To begin, what drew you to research the rare autoimmune disease, primary biliary cirrhosis (PBC), and what are your main aims and objectives?

I liked the thought of studying PBC as it represented an old-fashioned ‘who-done-it’ mystery. The development of auto-antibodies to a specific mitochondrial protein was an odd finding of completely unknown significance. That’s how we set off to characterise a virus in patients with PBC. Our aims and objectives now are to link this virus with the disease process.

What is currently known about PBC?

Little is known about the development of PBC. It is thought that the disease is triggered by an environmental agent that could either be bacteria, a virus or xenobiotic. It is also known that the risk of PBC increases tenfold for persons who have a first-degree sibling with the disease, so there clearly are heritable factors in the disease process. A number of genome-wide association studies have linked several genes with the development of PBC. With regard to environmental agents, our group has been working on the idea that primary biliary cirrhosis is in fact a form of viral cholangitis. However, this idea is highly controversial as others have been unable to reproduce our data. This notwithstanding, it must be noted that these scientists have not performed similar studies on the same tissues that we have used to show that virus causes disease.

Why has the existence of mammary tumour virus (MMTV)-like viruses in humans been the focus of heated debate for 40 years?

The mouse MMTV was first linked to breast cancer 40 years ago. Virus-like particles were found in the milk of patients with breast cancer, and subsequently virus proteins and nucleic acids were found in patient samples with breast cancer, as well as antibodies to this mouse betaretrovirus. However, the viral titres were low and many investigators were unable to confirm the findings. Consequently, heated debate has been ongoing for decades. One of the main complaints has been that we have been unable to move on, and so better techniques are required to prove that the virus truly infects humans.

Why did you use Combivir™ in your pilot studies and how effective are Truvada™ and Kaletra™ for patients with PBC?

We first tried Combivir because this was the drug that was being used for patients with HIV, and there were data to suggest that these drugs may be active against betaretroviruses as well. Combivir itself was not that good for treating patients with HIV, and we found that although treatment caused some reduction in liver tests, we were not able to show that Combivir could normalise liver function tests, which is a very stringent endpoint. We do not really know how effective Truvada and Kaletra are for patients with PBC but have found the combination works in mouse models of PBC associated with MMTV infection. There is one case in The Lancet where a patient with HIV and PBC normalised his liver tests over the year with this combination. We have one liver transplant patient who had very high liver tests, which subsequently normalised after treatment with a combination of Truvada and Kaletra. We are especially keen to treat this young patient, who has developed severe recurrent disease after a liver transplant.

Could you describe your greatest achievements to date? How close are you to developing effective therapies for patients with PBC?

Our greatest achievements to date have been finding the virus in patients; showing that the virus causes a specific phenotype in culture studies and in mice; and also finding medications to prevent the biliary disease in mice by reducing viral load. Now we need to show antiviral therapy works in patients.
PRESENTLY THERE IS no known cure for the rare autoimmune liver disease, primary biliary cirrhosis (PBC), a chronic condition that predominantly affects middle-aged women. In cases of PBC, the immune system attacks the interlobular bile ducts in the liver that drain bile into the extrahepatic bile ducts, gallbladder and then the duodenum. The resulting damage causes loss of the bile ducts and a dangerous build up of bile, leading to scarring of the tissue and loss of liver function. Current therapy relies on the use of ursodeoxycholic acid which acts to eliminate bile from the liver; however, a third of all patients receiving the treatment still require liver transplantation. Despite the rarity of PBC, 10 per cent of all patients in need of transplant have the disease, underlining the need for better therapies.

THE ENEMY WITHIN

Similar to other autoimmune diseases, PBC is poorly understood. Whilst it is widely believed that the condition is caused by a combination of genetic and environmental factors, a clear understanding of the disease has only recently been forthcoming. In 2003, at the same time the Human Genome Project was nearing completion, evidence began to emerge that the autoimmune response that leads to healthy tissue destruction by the immune system was linked to an inadequate response by the body to an unknown pathogen. The findings were reported by Professor Andrew Mason following extensive research by his team in the laboratories of the University of Alberta in Canada. Through collaboration with a worldwide group of heptologists and virologists, Mason, who has spent the last 15 years studying PBC, is beginning to shed light on the possible causes of the condition and the potential for radical new therapies.

Having found no sign of bacteria or microbes in the liver of PBC patients, the team used ‘representational difference analysis’, a technique used to discover Kaposi’s sarcoma virus, to uncover the presence of retroviral sequences. Retroviruses, such as HIV, integrate themselves into the DNA of their host, then replicate and spread around the body. Mason is unequivocal about the significance of the clinical observations from patients with AIDS: “Patients with HIV infection often make lots of auto-antibodies when their lymphocyte counts drop. So we now have an understanding that when the immune system’s function is diminished, autoimmune responses are more common,” he asserts.

Moreover, the researchers found that patients with PBC had antibodies to retroviruses and microscopic virus-like particles on the surface of the damaged bile ducts, furthering the suggestion of a link between a virus and a destructive autoimmune response. Mason’s team used a direct cloning approach to identify and characterise a full length human betaretrovirus (HBRV) in liver samples with PBC. It was found that the HBRV’s DNA sequence shared 95 per cent homology with the mouse mammary tumour virus (MMTV), a betaretrovirus linked with breast cancer in mice and the human mammary tumour virus.

Seemingly, Mason had unearthed early evidence linking virus infection to disease. However, he notes that the retroviral community remains unconvinced that HBRV can indeed infect humans: “In the 1990s, with the advent of polymerase chain reaction, Dr Beatrice Pogo found an MMTV-like sequence in patients with breast cancer. At that time, multiple groups were able to confirm her findings, but equally as many groups could not, and interest in this subject was lost”. In light of this, the team aims to provide layers of evidence linking virus infection with diseases and to develop new treatment modalities for autoimmune diseases.

