

Potential Honours Projects 2010 - Hepatitis B Virus Research Group

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The exact details of each project will be decided early in 2010, but projects will be offered in the 3 areas listed below. Students should feel free to contact Allison, Georget, Uwe or Feng for any additional information.

Project 1: Persistence of residual HBV and DHBV DNA following resolution of infection

Co-supervised by Dr Georget Reaiche
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Resolution of acute hepadnavirus infection was previously believed to result in the complete eradication of replicating virus. However, recent studies have shown that residual hepatitis B virus (HBV), woodchuck hepatitis virus (WHV) and duck hepatitis B virus (DHBV) DNA persists as covalently closed circular DNA (cccDNA) following resolution of infection.

The presence of residual HBV DNA has clinical implications as infection may reactivate following treatment with immunosuppressive drugs or following organ transplantation. HBV infection may result following transplantation of donor liver from residually infected patients. Understanding the mechanism of cccDNA persistence will allow the development of therapies for the successful elimination of residual HBV DNA.

This project will determine if new cccDNA synthesis can be blocked using a combination antiviral drugs as well as determining if cccDNA can be eliminated from the liver by increasing rates of hepatocyte death and promoting hepatocyte turnover. Molecular analysis of residual cccDNA, such as its sequence and transcriptional activity, as well as its ability to survive mitosis during cell division will be analysed in detail using the well established DHBV model. Methods that will be used in this project include; RNA and DNA extraction, DNA sequencing, PCR, quantitative PCR, RT-PCR, *in situ* PCR, FISH, histology and immuno-staining, various animal handling techniques.

Related journal articles:

1. Hu, K. Q. 2002. Occult hepatitis B virus infection and its clinical implications. *J Viral Hepat* 9:243-57.
2. Le Mire, M. F., D. S. Miller, W. K. Foster, C. J. Burrell, and A. R. Jilbert. 2005. Covalently closed circular DNA is the predominant form of duck hepatitis B virus DNA that persists following transient infection. *J Virol* 79:12242-52.
3. Menne, S., P. J. Cote, S. D. Butler, I. A. Toshkov, J. L. Gerin, and B. C. Tennant. 2007. Immunosuppression reactivates viral replication long after resolution of woodchuck hepatitis virus infection. *Hepatology* 45:614-22.
4. Michalak, T. I. 2000. Occult persistence and lymphotropism of hepadnaviral infection: insights from the woodchuck viral hepatitis model. *Immunol Rev* 174:98-111.
5. Michalak, T. I., C. Pasquinelli, S. Guilhot, and F. V. Chisari. 1994. Hepatitis B virus persistence after recovery from acute viral hepatitis. *J Clin Invest* 93:230-9.
6. Mulrooney-Cousins, P. M., and T. I. Michalak. 2007. Persistent occult hepatitis B virus infection: experimental findings and clinical implications. *World J Gastroenterol* 13:5682-6.

Project 2. The Human Hepatitis B Virus Receptor

Co-Supervised by Dr Uwe Stroehler

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350 million individuals are chronically infected with human hepatitis B virus (HBV) and it is estimated that 1 million people die annually from acute and chronic HBV infection. The HBV vaccine offers good protection against infection but does is ineffective in individuals who are already infected. Many fundamental questions about HBV remain unanswered including the identity of the cell receptor used by HBV to infect hepatocytes. Although a number of potential candidate receptors have been proposed such as the IgA binding receptor, and proteins found on HepG2 cell lines as well as various asialoglyco protein receptors (ASGRP), none of these have been shown to be essential for HBV entry into the cell (reviewed in Glebe and Urban 2007). This project will investigate interactions between HBV and hepatocytes using a number of techniques that might include: cloning and expression of HBV genes to produce purified histidine-tagged proteins, generation of specific antiserum and studies of interactions between hepatocyte proteins and HBV either as direct protein-protein interactions, or by immuno co-precipitation. Interacting proteins can then be identified by mass spectrometry or N-terminal sequencing. The proposed studies will help to identify the receptor used by HBV and may lead to studies of novel inhibitors of HBV infection.

1. Glebe, D. and Urban, S. (2007) Viral and cellular determinants involved in hepadnaviral entry. *World J. Gastroenterol.* 13: 22-38.

Project 3. Molecular adjuvants and DNA vaccines for DHBV infection

Co-supervised by Dr Feng Feng

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Current protective vaccines for human hepatitis B infection (HBV) have no therapeutic effect when used in chronically HBV infected patients. We recently developed and tested a DNA vaccine “prime” plus recombinant fowlpoxvirus (rFPV) “boost” vaccination strategy in duck hepatitis B virus (DHBV) infected ducks. The vaccination strategy included DNA vaccines and rFPV strains that expressed both DHBV surface and core antigens. Administration of the vaccines in conjunction with antiviral treatment with the Bristol-Myers Squibb antiviral drug, entecavir (ETV), eliminated DHBV-infected hepatocytes and prevented the development of persistent DHBV infection (Miller *et al.*, 2008) when the vaccination strategy was administered from the same time as virus infection. However, therapeutic protocols have so far not achieved successful therapy for persistent and established DHBV infection.

We now propose to improve the therapeutic DHBV vaccination strategies by the addition of potential molecular adjuvants. The molecular adjuvants that are being characterised and investigated include levamisole and duck interleukin-2 (DuIL-2), duck CD40 ligand (DuCD40L), duck interleukin-6 (DuIL-6) and duck tumour necrosis factor-alpha (DuTNF-a).

The project will involve characterisation of the molecular adjuvants *in vitro* followed by *in vivo* testing of their efficacy in DHBV-infected ducks. Effects of the vaccination protocols on DHBV infection will be performed by markers of DHBV infection in serum and liver using ELISA, immunostaining and quantitative PCR assays.

1. Miller DS, Boyle D, Feng F, Reaiche GY, Kotlarski I, Colonna R, Jilbert AR (2008). Antiviral therapy with entecavir combined with post-exposure "prime-boost" vaccination eliminates duck hepatitis B virus-infected hepatocytes and prevents the development of persistent infection. *Virology* **373** (2):329-41