

Hepatitis B virus genotypes: a South Pacific perspective

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Abstract

87- 91% but still, 0.6% of those that did respond to vaccination became infected. The infection rate of the vaccinated populations in the Pacific Islands ranged between 0.7 and 3.8%, which is comparable to Taiwan.

A vigorous polyclonal response This communication discusses the current status of research in the hepatitis B virus in relation to the South Pacific. The hepatitis B virus (HBV) is a small DNA virus - 3200 nucleotides. It has a circular genome and replicates through an RNA intermediate giving this DNA virus many characteristics similar to RNA viruses. Viral genomes can be single-stranded (+ or - sense) or double-stranded.

If not vaccinated, infants born to HBeAg positive mothers (i.e. with high viral titer) have a 90% chance of being infected and becoming HBV carriers themselves. Mutants that affect the major antigenic determinant in HBV surface antigens are probably responsible for HBV infection despite immunization and mutants in the polymerase protein may render HBV resistant to therapy with nucleoside analogs. Within HBV seven genotypes A-G have been reported that is, HBV genotype A (HBV_A), HBV genotype B (HBV_B) etc. HBV is endemic worldwide with an estimated that 5% of the worlds population being carriers.

An associated problem with HBV, in the South Pacific, is the hepatitis delta virus (HDV). HDV is a satellite viroid-like RNA virus that requires HBV for replication.

Before the introduction of vaccination programs carrier rates varied between 5 -30% in communities of these ethnic groups, and in some cases 80-90% of a community tested positive for HBV markers (i.e. were infected or had been infected). In Taiwan, of vaccinated babies born to HBV positive mothers, the proportion of those that responded to vaccination varied between will usually result in an acute infection and viral clearance. An associated problem with HBV, in the South Pacific, is the hepatitis delta virus (HDV). HDV is a satellite viroid-like RNA virus that requires HBV for replication. It can either co-infect with, or super-infect upon HBV infection resulting in acute infection and/or chronic infection respectively.

Introduction

Since the discovery in 1965 of the hepatitis B virus surface antigen, - the "Australian Antigen", the virus has been extensively researched. A great deal is known about its biology. It can be detected by serological tests, treated by drugs, and controlled through vaccination. Despite this, this superbly well-adapted parasite still persists. This communication discusses the current status of research in the hepatitis B virus in relation to the South Pacific. It considers the viral biology, as well as the different genotypes and their geographic distribution around the world. Finally it suggests direction for future research.

The hepatitis B virus (HBV) is a small DNA virus - 3200 nucleotides. It has a circular genome and replicates through RNA intermediate giving this DNA virus many

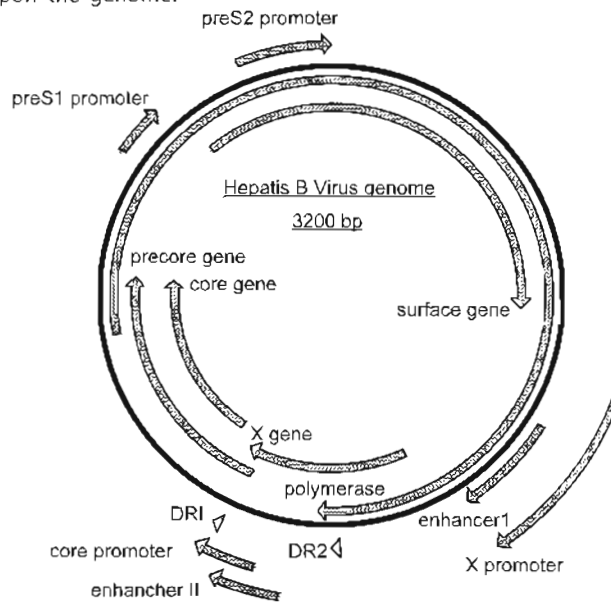
characteristics similar to RNA viruses. RNA viruses come in many shapes and sizes from 1300 nucleotides (e.g. hepatitis delta virus) to 15 000 nucleotides (e.g. measles), they may be singular (filamentous or circular), or segmented genomes (e.g. influenza A & B have 8 segments). Viral genomes can be single-stranded (+ or - sense) or double-stranded.

