

Developments in Basic Science Research

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The 13th Conference on Retroviruses and Opportunistic Infections featured a strong basic science program that provided new findings on the nature of HIV disease as well as surprises regarding the antiviral action of innate restriction factors.

Intrinsic Cellular Restrictions and Viral Replication

In the past couple of years, the identification of cellular proteins that potentially restrict infection of cells by primate lentiviruses has revealed the extent of the conflict between the virus and its host. The cellular and humoral arms of the immune system are generally considered to comprise the major defense strategy of the host against viruses such as HIV-1. However, it is now apparent that there are additional lines of defense against infection by primate lentiviruses and that cellular proteins including APOBEC 3 family members and TRIM 5 α are major players in an intrinsic antiviral defense strategy commonly referred to as innate cellular restriction. APOBEC 3 family members, and in particular APOBEC 3G and 3F, are cytidine deaminases that are packaged into virions and upon infection of a cell introduce catastrophic G-to-A hypermutations that compromise the integrity of the viral cDNA. The primate lentiviral Vif proteins counteract the cytidine deaminases by targeting them for proteosomal degradation. As a result, there is very little APOBEC packaging in virions and viral reverse transcription is not compromised.

Research from a number of laboratories has established a working model in which the enzymatic activity of APOBEC is necessary for its antiviral effect. To exert an antiviral effect, APOBEC must be packaged within virions. Studies summarized by Greene

and colleagues (Abstract 61) reveal a novel antiviral mechanism for APOBEC and one that may explain a poorly understood aspect of primate lentivirus biology. Dr Greene's group has identified a low molecular mass (LMM) form of APOBEC 3G that is enzymatically active and is present in the cytoplasm of resting CD4 + T cells and peripheral blood monocytes. Truly quiescent (G₀ stages of cell cycle) CD4 + T lymphocytes and peripheral blood monocytes have long been recognized as being refractory to productive HIV-1 infection. Studies presented by Dr Greene suggest that it is this enzymatically active LLM form of APOBEC 3G that accounts for the resistance of quiescent lymphocytes and monocytes to HIV-1 infection. When APOBEC 3G was silenced in quiescent lymphocytes using RNA interference, quiescent lymphocytes became susceptible to productive HIV-1 infection (Chiu et al, *Nature*, 2005). When quiescent lymphocytes are stimulated to enter cell cycle or when monocytes differentiate to macrophages, they become permissive to productive HIV-1 infection.

Dr Greene likewise demonstrated that lymphocyte stimulation and monocyte differentiation also result in incorporation of APOBEC 3G into a high molecular mass (HMM) RNA-protein complex that is enzymatically inactive. Resting tonsillar lymphocytes were also found to contain an HMM APOBEC 3G complex which would explain the ability of resting lymphocytes to support HIV-1 infection *in vivo*.

These experiments present a paradox. The current model is that APOBEC 3G must be packaged in virions to inhibit viral infectivity (by compromising reverse transcription). If APOBEC

3G is present as an HMM enzymatically inactive complex in cycling and permissive cells, why is it able to affect viral replication? The answer appears to be that APOBEC 3G is packaged as an HMM complex but the complex is subsequently degraded into an enzymatically active LMM form.

Dr Greene presented evidence that the viral RNAase H activity of reverse transcriptase is responsible for degrading the RNA that maintains the integrity of the HMM form of APOBEC 3G thereby reducing it to an LMM form that is enzymatically active. Dr Greene also presented the results of a proteomic approach that his laboratory has taken to characterize the components of the HMM complex of APOBEC 3G. More than 60 cellular proteins were associated with the HMM APOBEC 3G complex and were found to be similar in composition to a previously described high molecular weight complex known as a Staufen granule. Staufen is a protein that is involved in localizing small mRNAs during oogenesis and early central nervous system development in *Drosophila*. The mammalian Staufen protein harbors several conserved double-stranded mRNA-binding domains and forms granules that are transported to distal dendrites during neuronal maturation. These granules also colocalize with ribonuclear particles that transport small mRNAs to the dendrites. Collectively, these observations provide new and intriguing insights into the defenses that are levied against primate lentivirus infection.

