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Original Article

COVID-19 Vaccination Alters NK Cell Dynamics and Transiently Reduces HBsAg Titers Among Patients With Chronic Hepatitis B

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ABSTRACT

Coronavirus disease 2019 (COVID-19) vaccination may non-specifically alter the host immune system. This study aimed to evaluate the effect of COVID-19 vaccination on hepatitis B surface Ag (HBsAg) titer and host immunity in chronic hepatitis B (CHB) patients. Consecutive 2,797 CHB patients who had serial HBsAg measurements during antiviral treatment were included in this study. Changes in the HBsAg levels after COVID-19 vaccination were analyzed. The dynamics of NK cells following COVID-19 vaccination were also examined using serial blood samples collected prospectively from 25 healthy volunteers. Vaccinated CHB patients (n=2,329) had significantly lower HBsAg levels 1–30 days post-vaccination compared to baseline (median, −21.4 IU/ml from baseline), but the levels reverted to baseline by 91–180 days (median, −3.8 IU/ml). The velocity of the HBsAg decline was transiently accelerated within 30 days after vaccination (median velocity: −0.06, −0.39, and −0.04 $\log_{10}$ IU/ml/year in pre-vaccination period, days 1–30, and days 31–90, respectively). In contrast, unvaccinated patients (n=468) had no change in HBsAg levels. Flow cytometric analysis showed that the frequency of NK cells expressing NKG2A, an NK inhibitory receptor, significantly decreased within 7 days after the first dose of COVID-19 vaccine (median, −13.1% from baseline; $p<0.001$). The decrease in the frequency of $NKG2A^+$ NK cells was observed in the $CD56^{dim}CD16^+$ NK cell population regardless of type of COVID-19 vaccine. COVID-19 vaccination leads to a rapid, transient decline in HBsAg titer and a decrease in the frequency of $NKG2A^+$ NK cells.

Keywords: COVID-19 vaccines; Chronic hepatitis B; HBsAg; NK cells; NKG2A

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Conflict of Interest
Yun Bin Lee reports receiving research grants from Samjin Pharmaceuticals and Yuhan Pharmaceuticals. Yoon Jun Kim reports receiving research grants from Bristol-Myers Squibb, Roche, JW Creagene, Bukwang Pharmaceuticals, Handok Pharmaceuticals, Hanmi Pharmaceuticals, Yuhan Pharmaceuticals, and Pharmaking, and lecture fees from Bayer HealthCare Pharmaceuticals, Gilead Science, MSD Korea, Yuhan Pharmaceuticals, Samil Pharmaceuticals, CJ Pharmaceuticals, Bukwang Pharmaceuticals, and Handok Pharmaceuticals. Jung-Hwan Yoon reports receiving research grants from Bayer HealthCare Pharmaceuticals, Daewoong Pharmaceuticals, and Bukwang Pharmaceuticals. Jeong-Hoon Lee reports receiving research grants from Yuhan Pharmaceuticals, and lecture fee from GreenCross Cell, Daewoong Pharmaceuticals, and Gilead Korea. All other authors have declared that no conflict of interest exists.

INTRODUCTION

Chronic hepatitis B (CHB) is the most prevalent chronic viral infection globally (1). When hepatitis B virus (HBV) replication is suppressed with antivirals, the serum hepatitis B surface Ag (HBsAg) titer gradually decreases, and HBsAg-seroclearance can be achieved, though it is estimated to take 40 years in principle (2,3). In some cases, antiviral treatment precipitates HBsAg clearance, which may be induced by immune activation, leading to a functional cure. Patients who achieve HBsAg-seroclearance have a more favorable prognosis with a lower risk of developing hepatocellular carcinoma (HCC) than those who do not (3,4).

The use of coronavirus disease 2019 (COVID-19) vaccines was approved in December 2020 (5), and more than 12 billion doses have been administered globally (6). Neutralizing antibodies and memory T cells elicited by COVID-19 vaccination have been shown to play a pivotal role in protecting against COVID-19 infection and progression to severe disease (7).

COVID-19 vaccines have been the most commonly delivered vaccine worldwide since 2020, but the influence of COVID-19 vaccination on various chronic liver diseases is still unclear. Recent case reports and series suggest that COVID-19 immunization may enhance intrahepatic immune responses and lead to a decrease in HBsAg in CHB patients (8). Several cases of autoimmune hepatitis flare-up have been reported, which implicates COVID-19 vaccination in enhanced autoimmunity (9-11). Several cases of acute flare-up following COVID-19 vaccination have also been reported in patients with CHC or CHB who were not treated with antiviral agents (12,13), suggesting that COVID-19 vaccination may stimulate the inflammatory response against pre-existing hepatitis viruses. In the same context, we hypothesized that COVID-19 vaccination could affect the HBsAg titer of CHB patients who have suppressed serum HBV DNA with antiviral therapy by changing host immune responses.

In this study, we aimed to evaluate the dynamic change in HBsAg titer following COVID-19 vaccination in CHB patients whose HBV replication is suppressed by oral nucleos(t)ide analogues (NAs). In addition, we explored alterations in the NK cell population after COVID-19 vaccination.

MATERIALS AND METHODS

Study population

This study consisted of 2 parts: an investigation of the kinetics of HBsAg (the HBsAg kinetics study) and an analysis of immune cells after vaccination (the NK cell study). For the HBsAg kinetics study, consecutive patients with CHB negative for hepatitis B envelope antigen with serum HBV DNA suppressed below 1,000 IU/ml with NAs (e.g., tenofovir or entecavir) and whose HBsAg levels were measured at least 3 times between January 2019 and January 2022 at Seoul National University Hospital (Seoul, Korea) were eligible for inclusion. Patients were recommended to vaccinate in accordance with Korea Disease Control and Prevention Agency (KDCA) national vaccination guidelines, but some patients had not been vaccinated against COVID-19. Patients were classified into vaccinated and unvaccinated groups. The vaccinated group included patients who received one of the 4 most commonly used COVID-19 vaccines in Korea: BNT162b2 (Pfizer, New York, NY, USA), AZD1222 (AstraZeneca, Cambridge, UK), mRNA-1273 (Moderna, Cambridge, MA, USA), and JNJ-78436735 (Janssen, Beerse, Belgium). The date and type of COVID-19 vaccine were collected from the KDCA vaccination registry.