All of these viruses have a great potential to mutate, including HBV, creating quasi-species of related viral particles in an infected host. However, due to the compact nature of the HBV genome with up to three overlap-

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Fig. 1. Hepatitis B virus genome.

Illustrating overlapping gene products and sequences regulating gene expression. This results in complex mutational constraints upon the genome.



ping reading frames only the fit - biologically functional viruses - are infectious. See Figure 1 for a map of the hepatitis B virus genome.

Overlapping reading frames are common; they are found in over half of all viral genera and are superb adaptations for conserving genome size. The beauty of HBV (from the virus's point of view) is that it has the best of both RNA and DNA worlds. The infectious agent contains a partially double-stranded DNA genome, which is very stable. This stability increases viral infectivity, for example, the hepatitis B virus withstands prolonged desiccation in dust and is in consequence very much more infectious than HIV or HCV. So not only does this virus have a great potential to mutate whilst maintaining its genetic identity but also, it is highly infectious ¹.

Since the early 1970's molecular studies have searched for mutants and variants associated with the different clinical outcomes of the HBV. The most notable of these is a mutation at position 1896 of the virus genome creating a stop codon in the precore transcript. This transcript codes for the HBV e antigen (HBeAg), which is a serological marker for viral replication. A mutation at this position stops HBeAg expression and usually correlates with a drop in viral titer within the host. If not vaccinated, infants born to HBeAg positive mothers (i.e. with high viral titer) have a 90% chance of being infected

and becoming HBV carriers themselves. In contrast infants born to HBeAg negative mothers (i.e. with low viral titer) have only a 10% chance of becoming infected.

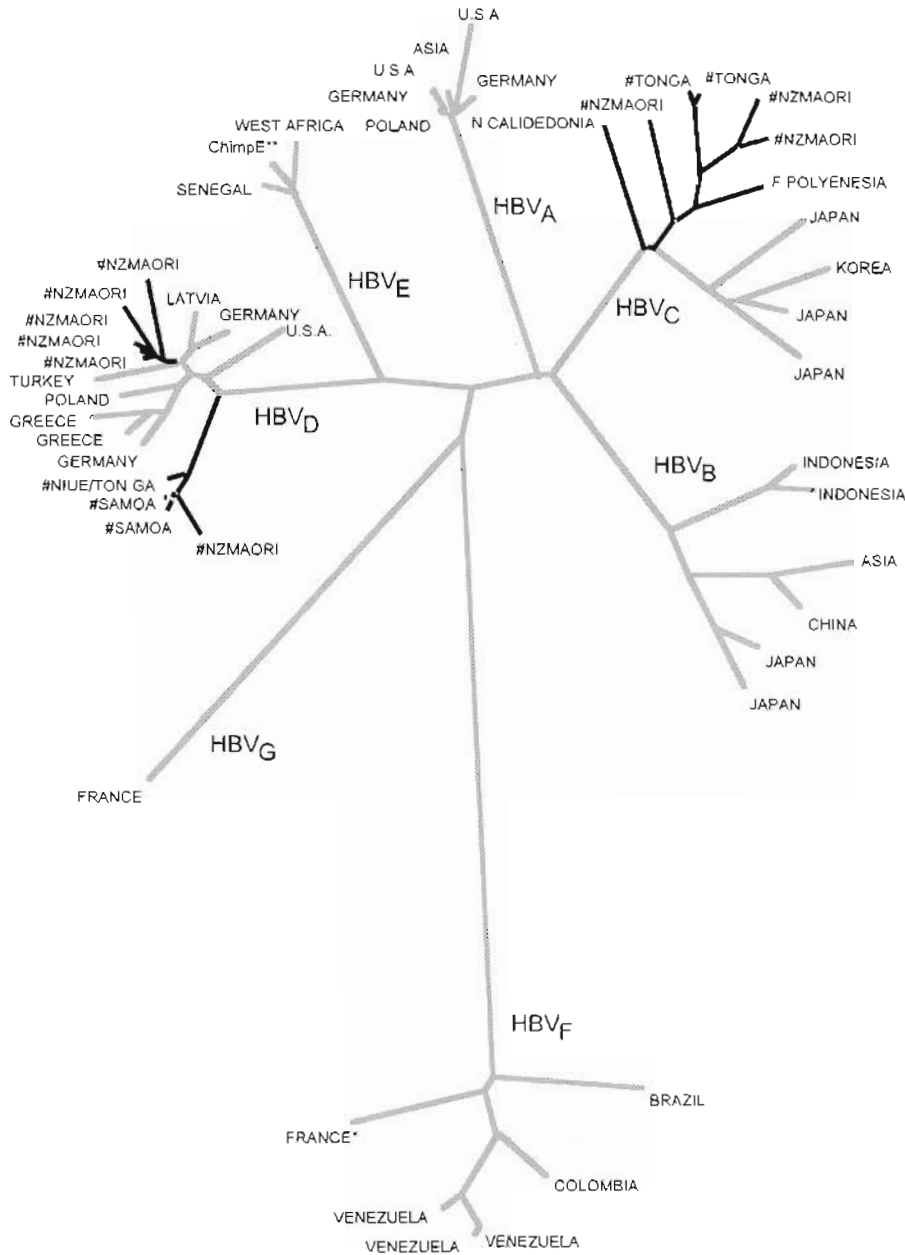
Mutants that affect the major antigenic determinant in HBV surface antigens are probably responsible for HBV infection despite immunization and mutants in the polymerase protein may render HBV resistant to therapy with nucleoside analogs.

