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A New Cellular System Opposing HIV Infection: Implications for Gene Transfer?

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One of the longest-standing mysteries in HIV research has been the function of the viral-encoded VIF protein. VIF is required for efficient viral infection *in vivo* [1–4], apparently because it overcomes the action of a host-encoded antiviral system [5,6]. A recent report in *Nature* describes a major breakthrough in understanding the cellular defense machinery that is overcome by HIV VIF [7]. In addition to its importance for HIV research, this discovery raises a variety of questions about whether manipulating this system might be helpful in optimizing gene transfer.

The *vif* gene is one of the "auxiliary" genes of HIV, which are defined as being dispensable for efficient replication in at least some settings in cell culture. *In vivo*, however, *vif* is clearly required, as indicated, for example, by the finding that simian immunodeficiency viruses (primate relatives of HIVs) lacking *vif* are greatly attenuated [1].

The outcome of infections with *vif*-negative viruses in different cell lines was intriguing and ultimately quite informative. In *vif*-permissive cells, HIV derivatives mutated for *vif* grow as well as wild-type virus. In *vif*-nonpermissive cells, growth is greatly abrogated, but in a selective fashion. The nonpermissive phenotype is only seen when *vif*-negative virus is produced from nonpermissive cells. When *vif*-negative virus is produced from permissive cells, then used to infect nonpermissive cells, the virus infection proceeds normally. Tests of primary human cells revealed that they were *vif* nonpermissive, suggesting that VIF acts against this system normally during HIV infection.

Elegant experiments from the laboratories of Malim and Kabat established that this could be explained by VIF overcoming a host-encoded antiviral system in virus producer cells. Both labs fused permissive cells with nonpermissive cells and asked whether or not the hybrid cells restricted growth of VIF-mutant HIV. They reasoned that if nonpermissive cells encoded an antiviral system, the fused cells should be nonpermissive because they would contain the interfering factor. The alternative was that nonpermissive cells might lack a factor required for efficient viral replication, in which case the fused cells would be permissive because the factor would be present. The experiments revealed that the fused cells were in fact nonpermissive, supporting the conjecture that restrictive cells express an antiviral system.

This finding launched a race to find the gene responsible. Beautiful work by the Malim laboratory reported in *Nature* [7] describes the isolation of a gene that determines the nonpermissive phenotype. Thanks to this work, not only is the gene now in hand, but its nature suggests a whole new dimension to host–virus interactions.

The details of Malim *et al.*'s strategy explain their success. They isolated cDNAs selectively expressed in nonpermissive cells using subtractive hybridization to remove cDNAs present in permissive cells. It was possible that expression of the key factor was induced by HIV infection, so they infected both the permissive and nonpermissive cells with HIV before preparing RNA from cells for analysis. Moreover, their choice of cells to study was inspired—they used two sublines of CEM cells that were found to differ by their permissive versus nonpermissive phenotype but were otherwise identical. Focusing on these two very closely related lines kept accidental differences between RNA populations minimal, allowing the difference accounting for the VIF phenotype to stand out more prominently over the background. The cDNAs unique to the nonpermissive cells were further tested for selective expression in the nonpermissive cells only. Several cDNAs survived this test and thus qualified as candidates for the cellular factor opposed by VIF.

Candidate cDNAs were then expressed in permissive cells and the effects on viral replication were assessed. One cDNA, named CEM15, showed the desired effect: it had no effect on replication of *vif*-plus virus but strongly inhibited *vif*-negative virus. Some of the other candidate cDNAs inhibited HIV replication, but not in a way that differed between *vif*-plus and *vif*-minus variants. The magnitude of the effect of CEM15 was very compelling—in one type of experiment, replication of *vif*-minus virus was inhibited by 97%, whereas that of the *vif*-plus virus was essentially unaffected. The data made a strong case that expression of CEM15 is responsible for the difference between permissive and nonpermissive cells.

The sequence of CEM15 revealed an altogether unexpected potential function. The gene had not been studied previously, but by homology analysis it seemed to encode a cytidine nucleoside/nucleotide deaminase similar to APOBEC-1 (the apolipoprotein B RNA editing

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enzyme) and phorbolin-1. One possible model is that CEM15 inactivates HIV-1 RNA by covalent modification of C residues, and VIF antagonizes this. Consistent with this possibility is the observation that VIF binds to RNA, potentially localizing all the players for efficient interaction. Other models also seem to be possible—for example, post-transcriptional RNA modification of some unknown cellular mRNA may be required for synthesis of the inhibitory factor.

Malim and colleagues show tasteful restraint in their speculation on possible models. After all, why should they talk in hypotheticals when their breakthrough gives them the tools to address these questions directly? We can be confident that the Malim laboratory and others will now be providing a steady stream of follow-up studies to determine how VIF works to antagonize function of the CEM15-dependent pathway.

These data provide food for thought for anyone interested in optimizing gene transfer. Might the CEM15 system antagonize the operation of other gene transfer systems? Might neutralization of CEM15 allow higher-level production of viral vectors, or perhaps production of vectors with fewer mutations in the transduced genes? Malim and coworkers have reported previously that VIF promotes replication of murine leukemia virus in human cells as well [8], suggesting that VIF might be a useful addition to cells producing MLV-based vectors, though another study disputed this finding [9]. The 293T cells often used for production of retroviral vectors are *vif*-permissive, so inactivation of the CEM15 system is not likely to be important in that setting. However, production of vectors from other cell types or multicycle replication of gene delivery vectors *in vivo* may be suppressed by CEM15. Given the intense interest in this area, the question of whether modulating the CEM15 system might facilitate gene transfer should soon be clarified.

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