S gene variation of HBV

W. F. Carman

Division of Virology, Institute of Biomedical and Life Sciences, University of Glasgow, UK.

Genotypes and subtypes

Based on an 8% nucleotide divergence (Okamoto *et al.* 1988 ; Norder *et al.* 1992a, 1992b), there are 6 genotypes of HBV, which tend to be distributed geographically. Four fundamental antigenic subtypes and 5 other sub-subtypes are antigenically defined, based on amino acid substitutions in the S protein. Residues 122 and 160 are critical, but variants such as at aalS9 and 126/127 allow full antigenicity (Norder *et al.* 1992a, 1992b). Genotypes and subtypes probably evolved in ethnic backgrounds over censures ; the selection pressure is unknown. The clinical importance (see below) of a variant of HBsAg may be different depending upon the subtype backbone. The genotypes do not appear to have any replication advantage over each other or different pathogenic potential, although there are hints that subtype adr may lead more often to long term carriage (Shiina *et al.* 1991) and genotype D may be associated with fulminant hepatitis more commonly than predicted from its prevalence in chronic carriers (M. Yasmin PhD thesis, 1997).

The biological significance of S variants of HBV

The S gene

The envelope proteins of HBV are three : large, middle and small. The small protein is also known as HBsAg. There is a region between aa 100 to 160 termed the major hydrophilic region (MHR), to which most of the anti-HBs stimulated by vaccine binds. Variants of this region have been well described in three clinical situations, described below.

Vaccine related escape variants of HBsAg

In studies of vaccinated children born to HBeAg positive mothers, about 15% become anti-HBc positive (evidence of infection, past or present). A proportion of these will be HBsAg positive ie they are carriers. About a half of those are infected with variants of the MHR, the most consistently observed being G145R (glycine to arginine at aa145 of HBsAg ; Carman *et al.* 1990). The mothers often have 145G, but a significant proportion have a minority species of R145. The four population-based studies from Singapore (Oon *et al.* 1995), Indonesia, the UK (Ngui *et al.* 1997) and the USA (Nainan *et al.* 1997) have had remarkably similar results.

Overall, around 15% of infants become infected and, of those, between 10 and 40% have S variants. There are also a number of individual case reports describing variants in vaccinated infants, mostly from the Far East (Okamoto *et al.* 1992a ; Fujii *et al.* 1992 ; Hino *et al.* 1995). The USA study showed, overall, that 0.8% % of vaccinated children born to HBeAg positive mothers were infected with 145R ; the other 4% with breakthrough were infected with 145G. Other variants also appear but, unlike 145R, often rapidly revert to the strain seen in the mother, indicating that 145R is not only replication competent, as shown in chimpanzees (Ogata *et al.* 1997), but its altered antigenicity gives it a significant survival advantage over 145G. Vaccination at birth is an ideal situation for selection of escape variants, being similar to liver transplantation, where high titre anti-HBs preparations (HBIG ; see below) is administered to prevent graft infection (Cariani *et al.* 1995 ; Hawkins *et al.* 1996 ; Carman *et al.* 1996). So far, variants have not been seen in children exposed to carriers (family and other close contacts). Much of the anti-HBs response is directed towards the second loop of the MHR, so it is expected that variants in this region arise after such highly focused immune pressure. I am convinced that the failure of the passive/active immunisation protocol to control variant infection is due to altered antigenicity. Perhaps the emergence of this variant is the result of an imbalance between the humoral and cytotoxic T cell immune responses. In acute infection, when variants do not become dominant, there is always a strong cytotoxic T cell response. It is not known if there is any specific host factor that predisposes individuals to selection of G145R. Phylogenetic analyses (J. Wilson *et al.*, unpublished observations) indicate that R145 strains from around the world are not closely related, so they can arise from almost any background. However, selection of G145R in some strains leads to a translational stop codon in the overlapping polymerase protein.

Correspondence : W. F. Carman, 1st Left, 148 Queens Drive Queens Park, G42 8QN Glasgow, UK.

Presented at the International Symposium on Viral Hepatitis beyond the Millennium Session of December 9, 1999.

Most cases have occurred in children with a low level of anti-HBs and the chimpanzee experiments indicate that high titres protect against infection with this variant (Ogata *et al.* 1994). We do not know whether the lower levels of immunity often seen in immunocompromised and older vaccinated patients will be aufficient to prevent infection. As 145G infection does occur in vaccinees with low levels of immunity, poorly immunised people may have even greater susceptibility to HBV variants.

