Development of Novel Vaccine Strategies for Duck Hepatitis B Virus Infection

A thesis submitted for the degree of

Doctor of Philosophy

as a portfolio of publications

by

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January, 2008

Abstract

Hepatitis B virus (HBV) is a life-threatening pathogen with major economic significance. Acute infection in adults is common, albeit usually self-limiting. Importantly, infection in infants typically results in chronic infection and increased incidence of hepatocellular carcinoma (HCC). Furthermore, the infectious carrier state is perpetuated in chronically infected individuals. Successful immuno-therapeutic vaccination would reduce the incidence of chronic infection and of HCC as well as reduce transmission of the disease.

Recovery from acute and chronic HBV infection typically occurs in the presence of robust antigen-specific humoral and cellular immune responses (CMI), whereas these responses are low or absent in chronically HBV-infected individuals. Therefore, it was hypothesised that effective stimulation of both humoral and CMI responses, in conjunction with currently available antiviral therapies, may contribute significantly to development of vaccines for treatment of chronic HBV infection.

The duck hepatitis B virus (DHBV) model of HBV infection was used to test novel vaccine strategies that could complement existing antiviral therapeutic approaches to treat chronically HBV-infected humans. To this end, three separate vaccine studies were conducted to investigate potential therapeutic regimes.

Methods to assess the efficacies of the vaccine strategies included immunoperoxidase detection of viral antigen and immune cell markers within the liver and development of sensitive assays to monitor levels of DHBV DNA, duck hepatitis B virus surface antigen (DHBsAg), antibodies to duck hepatitis B core (anti-DHBc) and surface antigens (anti-DHBs) in serum were developed and validated which allowed monitoring

of the kinetics of the humoral immune response following vaccination and the course and outcome of experimental DHBV infection.

The first vaccine study tested the protective efficacy of DNA vaccines encoding either the small form of DHBsAg (DHBs) protein or the larger antigen (DHBpre-S/S), These were administered to ducks at day 4 and 14 of age. On the same day as the second vaccination, ducks were challenged intravenously with DHBV. Immunoperoxidase staining of biopsy tissue collected at day 4 p.i. showed significant decreases in the number of DHBV infected hepatocytes in ducks receiving the DNA vaccines compared to the mock-vaccinated control ducks. Significant protection against development of chronic DHBV infection was observed in ducks vaccinated with DNA vaccines expressing either pre-S/S or S protein. Although anti-DHBs antibodies were not detected prior to DHBV challenge, the decrease in the percentages of DHBV-infected hepatocytes at day 4 p.i is suggestive that neutralisation of the inoculum by low-level anti-DHBs antibodies in cohort with CMI responses induced by vaccination were the most probable mechanisms of action.

The second vaccine study examined the protective efficacy of a novel whole-cell vaccine that expressed the DHBV core antigen (DHBcAg). Ducks were vaccinated on day 4 and 14 of age and DHBV challenge was administered 4 days later. Detectable anti-DHBc antibodies were generated as soon as 4 days after the initial vaccination suggesting that this regimen elicited increased immunogenicity than vaccination with DNA vaccines alone. In contrast to the first vaccine study with DNA vaccines expressing DHBsAg, no significant differences in the percentage of DHBV-infected hepatocytes were observed in biopsy tissue collected at day 4 p.i. This finding is confirmation that anti-DHBc antibodies were not neutralising to the initial DHBV

inoculum. However, significant protection against development of chronic DHBV infection was observed in the whole-cell vaccinated ducks suggesting that the mechanism of protection was consistent immune-mediated killing of DHBV-infected hepatocytes following CMI responses to determinants of DHBcAg.

The final vaccine study involved a combination strategy of antiviral drug Entecavir (ETV) and prime-boost vaccination with DNA vaccines and recombinant fowlpoxvirus (rFPV) expressing DHBV antigens. Immediately following DHBV infection, ducks were dosed by oral gavage with the antiviral drug Entecavir (ETV) and at the same time received the priming DNA vaccines encoding DHBV antigens. Seven days later the boosting vaccination consisting of recombinant fowlpox viruses (rFPV) also expressing DHBV antigens was administered. Extraordinary protection was observed, with 100% of ducks given combination therapy rapidly resolved their DHBV infection while 100% of non-treated ducks developed chronic infection. It was concluded that protection resulted from a combination of at least three factors. First, reduction and control of DHBV levels with the aid of ETV; secondly, stimulation of surface antigen-specific humoral immune responses resulting in neutralisation of newly produced virions; and finally, the combined up-regulation of CMI responses against DHBV core and surface antigens, resulting in elimination of infected hepatocytes.

The four manuscripts that comprise this thesis provide insights into the viral kinetics and immune responses that follow DHBV infection and/or vaccination of ducks. The results provide new directions for future vaccine studies aimed at developing effective treatments for chronic HBV infection.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis being made available at the University of Adelaide Library.

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Acknowledgements

First and foremost I would like to thank my supervisor Dr. Allison Jilbert for your support, guidance and encouragement to embark on a research career. You have taught me many things over the years for which I am forever grateful. My co-supervisors, Professor Christopher Burrell and Professor Ieva Kotlarski; to have you both, with Allison in the same room during lab meetings was sometimes frightening but always inspirational. I also thank Associate Professor Graham Mayrhofer for co-supervision in the later stages of my candidature and also for editing final versions of the immunology sections of the thesis.

