

## Basic Science Highlights

### Mario Stevenson, PhD

*The 14th Conference on Retroviruses and Opportunistic Infections generated a lot of excitement with the announcement of clinical studies employing the use of 2 new classes of antiretroviral drugs that target the viral integrase enzyme and the viral coreceptor CCR5. In addition, a number of presentations on cellular restriction factors provided surprises regarding the mechanism by which cellular restrictions antagonize viral infection. There was also much interest in studies presenting novel cellular cofactors of HIV-1 infection. The conference illustrated how basic science research is paying off. Essential steps in the viral life cycle, uncovered through basic research, are now being targeted by new classes of antiviral agents. In addition, basic science is unveiling potential new targets of antiretroviral therapy.*

#### Bench to Bedside

A number of presentations, including the plenary given by Edward Berger of the National Institutes of Health (NIH) (Abstract 12a) who was this year's recipient of the Bernard Field's Memorial Lectureship, illustrated how basic science has uncovered aspects of the biology of HIV-1 that are now being exploited through antiretroviral therapy. Dr Berger discussed his laboratory's role in identification of key cell surface molecules that are essential for HIV-1 infection. Shortly after the discovery of HIV-1 as the causative agent of AIDS, several research groups identified CD4 as an important cell surface protein that was needed for infection of a cell by HIV-1. However, it was apparent that although CD4 was necessary for HIV-1 infection of a cell, it was not sufficient and this suggested the existence of other cell surface proteins that may be required for virus infection.

In 1996, Dr Berger's laboratory identified a chemokine receptor named CXCR4 as a cell surface protein that, in addition to CD4, was required for HIV-1 infection. Research examining the infectivity of different HIV-1 isolates from lymphocytes and macrophages suggested that there may be more than 1 type of coreceptor. Around that time, research by Russo, Gallo, and colleagues suggested

that chemokines prevent HIV-1 infection of cells, including macrophages. Subsequently, Dr Berger's research group and independently several other research groups identified a chemokine receptor known as CCR5 as a principle coreceptor that, in addition to CD4, was required for infection of certain cell types including primary macrophages.

These research discoveries have had a fundamental impact on the field. The identification of viral coreceptors has furthered the understanding of how HIV-1 targets specific cell types and has provided important clues to underlying mechanisms that underscore viral pathogenicity. Importantly, the identification of these coreceptors revealed novel targets for therapeutic intervention of HIV-1 infection. In particular, CCR5 has long been recognized as a particularly attractive therapeutic target. Shortly after the identification of CCR5 as a viral coreceptor, research from the Aaron Diamond Research Center in New York demonstrated that individuals harboring a homozygous 32-base pair deletion in the CCR5 gene were highly resistant to HIV-1 infection. In addition, the lack of a functional CCR5 gene did not appear to affect the health of the individual. Therefore, since CCR5 was apparently dispensable, this provided hope that antiviral strategies that block CCR5 function would be well-tolerated by the host.

Presentations in session 33 provided exciting evidence that the CCR5 inhibitor maraviroc is very effective in impacting viral replication in antiretroviral therapy-experienced patients

(Abstracts 104aLB, 104bLB). There was also much excitement regarding salvage trials using another novel class of antiretroviral agent that targets the viral integrase enzyme. Integrase is encoded by the viral polymerase gene and integrase catalyzes the reaction by which viral cDNA is inserted into the host cell genome. Basic research demonstrated that this enzyme was essential for viral replication and also provided detailed insight into the mechanism by which integrase inserts viral cDNA into the cellular genome. This multistep process includes 2 catalytic reactions. The first involves 3'-endonucleolytic processing of the free ends of the viral cDNA and, in a second reaction, the viral cDNA is joined to cellular DNA.

Initial screening assays that interfered with the formation of complexes between integrase and specific DNA sequences in the viral long terminal repeat identified compounds that, unfortunately, were inactive against HIV-1 in culture. Second generation screening assays recapitulated the strand transfer reaction and inhibitors identified in these assays exhibited antiviral activity in culture.