There are a number of crucial questions. First is the current prevalence. Naturally occurring R145 strains have been reported (Carmen *et al.* 1995a ; Yamamoto *et al.* 1994 ; Nainan *et al.* 1997). On the other hand, large screening studies in Papua New Guinea, Sardinia and South Africa (Carmen *et al.* 1997b) failed to detect it. The second is whether there will be secondary spread in unvaccinated and vaccinated subjects. Unfortunately, this cannot be determined until the current generation of infected children reach sexual maturity. However, mathematical modelling (Wilson *et al.* 1998) indicates it will become the dominant viral strain. It will take a long time to emerge because, although highly contagious, HBV is not very infectious. However this slow shift means it will also take several decades to eradicate them, even with cross-reactive vaccines, because of the chronic nature of the infection. Third, how often will it cause disease ? 145R definitely causes chronic hepatitis ; the first reported vaccinated case was HBeAg positive and had chronic hepatitis. Whether novel vaccines such as those containing pre-S epitopes will resolve the issue is hard to predict ; however, one study in Indonesia would indicate not, as the incidence of vaccine variants in the MHR was just as high, even in the absence of HBIG administration (pressure) (Surya *et al.* 1996)

HBsAg variants and diagnostic assays

There are two possibilities to explain discrepancies in HBsAg reactivity between commercial assays. First, there may be very low levels of HBsAg. Second, they have variant sequences which are not recognised by the utilised antibodies. There are some good examples of variants that are not recognised by some commercial HBsAg assays. The most dramatic are insertions in the region between cysteines at aa121 and 124 (Carmen *et al.* 1995a ; Yamamoto *et al.* 1994 ; Hou *et al.* 1995). These appear to also have antigenic effects on distant epitopes within the MHR, explained by the interleaved nature of the linear and discontinuous epitopes in this antigenically complex region. It is not known whether these insertions affect neutralisation, so they may not be vaccine-related. Studies are only beginning to address the antigenic importance and epidemiology of such variants. However, it is clear that the harder one looks, the more will be found. For example (Carmen *et al.* 1997b) out of 2000 antenatal clinic attendees in Papua New Guinea, 5% of HBsAg positive subjects were negative in a widely used monoclonal based assay yet PCR positive. Over 50% of these discordant samples had rare or unique variants of the MHR.

HBsAg variants and HBIG therapy

The combination of high titre antibody (HBIG), low titre virus and a large number of susceptible cells (the new liver) is an ideal breeding ground for mutants. One can consider four scenarios after transplantation for chronic HBV. First, HBIG therapy is successful and patients are PCR negative. Second, patients receive HBIG but it is withdrawn because of side effects : mutant selection seems to be uncommon. Third, patients become HBsAg positive on therapy : such patients usually develop mutations in putative neutralising epitopes. G145R is by far the commonest variant observed to arise in this situation. It appears to arise de novo, because it is not often seen in the pre-transplant sample (Carmen *et al.* 1996 ; Protzer-Knolle *et al.* 1998). Withdrawal of HBIG often leads to reversion to the pre-transplant sequence (Ghany *et al.* 1998), intimating that the new viruses are not replicatively as efficient. It has been shown that HBIG does not bind to the selected viruses. Fourth, patients are PCR positive, but HBsAg negative, during therapy. Sometimes, a mutation that could effect secretion of HBsAg or has very significant effect on antigenicity is seen. Some approaches to diminish this selection pressure are to add nucleoside analogues such as lamivudine to HBIG or to add monoclonal antibodies which bind to the most important mutants along with HBIG. With the recent move to reduce use of human plasma derived products, monoclonal antibodies will probably become increasingly important.

Deletions and insertions in the pre-S regions during HBIG therapy can occur (Trautwein *et al.* 1996). Studies are underway to examine their in vitro effect and whether there is a significant amount of anti-pre-S in HBIG preparations.