COLLECTING THE EVIDENCE

Lymph nodes are considered a major reservoir for viruses. Initial studies of samples taken from the lymph nodes of PBC patients demonstrated that HBRV was present in around 75 per cent of cases. Also, PBC patients were found to make antibodies and auto-antibodies that react with betaretrovirus. Subsequently, the researchers have isolated an infectious betaretrovirus from the PBC patients’ lymph nodes and, in doing so, discovered that the virus could infect multiple cell types. After analysing lymph nodes and biliary epithelial cells for evidence of viral integration into the host genome, the team has derived concrete evidence for HBRV infection in the majority of PBC patients.

Using next generation DNA sequencing, the group’s collaboration with Dr Gane Ka-Shu Wong, also at the University of Alberta, led to the discovery of not only multiple betaretrovirus integration sites but also a site-specific sequence in the human genome – the first example of betaretrovirus integration. Mason expounds the magnitude of the results: “Detecting integration sites cannot easily be generated by artifactual processes. Therefore, they are considered gold standard. The implication of finding the HBRV integrated into the human genome is that the virus truly infects humans”.

The second layer of evidence centres on the link between betaretrovirus and a disease-specific phenotype. Utilising both cell culture and mouse models of PBC, Mason’s studies have shown a connection between viral infection and the production of anti-mitochondrial antibodies (AMAs). Introducing viral isolates to bile duct cells in culture led to the abnormal translocation of mitochondrial proteins to the cell surface, and it is believed that this process encourages production of AMAs by the immune system. Having identified that the presence of HBRV in PBC patients’ lymph nodes induces the disease-specific mitochondrial phenotype, the investigation has also highlighted the same aberrant expression of proteins in both mouse models and PBC patients. Indeed, MMTV was found to be integral in the development of autoimmune biliary disease in many of the spontaneous mouse models. The implication of this link is that betaretrovirus infection can be directly associated with intolerance to mitochondrial proteins.

Research at the University of Alberta is finding new evidence linking autoimmune disease with viral infection. This looks set to dispel the longstanding controversy surrounding the causal association of virus and disease present in PBC patients. The implications of this finding are potentially revolutionary, opening up new areas of investigation into the potential for radical new therapies.

Histological response of the same patient to antiviral therapy despite development of resistance.
**NEW THERAPIES**

Leading on from the research, pilot studies have been undertaken with the aim of demonstrating that antiviral combination therapy abrogates biliary disease. Treating PBC patients with the antiviral drugs zidovudine and lamivudine (Combivir™), the group has observed significant improvement to hepatic biochemistry and liver biopsy appearance. However, the researchers were unable to demonstrate a strong effect and found some signs of viral resistance to Combivir. As Mason explains, the results are tough to evaluate: "It is difficult to know what endpoints to choose for a study using antiviral therapy for patients with PBC. This is because we cannot measure the virus very well as it is very low level. Accordingly, we have to use surrogate endpoints such as reduction of liver tests".

Importantly, in silico studies have begun in collaboration with Dr Ram Samudrala of the University of Washington in the US to develop 3D models of drugs that can bind viral proteins, which they hope will identify better antiviral combinations that could block viral infection. Following tests using mouse models and cell cultures, the researchers are uncovering that, along with anti-retroviral therapy, combinations of reverse transcriptase inhibitors, such as tenofovir and emtricitabine (Truvada™), and HIV protease inhibitors, such as Kaletra™, may be effective at restoring liver function.

The ongoing work at the University of Alberta could signal a revolution in the treatment of PBC and other autoimmune diseases. As research continues into HBVR and the use of antiviral therapy, these groundbreaking findings have the potential for translation across the spectrum of disease. Mason, however, is understandably cautious: "I suspect our hypothesis will remain controversial and highly speculative until we can treat the disease with robust antiviral treatments and show that patients with PBC respond favourably to antivirals".

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**TRANSLATIONAL STUDIES TO TEST POTENTIAL THERAPIES FOR PATIENTS WITH PRIMARY BILARY CIRRHOSIS**

**OBJECTIVES**

To investigate the prevalence of the human betaretrovirus (HBVR) in patients with primary biliary cirrhosis (PBC). A causal association of virus and disease is being investigated using an in vitro model of PBC and a mouse model of disease, which is also being used to find novel combination antiviral therapies for patients with PBC.

**KEY COLLABORATORS**

- **Dr Gane Ka-Shu Wong**, University of Alberta, Canada
- **Dr Ram Samudrala**, University of Washington, USA

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**ANDREW MASON** is a Professor of Medicine and an Alberta Heritage Foundation for Medical Research Senior Scholar. He trained at the Liver Unit, King’s College Hospital, London, UK, and then moved to Washington University, St Louis, as a Gastroenterology Fellow to train in molecular virology. At Ochsner Clinic Foundation, New Orleans, USA, he became the Medical Director of Liver Transplantation and an Assistant Professor in the Department of Medicine at Tulane University Medical Center. He relocated to the University of Alberta, Canada, in 2002; he is currently the Director of Research for the Division of Gastroenterology and Director of The Applied Genomics Centre. Mason is also Principal Investigator for an international, multicentre, double blind, randomised controlled trial using highly active anti-retroviral therapy to treat patients with PBC. He co-directs a viral discovery programme in collaboration with Dr Gane KS Wong, using a metagenomic, deep sequencing approach.