Highlights of Basic Science Research

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The 9th Conference on Retroviruses and Opportunistic Infections arguably enjoyed its strongest showing in basic research. Some of the highlights included the identification of novel cellular factors that are essential for efficient HIV assembly and the identification of cellular ligands that may mediate the inhibitory action of the accessory protein Vif. Several presentations on the accessory proteins Nef and Vpr provided more insight into the possible mechanism of action of these enigmatic viral proteins. A general consensus has emerged regarding the important relationship between T-cell activation and susceptibility of the host cell to viral infection and replication. Several studies have begun to identify minimal states of T-cell cycle status that are sufficient for viral replication. Studies with dendritic cells have further pointed to the possible existence of a novel type of reservoir for HIV that surprisingly may not require the involvement of a proviral (ie, integrated) intermediate within the infected cell.

Virology

Following the transport of structural viral precursor proteins to the plasma membrane of the infected cell, virion proteins form immature assembly complexes that eventually detach from the cell. Proteolytic processing of structural virion proteins occurs predominantly after virus budding, leading to formation of the mature and infectious virion. Pioneering work by Carter and Wills has previously shown that the assembly and attachment of immature particles from the cell surface require sequences within the Gag polyprotein, otherwise known as late or L-domains. In HIV-1, the p6 domain of Gag contains the L-domain that facilitates virus particle budding. Previous studies using genetic screens have demonstrated that the p6 domain binds to a cellular protein called Tsg101. Studies presented at the conference have now provided definitive evidence that this cellular protein is essential for the final stages of particle budding and thus is an essential cofactor for viral replication.

Martin-Serrano and colleagues (Abstract 51) demonstrated that the Gag protein of HIV-1 and the matrix protein of Ebola virus bind Tsg101 in a 2-hybrid assay. Using a panel of HIV Gag p6 mutants, they demonstrated that infectious virion production was absolutely dependent upon the ability of p6 to bind Tsg101. A 4-amino acid (PTAP) motif was specifically required for interaction with Tsg101 and virion production. A similar motif was identified in the matrix protein of an Ebola virus. Importantly, in absence of the PTAP motif, the recruitment of Tsg101 to sites of viral assembly independent of Gag was sufficient to restore particle formation.

Demirov and colleagues (Abstract 52) extended this story by demonstrating that overexpressing a specific domain within Tsg101 potently inhibited virus particle production. The general requirement for this protein in retrovirus assembly was evidenced by its ability to impair the release of murine leukemia virus and Mason-Pfizer monkey virus particle production. The significance of this observation is that Tsg101 analogues represent potential specific inhibitors that target HIV particle production.

These studies complement published studies by Sundquist and colleagues (Garrus et al, *Cell*, 2001; Jenkins et al, J *Virol*, 2001) underscoring an essential role for Tsg101 in the assembly and production of HIV particles. The Sundquist group made use of a new technique in which specific RNAs can be targeted for degradation in the presence of small interfering RNAs. Sundquist and colleagues demonstrated that specific degradation of Tsg101 RNA within the cell by Tsg101-specific small interfering RNA rendered that cell incapable of manufacturing HIV virions. Collectively, these studies point to an exciting new target for antiretroviral intervention. These studies should spark efforts to identify dominant negative Tsg101 derivatives or nonfunctional mimetics that could target late stages in the viral life cycle.

Within the virus particle, viral nucleic acids are contained within a conical core comprised predominantly of the viral capsid protein. This core protects nucleic acids while in transit from the virus-producing cell to the new target cell. Upon infection of a new target cell, the viral core must be disassembled in order for viral nucleic acids to access cytoplasm of the target cell. Studies presented by Forshey and colleagues (Abstract 53) have identified mutations within the capsid that do not appear have any gross effects on the characteristic conical morphology of the core, but nevertheless markedly impair the ability of viral nucleic acids to undergo reverse transcription in the target cell. The investigators propose that these mutations affect the stability of the core and consequently may impair the function of the reverse transcription complex upon core disassociation. Thus, the capsid appears to control a crucial postentry step that influences the efficiency of viral DNA synthesis in target cells. Agents that affect core stability may have a utility in blocking establishment of infection in the cell.

