

New findings on blockage of HIV-1 RNase H activity

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The subject of HIV-1 inactivation is of obvious importance to a variety of AIDS-related disciplines. These include HIV therapeutics, but the topic also has relevance for HIV prevention efforts such as development of vaccines and microbicides.

The accompanying manuscript by Mathur *et al.* (pp. 000–000) demonstrates use of a novel approach to reduce infectious viral load in blood samples obtained from infected individuals. Notably, the authors have used an oligodeoxynucleotide, in the form of a RNA/DNA hybrid molecule, to activate the RNase H enzyme of HIV-1 and cause it to cleave viral RNA genomic material before the latter can be converted into viral DNA. As a result, the HIV replication cycle is interrupted at a very early stage. Using this approach, significant reductions in viral titer were obtained against viruses of diverse origin, including those of different subtypes as well as viruses that displayed resistance against a variety of antiretroviral drugs (ARVs). These findings were confirmed by both infectivity assay and, as well, by real-time quantitative PCR assay in which the investigators attempted and failed to find evidence of newly synthesized viral DNA in cell-free experiments.

It is important to understand that RNase H is a distinct enzymatic activity that is carried out by the viral reverse transcriptase enzyme. Although reverse transcriptase is responsible for the conversion of viral genomic RNA into viral DNA that subsequently becomes integrated into the host cell chromosome, the role of RNase H is to degrade the viral RNA template as this conversion to DNA takes place. This prevents viral RNA from being able to compete with viral mRNA that is ultimately generated

from integrated viral DNA and, thereby, serves to enhance viral replication. Indeed, RNase H activity is essential for viral replication, and mutations that eliminate RNase H activity also destroy HIV replication capacity [1]. The senior author of the current manuscript is prominently associated with some of the earliest work in the literature on the subject of retroviral RNase H [2].

Although the work is certainly of interest, it remains to be seen whether it will have practical application in regard to either HIV therapy or prevention research. In regard to therapeutics, it might be difficult to imagine the use of oligonucleotides as effective drugs, given that the latter are not easily bioavailable. Furthermore, although the RNase H enzyme of HIV-1 has long been targeted for drug discovery by both the pharmaceutical industry and academic scientists, no one has ever demonstrated success in regard to production of a nontoxic anti-RNase H small molecule that might potentially be bioavailable.

However, there can be little doubt that success at development and use of such a product would lead to drug resistance, likely based on mutations that would be selected out within the RNase H portion of the reverse transcriptase gene of HIV-1. Although such mutations might not impair either RNase H function or viral replication capacity, they might eliminate the ability of viral RNase H to recognize a small molecular inhibitor, in a manner not dissimilar to the mechanisms whereby the reverse transcriptase, protease, and integrase enzymes of HIV-1 can become resistant to ARVs [3]. It is not unlikely that HIV-1 might also become resistant over time to the RNase H activation approach used in this study to prevent viral replication [4]. Indeed, one must surmise

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that the HIV quasispecies within infected individuals already contains minority RNase H variants that possess mutations that might render the oligonucleotide activation approach noneffective, perhaps due to lesser ability to bind to RNase H itself [5]. It would have been interesting if the authors' culture systems had permitted the selection of such variants, but their experiments were not designed to deal with this issue.

The inactivation of HIV for purposes of vaccine or microbicide development or both, is a goal that has fallen into disfavour, in part, because of the failure of a number of phase III microbicide trials that were based on the notion that a suitable compound might inactivate HIV during sexual relations such that infection could no longer be transmitted. Other approaches involve use of the potential microbicide taurine chloramine as an inactivating agent that might give rise to a whole killed retroviral vaccine [6]. The problem, of course, will be to ensure that viral infectiousness has been completely destroyed, a difficult task, as well as to ensure that the use of such a vaccine product will generate protective immune responses, also a daunting challenge given the recent inability of vaccines that did engender an antiviral immune response to protect against sexual transmission of HIV-1.

In regard to microbicides, the failures of multiple nonspecific substances to protect women against HIV make it unlikely that anyone will want to try such an approach again soon, in spite of the fact that all these substances possessed the ability to kill HIV on contact as determined in biochemical and tissue culture systems [7].

In this context, it will require considerably more research to shed light on mechanisms whereby the activation of viral RNase H might be harnessed in a positive way. This notwithstanding, the current study does provide compelling new scientific information on the activation of RNase H as a potential step in the right direction that will hopefully lead to further advances aimed at control of HIV replication and transmission.

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