Basic Science Summary

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The scientific advances made in the year leading up to the 15th Conference on Retroviruses and Opportunistic Infections were overshadowed, to some extent, by setbacks in the AIDS vaccine research arena and in particular, the failure of the Merck STEP trial. Arguably, these disappointments were offset by strong advances that were being made in basic science and pathogenesis. In particular, recent discoveries into cellular factors that influence virus–host cell interplay and new insights into the mechanisms of viral pathogenesis were highlighted at the meeting. These research discoveries paint an optimistic picture regarding the development of new strategies to combat HIV and AIDS.

Cellular Restrictions and Viral Defenses

One of the most exciting areas of AIDS research in the past several years has been in cellular restrictions. This area of research was initiated with independent demonstrations by Malim's and Kabat's groups[1,2] that cells retained an activity that dominantly suppressed HIV-1 infection and that the HIV-1 accessory protein Vif counteracted the dominant restriction. Four years later, Malim and colleagues identified the nature of the restriction itself.[3] The restriction, called APOBEC 3G, is a cytidine deaminase that, when packaged into virions, causes extensive G-to-A hypermutation during reverse transcription of viral complementary DNA (cDNA). This compromises the stability of the viral cDNA as well as the functionality of the resulting provirus. To avoid this cellular restriction, primate lentiviruses have evolved a *vif* gene whose function is to target APOBEC 3 proteins for proteasomal destruction.

In 2004 the Sodroski laboratory identified a second cellular restriction termed TRIM 5α.[4] This protein targets the viral Gag polyprotein to exert a species-specific inhibitory effect on early events in virus infection. For example, the targeting of HIV-1 Gag by monkey TRIM 5α explains the resistance of cer-

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tain monkey cells to HIV-1 infection. Subtle amino acid changes can render Gag insensitive to TRIM 5α restriction.

Presentations by Guatelli (Abstract 104A) and Bieniasz (Abstract 114) described a third antiviral restriction that is counteracted by the accessory protein Vpu of primate lentiviruses. Research from a number of groups has revealed that in primary cells and in some cell lines, Vpu-defective viruses are unable to detach from the surface of the infected cell. Spearman's group[5] provided evidence that these cells contain a dominant restriction and that the Vpu protein counteracts the restriction.

At the conference, the Bieniasz and Guatelli groups independently revealed the nature of the cellular restriction that is counteracted by Vpu. In his plenary presentation, Bieniasz summarized recently published findings[6] that Vpu counters a cellular protein called CD317, or BST-2. Bieniasz's group has coined this protein a tetherin because it tethers fully formed virions to the surface of the infected cell. Their research has revealed that tetherin is an interferon-α inducible protein that causes retention of viral particles on the cell surface. These viral particles are subsequently endocytosed into CD317-positive cytoplasmic compartments. When tetherin was depleted by RNA interference, virus particle release was no longer Vpu-dependent. Bieniasz also demonstrated that Vpu colocalized with tetherin in infected cells. Guatelli, in his presentation, extended these findings and demonstrated that Vpu directly interacts with BST-2 and in the presence of Vpu,

surface levels of BST-2 are diminished. The BST-2 protein is a glycosylphosphatidylinositol (GPI)-anchored membrane protein of unknown function; BST-2 most likely, crosslinks cholesterol-rich virion membranes with the plasma membrane of the cell. Furthermore, the cellular expression of BST-2 appears to mimic the cells in which virus release is Vpu-dependent. Therefore, BST-2 expression is high in HeLa cells, in which virus production is Vpu-dependent, but low in HEK293 cells, in which Vpu is not required for efficient virus release. These presentations summarize exciting advances in understanding cellular restrictions against primate lentiviruses. That 2 of the 4 viral accessory proteins (Vif, Vpu) have evolved as counterdefenses to cellular restrictions illustrates how primate lentiviruses have responded to evolutionary pressure to evolve defense mechanisms to counter these cellular restrictions. Small-molecule inhibitors of these viral accessory proteins would be predicted to block the viral defense against the cellular restrictions, thereby rendering cells resistant to virus infection.