The theme of host-virus interactions that are essential for the function of viral proteins was illustrated in a number of presentations. Bushman (Abstract S1) discussed host cell factors that influence viral integration, and how the host cell deals with the presence of viral complementary DNA (cDNA). The Bushman group previously demonstrated that linear unintegrated HIV-1 cDNA promotes apoptosis. It is well established that some linear HIV cDNA molecules undergo circularization, and this process is thought to minimize induction of apop-

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tosis by linear cDNA molecules. Bushman and colleagues characterized preintegration complexes in which viral cDNA synthesis occurs and demonstrated that they contain the Ku protein that is involved in host cell nonhomologous DNA end-joining. These investigators have further demonstrated that this end-joining pathway is required for circularization of viral cDNA. Together, these studies demonstrate that the nonhomologous DNA end-joining pathway is exploited by HIV in order to circularize viral cDNAs that would otherwise induce host cell apoptosis. By circularizing cDNA molecules, the virus avoids inducing host cell apoptosis, thereby preserving the host cell to maximize virus output.

Kalpana and colleagues (Abstract S3) have identified a cellular protein that interacts with HIV-1 integrase and is known as integrase interactor-1 (Ini-1). This cellular protein, previously identified in yeast, is a chromatin remodeling protein that regulates transcription by repositioning nucleosomes. Studies in the Kalpana laboratory have demonstrated that Ini-1 is important for HIV assembly and particle production, in that expression of a fragment of Ini-1 carrying a minimal integrase interaction domain inhibits HIV particle production *in trans*. The Kalpana laboratory further demonstrated that Ini-1 carries a nuclear export signal that is masked in the context of the native protein and unmasked when downstream sequences are deleted. These investigators propose that the nuclear export activity is important for the ability of Ini-1 to regulate viral assembly, for example, through incorporation into virus particles.

Gomez and Hope presented evidence for differential interaction between actin, a cellular cytoskeletal protein, and the HIV Gag and matrix protein (Abstract 154-M). Within the virus-producing cell, matrix exists in the form of a Gag polyprotein that is processed after virus budding. Gomez and Hope demonstrated that actin exhibits a differential interaction with matrix and Gag, in that a mature matrix protein interacted with actin whereas matrix in the context of unprocessed Gag did not interact within actin. Although the significance of this observation is unclear, the investigators propose that the ability of matrix to interact with actin may be important for the ability of matrix to regulate early events in viral infection, ie, following initial entry into the new target cell. Presumably, such interactions would not be required in a virus-producing cell, which would explain the lack of an interaction between the Gag precursor and actin.

Accessory Genes

All members of the HIV-1/simian immunodeficiency virus$_{cpz}$ (SIV$_{cpz}$) lineage contain a *vpu* gene. However, *vpu* is absent from the genomes of HIV-2 and SIV that infect nonhuman African primates (SIV$_{sm}$, SIV$_{agm}$, SIV$_{syk}$, SIV$_{lhoest}$, and SIV$_{col}$). Courgnaud and colleagues (Abstract LB1) described molecular characterization of a novel SIV (SIV$_{gsn}$) from Cameroon, which contains a *vpu* gene, and which represents the first demonstration of a *vpu* homologue within a virus of the HIV-2/SIV lineage. Although HIV-2 and SIV lack a distinct *vpu* gene, they nevertheless contain Vpu-like activities in their envelope glycoproteins. Although Courgnaud and colleagues presented no evidence to suggest that their *vpu* homologue was functional with regard to certain Vpu activities (eg, CD4 down-regulation), it will be interesting to determine whether their novel SIV$_{gsn}$ variant contains duplicate Vpu-like activities (ie, within Vpu itself and within envelope).

> Highlights of basic research included identification of novel cellular factors essential for efficient HIV assembly and of cellular ligands that may mediate inhibitory action of Vif

The accessory protein, Vif, is essential for viral replication in primary cells and certain cell lines and is present in the genomes of all primate lentiviruses except equine infectious anemia virus. Certain cell lines, commonly referred to as permissive cells, have been identified that are permissive to infection by Vif-negative viruses. Studies examining the phenotype of heterokaryons among permissive and nonpermissive cells have demonstrated that nonpermissive cells (ie, those that will only replicate Vif-positive virus) contain an as yet unidentified cellular factor that inhibits the production of infectious virions. The current consensus is that lentiviruses have evolved a Vif protein to counteract this negative cellular factor, thereby preventing it from interfering with virus replication. Given the essential requirement for Vif in virus replication, there has been an intensive search for the negative cellular factor since it profoundly modulates viral infectivity.

