

Evolution of an arms race



Current treatments for HIV have serious side effects and cannot respond to the virus' high mutation rate. **Dr Linda Chelico** is taking an alternative approach to drug development by targeting cellular immune factors that could be potent restrictors of HIV



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How did your interest in the use of the APOBEC3 (A3) G host factor as an HIV-1 therapy target develop?

Although my current research has a clear goal to understand how we can use A3G as an HIV-1 therapy, my entrance in to this field was not so direct. My PhD research was focused on characterising the DNA repair systems of insecticidal fungi, with the goal of increasing fungal spore survival in agricultural ecosystems. However, I became more interested in the response of fungal spores that received large ultraviolet doses and used error prone DNA repair as a means of survival, despite the cost of acquiring additional mutations. I became increasingly interested in studying how directed mutagenesis can be used to an organism's advantage. This led me to conduct my postdoctoral research in the laboratory of a remarkable biochemist, Myron F Goodman, at the University of Southern California, USA, who had been studying targeted mutagenesis by the DNA deaminases A3G and AID. I focused on characterising the biochemistry of A3G, since the enzyme had not been previously studied *in vitro*. When I started my own lab in 2009, I expanded our

research to not only include biochemical analysis of all A3 enzymes, but also how they influence HIV-1 with an intent to develop strategies for their use as a therapeutic agent.

How does the HIV-1 protein, Vif, affect A3 enzymes?

In a laboratory setting, we study virus infectivity factor (Vif)-defective HIV-1 virions because Vif can inhibit the function of A3 enzymes. If we use an HIV-1 virus with a functional Vif protein, it will cause the ubiquitination and degradation of A3 enzymes and destroy their effects. In HIV-1 infected patients, the Vif gene will mutate and evolve and some viruses may become Vif defective, allowing A3 enzymes to restrict viral replication. However, it seems that HIV-1 can also find a middle ground where the Vif gene changes to only partially inhibit A3 function, thereby allowing a small amount of A3-induced mutagenesis of the HIV-1 genome that may facilitate evolution of HIV-1 over the course of the infection/disease.

What challenges have you encountered over the course of your project? How have you managed to overcome these?

Of the seven A3 enzymes, we found only four were easy to purify and use for *in vitro* work. The others have given us some difficulties in obtaining sufficient quantities for experiments. However, I have now been studying the biochemistry of A3 enzymes for nine years and have learned a few tricks for what these enzymes 'like', so for the most part we have been able to overcome these challenges.

How have your studies, which characterised the functional mechanisms of A3 enzymes, contributed to developing a host restriction factor-based HIV-1 therapy?

Our biochemical data have identified key amino acid determinants required for efficient

mutagenesis of the HIV-1 genome. These data can be used in the development of small molecules that bind A3G and block its interaction with Vif by ensuring that key functional amino acids are not obscured, thus negating A3G activity.

In what ways has a modelling approach to the co-evolution between HIV-1 and human A3 helped extend knowledge in this area? What are the key features of this 'arms race'?

The key feature of an 'arms race' between host and virus is co-evolution. This is imprinted in the genetic sequence by a process called positive selection. The virus is constantly adapting and changing. For the host to survive, it must counter this evolution by changing the coding sequence of its restriction factors. As a result, restriction factor genes show evidence of more rapid evolution in comparison to other types of genes.

Different Vif variants show different effectiveness in inducing the degradation of A3 enzymes. We are using this phenomenon as if each Vif variant may be a different snapshot of evolutionary time and trying to figure out why some work better than others. This will help us predict variations in the A3-Vif interface and how they would affect Vif-induced degradation of A3 enzymes.

Do you collaborate with other scientists in the course of your work?

Collaboration between different research groups, universities and governments is essential for combating global public health concerns. We have not established strong partnerships with other groups as our research is currently very basic, but as we progress towards more translational work we expect to also increase our collaborations with different scientists.

Tackling HIV with A3G

In the ongoing fight against HIV, researchers at the **University of Saskatchewan** are characterising the evolutionary 'arms race' between HIV and natural human deaminase enzymes, which are capable of stopping the virus in its tracks

THE IMPACT OF the HIV/AIDS pandemic is without precedent in human history. Currently 33 million people worldwide are infected with HIV-1, the more pathogenic form of the virus. In the three decades since the disease first emerged, much progress has been made in understanding its pathogenesis and HIV is now a chronic but manageable condition. However, the current recommended therapy of highly active anti-retroviral treatment (HAART), which uses a combination of drugs to inhibit essential proteins of the HIV-1 virus, has significant long-term side effects such as lipid abnormalities and cardiovascular risks.

