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Research article

Humoral immune response against SARS-CoV-2 and polyethylene glycol elicited by anti-SARS-CoV-2 mRNA vaccine, and effect of pre-existing anti-polyethylene glycol antibody in patients with hematological and autoimmune diseases

Taiki Hori [a], Taro Shimizu [b], Hidenori Ando [b], Naoto Okada [c], Hiroki Yamagami [a,d], Saya Yasui [a], Minae Hosoki [a], Akihiro Tojima [e], Toshiki Otoda [a,f], Tomoyuki Yuasa [a,f], Ken-ichi Aihara [a,f], Makoto Takishita [a], Sumiko Yoshida [d,h], Masahiro Abe [g], Tatsuhiro Ishida [b], Shingen Nakamura [f,*]

[a] Department of Internal Medicine, Anan Medical Center, Tokushima, Japan
[b] Department of Pharmacokinetics and Biopharmaceutics, Institute of Biomedical Sciences, Tokushima University Graduate School, Tokushima, Japan
[c] Pharmacy Department, Yamaguchi University Hospital, Yamaguchi, Japan
[d] Department of Hematology, Endocrinology and Metabolism, Tokushima University Graduate School of Biomedical Sciences, Tokushima, Japan
[e] Department of Rheumatology, Anan Medical Center, Tokushima, Japan
[f] Department of Community Medicine and Medical Science, Tokushima University Graduate School of Biomedical Sciences, Tokushima, Japan
[g] Department of Hematology, Kawashima Hospital, Tokushima, Japan
[h] Department of Clinical Research, National Hospital Organization Shikoku Medical Center for Children and Adults, Kagawa, Japan

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ABSTRACT

Background: The effects of vaccination are modified by hematological and autoimmune diseases and/or treatment. Anti-SARS-CoV-2 mRNA vaccine contains polyethylene glycol (PEG), it is largely unknown whether PEG influences the effects of vaccination or induces a humoral response. This study examined whether anti-PEG antibodies before vaccination (pre-existing) influenced the acquisition of SARS-CoV-2 antibodies and evaluated the relationship between the development of anti-SARS-CoV-2 antibodies and anti-PEG antibodies after SARS-CoV-2 vaccination in hematological and autoimmune diseases.

Methods: Anti-SARS-CoV-2 antibody IgG, anti-PEG IgG, and IgM titers were evaluated in patients with hematological and autoimmune diseases after the second dose of BNT162B2. Anti-PEG IgG and IgM titers were also measured before vaccination to examine changes after vaccination and the relationship with vaccine efficacy.

Results: In patients with hematological (n = 182) and autoimmune diseases (n = 96), anti-SARS-CoV-2 and anti-PEG antibody titers were evaluated after a median of 33 days from 2nd vaccination. The median anti-SARS-CoV-2 antibody titers were 1901 AU/mL and 3832 AU/mL in patients with hematological and autoimmune disease, respectively. Multiple regression analysis showed that age and days from 2nd vaccination were negatively associated with anti-SARS-CoV-2 antibody titers. Anti-CD20 antibody treatment was negatively correlated with anti-SARS-CoV-2

* Corresponding author. Department of Community Medicine and Medical Science, Tokushima University Graduate School of Biomedical Sciences, 3-18-15 Kuramoto-cho, Tokushima, 770-8503, Japan.
E-mail address: shingen@tokushima-u.ac.jp (S. Nakamura).

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antibody titers in hematological disease, and C-reactive protein (CRP) was positively correlated with anti-SARS-CoV-2 antibody titers in autoimmune disease. Baseline anti-PEG antibody titers were significantly higher in patients with autoimmune disease but were not correlated with anti-SARS-CoV-2 antibody titers. Patients with increased anti-PEG IgG acquired higher anti-SARS-CoV-2 antibody titers in patients with autoimmune disease.
Conclusions: Anti-SARS-CoV-2 antibody acquisition was suboptimal in patients with hematological disease, but both anti-SARS-CoV-2 antibody and anti-PEG IgG were acquired in patients with autoimmune disease, reflecting robust humoral immune response. Pre-existing anti-PEG antibody titers did not affect anti-SARS-CoV-2 antibody acquisition.

