Viral Attack Strategies

Multiple Pathways to Pandemic Preparedness

Molecular virologist François Jean is developing multiple approaches to stop the spread of life-threatening human viruses. His research focuses on enzymes that control both viral and host-cell pathways essential for infection, and he is developing inhibitors to interrupt these enzymatic pathways—and stop viruses in their tracks.

The potential threat of a viral pandemic has researchers and governments around the world working hard to keep one step ahead of new viruses and the latest mutations of existing ones. "Avian flu, HIV, hepatitis C, and West Nile viruses are emerging or re-emerging human-enveloped viruses associated with the world's most serious diseases," says François Jean, associate professor in Microbiology & Immunology at UBC.

In 1997, a strain of highly pathogenic (HP) avian flu, influenza A H5N1, made the genetic leap from chickens to humans in Hong Kong. Since that first H5N1 outbreak, H5 infA viruses have been reported in over 60 countries around the world, including Canada, where the first cases of avian H5 infA occurred in January 2009.

Over the last decade, new deadly strains of avian flu have been observed around the world. In February 2004, a low-pathogenic (LP) H7 avian infA strain (H7N3) was found in Canada in poultry on a commercial breeding farm east of Vancouver. Then the virus rapidly became highly pathogenic and spread to 42 farms and eleven backyards. The Government of Canada made a decision to destroy the poultry from the entire control area—an estimated 19 million birds—to keep the disease from spreading. Thus, a pandemic was averted—for the time being.

In his work, Jean leads an inter-institutional and multidisciplinary team that recently received $1.5 million from the Pandemic Preparedness Strategic Research Initiative of the Canadian Institutes of Health Research (CIHR). The largest award of its kind in Canada, it supports international collaborative research in influenza virus biology associated with human and avian flu. The team of world-class researchers from Canada, the US and China will study the genetics of H5/H7 infA viruses to understand how the disease manifests both in birds and humans, as well as the animal–human interface. This research includes co-supervision and inter-institutional training of graduate students and post-doctoral fellows in the HP H5/H7 infA virus field. "The results of our proposed study on the HP influenza A virus biology and pathogenesis will help to define new models for risk assessment, disease surveillance and pandemic preparedness," Jean says.

FINDER Functional Infectomics

François Jean is the scientific director of UBC's Facility for Infectious Disease and Epidemic Research (FINDER). Jean leads a major research initiative on the biology of risk group 3 (RG3) viruses of concern in Canada and around the world (e.g., influenza A H5N1 virus, West Nile virus, HIV-1, and SARS-CoV) in order to catalyze the discovery of novel classes of antiviral drugs. FINDER, funded by CFI and BCKDF infrastructure grants (Centre for Disease Modeling, $19.3 million, 2004), is equipping Canadian and international researchers with the resources to apply cutting-edge genomics, proteomics and imaging or "infectomics" tools to the research of RG3 pathogens.

Combating Re-emerging Viruses

West Nile virus (WNV), a member of the *Flaviviridae* family first discovered in 1937 in Africa, is now considered endemic in North America. Since its first appearance in New York in 1999, WNV has undergone a dramatic expansion of its ecological niche across Canada and the US, with several thousands of human cases across North America. While no cases have been seen in BC as yet, WNV has changed its genetic makeup over the last ten years of co-evolution in the Western hemisphere. The virus has evolved to spread into the brain (neuroinvasive) of the human host, causing encephalitis, which can be lethal.