Several aspects of the mechanism by which APOBEC 3 proteins are packaged in virions remain unanswered. Several studies (Abstracts 212, 213, 214, and 218) focused on a potential mechanism for APOBEC 3 packaging. Two main models were described that involve the interaction of APOBEC 3 with genomic viral RNA or the interaction of APOBEC with

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structural virion proteins including nucleocapsid and Gag. Although HIV-1 Vif, which is the viral counter defense against the antiviral activity of APOBEC 3 proteins, is a highly attractive target for therapeutic intervention, this target is yet to be exploited.

Abstract 200 identified Vif and APOBEC 3G peptides that inhibit the interaction of APOBEC 3G with HIV-1 Vif in vitro. Although these peptides are being used to derive structural and functional information about the mechanism by which these proteins interact, these peptides will be important reagents in the design of small molecule inhibitor screens for the identification of novel HIV-1 therapies.

TRIM 5 α is another recently identified cellular protein that restricts infection of a variety of viruses including HIV-1. The mechanism by which TRIM 5 α restricts infection is not fully understood. However, TRIM 5 α acts at an early stage in viral replication and most likely compromises the uncoating step of the virus in which genomic viral RNA is released into the cytoplasm after disassociation of the viral capsid core. Like APOBEC 3 proteins, TRIM 5 α exhibits species-specific antiviral activity. For example, HIV-1 infection is inhibited by TRIM 5 α from some monkey species but not by human TRIM 5 α . Intriguingly, owl monkey TRIM 5 α restricts HIV-1 because a portion of their TRIM 5 α , the B30.2 domain, was replaced by cyclophilin A through a pseudogene insertion. Cyclophilin A has previously been demonstrated to interact with the capsid protein of HIV-1. Therefore, fusion of cyclophilin with owl monkey TRIM 5 α allows cyclophilin A to tether TRIM 5 α to HIV-1 Gag. Studies presented in Abstract 59 examined, therefore, whether the B30.2 domain may be involved in mediating the interaction of TRIM 5 α proteins with capsid. By fusing different TRIM alleles to cyclophilin A, the investigators were able to examine the ability of other TRIM proteins to restrict HIV-1 infection. These studies also revealed that TRIM 5 α may exhibit an antiviral effect at 2 different stages in HIV-1

infection and that restriction factor binding has different mechanistic outcomes.

Abstract 207 presented information on the significance of TRIM 5 α cytoplasmic bodies. It has recently been suggested that TRIM 5 α localizes to cytoplasmic pods perhaps as high molecular weight complexes. However, the significance of this to restriction of HIV-1 infection is unknown.

In a study from Dr Hope's group, TRIM 5 α was fused to a fluorescent protein in order to study its subcellular localization. In the presence of a proteasome inhibitor, there was an increase in the size and decrease in the number of cytoplasmic bodies formed by fluorescent TRIM 5 α . However, expression and turnover of TRIM 5 α was not affected. Therefore, the proteasome appears to regulate the biology of TRIM 5 α .

Several studies (Abstracts 60, 201, 205, 206) presented evidence on the evolutionary consequences of TRIM 5 α variation. Genes encoding proteins that influence susceptibility to pathogens are predicted to be subject to rapid evolution. Malik and colleagues noted that evolution of TRIM 5 α as well as APOBEC 3G predates the evolutionary origin of primates (approximately 35 million years) and as a result predates the origin of primate lentiviruses. The authors suggest that endogenous retroviruses may have been responsible for the evolutionary pressure that drove TRIM 5 α and APOBEC 3G evolution. Current attempts to screen human populations for genetic variation in TRIM 5 α and other genes will lead to identification of novel restriction factors that provide defense against retroviruses and lentiviruses.

