency.

Abstract 257 presented evidence for establishment of latency in both naive and central memory T cells in a primary CD4+ T-cell model of HIV-1 latency. Previous studies have hypothesized that HIV-1 latency is established primarily in activated central memory T cells that enter a latent state upon return to quiescence. In these studies, resting naive CD4+ T cells were found to be relatively resistant to HIV-1 infection *in vitro*. Studies with lymphoid histocultures and studies in SIV macaque models have demonstrated infection in both naive and central memory cells. These results would argue that proliferation of the host cell is not required for the formation of a latent provirus.

CD4+ T cells from healthy donors were infected with HIV-1 NL4-3 and sorted into naive and memory cell subsets, which were then evaluated for integrated viral DNA by polymerase chain reaction and for replication-competent virus by limiting dilution coculture. Although central memory cells contained 2-fold more viral DNA than did naive cells, they were found to harbor up to 8-fold greater replication-competent virus. This evidence suggests that whereas both naive and central memory cells can become infected, many infection events in naive cells result in proviruses that are un-

able to promote replication upon cell stimulation.

Abstract 287 extended on this theme and demonstrated that X4-tropic but not R5-tropic viruses infect and integrate within naive CD4+ T cells, whereas R5-tropic viruses were able to infect and integrate only within memory cells. X4-tropic-infected naive cells produced low levels of Gag protein but did not release infectious virions while in the resting state. However, activation of these cells led to the production of infectious virus, suggesting that they harbor a latent infection. Furthermore, the authors found integrated viral DNA in sorted naive cells of 2 of 3 patients receiving antiretroviral therapy. These studies suggest that the naive CD4+ T-cell subset may be a long-lived reservoir of latent HIV-1 infection *in vivo*.

Abstract 258 presented evidence that myeloid dendritic cells engender signals to resting T cells that allow them to be infected and establish a latent infection. Resting CD4+ T cells were cocultured with myeloid dendritic cells and then infected with HIV. The number of latently infected cells that could be established in this coculture was over 20-fold higher than that achieved upon infection of resting CD4+ T cells alone. Increased CD69 expression was detected in CD4+ T cells after coculture with dendritic cells, but there was no evidence for expression of activation markers including HLA-DR or CD38. Separation of myeloid dendritic cells from resting CD4+ T cells using a semipermeable membrane before the CD4+ T cells were infected statistically significantly reduced the frequency of latently infected cells. However, the frequency was higher than that obtained with CD4+ T cells alone, suggesting the presence of soluble factors in dendritic cell-induced latency.

Abstract 259 presented the results of studies aimed at reactivating latent HIV-1 using a Jurkat cell-line model of HIV-1 latency. This latently infected cell line was used to screen 640 natural products for compounds that activate HIV-1 expression in this cell line. Nine compounds from 6 structurally diverse classes were found to reactivate the

expression of latent HIV-1. The mechanism by which these natural products reactivate latent HIV is under investigation. An important consideration in these experiments is how well the Jurkat cell-line model of latency recapitulates physiologic latency as it exists in resting CD4+ T cells *in vivo*.

Abstract 260 evaluated the ability of histone deacetylase inhibitors to reverse HIV-1 latency. Chromatin-associated transcriptional suppression is believed to be a mechanism to maintain transcriptional dormancy of the integrated HIV-1 genome. The investigational drug ITF2357 is an orally active histone deacetylase inhibitor approved for clinical trials in humans. Investigators used latently infected U1 and ACH2 cell lines, which have been shown previously to maintain HIV in a latent state, to evaluate the effects of ITF2357 on viral p24 production. ITF2357 was found to be highly active in inducing HIV-1 gene expression (as evidenced by p24 production) at clinically relevant concentrations. Therefore, this compound has the potential for purging HIV-1 reservoirs. However, as with the research presented in Abstract 259, the question remains as to whether U1 and ACH2 cell lines are valid models for viral latency as it exists *in vivo*.

While considerable effort has focused on characterization of the latent reservoir that persists during antiretroviral therapy, a continuing debate centers on whether ongoing viral replication may persist during antiretroviral therapy in some individuals. Ongoing viral replication would have the potential to replenish viral reservoirs including latent reservoirs. Sessions 26, 47, 64, and 65 contained studies that evaluated the impact of treatment intensification on viral reservoirs that persist during antiretroviral therapy. The rationale behind such studies is to evaluate whether treatment intensification can perturb such reservoirs.

