Repelling retroviruses

Dr Marc-André Langlois discusses his research into the immune response to retroviruses, as well as his new technique for screening DNA for rare mutated proviral sequences.

What are the overarching goals of your research into retroviruses?

Retroviruses are everywhere; all vertebrate animals (cats, dogs, birds, fish, primates and humans) have them encoded in their DNA. In fact, about 8 per cent of the human genome is composed of retroviruses. Most of these endogenous animal retroviruses are inactive through mutations and sequence deletions, however some can go on to infect new cells, new hosts and even different animal species. In addition to often being directly harmful to the cells they infect, some retroviruses are also known to cause cancer.

Compounded with the threat of retroviruses, mobile genetic elements such as retrotransposons, which make up as much as 30 per cent of the human genome, can also cause insertional and regulatory damage to our genes if not suppressed adequately. The link between retroviruses and retroelements is that they both replicate via a process called reverse transcription whereby single-stranded RNA is converted into double-stranded DNA.

My lab’s research is focused on better understanding the immune mechanisms that prevent the transposition of endogenous retroelements, and protect against the transmission of retroviruses, including HIV.

How will your investigations screening genomic DNA for rare hypermutated proviral sequences contribute to understanding the impact of APOBEC3-induced hypermutation on host-virus interactions?

DNA-mutating enzymes, such as APOBEC3 proteins, can act as double-edged swords in that they can help either the host or the pathogen given the right circumstances. If they hypermutate the viral DNA, then the outcome is the mutational inactivation of the virus – end of story. However, in a situation where APOBEC3 proteins are weakly expressed, they could introduce sublethal levels of mutations in the virus. These could create new genetic attributes that increase virulence, replicative fitness or even confer drug resistance.

Although the first two outcomes have not been formally proven to occur in HIV patients, some drug resistance mutations are associated with the activity of APOBEC3 proteins. Because infectious APOBEC3-mutated viruses constitute a small proportion of the overall virus population in an HIV-infected individual in the early stages of the infection, sensitive and high-throughput methods are required to detect them before they expand and proliferate. Early detection of drug-resistance mutations could guide clinicians in selecting the most effective antiviral drug combination for their patients in order to avoid therapy failure.

What specialised methods have you developed to study the restriction of retroviruses by APOBEC3 proteins?

Standard (Sanger) DNA sequencing is commonly used to analyse and screen for mutations made in viral DNA by APOBEC3 proteins. Although effective for a small number of samples, this method is relatively time-consuming, has to be outsourced and does not provide high-throughput capabilities. To address these issues, we adapted a current method used in genotyping called high-resolution melt (HRM) analysis for screening and quantifying APOBEC3-induced mutations. Because these mutations are C-to-T transitions (or G-to-A on the opposite strand), they lower the melting temperature of a given DNA fragment. By calibrating our assays with control samples with known numbers of mutations, we were able to develop an algorithm and protocol to both screen and quantify these types of mutations in DNA in a simple laboratory assay. The technique can easily be performed and will help identify rare APOBEC3-hypermutated sequences that have been linked to drug resistance in HIV patients.

Does the APOBEC3 enzyme family have additional roles in the human body beyond exerting innate antiretroviral immune activity against HIV and other retroviruses?

Whilst best known for their roles as retroviral (anti-HIV) restriction factors, the seven members of the human APOBEC3 family also have complementary and overlapping roles in conferring protection against endogenous retroelements (retrotransposons and endogenous retroviruses), against some DNA viruses and also against naked foreign DNA. In recent months, however, strong associations have been made between the expression and activity of APOBEC3B and several types of human cancers. One particularity of APOBEC3B is that it is expressed in the nucleus and can edit the human genome. Another member, APOBEC3A, is also expressed in the nucleus of some cell types but it not yet certain whether its expression and activity are linked to cancer. The evidence that these enzymes can modify the human genome suggests there could very well be other functions carried out by APOBEC3 proteins outside the confines of intrinsic immunity that we are still not aware of. It will be interesting to see what more we can learn about these fascinating enzymes in the coming years, and whether they will indeed inspire the design of successful therapeutic agents against HIV.
Spotlight on HIV restriction factors

Little is known of the body's specialised defences which act to counter retroviruses such as HIV. A team from the Department of Biochemistry, Microbiology and Immunology at the University of Ottawa is seeking to understand how retroviral restriction factors work, in the hope this knowledge will one day lead to more effective treatments.

VARIOUS HIV/AIDS DRUG treatments have greatly reduced mortality rates since the virus’ discovery in the early 1980s, yet the 30 million people currently infected continue to have a lowered life expectancy and virus-related chronic illnesses. HIV has proven difficult to counteract because, unlike the vast majority of disease-causing viruses, it is a retrovirus.

Retroviruses replicate in a process of reverse transcription, in which viral RNA is converted into DNA which is then irreversibly inserted into the host’s genome. The host then transcribes this DNA as if it were its own, and in doing so produces copies of the virus. These RNA copies of the viral genome are packaged into viral particles and finally released, free to infect other cells.

THE ANSWER INSIDE

Whilst many look to develop new drugs to attack the virus directly, some believe that answers can be found in the intrinsic response of the body's immune system to infection. There are four main protein families which form this defence: TRIM5α, BST-2/tetherin, SAMHD1 and APOBEC3 (A3). In contrast to slow immune responses such as the release of antibodies, these restriction factors are continuously produced by the cell so that upon entry, the virus can quickly be interrupted. As such, they are often said to form an ‘intrinsic’ immunity, always present in the cells. This immediate barrier is vital as retroviruses insert their DNA into the host genome quickly, in as little as eight hours, whilst antibody production is delayed.