Primate lentiviruses and lentivirus vectors infect nondividing cells. As a consequence, primate lentiviruses can infect terminally differentiated macrophages in the tissues. In contrast, only dividing cells are permissive to murine leukemia virus (MLV) infection. The prevailing hypothesis is that the lentiviruses infect nondividing cells because they harbor nucleophilic pro-

teins that allow the viral cDNA to translocate through the nuclear envelope in a nondividing cell. Although a number of nucleophilic viral proteins have been proposed (including Gag MA, Integrase, Vpr, and a triple-stranded cDNA intermediate known as the DNA flap), it is unclear which, if any, of these candidates dictates the ability of HIV-1 to infect nondividing cells. Studies presented in Abstract 58 provide evidence for an alternative model for the ability of HIV-1 to infect nondividing cells. Studies conducted by Emermen and colleagues demonstrated that transfer of the p12 and CA portions of Gag from MLV into HIV impaired the ability of HIV-1 to infect nondividing cells suggesting that p12 and CA distinguish this fundamental viral characteristic. The authors suggest a model in which capsid masks determinants within the viral genome that influence the ability to infect nondividing cells. They further suggest that the ability of HIV to infect nondividing cells may be dependent upon the uncoating step.

The identification of APOBEC 3G and TRIM 5 α as potent primate lentivirus restrictions has provided the impetus for research aimed at identifying novel additional restriction factors. Abstract 135 presented evidence for a novel restriction that potentially blocks HIV-1 infection. Kewalramani and colleagues expressed a cDNA library in cells permissive to HIV-1 infection in order to identify cells that acquired resistance to infection. One such HIV-1-resistant subclone contained a C-terminally truncated form of CPSF6 that restricted infection by X4- and R5-tropic HIV-1, HIV-2, and SIV but not by MLV. Propagation of HIV-1 in cells expressing truncated CPSF6 led to the emergence of CPSF6-resistant HIV-1 variants containing a mutation in *gag*. Truncated CPSF6 appeared to interfere with late steps in viral reverse transcription or with stability of nascent viral cDNA. The wild-type form of CPSF6 did not interfere with HIV-1 infection. Thus, the C-terminal truncation that was artificially created in CPSF6 during construction

of the cDNA library conferred antiviral activity upon CPSF6. Nevertheless, further understanding of the mechanism of antiviral restriction by truncated CPSF6 will provide important insight into the regulation of viral reverse transcription or cDNA stability and may ultimately reveal novel therapeutic targets in the viral replication cycle.

Viral Reservoirs and Mechanisms of Persistence and Latency

The ability of primate lentiviruses to persist within infected individuals reflects the life span of the cellular reservoirs that support HIV-1 replication. Although highly active antiretroviral therapy (HAART) has been effective in sustaining suppression of viral replication to below detectable levels for extended intervals, there is a rapid rebound of viremia if therapy is discontinued. The prevailing view is that HIV-1 becomes latent in a small population of memory lymphocytes and in this form is able to establish a life-long infection of the host. Within the latent state, the virus has been proposed to be transcriptionally silent.

Abstract 242 presented evidence that HIV-1 latency is determined post-transcriptionally. Primary resting CD4+ T lymphocytes were obtained from HIV-1-infected individuals on HAART who had undetectable viral loads. These cells were found to harbor low steady-state levels of full-length multiply spliced and unspliced HIV-1 RNA. Nevertheless, these cells did not produce virus. The authors found that the viral RNA was sequestered in the nucleus. The authors further demonstrated that expression of a polypyrimidine tract binding protein (PTB) in latently infected cells restored cytoplasmic accumulation of viral RNA and subsequent virus production. This study defines a novel mechanism for HIV-1 latency. This study may also aid in therapeutic strategies that attempt to purge latently infected cells by stimulating the exit of the virus from latency.

