Cytokine and Chemokine Responses in the Acute Phase of Hepatitis B Virus Replication in Naive and Previously Vaccinated Blood and Plasma Donors

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Background. Blood and plasma donor screening for hepatitis B virus (HBV) DNA, HBV surface antigen (HBsAg), and antibodies to surface (anti-HBs) and core (anti-HBc) antigens allows identification of individuals who acquired HBV despite previous HBV vaccination.

Methods. Of 14 HBV acute infection donor panels (HBV-DNA-positive/anti-HBc-negative), 6 donors were previously vaccinated (anti-HBs+). We investigated the differences in viral kinetics and immune responses in vaccinated and nonvaccinated individuals. Serial specimens were characterized for HBV DNA and serological markers and 39 cytokines.

Results. The rate of viral load increase was blunted, and virus was cleared more rapidly in vaccinated individuals (P = .004). In unvaccinated individuals, induced protein 10 (IP-10), interleukin 10 (IL-10), macrophage inflammatory protein 1β (MIP-1β), and soluble interleukin 2Rα (sIL-2Rα) levels were commonly elevated at the time of peak viremia. In contrast, vaccinated individuals had earlier peaks in IL-10 and IP-10 responses that occurred at much lower viral loads and coincided with anamnestic anti-hepatitis B surface (HBs) responses and clearance of viremia.

Conclusion. There is earlier engagement of innate and adaptive immunity in infected subjects with previous vaccination, possibly explaining suppressed viremia in vaccine breakthrough infections. Although breakthrough infections occur in partially protected vaccine recipients, vaccination likely contributes to early control of replication, limiting immune activation and preventing development of clinically significant acute and chronic HBV infection.

Keywords. hepatitis B virus; vaccination; cytokine; chemokine; acute infection; immunity.

Despite reduced transmission following implementation of an effective vaccine, hepatitis B virus (HBV) remains a significant global health problem. According to World Health Organization (WHO) statistics, 2 billion people have been infected with HBV, 350 million currently have chronic infections, and 4 million new clinical cases occur annually [1]. The kinetics of acute viremia, the subsequent detection of hepatitis B surface antigen (HBsAg) and then development of antibodies to core proteins (anti-HBc) and HBsAg (anti-HBs) during infection have been well described [2–5]. The initial immune recognition of HBV replication has a 10–12 week delay from the point of infectious exposure to detection of HBsAg in plasma and subsequent suppression of viral replication by adaptive immune responses [6]. During this time there are no clinically overt signs or symptoms, nor are there manifestations of viral or immune mediated hepatocyte damage, such as elevated transaminase levels in plasma. This prolonged “eclipse” period may be due to initial infection of a low number of hepatocytes, to early innate immunological control of viral spread, or to ineffective...
priming of an early immune response within the liver [7-9]. It is not until the accelerated ramp-up of viremia occurs weeks after infection that the adaptive immune response is triggered and detectable systemically, often associated with clinical acute hepatitis [10, 11].

The induction of an early immune response may be required for effective viral control and for preventing chronic infection. Recent studies have investigated this early period by using longitudinal panels of specimens from individuals with known dates of infection. This makes it possible to observe viral kinetics and circulating cytokine and cellular responses in an attempt to identify immunological profiles during subclinical infections [10, 11]. Shortly after the acute phase of infection, the virus is usually eradicated, but in a subset of patients HBV persists, leading to chronic infection and subsequent development of cirrhosis or hepatocellular carcinoma [12]. The viral and host mechanisms underlying differences between infections that resolve and those that become chronic are not well understood.

Since the 1980s, a prophylactic HBV vaccine has been available that induces strong humoral responses against the surface antigen after a 3-dose regimen. The HBV vaccine has been very successful at preventing infection when a protective antibody titer of 10 mIU/mL or greater is achieved, with >90% decreases of infection rates in infants and children [13], and consequent declines in late-stage complications of HBV infection, including cirrhosis and hepatocellular carcinoma. Nevertheless, HBV infection still does occur in vaccinated individuals following high-risk exposures, as evidenced by anti-HBc seroconversion in long-term follow-up studies [14-18]. More recently, vaccine breakthrough infections have been documented in blood donors with histories of previous vaccination and low levels of vaccine-induced anti-HBs who are detected as HBV DNA positive by screening using sensitive nucleic acid amplification technology (NAT) tests in parallel with HBV serological screening [4, 14, 19].

