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J Clin Invest. 2018;128(10):4257-4259. <https://doi.org/10.1172/JCI124098>.

Commentary

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Entering the spotlight: hepatitis B surface antigen-specific B cells

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Hepatitis B virus-specific (HBV-specific) T cells have been identified as main effector cells in HBV clearance. In contrast, B cells producing neutralizing antibodies against the HBV surface antigen (HBsAg) have been studied in little detail, mainly due to methodical limitations. In this issue of the *JCI*, two reports use a new technique to specifically detect and characterize HBsAg-specific B cells *ex vivo*. Indeed, these cells are present, but show phenotypic alterations and impaired function during acute and chronic HBV infection. Thus, HBsAg-specific B cells are a novel attractive target for antiviral strategies toward functional cure of chronic HBV infection.

Hepatitis B surface antibody in history and treatment

A fourth of the world's population has been infected with hepatitis B virus (HBV), and an estimated 250 million people are chronically infected, putting them at risk for progressive liver disease, liver cirrhosis, liver failure, and hepatocellular carcinoma. Of adults infected with HBV, 95% clear the virus spontaneously, while 90% of newborns develop chronic infection. Since HBV infection does not induce an interferon response, HBV is a stealth virus with respect to the innate immune system (1, 2). Therefore, HBV clearance depends on a successful adaptive immune response. Indeed, a multispecific and vigorous HBV-specific CD8⁺ T cell response has been associated with viral clearance (3, 4), while HBV-specific CD8⁺ T cell exhaustion is a hallmark of persistent infection (5-7). Less attention, however, has been paid to the role of neutralizing antibodies targeting the envelope protein of HBV, the HBV surface antigen (HBsAg). Indeed, the hepatitis B surface antibody (anti-HBs) is only detectable in the blood of HBV-infected individuals after the loss of HBsAg, either upon the resolution of

acute infection or in case of the rare event of spontaneous or drug-induced clearance of chronic HBV infection. Thus, anti-HBs is seen as not playing a major role in HBV clearance. Anti-HBs, on the other hand, has been established as a diagnostic tool, indicating protective immunity after acute-resolving HBV infection or vaccination as well as “functional cure” of chronic HBV infection (HBsAg to anti-HBs seroconversion). There are, however, several clinical observations that argue against such a minor role of anti-HBs. First, anti-HBs contained in hepatitis B immunoglobulin (HBIG) is well known to prevent vertical HBV transmission from infected mothers to newborns as well as reinfection of liver transplants in previously HBV-infected hosts. Second, B cell-depleting drugs used in oncology and rheumatology, such as the anti-CD20 antibody rituximab, induce HBV reactivation in HBV carriers and even in patients with “resolved” HBV in whom nuclear HBV cccDNA persists as a viral reservoir for decades (8). These clinical observations indicate that anti-HBs has a more prominent role than previously acknowledged. Indeed, anti-HBs can block HBV cell entry, either by block-

ing the binding site of HBsAg for the cell receptor recently identified to be sodium taurocholate cotransporting polypeptide (NTCP) or by binding to the “antigenic loop” of HBsAg that is involved in interaction with heparan sulfate proteoglycan (HSPG) in a prereceptor step of cell entry. In addition to direct inhibition of cell entry, anti-HBs also mediate endocytosis and subsequent neutralization of virions as well as Fc-mediated phagocytosis and killing of infected cells. In addition, anti-HBs, HBsAg, and additional components build immune complexes that can activate HBV-specific T cells (9).

HBsAg-specific B cells in acute and chronic HBV infection

Given these important antiviral functions of anti-HBs, a main open issue in HBV immunobiology is the reason for the lack of detectable anti-HBs in both acute and chronic HBV infection prior to HBsAg loss. To date, two plausible hypothetical explanations for the lack of anti-HBs have been put forth. First, it was suggested that anti-HBs is produced by HBsAg-specific B cells in acute and chronic HBV infection, but is depleted by the large number of circulating HBsAg-containing subviral particles that outnumber complete virions by an order of magnitude. This hypothesis was supported by the finding that complexes of anti-HBs with circulating HBsAg are not recognized by current diagnostic assays, but can be detected in chronically infected patients using highly sensitive immunoassays (10). Additionally, there were early and have been more recent reports of HBsAg-specific B cell dysfunction, especially in chronic HBV infection. Indeed, B cells isolated from patients with chronic HBV infection showed a reduced proliferative capacity and were not able to produce anti-HBs upon stimulation. This functional defect reverted after the rare event of HBsAg loss during chronic infection (11, 12). In the current issue of the *JCI*, Salimzadeh and Le Bert et al. from the

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Conflict of interest: The authors have declared that no conflict of interest exists.