A somewhat different take on the mechanism by which HIV-1 persists in

the presence of therapy was presented in Abstract 168. Chun and colleagues have been examining virus activity in aviremic individuals on long-term suppressive HAART. In contrast to current models suggesting that the virus is harbored in a latent state, Chun presented evidence that highly virologically suppressed individuals harbor activated lymphocytes that chronically produce infectious HIV-1. Furthermore, phylogenetic analysis of viral sequences in the activated lymphocyte population and in resting cells indicated that viruses released from activated cells were infecting resting cells even in the presence of HAART. In contrast to the current latency models, this suggests that viral replication may persist in the face of HAART and that ongoing infection may continually replenish viral reservoirs. This study has several important implications. The ability of the virus to replicate in the presence of HAART may indicate that current therapies are not completely suppressive. If this is the case, therapeutic intensification by, for example, incorporating the next generation of drugs (coreceptor inhibitors and integrase inhibitors) into regimens may begin to address the question of whether therapy intensification interrupts viral persistence.

Activated memory CD4+ T lymphocytes that coexpress CCR5 are considered to be the principle targets for HIV-1 and SIV replication in the host. In humans, the majority of these cells are contained within the intestinal tract and, as recently demonstrated by several groups (for a review, see Veazey et al, *Nat Med*, 2005), massive acute viral replication in the intestine profoundly depletes these cells within a few weeks of infection. As a consequence, acute infection and viremia is considered to be a pivotal event in the natural history of HIV-1-mediated immunodeficiency. Paradoxically, SIV infection of natural monkey hosts such as sooty mangabeys is non-pathogenic despite significant viremia and lymphocyte turnover. In contrast to humans, CCR5-expressing memory CD4+ cells are rare in the gut of sooty mangabeys.

Therefore, to examine whether there was a rate-limiting number of permissive cells for SIV infection that might explain why they exhibit a non-pathogenic infection, several studies (Abstracts 36, 37, 40, and 167) examined whether nonpathogenic infection exhibited any of the profound and acute intestinal lymphocyte depletion characteristics of pathogenic HIV-1 infection. Remarkably, analysis of naturally SIV-infected sooty mangabeys after acute infection revealed a similar massive depletion of CD4+ T lymphocytes in the mucosal lymph node tissue. A similar pattern of massive intestinal lymphocyte depletion was observed in SIV-infected African green monkeys in which SIV is non-pathogenic. Therefore, lack of disease in natural SIV infections cannot be explained by limited availability of permissive substrates for viral replication in the gut, and profound and acute lymphocyte depletion is a characteristic of both nonpathogenic SIV infection as well as pathogenic SIV and HIV-1 infection. This research is sure to drive an intense investigation into why lymphocyte destruction can be tolerated in naturally infected monkeys since this will further our understanding of why HIV-1 is pathogenic.

In addition to CD4, HIV-1 infection of a cell requires coreceptor molecules of which CCR5 and CXCR4 are the most widely used. Viruses are classified according to X4 (CXCR4-using) or R5 (CCR5-using) variants with R5 variants being described as macrophage tropic. Abstract 134 presented evidence that challenges this.

Instead of virus isolation, Clapham and colleagues directly amplified envelope genes from various tissue sources of infected individuals and examined the in vitro tropism of viruses that contain these chimeric virus envelopes. The investigators demonstrated that viruses harboring R5 envelopes differed from one another by more than 1,000-fold in their ability to infect primary macrophages. Instead, the ability to exploit low levels of CD4 was a more accurate determinant of macrophage tropism. Furthermore, there was a

striking absence of macrophage-tropic viruses in lymph nodes, blood, and semen. Therefore, macrophage-tropic viruses may define a subset of viral variants that are specifically restricted to certain tissues such as the brain. In contrast, R5 tropism likely reflects tropism for lymphocytes that express varying amounts of CCR5. The classification of viruses on the basis of R5 and X4 tropism warrants re-evaluation.

Financial Disclosure: Dr Stevenson has no financial affiliations with commercial organizations that may have interests related to the content of this article.

A list of all cited abstracts appears on pages 63 to 70.

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