Commentary
Hepatitis B antiviral resistance and vaccine escape:
two sides of the same coin

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See article by Sloan and colleagues on pp 439–447 of this issue.

Hepatitis B virus (HBV) remains a major global health concern, even though the availability of an effective commercially available vaccine comprising yeast-derived recombinant hepatitis B surface antigen (HBsAg) protein has provided an effective means of preventing infection. The beneficial effect of HBV vaccination programs has become evident in several regions of the world where HBV endemicity is high. In the 15 years of follow up after the introduction of vaccination programs in Taiwan a marked reduction in infant mortality, HBsAg prevalence and incidence of liver cancer have all been reported [1]. The introduction of universal HBV vaccination in Italy also resulted in a dramatic decline in the incidence of acute hepatitis B and in the prevalence of HBsAg [2]. Similar reductions in the incidence of HBV have now also been reported from Alaska [3], South Africa [4] and the Pacific Islands [5], attesting to the success of HBV vaccination programs in curbing the prevalence of HBV infection.

The efficacy of the hepatitis B vaccine can be attributed to its ability to induce protective neutralizing antibodies to an antigenic region of the HBsAg protein referred to as the ‘a’ determinant [6–8]. An important concern for HBV immunization programmes is what effect the emergence of hepatitis B variants harbouring altered HBsAg proteins with reduced antigenicity and the ability to escape neutralization may have on the long-term success of these programmes. It is clear that these variants are selected following immunization of infected individuals. The ‘a’ determinant is a highly conformational region of the HBsAg protein and mutations in and around the ‘a’ determinant have been shown to alter the antigenicity of the HBsAg protein and, consequently, antibodies against HBsAg (anti-HBs) fail to neutralize HBV [9–13] resulting in infection of vaccinated individuals [11,14]. In fact, a mutation of even a single amino acid residue within this region is sufficient to result in failure of anti-HBs to neutralize the virus. Some of the commonly reported HBsAg mutations with the potential to escape neutralization by vaccine-induced antibody include sG145R, sD144A, sP142S, sK141E, sQ129H, sI126N/A and sM133L [10,12]. The sG145R variant has been associated with high levels of viraemia and persistence for up to 8 years, suggesting that once such viruses emerge in a population they are likely to persist for extended periods.

In Taiwan, a country with a high HBV prevalence and a universal hepatitis B vaccination programme that has been continuing for over 15 years, up to 28% of children carrying HBV harbour HBsAg mutants [14]. The introduction of hepatitis B immunization programmes in other countries of high HBV endemicity has resulted in similar increases in the incidence of HBV variants with antigenically altered HBsAg protein [15,16]. However, the emergence of these viruses to date has not resulted in an apparent failure of HBV vaccination programs.

Despite the success of HBV vaccination programmes there are a number of important virological factors that may have a further negative effect on the long-term success of these programmes and warrant consideration. The genome of hepadnaviridae, including HBV, is organized into compact overlapping reading frames. In addition, hepadnaviridae utilize an error-prone reverse transcription step in the replication of the viral genome. This process results in the selection of viral quasispecies that contain mutations within the viral genome, some of which are functionally significant; changes in the overlapping genes and their translational products can also result in changes in the functional properties of mutated viral proteins [17,18]. The complete overlap of the gene encoding HBsAg (the
S gene) by the polymerase (pol) gene creates a unique situation in which a change within the pol gene following nucleoside analogue therapy might result in structural changes in the HBsAg protein and a subsequent reduction in the antigenicity of the protein [18]. Lamivudine-associated resistance mutations in the polymerase are associated with changes in the HBsAg protein [19,20] with a consequent reduction in antigenicity of the HBsAg protein that is comparable to that of vaccine escape HBsAg mutants [18]. Thus, lamivudine-associated drug-resistant isolates have the potential to become vaccine escape mutants. The reverse is also true: where changes within the S gene selected as a consequence of HBV vaccination or by treatment with hepatitis B immunoglobulin (HBIg) can produce a functionally significant alteration of the viral polymerase and influence the viral replication potential to become vaccine escape mutants. The authors not only clearly demonstrate that mutations arising from nucleoside analogue therapy are sufficient to cause changes within the HBsAg protein and its antigenicity, but also show that a combination of mutations can reduce or restore antigenicity.