1. Introduction

In December 2019, pneumonia of an unknown cause occurred in Wuhan City, Hubei Province, China, where severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection was reported [1,2]. The infectious disease caused by SARS-CoV-2 is called coronavirus disease 2019 (COVID-19); it has spread worldwide [3].

When patients with hematological malignancies are infected with SARS-CoV-2, they have poor outcomes due to COVID-19, immune dysfunction from the malignancy itself, or treatments such as chemotherapy, immunosuppressants, and hematopoietic stem cell transplantation. The risk of death from COVID-19 in adult patients with hematological malignancies was reported to be as high as 30–34 % [4,5]. COVID-19 has a higher mortality rate in rheumatic disease with comorbidities than in the general population [6]. Additionally, the effects of vaccination may be hampered in patients with autoimmune diseases, even though immunosuppressive therapies have been used to treat these diseases. Therefore, patients with both hematological and autoimmune diseases are concerned about the effects of vaccination because they are immunosuppressed by the disease itself and/or its treatment.

Messenger RNA (mRNA) vaccines against SARS-CoV-2 were the first practical vaccines in human history and are highly effective in preventing COVID-19. They reduced COVID-19-associated hospitalization and death. The mRNA vaccine introduces a synthetically created fragment of the viral RNA sequence into the vaccinated individual. Dendritic cells present viral antigens produced by the host cells to T and B cells and trigger the production of specific antibodies [7]. This vaccine induces antibodies against spike proteins which are a part of the SARS-CoV-2 receptor-binding domain.

Conversely, the mRNA vaccine against SARS-CoV-2 contains polyethylene glycol (PEG) on the surface of the lipid nanoparticle (LNP), which is suspected as one of the causative agents of anaphylaxis [8,9]. PEG has been known as the cause of an unexpected immunogenic response named the accelerated blood clearance (ABC) phenomenon, resulting in the increased clearance and reduced efficacy of PEG-conjugated drugs such as PEGylated recombinant factor VIII products and pegylated granulocyte colony-stimulating factor [10–12]. However, it is not well known whether the presence or absence of anti-PEG antibodies before vaccination affects the vaccine efficacy or whether they are induced after vaccination. Therefore, in the present study, we investigated the relationship between the acquisition of anti-SARS-CoV-2 antibodies and anti-PEG antibodies after SARS-CoV-2 vaccination and whether anti-PEG antibodies before vaccination influence the degree of the acquisition of SARS-CoV-2 antibodies in hematological and autoimmune diseases.

2. Materials and methods

2.1. Study design, participants, and ethics statement

In this cross-sectional study, we consecutively recruited 278 Japanese outpatients or inpatients with hematological and autoimmune diseases (125 men and 153 women). All participants were older than 20 years and were recruited consecutively from the Department of Internal Medicine at Anan Medical Center (Anan, Tokushima, Japan) and the Department of Hematology at Tokushima University Hospital (Tokushima, Tokushima, Japan) between April 2021 and December 2021. The exclusion criteria were as follows: 1) patients infected with SARS-CoV-2, 2) patients who did not receive a second vaccination. All participants enrolled in this study underwent a standardized interview and physical examination. Body mass index (BMI) was used as an index of obesity. Antibody titers against the SARS-CoV-2 spike protein in serum were measured 7 days or later after the second vaccination (BNT162b2). Disease duration, treatment within 6 months before vaccination, and days from vaccination to measurement were surveyed from the medical chart. Blood samples were collected and used to determine blood cell counts [white blood cells (WBCs), lymphocytes (Lym), hemoglobin (Hb), platelets (Plt)], and serum biochemical parameters, including creatinine (Cr) and C-reactive protein (CRP). Measurement of anti-PEG antibody levels was performed only in individuals whose serum was available within the scope of routine practice and not for a specific patient group.

Our study followed the institutional guidelines of the Anan Medical Center, Tokushima University. It was approved by the Institutional Review Boards of Anan Medical Center (approval number 202102) and Tokushima University (approval number 3987-1). Informed consent was obtained from all patients in accordance with the Declaration of Helsinki.

