Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.
Hepatitis B Virus:
A Longitudinal Study

A thesis presented in partial fulfilment of the requirements for the degree of
Masters of Science
At
Massey University,
Palmerston North, New Zealand

Katherine Graciosa Blake-Palmer

2002
Abstract

Hepatitis B Virus (HBV) is a member of the hepadnavirus family. Viruses from this family infect primate, rodent and avian species. Wild type HBV virions consist of partially double-stranded circular DNA which is converted into covalently closed circular molecules in nuclei upon infection into host cells. The HBV genome is about 3.2kb in size and consists of four transcripts encoding the surface, core, polymerase and X proteins, in overlapping reading frames. HBV infection causes a variety of liver diseases in humans, for example, liver cirrhosis and hepatocellular carcinoma. Clinical manifestations range from asymptomatic to acute. The outcome of acute hepatitis B infection may be influenced by host factors some of which are controlled by the Major Histocompatibility Complex (MHC). In humans the MHC is known as the Human Leukocyte Antigen (HLA) region. Accordingly, the individuals involved in this study were HLA typed.

The aim of this study is to investigate HBV DNA differences in three different clinical types of hepatitis B disease over a 15 year period, and to determine if there is a correlation between specific HBV variants and particular clinical states. In 1985, 93% of the population of Kawerau (7,901) was tested for HBV, those found to be positive (519) have been monitored ever since. In 1998, individuals that fitted our requirements were invited to participate in our study. HBV DNA was extracted from blood samples and complete genomes sequenced, over 120,000 nucleotides were sequenced. Differences in HBV genomes sequenced between clinical types and HBV genotypes were compared. HLA alleles between the different clinical types were compared, as well as comparing HBV infected individuals with the general New Zealand population. The overall project is a major one and the results of this thesis get it well underway.
Acknowledgements

I would like to thank Dr Chris Moyes of the Whakatane Child Health and Hepatitis Foundation for providing the hepatitis B virus samples.

Thank you David Penny for your supervision and much-needed assistance in writing this thesis.

Abby Harrison – thanks so much for your continued support throughout these last two years!

Thanks also to Trish McLenachan for all your help in the lab.

Thanks also to Rissa Otta for your help with the statistical analysis.

A big thanks to all my family and friends for your support outside of the lab – everybody needs to escape now and then!
# Contents

Abstract ii  
Acknowledgements iii  
Table of Contents iv  
List of Figures vii  
List of Tables viii

## Chapter 1 Introduction

1.1 General Introduction to hepatitis B virus 1  
1.1.1 Virus lifecycle 2  
1.1.2 Viral Infection 5  
1.1.3 Ways to combat virus 7  
1.2 Introduction to the Human Leukocyte Antigen Complex 8  
1.2.1 Function of the immune system 8  
1.2.2 HLA and HBV 9  
1.2.3 Linkage disequilibrium 10  
1.2.4 HLA and the Pacific 11  
1.3 Aim of thesis 13

## Chapter 2 Characteristics of Viral Genome

2.1 Precore/Core gene 14  
2.2 X gene region 20  
2.3 Polymerase gene 22  
2.4 Surface gene 24  
2.4.1 Serotypes and Genotypes 25  
2.4.2 S protein 28  
2.4.3 M protein 30  
2.4.4 L protein 30  
2.5 Quasispecies Distribution 32
Chapter 3 Materials and Methods

3.1 DNA extraction 33
3.2 PCR amplification 34
3.3 Primer design 35
3.4 Confirmation of PCR product 36
3.5 PCR template purification 37
   3.5.1 Shrimp Alkaline Phosphatase /Exonuclease I digest 37
   3.5.2 Rapid gel extraction 37
3.6 DNA quantification 37
3.7 Cloning 38
   3.7.1 Ligation 38
   3.7.2 Transformation 38
   3.7.3 Recombinant DNA extraction 39
3.8 Sequencing 40
   3.8.1 Precipitation 40
3.9 Sequence analysis 41
3.10 HLA typing 41

Chapter 4 Results and Discussion

4.1 Genome sequences 42
   4.1.1 HBV sequencing results 42
   4.1.2 X gene 49
   4.1.3 Precore/Core gene 51
   4.1.4 Surface gene 56
   4.1.5 Polymerase gene 60
   4.1.6 1985 and 1998 comparisons 63
4.2 HLA results 65
   4.2.1 Kawerau HLA samples 65
   4.2.2 Comparing Kawerau samples with other NZ populations 70
Chapter 5 Summary and Conclusions