We hypothesized that previous vaccination would lead to suppression of viral replication kinetics by earlier induction of the innate and adaptive immune responses compared to infections in unvaccinated individuals. To investigate this hypothesis, vaccinated, previously uninfected individuals were identified by the presence of anti-HBs and lack of anti-HBc in index donation samples that tested positive for HBV DNA. Levels of viral DNA, HBsAg, anti-HBs, and anti-HBc, and a panel of cytokines were measured to demonstrate the evolution of HBV DNA, serological and immunological markers during acute infection in vaccinated compared to unvaccinated individuals. Our study extends the current understanding of early immune responses to HBV in unvaccinated individuals while providing insight into which cytokines/chemokines are induced during a virological challenge in individuals who had already been vaccinated, thereby providing a model for an effective, albeit nonsterilizing, vaccine-induced immune response [20].

PATIENTS AND METHODS

Study Samples

All testing was performed on de-identified repository specimens from consented blood and plasma donors and therefore not considered human subjects research. Longitudinally collected acute HBV infection panels were obtained from 3 sources (Table 1). The first source panels were identified by testing the first panel member for anti-HBs in 55 HBV plasma donor seroconversion panels provided to us by Zeptometrix Corporation and SeraCare Life Sciences. The serial plasmapheresis units from these donors had been collected prior to HBsAg positivity and held in quarantine at −20°C. When these donors tested positive for HBsAg, they were informed of their test results and counseled to follow up with local healthcare providers, and no further plasma units were collected. The existing donations were compiled into plasma seroconversion panels so that investigators could evaluate performance of HBV screening assays [5]. A total of 55 HBsAg seroconversion panels were available from these sources and screened for evidence of previous HBV vaccination by testing the first available samples in each panel for anti-HBs; 2 panels were identified as vaccine breakthrough cases and acquired for further study. The second source consisted of 8 plasma donor HBV seroconversion panels from Abbott Laboratories, which did not have anti-HBs reactivity preceding HBV DNA or HBsAg detection and reflected the dynamics of HBV infection in nonvaccinated subjects. Five of these panels were sourced and characterized by Abbott. Three of the panels were commercially available HBV seroconversion panels from BIOMEX GmbH (Heidelberg Germany), which Abbott provided for this study. Both of these sets of plasma donor panels were further characterized by PRISM HBsAg, PRISM HBc, AxSYM AUSAB, Corzyme (Abbott Laboratories, Abbott Park, IL) or DiaSorin assay for total anti-HBc, and by Abbott RealTime HBV (Abbott Laboratories, Abbott Park, IL). Results are expressed as S/CO values for PRISM HBsAg and the anti-HBc assays. For the data shown in the table and figures, the anti-HBc results were transformed to CO/S values. The detection limit of the RealTime HBV assay used in this study was 10 IU/mL. The third source of samples was the American Red Cross, from which serial samples from 4 HBV NAT yield blood donors were analyzed. All panels were defined as vaccine breakthrough infections based on the donors testing HBV DNA positive during blood donation screening (Procleix Ultro Hologic/Gen-Probe; HBV DNA lower limit of detection 11 IU/mL), seronegative for HBsAg (lower limit of detection, 0.10 ng/mL or 0.018 IU/mL) and anti-HBc (Abbott PRISM HBsAg and PRISM HBc), and positive for anti-HBs (BioRad). Additional testing of these donors included repeat
NAT and serology on residual index and follow-up samples and further testing for HBV DNA confirmation with the COBAS AmpliScreen HBV Test (Roche) with the 1 ml extraction method (95% lower limit of detection, 5 IU/mL) [4].