The fruits of these efforts were revealed in presentations in session 33 (Abstracts 105aLB, 105bLB) and in session 40 (Abstract 143LB) where results of the effect of integrase inhibitors on viral replication in antiretroviral therapy-experienced patients were presented. Clinical trials with the CCR5 and the integrase inhibitors in antiretroviral therapy-experienced patients demonstrated that these agents are effective against viruses which are highly resistant to other classes of inhibitors. These results have generated optimism in the field since they represent new additions to the armamentarium being used in the treatment of HIV-1 infection.

#### Cellular Restrictions

Primate lentiviruses like HIV-1 operate on a limited genetic budget. Therefore, these viruses commandeer cellular func-

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tions that serve as cofactors at various stages in the viral replication cycle. However, it is clear that some cellular proteins with which the virus interacts do not cooperate in viral replication but rather, antagonize viral replication. APOBEC 3G was the first cellular protein shown to antagonize primate lentivirus replication. A number of APOBEC 3 family members and in particular, APOBEC 3G and 3F, have been shown to antagonize replication of a variety of lentiviruses and retroviruses as well as unrelated viruses such as hepatitis B virus. In the case of primate lentiviruses, APOBEC 3G exhibits potent antiretroviral activity but this is counteracted by the viral *vif* gene. *Vif* antagonizes the antiviral activity of APOBEC by promoting its ubiquitylation and subsequent proteasomal degradation.

APOBEC 3G restriction has received a considerable amount of research attention. As a result, detailed insight has been gathered into how *Vif* promotes ubiquitylation of APOBEC proteins. However, the picture is less clear regarding the mechanism by which APOBEC proteins antagonize viral replication. At the conference, a number of presentations focused on the interplay between APOBEC and *Vif* (Abstracts 20, 49, 107, 184, 188, 325, and others). APOBEC 3G and 3F are cytidine deaminases that are packaged into viral particles during virus assembly. When those particles infect a new cell, and viral cDNA is being reverse transcribed, it is thought that these enzymes edit the viral genome during the reverse transcription process such that the synthesized cDNA contains extensive G-A mutations. These mutations are then thought to compromise the integrity of the viral cDNA both in terms of stability and its ability to encode functional viral transcripts. This mechanism of antiviral activity by the APOBEC proteins was questioned by the demonstration that APOBEC 3G mutants lacking a functional enzymatic site still retained antiviral activity.

Abstracts 204 and 211 extended on the theme that the antiviral activity of APOBEC 3 proteins is independent of cytidine deaminase activity. Abstract 211 examined mutant APOBEC 3G and

3F proteins with altered N-terminal or C-terminal cytidine deaminase motifs. APOBEC 3G and 3F mutants lacking cytidine deaminase activity were still fully able to function as antiviral factors. Importantly, the antiviral activity was strongly correlated with inhibition of reverse transcription. Similarly, Abstract 204 examined the cytidine deaminase activity and antiviral activity of a large panel of APOBEC 3G mutants in order to identify regions of APOBEC 3G which are important for antiviral activity. Collectively, these studies suggest that the antiviral activity of APOBEC 3 proteins might involve inhibition of reverse transcript accumulation and that this inhibition is independent of cytidine deamination.

A number of APOBEC 3 proteins have now been demonstrated to inhibit retrotransposition of LTR- and non-LTR retrotransposons. Abstract 188 examined whether the ability of APOBEC 3 proteins to inhibit retrotransposition required a catalytically functional active site on the APOBEC 3 protein. Mutations in 1 or both of the active sites of human APOBEC 3B, 3F and 3G were examined for cytidine deaminase activity and ability to inhibit retrotransposition. This study concluded that the active sites of APOBEC 3 proteins are required to inhibit retrotransposition. Abstract 19 presented evidence that HIV-1 infection of primary CD4+ T-cells *in vitro* actually induced retrotransposition and the progressive accumulation of both LTR and non-LTR gene elements. Future studies should determine whether the induction of retrotransposition activity by HIV-1 infection plays a role in HIV-1 mediated immunopathogenesis.