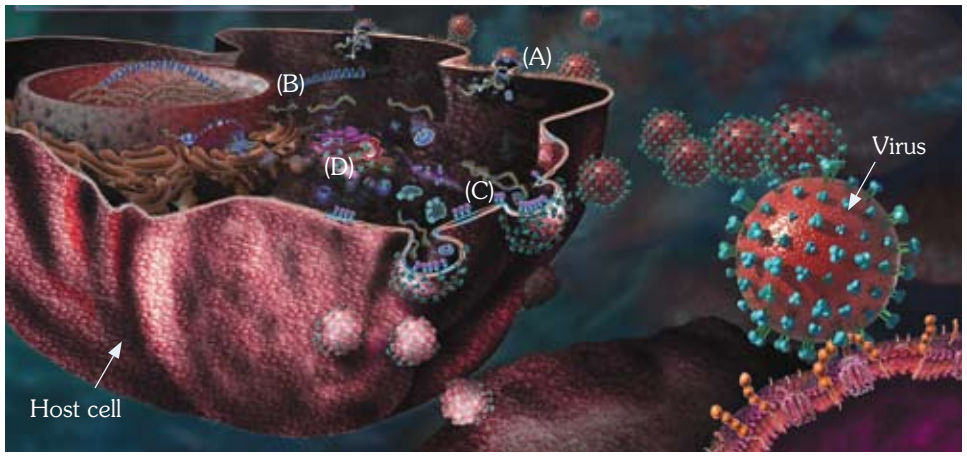
Another member of the *Flaviviridae* family, hepatitis C (HCV), identified 20 years ago, is continuously spreading around the world and has reached epidemic proportions. An estimated three percent of the world population is infected and around 170 million people are chronic carriers, including approximately 240,000 Canadians. HCV is referred to as the silent killer, since both acute and chronic HCV have no symptoms. However, persistent infection can result in liver disease and liver cancer.

"Despite the increased threat of these viruses around the world, our arsenal of treatment against *Flaviviridae* (HCV, WNV), *Orthomyxoviridae* (infA), and *Retroviridae* (HIV-1) is very limited," Jean says. "In the case of anti-*Flaviviridae* drugs, the current multi-drug regimen for HCV is not very effective against the most predominant HCV genotype circulating in North America, Japan and Western Europe. Anti-WNV drugs are just not available yet. And although anti-infA and anti-HIV drugs were initially successful, their therapeutic application is now seriously compromised by the rise of resistant viral strains around the world."

Jean and his staff are working on several lines of attack simultaneously: investigating inhibitors of both viral enzymes and host cell enzymes, and identifying both small-molecule and protein-based antiviral agents. "We are testing all of these approaches side by side and trying to find connections and synergies," he says. With multiple strategies for both viral and host-cell-directed targets, they hope to discover new antiviral agents and combination therapies that are more effective, less toxic and less susceptible to viral resistance than the current regimens.

Marine Compounds Stymie Viral Attack

During 2003, severe acute respiratory syndrome (SARS) spread quickly in 29 countries, including Canada, causing over 8,000 probable cases worldwide and more



Artwork: Sayo-Art

Discovery of Novel Antiviral Targets and Class of Antiviral Drugs. *On this HIV life cycle representation, (A) to (D) indicate Jean's research into various viral and host cell pathways and his strategy goals for developing inhibitors that eventually stop infection. (A) Virus adsorption, virus-cell fusion. Goal: co-receptor antagonists, fusion inhibitors. (B) Reverse transcription (RT), integration, transcription. Goal: RT inhibitors, integrate inhibitors. (C) Translation, proteolytic cleavage, assembly, budding. Goal: novel class of viral protease inhibitors. (D) Cell protease. Goal: novel class of cellular protease inhibitors. Outcome: new combination therapy (C, D).*

than 700 deaths. "At this point, it appears that the SARS coronavirus (SARS-CoV) is in remission, but it continues to exist in animals and could well re-emerge in the human population again," Jean says.

In 2004, Jean's research group initiated a pilot project with Raymond Andersen at UBC to discover new anti-SARS small molecules derived from natural marine extracts. "Nature has been generating all sorts of metabolites over millions of years, and the biodiversity is phenomenal. We just cannot reproduce that with synthetic chemistry," says Jean. Funded by CIHR and NCE/PENCE, the studies resulted in the development of a new high-throughput (HT) fluorescence-based assay, which is now used to identify small-molecule anti-SARS agents from marine organisms. Using the HT screening assay platform, the team identified a new inhibitor, marine compound 8 (MC8) in the marine sponge *Axinella corrugata*. Studies performed with Richard Kao at the biocontainment level-3 laboratory at the University of Hong Kong have confirmed the existence of MC8 anti-SARS activity in the cell-based system of SARS-CoV infection. "Our results have triggered considerable research in the potential of marine-based compounds to work as viral protease inhibitors with good inhibitory activity and low toxicity in human cells," Jean says.