Furthermore, HAART cannot cure patients of HIV since the virus can infect cells latently. It remains dormant in CD4+ cells, where it cannot productively replicate, but can exist integrated in the host DNA. If the resting CD4+ cell is activated then the HIV becomes able to replicate and can reinstate infection of the host. HIV patients will not be cured until a treatment can be designed to simultaneously activate all latent virus, so that antiretrovirals can successfully purge it from the infected person.

NATIVE IMMUNE FACTORS

An alternative approach to developing HIV-1 therapeutics is to take advantage of native cellular immune factors that naturally limit replication. These 'host restriction factors' can restrict the intracellular HIV-1 lifecycle at specific steps but are often thwarted by HIV-1 accessory proteins designed to overcome these barriers. Researchers at the University of Saskatchewan, which is located in a region of Canada where HIV-1 is especially prevalent, are now characterising some of these host restriction factors and investigating the consequences to other host cells of using them as an HIV therapy. Led by Dr Linda Chelico, the team's research builds upon previous work that identified APOBEC3 (A3) enzymes. This is a family of host factors that can restrict HIV-1 infection of CD4+ T cells when the infection is defective in virus infectivity factor (Vif) – a protein found in HIV which is essential for its replication.

Although the biology of A3 enzymes is well understood, detailed information on their biochemistry and molecular mechanisms of restriction is lacking. Chelico's goal is to study the molecular 'arms race' between HIV-1 and the human A3 interface, and her lab's research is targeted at understanding the co-evolution of the viral strategies and host defences in order to model how the virus and host respond to one another. She elucidates: "We are working on characterising all seven A3 enzymes (A3A-D and F-H) in order to

determine how each one differs in its inherent biochemical characteristics to perform certain actions necessary for restriction of HIV-1; such as binding RNA, binding single-stranded DNA (ssDNA), scanning ssDNA and deamination activity". Indeed, the group has established a model where the mechanism by which the A3 enzymes interact with ssDNA – how they scan the DNA in search for cytosine residues to deaminate – determines their effectiveness in HIV restriction.

A3G

Chelico and her team have been focusing their efforts on the A3G enzyme, which has shown promise as the most effective host restriction factor. It is able to specifically induce mutagenesis of ssDNA, reverse transcribed from the HIV-1 RNA genome, without interfering with the host's DNA replication processes. A3G is localised to the cytoplasm in normally replicating cells protecting the host's DNA from mutagenic A3G-catalysed deaminations of cytosine to uracil. This is different to other A3 enzymes, which can enter the nucleus and deaminate cytosine residues in host DNA when it becomes single stranded during replication or transcription. "For example, A3B deamination activity has been associated with multiple cancers

and A3A has been found to induce mutagenesis of genomic DNA in different cell types. However, the physiological function of A3 enzymes is thought to be restriction of retrotransposon activity (retrotransposons have a similar reverse transcription state to HIV-1), so their benefits outweigh their risks in human cells," Chelico adds.

