The Enigma of Concurrent Hepatitis B Surface Antigen (HBsAg) and Antibodies to HBsAg

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(See the article by Zhang et al. on pages 1161–9)

Hepatitis B virus (HBV) infection continues to be a major health problem worldwide. The large-scale immunization programs for persons in high-risk groups, newborns, and adolescents have greatly reduced the incidence of new infection, but the ~400 million HBV carriers with HBV surface antigen (HBsAg) in the blood remain a burden that will dwindle only within decades. Furthermore, an unknown disease potential is hidden in the carriers of “occult” HBV infection. In fact, the majority of people with “resolved” HBV infection (i.e., 40% of the world population) harbor the virus intrahepatically, but its replication is controlled by cytotoxic T lymphocytes, and its spread is blocked by the host’s neutralizing antibodies to HBsAg (anti-HBs). Anti-HBs are also the major protective component of vaccine-induced immunity. In view of their important protective role, occurrence of anti-HBs in HBsAg-positive patients with active chronic HBV infection is extremely puzzling. This phenomenon has been known since at least 1976 [1, 2].

Patients with chronic HBV infection may test persistently positive for HBsAg and anti-HBs without significant change in the infection status. This paradoxical pattern led to a seemingly obvious explanation: the subtype of the HBsAg and anti-HBs were heterologous. HBsAg subtype determinants were first described as allelic exclusive determinants d or y, in addition to the common HBsAg determinant a. Thus, HBsAg/ad was accompanied by anti-HBs/y and vice versa [1–4]. These findings were later confirmed for the second pair of subtype determinants, w and r, in patients in East Asia [5]. These studies from the 1970s and 1980s suggested that anti-HBs in HBV carriers had no significant protective or pathogenic effect and were usually associated with high replicative activity. It was speculated that the production of anti-HBs was not completely blocked in chronic HBV carriers, but B cells encoding high-affinity antibodies to the carrier’s own HBsAg would be somehow ineffective, whereas B cell clones encoding antibodies with low affinity to the homologous HBsAg could expand and express their antibody [6]. These anti-HBs would not be bound to the carrier’s own HBsAg but to HBsAg with slightly different antigenicity. The possibility that the heterotypic anti-HBs were induced by superinfection with a second HBV strain was dismissed by most studies.

After the discovery of vaccine-induced HBsAg escape mutants in 1990, another explanation became likely and could be tested experimentally. Several studies employing PCR amplification and sequencing of the gene for HBsAg (S gene) suggested that there were indeed escape mutations in the HBsAg if anti-HBs were concomitantly present. These studies, however, suffered from low numbers of patients and lack of adequate control subjects. This criticism is not applicable to the recent study from Lada et al. [7] that compared the rate of amino acid exchanges in the S genes from chronic HBsAg carriers with and without concomitant anti-HBs. These authors indeed found an enhanced number of mutations (9.5% in anti-HBs carriers vs. 2.4% in those without anti-HBs) in the HBsAg a-determinant in anti-HBs-positive carriers and concluded that anti-HBs would significantly favor selection of escape mutants. The previous conclusion that concomitant anti-HBs were not protective seemed to be disproved, but in this issue of Clinical Infectious Diseases, Zhang et al. [8] quite convincingly demonstrate that chronic HBsAg carriers with anti-HBs did not have significantly more mutations than well-matched carriers without anti-HBs. In fact, the proportion of mutations in the a-determinant was very low (0.7%) and restricted mostly (in 0.5%) to position 126, which seems to be polymorphic even without immune selection. Furthermore, the authors confirmed
the previous observations that anti-HBs present in patients with chronic HBV infection had low affinity and were directed to a heterologous HBsAg subtype, whereas anti-HBs in vaccinated persons had high affinity and bound to all 3 HBsAg subtypes (adw, adr, and ayw).