Several studies focused on cellular factors that play a role in *Vif*-APOBEC interplay. Abstract 20 provided evidence that 7SLRNA interacts with an APOBEC 3G and is preferentially packaged into HIV-1 particles as a result of this interaction. Reduction of 7SLRNA packaging impaired the packaging of APOBEC 3G and its antiviral function. To exert their antiviral activity, APOBEC 3 proteins must be packaged within virus particles. However, precise information on the mechanism by which

APOBEC 3 proteins are packaged within virions is not yet available. Furthermore, retroviruses such as murine leukemia virus (MLV) are sensitive to inhibition by mouse APOBEC 3. However, MLV does not harbor a known *vif* gene. Nevertheless, MLV appears to avoid restriction by mouse APOBEC 3 by excluding it from the virion.

Abstract 206 examined determinants that are important for APOBEC 3 packaging. The study demonstrated that both mouse and human APOBEC 3 proteins can be incorporated into HIV-1 Gag virus-like particles and that the nucleocapsid sequence within HIV-1 Gag is important for this packaging. Interestingly, chimeric proteins in which mouse APOBEC 3 fragments were fused to human APOBEC 3 fragments were packaged within MLV particles. Therefore, these chimeric proteins were able to overcome the mouse APOBEC 3 exclusion mechanism that is present in MLV. Abstract 49 characterized the subcellular localization of APOBEC 3G. This study presented evidence that APOBEC 3G localizes to specialized cytoplasmic compartments of mammalian cells, which are known as mRNA processing (P) bodies. P bodies are involved in degradation and storage of cellular mRNA. The study examined the colocalization of P body components with APOBEC 3G. The study concluded that the strong degree of colocalization between APOBEC 3G and P body components assigns a P body localization to APOBEC 3. Additional studies are underway to determine whether APOBEC 3G proteins that are contained within P bodies exhibit antiviral effects.

The cytoplasmic body component TRIM 5 $\alpha$  was identified 3 years ago as a protein that restricts HIV-1 infection of Old World monkey cells. Since that finding, a number of investigators have turned their attention to deciphering the mechanism by which TRIM 5 $\alpha$  proteins impact virus infection, and a number of presentations on this topic were featured at the conference.

Abstract 16 presented insight regarding the antiviral mechanism of TRIM 5 $\alpha$  restriction. Current models suggest that TRIM 5 $\alpha$  interacts with

capsid proteins of incoming virions. This interaction is thought to interrupt efficient uncoating of viral nucleic acid that subsequently leads to an inhibition of reverse transcription. Data presented in Abstract 16 presented evidence for a role of the proteasome in the antiviral mechanism of TRIM 5 $\alpha$  restriction. The authors examined the fate of GFP-VPR-labeled virus particles in cells over-expressing monkey TRIM 5 $\alpha$  and observed that proteasome inhibition led to the association of TRIM 5 $\alpha$  cytoplasmic bodies with ubiquitin and proteasomal subunits. The authors proposed a model in which TRIM 5 $\alpha$  proteins encapsulate the incoming viral core and alter trafficking away from the nucleus and to the proteasomal degradation pathway.

In Abstract 216, the effect of TRIM 5 $\alpha$  on the fate of capsid during early steps of the infection was examined. Previous studies have suggested that TRIM 5 $\alpha$  accelerates uncoating of the viral core. Data presented in Abstract 216 suggested that in restrictive cells (which express TRIM 5 $\alpha$ ) capsid undergoes a slow yet measurable degradation whereas it remains intact in nonrestrictive cells. The authors suggest that TRIM 5 $\alpha$  directs viral cores to a vesicular compartment for degradation.