Cellular Cofactors in the Viral Replication Cycle

Presentations describing new cellular cofactors of HIV-1 replication were also highlights of the conference. Primate lentiviruses have a limited genetic repertoire comprising only 9 genes. Therefore, primate lentiviruses commandeer cellular proteins to complete certain aspects of their replication cycle. For example, these viruses use cell-surface proteins such as CD4, CCR5, and CXCR4 to gain entry to the host cell. An exciting presentation by Brass (Abstract 104bLB) summarized his group's recent published study,[7] which identified numerous host proteins required for HIV-1 infection. Brass and colleagues employed a functional genomic screen to survey proteins of the cell that were necessary for early and late events in

HIV-1 replication. In their screen, they transfected HIV-1-susceptible indicator cells with short interfering RNA (siRNA) pools. These cells were then infected with HIV-1. Supernatants of the transfected cells were recovered and used to initiate fresh infections of indicator cells. This 2-step process was used to reveal genes that were important for early events in viral replication as well as late events in the viral life cycle.

The approach identified over 250 host factors (referred to by the group as HIV-dependency factors, HDFs). Thirty-six host factors identified in the screen were previously implicated in HIV-1 biology (genes such as *CD4, CXCR4, nuclear factor [NF]-κB*). The remaining 237 genes were novel, and more than 100 of these revealed a phenotype when silenced by 2 or more individual siRNAs. The validity of the observations was increased by the fact that some HDFs found to be important for HIV replication were part of the same macromolecular complex. For example, 4 of 6 subunits of the nuclear pore complex *nup160* subcomplex were identified as HDFs. The involvement of the nuclear pore complex in HIV-1 infection is not surprising because a long-recognized feature of primate lentivirus infection is the ability to translocate through the nuclear pore envelope. This is a property that has been considered necessary for the ability of primate lentiviruses to infect nondividing cells. Therefore, silencing of nuclear pore complex components likely prevented efficient translocation of viral reverse transcription complexes from the cytoplasm to the nucleus.

Three late-acting HDFs were found to encode enzymes involved in the glycosylation of cellular proteins. Again, this is not surprising because the viral envelope protein is heavily glycosylated, and this modification is necessary for envelope function. However, the screen by Brass and colleagues revealed some unexpected HDFs. For example, several factors involved in autophagy, which is essential for the degradation and recycling of cellular components, were required for HIV-1 infection. This finding is surprising because no published scientific data link the HIV-1 rep-

lication cycle with autophagy. Similarly, RAB6 and VPS53, which are important for retrograde vesicular transport, were necessary for HIV-1 infection but were dispensable for murine leukemia virus (MLV) infection and for HIV-1 infection by the endocytic route.

Further insight into fundamental steps in HIV-1 replication were also revealed by the demonstration by Brass and colleagues that the karyphenin transportin3 and RAN BP2 were required for HIV-1 infection. The RAN BP2 protein is large and lies on the cytoplasmic side of the nuclear pore. This protein contains phenylalanine-glycine (FG)-domains. Current models of nuclear import suggest that nuclear pore filaments capture proteins to be imported, and these proteins "slide" down the FG domains toward the nuclear pore itself. Nuclear pore proteins also contain FG domains, which then capture the importing cargo. Transportin is a nuclear importer shown to be important for recruiting serine arginine splice factors to the nucleus. It was required for HIV-1 entry and for HIV-1 entry by the endocytic route but was dispensable for MLV infection. This information fits current models of lentivirus biology, in which lentiviruses have the capacity to translocate across the nuclear envelope, whereas simple retroviruses such as MLV cannot.

These observations underscore a model in which HIV-1 usurps transportin3 and proteins of the nuclear pore complex to shuttle into the nucleus of the nondividing cell. Future research will seek to determine which HIV-1 proteins interact with these nuclear pore constituents. More importantly, the identification of more than 200 novel cellular cofactors is an important advancement because each cellular cofactor represents a potential point of intersection with which to truncate HIV-1 replication. For example, small-molecule inhibitors of HIV-1 envelope–CCR5 interaction are being exploited clinically for the treatment of HIV-1 infection. The challenge will be to use this information to accelerate the development of novel HIV-1 inhibitors.