Studies presented by Madan and colleagues (Abstract S2) pointed to one potential negative cellular factor that is targeted by Vif. Using a yeast 2-hybrid system, Madan and colleagues identified a nuclear body protein (Sp140) as a binding partner of Vif. Sp140 exists in multiple isoforms. The expression of Sp140 correlated with the Vif permissivity phenotype in that permissive cells that do not require Vif did not express Sp140, but nonpermissive cells that do require Vif for virus replication did express Sp140. Mutations that inactivate the biologic activity of Vif were also shown to impair the association of Vif with Sp140. HIV infection caused redistribution of Sp140 from nuclear bodies to the cytosol. Interestingly, Sp140 is related to the progressive multifocal leukoencephalopathy (PML)-associated nuclear body protein Sp100, and since PML has previously been implicated in defenses against unrelated viruses, this may represent a general antiviral defense mechanism of the cell. Madan and colleagues proposed that Sp140 or one of its isoforms may potently inhibit HIV and that Vif counteracts this inhibitory activity, thereby facilitating viral replication in primary cells.

Additional candidate Vif-interacting proteins were described by Sheehy and Malim (Abstract 55). Those investigators

took an approach very different from that of Madan and colleagues in that they identified 2 T-cell lines that, although genetically similar, were permissive and nonpermissive with respect to Vif function. Using a polymerase chain reaction (PCR)-based cDNA subtraction technique, Sheehy and Malim identified approximately 20 cDNAs that are expressed primarily in nonpermissive cells. Two of these cDNAs exhibited a suppressive effect on HIV replication when expressed in T-cell lines. At present, it is unclear whether either of these cDNAs are targeted by Vif. Nevertheless, this provides intriguing evidence for an intrinsic host defense mechanism against viruses such as HIV that may be exploited in order to reduce the susceptibility of the host cell to viral infection and replication.

Klein and Lingappa (Abstract 54) identified a Vif-associated protein (HP68) that appears to participate in viral capsid formation. HP68 was originally identified as a cellular factor that associates with HIV-1 Gag polyproteins, and dominant-negative variants of HP68 were found to interfere with posttranslational events in capsid formation. Subsequent studies by Klein and Lingappa demonstrated that HP68 also interacts with Vif. The significance of this interaction to Vif function is at present unclear.

All HIV-1 and HIV-2 variants and some strains of SIV contain a *vpr* gene within the so-called central viral region, which is a genomic region overlapping the *vif* and *tat* open reading frames. Members of HIV-2/SIV_{sm} lineage also contain a *vpx* gene. Three activities associated with HIV-1 *vpr (*induction of cell-cycle arrest, promotion of nuclear import of viral reverse transcription complexes, and association with the DNA repair enzyme uracil DNA glycosylase [UDG]), are segregated between the *vpr* and *vpx* genes of HIV-2/SIV_{sm}. That is, import activity is associated with SIV *vpx* whereas cell-cycle arrest and UDG association are activities exhibited by SIV *vpr*.

Goh and colleagues (Abstract 57) provided detailed information on the mechanism through which Vpr affects G_2 arrest. Goh and colleagues demonstrated a specific association of HIV-1 Vpr with the cellular phosphatase Cdc25C, which is an important regulator of cell cycle progression. The interaction of Vpr with Cdc25C specifically impaired the ability of this phosphatase to dephosphorylate p34Cdc2, which is normally necessary for progression into mitosis. These studies elucidate the mechanism of cell-cycle arrest by Vpr.

Evidence that cell-cycle arrest occurs within infected cells in vivo was presented by Sherman and colleagues (Abstract 58). These investigators used an intracellular p24 staining protocol to identify infected peripheral blood mononuclear cells from patients with primary HIV infection and high viral loads. They demonstrated that a large percentage of p24-positive, activated T cells obtained from infected individuals were arrested or paused at the G_2/M phase of the cell cycle, while p24-negative cells from the same patients had a normal cell-cycle profile. This study provides formal in vivo evidence that HIV infection of the host cell interferes with its cell cycle progression, and although it is not possible to confirm that this is strictly Vpr-dependent, this delayed cell-cycle progression is most likely of consequence of *vpr* expression.

