Uncover the immune biomarkers underlying hepatitis B e antigen (HBeAg) seroconversion: A need for more translational study

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The Hepatitis B e antigen (HBeAg) is a non-particulate secretory protein, which is not essential for viral assembly or replication but is important for the establishment of persistent infection in vivo. A recent study suggested that it down-regulates the innate immune response to infection and function as a T cell tolerogen [1]. For patients with chronic HBeAg positive chronic hepatitis B infection (CHB), HBeAg seroconversion, defined as loss of HBeAg with the appearance of anti-HBe, is often associated with clinical remission and a transition to inactive liver disease [2]. Accompanying HBeAg seroconversion, there is a reduction in liver fibrosis, a lower incidence of cirrhosis and hepatocellular carcinoma. HBeAg seroconversion, whether spontaneous or treatment-induced, is also associated with a higher probability of hepatitis B surface antigen (HBsAg) loss and seroconversion, which is considered to be a more permanent clinical remission of liver disease. Thus, the achievement and maintenance of HBeAg seroconversion, in association with polymerase chain reaction-undetectable hepatitis B virus (HBV) DNA levels, are an important goal in the management of patients with HBeAg-positive CHB. Based on evidence demonstrating HBeAg seroconversion as an important hallmark event of a durable clinical remission of liver disease, major liver societies treatment guidelines have adopted HBeAg seroconversion with sustained suppression of HBV DNA as an end point for treatment in patients with HBeAg-positive CHB who do not have cirrhosis or decompensated liver disease.

To date, there are seven registered treatments for CHB, two immunomodulatory agents – conventional interferon-α and pegylated interferon α2a – and five nucleos(t)ide analogues (NUCs) – lamivudine, adefovir dipivoxil, telbivudine, entecavir, and tenofovir disoproxil. Among the various forms of treatment, one-year treatment with pegylated interferon α2a yielded the highest rate of HBeAg seroconversion (around 40%) and among them, one-tenth further benefit from HBsAg seroconversion. On the other hand, treatment with NUCs achieved a much lower rate of HBeAg seroconversion and hence more prolonged therapy is required in CHB patients treated with NUCs. However, with more prolonged NUCs therapy, the treatment costs escalate and it is not uncommon, as a practicing clinician, to see patients with premature termination of NUCs, due to financial reasons or non-compliance. This can result in serious liver-related morbidity and mortality. It is thus of paramount importance to explore new treatment methods to hasten and increase the rate of sustained HBeAg seroconversion in chronic HBeAg positive patients [2].

In order to improve the rate of HBeAg seroconversion, it is urgently necessary to uncover its underlying mechanisms. In this aspect, immunological studies on patients before, during, and after sustained HBeAg seroconversion are of great importance. During the course of HBeAg seroconversion in CHB patients treated with antiviral therapy, many important immunological changes have been observed, reflecting the importance of restoration of the host immunity against HBV in HBeAg seroconversion. Through comprehensive immunological analysis, frequency of plasmacytoid dendritic cells (pDCs), Toll-like receptors (TLRs), and programmed death-1 (PD-1) has been related to HBeAg seroconversion. Notably, the latter two have been demonstrated to be regulated by the presence of HBeAg. These studies have provided some potential immunological biomarkers to predict HBeAg seroconversion [3].

In keeping with the decisive role of cytokines in initiating and shaping immune-related pathologic responses to chronic viral infection, interleukin (IL)-10, and IL-12 have been linked to HBeAg seroconversion. These important findings were based on careful analysis of serially collected clinical samples before, during and after HBeAg seroconversion in HBeAg-positive CHB patients. A substantial increase of bioactive IL-12 and Th1 cytokines has been associated with HBeAg seroconversion in HBeAg-positive CHB patients treated with interferon-α. Importantly, the peak of IL-12 occurred either before or simultaneously with hepatitis B e seroconversion [4]. It has also been shown that higher levels of serum IL-12 and IL-10 were associated with early, spontaneous HBeAg seroconversion in HBeAg-positive CHB patients [5].

In this issue of the Journal, Ma et al. explored the role of IL-21 in HBeAg seroconversion. They studied seventy-five patients with HBeAg-positive CHB from China who participated in a phase IV,
Step 1: Transient and reversible changes in immune markers

Step 2: T cell responses |
| pDC/mDC |
| Treg/PD-1 |
| IL-12, IL-10, IL-21 |
| others? |

Step 3: ?