In session 31, several presentations focused on cellular proteins that influ-

ence HIV–host cell interactions. For example, Hakata (Abstract 100) described the role of the cellular proteins DCAF1 and DDB1 in the activity of the HIV-1 accessory protein Vpr. Several activities have been described for the HIV-1 Vpr protein, including enhancement of macrophage infection, cell cycle arrest, and association with DNA repair enzymes. Hakata presented evidence that the interaction of Vpr with proteins such as DDB1 is species-specific in that the Vpr of simian immunodeficiency virus of African green monkeys (SIV_{agm}) was unable to bind human DDB1. As a consequence, Vpr of SIV_{agm} was unable to induce cell-cycle arrest in human cells. This underscores many recent studies implicating DDB1 as a cellular cofactor necessary for induction of cell-cycle arrest by Vpr.

In another presentation (Abstract 150), de Noronha described the identification of proteins that interact with HIV-1 Vpr to mediate its ability to induce cell-cycle arrest. His group's studies also revealed an association between HIV-1 Vpr and a previously described Vpr binding protein (originally Vpr BP, now called DCAF1) that forms part of a ubiquitin-ligase complex. This suggests that Vpr commandeers the ubiquitin-ligase complex perhaps to degrade other cellular proteins; however, the identities of cellular proteins that may be targeted for degradation by Vpr are not known. The ability of Vpr to induce cell-cycle arrest was inhibited when DDB1 was depleted, suggesting that the protein targeted for degradation by Vpr is required for normal cell-cycle progression.

The presenter also raised the possibility that Vpr may target DNA repair enzymes for degradation. Previous studies have suggested that Vpr associates with and promotes degradation of the DNA repair enzyme uracil DNA glycosylase to prevent destruction of APOBEC-3-edited cDNA. He presented evidence that overexpression of DDB1, a protein that binds Vpr, impaired turnover of uracil DNA glycosylase and promoted its redistribution to the cell nucleus. With his colleagues, de Noronha suggests a model in which uracil DNA glycosylase associates normally with the DCAF1, DDB1, CUL4A ubiquitin-li-

gase complex and is targeted for proteasomal destruction upon ubiquitylation by this complex. In the presence of Vpr, however, association of uracil DNA glycosylase with the ubiquitin-ligase complex is enhanced, thereby augmenting degradation or nuclear shuttling of the glycosylase.

Viral Navigation Through the Cell

New insight was provided in session 15 into how HIV-1 navigates through the cell and between cells. After infection of cells by HIV-1, reverse transcription of viral cDNA is initiated in the cytoplasm. Nascent viral cDNA then translocates to the nucleus in the context of a high-molecular-weight nucleoprotein complex (commonly referred to as the reverse transcription complex). In the nucleus, a derivative of the reverse transcription complex (commonly referred to as the preintegration complex) binds to chromatin and catalyzes integration of viral cDNA with host cell DNA.

The diameter of the reverse transcription complex has been estimated to be approximately 30 nm, whereas the diameter of the cell is nearer 20 μm to 30 μm. Therefore, the voyage that the viral reverse transcription complex takes from the point of virus entry at the cell membrane to the host cell nucleus can be compared to the movement of a soccer ball across a soccer field. Thus, the virus likely uses a road map to navigate through the cell in an orderly fashion.

In her presentation (Abstract 49), Arhel provided a summary of current knowledge about the road map used by viruses such as HIV-1 to navigate from the plasma membrane to the nuclear envelope. She presented a model in which reverse transcription complexes deposited in the cytoplasm upon infection of the cell rapidly contact microtubules and then transit to actin filaments. These reverse transcription complexes then move along actin filaments at a speed of approximately 1 μm/s toward the nuclear membrane. At the nuclear membrane, a moiety on viral cDNA, referred to as the central

DNA flap, promotes the maturation of reverse transcription complexes to the preintegration complexes by prompting dissociation of the capsid shell. These events are prerequisites for the ability of the reverse transcription complex to translocate through the nuclear pore complex. Arhel and colleague Chameau have used electron microscopy to provide images of reverse transcription complexes docked at a nuclear pore complex.

