

Why HIV Cannot Infect Resting CD4 T-Cells

The Gladstone Connection

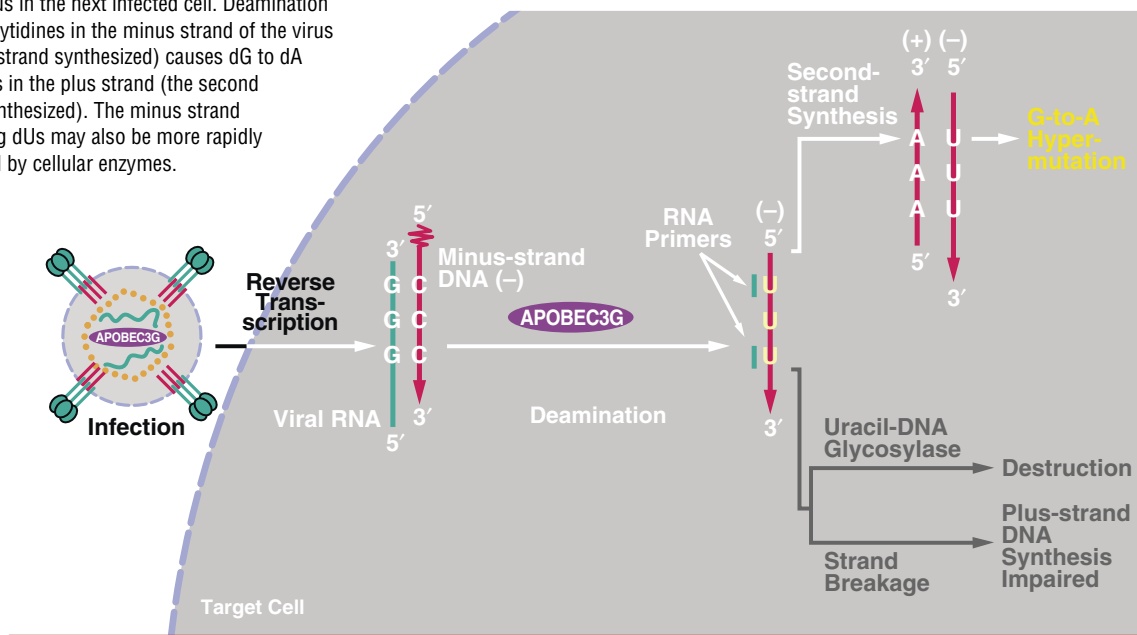
For more than 20 years, HIV biologists have puzzled over why resting, in contrast to activated, CD4 T-cells so effectively resist HIV infection. Is this highly effective block due to an active inhibitory mechanism, or simply the lack of a key factor or nutrient in these cells? If the former, it might open the door to an entirely new strategy for blocking the growth of HIV in cells where the virus normally grows well.

A3G—A New Player in HIV Biology

Recent studies in Dr. Warner Greene's laboratory at the Gladstone Institute of Virology and Immunology have shed new light on this question. As reported in the journal *Nature*, an active defense mechanism is involved, and a key player in this defense is an antiviral host factor termed APOBEC3G (A3G).

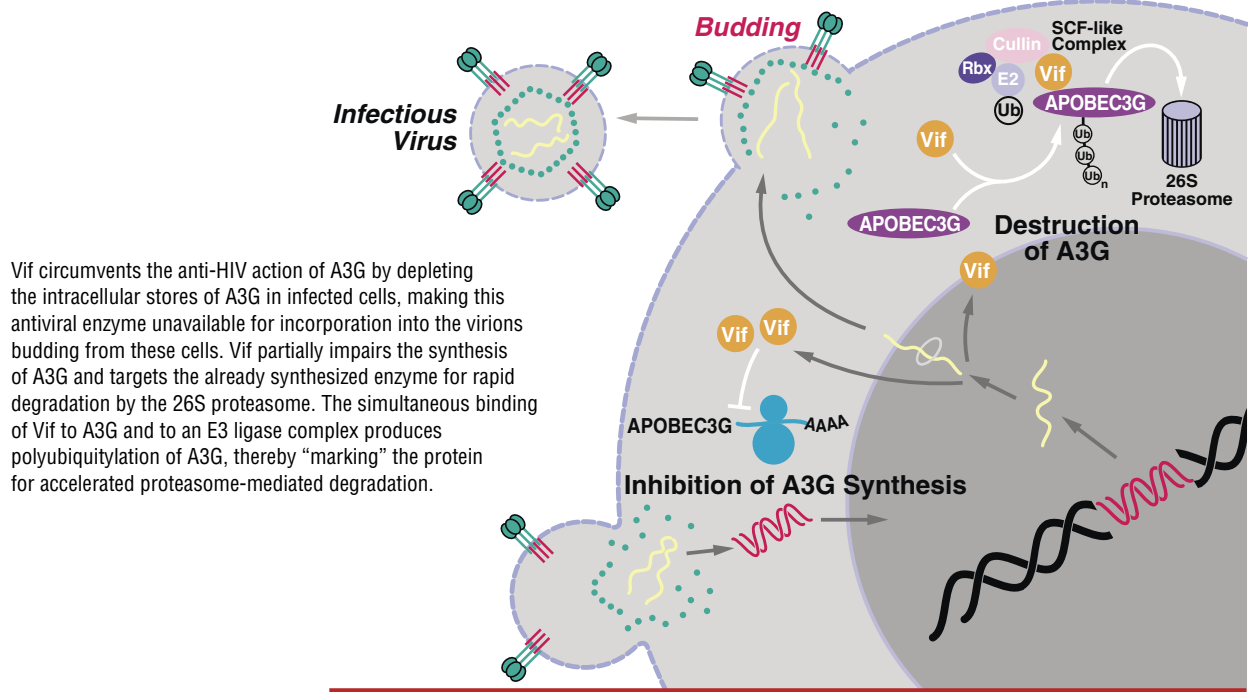
The A3G story began in 2001 with the molecular cloning of this factor by Dr. Ann Sheehy, a postdoctoral fellow working with Dr. Michael Malim at the University of Pennsylvania. Its isolation galvanized the field. Many laboratories rapidly joined the effort to understand how the anti-HIV factor works. In rapid-fire order, four groups published papers in *Science*, *Nature*, and *Cell* identifying A3G as a DNA mutator enzyme (more precisely a deoxycytidine deaminase). In brief, these studies revealed that A3G is incorporated into budding virions and exerts its antiviral action in the next infected cell by mutating nascent viral DNAs formed during the course of reverse transcription.