I also wish to thank our collaborators who helped to make this work possible. Dr. Michael Halpern for innovative ideas about the whole cell vaccines, Dr. Richard Colonno and Bristol-Myers Squib for generously providing the Entecavir, Dr. David Boyle and Dr. Barbara Coupar for the derivation of recombinant fowl pox viruses and Dr. Bill Mason for critical review of the manuscripts.

Whilst conducting these studies I was supported by a Royal Adelaide Hospital Dawes Scholarship for funding for which I am grateful. I also thank the School of Molecular and Biomedical Science and the Institute of Medical and Veterinary Science (IMVS) for other resources relating to my project.

To all past and present members of the Hepatitis Laboratory and the Discipline of Microbiology and Immunology who made research both interesting and fun, thank you. In particular, I thank Ed Bertram for his input with the manuscript in Chapter 2 of this thesis and Cathy Scougall for re-counting hundreds of stained slides and editing the final version of the thesis.

Finally, an extra special thanks to Georget Reaiche for understanding, love and support during the final stages of my candidature and also my daughters Jacqui and Elise for the real pleasures in life.

Publications and patent arising

Publications

I. Darren S. Miller, Edward M. Bertram, Catherine A. Scougall, Ieva Kotlarski, and Allison R. Jilbert. Studying host immune responses against duck hepatitis B virus infection. *Methods Mol Med* 2004 96:3-26.

II. Darren S. Miller, Ieva Kotlarski and Allison R. Jilbert. Vaccination of ducks with a whole-cell vaccine expressing duck hepatitis B virus core antigen elicits antiviral immune responses that enable rapid resolution of *de novo* infection. *Virology* 2006 348:297-308.

III. Darren S. Miller, Michael Halpern, Ieva Kotlarski and Allison R. Jilbert. DNA vaccines expressing the duck hepatitis B virus surface proteins lead to reduced numbers of infected hepatocytes and protect ducks against the development of chronic infection in a virus dose-dependent manner. *Virology* 2006 351:159-69.

IV. Darren S. Miller, David Boyle, Feng Feng, Georget Y. Reaiche, Ieva Kotlarski, Richard Colonno and Allison R. Jilbert. 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*, 2008 373:329-341.

Patent

I. Miller D. S., Kotlarski I., Burrell C. J., and Jilbert A. R. **"Combination Treatment". *PCT/AU2006/000828***

Conference presentations and abstracts arising

Miller D. S., Kotlarski I., Jilbert A. R. Development of a novel whole-cell DNA vaccine for duck hepatitis B virus (DHBV) infection. 2004 Australian Centre for Hepatitis Virology & HIV Virology, National Scientific Workshop, Barossa Valley 25th-27th June, 2004

Miller D. S., Kotlarski I., Jilbert A. R. Development of a novel whole-cell-DNA vaccine for duck hepatitis B virus (DHBV) infection: The Molecular Biology of Hepatitis B Viruses, Marine Biological Laboratory Woods Hole, Massachusetts USA. 24-27th October, 2004.

Miller D. S., Kotlarski I., Jilbert A. R. Development of a novel whole-cell-DNA vaccine for duck hepatitis B virus (DHBV) infection. Australian Society of Immunology: Student Meeting, Adelaide, South Australia. October, 2004.[*1]

Miller D. S., Kotlarski I., Jilbert A. R. Development of a novel whole-cell-DNA vaccine for duck hepatitis B virus (DHBV) infection. The Australian Society for Medical Research, South Australian Annual Meeting. June, 2005.[*2]

Miller D. S., Kotlarski I., Colonno R., Boyle D., Jilbert A. R. Entecavir and prime-boost vaccination strategies for hepatitis B virus infection. The Molecular Biology of Hepatitis B Viruses. University of Heidelberg, Germany. September, 2005.

Miller D. S., Kotlarski I., Colonno R., Boyle D., Jilbert A. R. Entecavir and prime-boost vaccination strategies for hepatitis B virus infection. Poster presentation. 3rd Australian Virology Group Meeting, Phillip Island, Australia. December, 2005.[*3]

Miller D. S., Kotlarski I., Colonno R., Boyle D., Jilbert A. R. Entecavir and prime-boost vaccination strategies for hepatitis B virus infection. Australian Society of Immunology, Student meeting. Adelaide, December, 2005.[*4]

Miller D. S., Kotlarski I., Colonno R., Boyle D., Jilbert A. R Entecavir and prime-boost vaccination strategies for hepatitis B virus infection. 1st Australian Vaccines & Immunotherapeutics Development, Melbourne, Australia. May, 2006.

Awards arising

*[1] Best Oral Presentation. Australian Society of Immunology: Student Meeting, Adelaide, South Australia. October, 2004

*[2] Best Oral Presentation. Student Biotechnology Award. The Australian Society for Medical Research, South Australian Meeting. June, 2005.

*[3] Best Poster Presentation. 3rd Australian Virology Group Meeting, Phillip Island, Australia. December, 2005.

*[4] Runner-up, Best Student Oral Presentation. Australian Society of Immunology, Student meeting. December, 2005.