References

1. ALEXOPOULOU A., OWSIANKA A.M., KAFIRI G., DOURAKIS S.P., CARMAN W.F., HADZIYANNIS S.J. Core variability does not affect response to interferon alpha in HBeAg negative chronic hepatitis B. *Journal of Hepatology*, 1998, **29** : 345-351.
2. CARIANI E., RAVAGGI A., TANZI E., ROMANI L., FIORDALISI G., BELLATI G., CACCAMO L., GALMARINI D., ALBERTINI A., ZANETTI A. Emergence of Hepatitis B virus S gene mutant in a liver transplant recipient. *J. Med. Virol.*, 1995, **47** : 410-415.
3. CARMAN W.F., KORULA J., WALLACE L., MACPHEE R., MIMMS L., DECKER R. Fulminant reactivation of hepatitis B due to envelope protein mutant of HBV that escaped detection by monoclonal HBsAg ELISA. *The Lancet*, 1995a, 345, 1406-1407.
4. CARMAN W.F., TRAUTWEIN C., VAN DEURSEN F.J., COLMAN K., DORNAN E., MC INTYRE G., WATERS J., KLIEM V., MULLER R., THOMAS H.C., MANNIS M.P. Hepatitis B virus envelope variation after transplantation with and without hepatitis B immune globulin prophylaxis. *Hepatology*, 1996, **24** : 489-493.
5. CARMAN W.F., TRAUTWEIN C., VAN DEURSEN F.J., COLMAN K., DORNAN E., MCINTYRE G., WATERS J., KLIEM V., MULLER R.,