A3G is incorporated into budding HIV virions and induces lethal mutations during reverse transcription of the virus in the next infected cell. Deamination of deoxycytidines in the minus strand of the virus (the first strand synthesized) causes dG to dA mutations in the plus strand (the second strand synthesized). The minus strand containing dUs may also be more rapidly destroyed by cellular enzymes.

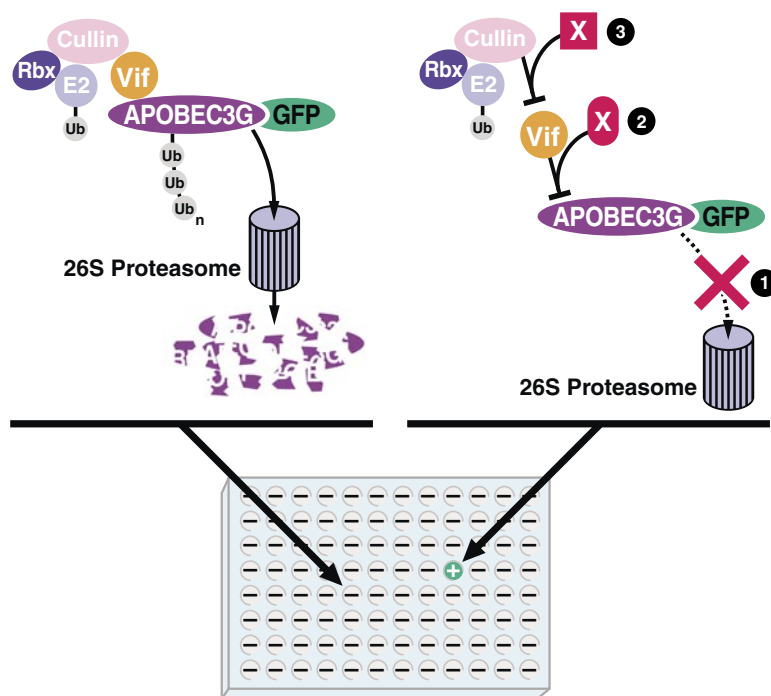


New Tricks from an Old Protein

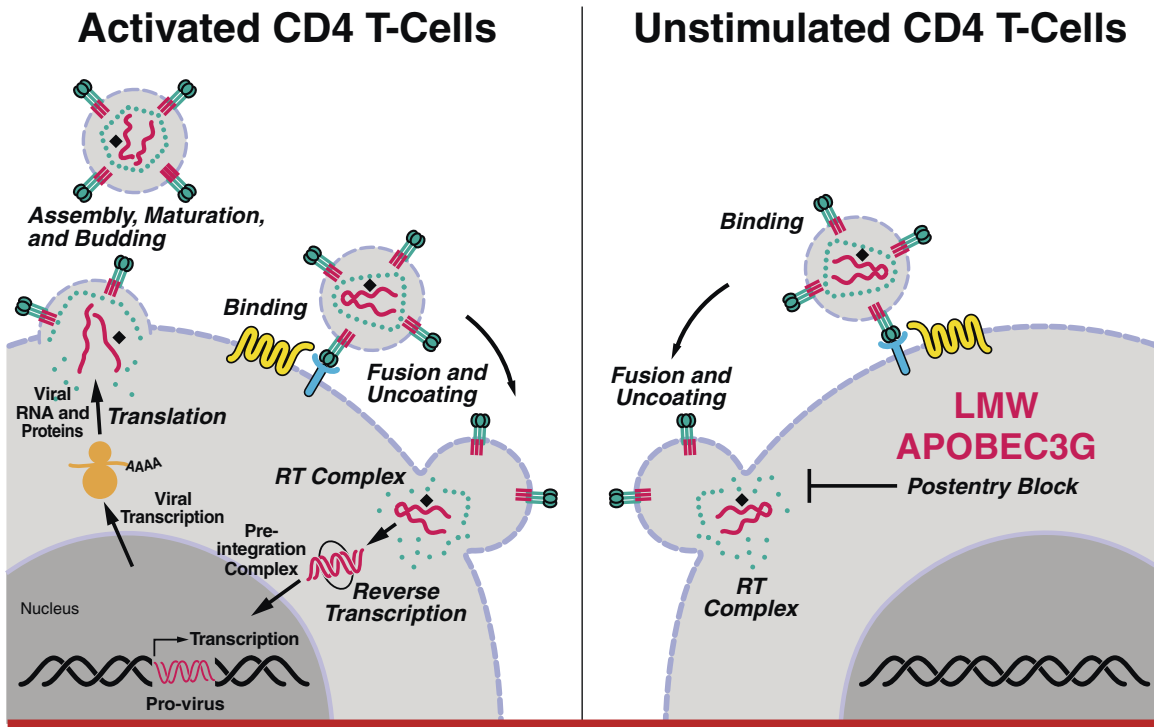
However, HIV does not take these antiviral effects of A3G lying down. Instead, it dedicates one of its nine genes to neutralize the threat posed by A3G. The Greene laboratory was the first to describe how Vif overcomes A3G's antiviral effects. They showed that this viral protein both targets A3G for rapid destruction in the proteasome (a cellular "garbage disposal" that normally eliminates unwanted or expired proteins) and partially inhibits the synthesis of new A3G proteins. In combination, these two actions effectively deplete the intracellular stores of A3G, making it unavailable for incorporation into new virions—no enzyme, no virion incorporation, no DNA mutation, and no antiviral effect.



Vif circumvents the anti-HIV action of A3G by depleting the intracellular stores of A3G in infected cells, making this antiviral enzyme unavailable for incorporation into the virions budding from these cells. Vif partially impairs the synthesis of A3G and targets the already synthesized enzyme for rapid degradation by the 26S proteasome. The simultaneous binding of Vif to A3G and to an E3 ligase complex produces polyubiquitylation of A3G, thereby "marking" the protein for accelerated proteasome-mediated degradation.



Vif-A3G is an exciting new target for anti-HIV drug discovery. Millions of small molecules could be rapidly screened in an assay developed in the Greene laboratory. This assay involves the coexpression of Vif and A3G tagged with green fluorescence protein (GFP) in cells. Normally, Vif induces proteasome-mediated degradation of the A3G-GFP fusion protein, leading to little or no GFP epifluorescence