Although studies addressing this question have been hampered by a lack of sensitive assays with which to probe the viral reservoirs, investigators have been employing a variety of surrogate markers including immune

activation, ultrasensitive viremia assays, and assays of unintegrated viral DNA. The original studies of Giorgi and colleagues presented evidence that immune activation is an accurate surrogate of viral replication and T-cell depletion in HIV-1-infected individuals.¹¹ Pathogenic lentivirus infection is associated with elevated levels of immune activation that is reduced but not normalized by suppressive antiretroviral therapy. However, it is not known whether the elevated immune activation that persists during suppressive antiretroviral therapy is a result of ongoing viral replication.

A number of studies have revealed that most HIV-1-infected individuals receiving suppressive antiretroviral therapy exhibit a low level of viremia—several copies of viral RNA per milliliter of plasma. The origin of this low-level viremia is unclear, including whether it is a result of ongoing viral replication or the steady production of viral particles from persistent reservoirs. Data presented in Abstracts 279 and 280 indicated that antiretroviral therapy intensification with the integrase inhibitor raltegravir did not change the level of persistent low-level viremia in HIV-1-infected individuals receiving suppressive antiretroviral therapy. Similarly, Abstracts 101LB, 282, and 283 failed to observe an impact of raltegravir (Abstract 101LB) or maraviroc (Abstracts 282, 283) intensification on immunologic responses including immune activation markers (CD38+ or HLA-DR+ CD8+ cells). Therefore, these studies suggest that persistent immune activation in patients receiving antiretroviral therapy is unlikely to be the result of ongoing viral replication.

In contrast, Abstract 100LB presented evidence that raltegravir intensification of a suppressive antiretroviral therapy regimen reduced the extent of immune activation (measured by CD38+ or HLA-DR+ CD8+ cells) and impacted episomal viral cDNA dynamics in approximately 30% of patients. However, there was no effect of intensification on low-level viremia in these individuals. Episomal viral cDNA is a dead-end product of viral infection. However, these viral cDNA forms are

dynamic and turn over in vivo. As such, they are indicative of recent infection events. Upon intensification with raltegravir, there was a dramatic and transient increase in the frequency of episomes by 2 weeks to 4 weeks postintensification.

Because the formation of episomal cDNA requires de novo infection and reverse transcription, this evidence indicates that infection continues despite antiretroviral therapy in a statistically significant percentage of infected individuals. Furthermore, individuals with detectable episomal cDNA were more likely to have elevated levels of activated CD8 cells, suggesting that ongoing viral replication might be a contributing factor to immune activation in patients receiving antiretroviral therapy. Although these observations are provocative, they require confirmation in other cohorts undergoing suppressive antiretroviral therapy.

Abstracts 284 and 285 presented evidence that intensification with a coreceptor antagonist (maraviroc) reduced the size of the latent reservoir and residual viremia (Abstracts 284) and reduced the level of immune activation markers on both CD4 and CD8+ cell subsets (Abstracts 284 and 285). The basis for the effect of maraviroc on the latent reservoir is unclear. One hypothesis is that maraviroc engenders a signal through CCR5 that reverses viral latency. The ability to detect an increase in viremia as well as episomal cDNA in some of these individuals after maraviroc intensification is consistent with this hypothesis because reversal of viral latency and production of infectious virus could promote new infection events. Regardless of the mechanism, if these studies are reproduced, CCR5 inhibitors could represent one possible approach to purging the reservoirs that persist in the face of antiretroviral therapy.

Mechanisms of Immunopathogenesis

Studies investigating underlying mechanisms governing pathogenic lentivirus infection continue to remain a strong component of the conference.

In his plenary presentation (Abstract 73), Silvestri provided an update on factors that distinguish pathogenic and nonpathogenic lentivirus infections. Pathogenic lentivirus infection, as exemplified by HIV-1 infection of humans or SIV infection of rhesus macaques (SIVmac), involves high-level viral replication, accelerated CD4+ T-cell turnover, and elevated levels of immune activation. Studies from several groups have demonstrated that nonpathogenic infection, as exemplified by SIV infection of sooty mangabeys (SIVsm) or African green monkeys (SIVagm), exhibits all of the characteristics of pathogenic infection with the exception of immune activation.

Studies over the past several years have focused on processes that drive immune activation. The consensus is that loss of mucosal integrity, as a result of virus replication, permits translocation of bacterial products (lipopolysaccharides, LPS), which drives immune activation and further enhances conditions for viral replication. Previous studies by groups led by Brenchley, Douek, and others^{12,13} have suggested that infection and depletion of T_H17 cells, which are essential in host immunity against microbial pathogens, are direct causes of impaired mucosal integrity. In nonpathogenic infection, there is no apparent loss of T_H17 cells.