In Abstract 185, evidence for the ability of human TRIM 5 $\alpha$  to restrict an extinct retrovirus was presented. Throughout the last 30 million years, TRIM 5 $\alpha$  has been evolving under extreme positive selection in the primate lineage. It has been proposed that this rapid evolution has been driven by conflicts with retroviruses. Chimpanzees contain approximately 150 copies of a retrovirus called PtERV that is now endogenous. However, this retrovirus is absent from the human genome. The authors examined whether the absence of PtERV from the human genome may have been due to possible protection of the human genome by TRIM 5 $\alpha$ . The authors constructed chimeric viruses containing the P12 and capsid regions of PtERV in the backbone of MLV. They found that human TRIM 5 $\alpha$  potentially restricts PtERV capsid suggesting that the evolution of TRIM 5 $\alpha$  may have protected humans

from infection when PtERV was exogenous some 3 to 4 million years ago.

### Cellular Cofactors

Presentations that provided evidence for novel cofactors of HIV-1 replication received a lot of interest at the conference. Several years ago, a couple of research groups identified LEDGF/p75 as a binding partner of HIV-1 integrase in human cells. In the past year, several groups have demonstrated a convincing role for this protein in viral replication.

Abstract 15 presented an overview of how LEDGF/p75 may impact viral replication. The authors presented a model in which LEDGF/p75 helps to tether integrase within the viral pre-integration complex to chromosomes. The study provides the rationale for the development for small molecule inhibitors which interrupt the interaction between integrase and LEDGF/p75. In order for viruses to assemble at the plasma membrane, newly synthesized retroviral Gag polyproteins are directed to the plasma membrane as a result of the membrane binding activity of the matrix domain of Gag. This domain contains an N-terminal myristyl group that can exist in both sequestered and exposed conformations.

Abstract 46 presented evidence that phosphatidylinositol-(4,5)-bisphosphate acts as a trigger of the myristyl switch. This factor is abundant in the inner leaflet of the plasma membrane and the findings present a mechanism for the specific targeting of HIV-1 Gag to membranes enriched in this phosphoinositide.

Abstract 47 presented evidence for a new cellular cofactor (TIP47) as being essential for the incorporation of HIV-1 Env glycoprotein into viral particles. During virus assembly, viral Env glycoprotein is specifically incorporated into virions but the mechanism by which Env associates with Gag particles is unknown. Research presented in Abstract 47 indicated that TIP47 promotes physical association between HIV-1 Gag and Env proteins during virus assembly. SiRNA-mediated silencing of TIP47 prevented the association of Gag with Env. The results

further suggest that TIP47 interacts with the matrix domain of Gag. Since TIP47 appears essential for the incorporation of Env into virus particles and for HIV-1 infectivity, the interaction of TIP47 with Gag or Env is an attractive antiviral target.

Lentiviruses like HIV-1 can efficiently infect nondividing cells. However, the viral and cellular determinants that promote the nuclear uptake of viral reverse transcription complexes remain obscure. In Abstract 48, evidence was presented that a truncated form of the cellular protein CPSF6 exhibits an antiviral effect by opposing the nuclear entry of HIV-1. When HIV-1 variants resistant to the antiviral activity of CPSF6 were derived, mutations in capsid were found to confer resistance. Interestingly, some of these mutants were impaired in their ability to infect nondividing HeLa cells but only slightly affected in their ability to infect nondividing macrophages.

In contrast, other mutations in CPSF6-resistant HIV-1 CA did not affect infection of growth-arrested HeLa cells but blocked infection of macrophages. The authors speculate that these mutations could influence the ability of capsid to dissociate from HIV-1 reverse transcription complexes and that truncated CPSF6 or its cofactors aid in this dissociation. This model suggests that nuclear entry of HIV-1 is in part regulated by the dissociation of capsid from the reverse transcription complex. The study also illustrates that caution should be exerted when using artificially growth-arrested cell lines as surrogates for nondividing macrophages.

The genomes of metazoans, including humans, encode short regulatory RNAs known as Micro RNAs (miRNAs). These miRNAs are expressed as long, capped, polyadenylated transcripts that are processed by the RNase III enzymes Droscha and Dicer to generate mature 22-nt miRNAs. The miRNAs are then incorporated into the RNA-induced silencing complex (RISC) to guide RISC to complementary mRNA. The target mRNA is then translationally repressed.

