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Visions & Reflections

The HIV-1 Vif protein: a paradigm for viral : cell interactions

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Rarely are the functions of retroviral proteins determined within a few years of their discovery and analysis. Nonetheless, one of the viral proteins of human immunodeficiency virus type I (HIV-1) has remained, over the past 18 years, especially enigmatic and confusing. Since its discovery in the mid 1980s, when the gene was at first entitled *sor* [1, 2], the function of the product of this particular open reading frame of HIV-1 posed a mystery. The gene product was later renamed Vif (virion infectivity factor), and is a 23-kDa basic protein, found to represent a common accessory gene product in all primate immunodeficiency viruses. Unlike other HIV-1 accessory proteins, Vif was unique in its phenotype, both qualitatively and quantitatively. First, if the *vif* gene was mutated in HIV-1 strains, and human peripheral blood T lymphocytes or monocytes/macrophages, the primary cell reservoirs for HIV-1 in vivo, were infected with this mutant virus, the virions produced were nearly completely defective (i.e., dead) [3]. This is unique, as mutation of other HIV-1 accessory genes, such as Nef, Vpr, and Vpu, leads to debilitated but certainly reproducing viruses which replicate in most human cell types. Second, the growth of HIV-1 mutant viruses in which Vif was knocked out was also unique, being cell type specific [4]. In essence, there were found to be select cell types from which the mutant virus grew (i.e., producer lines) yielding virus progeny that were non-infectious, with poor reverse transcriptase activity [3, 5, 6], regardless of the target or recipient cells. These producer cells (e.g., H9 and CEM T cell lines) were described as ‘non-permissive’ or restrictive for Vif mutant viral progeny generation. Vif appeared to be required in non-permissive cells at the time of virion production. Other selected cell lines did not seem to require Vif for producing infectious and replicating virions, regardless of the target, and were called ‘permissive.’ As such, the Vif-negative viral phenotype was based on the producer rather than the target cell type (see fig. 1).

Over the last decade or more, numerous laboratories worldwide have tried to solve this riddle. But the conun-

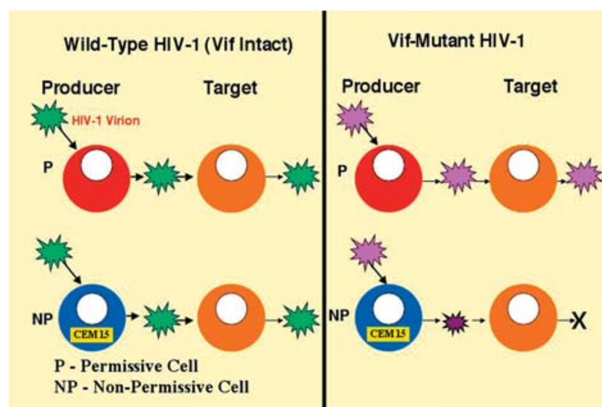

Figure 1. Inhibition of Vif-deleted HIV-1 by CEM15. This schematic diagram illustrates the phenomenon of Vif mutant viruses leading to non-infectious progeny from non-permissive producer cell types, which express the host gene product CEM15 (APOBEC-3G). Intact, infectious viral progeny are produced in permissive producer cell types, which do not express the CEM15 protein. Of note, wild-type HIV-1 with an intact Vif can produce infectious progeny from all cell types whether they express CEM15 or not. This is presumably due to the as yet undescribed molecular mechanism by which Vif usurps the inhibitory role of CEM15 on infectivity of progeny HIV-1 virions. CEM15, without Vif antagonism, leads to hypermutation of the viral DNA [18–21].

drum continued. Studies have shown that Vif binds to HIV-1 RNA but the mechanisms by which this might lead to the particular viral phenotype of Vif mutant viruses remained unclear [7–9]. A breakthrough came in 1998, when two groups demonstrated that there seemed to be an endogenous cellular cofactor inhibitor of HIV-1, which was overcome by the viral Vif protein [10, 11]. This factor appeared to alter Vif mutant HIV-1 at the late stages of the viral life cycle in non-permissive producer cells. These findings were demonstrated using heterokaryon formation between permissive and non-permissive cell-types.

However, the field again stalled somewhat until an article by Sheehy et al. was published [12]. This laboratory demonstrated that there appeared to be a specific human gene product which inhibits HIV-1 infection but whose effects are suppressed by the lentiviral Vif protein.

Utilizing a novel polymerase chain reaction (PCR)-based cDNA subtraction strategy with non-permissive and permissive cell lines, which were genetically related but had different permissivity for Vif function, a cellular inhibitor representing a cofactor for the Vif phenotype was demonstrated to exist. It was initially named CEM15, based on the cell line from which it was cloned. The authors did a relatively comprehensive job demonstrating CEM15 presence in all non-permissive cells, but its absence in all permissive cells. Of great importance in demonstrating the specificity of this effect were the experiments which demonstrated that transfecting CEM15 in permissive cells inhibited HIV-1 infectivity when the infecting viral strain was mutated for the Vif open reading frame, but not when the virus was wild type. There appeared to be an unarguable correlation between the production of CEM15 and the demonstration of Vif-negative, non-permissivity (see fig. 1). These data suggest that this cellular protein represents one of probably multiple host-mediated mechanisms which can inhibit retrovirus replication [13], but which in this case, is overcome by the HIV-1 protein Vif.