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Abbreviations

CHB, chronic hepatitis B; CI, confidence interval; COVID-19, coronavirus disease 2019; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; ICI, immune checkpoint inhibitor; ICS, intracellular cytokine staining; IQR, interquartile range; KDCA, Korea Disease Control and Prevention Agency; KIR, killer-cell immunoglobulin-like receptor; NA, nucleos(t)ide analogue; OR, odds ratio; TKI, tyrosine-kinase inhibitor; TIGIT, T cell immunoreceptor with immunoglobulin and ITIM domain.

Author Contributions

Writing - original draft: Shin H, Lee HS, Shin EC, Lee JH; Writing - review & editing: Noh JY, Koh JY, Kim SY, Park J, Chung SW, Hur MH, Park MK, Lee YB, Kim YJ, Yoon JH, Ko JH, Peck KR, Song JY, Shin EC.

The unvaccinated group included patients who were never immunized with a COVID-19 vaccine. The study protocol was approved by the Institutional Review Boards of Seoul National University Hospital (No. H-2206-096-1332). Informed consent was waived because of the retrospective and anonymized nature of the data.

For the NK cell study, a pre-established prospective cohort of healthy volunteers who donated peripheral blood samples, which were drawn once on the day of vaccination and every 3–16 days for 3 months after vaccination, was used. The study protocol was approved by the Institutional Review Boards of Korea Advanced Institute of Science and Technology (Daejeon, Korea; No. 21-379), Samsung Medical Center (Seoul, Korea; No. 2021-01-165), and Korea University Guro Hospital (Seoul, Korea; No. 2021GR0099). Informed consent was obtained from all participants before enrollment.

HBsAg measurement

HBsAg levels were measured using the Elecsys HBsAg II assay (Roche Diagnostics, Basel, Switzerland) with the Modular Analytics E170 system (Roche Diagnostics) and/or ARCHITECT HBsAg Qualitative assays (Abbott Diagnostics, Chicago, IL, USA) with the Architect i2000SR analyzer (Abbott Laboratories, Chicago, IL, USA). When the output value was given in a signal-to-cutoff ratio, the relevant conversion formula was employed to convert the units to IU/ml (14).

Day 0 (index date) was defined as the day on which the first COVID-19 vaccination was received by the vaccinated patients. For the unvaccinated patients, day 0 was allocated as June 17, 2021, which was the median date of the first dose of COVID-19 vaccine among the vaccinated patients. The difference in HBsAg levels at set timepoints from baseline (i.e., HBsAg value last obtained before day 0) was determined for each patient. To interpret the kinetics of HBsAg, changes in median HBsAg values at specific timepoints (i.e., days −360 to −180, days −180 to −1, days +1 to +30, days +31 to +90, and days +91 to +180) were evaluated and compared to baseline values. Negative and positive values presented in the expression of time intervals indicate before and after day 0, respectively.

Isolation of PBMC

We used Lymphocyte Separation Medium (Corning Inc., Corning, NY, USA) to isolate PBMCs by density gradient centrifugation. After isolation, PBMCs were cryopreserved in FBS (Corning Inc.) containing 10% DMSO (Sigma-Aldrich, St. Louis, MO, USA) until use.

Multi-color flow cytometry

Multi-color flow cytometry was performed to examine the phenotypes of NK cells. Thawed PBMCs were stained with fluorochrome-conjugated antibodies against surface markers for 15 min at room temperature. LIVE/DEAD red fluorescent reactive dye (Invitrogen, Waltham, MA, USA) was used to exclude dead cells. Stained cells were analyzed using the BD LSR II Flow Cytometer (BD Biosciences, Franklin Lakes, NJ, USA) and data analyzed using FlowJo software (FlowJo LLC, San Francisco, CA, USA). **Supplementary Table 1** list all of the antibodies used for flow cytometry.

Intracellular cytokine staining (ICS)

ICS assays were performed to examine HBV-specific T-cell responses using PBMC samples from 2 HBV-infected donors (one vaccinated with BNT162b2 and the other with AZD1222). Thawed PBMCs were stimulated with the 1 μg/ml overlapping peptides of HBsAg (PM-HBV-LEP) or HBcAg (PM-HBV-CP) (JPT, Berlin, Germany) and 1 μg/ml anti-human CD28

and CD49d monoclonal antibodies for 6 h at 37°C. Although HBV in South Korea is mostly genotype C (15), we used overlapping peptide pools of genotype A2 in the current study. Amino acid sequences of HBsAg and HBcAg exhibit 92.5% and 94.6% homology between genotype A2 and C, respectively. One hour after the initial stimulation, brefeldin A (GolgiPlug; BD Biosciences) and monensin (GolgiStop; BD Biosciences) were added. Negative controls were cultured with DMSO and anti-CD28/CD49d. The cells were collected and stained with fluorochrome-conjugated antibodies for surface markers. Cells were permeabilized with the FoxP3 staining buffer kit (Invitrogen), and then further stained for cytokines.

