Potential threat of drug-resistant and vaccine-escape HBV mutants to public health

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Introduction

Concerns were raised towards the end of the previous century over reports of new and unusual HBV mutants emerging from children given immunoprophylaxis against hepatitis B and from patients with chronic hepatitis B (CHB) treated with the synthetic nucleoside analogue lamivudine (3TC) [1,2]. Although direct antiviral therapy using 3TC had been efficacious in suppressing HBV replication and was leading to marked reduction in the risk of cirrhosis and hepatocellular carcinoma [3], clinical benefits were mitigated by the rapid development of resistance against 3TC. From the public health standpoint, widespread 3TC usage was transforming CHB patients into carriers of drug-resistant HBV mutants. At the turn of the century, the European Union, recognizing the potential threat these mutants posed to clinical practice and public health, funded a multinational collaborative project to conduct virological and clinical research and to launch pilot surveillance programmes [4]. Since then, nucleoside analogues (adefovir [ADV], entecavir [ETV] and tenofovir [TDF]) and the newer nucleotide analogue (telbivudine) have been introduced to complement or substitute for 3TC. These drugs have the capacity to generate point mutations distinct from 3TC and also to select for cross-resistance [5]. Although the rates of mutations generated by them are considerably slower than 3TC, their long-term usage by CHB patients (some of whom would have previously been exposed to 3TC) and by those undergoing liver transplantation (who are additionally given hepatitis B immunoglobulin) has created fresh evolutionary milieus from which multidrug-resistant HBV mutants are now emerging [6,7]. Therapeutic and public health challenges are thereby compounded, to which the World Health Organization has recently been alerted [8].

In June 2009, the US Centers for Disease Control and Prevention convened a symposium entitled ‘Drug-resistant and vaccine-escape hepatitis B virus mutants: emergence and surveillance’. During the symposium the following issues amongst others were deliberated. In what settings do HBV mutants arise following vaccination and drug therapy? How transmissible are they? What type of patients are at risk of becoming their transmitters? If infected, would the course of acute hepatitis B or CHB be altered? How susceptible are people already vaccinated against hepatitis B to infection by the mutants? If these susceptible people – whether vaccinated or not – are exposed to these mutants, to what extent would the course of infection differ from infection by wild type? How might the spread of emergent mutants be expeditiously tracked? Topical updates were presented by US Centers for Disease Control and Prevention and international experts engaged in viral hepatitis surveillance, the care of patients with CHB, and the virological and computational studies of vaccine-escape and drug-resistant HBV mutants. This Spotlight issue of Antiviral Therapy features reviews that highlight those presentations.
HBV S-gene mutants with mutations in the ‘a’ determinant

The contribution by Stephen A Locarnini and Lilly Yuen [9] provides an overview of the molecular genesis of the mutations. The S gene of HBV encodes the viral envelope, which is also called the hepatitis B surface antigen (HBsAg). HBsAg elicits strong neutralizing antibody responses and is the principal immunogen of hepatitis B vaccines. The immunodominant portion of HBsAg is the ‘a’ determinant, whose locus maps to codons 121–147 of the S gene. HBV variants with mutations therein that specify amino acid changes have the propensity to evade binding to vaccine-induced antibodies: they can accordingly behave as vaccine-escape mutants. Such mutations classically arise from two settings: infants born to women with persistent HBV infection who after birth are provided passive-active anti-HBV immunoprophylaxis and CHB patients given hepatitis B immunoglobulin following liver transplantation.

The review by Mei-Hwei Chang [10] on the phenomenal success of the universal hepatitis B immunization programme in Taiwan provides the backdrop from which the extent of the emergence of these ‘a’ determinant mutants – which have led to breakthrough infection among vaccinated children – should be viewed. Close surveillance determined that although the rates of detection of the mutants has increased, that increase nevertheless occurred pari passu to the marked decline in the HBsAg-carrier rate among vaccinees. These data from Taiwan, while reassuring, underscore the importance of maintaining high-coverage hepatitis B vaccination programmes and having effective HBV surveillance schemes in place.