In his plenary, Dr Bryan Cullen (Abstract 112) overviewed what is cur-

rently known regarding the cellular machinery involved in the synthesis of cellular miRNA. He presented evidence that several DNA viruses including Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated herpes virus (KSHV) encode viral miRNAs that may target host genes involved in antiviral defense. Dr Cullen presented evidence that RNA viruses such as HIV-1 and hepatitis C virus do not generate detectable miRNAs in infected cells.

The identification and characterization of viral miRNAs encoded by EBV and KSHV were further expanded upon in Abstract 18. Future studies will identify how these viral miRNAs regulate viral replication in the infected cell. Recent studies have suggested that HIV-1 Tat protein can functionally suppress RNA silencing. Data presented in Abstract 217 presented evidence to the contrary and argued that Tat and related human transactivators do not act as suppressors of the RNA silencing machinery.

### Regulation of Virus Replication

The establishment of a latent infection in memory CD4+ T-lymphocytes has been proposed to represent the biggest obstacle to HIV-1 eradication in infected individuals. However, understanding the actual mechanism of latency has been difficult because of a lack of physiologic models which faithfully recapitulate viral latency in vitro.

Abstract 228 presented evidence that the maintenance of a latent state is governed by transcriptional interference. Several groups have presented evidence that latent viral genomes are located primarily within introns of active host genes in vivo. This raises a possibility that active genes may be transcriptionally interfering with the transcription of the integrated provirus. To examine this, the authors constructed an actively transcribed HPRT gene containing an HIV-1 vector within a specific intron. The authors observed that HPRT transcription negatively regulated HIV-1 gene expression implying that integration of proviruses into transcriptionally active genes may promote the establishment of a latent provirus.

Abstracts 274 and 275 examined conditions under which nonproliferating lymphocytes can become latently infected. In Abstract 274, the authors demonstrated that enhanced transduction and addition of deoxynucleosides enhanced the efficiency of integration in resting lymphocytes. This argues that limiting substrate rather than postentry blocks may limit integration into resting CD4+ lymphocytes. Abstract 275 examined how the level of CD4+ T-cell activation at the point of infection dictates whether a latent or persistently infected cell will subsequently be established. The results suggested that cells with the lowest division rate were more likely to survive infection and return to a quiescent state that harbored latent, persistent infection.

Abstracts 227 and 258 examined Toll-like receptor (TLR) ligands for their ability to modulate infection of CD4+ T-lymphocytes. Data presented in Abstract 227 presented evidence that TLR stimulation of CD4+ T-lymphocytes led to activation of viral gene expression. Therefore, TLR regulation, which has previously been shown to be important in macrophage function, may also impact the dynamics of T-cell reservoirs of HIV-1. In Abstract 258, CCR7 ligands CCL19 and CCL21 were found to render resting CD4+ T-lymphocytes highly permissive to HIV-1 infection. However, although there was a high degree of HIV-1 integration, virus production was at a low level. Therefore, these CCR7 ligands may contribute to the induction of a latent HIV-1 infection in resting CD4+ T-lymphocytes.

Although CD4+ T-lymphocytes and macrophages are considered the principle targets for HIV-1 infection in tissues, a variety of studies have suggested that other cell types may act as reservoirs of HIV-1 replication. Abstract 167 presented evidence that primary human eosinophils are susceptible to infection by X4-tropic HIV-1.

Abstract 273 presented evidence that CD34+ hematopoietic stem cells are susceptible to HIV-1 infection. The study showed that CD34+ stem cells were susceptible to HIV-1 infection and, despite infection, were able to survive and proliferate. The infection

of such cells could have important implications for HIV-1 persistence and for strategies to eradicate HIV-1.

Abstract 276 characterized the cell subset in the CD16 monocyte population that is susceptible to HIV-1 infection. Previous studies have shown that viral DNA can be detected in monocytes from HIV-1 infected patients on antiretroviral therapy. Levels of viral DNA in CD16+ and CD16- monocytes were compared and the authors observed that CD16+ monocytes were preferentially infected at levels compatible to resting memory T-cells. The authors suggest that CD16- monocytes may restrict HIV-1 infection because they contain active low-molecular mass APOBEC 3G complexes and may also exhibit limiting amounts of CD4 and CCR5 expression as compared with CD16+ monocytes.