Statistical analysis

Statistical analysis was performed with the Student's *t*-test, Mann-Whitney *U* test, ANOVA, and Kruskal-Wallis test to analyze quantitative variables, whereas the χ^2 test and Fisher test were used for qualitative variables. Univariable analysis included baseline characteristics (age and sex) and clinical characteristics (liver cirrhosis, current or previous HCC, and comorbidities). Nonparametric continuous variables are presented as medians with interquartile range (IQR) unless otherwise specified. Categorical variables are presented as absolute cases and/or percentages. Odds ratios (ORs) are presented with 95% confidence intervals (CIs). All data were analyzed using SPSS version 26 (IBM, Armonk, NY, USA), R statistics version 4.2.0 (The R Foundation, Vienna, Austria), and GraphPad Prism version 8.4.2 (GraphPad Software, San Diego, CA, USA). Two-sided p-values were determined in all analyses. The $p<0.05$ was considered significant.

RESULTS

Baseline characteristics

A total of 2,797 patients were enrolled in the study (**Supplementary Fig. 1**), and their baseline characteristics are presented in **Table 1**. Among 2,329 patients who received a COVID-19 vaccination, 994 patients were immunized with BNT162b2, 298 with AZD1222, 174 with mRNA-1273, and 3 with JNJ-78436735. Heterologous boosting vaccines were administered to 842 patients, the majority of whom were immunized with 2 doses of AZD1222 and received a third dose as mRNA vaccine (i.e., BNT162b2 and mRNA-1273) (**Table 2**). Eighteen patients received only the first shot of BNT162b2, AZD1222, or mRNA-1273 without a recommended second shot. A total of 468 patients were not vaccinated against COVID-19 throughout the observation period and were classified as the unvaccinated group (**Supplementary Table 2**). HBsAg kinetics were studied between January 2019 and January 2022. During this time span, the cumulative rate of COVID-19 infection, as evaluated by positivity for anti-nucleocapsid antibody, was <1% in South Korea (16,17), indicating that cases of unreported infection were rare among the population.

HBsAg kinetics after COVID-19 vaccination

A total of 21,519 serum HBsAg measurements from 2,797 CHB patients were obtained and changes in HBsAg titers analyzed at multiple timepoints compared to baseline levels. In the vaccinated group (**Fig. 1A**), HBsAg levels decreased significantly in the period +1 to +30 days (median, −21.4 [IQR, −65.8 to +3.8] IU/ml from baseline; $p<0.001$), +31 to +90 days (median, −13.2 [IQR, −57.2 to +10.3] IU/ml from baseline; $p<0.001$), and +91 to +180 days (median, −4.4 [IQR, −48.7 to +30.3] IU/ml from baseline; $p<0.001$) compared to baseline. There was a tendency for gradual recovery to baseline HBsAg levels in the period +91 to +180 days. In contrast, in the unvaccinated group, no significant change occurred in HBsAg titer between +1

Table 1. Characteristics of patients with CHB

Characteristic	Value (n=2,797)
Age (years), median (IQR)	62.0 (55.0–68.0)
Male	1,872 (66.9)
Liver cirrhosis	1,132 (40.5)
HCC	1,393 (49.8)
Anti-HBV antivirals	
ETV	1,402 (49.8)
TDF	1,085 (38.8)
TAF	115 (4.1)
Others*	195 (7.0)
COVID-19 vaccine	
Fully vaccinated with	
BNT162b2	994 (35.5)
AZD1222	298 (10.7)
mRNA-1273	174 (6.2)
JNJ-78436735	3 (0.1)
Heterologous vaccinated†	842 (30.1)
Not fully vaccinated	18 (0.6)
HBsAg >100 IU/ml‡	1,618 (57.8)

Values are number (%) unless otherwise noted.
ETV, entecavir; TDF, tenofovir disoproxil fumarate; TAF, tenofovir alafenamide; BNT162b2, COVID-19 vaccine from Pfizer; AZD1222, COVID-19 vaccine from AstraZeneca; mRNA-1273, COVID-19 vaccine from Moderna; JNJ-78436735, COVID-19 vaccine from Janssen.
*Including cases taking a combination of several antivirals or changing the antivirals during the observation period.
†Two or more inoculations with 2 or more vaccines.
‡Based on initial values measured during the observation period.

Table 2. Type of vaccine use for each COVID-19 vaccination dose

Dose	Value
1st dose	2,329
AZD1222	1,110 (47.7)
BNT162b2	1,006 (43.2)
mRNA-1273	177 (7.6)
JNJ-78436735	36 (1.5)
2nd dose	2,309
BNT162b2	1,083 (46.9)
AZD1222	1,014 (43.9)
mRNA-1273	176 (7.6)
JNJ-78436735	36 (1.6)
3rd dose	1,511
BNT162b2	1,041 (68.9)
mRNA-1273	470 (31.1)

Values are presented as number (%).
BNT162b2, COVID-19 vaccine from Pfizer; AZD1222, COVID-19 vaccine from AstraZeneca; mRNA-1273, COVID-19 vaccine from Moderna; JNJ-78436735, COVID-19 vaccine from Janssen.

to +30 days (median, −0.01 [IQR, −0.08 to +0.03] $\log_{10}$ IU/ml from baseline; p=1.00; **Fig. 1B**). The velocity of HBsAg decline was accelerated between +1 to +30 days after the first dose of COVID-19 vaccine (median, −0.39 [IQR, −0.88 to +0.12] $\log_{10}$ IU/ml/year vs. −0.06 [IQR, −0.16 to +0.01] $\log_{10}$ IU/ml/year; p<0.001), then gradually reverted to the velocity of pre-vaccination periods (median, −0.17 [IQR, −0.55 to +0.11] $\log_{10}$ IU/ml/year at days +31 to +90; −0.04 [IQR, −0.18 to +0.08] $\log_{10}$ IU/ml/year at days +91 to +180; p<0.001 by Kruskal-Wallis test). The rate of HBsAg decline between +91 to +180 days was comparable to previous studies in patients with low HBV viral load (18,19). In addition, similar changes were maintained when comparing all measured HBsAg values (median, −0.39 [IQR, −0.88 to +0.12] $\log_{10}$ IU/ml at days +1 to +30; p<0.001; **Supplementary Fig. 2**). In subgroups classified by the type of vaccine received, similar trends in HBsAg decline at +1 to +30 days and following recovery were observed. However, the trend toward faster HBsAg recovery with the mRNA