Vpr is a virion protein, a feature that supports the notion that Vpr acts at an early stage in viral infection (ie, prior to de novo synthesis of viral proteins) to regulate viral replication. The paradox is that Vpr is also a nuclear protein, and an unresolved issue is why the nuclear localization of Vpr does not interfere with its virion incorporation. Studies described by Elder and colleagues (Abstract 140-M) suggest that Vpr localization is strongly affected by the presence of other genes previously shown to interact with Vpr. Elder and colleagues have been studying Vpr activity in the fission-yeast model system. As discussed previously, Vpr binds to the DNA repair enzyme UDG. In work by the Elder laboratory, overexpression of UDG redirected Vpr from the nuclear envelope into the nucleus or to the mitochondria, depending on which form of UDG was over-expressed.

Vpr has previously been shown to interact with *14-3-3* proteins, which are involved in cell-cycle regulation. Overexpression of RAD25, which is a yeast homologue of human 14-3-3 genes, redirected Vpr from nuclear envelope to the cytoplasm. Recently, Green and colleagues (de Noronha et al, *Science*, 2001) described how expression of Vpr can cause blebbing of the nuclear envelope, a property that may facilitate nuclear uptake of viral reverse transcription complexes. Interestingly, Vpr also induced nuclear blebbing in fission-yeast. Elder and colleagues propose that Vpr localization may depend upon coordinated interaction with cellular proteins that alter the subcellular distribution of Vpr, thereby allowing it to participate in multiple steps in the viral replication cycle.

Tsopanomichalou and colleagues (Abstract 141-M) used a yeast 2-hybrid system to identify cellular proteins that interact with Vpr. They identified isoforms of 14-3-3 as being able to interact with Vpr. Vpr and 14-3-3 could be coimmunoprecipitated from cell extracts. Since 14-3-3 proteins are involved in cell-cycle regulation, the authors propose that interaction between these proteins may interfere with the function of Cdc25C, thereby affecting G_2/M arrest in the infected cell.

Other potential mechanisms through which HIV-1 Vpr affects cell-cycle arrest were described. Sawaya and colleagues (Abstract 144-M) described the interaction of Vpr with p21, a cellular protein implicated in cell-cycle arrest. The investigators demonstrated that Vpr interacts directly with p21 and that overexpression of Vpr impaired p21-mediated cell-cycle arrest. Roshal and colleagues (Abstract 145-M) described the interaction of Vpr with ATR, a member of the PI3 kinase family. Inhibition of ATR function led to reduction in Vpr-induced cell-cycle arrest.

Two studies addressed the biologic significance of natural polymorphisms in Vpr alleles obtained from infected individuals. Tungaturthi and colleagues (Abstract 143-M) examined the impact of naturally occurring mutations, identified in Vpr alleles from diverse clades, on various aspects of Vpr function. Polymorphisms, particularly within turn regions of Vpr, markedly compromised Vpr stability, and some polymorphisms redistributed Vpr from the nucleus to the cytoplasm. The significance of these polymorphisms in terms of viral fitness are as yet unclear.

Lum and colleagues (Abstract 146-M) described experiments that draw a

provocative link between the Vpr and host-cell cytopathicity. They examined the in vitro properties of Vpr proteins obtained from HIV-1-infected patients who are long-term nonprogressors (LTNPs) with normal coreceptor alleles. Interestingly, they observed that peptide derivatives of Vpr alleles from LTNPs inefficiently induced host-cell apoptosis compared with wild-type peptides. The investigators identified an R77Q mutation in Vpr that was present at high frequency in the LTNP cohort. In vitro, Vpr peptides containing the R77Q polymorphism induced lower levels of apoptosis and caspase activation than peptides derived from a wild-type Vpr. The investigators propose that polymorphisms within Vpr that impact its apoptotic activity may contribute to the LTNP phenotype.