2.2. Antibody titer measurement

Residual serum was collected after routine medical practice and stored at −20 °C until measurement assay. Measurement of antibody titer against SARS-CoV-2 spike protein was outsourced and conducted at labo of BML Inc (Tokyo, Japan) according to the manufacturer's standard procedure, while antibody titer against PEG was measured by our laboratory circumstance. Antibody titer against SARS-CoV-2 spike protein in the serum was measured using ARCHITECT® analyzer i 2000SR by chemiluminescent immunoassay method with ARCHITECT SARS-CoV-2 IgG II Quant measuring kit at the labo of BML, INC (Tokyo, Japan). ARCHITECT SARS-CoV-2 IgG II Quant (Abbott) was characterized by the ability to identify high- and low-titer plasma for wild-type and variant SARS-CoV-2 [13], and correlates with other assays [14,15]. 1 binding antibody unit (BAU)/mL corresponds to 0.142 Abbott arbitrary units (AU)/mL, and the antibody positivity threshold was set at 50 AU/mL [16].

The antibody titer against PEG was measured using an enzyme-linked immunosorbent assay (ELISA), which was employed to detect anti-PEG IgM and IgG, as previously described [11]. Briefly, a 96-well plate (Evergreen, CA, USA) coated with 50 μL/well $mPEG_{2000}$-DSPE (20 nmol in ethanol) for 2 h at 37 °C was blocked with 200 μL/well of 5 % BSA/0.05 % CHAPS Tris-buffered saline (blocking buffer). After washing, serum samples diluted in blocking buffer were added to each well. After incubation, horseradish peroxidase-conjugated goat anti-human IgM or IgG antibodies (Bethyl Laboratories, Montgomery, TX, USA) were added. After washing, 3,3′,5,5′-tetramethyl-benzidene was added, and the reaction was stopped using 2 M H_2SO_4. The absorbance of each well was measured at 450 nm using a microplate reader (TECAN, Männedrof, Switzerland). Mouse monoclonal antibodies, either anti-PEG IgM (HIK-M11) or anti-PEG IgG (HIK-G11), were used instead of serum samples and then detected with either HRP-conjugated goat anti-mouse IgM or IgG antibody (Bethyl Laboratories) to generate standard curves. The concentrations of anti-PEG IgM and IgG in serum samples were calculated using each standard curve, and 100 ng/mL of anti-PEG antibody was set to 1 U/mL.

2.3. Statistical analysis

Normally distributed continuous data are presented as means ± standard deviation (SD). Skewed continuous data were presented as medians and interquartile ranges (IQR). Categorical variables were compared using the χ^2 test or Fisher's exact test. For comparisons between two groups, we performed the Mann–Whitney *U* test or Student's *t*-test for numeric variables depending on the distribution of the variable.

We compared antibody titers against the SARS-CoV-2 spike protein between patients with hematological and autoimmune diseases using the Mann–Whitney *U* test. In the two patient groups, the degree of association of antibody titer against SARS-CoV-2 spike protein with each variable, including sex, age, BMI, WBCs, lymphocytes, Hb, Plt, Cr, CRP, days from second vaccination, and drugs taken within 6 months, was determined using simple linear regression analysis. The Mann–Whitney *U* test was used when comparing antibody titers against the SARS-CoV-2 spike protein with and without drugs such as anti-CD20 antibodies that are not continuous variables. In addition, multiple linear regression analysis was performed with the significant variables determined using the simple linear regression analysis. Multicollinearity was also evaluated in each multiple regression.

The levels of anti-PEG IgM and IgG were compared in patients with hematological and autoimmune diseases using the Mann–Whitney *U* test. We investigated the relationship between the acquisition of anti-SARS-CoV-2 antibodies and baseline anti-PEG antibodies after SARS-CoV-2 vaccination using simple linear regression analysis. Comparisons of anti-PEG antibody titers before and after vaccination were performed considering the number of days after the vaccination. Anti-SARS-CoV-2 antibody titers were compared between patients with and without increases in anti-PEG IgG and IgM using analysis of covariance with the number of days after vaccination as a covariate.

Analyses were performed using Excel (Microsoft Office Excel 2019; Microsoft, Richmond, CA, USA), GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA) R (The R Foundation for Statistical Computing, Vienna, Austria), and EZR [17] (Saitama Medical Center, Jichi Medical University, Saitama, Japan). Statistical significance was set at $p < 0.05$.