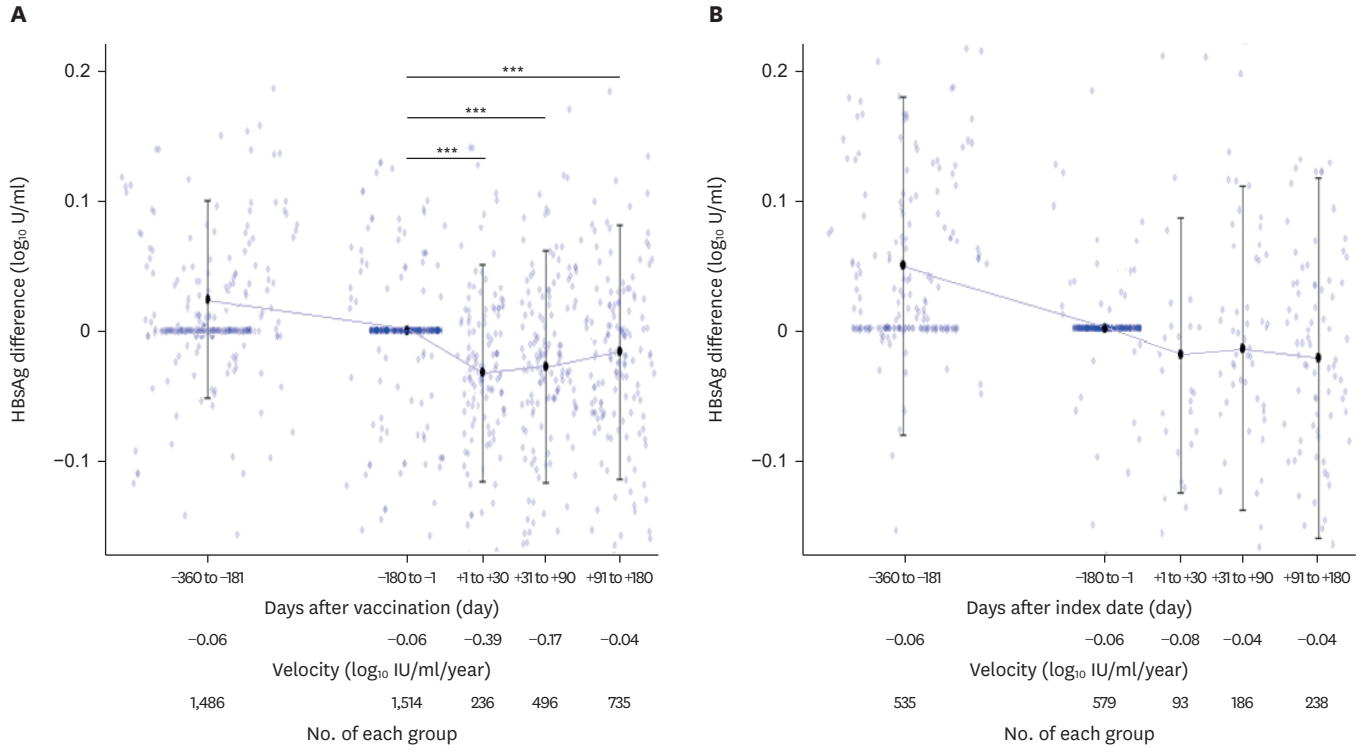

Figure 1. Median HBsAg differences from baseline in each patient following COVID-19 vaccination in vaccinated patients or after the index date in unvaccinated patients. HBsAg titers were measured and compared to baseline values in each patient. (A) Plot of the differences in HBsAg from baseline and the time interval between the date of the test and the date of the vaccination for each vaccinated patient. The length of the time following vaccination was grouped into 5 periods: days −360 to −181, −180 to −1, +1 to +30, +31 to +90, and +91 to +180. (B) Plot of the differences in HBsAg from baseline and test date from the index date in each unvaccinated patient. Time after index date was grouped into 5 periods: days −360 to −181, −180 to −1, +1 to +30, +31 to +90, and +91 to +180. If time groups included multiple samples from the same patient, the median value was used in the analysis. Dots and error bars represent median and IQR.
***p<0.001 according to a Wilcoxon signed-rank test for paired groups and Mann-Whitney *U* test for unpaired groups.

vaccine was not statistically significant (**Supplementary Fig. 3**). In addition, in subgroups of patients with liver cirrhosis (median, −0.23 [IQR, −0.78 to +0.31] $\log_{10}$ IU/ml +1 to +30 days from baseline), HCC (median, −0.28 [IQR, −0.61 to +0.05] $\log_{10}$ IU/ml +1 to +30 days from baseline), and liver transplantation (median, −0.24 [IQR, −0.65 to +0.18] $\log_{10}$ IU/ml +1 to +30 days from baseline) also maintained similar trends as the HBsAg kinetics of the entire cohort (all p<0.001 by Wilcoxon signed-rank test). A total of 11 patients received tyrosine-kinase inhibitors (TKIs) or immune checkpoint inhibitors (ICIs) within 3 months prior to COVID-19 vaccination. In a subgroup of patients without a history of liver transplantation, patients who were not treated with a TKI or ICI also maintained comparable HBsAg kinetics (median, −0.44 [IQR, −0.98 to +0.11] $\log_{10}$ IU/ml +1 to +30 days from baseline; p<0.001 by Wilcoxon signed-rank test).