THOMAS H.C., MANNIS M.P. Hepatitis B virus envelope variation after transplantation with and without hepatitis B immune globulin prophylaxis. *Hepatology*, 1996, **24** : 489-493.
6. CARMAN W.F., VAN DEURSEN F.J., MIMMS L.T., HARDIE D., COPPOLA R., DECKER R., SANDERS R. The prevalence of surface antigen variants of hepatitis B virus in Papua New Guinea, South Africa and Sardinia. *Hepatology*, 1997b, **26** : 1658-1666.
7. CARMAN W.F., ZANETTI A.R., KARAYIANNIS P., WATERS J., MANZILLO G., TANZI E., ZUCKERMAN A.J., THOMAS H.C. Vaccine-induced escape mutant of hepatitis B virus. *Lancet*, 1990, **336** : 325-329.
8. FUJII H., MORIYAMA K., SAKAMOTO N., KONDO T., YASUDA K., HIRAIZUMI, Y., YAMAZAKI M., SAKAKI Y., OKOCHI K., NAKAJIMA E. Gly 145 to Arg substitution in HBs antigen of immune escape mutant of hepatitis B virus. *Biochem. Biophys. Res. Comm.*, 1992, **184** : 1152-1157.
9. GHANY M.G., AYOLA B., VILLAMIL F.G., GISH R.G., ROJTER S., VIERLING J.M., LOK A.S.F. Hepatitis B Virus S Mutants in Liver Transplant Recipients Who Were Reinfected Despite Hepatitis B Immune Globulin Prophylaxis. *Hepatology*, 1998, **27** : 213-222.
10. HAWKINS A., GILSON R., GILBERT N., WREGHIU T., GRAY J., BOA I.A.-D., TEDDER R., ALEXANDER G. Hepatitis B virus surface mutations associated with infection after liver transplantation. *J. Hepatol.*, 1996 : 8-14.
11. HINO K., OKUDA M., HASHIMOTO O., ISHIKO H., OKITA K. Glycine-to-arginine substitution at codon 145 of HBsAg in two infants born to hepatitis B e antigen-positive carrier. *Dig. Dis. Sci.*, 1995, **40** : 566-570.
12. HOU J.L., KARAYIANNIS P., THOMAS H.C., LUO K.X. *et al.* A unique insertion in the S gene of surface antigen negative HBV in Chinese carriers. *Hepatology*, 1995, **21** : 273-8.
13. NAINAN O.V., STEVENS C.E., TAYLOR R.E., MARGOLIS H.S. Hepatitis B Virus (HBV) Antibody Resistant Mutants Among Mothers and Infants with Chronic HBV Infection. *In* : RIZZETTO M., PURCELL R.H., GERIN J.L., VERME G. (eds). Viral Hepatitis and Liver Disease. Edizioni Minerva Medical Turin, 1997 : 132-134.
14. NGUI S.L., O'CONNELL S., EGLIN R.P., HEPTONSTALL J., TEO C.G. Low Detection Rate and Maternal Provenance of Hepatitis B Virus S Gene Mutants in Cases of Failed Postnatal Immunoprophylaxis in England and Wales. *J. Infec. Dis.*, 1997, **176** : 1360-5.
15. NORDER H., COUROUCÉ A.-M., MAGNIUS L.O. Molecular basis of hepatitis B virus serotype variations within the four major subtypes. *J. Gen. Virol.*, 1992, **73** : 3141-3145.
16. NORDER H., HAMMAS B., LÖFDAHL S., COUROUCÉ A.M., MAGNIUS L.O. Comparison of the amino acid sequences of nine different serotypes of hepatitis B surface antigen and genomic classification of the corresponding hepatitis B virus strains. *J. Gen. Virol.*, 1992b, **73** : 1201-1208.
17. OGATA N., MILLER R.H., ISHAK K.G., PURCELL R.H. The complete nucleotide sequence of a pre-core mutant of hepatitis B virus implicated in fulminant hepatitis and its biological characterization in chimpanzees. *Virology*, 1993, **194** : 263-276.
18. OGATA N., ZANETTI A.R., YU M., MILLER R.H, PURCELL R.H. Infectivity and pathogenicity in chimpanzees of a surface gene mutant of hepatitis B virus that emerged in a vaccinated infant. *J. Infec. Dis.*, 1997, **175** : 511-523.
19. OKAMOTO H., TSUDA F., SAKUGAWA H., SASTROSOEWIGNJO R.I., IMAI M., MIYAKAWA Y., MAYUMI M. Typing hepatitis B virus by homology in nucleotide sequence : Comparison of surface antigen subtypes. *J. Gen. Virol.*, 1988, **69** : 2575-2583.
20. OKAMOTO H., YANO K., NOZAKI Y., MATSUI A., MIYAKI H., YAMAMOTO K., TSUDA F., MACHIDA A., MISHIRO S. Mutations within the s gene of hepatitis-B virus transmitted from mothers to babies immunized with hepatitis-B immune globulin and vaccine. *Paediatric Res.*, 1992a, **32** : 264-268.
21. OON C.-J., LIM G.-K., YE Z., GOH K.-T., TAN K.-L., YO S.-L., HARRISON T.J., ZUCKERMAN A.J. Molecular epidemiology of hepatitis B virus vaccine variants in Singapore. *Vaccine*, 1995, **13** : 699-702.
22. PROTZER-KNOLLE U., NAUMANN U., BARTENSCHLAGER R., BERG T., HOPF U., BUSCHENFELDE K.-H., NEUHAUS P., GERKEN G. Hepatitis B Virus With Antigenically Altered Hepatitis B Surface Antigen Is Selected by High-Dose Hepatitis B Immune Globulin After Liver Transplantation. *Hepatology*, 1998, **27** : 254-263.
23. SHIINA S., FUJINO H., UTA Y., TAGAWA K., UNUMA T., YONEYAMA M., OHMORI T., SUZUKI S., KURITA M., OHASHI Y. Relationship of HBsAg Subtypes with HBeAg/anti-HBe Status and Chronic Liver Disease. Part I : Analysis of 1744 HBsAg Carriers. *The American Journal of Gastroenterology*, 1991, **86** : 866-871.
24. SURYA L.G.P., KISHIMOTO S., SUDARYAT S., TSUDA F., HAMID A., TAKAHASHI K., SUWIGNYO S., HASHIMOTO K., MULYANTO, MISHIRO S. Prevention of Mother-to-Infact Transmission of Hepatitis B Virus with Use of a Pre-S2-Containing Vaccine in Bali, Indonesia. *Vaccine Research*, 1996, **5** : 203-213.
25. TRAUTWEIN C., SCHREM H., TILLMANN H.L., KUBICKA S., WALKER D., BÖKER K.H.W., MASCHEK H.-J., PICHLMAYR R., MANNS M.P. Hepatitis B virus mutations in the preS genome before and after liver transplantation. *Hepatology*, 1996, **24** : 482-488.
26. WILSON J.N., NOKES D.J., CARMAN W.F. Current status of HBV vaccine escape variants — a mathematical model of their epidemiology. *Journal of Viral Hepatitis*, 1998, Vol. 5, No. **32** : 25-30.
27. YAMAMOTO K., HORITA M., TSUDA F., ITOH K., AKAHANE Y., YOTSUMOTO S., OKAMOTO H., MIYAKAWA Y., MAYUMI M. Naturally occurring escape mutants of hepatitis B virus with various mutations in the S gene in carriers seropositive for antibody to hepatitis B surface antigen. *J. Virol.*, 1994, **68** : 2671-2676.
28. YASMIN M. Molecular biology of fulminant hepatitis B viruses. PhD Thesis, University of Glasgow. April 1997.