Many activities have been described for the accessory protein Nef, including down-regulation of cell surface receptors CD4 and class I major histocompatibility complex (MHC), modulation of host cell activation pathways, activation of chemokine genes in macrophages, and increase in susceptibility of suboptimally activated T cells to viral infection. Pillai and colleagues (Abstract 60) examined whether Nef alleles obtained from 2 different anatomical compartments, namely the plasma and central nervous system, would vary with regard to class I MHC down-regulation. It has been proposed that down-regulation of class I MHC by Nef suppresses recognition of the infected cell by cytotoxic T lymphocytes (CTLs). The investigators reasoned that there may be less pressure to maintain such a function in the central nervous system, where there is less CTL surveillance. Nef alleles were obtained from cerebrospinal fluid in plasma by reverse transcriptase PCR and multiple Nef alleles were examined for their ability to down-regulate class I MHC. The authors observed clustering of cerebrospinal fluid and plasma Nef sequences, but this was not due to differences in ability to down-regulate class I MHC. It is unclear whether these cerebrospinal fluid and plasma Nef alleles exhibit differences in other Nef-associated activities.

Saksela (Abstract S4) examined the impact of Nef on host-cell activation status. Until recently, scientists thought that permissiveness to productive HIV infection required T cells beyond the G_1b phase of the cell cycle. A number of studies have shown that the cycling T cells provide a more efficient environment for reverse transcription and nuclear translocation of viral cDNA. Therefore, much activity has focused on the potential role of Nef in augmenting T-cell activation status, thereby improving conditions for viral replication. Studies by Saksela and colleagues have focused on the mechanism through which Nef activates T-cell signal transduction. The investigators described an interaction between Nef and the p21-activated kinase-2 (PAK-2). There is extensive biochemical evidence that Nef activates PAK-2, but formal evidence that this interaction directly promotes host-cell activation is still pending.

Viral Replication Cycle

All lentiviruses and retroviruses encapsidate 2 copies of genomic viral RNA within each virus particle. Although there is detailed mechanistic information on the mechanism of RNA packaging specificity in retroviral systems, features governing specific packaging of HIV-1 genomic RNA are less clear. Studies presented by Russell and colleagues (Abstract 163-M) investigated the contribution of the so-called dimerization initiation site (DIS) to packaging of HIV-1 genomic RNA. Previous studies have indicated that this sequence promotes dimerization of genomic viral RNA prior to encapsidation. Russell and colleagues demonstrated that viruses carrying mutations at the DIS still retained significant levels of dimerized RNA. In contrast, viruses carrying deletions within the poly (A) and U5-PBS motifs at the 5' end of the viral genome exhibited reduced levels of dimeric RNA and severely delayed replication kinetics. These studies shed new light on the complex nature of HIV RNA dimerization and encapsidation.

In an extension of these studies, Whitney and colleagues (Abstract 162-M) examined the contribution of sequences in *gag* and in the DIS to dimerization and packaging of SIV genomic RNA. Mutations within stem-loop 1, a sequence containing the DIS, abrogated RNA dimerization, while the stability as well as incorporation of dimers was affected by sequences within *gag*.

Several groups have exploited genomic approaches to identify genes that are regulated after HIV infection. Ottones and colleagues (Abstract 166-M) examined the expression levels of genes upon HIV infection of macrophages. Interestingly, some genes previously shown to be implicated in host resistance to viral infection were up-regulated by HIV infection. For example, the *MxA* and *MxB* genes, which have been shown to interfere with the trafficking and transcriptional activity of viral ribonucleoprotein protein complexes, were up-regulated by HIV infection. Similarly, expression of the *NOD2* gene (the macrophage-specific homologue of *NOD1* that regulates apoptosis and NF-κB activation pathways) was up-regulated by HIV infection. These studies suggest an intriguing activation of host-cell defense mechanisms against viruses by the process of viral infection itself. It will be interesting to identify the mechanism through which HIV triggers increased expression of these antiretroviral gene products.

Van't Wout and colleagues (Abstract 168-M) examined genes that were up-regulated upon HIV infection of a human T-cell line. The investigators used a pseudo-type virus, which would bypass any potential signaling upon HIV binding to CD4 and coreceptor molecules. The investigators reported an up-regulation of several genes involved in sterol synthesis. Studies by the Hildreth group (Liao et al, *AIDS Res Hum Retroviruses*, 2001) have previously demonstrated that cholesterol present within lipid rafts promotes viral infectivity, most likely by influencing the fluidity of the viral membrane. Van't Wout and colleagues proposed that stimulation of sterol synthesis may lead to increased levels of cholesterol in the cell membrane and ultimately promote the infectiousness of viral particles emerging from that cell.