The vaccinated group experienced significantly frequent HBsAg-seroclearance during the 24-wk post-vaccination period compared to the pre-vaccination period (0.5% [12 of 2,326] vs. 0.1% [3 of 2,329]; OR, 4.02 [95% CI, 1.13 to 14.27]; p=0.03; **Table 3**). In particular, 7 of 12 cases of post-vaccination HBsAg-seroclearance occurred after administration of the first vaccine dose. Rapid HBsAg decline (defined as >0.5 $\log_{10}$ IU/ml/year) occurred more frequently during the 24-wk post-vaccination period than during the pre-vaccination period (15.5% [361 of 2,329] vs. 10.5% [244 of 2,329]; OR, 1.57 [95% CI, 1.32 to 1.86]; p<0.001; **Table 3**). **Supplementary Table 3** shows the rapid decline in HBsAg compared to subgroups divided according to the presence of HCC (**Supplementary Fig. 4A**), presence of HCC and baseline

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Table 3. Comparison of HBsAg-seroclearance and rapid HBsAg decline in vaccinated patients with or without HCC according to baseline HBsAg level and fibrosis score measured by transient elastography

Characteristic	HBsAg-seroclearance		Rapid HBsAg decline		Number	OR (95% CI)	p
	(+)	(–)	(+)	(–)			
Pre-vaccination	3	2,326			2,329		
Post-vaccination							
After 1st vaccination	7	2,319			2,326	2.34 (0.60 to 9.06)	0.220
After all vaccination	12	2,314			2,326	4.02 (1.13 to 14.27)	0.030
Total					2,329	1.57 (1.32 to 1.86)	<0.001
Pre-vaccination			244	2,085			
Post-vaccination			361	1,968			
HCC (+)					1,146	1.32 (1.03 to 1.70)	0.030*
Pre-vaccination			122	1,024			
Post-vaccination			156	990			
HCC (–)					1,183	1.82 (1.43 to 2.32)	<0.001*
Pre-vaccination			122	1,061			
Post-vaccination			205	978			

*$p_{interaction}$=0.43.

HBsAg level >100 IU/ml (**Supplementary Fig. 4B**), and fibrosis as measured by transient elastography (**Supplementary Fig. 4C**). The distribution of patients within each category of HBsAg titer over time is shown in **Supplementary Fig. 5**.

NK cell dynamics after COVID-19 vaccination

Next, we examined if COVID-19 vaccination induces immunological changes that might be related to the transient decrease in HBsAg levels. We examined the frequency and phenotype of NK cells, which can exert direct effector functions against HBV (20,21), using 166 serial PBMC samples from 25 healthy participants (11 participants vaccinated with BNT162b2 and 14 with AZD1222). After the initial COVID-19 vaccination, PBMC samples were obtained every few days (median interval, 6 [IQR, 3 to 16] days) from day 0 to day 72. We examined the frequency of $CD56^{+}CD3^{-}$ NK cells by flow cytometry (**Supplementary Fig. 6**). We also examined the frequency of $CD56^{dim}CD16^{+}$ and $CD56^{bright}CD16^{-}$ NK cells, which are known as cytolytic and cytokine-producing NK cells, respectively (22-24). We found that their frequencies among total lymphocytes were not significantly changed by COVID-19 vaccination (**Fig. 2A-C**).

Next, we examined the expression of inhibitory NK cell receptors (i.e., NKG2A, killer-cell immunoglobulin-like receptors [KIRs], and T cell immunoreceptor with immunoglobulin and ITIM domain [TIGIT]) and activating NK cell receptors (i.e., NKp30, NKp46, and NKG2C). The frequency of $NKG2A^{+}$ cells among NK cells was significantly reduced at +1 to +7 days (median, –13.1% from baseline [IQR, –25% to 0%]; p=0.0009) and recovered to baseline levels at +8 to +30 days (median, –5.7% from baseline [IQR, –22% to +22.4%]; p=0.96) and +31 to +72 days (median, –9.8% from baseline [IQR, –30.4% to +18.8%]; p=0.11; **Fig. 2D**, **Supplementary Fig. 7A**). However, there was no significant change in the frequency of NK cells expressing KIRs, TIGIT, NKp30, NKp46, or NKG2C (**Fig. 2E-I**, **Supplementary Fig. 7B-F**).

Next, we examined the frequency of $NKG2A^{+}$ cells in $CD56^{dim}CD16^{+}$ and $CD56^{bright}CD16^{-}$ NK cell populations. In the $CD56^{dim}CD16^{+}$ NK cell population, the frequency of $NKG2A^{+}$ cells decreased at +1 to +7 days (median, –21% from baseline [IQR, –27.3% to –12.3%]; p<0.0001) and at +31 to +72 days (median, –15% from baseline [IQR, –32.7% to –1%]; p=0.0002), though this decrease transiently disappeared at +8 to +30 days (median, –10% from baseline [IQR, –19.5% to +10%]; p=0.43; **Fig. 3A**). In the $CD56^{bright}CD16^{-}$ NK cell population, there was no significant change in the frequency of $NKG2A^{+}$ cells (**Fig. 3B**).