Cytokine Multiplex Testing

Panels were assayed using a high-sensitivity Milliplex kit (Millipore, Billerica, MA) with antibody coated beads for detection of GM-CSF, interferon γ (IFN-γ), interleukin 10 (IL-10), interleukin 12 (IL-12; p70), interleukin 13 (IL-13), interleukin 1 β (IL-1β), interleukin 2 (IL-2), interleukin 4 (IL-4), interleukin 5 (IL-5), interleukin 6 (IL-6), interleukin 7 (IL-7), interleukin 8 (IL-8), and tumor necrosis factor α (TNF-α), and a standard-sensitivity Milliplex Map kit (Millipore) for epidermal growth factor (EGF), eotaxin, fibroblast growth factor 2 (FGF-2), fractalkine, Flt-3 ligand, GRO, G-CSF, interferon α2 (IFN-α2), interleukin 1α (IL-1α), interleukin 1β (IL-1β), interleukin 3 (IL-3), interleukin 9 (IL-9), interleukin 12 (IL-12; p40), interleukin 15 (IL-15), interleukin 17 (IL-17), interferon gamma induced protein 10 (IP-10), monocyte chemotactic protein 1 (MCP-1), monocyte chemotactic protein 3 (MCP-3), macrophage-derived chemokine (MDC), macrophage inflammatory protein 1α (MIP-1α), macrophage inflammatory protein 1β (MIP-1β), soluble interleukin 2 (sIL-2) receptor α (Rα), transforming growth factor α (TGα), tumor necrosis factor β (TNF-β), vascular endothelial growth factor (VEGF), and soluble CD40 ligand (sCD40L). Testing was performed following the manufacturer’s protocols. Panel longitudinal samples were run on the same plate and in sequence for consistency, but without knowledge of the vaccination or HBV marker status. Standard curves and samples were tested in duplicate. Results were acquired on a Luminex 100 analyzer (Luminex, Austin, TX) using Bio-Plex manager software (Bio-Rad, Hercules, CA), and study plates were compiled using Data Pro (Bio-Rad).

Statistical Analysis

Throughout analyses subjects were divided into vaccinated and nonvaccinated groups. Viral loads were log-transformed and linear regressions were used to calculate HBV doubling times (DT) during the ramp-up phase for each subject as published elsewhere [5, 21]. Based on these analyses, we further estimated the days that viral loads in each subject would have reached a level of 50 IU/mL and then assigned this time point as day 0 (D0) to normalize sample time-points for the longitudinal synchronization of cytokine responses and other virological parameters. Heat maps showed the differences across various cytokines at different time points by normalizing data and computing Z scores depicting strength of response by a spectrum of color from red (strong response) to yellow (weak response). Analysis of variance, t-test or Mann–Whitney, and regression were applied to compare differences or identify...
correlations between groups or variables. P values were computed and then adjusted to account for false discovery rates (FDR) by the Benjamini and Hochberg controlling procedure [22]. Statistical significance was defined by $P < .05$ and FDR $< .01$. R/Bioconductor and Prism 5.0 software were used for statistical analyses.

RESULTS

Study Subjects

We studied 14 acute HBV infection series from paid plasma donors ($N = 10$) and volunteer blood donors ($N = 4$). The plasma donor panels, which had a mean of 24 serial samples (range 9–31; see Table 1), were originally collected before FDA-approved NAT screening assays were developed and implemented. Consequently, samples from each donor had been prospectively screened for HBsAg and subsequently characterised for anti-HBc, anti-HBs and HBV viral load.

Eight of the 10 plasma donor seroconversion panels had acute HBV infection without evidence of prior vaccination; these included 7 panels with long follow-up periods that demonstrated clearance of HBsAg and seroconversion to anti-HBc and then anti-HBs, and 1 panel from a donor who developed chronic hepatitis B infection evidenced by persistent HBV DNA and HBsAg for longer than 6 months with seroconversion to anti-HBc but not anti-HBs.

We studied 6 individuals who were identified as having confirmed or probable HBV vaccine breakthrough infections. Based on anti-HBs screening of the first samples of 55 plasma donor panels, we identified 2 plasma donor panels with evidence of probable vaccine breakthrough infection (ie, the presence of anti-HBs and HBV DNA in the absence of anti-HBc on the first available plasma donation sample, followed by the appearance of HBsAg on subsequent donation-derived samples). These 2 panels had 9 and 26 longitudinal samples (Table 1). There were 4 additional previously vaccinated whole blood donors who were identified by NAT testing as having HBV vaccine breakthrough infections, as reported elsewhere [4]. These blood donor vaccine breakthrough infection panels had an average of 10 serial samples (range, 8–13) that were taken at convenient time intervals following HBV DNA detection on index donations.

Replication Kinetics in Vaccinated vs Nonvaccinated Subjects

To confirm and extend prior observations of blunted acute phase viremia in previously HBV vaccinated subjects [4], viral load and HBsAg were measured longitudinally (Figure 1). Doubling times (DTs; ie, the time in days for the plasma HBV viral load to double in concentration) were calculated to determine if DTs differed based on prior vaccination status. Of the 14 total individuals for whom serial samples were available, one vaccinated case that had no change in viral load over time was excluded from the DT calculation for the overall vaccinated group. The average DT for the nonvaccinated donors was 2.7 days (range, 2.0–4.2 days), consistent with previous studies [5, 21, 23], whereas ramp-up viremia was significantly slower with an average DT of 14.0 days (range, 6.7–30.3 days) for the 5 vaccinated individuals with estimated DTs ($P = .004$). Thus, the rate of increase in viral DNA was suppressed in infected donors with preexisting vaccination compared to nonvaccinated individuals (Tables 2) [5].