Reference information: *J Clin Invest*. 2018;128(10):4257-4259. <https://doi.org/10.1172/JCI124098>.

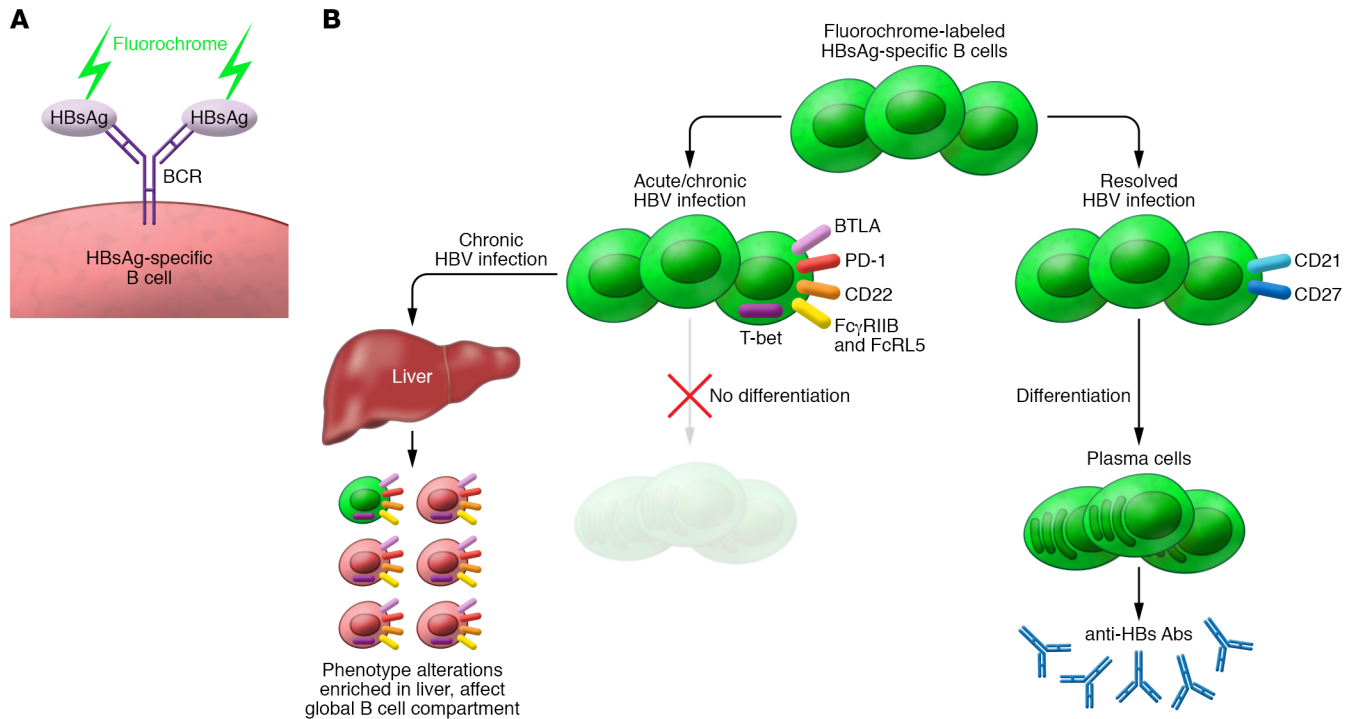


Figure 1. HBsAg-specific B cells in HBV infection. (A) Fluorochrome-labeled HBsAg binds to the HBsAg-specific B cell receptor (BCR) of HBsAg-specific B cells, allowing their detection and characterization directly ex vivo. Using this approach, phenotypical as well as functional differences between HBsAg-specific B cells in acute and chronic infection versus resolved infection were identified. (B) In acute and chronic infection, HBsAg-specific B cells are present in similar frequencies compared with their presence in resolved infection; however, they display low expression of CD21 and CD27 (consistent with atypical memory B cells) as well as a high expression of inhibitory receptors, such as PD-1, BTLA, CD22, Fc γ RIIB, and FcRL5. They also highly express the transcription factor T-bet and are not able to differentiate into anti-HBs-secreting plasma cells. These phenotypical alterations are enriched in the liver. However, they are not restricted to HBsAg-specific B cells, but affect the global B cell compartment of patients with acute or chronic HBV infection.