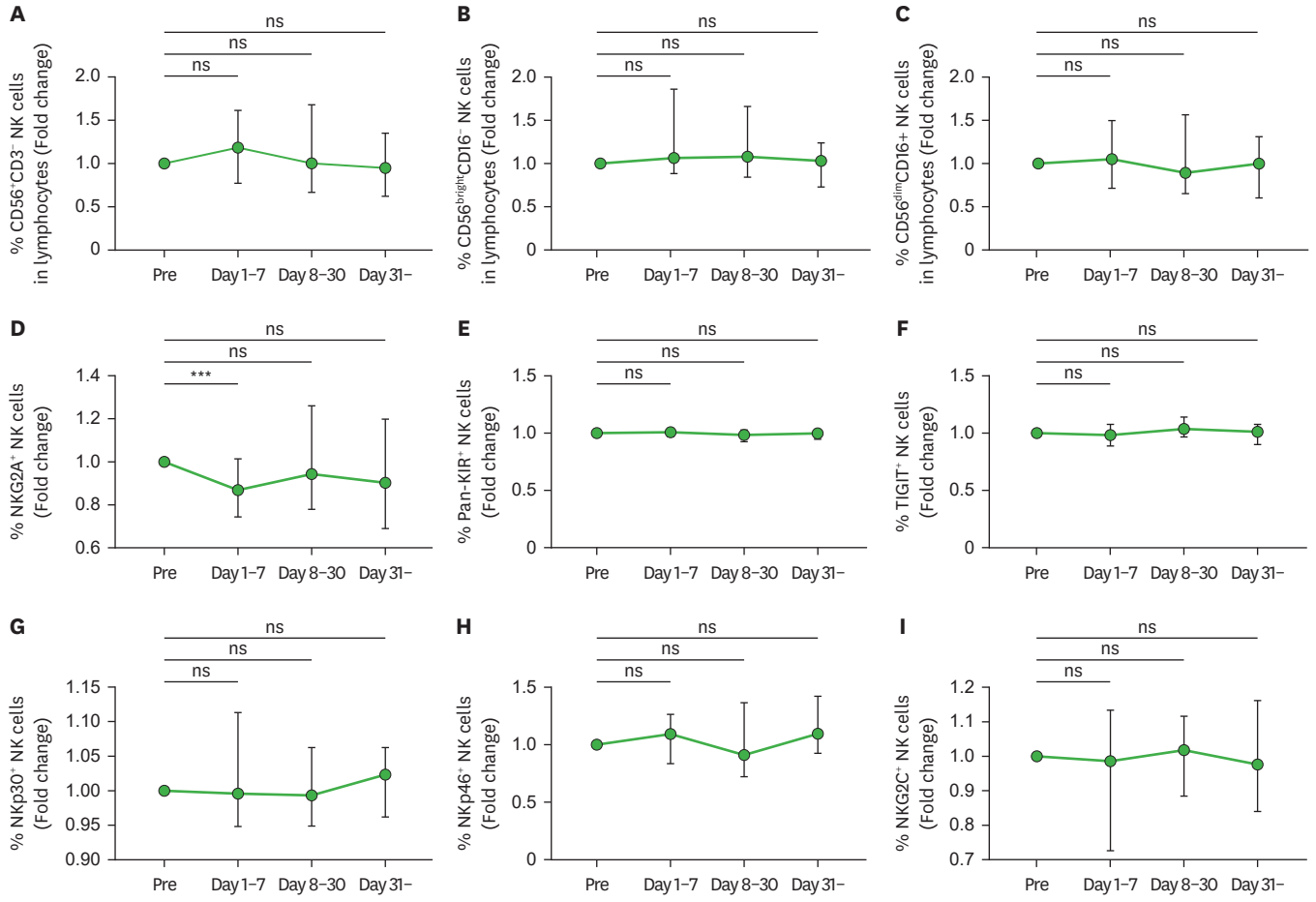

Figure 2. Frequency and phenotype of peripheral blood NK cells after COVID-19 vaccination. The frequency and phenotype of NK cells were examined using 166 serial PBMC samples from 25 healthy participants (11 participants vaccinated with BNT162b2 and 14 with AZD1222). Time after vaccination was grouped into 3 periods: days +1 to +7, +8 to +30, and +31 to +72. If time groups included multiple samples from the same participant, the median value was used in the analysis. (A) Changes in the relative frequency of $CD56^{+}CD3^{-}$ NK cells, (B) $CD56^{bright}CD16^{-}$ NK cells, and (C) $CD56^{dim}CD16^{+}$ NK cells among lymphocytes (n=25) compared to baseline. (D) Changes in the relative frequency of NK cells expressing NKG2A, (E) KIRs, (F) TIGIT, (G) NKp30, (H) NKp46, and (I) NKG2C among total NK cells (n=25) compared to baseline. Dots and error bars represent median and IQR.
ns, not significant.
***p<0.001 according to Wilcoxon signed-rank test for paired groups and Mann-Whitney *U* test for unpaired groups.

We analyzed the frequency of $NKG2A^{+}$ cells in the $CD56^{dim}CD16^{+}$ NK cell population among subgroups of patients vaccinated with BNT162b2 (**Fig. 3C**) and AZD1222 (**Fig. 3D**). A significant decrease in the frequency of $NKG2A^{+}$ cells was observed at +1 to +7 days in both subgroups (BNT162b2: median, −24.2% from baseline [IQR, −28% to −12.2%]; p=0.01 and AZD1222: median, −18.1% from baseline [IQR, −25.8% to −13.6%]; p=0.02). Similar changes were observed at +30 to +72 days in the BNT162b2 (median, −10.3% from baseline [IQR, −30.4% – −2%]; p=0.01) and AZD1222 (median, −15% from baseline [IQR, −31.7% to −2.8%]; p=0.048) subgroups. Given that NKG2A blocking antibodies reduce HBsAg titers in a mouse model of HBV infection (25), the current findings suggest that the significant decrease in the frequency of $NKG2A^{+}$ cells may be related to the reduction in HBsAg after COVID-19 vaccination. Next, we examined the frequency of $NKG2A^{+}$ cells using PBMC samples from 2 HBV-infected donors (one vaccinated with BNT162b2 and the other with AZD1222). The frequency $NKG2A^{+}$ cells among total NK cells and $CD56^{dim}CD16^{+}$ NK cells tended to be reduced at +1 to +7 days (median, −13.1% from baseline in total NK cells, −22.5% from baseline in $CD56^{dim}CD16^{+}$ NK cells; **Supplementary Fig. 8**). This result