Upon insertion, retroviruses can cause disease – such as AIDS in the case of HIV, or even sometimes cancer – and if inserted into the genome of a germ cell can result in the provirus being passed on to offspring. Luckily, restriction factors are highly effective and there are only two retroviruses that are known to cause disease in humans – human T-lymphotropic virus (HTLV) and HIV.

RESTRICTION FAMILY

Dr Marc-André Langlois and his team at the University of Ottawa in Canada focus their work on the antiretroviral APOBEC3 protein family. These seven highly conserved proteins have been defending humans throughout their evolution, thwarting the action of the vast majority of retroviruses. One of them in particular – APOBEC3G – has been widely studied and found to be heavily involved in the body's response to HIV, acting on the virus before it has a chance to irreversibly integrate into the host cell’s DNA.

Langlois and his team have sought to further understand the two main mechanisms by which APOBEC3G prevents the spread of HIV: those which involve deamination and those which occur independently of such processes.

DEACTIVATE, DEAMINATE

It is largely believed that mutagenic deamination is the main mechanism that confers APOBEC3 proteins their potent effect. In this pathway, APOBEC3 proteins cause mutations in single-stranded DNA intermediates of reverse transcription, significantly altering the proviral DNA which is inserted into the host genome, making it dysfunctional.

This deamination process involved the APOBEC3G-mediated removal of the amine group from position four of cytosine, in the minus-strand of viral DNA, resulting in a base change to uracil. Because APOBEC3G induces extensive DNA deamination, the process is known as hypermutation. The complementary base pairs in the plus-strand proviral DNA are thus mutated from G to A, resulting in stop codons or genes which code for dysfunctional proteins. Such hypermutations lead to the viral offspring becoming non-infectious or non-functional. Remnants of ancient germline infections carrying mutated DNA from this process can be observed scattered across the genome of all mammals, including humans.

In order to circumvent the deaminating defences of APOBEC3 proteins, HIV has evolved to possess the accessory protein Vif. Vif binds to several APOBEC3 proteins including APOBEC3G, leading to their cellular degradation. If drug therapies can prevent their interaction with Vif, the body may thus be able to resist HIV infection, as Langlois elaborates: "If successful, these strategies would constitute a new type of antiviral therapy that enables naturally expressed host restriction factors to do their job, rather than drugs directly targeting the virus”.

Possessing only one APOBEC3 protein, mice provide a simplified in vivo model for the study of this viral defence protein within the broader context of the immune system. Langlois’ team has studied deamination by APOBEC3 in mice, finding that some retroviruses are targeted for restriction whilst others are left alone. Seeking to understand the reason for this specificity, they investigate how certain genetic attributes of mouse retroviruses influence sensitivity to APOBEC3, knowledge which can be translated to the study of human APOBEC3 proteins.

LURKING IN THE SHADOWS

However, the research largely focuses on the mechanisms of APOBEC3 proteins which do not rely on this process of deamination. Such mechanisms interfere with viral infection in its early stages, such as the reverse transcription of viral RNA into DNA, but are currently poorly understood. The team’s...
investigations have shed light on these mechanisms, suggesting that they are, in fact, highly important in the fight against retroviruses.

In their quest for understanding, Langlois and his team have already shown that restriction depends on APOBEC3G’s ability to bind to cellular RNA. APOBEC3G accumulates in the cytoplasm in large ribonucleic complexes in which the deaminating function of the proteins is inactivated. Eliminating the RNA binding properties of APOBEC3G by mutating particular amino acids, preventing these complexes from forming, causes all deamination-independent restricting properties of the protein to disappear. This significantly hampers the cell’s defences in the early stages of infection. The researchers have thus shown that RNA binding is vital for preventing the replication of retroviruses. They have also revealed that these deamination-independent mechanisms are responsible for interfering with the production of proviral DNA and its integration into the host’s genome, whilst APOBEC3G’s mutating activity has only a minor role to play in the early stages of infection.

Having established this link, the team hopes to delve further into the precise mechanism behind it. Exploring this relationship between APOBEC3G’s restrictive capabilities and its ability to bind to RNA provides a lead that could eventually result in better treatments, as Langlois explains: “We believe that these deamination-independent restriction mechanisms could be exploited to develop new therapeutic approaches against HIV infection, but only if we have a better understanding of how they work”.

KNOWLEDGE BUILDS TOOLS

The scope of the team’s work encompasses many different aspects of APOBEC3G. In the attempt to create lethal mutations in the viral DNA, APOBEC3 proteins can sometimes help HIV evolve and develop drug resistance. Hence, Langlois’ group has developed a method based on high-resolution melt analysis to provide a sensitive, high-throughput tool to detect these mutations that can be used in a clinical setting. This is another facet of the team’s output which, as a whole, focuses on broadening our understanding of these restriction factors and the body’s intrinsic immune system. Rather than attacking HIV directly through drugs, they hope that the knowledge they uncover will help guide strategies that facilitate the body’s own defences. In this way, it may one day be possible to prevent HIV infection from ever occurring.

Restriction of HIV infection by human APOBEC3 proteins.