In this issue of Antiviral Therapy, Sloan and colleagues describe HBV mutants selected as a consequence of antiviral therapy that display altered HBsAg protein ‘a’ determinants and have the ability to evade neutralizing antibodies. By using recombinant HBsAg proteins produced in mammalian cells these investigators report a comprehensive analysis of the effect of mutations in pol selected by commonly available antiviral agents (lamivudine, entecavir and adefovir) on the antigenicity of HBsAg protein.

The recombinant HBsAg proteins harbouring antiviral-selected changes were tested for their ability to bind to a panel of well-characterized monoclonal antibodies. These antibodies were known to recognize distinct epitopes in the first and second loops of the ‘a’ determinant. By using an HBsAg capture ELISA and calculating a ‘binding ratio’ the authors were able to derive a method for determining the area under the curve (AUC) for the binding of each monoclonal antibody against wild-type and mutant HBsAg proteins. Although this method did not assess virus neutralization, it did provide a very useful measure of the strength of the antibody–antigen binding. In so doing, the authors were able to make meaningful comparisons between the antibody-binding patterns of wild-type and antiviral-selected HBsAg mutant proteins.

The authors demonstrated that the antibody binding of the antiviral-selected variants rtF166L/sF158Y (a lamivudine-associated compensatory polymerase mutation) and rtI169T/sF161L (associated with entecavir resistance) with single mutations in HBsAg was reduced to levels comparable to those reported in the classical G145R vaccine-associated escape mutant. Antibody binding to epitopes in the first or second loop or to both loops of the ‘a’ determinant was also reduced with the rtM204V/sI195M and rtV173L/sE164D lamivudine-selected mutants.

Far more disconcerting was the observation that the combination of rtM204V/sI195M with the rtSilent/sD144E (antibody escape) mutation caused a reduction in antibody binding as compared with the rtSilent/sD144E mutation alone, which had epitope binding comparable with wild-type HBsAg. In addition, the antibody binding to this combination mutant by one of the monoclonal antibodies tested was completely abolished. Thus, the authors have shown that it is possible to convert an HBsAg mutant that is recognized by anti-HBs to one that is potentially no longer able to be neutralized.

Interestingly, the authors also show that the combination of rtM204V/sI195M with either the rtF166L/sF158Y or the rtR153Q/sG145R mutations resulted in a restoration of antibody reactivity to epitopes in the second loop of the ‘a’ determinant. The findings of Sloan and coworkers confirm previous reports demonstrating that HBsAg bearing rtM204V/sI195M together with the rtV173L/sE164D mutation has an antibody–epitope binding profile similar to the rtR153Q/sG145R vaccine escape-associated mutant [18]. The authors not only clearly demonstrate that mutations arising from nucleoside analogue therapy are sufficient to cause changes within the HBsAg protein and its antigenicity, but also show that a combination of mutations can reduce or restore antigenicity.

What could the implications of these findings be for the broader global HBV vaccination programmes? These programmes have now been in place in several regions of South East Asia for well over 15 years. Although these have been beneficial in reducing HBsAg prevalence and the incidence of hepatocellular carcinoma, they have also caused the emergence of viruses containing HBsAg protein mutations. On the basis of previous mathematical models, it is likely that HBV with mutated HBsAg proteins will become widespread in countries with high endemicity for HBV [21]. Although most of these mutant viruses are likely to be of little significance, and their emergence has not resulted in the failure of these vaccination programs so far, the introduction of nucleos(t)ide monotherapy for the treatment of chronic hepatitis B infection in many of these South East Asian countries is creating an environment that is well suited for the emergence of transmissible drug-resistant mutants for which the current vaccine may be poorly effective. Sloan and colleagues have shown that the combination of the lamivudine-associated mutation rtM204V with an HBsAg mutant sD144E was sufficient to convert the antibody-binding profile of the latter from a wild-type pattern to that of the classical vaccine escape mutant sG145R. It is plausible that in these populations the co-emergence of antiviral therapy resistant HBV and viruses harbouring HBsAg protein mutants will
provide a natural ‘mixing pool’ for the more frequent selection of viruses that are no longer neutralized by anti-HBs antibody.