Molecular studies have identified many other important mutations such as the vaccine escape mutants identified in Papua New Guinea, that Carmen *et al* 1997 ² described. In general though, mutations that affect HBeAg synthesis, and novel binding sites of the hepatocyte nuclear-factor 1 (in the core promoter) or large genome deletions have all been associated with severe liver disease - though not in any consistent manner. Mutants that affect the major antigenic determinant in HBV surface antigens are probably responsible for HBV infection despite immunization and mutants in the polymerase protein may render HBV resistant to therapy with nucleoside analogs. Not surprisingly, considering the nature of the virus, there has been many of these types of mutations reported ³.

HBV, though a unique human virus, belongs to the hepadnaviridae family that infects a range of primates (humans, gibbons, chimpanzees, orangutans, gorillas and woolly monkeys), rodents (woodchucks and ground squirrels) and birds (heron, duck and geese). Within HBV seven genotypes A-G have been reported that is, HBV genotype A (HBV_A), HBV genotype B (HBV_B) etc (see Figure 2).

Fig. 2. Hepatitis B virus genotypes A-G

Neighbor-joining tree demonstrating the seven HBV genotypes, highlighting the unique HBV_C Polynesian cluster as well as the Polynesian HBV_D clusters.



* This individual was presumably infected in South America.
 ** This chimp was presumably infected from humans in Africa.

The genotypes have different geographic distributions, and apparently different primary means of transmission. Genotype A and D predominate in Europe and European populations (although both are found world-wide). In European communities the primary route of transmission is horizontal, through adult populations. Genotypes B and C are found in South East Asia and the Pacific, in these peoples vertical transmission (i.e. perinatal) or early child-

hood transmission predominates. Prior to immunization, up to 80% of children in these areas tested positive for HBV markers. Genotype E is localized to Africa, which is also an area of endemic infection where most individuals are infected at birth or in childhood. Genotype F accounts for about 70% of infections in South America and has been suggested to occur in French Polynesia. Genotype G, only recently detected, appears to be European in origin.