Individual Cytokine Responses and Kinetics after

Overall, 39 cytokines were measured for all subjects. Figure 2 illustrates the dynamics of immune responses relative to viral parameters for a representative unvaccinated resolved infection case (Abbott-02; Figure 2A) and a vaccine-breakthrough case (Zepto-01; Figure 2B), as well as the single case of acute infection that progressed to a chronic carrier (Abbott-01; Figure 2C). When cytokine responses for each individual were graphed to investigate responses over time, we found that peak responses occurred soon after peak levels of HBV DNA in unvaccinated individuals (Figure 3). Specifically, IP-10, sIL-2Ra, MIP-1β, and IL-10 cytokine responses were synchronized at the time of peak viremia in the unvaccinated individuals. Although these same cytokines were also elevated during the suppressed ramp-up viremia phase in the vaccinated group, these cytokine elevations occurred prior to peak viremia and only 2 individuals had time points collected that show distinct peak elevations in their cytokine responses (Figures 3 and 4).

Earlier Immune Induction with Vaccination

To align cytokine responses with viral replication dynamics, each subject’s data points were examined relative to the imputed viral load of 50 IU/mL, and this time point was set as day 0 ($D_0$). We used IP-10 and IL-10 for comparison because these cytokines had been previously shown to be induced during acute viral infections [10] and were also consistently elevated in the unvaccinated donors in our study. In the unvaccinated group (Figure 3), the IP-10 response peaked at a mean of 64 days (95% confidence interval [CI], 45, 82) after $D_0$ and the IL-10 response peaked at 78 days (95% CI, 60, 96) after $D_0$. In the individual (Abbott-01) who became a chronic carrier, the initial IP-10 response peaked at day 89, more than 3 weeks after the unvaccinated HBV-infected individuals who cleared HBsAg, and other cytokine responses (IFN-γ, IL-5, IL-6, IL-13, and TNF-α) were elevated long after the acute phase of infection and immune response had subsided in the individuals who cleared viremia and seroconverted to anti-HBs (Figure 2C).

We hypothesized that subjects who were previously vaccinated would mount vigorous immune responses prior to or very early in the HBV ramp-up phase. Temporal analysis of cytokine responses was more difficult to characterize in the previously vaccinated subjects because we did not know the true
date of infection, and they were all viremic at the first available time point. Nonetheless we found that subject Zepto-01 had a distinct peak in IP-10 around 1 day after the imputed VL was 50 IU/mL (Figure 4). A similar analysis for subject Zepto-02 revealed peak levels for IP-10, IL-10, and MIP-1β around 35 days after D₀. The remaining 4 vaccinated subjects did not show the peaks in cytokine levels that were typically seen in unvaccinated subjects.

To further elucidate the timing of immune responses in relation to viral load, we quantified the change in time (ΔT) from the day of peak viral load to the day in peak cytokine. Although we did not have peak viral load information for the vaccinated individuals because the viral loads were still increasing at the final time point followed by negative DNA results, we chose the last DNA-positive time point as the time point for comparison. We found that the median ΔT for the IP-10 responses in unvaccinated individuals was 7 days (95% CI, 22, 26) after peak in viremia, whereas the 2 vaccinated individuals had peak elevations in IP-10 39 days (−64, −14) before the final detectable VL measurement. We found that the median ΔT for the IL-10 responses in unvaccinated individuals was 7 days (95% CI, 0, 26) after peak in viremia, whereas the 2 vaccinated individuals had