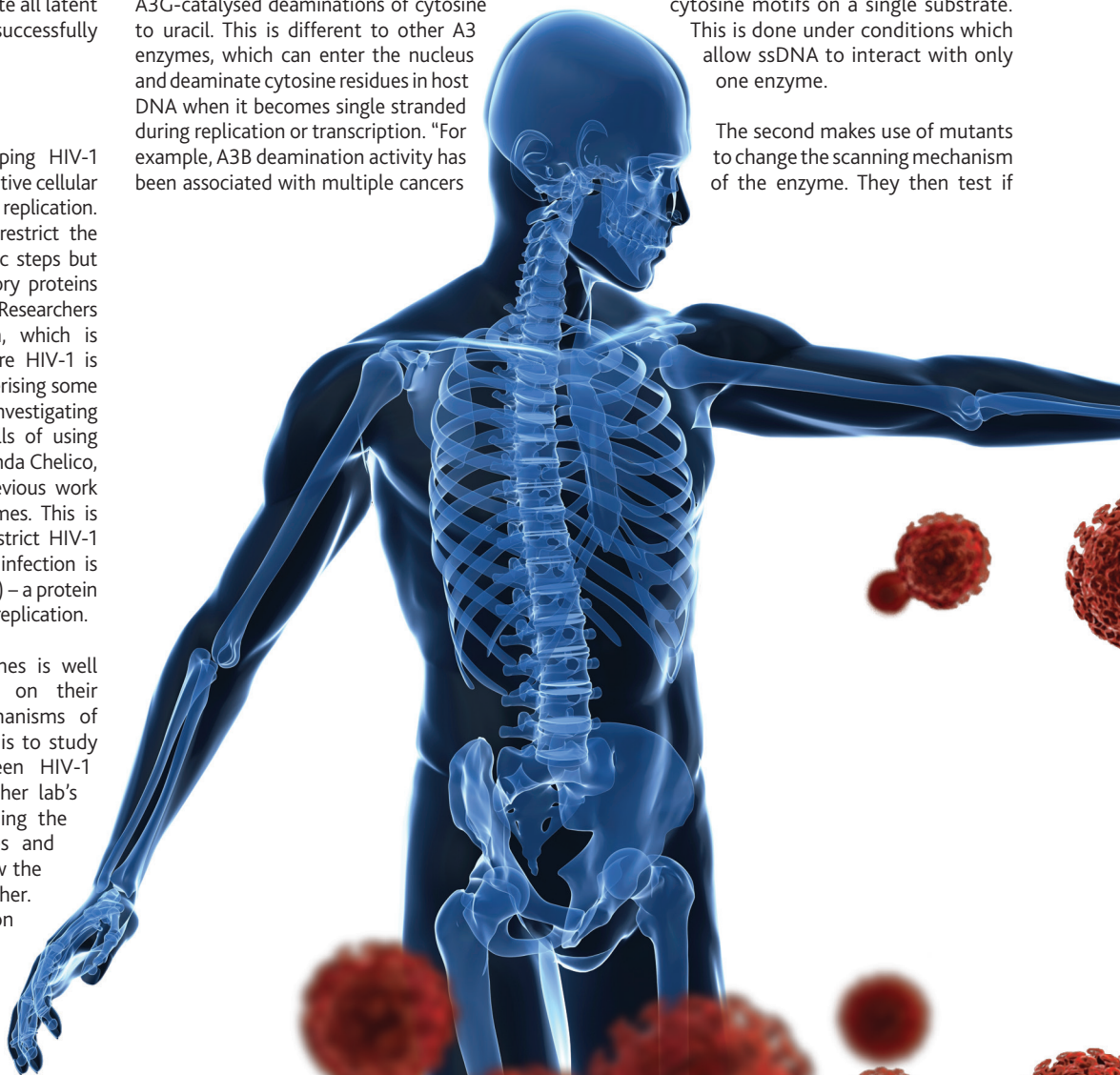
With regard to HIV-1, A3G that escapes Vif is able to enter the assembling virus particle through a binding interaction with RNA. After the virus enters the next target cell, A3G exerts its antiviral function during the reverse transcription process within the viral capsid. A3G can bind both RNA and ssDNA, but is only catalytically active on the latter.