Table 1. Hepatitis B vaccination statistics for Pacific Islands**

Country	Year	% Vaccinated	% Vaccine responders	% Vaccinated 3 doses	Infected -2 doses	% of study infected
Fiji	1998	98	79	-	-	0.7
Vanuatu	1998	75	79	-	-	3.0
Kiribati	1998	89	49	-	-	3.8
Tonga	1998	93	62	-	-	3.8
<i>Study total *</i>	<i>1998</i>	-	-	<i>0.41</i>	<i>0.06-2.89</i>	-
America Samoa**	1991	82	86	-	-	2.0
America Samoa **	1995	87	95	-	-	3.0
CNMI	1995	94	-	-	-	2.5
FSM	1994	82	-	2.6	-	5.0
Chuuk	1992	40 ***	-	-	9	11.0
Palau	1995	-	93	7.3	-	-
Taiwan	1990	83	87	0.6	-	2.4
xx	<i>Vaccination statistics for Pacific Island populations, including Chinese, Melanesian, Polynesian and Micronesian communities</i>					
*	<i>These values were taken from the total population sampled in the study of Wilson N. et al 2000⁵.</i>					
**	<i>Two studies were undertaken, one in 1991 and the other in 1995, both are reported here.</i>					
***	<i>This value appears to be an estimate as not all records were found.</i>					
-	<i>Data not available.</i>					

Transmission data for genotypes F and G are currently unavailable⁴.

We have recently sequenced the complete genomes of 14 hepatitis B viruses from patients of Polynesian descent. Our results found the presence of both C and D genotypes. The most striking result of our research is that all our HBV_C samples from New Zealand (including people of Tongan and Samoan descent) form one clade (or cluster), that is unique to the South Pacific. See *Figure two*. This clade is distinct, though closely related to the HBV_C genotypes of South East Asia. Combined with the knowledge that hepatitis B in the Pacific is largely transmitted in childhood causing a latent infection these results is consistent with HBV being indigenous to the region. We have also found several HBV_D genotypes, which cluster into two sub-groups. One subgroup is distinct, but the other is not and it could be a recent introduction, though from where is uncertain.

HBV is endemic worldwide with an estimated that 5% of the worlds population being carriers. It is of concern that at least 77% of these carriers are in South East Asia and the South Pacific (WHO 1983). Melanesian, Micronesian, Polynesian and Taiwanese populations share similar viral disease characteristics and statistics. Before the introduction of vaccination programs carrier rates varied between 5 -30% in communities of these ethnic groups, and in some cases 80-90% of a community tested positive for HBV markers (i.e. were infected or had been infected). Interestingly although overall carrier rates are usually similar within each ethnic group in a region, at a village level there is great variation between communities. Rates vary much more between ethnic groups; for example,

Melanesian Fijians demonstrated a carrier rate of 18%, which contrasted to Indian Fijians who demonstrated a carrier rate of 1.8%. Similar statistics have been shown between New Zealand Maori and New Zealand Europeans as well as Australian Aborigines and Australian Europeans. Not only do the indigenous peoples of this region share similar patterns of carrier rates but they also appear to respond similarly to vaccination.

In Taiwan, of vaccinated babies born to HBV positive mothers, the proportion of those that responded to vaccination varied between 87- 91% but still, 0.6% of those that did respond to vaccination became infected. Moreover, a carrier rate of 2.4% was estimated for a vaccinated Taiwan population (based on those that did not respond to vaccine and those that responded yet still became infected)⁵ See *table one*. In a recent study of Fiji, Kiribati, Tonga and Vanuatu 48-79 % of those vaccinated responded to the vaccine and of the infants receiving complete vaccination (3 doses) 0.41% became infected, See *Table 1*. In addition it was found that under the current immunization programs 33% of babies born to HBeAg positive mothers were NOT being protected against HBV. These infants were not vaccinated, did not receive the recommended three doses, or failed to respond to the vaccine. This is an issue that needs to be addressed⁶. The infection rate of the vaccinated populations in the Pacific Islands ranged between 0.7 and 3.8%, which is comparable to Taiwan.