Viral Dissemination Between Cells

Hope, in his introductory comments to the session, summarized current models of how HIV-1 virions are transmitted between cells. Previously, it was assumed that in HIV-1-infected individuals, viral particles produced by infected cells entered body fluids, where they randomly encountered new target cells. However, studies from the research groups of Martin and Ho demonstrated that cell-free simian immunodeficiency virus (SIV) particles, when injected into monkeys, were rapidly cleared from body fluids to the extent that cell-free virions had a half-life of minutes. For this reason, many investigators have favored the model in which viruses spread in the tissues between cells that are in close contact. Hope presented microscopic evidence for the transmission of viruses between cells through a virologic or infectious synapse.[8] These synapses comprise existing cellular pathways involved in antigen presentation and T-cell communication (for review see Jolly and Sattentau[9]).

It is now apparent that a variety of retroviruses and lentiviruses exploit receptor-containing adhesive junctions formed between cells in order to pass directly from infected to uninfected immune cells. Mothes (Abstract 50) presented live images of retroviruses transmitting between cells and summarized exciting new published information on the role of nanotubes in the transmission of HIV-1 between cells.[10] He and his group have been investigating why the infectivity of HIV-1 is 2 to 3 $\log_{10}$ higher when cells are in

contact and whether movement between cells is through diffusion or a direct process. For example, in the case of human T-lymphotrophic virus 1, there is an almost complete lack of virus-particle release into cultured fluids, yet the virus is able to spread efficiently between cells. Using fluid fluorescently labeled MLV as a model system, Mothes and colleagues obtained evidence that viruses move from infected to uninfected cells through filopodia. These filopodia are thin membrane projections along which viral particles "surf" unidirectionally toward the uninfected cell. Filopodia are normally short-lived structures; however, during the transmission of viral particles, they appear to form stable bridges between infected and uninfected cells. Virus particles can take in the order of 20 minutes to move along filopodia that connect the infected and uninfected cells. These filopodia can extend 10 μm to 20 μm from the surface of the infected cell.

The presence of filopodial bridges between infected and uninfected cells is dependent upon the presence of the viral envelope in the infected cell and the transmembrane receptor on the target cell. Although filopodia appear as rodlike membrane protrusions, virus particles surf on the outside of the filopodia during cell-to-cell transmission. The HIV-1 transmits across filopodia formed between T cells.[10] Also, nanotubes physically connect T cells over long distances and offer a route of transmission for HIV-1. Nanotubes have been shown to connect many cell types and allow transmission of calcium signals. They are formed when T cells make contact and subsequently part, and they can extend several cell diameters (100 μm). As with the distribution of MLV through filopodia, HIV dissemination through nanotubes is receptor dependent and occurs at a rate of approximately 0.08 μm/s. Therefore, membrane nanotubes provide a novel route for cell-to-cell dissemination of HIV-1. Nanotubes most likely avoid the rate-limiting diffusion step of cell-free virus spread, and further, may help minimize exposure of the virus particle to neutralizing antibody.

Inhibitors and Enhancers of Viral Infectivity

Kirchhoff, in his plenary presentation (Abstract 66), described the presence of natural enhancers and inhibitors of HIV-1 infectivity. He and his colleagues undertook a systematic analysis of compounds present in human body fluids that could influence HIV-1 infectivity. The investigators obtained a peptide library from large volumes of hemofiltrate obtained from individuals with renal failure. This hemofiltrate contains toxins as well as substances with a molecular rate less than 30 kDa. The peptide library obtained from the hemofiltrate was then screened for anti-HIV-1 infectivity.

A 20-amino-acid fragment of α_1-antitrypsin, termed VIRIP, was identified as a potent inhibitor of HIV-1 replication. The protein α_1-antitrypsin is present in large quantities in individuals with infection and inflammation. Its main function is to inhibit neutrophil elastase in the lung and the liver. The peptide VIRIP was active against all HIV-1 and SIV isolates and inhibited viral entry.[11] Although the inhibitory concentration of the initial VIRIP was in the micromolar range, structure-activity-relationship analysis led to the development of analogues with greatly increased antiviral potency. Importantly, VIRIP was active against HIV-1 variants resistant to other types of entry inhibitors such as T20. These studies suggest that VIRIP may target a novel step in HIV-1 entry before or shortly after insertion of the fusion peptide of HIV-1 envelope into the target cell membrane.