How can the contradicting results of Lada et al. [7] and Zhang et al. [8] be explained? One important difference is the selection of patients. Zhang et al. [8] analyzed only HBeAg-positive HBV carriers with elevated transaminases and HBV loads >10⁵ copies/mL. All were obviously from China and had HBV genotype C or B. In contrast, Lada et al. [7] included 5 HBeAg-negative carriers in their study cohort of 14 patients, 7 of whom had HBV genotypes other than B or C. They did not comment on the disease activity, but 4 patients had low viral loads (<10⁵ copies/mL). Thus, Lada et al. [7] may have included patients who were in the process of eliminating HBV, whereas the study by Zhang et al. [8] included only patients with persistently high viral loads and a high degree of immune tolerance to HBV infection. Lada et al. [7] also included HBeAg-negative patients with low viral loads in their anti-HBs-negative control group, but one may presume that anti-HBs may contribute to immune selection only if HBV elimination is already taking place, as was probably the case in some of the patients in the study by Lada et al. [7] but in none in the study by Zhang et al. [8].

The heterogeneity of patients with concurrent HBsAg and anti-HBs had already been reported by Shiels et al. in 1987 [4]. They found this serological pattern more often in HBeAg-positive carriers with chronic active disease than in carriers with mild or no disease or in patients with acute HBV infection. Thus, the patient group studied by Zhang et al. [8] may be more typical. In my own limited experience (unpublished data), carriers with HBsAg and anti-HBs have little variation in the S gene if the viral load is high and greater variability if the viral load is low, leading to selection of escape mutants. The usual low levels of escape mutants have probably contributed to the fact that, until now, they have not spread to the general population. However, in patients experiencing immune suppression, escape mutants may reactivate and reach very high levels (>10⁶ copies/mL) in the presence of anti-HBs [9, 10]. It is not known whether this very special type of immunocompromised patient was included in the study by Lada et al. [7] or in other studies reporting a high frequency of escape mutations in patients with HBsAg and anti-HBs.

Theoretically, one would expect that concurrent HBsAg and anti-HBs would occur regularly during convalescence from acute HBV infection. However, as described in textbooks, the opposite is the case. After disappearance of HBsAg from the serum, it usually takes several weeks or even months until anti-HBs become detectable. One reason for this is probably that, during the early phase of acute infection, the levels of anti-HBs are low, compared with the levels of HBsAg. One IU of anti-HBs is able to bind to 0.9 μg of HBsAg [11]. However, anti-HBs titers in the convalescence phase usually remain <1000 IU/L of serum [11], whereas levels of HBsAg in the early acute or chronic phase of HBV infection are typically 10,000–100,000 μg/L. Levels of anti-HBs were also mostly <1000 IU/L in the study by Zhang et al. [8]. The study did not report the level of HBsAg, but there is no indication to assume that an HBeAg-positive carrier with a high viral load had lower than usual HBsAg concentrations. Thus, anti-HBs reacting with the homologous HBsAg, if present, would be bound and would never become detectable. Zhang et al. [8] argue against that theory, because they did not find a decrease in the HBsAg level after removing hypothetical immune complexes from their samples. However, if there is an excess of HBsAg, no significant decrease would be observed. In fact, immune complexes of HBsAg and anti-HBs have been found in the majority of patients with chronic HBV infection and high viral loads, irrespective of concomitant reactivity of anti-HBs [12].

Furthermore, immune complex diseases, such as arthritis, glomerulonephritis, periarteritis nodosa, and Giannotti-Crosti syndrome, are known as extrahepatic manifestations of highly replicative HBV infection, and HBsAg has been detected in the lesions [13]. Thus, one may speculate that the small proportion of HBsAg carriers who also have detectable anti-HBs is only the tip of the iceberg and, possibly, most carriers have some kind of antibodies against the HBV surface proteins, but these are masked by the excess of HBsAg. In addition to the “normal” anti-HBs, these antibodies may be directed against the pre-S domains of the viral surface proteins [5]. The weakness of this immune response is that it is quantitatively and/or qualitatively insufficient to overcome chronic infection. On the other hand, it could be a basis for future immune therapeutic attempts to expand the antibody response to curative levels.

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References

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