Studies in America Samoa, Chuuk State, Republic of Palau, Pohnpei (Federated States of Micronesia) and Commonwealth of Northern Mariana Islands (CNMI) showed that in America Samoa and Palau 94% responded to

vaccine. However, in Palua 7.3% of fully vaccinated infants still became infected. This study also found that in Chuuk, for example, 11% of those who only received two doses of vaccine became infected. 25% of those that received only one dose became infected and 60% of unvaccinated children became infected⁷. Clearly the unvaccinated, the non-responders to vaccine, and a small proportion of vaccine responders, are still at risk of HBV infection.

It is well recognized that the difference between acute and chronic infection is dependant on the host immune response. A vigorous polyclonal response will usually result in an acute infection and viral clearance. In contrast a weak monoclonal response may lead to chronic infection. This has interesting implications, clearly infant infection has been a strong component of the high carrier rate in the Pacific region but it may have been further compounded by genetics of the local populations' immune system (e.g. lack of alleles that help clear the virus).

An associated problem with HBV, in the South Pacific, is the hepatitis delta virus (HDV). HDV is a satellite viroid-like RNA virus that requires HBV for replication. It can either co-infect with, or super-infect upon HBV infection resulting in acute infection and/or chronic infection respectively. HDV infection is very serious, it is not easily treated, chronic infections are rarely cleared and HDV infection increases the chances of both fulminant hepatitis and morbidity dramatically. Because of the HBV burden in these regions and the frequent travel between islands, HDV infection is a real concern. It is recognised that there is a strong interplay between the two viruses and the host immune genes; both viruses are capable of rapid mutation⁸.

Despite the great wealth of knowledge about the biology of this well adapted parasite, as outlined in this communication, there are clearly issues with HBV that still need to be addressed (particularly in the South Pacific). In future research there needs to be a DUAL focus on the genetic variations of the viruses and the host immune systems (human leukocyte antigens) associated with differences of transmission and chronicity. This focus should also be applied to immunization, as not all vaccinated infants are being protected. Also, differences in vaccination practices need to be investigated as different programs show different levels of failure. HBV, with its RNA intermediate phase, has a great potential to mutate and viral mutations are capable of escaping the current vaccines and immunoassays used for blood screening. This also requires research. Though great strides in research have been achieved with HBV it is still a signifi-

cant problem for many of the worlds populations, so that research into HBV has the potential to improve the health of many millions.

References

1. Evans S and Kaslow R.A. (1997). *Viral Infections of Humans: Epidemiology and Control*. Plenum Medical Book company. New York and London.
2. Carmen W.F., van Deursen F.J., Mimms L.T., et al (1997). The prevalence of surface antigen variants of hepatitis B virus in Papua New Guinea, South Africa and Sardinia. *Hepatology*. 26: 1658-1666.
3. Grethe S., Monazahani M., Bohme I., and Thomssen R. (1998). Characterization of unusual escape variants of hepatitis B virus isolated from a hepatitis B surface antigen-negative subject. *Journal of Virology*. 72(9) 7692-7696.
4. Stuyver L, Gendt S.D., Van Geyt C., et al (2000). A new genotype of Hepatitis B virus: complete genome and phylogenetic relatedness. *Journal of General Virology*. 81: 67-74.
5. Sung J.L. and the Asian Regional Study Group. (1990). Hepatitis B virus eradication strategy for Asia. *Vaccine*. 8 suppl. S95-S99.
6. Wilson N., Ruff T.A., Rana B.J., et al. (2000). The effectiveness of the infant hepatitis b virus immunisation program in Fiji, Kiribati, Tongas, and Vanuatu. *Vaccine*. 18: 3059-3066.
7. Mahoney F.J., Woodruff B., Auerbach S., et al. (1996). Progress on the elimination of hepatitis B virus transmission in Micronesia and American Samoa. *Pacific Health Dialog*. 3. (2) 140-146
8. Hourieux C., Sureau C., Poisson F., et al. (1998). Interaction between hepatitis delta virus-encoded proteins and hepatitis B virus envelope protein domains. *Journal of General Virology*. 79: 1115-1119.

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