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Persistent viral infection

Dr Alain Lamarre holds the Jeanne and J-Louis Lévesque Chair in Immunovirology. In this interview, he discusses his studies of natural antibodies and their role in persistent viral infection

What are your overarching research interests and goals?

Our primary focus is to better understand the mechanisms used by certain viruses to escape the immune response and establish persistence. Of these, our main interest is the hepatitis C virus (HCV), which infects the liver and leads to life-threatening complications in a significant number of patients. More recently, we have begun to study the immune response to certain tumours, as there are a number of similarities between persistent viruses and cancer in terms of how they evade recognition by the immune system. Using this knowledge, we are developing new vaccines and treatments against persisting viruses and cancer.

How and why are you studying the role of natural antibodies in the immune response to persisting viruses?

We tend to think of antibodies as immune mediators that are induced following infection with a pathogen, leading to its elimination. Whilst this is true for most acute infections, persisting viruses such as HCV and HIV often do not lead to the development of antibodies able to eradicate the infection. These infections require the development of cellular immunity, but HCV and HIV have evolved to avoid recognition by immune cells and so can establish persistent infection.

Natural antibodies, which are present even before exposure to a pathogen, represent a link between the innate and adaptive immune systems, which facilitate the development of cellular immune responses. We have demonstrated that natural antibodies have a significant impact on the induction of T cell responses in a persistent viral infection model in mice. We did so using transgenic and knockout mice models with different degrees of antibody diversity, and now aim to validate these results in humans.

What makes you particularly interested in the pathology of HCV?

HCV infection is a major global health problem; around 3 per cent of the world's population is infected with the virus. In 75-85 per cent of individuals the infection becomes chronic, 10-20 per cent develop liver cirrhosis within 10-20 years of infection, and 5-10 per cent of those develop hepatocellular carcinoma. It is becoming increasingly clear that the strength and breadth of the immune response which is induced soon after infection is pivotal to viral clearance.

Are there any particular challenges associated with this field?

Acute HCV infection is asymptomatic in the majority of cases and infection is diagnosed when symptoms appear, often decades after infection. This makes the study of acute HCV infection particularly challenging.

Could you provide an insight into the novel vaccination platform under development based on virus-like particles of the papaya mosaic virus (PapMV)?

We have found that PapMV nanoparticles, produced from a plant virus, are highly immunogenic in mice. PapMV can be used as a vaccine delivery platform or an adjuvant to enhance immune responses to co-administered antigens or vaccines. We have recently demonstrated that the immunogenic properties of PapMV nanoparticles reside in their ability to efficiently activate the immune system through recognition by toll-like receptor 7 (TLR7), a pathogen-associated molecular pattern receptor involved in antiviral immune responses. This technology is due to enter a phase I clinical trial in early 2014, and phase I-II later in the year.

What recent findings have you made?

We recently discovered a previously unrecognised link between the complement system and natural killer (NK) cells in the induction of an anti-tumour immune response.

Complement proteins are soluble mediators of the innate immune system in serum, which participate in the clearance of certain pathogens and induce inflammation. We found that the complement system restricts NK cell availability in the tumour tissue. NK cells normally participate in the induction of a tumour-specific immune response via tumour cell lysis. Inhibiting complement could thus provide a potential therapeutic target in cancer patients to improve anti-tumour immune responses.

Looking ahead, what are the next steps for your research?

Until recently, we have been focused on the discovery of fundamental mechanisms which participate in the induction of successful immune responses against viruses and tumours, mostly using murine experimental models. We are now moving towards translating these discoveries to human disease. Although there are huge challenges in studying human immune responses, we believe we can make great progress in the development of novel treatments and vaccines against persistent viruses and cancer.

Tackling Hepatitis C

The **INRS-Institut Armand-Frappier Research Centre** is at the forefront of viral research. Researchers are developing a novel vaccination platform for chronic viral infection, focusing on the hepatitus C virus

VIRUSES PRESENT A great challenge to scientists as their infectivity can at times seem capricious. Some are able to be controlled with relative ease, such as influenza, whilst others are much more difficult to eradicate, such as human immunodeficiency virus-1 (HIV-1) and hepatitis C virus (HCV). It is unclear why certain viruses persist whilst others do not, and why some of us are more resistant than others. Based at the Institut national de la recherche scientifique (INRS), Dr Alain Lamarre and his team are seeking to tackle this challenge, with a focus on HCV.

HCV is primarily spread through blood-to-blood contact and more than 170 million people worldwide are infected with the virus. 20 per cent of infected individuals never display any symptoms and are able to clear the virus whilst remaining unaware of any infection. The remaining 80 per cent usually do not display any symptoms at the point of infection but will never eliminate the virus, which effectively lies dormant until symptoms appear much later.