Figure 1. Synchronized viral growth responses show slower doubling time in vaccinated individuals. The viral kinetics were graphed, normalizing D₀ for all panels to the day that VL was 50 IU/mL. Vaccinated individuals are in red and nonvaccinated individuals are in black. HBV DNA and HBsAg were graphed using this synchronization. The viral kinetics show a much slower doubling time for the VL in the previously vaccinated individuals. Two vaccinated donors (Zepto-01 and Zepto-02) had HBsAg growth rates similar to unvaccinated donors. Except for one additional donor (ARC-05) who had delayed detection of HBsAg, donors (ARC-03, ARC-04, and ARC-06) all had HBsAg below the threshold of detection. Abbreviations: D₀, day 0; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; VL, viral load.
individuals in our study, the longitudinal study design allowed the date of the infectious exposure to be unknown in all of the matched peripheral blood mononuclear cells to study the cellular immune responses. Although vaccine-induced pre-existing immunity shows early innate and adaptive immune responses that may be specific to infections that occur despite partial vaccine-induced protection. Although the date of the infectious exposure was unknown in all of the individuals in our study, the longitudinal study design allowed for interpolation of the time at which the viral load crossed a threshold of 50 IU/mL. This made it possible for us to synchronize the panels and analyze the viral and immunological kinetics relative to a defined low-viral load threshold [5]. After normalizing the timelines we found that the unvaccinated individuals had similar viral kinetics, with an average DT of around 3 days, which is very similar to DTs previously reported by our group and Japanese Red Cross investigators based on analyses of larger numbers of plasma and blood donor acute HBV infection panels derived from nonvaccinated individuals [5, 21, 23]. Conversely, the vaccinated donors showed much slower viral kinetics over this same pre-HBsAg stage of infection, with a DT of approximately 14 days. This slower viral DT was likely due to the earlier induction of innate and adaptive immune responses and/or the presence of low levels of nonsterilizing but partially neutralizing anti-HBs antibodies as a result of vaccination acting in suppressing HBV production from hepatocytes and/or clearing plasma virus. In these cases, ramp-up viremia was moderated and fully cleared soon after infection, often without development of detectable levels of HBsAg.

We investigated the effect of vaccination on viremia and immune responses by qualitatively comparing cytokine responses in unvaccinated and previously vaccinated individuals. Once we synchronized the time courses of the panels, immune responses in the unvaccinated group also synchronized, and there were distinct peaks in IP-10, sIL-2Rα, MIP-1β and IL-10 that coincided with or occurred immediately subsequent to peak viremia (Figure 3). In the vaccinated group, the immune responses were not synchronized; however, there were increases in many of the same cytokines and chemokines (Figure 4) that occurred during very early infection based on coinciding HBV DNA levels. The earlier induction of the same cytokine responses in the vaccinated group suggests that they have vaccine induced immunological memory and that a lower activation threshold enables faster immune responses with earlier control of viremia. These results suggest that these early immune responses may be involved in the resolution of viremia. Thus, an earlier and more substantial engagement of host immunity in pre-vaccinated individuals with vaccine-breakthrough infections could explain the suppressed viral load profile with longer DTs and generally undetectable HBsAg.

Table 2. Doubling Time Calculations

<table>
<thead>
<tr>
<th>Unvaccinated/ Vaccinated</th>
<th>No. of Samples</th>
<th>Doubling Time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unvaccinated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abbott-01</td>
<td>10</td>
<td>2.7</td>
</tr>
<tr>
<td>Abbott-02</td>
<td>8</td>
<td>2.4</td>
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<tr>
<td>Abbott-03</td>
<td>16</td>
<td>2.9</td>
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<td>Abbott-04</td>
<td>5</td>
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<td>8</td>
<td>2.4</td>
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<tr>
<td>Abbott-06</td>
<td>8</td>
<td>2.2</td>
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<tr>
<td>Abbott-07</td>
<td>7</td>
<td>4.2</td>
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<tr>
<td>Abbott-08</td>
<td>15</td>
<td>2.8</td>
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<tr>
<td>Mean unvaccinated (95% CI)</td>
<td>2.7 (2.68–3.12)</td>
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| Vaccinated               |                |                      |
| Zepto-01                 | 26             | 30.3                 |
| Zepto-02                 | 9              | 6.7                  |
| ARC-03                   | 2              | 6.8                  |
| ARC-04                   | 6              | 17.0                 |
| ARC-05                   | NA             | NAb                 |
| ARC-06                   | 3              | 9.4                  |
| Mean vaccinated (95% CI) | 14.0 (9.95–18.13) |                      |

Abbreviations: CI, confidence interval; NA, not available.

a Number of “ramp-up” samples contributing to DT calculation.

b NA, not applicable and represents a case in which we were not able to perform doubling time calculations because there were too few timepoints with detectable HBV DNA and less than a 1 Log change in values.