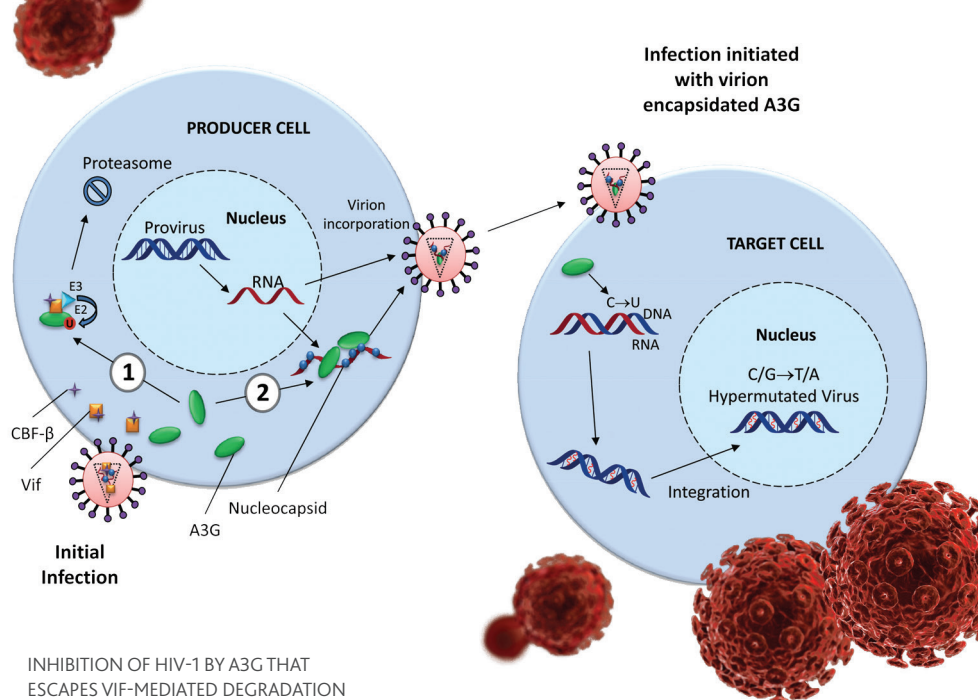
NOVEL INVITRO SYSTEMS

Chelico and her colleagues are employing three systems in their lab. The first uses purified proteins to study movement of ssDNA by tracking deamination events of two cytosine motifs on a single substrate.

This is done under conditions which allow ssDNA to interact with only one enzyme.

The second makes use of mutants to change the scanning mechanism of the enzyme. They then test if





these differences in processivity between the wild type enzyme and mutants (or different A3 enzymes) are significant during reverse transcription. To do this, they have designed a novel *in vitro* system in which A3 enzymes, prebound to RNA in the presence of reverse transcriptase and nucleocapsid, as in a virus particle, need to wait for reverse transcriptase to synthesise the first strand of DNA in order for deaminations to occur.

Additionally, the researchers have engineered a specific viral sequence into their system in order to initiate second strand DNA synthesis

in the same reaction, without the addition of other components. This allows them to test if differences in processivity matter in a complex and dynamic system. "We have found that processivity does make a difference to mutational efficiency and our results support the hypothesis that A3G is the most potent restrictor of HIV-1 due to an optimal processive scanning mechanism," Chelico adds.

DRUG DESIGN

Whilst current HIV treatment strategies rely on inhibiting key enzymes of HIV such as reverse transcriptase, HIV-protease, or integrase, these enzymes can develop resistance to the drugs because of the high mutation rate of HIV-1. Chelico explains: "The idea with A3G is that we may be able to disrupt the interaction between A3G and Vif, thus allowing A3G to do its job and restrict HIV-1 replication. If this is accomplished by designing a drug that binds Vif and blocks the interaction with A3G then the same issue of resistance development will be encountered. However, if we can design a drug that binds the human counterpart, A3G – that is less prone to rapid mutational evolution – then perhaps the treatment can maintain effectiveness for longer".

Chelico's work could lead to powerful, novel alternatives for treating HIV-1 but first, she and her team need to establish the biochemical determinants of efficient restriction of HIV-1 by A3G so that they can avoid changing the function of A3G when bound by a drug. They also need to further understand the interplay between HIV-1 and A3G, since the mechanism by which it inhibits HIV-1 is through mutagenesis. "If A3G does not induce enough mutagenesis of the HIV-1 genome, it may help the virus to evolve and we want to avoid that situation. Our research focuses on potential therapeutic avenues but we are also trying to understand how things could 'go wrong' so that we can circumvent such situations," Chelico concludes.

INTELLIGENCE

THE VIRUS INFECTIVITY FACTOR (VIF) OF HIV-1: MECHANISMS OF INHIBITING ANTIVIRAL APOBEC3 IMMUNE FACTORS

OBJECTIVES

To study the molecular 'arms race' between HIV-1 and the human A3 interface. The specific goals are to biochemically characterise the APOBEC3 enzymes to determine the intrinsic properties that enable efficient restriction of HIV-1, to determine how Vif interacts with APOBEC3 enzymes and inhibits their function, and to determine the off target consequences of having DNA mutating enzymes expressed in human cells.

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DR LINDA CHELICO is an assistant professor in the Department of Microbiology and Immunology at the University of Saskatchewan where she has been since 2009 when she established her own laboratory. After completing a BSc degree and PhD in Applied Microbiology – investigating the DNA repair systems of insecticidal fungi – at the University of Saskatchewan, she moved to the University of Southern California to carry out postdoctoral research in the lab of the biochemist Myron F Goodman.