Chronic HCV infection gradually destroys liver cells such that 10-30 years after infection, patients develop cirrhosis or liver cancer, killing over 350,000 people every year. Despite the widespread nature of the infection and its devastating consequences, the virus remains poorly understood and there is no vaccine. Moreover, the current treatment options available are unsatisfactory. Interferon and ribavirin medications are not particularly effective, and although transplants are available, the virus will re-attack the transplanted organ, making repeat transplants necessary.

VIRAL PUZZLE

So why are some people unaffected by HCV infection, whilst others suffer damaging consequences? Lamarre is attempting to solve this conundrum by studying the mechanisms behind the virus's deadly actions. Yet this is fraught with problems. By way of example, HCV is almost impossible to cultivate *in vitro*, and most animals are insusceptible to it. The research is further complicated by the unusual pathology of the virus.

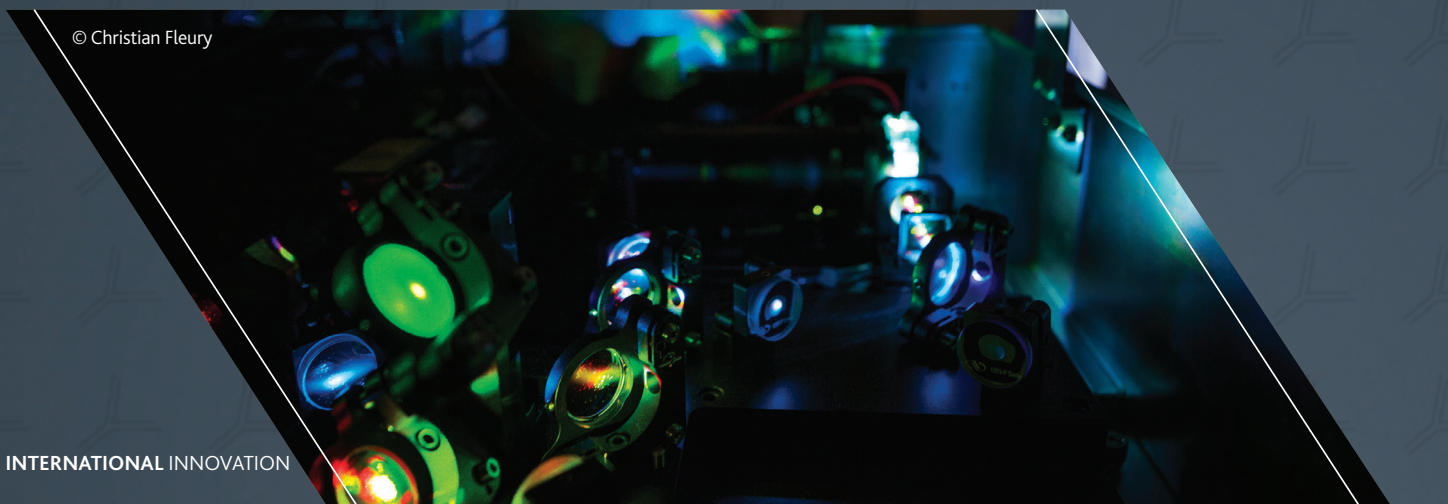
Lamarre is confident, however, that solving this mystery, in spite of the difficulties, is the key to understanding the pathogenicity of HCV. This will hopefully lead to the improved treatment, and perhaps even prevention, of HCV and other chronic viral infections.

RESEARCH ARMS

The research is split between four main areas: the role of natural antibodies in the adaptive immune response against HCV; the delayed appearance of neutralising antibodies in chronic viral infections; vaccination strategies for persistent viral infections; and viral oncolysis.

The team's primary hypothesis concerns natural antibodies, which are produced by B1 lymphocytes and act as agglutinants to form immune complexes with numerous different pathogens. Lamarre theorises that people with a greater repertoire of antibodies preferentially hold these complexes in lymphoid organs and initiate an adaptive immune response via cytotoxic T cells, a process which is undermined in those with a weaker repertoire. To study the action of these natural antibodies, the researchers use the lymphocytic choriomeningitis virus (LCMV), a mouse virus which is thought to be the best model of HCV infection in humans. Their investigations have shown that individuals with a larger repertoire of natural antibodies do in fact launch a stronger immune response, which prevents the infection from spreading.