DISCUSSION

Vaccination effectively prevents the large majority of symptomatic and asymptomatic acute HBV infections as well as chronic HBsAg+ infections, but studies show that breakthrough infection can still occur [4, 15]. Although we conducted this study using historic plasma specimens from incident HBV infection in blood and plasma donors, we did not have matched peripheral blood mononuclear cells to study the cellular immune response in more detail. However, our analysis of the dynamics of viral and serological markers in the context of vaccine-induced pre-existing immunity shows early innate and adaptive immune responses that may be specific to infections that occur despite partial vaccine-induced protection. Although the date of the infectious exposure was unknown in all of the individuals in our study, the longitudinal study design allowed elevations in IL-10 39 days (95% CI, −64, −14) before the final detectable VL measurement. The difference in times from peak or final viral loads to peaks in cytokines between the nonvaccinated and vaccinated cases were statistically significant ($P = .03$).

850 • JID 2014:209 (15 March) • Keating et al
they found that individuals with acute HBV infection had no significant increases in IL-15, IFN-α, IFN-λ during the peak in viremia [11]. In that study, and similar to our findings, IL-10 peaked around the time viremia peaked, potentially indicating support of a Th2 response for antibody production to control virus.

One major difficulty in conducting longitudinal studies of acute infection is the timing of the blood draws. Although it is sometimes possible to obtain blood samples from volunteers at similar time points (eg, from regular plasma donors), those time points may not be optimized for the course of the disease. Differences in the results seen with nonvaccinated and vaccinated infected donors may be due partially to the variation in timing of sample collection. The panels we analyzed came from plasma donation sites with twice weekly donations and one blood donation group where donors were enrolled and followed at variable intervals relative to documentation of HBV infection. We could normalize these differences by synchronizing

Figure 2. Representative cytokine responses during acute HBV infection. Thirty-nine cytokines, chemokines, and growth factors were measured in longitudinal samples and graphed by heat map alongside clinical viral load, HBsAg, anti-HBc and anti-HBs levels. A, A panel from an unvaccinated donor with acute HBV infection shows several biomarkers peak after ramp-up in viremia and HBsAg, including IP-10, IL-10, IL-2, IL-5, TNF-α, and MIP-1β. B, A panel from a previously vaccinated HBV-infected individual shows peak immune responses before viral ramp-up for IP-10, IL-10, IL-2, IL-8, IL-12 (p70), IFN-γ, GM-CSF, TNF-α, IL-1α, IL-1β, MCP-1, MCP-3, TGF-α and sIL-2Ra. C, Panel cytokine responses from an individual with acute HBV infection who did not clear HBV shows sustained increases in inflammatory and Th2 cytokines IP-10, IL-1β, IL-2, IL-5, G-CSF, IFN-α2, and MCP-1. Abbreviations: G-CSF, granulocyte colony stimulating factor; GM-CSF, granulocyte macrophage colony stimulating factor; HBc, hepatitis B core; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; IFN, interferon; IL, interleukin; IP, interferon gamma induced protein; MCP, monocyte chemotactic protein; MIP, monocyte inducible protein; TGF, tumor growth factor; TNF, tumor necrosis factor.
the viral kinetics and consequently study the immune response relative to viral parameters. Although this helped us to compare cytokine responses, intermittent blood draw timing possibly missed some transient immune responses. Our study nonetheless revealed significantly elevated immune responses during acute infection in nonvaccinated and vaccinated individuals, and we demonstrated that although HBV vaccination may not be “sterilizing” in all cases, when breakthrough infections occur they are characterized by earlier immune responses in the context of suppressed viral replication dynamics.

In conclusion, our study shows that earlier initiation of immune responses may mediate vaccine-induced protection preventing symptomatic acute and chronic HBV infections in individuals with HBV vaccine breakthrough infections. We identified common early induction of several cytokines and chemokines (ie, IP-10, sIL-2Rα, MIP-1β and IL-10) that may
play a role in triggering earlier adaptive cellular responses, thus regulating inflammation and inducing anamnestic anti-HBs antibodies to help control viremia. Further studies of viral control and immune response in the context of pre-existing vaccination are needed to fully understand mechanisms underlying vaccine breakthrough infection such as incomplete seroconversion, waning anti-HBs, or prevalence of diverse HBV genotypes. Finally, studies elucidating consequences of vaccine
breakthrough infection should be conducted to determine if there is persistence of HBV after breakthrough infection and if these individuals are susceptible to reactivation in the context of immunosuppression as in classically resolved HBV infections [24].

Notes

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Potential conflicts of interest. M. K. and C. M. are employed by Abbott Laboratories. M. B. was a member of Gen-Probe (now Hologic) Scientific Advisory Committee. All other authors report no potential conflicts.

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