Fig. 1. Immune markers correlate with HBeAg seroconversion. Three stages represent the efficacy of anti-HBV therapy: (1) complete virus suppression (viral load drops below detectable level) together with ALT normalization. In general, there is a transient or reversible change of enhanced immune response during this period; (2) HBeAg seroconversion; and (3) HBsAg seroconversion. Immune markers, such as T cell responses, pDC/mDC numbers, Treg numbers, PD-1 expression, IL-12, IL-10, IL-21 are closely associated with HBeAg seroconversion. However, there are still short of immune markers to predict the HBsAg seroconversion, the “almost cured” clinical status.

Antiviral therapy

HBeAg-positive CHB patients

HBV DNA suppression
ALT normalization

HBeAg seroconversion

HBsAg seroconversion

multi-center, open-label clinical trial of telbivudine (CLDT600ACN07T). They measured serial serum IL-21 levels by enzyme linked immunosorbent assay and the proportions of T-cells producing IL-21 and/or expressing PD-1 in peripheral blood mononuclear cells, longitudinally during treatment by intracellular cytokine staining and flow cytometry. They found that serum IL-21 levels at week 12 of treatment independently predicted HBeAg seroconversion in the first year of treatment. A week 12 serum IL-21 concentration of less than 51.4 pg/ml gave a negative predictive value for HBeAg seroconversion of 95%. However, the decreases in PD-1 expression on CD4+ and CD8+ T cells during the first 12 weeks of telbivudine treatment were not correlated with changes in serum IL-21 levels [6]. In a mouse model, IL-21 has been shown to be an essential component of CD4+ T cell help for CD8+ T effector cells in the control of chronic viral infection [7] and CD4+ T cell help has been shown to play an important role in restoration of host immune response to CHB. In addition, it has been shown that IL-21 increases B cell proliferation after activation via B cell receptor plus T cell derived co-stimulatory signals. More importantly, IL-21 counteracts the Treg-mediated suppression of human CD4+ T lymphocytes and regulates the maturation, growth and cytolysis activity of natural killer (NK) cells and the proliferation of NKT cells [8]. NK cells are abundant in the liver and serve as a major innate immune component against microbial infection. Recently, in CHB patients, hepatic NK cells were found to be activated and preferentially skewed toward cytolytic activity, which depends on an imbalanced cytokine milieu and correlates with liver injury during chronic HBV infection. Taking together, these results explained why week 12 serum IL-21 is identified as an immune biomarker for HBeAg seroconversion.

With this important study conducted by Ma et al., more questions than answers have been raised. Currently, there are data, though not head-to-head comparison, suggesting a higher rate of HBeAg seroconversion in HBeAg-positive patients treated with telbivudine than other NUCs (lamivudine, adefovir dipivoxil, and entecavir). However, whether this IL-21 increase is specific to telbivudine treatment in CHB patients needs to be understood. Is this enhanced IL-21 level important in interferon-α treated CHB patients? As increased IL-21 can stimulate host immune response like boosting the T-cell response or B cell response, it will be of great interest to understand whether its effect is mediated via immunological changes such as decrease of PD-1, or Treg or DC.

HBeAg seroconversion confers favorable outcomes together with significant immune alterations in vivo. Due to the versatile nature of virus-host immune interaction, it is reasonable to speculate that just measuring one immunological parameter, such as the frequency or function of a particular immunocyte or the concentration of a single cytokine, will not be sufficient to correctly identify the biomarker of clinical relevance in CHB patients. Hence, future studies should figure out a unique panel of immune biomarkers for the evaluation of the efficacy of the various anti-HBV therapies and the clinical outcome for CHB patients. Though HBeAg seroconversion is a critical prerequisite for the clearance of HBsAg, there is a lack of immune markers to predict the HBsAg seroconversion (Fig. 1). In particular, no available immune markers have been found to reflect the dynamic alteration of HBsAg loss together with the appearance of HBsAb in patients with HBsAg seroconversion. To better optimize antiviral therapy, there is a call for more translational study. More studies, based on serially collected clinical samples in well-designed prospective clinical trials, will be required to obtain comparable data and reference values for surrogate immune markers with relevance to an ideal treatment end-point (HBsAg seroconversion). In addition, it is necessary to develop prognostic scores or formulas on the combination of some immune markers, physiological, and biochemical indexes to help physicians make decisions on who should be treated, how to treat, and when to stop treatment.
Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

References


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