Kirchhoff and colleagues then used a similar approach to screen a semen-derived peptide library for novel inhibitors of HIV-1 infection. Although the goal was to identify an inhibitor of HIV-1 infection in semen, all of the peptide pools analyzed failed to significantly inhibit HIV-1 infection. In contrast, 1 of the peptide fractions markedly enhanced HIV-1 infection. This fraction contained a 34- to 40-amino-acid fragment of prostatic acid phosphatase (PAP). Although freshly diluted synthetic PAP fragments were inactive against HIV-1 infection, these became active after overnight incubation. Electron microscopy of the active form revealed the presence of amyloid fibrils of PAP. These fibrils were coined semen enhancer of virus infection (SEVI) and were found to capture virus particles and mediate their attachment to the surface of target cells. The SEVI enhanced both R5 and X4 HIV-1 infection of lymphocytes and macrophages. The enhancing effect of SEVI was greatly manifested during infection with low amounts of input virus. Remarkably, SEVI enhanced HIV-1 infection of lymphocytes and cells of the CEM cell line by up to 400,000-fold. This meant that in the presence of SEVI, 1 to 3 HIV-1 particles was sufficient for productive infection.[12] This study has important implications for the understanding of HIV-1 transmission at mucosal surfaces and for the development of microbicide. It will be important to identify the protease that clears PAP to generate the active peptide because interfering with peptide formation as well as amyloid fibril formation could represent a strategy for prevention of HIV-1 transmission across mucosal surfaces.

Underlying Mechanisms of Viral Pathogenicity

Exciting research aimed at understanding the underlying mechanism of pathogenic lentiviral infections was represented at the meeting in sessions 11 and 36. Pathogenic lentivirus infection (eg, HIV-1 infection of humans, SIV infection of rhesus macaques) is reflected by high-level viremia, accelerated CD4+ lymphocyte turnover, and increased immune activation. In contrast, nonpathogenic lentivirus infections (eg, SIV infection of sooty mangabeys) is reflected by high-level viremia and accelerated CD4+ lymphocyte turnover but normal levels of immune activation. Therefore, the extent of immune activation appears to be a distinguishing feature between pathogenic and nonpathogenic lentivirus infection.

For this reason, mechanisms driving immune activation have been of intense interest to AIDS investigators because this area may hold the key to how HIV-1 causes disease. At this year's conference, research was presented to suggest that immune activation is driven by translocation of bacterial products across mucosal surfaces. At last year's conference (2007), several presentations revealed the rapid destruction of gut lymphoid tissue during acute HIV-1 infection. Those studies showed that CD4+, CCR5+ memory T cells were rapidly depleted as a consequence of HIV-1 infection.

This year, 4 presentations (Abstracts 115, 116, 117LB, 374) provided evidence that a subset of these cells ($T_H 17$-CD4+ T cells) are preferentially depleted in the gastrointestinal (GI) tract of HIV-1–infected humans but not SIV-infected sooty mangabeys. The significance of this finding is that $T_H 17$ cells produce interleukin 17 (IL-17), which is thought to be important for antibacterial immunity. This cytokine is responsible for recruiting neutrophils, inducing the proliferation of GI enterocytes, and inducing the production of antibacterial defensins. The $T_H 17$ cells were found to be preferentially located in the GI tract but present at very low frequencies in blood. Therefore, the destruction of $T_H 17$ cells may undermine the ability of the immune system to control microbial agents. Furthermore, if IL-17 plays an important role in the proliferation of GI enterocytes, loss of the $T_H 17$-CD4+ subset may compromise the integrity of mucosal surfaces and permit translocation of bacterial products from the gut lumen into mucosal tissue.

This hypothesis was supported by 2 presentations (Abstracts 119, 377) that demonstrated a direct correlation between the extent of immune activation and the presence of bacterial products such as lipopolysaccharide or bacterial DNA. Because activated lymphocytes are preferred targets for HIV-1 infection, the increased immune activation driven by microbial translocation would serve to enhance conditions for viral replication and spread. These observations have very important implications for HIV-1 pathogenesis and the treatment of HIV-1 infection. If damage to the mucosal barrier and microbial translocation are triggering events in pathogenic in-

fection, strategies aimed at preserving mucosal activity and neutralizing microbial products would be predicted to limit the extent of pathogenicity. Next year's conference is likely to provide a forum for the presentation of research findings based on these therapeutic strategies.

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A list of all cited abstracts appears on pages 69-77.

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