in the cells (1). A small molecule (X) that prevents Vif from binding to A3G (2) or recruiting the key cellular E3 ligase (3) would result in the accumulation of A3G-GFP within cells, causing them to turn green. A robotic high-throughput version of the assay could survey small-molecule libraries for active molecules.



The LMW form of A3G found in unstimulated CD4 T-cells plays a key role in blocking HIV growth in these cells. Activation of these cells relieves the block by recruitment of A3G into inactive HMW complexes.

deamination. Rather, it seems likely that the ability of LMW A3G to bind RNA may play an important role in restricting the function of the reverse transcription complex. The finding that incoming virions contain little if any Vif and the fact that Vif is only produced much later in the HIV life-cycle highlight another key feature of this postentry block: this antiviral function of A3G is very active against wildtype versions of HIV.

New Insights and New Possibilities

These findings provide at least part of the answer to the 20-year riddle of why resting CD4 T-cells are so resistant to HIV. LMW A3G is the key. This antiviral enzyme actively inhibits growth of the virus principally by delaying reverse transcription in the absence of overt mutation of the reverse transcripts formed. These studies raise an important question: Can we harness this new information to produce a novel form of antiviral therapy? One possibility would be to identify small molecules that disassemble the HMW A3G complex, thereby creating in highly permissive cells the same antiviral shield that protects resting CD4 T-cells. While exciting, we must consider the fact that the A3G is inserted into an inactive HMW complex for a reason, possibly to protect the cell's own DNA from mutation during cell division.

In summary, A3G forms an exciting new focus in HIV biology. Scientists at the Gladstone Institute of Virology and Immunology have been deeply involved in elucidating the mode of action of A3G, and they will continue to be on the cutting edge of this and other studies that could eventually lead to novel HIV treatments.