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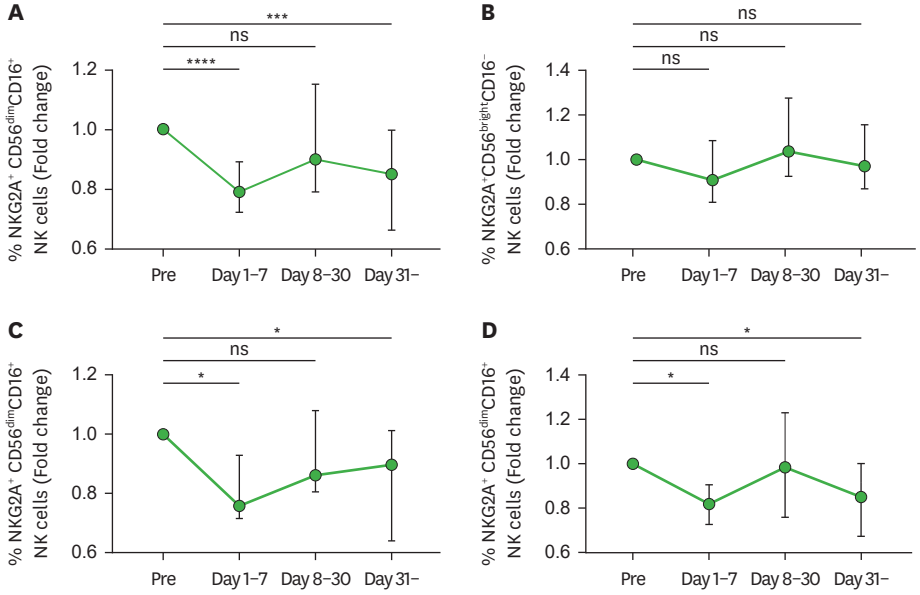

Figure 3. Frequency of NKG2A$^+$ cells in CD56^{dim}CD16$^+$ and CD56^{bright}CD16$^-$ NK cells after COVID-19 vaccination. Time after vaccination was grouped into 3 periods: days +1 to +7, +8 to +30, and +31 to +72. If time groups included multiple samples from the same participant, the median value was used in the analysis. (A) Changes in the relative frequency of NKG2A$^+$ cells among CD56^{dim}CD16$^+$ NK cells and (B) CD56^{bright}CD16$^-$ NK cells (n=25) compared to baseline. (C) Changes in the relative frequency of NKG2A$^+$ cells in CD56^{dim}CD16$^+$ NK cells among subgroups of BNT162b2 (n=11) and (D) AZD1222 (n=14) compared to baseline. Dots and error bars represent median and IQR. ns, not significant.
$^*p<0.05$, $^{***}p<0.001$, $^{****}p<0.0001$ according to Wilcoxon signed-rank test for paired groups and Mann-Whitney *U* test for unpaired groups.

indicates that the frequency of NKG2A$^+$ cells decreases early after COVID-19 vaccination irrespective of HBV infection.

We also examined HBV-specific T-cell responses before and after COVID-19 vaccination by performing IFN-γ ICS assays using PBMC samples from 2 HBV-infected donors (one vaccinated with BNT162b2 and the other with AZD1222). Prior to vaccination, IFN-γ$^+$ cells against HBsAg and HBcAg were barely detected among CD4$^+$ and CD8$^+$ T cell populations in both donors, and the same result was obtained after vaccination (**Supplementary Fig. 9**), indicating that HBV-specific T-cell responses may not be related to the transient reduction in HBsAg following COVID-19 vaccination.

DISCUSSION

In this study, we investigated the impact of COVID-19 vaccination on changes in HBsAg levels. After COVID-19 vaccination, HBsAg rapidly decreased to significantly lower levels within 30 days compared to the pre-vaccination value, but tended to revert to baseline 91–180 days post-vaccination. In a longitudinal analysis of NK cell dynamics in COVID-19-vaccinated healthy subjects, NKG2A$^+$ NK cells decreased significantly within 7 days after the initial COVID-19 vaccination. Considering that NKG2A is an immune inhibitory immune checkpoint (26), these results may collectively suggest that COVID-19 vaccine-induced HBsAg decline may be related to the decrease in the frequency of NKG2A$^+$ NK cells among CHB patients treated with antiviral agents.

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NK cells, which are at the forefront of innate immunity, are regarded as crucial antiviral effector cells against HBV or HCV (25,27,28), and the significance of NK cells in immune responses against COVID-19 has recently attracted attention (7,29-31). Our current study demonstrated that the decrease in HBsAg corresponded well with the decrease in the frequency of NKG2A$^+$ NK cells. Several studies have demonstrated that mice infected with HBV or HCV have an increased proportion of NKG2A$^+$ NK cells relative to total NK cells, and that anti-NKG2A antibodies may reduce the HBsAg titer or viral titers (25,28). In addition, there was a positive correlation between NKG2A expression and viral load in CHB patients, and blocking NKG2A$^+$ CD56^{dim} NK cells *in vitro* produces IFN-γ, which reduces viral replication (25,32). Recently, anti-NKG2A monoclonal antibodies have been applied in immuno-oncology and are expected to have a role in CHB treatments (25,33,34). The decrease in HBsAg (i.e., −21.4 IU/ml or −0.39 $\log_{10}$ IU/ml/year at 1–30 days) following COVID-19 vaccination may not be clinically significant, although statistically significant. It will be interesting to study if anti-NKG2A monoclonal antibodies can block the inhibitory effect of NKG2A and decrease HBsAg titers. In the current study, a decreased frequency of NKG2A$^+$ cells was observed in the CD56^{dim}CD16$^+$ NK cell population but not in the CD56^{bright}CD16$^-$ NK cell population. In humans, CD56^{dim}CD16$^+$ NK cells are more abundant than CD56^{bright}CD16$^-$ NK cells, and CD56^{dim}CD16$^+$ and CD56^{bright}CD16$^-$ NK cells were originally known as cytolytic and cytokine-producing NK cells, respectively (22-24). However, CD56^{dim}CD16$^+$ NK cells can also produce large amounts of IFN-γ (35). With reduced NKG2A expression, CD56^{dim}CD16$^+$ NK cells may exert enhanced effector functions, including cytotoxicity and IFN-γ production. Considering a non-cytolytic HBV control mechanism of IFN-γ (36), IFN-γ may be responsible for a decrease in HBsAg without acute flare-up of hepatitis after COVID-19 vaccination, which was observed in the current study.