Several groups examined the potential impact of HIV infection on cellular signaling pathways. Previous studies have suggested that HIV interaction with CCR5 and CXCR4 initiates a signaling cascade, which may increase the susceptibility of the cell to infection. On the other hand, signaling itself is not

required for HIV entry since signaling-defective coreceptor molecules still permit efficient HIV entry. Francois and Klotman (Abstract 171-M) described the activation of the phosphatidylinositol 3-kinase (PI3-kinase) pathway both by soluble HIV gp120 and by virion-associated gp120. Signaling was observed both with the R5 and X4 gp120 molecules. Interestingly, those investigators presented evidence that activation of PI3-kinase signaling enhanced infection, in that treatment of macrophages and T cells with a PI3-kinase-specific inhibitor suppressed viral infection. The basis of this effect is unclear.

Del Corno and colleagues (Abstract 186-M) examined the consequences of gp120 signaling through CCR5 and CXCR4 on macrophages. Macrophages express both CCR5 and CXCR4, but infection of those cells through CXCR4 is very inefficient. Those investigators have shown previously that gp120 activates several signaling molecules through CCR5 and CXCR4 on macrophages. They have now extended those observations to examine the ability of virion-associated envelope to initiate signaling in macrophages. Evidence was presented that soluble gp120 activates the tyrosine kinase Pyk2 and several MAP kinases. By comparison, whole virions elicited Pyk2 phosphorylation approximately 300-fold more efficiently than monomeric gp120. Since MAP kinases have been shown to regulate the activity of chemokine genes, Del Corno and colleagues propose that this signaling cascade may activate leukocytes and promote leukocyte migration processes that may be operative in HIV-mediated neuropathogenesis.

Vasudevan and colleagues (Abstract 85) presented intriguing evidence that gp120 signaling through CCR5 may significantly impact the ability of resting cells to support HIV infection. As discussed previously, truly resting T cells are refractory to HIV infection in vitro. Nevertheless, observations in acutely infected monkeys and humans and experiments conducted with tonsilar histocultures suggest that under certain conditions, HIV can infect cells of a minimally activated phenotype. Vasudevan and colleagues established resting memory T cells from normal donors and challenged these T cells with R5 HIV or X4 HIV. Those cells were then activated with the chemokine MIP-1β or with R5 or X4 virions. The investigators demonstrated that resting memory T cells were infected by R5 HIV but not X4 HIV. Infection by R5 HIV correlated with activation of the protein tyrosine kinase, Pyk2. Importantly, a wild-type but not signaling-defective CCR5 molecule was able to promote susceptibility of resting memory T cells to HIV infection. Collectively, these data suggest that HIV binding to CCR5 generates a signal that increases a threshold for infection in resting memory T cells. The physiologic changes that occur in these T cells that allow them to support HIV infection await characterization. Previous work by Spina and colleagues (*J Virol*,1995) similarly established that resting T cells could be infected but not produce virions unless they were subsequently activated.

Host-Virus Interactions

An important task in basic HIV research is the identification of the mechanism of HIV latency and its contribution to maintaining viral persistence in the face of highly active antiretroviral therapy. Several presentations pointed to the existence of a potential new form of latency, one that may present a significant obstacle to long-term control of HIV infection. Richman (Abstract S21) discussed the possible existence of a long-lived reservoir of HIV virions on follicular dendritic cells (FDCs). He discussed recent studies by Smith and colleagues (*J Immunol*, 2001) that examined the stability of viral particles in a non-permissive murine model. Those investigators demonstrated that HIV virions were stable for upwards of 9 months when trapped on murine FDCs in vivo.

The mechanism by which virion integrity is preserved in association with FDCs is unclear, although some insight was provided in a talk by Pope (Abstract L2). She examined distribution of virions in pulsed, mature, and immature dendritic cells, and demonstrated that virions exhibit a vacuolar distribution in mature dendritic cells, but tend to accumulate at the plasma membrane in immature dendritic cells. The nature of this vacuolar reservoir is unclear. Clearly, it does not represent an endosome since the pH of these endosomes would rapidly inactivate viral infectivity. These studies have important implications for antiretroviral therapy. The half-life of virions in association with dendritic cells or FDCs is unclear. However, since these virions do not necessarily require a proviral intermediate, the establishment of these vacuolar reservoirs may be insensitive to current antiretroviral agents and could significantly hamper efforts to eradicate reservoirs of infectious HIV in infected individuals.