5.1 Summary of HBV sequencing results 77
5.2 HLA results summary 79
5.3 Conclusions 80

Bibliography 81

Appendices (see CD for appendices)

   Appendix A
   Appendix B
List of Figures

Chapter 1 Introduction
1.1 Hepatitis B virus genome 1
1.2 Virus lifecycle 4

Chapter 2 Breakdown of Genes
2.1 Mis-sense mutations 18
2.2 S protein 29
2.3 M protein 30
2.4 L protein 31

Chapter 4 Results and Discussion
4.1 Gel photos of Polymerase gene 45
4.2 Schematic of Surface gene 56
List of Tables

Chapter 1 Introduction
1.1 Hepatitis B transcripts 3
1.2 NZ ethnicity 11

Chapter 2 Breakdown of the Genes
2.1 The functions of the X-protein 21
2.2 Geographical distribution of HBV genotypes 27
2.3 Amino acid residues specifying determinants of HBsAg 27

Chapter 3 Material and Methods
3.1 Primers 36

Chapter 4 Results and Discussion
4.1 Clinical groups in the 1998 samples, by viral genotype 43
4.2 Summary of sequencing results for the 1998 samples, ++ clinical type 43
4.3 Cloned regions from 1998 samples 44
4.4 Gene regions that were amplified of the 1985 samples 46
4.5 Summary of sequencing results for the 1985 samples 47
4.6 Samples which were sequenced over 70% 48
4.7 The number of samples with mutation(s) for the X-gene region 49
4.8 The number of samples with mutations in the function areas of the PreC/C gene region 52
4.9 The samples with mutations in the functional areas of the Surface gene region 58
4.10 Overall single nucleotide mutation rate for the different Polymerase domains 61
4.11 Mutation frequencies for each domain in the Polymerase region 62
4.12 Number of samples with single nucleotide mutations in the different P gene domains 62
4.13 The number of single base-pair differences and indels observed
between 1998 and 1985 transcripts of the same sample

4.14 Allele family frequency for each HLA gene

4.15 HLA allele family frequencies for each of the clinical states of HBV infection

4.16 Genotype frequencies for each of the clinical states

4.17 Chi square results for the haplotype data

4.18 Table converting the serological names for HLA alleles to SBT equivalents

4.19 HLA-A allele frequencies for New Zealand populations

4.20 HLA-B allele frequencies for New Zealand populations

4.21 HLA-C allele frequencies for New Zealand populations

4.22 HLA-DRB1 allele frequencies for New Zealand populations

4.23 HLA-DQB1 allele frequencies for New Zealand populations

4.24 Haplotype frequency for NZ Maori and NZ European and Kawerau samples

Appendix A

A.1 Mutations in the X gene region

A.2 Mutations in the Precore/Core gene region

A.3 Mutations in the S gene region

Appendix B

B.1 Haplotype frequencies of HLA-A and HLA-B alleles for ++ clinical types

B.2 Haplotype frequencies of HLA-A and HLA-B alleles for +- clinical types

B.3 Haplotype frequencies of HLA-A and HLA-B alleles for -- clinical types

B.4 Haplotype frequencies of HLA-A and HLA-B alleles for all clinical types

B.5 Haplotype frequencies of HLA-A and HLA-C alleles for ++ clinical types

B.6 Haplotype frequencies of HLA-A and HLA-C alleles for +- clinical types

B.7 Haplotype frequencies of HLA-A and HLA-C alleles for -- clinical types

B.8 Haplotype frequencies of HLA-A and HLA-C alleles for all clinical types

B.9 Haplotype frequencies of HLA-B and HLA-C alleles for ++ clinical types

B.10 Haplotype frequencies of HLA-B and HLA-C alleles for +- clinical types
B.11 Haplotype frequencies of HLA-B and HLA-C alleles for -- clinical types
B.12 Haplotype frequencies of HLA-B and HLA-C alleles for all clinical types
B.13 Haplotype frequencies of HLA-DRB1 and HLA-DRQ1 alleles for ++ clinical types
B.14 Haplotype frequencies of HLA-DRB1 and HLA-DRQ1 alleles for +- clinical types
B.15 Haplotype frequencies of HLA-DRB1 and HLA-DRQ1 alleles for -- clinical types
B.16 Haplotype frequencies of HLA-DRB1 and HLA-DRQ1 alleles for all clinical types