The absence of acute flare-up can be explained by another way. Notably, serum HBV replication was well suppressed already at the index date in all included patients, which may prevent acute flare-up of CHB after COVID-19 vaccination. However, as noted in the previous case reports and series (11-13,37,38), patients whose chronic viral hepatitis or autoimmune hepatitis was not well controlled by antiviral agents or immune suppressants may experience acute flare-up of underlying liver disease following COVID-19 vaccination. These phenomena can be partially explained by immune reconstitution inflammatory syndrome, known as paradoxical worsening of infectious diseases after immune reconstitution (39,40). Thus, for patients who have chronic viral hepatitis or autoimmune hepatitis of which the activity is uncontrolled, COVID-19 vaccine should be administered with caution after assessing the risks and benefits.

Our study has a limitation. Due to the retrospective nature of the HBsAg kinetics study, we were unable to collect HBsAg titers at the index date; thus, baseline values were assigned as the last value obtained before vaccination, and the differences from baseline values were compared in the study. Nevertheless, due to the large sample size, HBsAg changes could be confirmed even when just compared to the HBsAg values tested within 30 days before vaccination. In addition, evaluation of intrahepatic immune cell activities in liver tissue, which was not performed in the present study, could potentially provide evidence for the association between COVID-19 vaccination and HBsAg kinetics.

In conclusion, COVID-19 vaccination was associated with a transient decrease in HBsAg titers in CHB patients treated with antivirals, regardless of the type of vaccine. In addition, COVID-19 vaccination transiently reduced the frequency of NKG2A$^+$ NK cells, which might be related to the decrease in HBsAg titers.

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SUPPLEMENTARY MATERIALS

Supplementary Table 1
A list of flow cytometry antibodies

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Supplementary Table 2
Characteristics of vaccinated and unvaccinated patients with CHB

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Supplementary Table 3
Comparison of rapid HBsAg decline in patients with or without HCC according to baseline HBsAg level and fibrosis score measured by transient elastography

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Supplementary Figure 1
Flow chart of patient selection.

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Supplementary Figure 2
All HBsAg differences from baseline for each patient following COVID-19 vaccination. HBsAg titers were measured and compared to baseline values in each vaccinated patient. All measurements of HBsAg were included in the analysis. The length of the time following vaccination was grouped into 5 periods: days −360 to −181, −180 to −1, +1 to +30, +31 to +90, and +91 to +180. Dots and error bars represent median and IQR.

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Supplementary Figure 3
HBsAg differences from baseline in (A) each type of vaccine and (B) heterologous or homologous vaccination. HBsAg titers were measured and its median values were compared to baseline values in each vaccinated patient. The length of the time following vaccination was grouped into 5 periods: days −360 to −181, −180 to −1, +1 to +30, +31 to +90, and +91 to +180. Dots and error bars represent median and IQR.

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Supplementary Figure 4

Ratio of the incidence of rapid HBsAg decline in CHB patients. (A) Patients with HCC. (B) Patients with HCC and baseline HBsAg >100 IU/ml. (C) Patients with fibrosis as measured by transient elastography.

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Supplementary Figure 5

Distribution of HBsAg levels after first COVID-19 vaccination. Percentage of patients is on the left.

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Supplementary Figure 6

Gating strategy for $CD56^{+}CD3^{-}$ NK cells, $CD56^{bright}CD16^{-}$ NK cells, and $CD56^{dim}CD16^{+}$ NK cells. Representative figure showing the gating strategy for $CD56^{+}CD3^{-}$ NK cells, $CD56^{bright}CD16^{-}$ NK cells, and $CD56^{dim}CD16^{+}$ NK cells.

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Supplementary Figure 7

Gating strategy for phenotypic analysis of NK cells. Representative figure showing the gating strategy for NK cells expressing NKG2A (A), KIRs (B), TIGIT (C), NKp30 (D), NKp46 (E), and NKG2C (F).

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Supplementary Figure 8

Frequency of $NKG2A^{+}$ cells in total NK and $CD56^{dim}CD16^{+}$ NK cells after COVID-19 vaccination from 2 HBV-infected donors. Time after vaccination was grouped into 3 periods: days +1 to +7, +8 to +30, and +31 to +72. If time groups included multiple samples from the same participant, the median value was used in the analysis. (A) Changes in the relative frequency of $NKG2A^{+}$ cells among total NK cells and (B) $CD56^{dim}CD16^{+}$ NK cells (n=2) compared to baseline.

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Supplementary Figure 9

HBV-specific T-cell responses after COVID-19 vaccination. ICS was performed to examine the frequency of $CD4^{+}$ or $CD8^{+}$ T cells responding to HBcAg and HBsAg using PBMC samples from HBV-infected donors. Representative figures showing IFN-γ^{+} cells among $CD4^{+}$ (A) or $CD8^{+}$ (B) T cells in CHB patients who received BNT162b2.

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