To further compound this issue are reports that HBV is able to develop the compensatory rtV173L mutation in the polymerase protein [22], which restores the replication phenotype of lamivudine-resistant HBV [17,23]. The effect of selecting antiviral-resistant HBV in an endemic population with a high frequency of viruses carrying HBsAg protein mutations could be the emergence of HBV that is resistant to antiviral therapy with the potential to replicate like wild-type HBV and escape neutralization by vaccine-induced anti-HBs.

How could such a situation be prevented? One approach would be to prevent the emergence of antiviral-resistant HBV in the first place. Interferon-based treatment for chronic hepatitis B is effective and also reduces the long-term risk of developing hepatocellular carcinoma [24]. Moreover, it is not associated with the development of antiviral resistance [25,26]. Alternatively, treatment could be initiated with nucleos(t)ide analogues that are far more potent than lamivudine [27,28], such as entecavir or telbivudine [27,29,30]. However, in patients with pre-existing lamivudine resistance treatment failure with these agents is not infrequent [28].

Another alternative is to commence treatment with combination antiviral therapy to maximize viral suppression, thus reducing the likelihood of the emergence of mutations. Even though the combination of pegylated interferon and lamivudine [31–33] has not been shown to improve virological response, the combination does result in potent viral suppression and higher HBsAg seroconversion rates and the emergence of lamivudine-resistant virus is infrequent compared with lamivudine monotherapy. The combination of adefovir and pegylated interferon has also been shown to significantly reduce intrahepatic HBV covalently closed circular DNA and to produce higher HBsAg seroconversion than might be expected with nucleos(t)ide analogues alone [34]. However, this beneficial effect was lost following discontinuation of pegylated interferon and continuation with adefovir monotherapy [35].

In addition, in a recent retrospective study of patients with lamivudine-resistant HBV who were either swapped to adefovir alone or had adefovir added to lamivudine, the rates of virological breakthrough (9% versus 2%) and adefovir genotypic resistance (5% versus 0.8%) after 2 years of treatment were significantly lower in patients on combination therapy [36]. By continuing patients on combination adefovir and lamivudine the low cumulative rate of resistance to adefovir was maintained for up to 4 years [37]. Also, in a recent prospective trial of adefovir alone versus adefovir in combination with lamivudine in patients with HBeAg-negative chronic hepatitis B, adefovir resistance developed only among patients treated with adefovir monotherapy (22%) [38]. These studies show that combination therapy serves a potentially important role in preventing the selection of HBV antiviral therapy resistance.

Whilst combination antiviral therapy has not been shown to increase HBeAg seroconversion rates and the long-term virological response has yet to be proven, its ability to maximally suppress viral replication is likely to prevent the emergence of nucleos(t)ide-resistant virus. In doing so, combination therapy could prevent the emergence of replication-competent, antiviral therapy resistant, vaccine escape HBV mutants particularly in countries of high HBV endemicity. With the potential threat that such viruses would pose for vaccination and antiviral treatment strategies alike, it is of paramount importance that surveillance programmes are established to monitor the emergence and clinical effect of these viruses. It is difficult at present to determine whether or not these viruses will pose a genuine public health risk to the long-term success of both vaccination programmes and efficacy of antiviral treatment strategies based on monotherapy with nucleos(t)ide analogues. However, the natural experiment to determine the final answer to this question may well have commenced on a global scale.

Disclosure statement

The author declares no conflicts of interest.

References