Current models of viral latency suggest that resting T cells harbor integrated viral genomes that are transcriptionally silent. Studies presented by Chun and colleagues (Abstract 493-M) suggest that latently infected T cells may not be as virally inactive as previously thought. They examined whether the latent viral reservoir is actually capable of manufacturing HIV virions in patients on potent antiretroviral therapy. Resting CD4+ T cells were isolated from 6 viremic and 7 aviremic patients. In the absence of activating stimuli, resting T cells from all viremic patients produced readily detectable levels of viral particles. Cultures from a small number of aviremic patients on therapy were also capable of shedding viral particles. These studies challenge the prevailing view that HIV latency involves an inactive proviral state. These studies further underscore the notion that full cell-cycle progression is not required either for HIV infection or for virion production.

HIV Envelope and Receptors

The envelope glycoproteins on the surface of HIV virions interact sequentially with CD4 and a 7-transmembrane coreceptor on the surface of cells. These events trigger fusion of cell and viral membranes, allowing entry of the virus core into the cytoplasm of the cell. The mechanisms and intermediate structures involved in this process are becoming better understood. Several therapeutic strategies that target these entry events are currently being developed.

Wyatt (Abstract L4) gave an overview and update on the gp120 structure. He has studied the cavity or hollow into which the N-terminus of CD4 binds. The

aromatic ring on the F43 of CD4 fills a second, smaller cavity close by on gp120. The site around the F43 cavity is highly conserved. The first hollow appears to allow in the IgG-like N-terminal domain of CD4 but is too small for most dimeric antibodies. IgGB12 is an antibody that does seem to be able to get into the hollow, although the mechanism is not known. Wyatt and colleagues measured entropy and enthalpy during CD4 binding. Results indicate that there are substantial rearrangements in the gp120 core when CD4 binds. These experiments included gp120 without V1, V2, and V3 and indicate that rearrangements do not just involve movement of the variable loops to expose a coreceptor binding site. The gp120 is floppy before CD4 is bound, and some mutations that partially push gp120 into the activated state were described (375S/W). A glycosylation site at the base of V3 (N301R) severely influences sensitivity to neutralizing antibodies. When this site is mutated, YU2 becomes more sensitive to F105, 15e (CD4bs monoclonal antibodies), and 39F (V3 loop monoclonal antibody), but not to 2F5 (gp41). The former three do not usually neutralize YU2. These monoclonal antibodies bind equally to YU2wt and YU2 with the N301R mutation. The mechanism of neutralization is therefore unclear.

Hoxie and colleagues (Abstract 81) reported a frameshift mutation in the cytoplasmic tail of HIV-1, which results in a stop codon. Amazingly, this results in exposure of CD4-induced epitopes on gp120. The implications of these results are unclear, but they show that there is "communication" between gp120 outside and gp41 domains inside the particle or cell.

Richman and colleagues (Abstract LB5) followed neutralizing antibody response over time in 14 infected individuals. Reporter viruses were used that carried envelopes PCR-amplified from plasma RNA at different times. Most patients generated strong neutralizing responses to autologous virus; however, the rate of viral escape was remarkable. Envelopes were resistant to neutralization by concurrent serum samples but were sensitive to serum samples taken at later time points. The data presented were very clear and it is strange that such lucid observations have not been made previously. The new observations were made possible by the new entry assay utilizing envelope amplified from plasma RNA. Previous assays of neutralizing antibody have been hampered by the difficulty of isolating, propagating, and titering multiple virus isolates.

All HIV and SIV strains bind CD4, and it is therefore hoped that reagents that inhibit this interaction will be active against divergent viruses. Colonno and colleagues (Abstract 9) reported that BMS-806 (molecular weight, ~400) blocks infection by a panel of HIV-1 isolates including subtypes A, B, C, and D; however, subtypes E, F, G, and O are resistant. BMS-806 binds gp120 and competes with CD4 for binding. Virus variants selected for resistance to BMS-806 carried mutations around the CD4-binding site consistent with this gp120 site as the target for BMS-806. It is hoped that BMS-806 will eventually lead to derivatives that block more diverse HIV isolates.