Table 1
Patient characteristics.

	Total	Hematological disease	Autoimmune disease	p value
Number of Subjects	278	182	96	
Male (n, (%))	125 (45.0)	100 (54.9)	25 (26.0)	<0.001
Age (years)	71 [62–78]	71 [63–78]	70 [62–78]	0.685
BMI (kg/m2)	22.4 [20.3–24.8]	22.3 [20.0–24.8]	22.7 [20.5–25.3]	0.211
WBC (× 103/μL)	5.5 [4.3–7.0]	5.1 [4.1–6.8]	5.7 [4.4–7.4]	0.072
Lym (× 103/μL)	1.5 [1.1–2.0]	1.4 [1.0–1.8]	1.7 [1.3–2.3]	<0.001
Hb (g/dL)	12.5 [11.4–13.6]	12.5 [11.1–13.8]	12.7 [11.9–13.4]	0.429
Plt (× 103/μL)	215 [165–257]	205 [157–248]	224 [184–272]	0.012
Cr (mg/dL)	0.78 [0.63–1.00]	0.85 [0.67–1.06]	0.67 [0.57–0.83]	<0.001
CRP (mg/dL)	0.12 [0.06–0.25]	0.11 [0.05–0.23]	0.14 [0.06–0.32]	0.124
Days from second vaccination (day)	33 [21–52]	34 [21–57]	30 [19–44]	0.057
Antibody against SARS-CoV-2 spike protein positive (n, (%))	240 (86.3)	148 (81.3)	92 (95.8)	0.001
Antibody titer against SARS-CoV-2 spike protein (AU/mL)	2519 [552–7521]	1901 [227–6680]	3832 [1072–8534]	0.003

3. Results

3.1. Patient characteristics

The physical and laboratory characteristics of the participants are presented in Table 1 and Supple. Table 1. There were 182 patients with hematological disease: malignant lymphoma (n = 66), plasma cell tumor (n = 39), myeloproliferative neoplasm (n = 18), HIV infection (n = 13), myelodysplastic syndrome (n = 9), idiopathic thrombocytopenic purpura (n = 8), acute leukemia (n = 7), chronic myeloid leukemia (n = 5), aplastic anemia (n = 4), autoimmune hemolytic anemia (n = 3), others (n = 10). Also, there were 96 patients with autoimmune disease: psoriatic arthritis (n = 34), rheumatoid arthritis (n = 23), psoriatic/rheumatoid arthritis (n = 10), rheumatoid arthritis/spondyloarthritis (n = 6), Crohn's disease (n = 4), spondyloarthritis (n = 3), ulcerative colitis (n = 2), autoimmune hepatitis (n = 2), psoriatic arthritis/polymyalgia rheumatica (n = 2), others (n = 10). Patients with hematological disease had higher levels of Cr (0.85 mg/dL [0.67–1.06] vs. 0.67 mg/dL [0.57–0.83]; $p < 0.001$) and a higher percentage of males (54.9 % vs. 26.0 %; $p < 0.001$). Patients with autoimmune disease had higher levels of Lym (1.4×10^3/μL [1.0–1.8] vs. 1.7×10^3/μL [1.3–2.3]; $p < 0.001$), and Plt (205×10^3/μL [157−248] vs. 224×10^3/μL [184−272]; $p = 0.012$) than those with hematological disease. There were no significant differences in age, BMI, WBC count, Hgb, CRP, or days from the second vaccination between patients with hematological and autoimmune diseases.

3.2. Humoral response against SARS-CoV-2 mRNA vaccination in hematological and autoimmune diseases

Commercial anti-SARS-CoV-2 receptor binding domain antibody test correlates with a chemiluminescent reduction neutralizing test [18]. Therefore, we conducted subsequent experiments using this assay. The hematological disease group had a lower percentage of antibody-positive patients than the autoimmune disease group after the second dose of vaccine (hematological disease, 148 (81.3 %) vs. autoimmune disease, 92 (95.8 %); $p = 0.001$) (Table 1). Lower antibody titers were observed in the hematological disease group than in the autoimmune disease group (hematological disease 1901 AU/mL [227.0–6680] vs. autoimmune disease 3832 AU/mL [1072–8534]; $p = 0.003$) (Fig. 1(a)).

