Hepatitis B Virus infection in the South Pacific

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This thesis is dedicated to the scent of Sunshine.

Abstract

An exploratory study into the genetics of the hepatitis B virus and its human host in the South Pacific was undertaken to determine direction for future research. This virus is a serious health concern, especially for the indigenous people of this region. The DNA sequence of 14 complete and 2 partial virus genomes were obtained. The viral sequence mutations were investigated and compared with a collected database and current literature. Phylogenetic analysis of the viral sequences was carried out using version 4.64 of PAUP* and SplitsTree. Using the new sensitive method of sequence based typing, HLA-DPA1 allele's were typed in 51 unrelated Trobriand Islanders.

The viral genomes displayed a great deal of variation with many interesting mutations. The results highlight the affect of quasi-species distribution within a host. Phylogenetic analysis identified two hepatitis B genotypes within the South Pacific, HBV-**C** and HBV-**D**. However, the genotype common in northern Europe (HBV-**A**) was not found. The phylogenetic analysis presented a pattern of evolution that resembled that of its human host. The Trobriand Islanders were found to be an extremely homogeneous population, with 86% homogenous for the HLA-DPA1*02022 allele.

The study proved to be very informative, providing the directions of research we aimed for. The Hepatitis B samples demonstrated an interesting pattern of evolution that parallels that of its host supporting a co-evolutionary relationship between host and pathogen, thus hepatitis B appears to be indigenous in the South Pacific. We are presently establishing research to further investigate this pattern by analysing viral samples from Fiji. We have also established research that will investigate the rate of evolution of this virus. The sequenced based typing method proved to be very informative with the ability to detect new alleles. The allele frequency obtained from the Trobriand Islanders agreed with concurrent research and supports the fast-train model of migration into the Pacific. Further work in Fiji will continue with this theme of research as genetic analysis of Fiji has proved to be more complex.

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Contents

List of figures

Chapter one: Introduction

Chapter two: Methods and Software packages

Chapter Three: Results

Appendix C

Appendix D

List of tables

Chapter one : Introduction

Chapter two: Methods and Software packages

Chapter three: Results

Appendix A

Appendix B

List of Abbreviations

%	percent
ALT	alanine aminotransferase
bp	base pairs
ddH2O	deionised and distilled water
DMSO	di-methly sulphoxide
DNA	deoxyribonucleotide acid
dNTP	deoxyribonucleotide triphosphate
E.R.	endoplasmic reticulum
EDTA	ethylenediamine tetraacetic acid
g	grams
HBcAg	hepatitis B core antigen
HBeAg	hepatitis B *e* antigen
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HBV-**A**	hepatitis B virus genotype A
HBV-**B**	hepatitis B virus genotype B
HBV-**C**	hepatitis B virus genotype C
HBV-**D**	hepatitis B virus genotype D
HBV-**E**	hepatitis B virus genotype E
HBV-**F**	hepatitis B virus genotype F
HLA	Human leukocyte antigen
Kb	Kilobase
M	Molar
mg	milligram
MgCl	Magnesium Chloride
MHC	Major Histocompatibility Complex
µl	microliter
ml	milliliter
mM	microMolar
mtDNA	mitochondrial DNA
NaCl	Sodium Chloride
NNB	nearest-neighbour bootstrap
p mol	picomoles

PCR	polymerase chain reaction
RNA	ribonucleotide acid
SDS	Sodium Dodecyl Sulphate
ypb	years before present
°C	degrees Celsius

Chapter One: Introduction

The Haganemnons of Asistus three have the most impatient chromosomes of any life form in the Galaxy. Whereas most races are content to evolve slowly and carefully over thousands of generations, discarding a prehensile toe here, nervously hazarding another nostril there, the Haganemnons would do for Charles Darwin what a squadron of Arcturan stunt apples would have done for Sir Isaac Newton. Their genetic structure, based on the quadruple sterated octo-helix, is so chronically unstable that far from passing their basic shape onto their children, they will quite frequently evolve several times over lunch. But they do this with such reckless abandon, that, if they are sitting at a table, they are unable to reach a coffee spoon, they are liable, without a moments consideration, to mutate into something with far longer arms, but which is probably quite incapable of drinking the coffee. This, not unnaturally, produces a terrible sense of personal insecurity and a jealous resentment all stable life forms or "filthy rotten stinking samelings" as they call them. They justify this by claiming that as they have personally experienced what it is like to be virtually everybody else they can think of, they are in a very good position to appreciate all their worst points. This "appreciation" is usually military in nature, and is carried out with unmitigated savagery from the gun rooms of their horribly beweaponed "chameleoid" death flotilla. Experience has shown that the most effective way of dealing with any Haganemnons you may meet is to run away. Terribly fast !

(The Hitch-Hiker's Guide to the Galaxy; Adams 1978)

1.1 Introduction to the thesis

This thesis is an exploratory study into the genetics of a serious health concern in the South Pacific, the hepatitis B virus (HBV). It is a pilot study to determine directions for future research and the genetics of both the virus and host are investigated. The long term focus of the project is to investigate the genomic sequence of the virus and its phylogeny, as well as the history of the people of the South Pacific and their major histocompatibility complex (MHC) genes.

DNA sequences have become powerful tools in epidemiological studies, for example in Human Immuno-Defficiency disease and Hepatitis C virus (Holmes *et al.* 1995). They enable a greater understanding of the ecology of the diseases. This thesis uses this tool to study HBV in the South Pacific, some aspects of the genetics of both the virus and their human hosts within the Pacific will be investigated. Complete viral genomes from infected Polynesians will be sequenced and analysed, and a start will be made on human leukocyte antigen (HLA) typing, using a new sequence based method.

HBV is the smallest-double stranded DNA virus known to infect humans; it contains multiple overlapping reading frames and replicates through an RNA intermediate by reverse transcriptase. The lack of proof-reading ability of this reverse transcriptase accounts for the high mutational rate of this virus however, the overlapping reading frames place constraints on viable replicants, which restricts the viral evolution. Combined, these factors give HBV a unique mode of evolution which, along with the viral/host evolutionary relationship, remains unresolved (Miller and Robinson 1986; Gojobori *et al.* 1990; Bollyky and Holmes 1998). Co-evolution, cross-species, and recent transfer hypotheses have been suggested but current more sophisticated studies are inconclusive (Mandart *et al.* 1984; Orito *et al.* 1989; Norder *et al.* 1996; Bollyky and Holmes 1998). Clarification of these relationships is of interest, for not only is the molecular biology of the hepatitis B virus unique, but also as it is the major cause of hepatocyte carcinoma world wide, it is a serious health concern.

Infection with HBV can result in a range of clinical states ranging from asymptomatic to fatal. The differences appear to be related to genetics of both the host immune system and the virus (Chisari and Ferrari 1995; Milne *et al.* 1995; Gust 1996). It is estimated that 75% of the world's estimated carriers are within the Western Pacific and South East Asia. Here, where the virus is hyper-endemic (that is, there is a very high proportion of carriers in the population see 1.2.3), patterns of infection vary considerably between villages, cities, countries and ethnic groups (Gust 1996). This observation has relevance when it is noted that archaeological, linguistic and biological studies have shown human migrations through the Near and Far Oceania to be of two separate lineages (Hill and Serjeantson 1989; Kirch 1997; Spriggs 1997). To date little research has been undertaken to clarify the level of genetic admixture between these populations — a relevant issue when considering apparent mode of disease transmission and clearance.