There is a proliferation of development of small molecules that target coreceptors. Some are now in phase 1 and 2 clinical trials. Reynes and colleagues (Abstract 1) reported that SCH-C, an investigational CCR5 inhibitor, was safe and well tolerated in 12 HIV-seropositive adults who took the drug orally every 12 hours for 10 days. Ten of 12 subjects showed at least a 0.5-$\log_{10}$ reduction in plasma HIV-1 RNA level, with 4 achieving a reduction of more than 1 $\log_{10}$. There was a prolonged effect on plasma HIV-1 RNA level before a rebound following cessation of treatment. In vitro inhibition experiments showed that a large range of R5 viruses of different subtypes are sensitive to SCH-C, but 1 Russian strain was largely resistant despite apparently using CCR5 only as a coreceptor.

Schols and colleagues (Abstract 2) described dose-escalating treatment of 40 HIV-seropositive individuals with AMD3100, a CXCR4 inhibitor, administered over 10 days by continuous infusion (AMD3100 is not bioavailable). One patient who carried X4 virus at the start and finish of the treatment benefited from nearly a 1-$\log_{10}$ reduction in plasma HIV-1 RNA level. Other patients who carried R5 as well as X4 or R5/X4 envelopes at the start had only R5 envelopes at the end of treatment. Other small-molecule inhibitors specific for CCR5 were described in the poster session on entry inhibitors, including spirodiketopiperazine derivatives and SCH-D, a more potent version of SCH-C (Abstracts 396, 402, and 404). These compounds hold great promise for therapy.

Steffens and colleagues (Abstract 84) looked at the location and mobility of CD4 and CCR5 on HOS cells. CD4 was located on actin-dependent structures (eg, microvilli on projections) and restricted to small spots. CCR5 was expressed slightly differently on the leading edges of cells and where membrane activity was occurring. This group then used fluorescence recovery after photobleaching to look at the mobility of receptors on the cell surface. A fluorescent area on the cell surface was photobleached to darkness and then the recovery of fluorescence due to fluorescent receptors moving back into the dark area was measured. CCR5 was highly mobile and fluorescence was completely restored in 20 seconds. Recovery was completely blocked if cholesterol was depleted. CD4 recovered much more slowly over 5 minutes.

HIV Origins

The 3 HIV-1 groups (M, N, and O) were derived from 3 individual zoonoses from a naturally infected primate reservoir. Closely related SIV_{cpz} strains harbored in chimpanzees are the most likely origin of HIV-1. However, very few SIV-seropositive chimpanzees have been identified, with most belonging to the *Pan troglodytes troglodytes* subspecies. These viruses cluster with HIV-1 subgroup N rather than with M, the cause of the HIV-1 global epidemic. Hahn (Abstract L1) described how noninvasive techniques are being used to survey SIV-specific antibodies and viral sequences in chimpanzee urine and fecal samples collected in the field. Hahn's group has now surveyed 2 more of the 4 chimpanzee subspecies, including *P troglodytes verus* in Cote d'Ivoire and *P troglodytes schweinfurthii* in Gombe Park, Tanzania, at the eastern end of their habitat. Of 58 chimpanzees, only 1 *P troglodytes schweinfurthii* from Gombe was SIV-seropositive, although a second seropositive chimpanzee was subsequently identified from a more focused

survey at Gombe. Sequences from these 2 animals clustered with the single previous *P troglodytes schweinfurthii* SIV and not with the *P troglodytes troglodytes* SIV or with HIV-1. SIV_{cpz} in *P troglodytes troglodytes* is therefore still the main candidate as the source of HIV-1. However, the seroprevalence of SIV_{cpz} in chimpanzees is remarkably low, and the possibility of an alternative primate species harboring the progenitor of HIV-1 is still open. Hahn also reported a survey of SIV in primate bushmeat sold at African markets and supermarkets. Meat from 13 of 16 different primate species was seropositive for SIV. Five new SIV lineages were discovered and 4 new species were identified as harboring SIV. Clearly, the potential for further zoonoses remains.

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Additional Suggested Reading

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