"We have since tested a wide range of marine-based compounds against other important viral proteases and recently discovered two nanomolar inhibitors, compound 11 (C11) and compound 21 (C21), that are selective against the *Flaviviridae* HCV NS3 protease," Jean notes. HCV NS3 protease as an effective target for anti-HCV therapy was recently validated in a clinical setting by three research teams using competitive small-molecule protease inhibitors (PIs). "Given the limited number of such inhibitors in clinical trials, as well as the likelihood of eventual resistance to monotherapy, there is an important need to identify and develop a new class of small-molecule HCV NS3 PIs," Jean says.

His lab's C11/C21 analogs were recently patented with UBC's University-Industry Liaison Office. Their work on HCV NS3 PIs is supported by a Proof of Principle phase I CIHR grant. The goal is to scale up synthesis of C11/C21 to demonstrate their anti-HCV properties using cell-based systems of HCV replication and infection. This project also aims to identify C11/C21 analogs through a virtual screening strategy, using a powerful *in silico* chemical library screening approach.

Innovative Targeting of Host Enzymes

Worldwide nearly half of all antiviral drugs available are small-molecule inhibitors that target virally encoded enzymes (e.g., protease, helicase, polymerase, reverse transcriptase). Yet drug-resistant viruses continue to evade treatment, leaving few or no alternative therapeutic agents available. Although combination therapies have reduced the number of emerging resistant variants over the last decade, new global antiviral strategies are required to aid in the creation of therapeutics with novel mechanisms of action. Jean's team is proposing to shift the current paradigm on global antiviral strategies towards the exploration of host-directed drug targets to create a new class of therapeutic agents.

The team's primary goal, funded by CIHR, is to develop new drug leads that target recently discovered host enzymes from the proprotein convertase (PC) family. These host enzymes are essential to viral infection for a number of serious human viruses such as HIV-1, HP H5/H7 infA and WNV. "Our hypothesis is that because the therapeutic targets are cellular enzymes, resistance by mutation of the virus is highly unlikely," Jean says. "By developing selective and potent cellular enzyme inhibitors as antiviral drugs, my lab is pioneering a new global antiviral strategy directed at inhibiting the host-mediated activation of viral glycoproteins in the secretory pathway of the cells, an essential processing event for virus infectivity."

The results of a series of recent studies performed with a protein-based inhibitor of PCs discovered in the Jean lab, Spn4A, demonstrate that developing novel host-directed broad-spectrum biopharmaceuticals is achievable. Team member Vesna Posarac, in collaboration with Peter Cheung at the BC Centre of Excellence in HIV/AIDS at UBC, recently demonstrated the anti-HIV properties of Spn4A in cultured cells.

Team Members and Research Funding

The Jean lab team (2008/09) includes graduate students Heather Braybrook, Stephanie Condotta, Christine Lai, Emma-Kate Loveday, Andrea Olmstead, and Meera Raj; post-doctoral fellow Julius John; lab technicians Martine Boutin and Ingrid Hao; Co-op students Pamela Lincez and Vanessa Silva; and research assistant Steven McArthur. Morgan Martin and Vesna Posarac graduated in 2008. Research in the Jean lab is currently supported by three CIHR operating grants (F. Jean, PI). The trainees are supported by UBC, CIHR, CIHR-TRID, and MSFHR.

New Appointments

Professor Doug Bonn was appointed head of the Department of Physics & Astronomy January 1, 2009. His administrative background includes serving as chair of the university's Senior Appointments Committee. Bonn's research into the electromagnetic properties of high-temperature superconductors has helped make UBC a world leader in the area. His main focus involves unravelling the origin of high-temperature superconductivity—a major unsolved problem in condensed matter physics. Bonn was awarded a Killam Research Prize in 1999 and, in 2005, shared the NSERC Brockhouse Canada Prize with colleagues Walter Hardy and Ruixing Liang. "Since joining UBC in 1989, Bonn has made many valuable contributions to the department, the Faculty of Science and the university," notes dean Simon Peacock. Bonn succeeds physics professor Jeff Young, who provided five years of excellent leadership. www.physics.ubc.ca