HBV S-gene mutants with mutations outside the ‘a’ determinant

The HBV genome is organized such that the P gene overlaps the S gene. One outcome of this overlap is that mutations in the P gene selected under pressure by drugs that suppress HBV polymerase activity can lead to corresponding mutations in the S gene. The typical primary mutation associated with HBV resistance to 3TC (rtM204V/sI195M) is located >50 nucleotides downstream of the ‘a’ determinant locus. Secondary (compensatory) mutations, rtV173L/sE164D and rtL180M, encroach onto the locus, but are not sites within it. Some of the mutations associated with resistance to ADV (rtA181/sL173F) and ETV (I169T/sF161L and rtT184SL/sL176V) are also near to but not located in the locus. Nonetheless, several of these mutations – singly, in combination with other S- or P-gene mutations and in combination with the classical vaccine-escape mutations – can profoundly abrogate binding of HBsAg to anti-HBsAg polyclonal antibodies and to monoclonal antibodies raised against ‘a’ determinant epitopes [11,12]. Evidently, the conformational interactions of amino-acid residues in the ‘a’ determinant have complex roles in determining the overall immunogenicity of HBsAg, predisposing virions that are coated with the mutated protein to evade binding to and therefore escape neutralization by vaccine-induced antibodies. Locarnini and Yuen [9], and Chau-Ting Yeh [13] in their contributions have conferred foreboding acronyms on these HBV S-/P-gene overlap mutants: ADAPVEMs for antiviral-drug-associated potential vaccine-escape mutants and ADASMs for antiviral-drug-associated S-gene mutants, respectively. Saleem Kamili [14] reviews experimental data in the chimpanzee model that suggested the vaccinated host can be susceptible to infection by the commonly encountered ADAPVEM/ADASM – the 3TC-resistance-associated, ‘triple’ mutant, which bears the rtV173L/sE164D, rtL180M and rtM204V/sI195M mutations.

No reports of infection by ADAPVEMS/ADASMS have yet to be reported in human vaccinees. Transmissions of drug-resistance-associated HBV mutants to unvaccinated humans have been reported; however, the cases identified were infected by the 3TC-resistance-associated rtM204V/sI195M mutant [15,16]. Although it is unknown if the infecting HBV variants were naturally occurring or had been generated in hosts previously exposed to 3TC, that all the cases were infected with HIV raises concerns over the susceptibility of HIV-infected individuals to infection by drug-resistant HBV mutants and also the extent to which the HBV–HIV-coinfected state might be conducive to their emergence. Chloe L Thio [17] and Karine Lacombe and colleagues [18], in their reviews of the adverse clinical and virological events experienced by HBV–HIV-coinfected patients in the US, Australia and Europe, highlight the higher rate and faster appearance of antiviral-resistant HBV mutations in coinfected patients given 3TC for CHB compared with treated HBV-monoinfected patients. Prior 3TC exposure also predisposes to higher rate of ETV resistance in HBV–HIV-coinfected compared with HBV-monoinfected patients.

The HBV–HIV coinfected state might, from a global perspective, also undermine the efficacy of hepatitis B vaccination programmes. Many developing countries that had long been hyperendemic or endemic for HBV are the ones where HIV is concurrently epidemic. Table 1 summarizes data from serosurveys of HBsAg among therapy-naive, HIV-infected individuals conducted in three representative countries: Thailand, Nigeria and Brazil. Although the HBsAg seropositivity rates vary depending on the risk of HBV infection of the study populations, sampling error and the
performance of the HBsAg assay used, people who are HBV–HIV-coinfected should be considered to constitute a sizeable reservoir of HBV infection. It is from this context that the increasingly widespread usage of 3TC-containing antiretroviral therapy (ART) might be viewed to pose a challenge to public health: by potentiating the emergence of resistant mutants, particularly those that bear ADAPVEM/ADASM traits. For 3TC is, among the nucleoside analogues that are highly effective against HIV, the cheapest to manufacture and so has become the mainstay of fixed-dose ART combinations (for example, as Triomune or Combivir) for use in mass treatment programmes. Adrian M Di Bisceglie and colleagues [19] review the HBV–HIV coinfection situation in South Africa and summarize data from preliminary studies that were conducted there on naturally occurring and ART-associated HBV mutants. Recently, the World Health Organization, in recognition of the special clinical and public health status of HBV–HIV coinfected individuals, has updated ART guidelines recommending that coinfected patients be treated with TDF and 3TC or emtricitabine [20]. Pre-therapy serological testing for HBsAg is presumed. That, together with the need to use TDF and substitute emtricitabine for 3TC, can be cost-prohibitive for countries with lesser resources.