CEM15 is interesting because it has significant amino acid identity to two important human proteins, APOBEC-1, a cytosine deaminase that is the catalytic subunit of an mRNA-editing enzyme [14], and also to a novel phorbol-induced protein, Phorbolin-1. Of importance is the demonstration that there is a zinc-coordinating motif in each of these proteins, and which is important in cytosine deaminases in virtually all organisms across the phylogenetic scale. CEM15 has recently been shown to be a cytidine deaminase, and has been renamed APOBEC-3G [15]. In addition, Vif clearly binds to viral genomic RNA [7–9], and some-what controversial, recent data suggest that Vif [16], as well as CEM15 [12], are incorporated into HIV-1 virions. Thus, the effects of CEM15 on HIV-1 replication and its interactions with Vif may occur in virion particles.

The paper by Sheehy et al. [12] took us a giant leap forward in exploring one of the 'final frontiers' in HIV-1 molecular virology. It was critical to try to understand whether CEM15 binds directly to Vif intracellularly or operates in another fashion. Unfortunately, the specific molecular mechanisms of the effects of CEM15 on mutant Vif HIV-1 viruses were not demonstrated by this initial article. However, in a remarkably comprehensive study of HIV-1 Vif, Vif-negative virions were demonstrated to be essentially indistinguishable morphologically and biochemically from virions with a genome with an intact *vif* open reading frame (17).

Very recent studies, including ones from our Center, have suggested that in the absence of Vif, CEM15 acts as a cytosine deaminase on the first reverse transcribed strand (i. e., viral DNA), inducing C to U changes and hypermutation, both in HIV-1 and murine leukemia virus [18–21]. Thus, at least one molecular mechanism accounting for the Vif phenotype appears to be the inhibition of CEM15-induced hypermutation. Processes by which Vif inactivates CEM15 are now areas of on-going research.

These findings suggested that CEM15 may be one example of what promise to be several cellular inhibitory molecules which naturally decrease HIV-1 replication in certain specific cell types, both in the pre- and post-integration phases of the viral life cycle. Human resting CD4+ T lymphocytes, although a key reservoir for HIV-1 in vivo, are extremely difficult to infect with cell-free HIV-1, and have a variety of cell inhibitory mechanisms which decrease the stability and activity of the viral pre-integration complex after virion binding and internalization. In addition, recent data on RNA interference suggest that this too may represent a host cell mechanism for inhibiting primate lentiviral infection [22, 23].

Certainly, HIV-1 Vif and its interactions, direct or indirect, with CEM15 are a unique site for anti-retroviral drug discovery. As mentioned above, unlike the other accessory proteins of HIV-1, when Vif is knocked out, reasonably "dead" virion progeny are produced in the major cell types in which HIV-1 lurks in vivo. Thus, the robustness of the Vif-negative phenotype illustrates its promise as a site for potential small-molecule inhibitors. The described multimerization of Vif [24, 25], required for its function in non-permissive cells, also suggests its potential utility for anti-HIV-1 approaches. Further detailed understanding of the mechanisms by which CEM15 directly inhibits HIV-1 replication, and precisely how this is overcome by Vif, will be critical in rational drug design for this viral accessory protein, whose utility as a drug target has been opened up by the recent exciting findings.

Recent studies have also suggested that Vif may interact with a variety of other cellular proteins. These include the tyrosine kinase, Hck, as well as a lymphocyte-specific nuclear body protein, Sp140 [26, 27]. Both have been suggested to have functional roles in inhibiting HIV-1 replication which are possibly counteracted by Vif. It is formally possible that Vif may bind and be functionally

relevant in inhibiting other inhibitors of HIV-1 replication in addition to CEM15. Whether CEM15 is only part of the answer to the complicated phenotype of Vif during HIV-1 infection will require detailed further investigations. In addition, an interesting study has now also suggested that intravirion processing of HIV-1 Vif by the HIV-1 protease may correlate with function [16]. At this point, how this processing of Vif within the virion would have effects on the inhibitory molecules such as CEM15, Hck, or Sp140 in the producer cell lines is not clear. Novel methods to inhibit HIV-1 replication by targeting Vif remain critical areas of investigation [28], even before the exact molecular mechanisms of Vif:host cell protein interactions are elucidated.

The last 2 years have witnessed remarkable progress in the attempt to understand the molecular mechanisms of HIV-1 Vif in the viral life cycle. It remains an excellent paradigm for the ability of viruses in general and retroviruses in particular to counteract cellular inhibitors of viral replication. Vif can be considered an example of the new paradigm, namely that intracellular inhibition of viral replication may occur at the same time that viruses are parasitizing other host cell proteins during their life cycle. Through a detailed understanding of Vif function we will very likely be given important insights into approaches to render cells innately immune to many pathogenic viruses.

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