Craig Hart was appointed director of UBC's Mineral Deposit Research Unit (MDRU) and grant-tenured associate professor in the Department of Earth & Ocean Sciences January 1, 2009. UBC alumnus Hart returned to UBC from the University of Western Australia (Perth), where he was a senior research fellow at the Centre for Exploration Targeting. He completed his PhD at the University of Western Australia (2004) working on the Tintina Gold Belt in the Yukon and Alaska, research he began as a geologist for the Yukon Geological Survey. "On behalf of the board, I welcome Craig back to Canada," notes Ian Graham, chair of the MDRU board of directors. "We look forward to working with him to continue the research and training excellence that has been established at MDRU through the previous directors." Hart succeeds Richard Tosdal, who spent nine years at the helm of MDRU. www.mdru.ubc.ca

Tara Ivanochko was appointed an instructor in the Department of Earth & Ocean Sciences January 1, 2009. Ivanochko, who teaches environmental science, provides a direct connection between the curriculum content and events that are timely or significant in our daily lives. She motivates her students to understand the science underlying current world issues. Ivanochko's research looks into the mechanisms by which the tropical oceans exert influence on high-latitude climate change and vice versa. She reconstructs paleoenvironments and investigates climate change on decadal to millennial time scales. Ivanochko graduated with a BSc in Biology and Oceanography and an MSc in Marine Geochemistry from UBC, and earned her PhD in Paleoclimatology from the University of Edinburgh, UK. She was a research associate at UBC 2006 to 2008. www.eos.ubc.ca/about/faculty/T.Ivanochko.html

Allan Berezny was appointed assistant dean of Development for the UBC Faculty of Science February 1, 2009. He attended McGill University (BA and MA) and Queen's University (MPA) and has worked in the field of university development at McGill, the University of Victoria, Cardiff University (Wales), and the University of Northern BC. Throughout his career, Berezny has been active in securing funding for large initiatives. These include scientific and medical research projects involving agencies and donors in North America, Europe and Asia. Berezny looks forward to helping advance the work of researchers in UBC Science. For further information on how he and his team can be of assistance, contact him at 604-822-8686 or at allan.berenzny@ubc.ca. science.ubc.ca/support/giving

Quentin Cronk joined the department of Botany as full professor in December 2008. He came to Canada from his position as Reader in Vascular Plant Systematics at the University of Edinburgh, UK, in 2002, when he was appointed professor and director of UBC's Botanical Garden and Centre for Plant Research. After six very successful years at the helm of the Botanical Garden, he stepped down from the position in order to devote more time to research. Cronk graduated with a BA Natural Sciences (Botany) and earned his PhD Botany from Cambridge University, UK. His appreciation for the vast diversity in plants and the impact it has on our lives and the lives of other organisms on the planet motivates Cronk's research into how this variation evolved at all levels—from the molecular to the ecosystem. www.botany.ubc.ca/people/faculty.html

Nigel Lockyer, director of TRIUMF (since May 2007), joined the Department of Physics & Astronomy as full professor July 1, 2008 (BSc Physics, York University, Toronto, ON; PhD Physics, Ohio State University, Columbus, USA). Lockyer came to Canada from the University of Pennsylvania (Philadelphia, USA), where he focused on high energy particle research, reaching into neighbouring areas such as accelerator science and medical physics. Owned by seven Canadian universities and located on the UBC Vancouver campus, TRIUMF is Canada's National Laboratory for Particle and Nuclear Physics. With 55 partner institutions and an international user community of nearly 1,000 scientists, it is one of the world's leading subatomic physics laboratories. www.triumf.ca

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Another team member, Heather Braybrook, is testing the efficacy of these PC inhibitors against HP H5 infA viruses, and the preliminary results are very promising.

The key to developing host enzyme inhibitors, they have found, is to knock down the target enzyme rather than knock it out, or eliminate it completely. "Just a reduction in the actual activity levels of the cellular enzyme can trigger the effect you are looking for while still allowing it to perform its natural biological function, and this reduces the risk of side effects," says Jean.

For Jean, developing effective, non-toxic antiviral drugs requires the best of multiple approaches. "The most effective line of defence might be a combination of small molecules and protein-based inhibitors, or a combination of inhibitors that target both viral and cellular enzymes," he says.

"What makes this research so exciting and challenging is that there are always surprises." ■