Some cell types may not be directly susceptible to HIV-1 infection but may assist in the dissemination of the virus to other cell types. Previous studies have shown that dendritic cells have the capacity to capture HIV-1 particles and present them to CD4+ T-cells.

Abstract 202 presented evidence that Langerhans cells (LCs), which are a subtype of dendritic cells, can capture HIV-1 particles and transmit internalized particles to CD4+ T-cells in trans. Evidence was presented that viral particles are directed to an intracellular compartment that was positive for tetraspanin markers including CD81. The major histocompatibility complex-like protein CD1A appeared to play an enhancing role in HIV infectivity. This suggests that HIV traffics through a CD1A+ compartment in LCs and that this trafficking is important for transinfection.

### Pathogenesis

Several presentations highlighted the role of viral determinants in disease pathogenesis. The viral accessory protein Nef has been shown to exhibit a variety of functions. Among these activities, Nef has been shown to down modulate T-cell receptor-CD3 complexes from infected cells thereby restrict-

ing their ability to respond to stimulation and activation-induced cell death.

In Abstract 156, data were presented to suggest that the ability to down-regulate TCR-CD3 and to block apoptosis is only exhibited by *nef* alleles of viruses that exhibit nonpathogenic infections. In contrast, *nef* alleles from HIV-1 and subset of closely related simian immunodeficiency virus (SIV) lack the ability to down-regulate TCR-CD3. The authors suggest that differences in *nef* function may contribute to the high levels of immune activation and apoptosis that characterize pathogenic HIV-1 infection. The authors further suggest that the ability of naturally infected monkeys to maintain high viral loads in the absence of pathogenicity may be due to the ability of Nef to prevent high levels of immune activation and apoptosis.

Individuals infected with HIV-1 variants containing defective alleles *nef* have been shown to exhibit a long-term, non-progressive HIV-1 infection. Abstract 250 examined 6 individuals with Nef-deleted viruses who had long-term, non-progressive infection, 4 of whom eventually had HIV disease progression. Individuals harboring Nef-defective viruses who subsequently exhib-

ited HIV disease progression harbored viruses with extended coreceptor use to CXCR4. Therefore, despite low levels of viral replication in these individuals harboring Nef-defective viruses, there was sufficient ongoing replication to allow envelope evolution and expanded coreceptor use that may ultimately be associated with disease progression.

In Abstract 269, *vpu* and envelope genes in 240 HIV-1-infected women from a high-risk commercial sex worker cohort were examined. Of the 240 samples examined, 16 contained an incomplete *vpu* gene and 13 of these also lacked a complete envelope gene. In addition, the incidence of *vpu* and envelope defective proviruses increased between 2000 and 2004 suggesting that these isolates are becoming more prevalent. Patients harboring proviruses with defective *vpu* and envelope genes had higher CD4+ cell counts and lower plasma viral load than patients harboring intact proviruses. In Abstract 304, evidence was presented that TLR ligands induced the expression of activation markers on CD4+ and CD8+ T-cells. The authors propose that microbial products can induce T-cell activation and turnover. Therefore, heightened translocation

of microbial products through gut mucosa could contribute to exaggerated immune activation that underscores chronic HIV-1 infection (see also Abstract 227).

In approximately 50% of patients, disease progression is reflected by an emergence of CXCR4-utilizing viruses. Abstract 252 examined the timing of HIV-1 coreceptor usage in subjects with rapid and slow rates of CD4+ T-lymphocyte depletion. Results indicate that the emergence of X4 viruses is associated with the rapid loss of CD4+ T-cells but that this emergence occurs after rapid CD4+ T-cells loss begins. This would suggest that the emergence of X4 variants is a result of rather than a cause of rapid CD4+ cell depletion.

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**A list of all cited abstracts appears on pages 83 to 91.**

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