3.3. Humoral response-associated factors against SARS-CoV-2 mRNA vaccine in hematological disease

The antibody-positive rates and antibody titers for each underlying disease and treatment are shown in Supple. Table 1. All patients treated with anti-CD20 monoclonal antibodies within 6 months were antibody negative. Simple linear regression analysis of all participants showed that age and days from the second vaccination were negatively correlated with antibody titers ($Y = -139.3X+14244$, $R^2 = 0.07740$, $p < 0.001$, and $Y = -64.10X+7320$, $R^2 = 0.06668$, $p < 0.001$, respectively) (Fig. 1 (b) and (c)). Patients who used anti-CD20 antibodies within 6 months had significantly lower antibody titers (0 AU/mL [0–0] vs. 2491 AU/mL

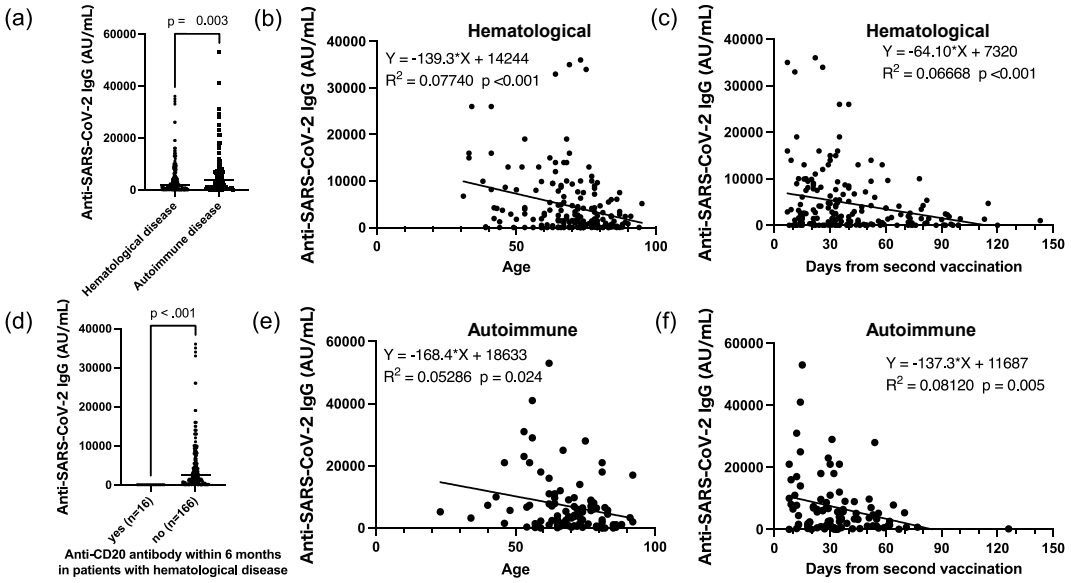

Fig. 1. Humoral response against SARS-CoV-2 mRNA vaccination in hematological and autoimmune diseases (a) Antibody titers against anti-SARS-CoV-2 obtained by vaccination in hematological and autoimmune diseases. (b) Antibody titers by age in hematological disease (c) Antibody titers by days from second vaccination in hematological disease (d) Antibody titers in patients with hematological disease with or without anti-CD20 antibody therapy within the last 6 months. (e) Antibody titers in autoimmune disease by age. (f) Antibody titers in autoimmune disease by days from second vaccination.

[651–7568]; $p < 0.001$) (Fig. 1(d)). Multiple regression analysis showed that the clinical factors determining antibody titers were age ($p < 0.001$), days since the second vaccination ($p = 0.001$), and the presence of anti-CD20 monoclonal antibodies ($p = 0.013$) (Table 2).

3.4. Humoral response-associated factors against SARS-CoV-2 mRNA vaccine in autoimmune disease

The antibody-positive rates and antibody titers according to the underlying disease and treatment are shown in Supple. Table 1. Simple linear regression analysis for the entire series of participants showed that age and days since the second vaccination were negatively correlated with antibody titers ($Y = -168.4X+18633$, $R^2 = 0.05286$, $p = 0.024$ and $Y = -137.3X+11687$, $R^2 = 0.08120$, $p = 0.005$, respectively) (Fig. 1 (e) and (f)). Multiple regression analysis showed that the clinical factors determining antibody titers were age ($p = 0.024$), CRP level ($p = 0.035$), and days from the second vaccination ($p = 0.004$) (Table 2).