HBV variants bearing the classic ‘a’ determinant mutations and those that are ADAPVEMs/ADASMs, in being able to affect immune or vaccine escape, may by corollary evade serological detection. HBsAg has historically been the analyte of choice whenever the presence of active HBV infection needs to be serologically sought, on account of its abundance in the blood of HBV-infected hosts that stems from its exuberant shedding from the liver. Serological assays using monoclonal antibodies that have poor affinity to mutant HBsAg would not detect it. Nonetheless, assay manufacturers are aware of the potential for such serological escape and are adept at constructing better performing assays using antibody-capturing cocktails comprising monoclonals that can bind well to the mutants – as long as the mutations have been predefined and characterized. Yeh’s [13] review includes discussion of HBV variants with mutations outside the ‘a’ determinant whose HBsAg are serologically occult: some bear mutations (for example, sP120S/T) that result in poor affinity between HBsAg to anti-HBsAg antibodies, and others whereby HBsAg cannot be expressed because mutations have led to truncation or impaired secretion of the S-gene-encoded products. One particular mutant (rtA181T/sW172*), which is resistant to 3TC, telbivudine and ADV, has been shown to confer tumourigenicity in nude mice. Table 2 shows the clinical and surveillance settings where mutant HBV may miss being detected.

From genotype to phenotype

The review by Yury Khudyakov [21] offers fresh perspectives from which to view the emergence of iatrogenic mutants. Alterations in the biological properties

<table>
<thead>
<tr>
<th>Country/locality</th>
<th>Study period</th>
<th>Type of study population</th>
<th>Patients, n</th>
<th>HBsAg-seropositive, %</th>
<th>Reference</th>
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<td>Thailand</td>
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<td>[27]</td>
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<td>20.6</td>
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<tr>
<td>Nasarawa</td>
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<td>Prisoners</td>
<td>54</td>
<td>23</td>
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<td>1,603</td>
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<tr>
<td>Cuiba</td>
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<td>851</td>
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HBsAg, hepatitis B surface antigen.
of HBV as they relate, for example, to replication and transmissibility, need not per se be due to point mutations in specific genes, but could also be outcomes of epistatic connectivity networking with pre-existing genomic sequences and with changes selected in other genomic regions following drug exposure or immune attack. The rising number of mutants and the accompanying, increasingly complex spectra of viral genetic changes warrant the establishment and curatorship of databases capacious enough to archive sub-genomic as well as whole-genome-wide nucleotide and cognate amino acid sequences. Carla Kuiken [22] in her contribution suggests approaches by which such databases might be created, accessed and annotated. Surveillance for HBV mutants need not be entirely genotypically based, however. David Durantel [23] presents new and promising laboratory methodologies that pave the way for replication-fit of HBV mutants to be more routinely measured. Daniel J Tenney [24] in his review provides a window into how phenotypic changes of newly emerging HBV mutants might be evaluated and monitored. Their contributions are a fitting reminder that ‘resistance’ is, after all, effected at the level of the phenotype, not the genotype.

Conclusion

We hope this collection of contributions provides a flavour of the proceedings of the symposium. We commend it as constituting a corpus of work that would facilitate further deliberations into how mutants might interfere with the efficacy of antiviral therapy, alter the natural history of hepatitis B, undermine the success of hepatitis B vaccination programmes and become further transmitted.

Disclosure statement

The authors declare no competing interests.

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