Silvestri presented evidence that the acute infection phase is remarkably similar in pathogenic and nonpathogenic infections. After acute infection, there is a generalized immune activation and massive viremia that lead to rapid depletion of gut CD4+ T cells. Silvestri presented data that similar events occur in the nonpathogenic infection of sooty mangabeys. However, analysis of cellular transcriptome profiles indicated that a number of cellular genes involved in immune activation are upregulated in both pathogenic and nonpathogenic infections. This activation profile was resolved following the acute infection phase in sooty mangabeys but persisted in pathogenic infection. Silvestri proposed that the rapid resolution of immune activation following the acute

phase of infection contributes to the nonpathogenic state.

Silvestri also presented evidence that central memory T cells, which are a self-renewing source of T effector memory cells targeted by the virus, are protected from infection in natural SIV hosts. Studies from the research group led by Picker have suggested that the depletion of T central memory cells is crucial for progression to AIDS in SIV-infected rhesus macaques. Silvestri presented evidence that CCR5 expression on central memory T cells from sooty mangabeys is extremely low relative to CD4+ T cells from nonnatural hosts. These cells were also found to be relatively resistant to SIV infection compared with T central memory cells from rhesus macaques. These results support a model in which both rapid resolution of immune activation after acute infection as well as target cell restriction may be contributing factors that protect natural hosts of SIV from CD4+ T-cell depletion and AIDS.

Continuing with this theme, Abstract 44 presented evidence that increased microbial translocation and loss of natural killer (NK) cells are associated with rapid SIV disease progression in pigtail macaques. The authors measured peripheral and gastrointestinal tract lymphocyte function, activation, and turnover as well as microbial translocation in the gastrointestinal tract of pigtail macaques and rhesus macaques at various stages of disease progression.

The degree of damage to the gastrointestinal tract was found to correlate strongly with the LPS level in the colon, which in turn correlated with the amount of immune activation in the colon. Colon NK cells were able to produce interleukin-17 (IL-17), which is important for maintenance of gut enterocytes and mucosal integrity. The frequency of IL-17-producing NK cells was found to correlate negatively with the extent of damage to the colon. These data indicate that continued damage to gut epithelia may drive rapid disease progression after SIV infection and further suggests that local NK cells within the gut are necessary to maintain mucosal integrity. Therefore,

NK cells may be crucial to preserving gut integrity and limiting microbial translocation and immune activation.

Abstract 96LB presented evidence for the existence of defective CCR5 molecules in sooty mangabeys that did not support SIV infection. The authors identified a novel 2-base-pair deletion in the sooty mangabey CCR5 gene that results in a frame shift of the fourth transmembrane domain. This mutant CCR5 molecule was not expressed at the cell surface, nor did it support SIV entry. There appeared to be little selection pressure for or against the mutant allele in sooty mangabeys from the Yerkes National Primate Research Center, where the allele was detected at a frequency of approximately 25%. Because sooty mangabeys support robust HIV infection, these results indicate that CCR5-independent entry pathways are being used by SIVsmm in sooty mangabeys. The use of alternative pathways may play a role in protecting critical target cells that restrict pathogenesis in natural SIV infection.

Data presented in Abstract 93 suggest that the whey acidic protein family member WFDC1/ps20 is an HIV-1 permissivity factor on CD4+ T cells. The authors demonstrated that CD4+ T cells can be segregated into ps20^{high} and ps20^{low} subsets and that ps20^{high} clones were preferentially susceptible to HIV-1 infection. To determine whether preferential infection and depletion of ps20^{high} CD4+ T cells in vivo could lead to a drop in circulating ps20 levels, the authors examined plasma levels of ps20 in infected and uninfected individuals. Statistically significant differences in ps20 levels were observed between healthy volunteers

and HIV-1-seropositive individuals; statistically significantly lower ps20 levels were found in control subjects versus infected individuals and in elite controllers versus chronic HIV-1-seropositive individuals. Therefore, HIV-1 infection is associated with a reduction in levels of circulating ps20, and plasma ps20 level is a novel correlate of viremia. It remains to be determined whether ps20 levels can be normalized after antiretroviral infection and whether reduction in ps20 levels correlates with other immunologic markers of AIDS progression.

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A list of all cited abstracts appears on pages 93-99.

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