Another part of the project focuses on both the viral and cellular mechanisms that postpone the immune response to persistent infection. Viruses which cause non-persistent infections lead to the rapid production of neutralising antibodies, a process which is delayed in infections that cause chronic disease. Again, using mouse models and LCMV, Lamarre hopes to develop a method of stimulating the immune response.

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The investigators are also devoted to creating new vaccination strategies for persistent viral infections. The aim of this project, conducted in collaboration with the Centre de Recherche en Infectiologie in Quebec is to develop vaccines using papaya mosaic virus (PapMV). It is completely innocuous to humans but nevertheless generates a strong immune response. Lamarre believes that by grafting segments of pathogenic antigens onto PapMV, it will stimulate the immune system to produce antibodies and stimulate T-cells to fight those pathogens.

> It is unclear why some viruses persist whilst others do not, and why some of us are more resistant than others

Lamarre's group hypothesises that the reason some individuals can fight HCV whilst others are susceptible is largely due to the range of antibodies produced during the initial stages of the infection or even before. They believe that these antibodies may redirect the virus towards lymphoid organs where a more efficient antiviral immune response can be mounted. Technical limitations may be the reason preventing this question being properly addressed to date, but Lamarre is confident that this is now changing: "Recent advances have permitted the development of new tools to address the role of natural antibodies in HCV clearance," he comments. "We are using novel infectious HCV systems and highly powerful sequencing methods to properly address the role of antibody diversity in HCV immunity." His team is collaborating with hepatologists at Hôpital Saint-Luc, Canada's leading research centre for Hepatitis C. Having access to cohorts of individuals at high risk of infection, they take blood samples before and early after infection to identify the antibodies active in the innate immune system response.

FIGHTING CANCER

Despite their toxic connotations, viruses can also be helpful by killing cancer cells. Although viruses have been used to fight cancer for over a century, only in recent decades has oncolytic virus (OV) therapy moved towards structured clinical development. OV therapy is based on the principle that certain viruses preferentially infect tumour cells over healthy cells. However, it is only recently that the role of the immune system in this process has been fully considered, and the question is key to Lamarre's exploration of virus pathology: "Using state-of-the-art immunological techniques and experimental viral and tumour models, we are analysing the mechanisms that favour the development of a successful anti-tumour immune response following treatment with OV," he elucidates. Specifically, he is studying the possibility of treating melanomas using vesicular stomatitis virus. Trials in mice have been very promising, and it is hoped the project will give rise to pioneering clinical applications.

VACCINATION IN SIGHT?

The big question for the future is whether we will be able to protect ourselves against HCV. Lamarre foresees that in the short to medium term more effective antiviral drugs will be developed, in a similar way to breakthroughs for HIV treatment in recent years. However, whilst the mechanisms of the virus remain enigmatic it is unlikely that a vaccine is imminent.

Aided by his partnerships with Hôpital Saint-Luc, the Centre de Recherche en Infectiologie and Université du Québec à Montréal (UQAM), Lamarre is continuing his research into HCV. He believes he will soon be able to confirm that the findings in humans are the same as in mice, which may lead to an ability to strengthen human immune weaknesses. The PapMV vaccine platform has been patented by Folia Biotech. The company is currently conducting preclinical trials on various vaccines based on this technology and will soon initiate clinical testing, but the possibilities are vast and Lamarre has high hopes of commercial vaccines for HCV and cancer.

INTELLIGENCE

MECHANISMS INVOLVED IN THE ESTABLISHMENT OF VIRAL PERSISTENCE

OBJECTIVES

To understand the mechanisms that permit the establishment of persistent virus infections and of tumours. The four main projects involve: 1) understanding the role of natural antibodies in promoting acquired immune responses to persisting viruses; 2) characterising the mechanisms involved in the establishment of viral persistence; 3) developing a novel vaccination platform against chronic viral infections and cancer; and 4) analysing the tumour-specific immune response generated following oncolytic virus therapy.

KEY COLLABORATORS

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DR LAMARRE RECEIVED his PhD in Immunovirology from the Institut Armand-Frappier in 1996 and then completed a postdoctoral fellowship with Nobel laureate Rolf Zinkernagel at Zurich's Institute of Experimental Immunology (1997-2002). He then returned to Canada to head the Immunovirology Laboratory of the INRS-Institut Armand-Frappier Research Centre where he holds the Jeanne and J-Louis Lévesque Chair in Immunovirology. He was a CIHR New Investigator Award recipient from 2003-2008. He is Chair of the National Organizing Committee of the 16th International Congress of Virology that will be held in Montreal, Canada from 27 July-1 August, 2014 (www.montrealiums2014.org). The main focus of his laboratory is to better define the mechanisms that permit the establishment of persistent viral infections.

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