Bertoletti laboratory (13) and Burton et al. from the Maini laboratory (14) addressed the issue of lack of anti-HBs during acute and chronic HBV infection by making use of a new technique to detect HBsAg-specific B cells. Specifically, they utilized fluorochrome-labeled recombinant HBsAg as “bait” to detect and characterize HBsAg-specific B cells by FACS analysis ex vivo. Strikingly, by using a similar approach independently, the two groups came to the very same results (Figure 1): first, HBsAg-specific B cells are present in acute, chronic, and resolved HBV infection in similar frequencies. Second, HBsAg-specific B cells isolated from patients with acute as well as chronic, but not resolved, HBV infection are unable to mature into anti-HBs-secreting cells in vitro. Third, in phenotypical analyses, these HBsAg-specific B cells in acute and chronic HBV infection resemble “atypical memory B cells” that are characterized by low expression of CD21 and CD27, but high expression of inhibitory markers, such as PD-1, and the transcription factor

T-bet (15). Fourth, Burton et al. demonstrated that these HBsAg-specific atypical memory B cells accumulate in the liver, the site of infection and inflammation of HBV-infected patients. Fifth, this phenotype and functional impairment are not restricted to HBsAg-specific B cells, but also affect the global B cell population in HBV-infected patients, a finding with so far unclear implications that needs further clarification. Sixth, the function of HBsAg-specific B cells can be partially restored in vitro by specific culture conditions, such as PD-1 blockade (13, 14) or the addition of IL-2, IL-21, and CD40 ligand-expressing (CD40L-expressing) feeder cells (13).

Opening the doors for B cell-targeted research in hepatitis B

These results clearly argue for B cell dysfunction rather than antibody depletion as the main reason for the lack of anti-HBs in acute and chronic HBV infection. Thus, these findings also open the door for B cells as targets of novel antiviral strategies aiming at a functional cure of chronic HBV

infection (seroconversion from HBsAg to anti-HBs). Indeed, none of the currently developed antiviral strategies, including viral entry inhibitors, viral capsid release inhibitors, immune modulators, or therapeutic vaccination, achieve functional cure in a substantial number of patients, even if used in combination with the currently approved antiviral compounds — pegylated IFN- α and nucleoside/nucleotide analogs, such as tenofovir or entecavir (16). It is important to note that the current studies by Salimzadeh and Le Bert et al. and Burton et al. are not the completion, but just the beginning of B cell-targeted research in the field of hepatitis B, similar to the emerging T cell research after the broad introduction of peptide/MHC tetramers nearly two decades ago (17). Indeed, many open issues remain, such as the cellular and molecular mechanisms leading to HBsAg-specific B cell dysfunction, e.g., the involvement of impaired follicular helper T (Tfh) cells (18, 19) and the mechanisms and (clinical) consequences of impairment of the global B cell population as well as

the optimal strategy for restoring HBsAg-specific B cell function on the way to functional cure of chronic HBV infection. Of note, PD-1 blockade enhances antiviral B cell immunity against SIV in macaques in vivo (20). Specifically, it will be important to identify the best intervention for the restoration of B cell function, e.g., targeting of single versus multiple inhibitory receptors or choice of the most promising cytokine and feeder cell to be used in vivo and/or for adoptive cell transfer. With these important questions and exciting opportunities, HBsAg-specific B cells are expected to be in the spotlight for the next several years.

Acknowledgments

This work is supported by the Deutsche Forschungsgemeinschaft: SFB1160, Immune-mediated pathology as a consequence of impaired immune reactions (IMPATH), project 8 (to RT) and project 10 (to CNH); and TRR-179, Determinants and dynamics of elimination versus persistence of hepatitis virus infection, project 1 (to RT) and project 2 (to CNH).

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