3.5. Association between baseline anti-PEG antibody and acquired humoral response against SARS-CoV-2 vaccination in patients with hematological and autoimmune diseases

Baseline anti-PEG antibody titers were measured in 60 patients with hematological disease and 42 with autoimmune disease. Baseline anti-PEG IgM and IgG titer were significantly higher in autoimmune disease (IgM: 1.89 U/mL [0.87–2.48] vs. 2.76 U/mL [1.14–4.47]; $p = 0.017$, IgG: 144.80 U/mL [116.50–187.80] vs. 456.10 U/mL [292.50–594.20]; $p < 0.001$) (Fig. 2 (a) and (b)). Baseline anti-PEG IgM and IgG levels did not correlate with anti-SARS-CoV-2 IgG antibody titers after vaccination in patients with hematological or autoimmune diseases (Fig. 2 (c), (d), (e), (f)).

3.6. Humoral response against PEG after vaccination

Anti-PEG antibody titers before and after 2nd vaccination were measured in 49 patients with hematological disease and 42 with autoimmune disease. Anti-PEG antibody IgM titers were increased after vaccination in patients with hematological disease (1.88 U/mL [0.75–2.48] vs. 1.96 U/mL [0.84–2.87]; $p = 0.008$); there was no significant difference in patients with autoimmune disease (2.76 U/mL [1.14–4.47] vs. 2.75 U/mL [1.59–6.57]; $p = 0.183$). Anti-PEG antibody IgG titers were decreased after vaccination in patients with hematological and autoimmune diseases (hematological 155.10 U/mL [126.00–216.90] vs. 128.30 U/mL [85.40–171.50]; $p < 0.001$, autoimmune 456.10 U/mL [292.50–594.20] vs. 377.00 U/mL [273.20–465.00]; $p < 0.001$) (Supple. Fig. 1 (a) and (b)). When we compared patients with and without an increase in anti-PEG IgM or IgG titers after vaccination, anti-SARS-CoV-2 antibody titers were not associated with changes in anti-PEG antibodies in patients with hematological disease (Supple. Fig. 2 (a)). However, patients with autoimmune disease who had elevated anti-PEG IgG levels after vaccination had significantly higher anti-SARS-CoV-2 antibody titers (median 2686 U/mL [1069–6949] vs. 22,000 U/mL [6353–29,500]; $p < 0.001$) (Supple. Fig. 2 (b)). To analyze the effect of the difference in the timing of collecting serum samples after vaccination, further studies were warranted. Supple. Fig. 3 visualizes the baseline anti-PEG antibody and anti-PEG antibody after the second vaccination. The anti-PEG IgM titers in some patients with hematological disease were elevated around 30 days after the second vaccination (Supple. Fig. 3 (a)); however not all patients had baseline or acquired anti-PEG IgM in autoimmune disease (Supple. Fig. 3 (c)). Patients with high baseline anti-PEG IgG did not necessarily have elevated levels after the second vaccination in both hematological disease (Supple. Fig. 3 (b)) and autoimmune disease (Supple. Fig. 3 (d)). Supple. Fig. 4 shows a diagram of anti-SARS-CoV-2 IgG antibody titers and days from the second vaccination depending on the with or without increases in anti-PEG antibody titers. All p-values for interactions between group variables and covariates were >0.05. We performed an analysis of covariance with the number of days after vaccination as a covariate. In patients with hematological disease, the with or without increases in anti-PEG IgM ($p = 0.276$) and IgG ($p = 0.678$) were not correlated with anti-SARS-CoV-2 IgG antibody titers (Supple. Fig. 4 (a) and (b)). In patients with autoimmune disease, with or without an increase in anti-PEG IgM was not correlated ($p = 0.970$) (Supple. Fig. 4 (c)). Patients with increased anti-PEG IgG titers had significantly higher anti-SARS-CoV-2 IgG antibody titers even when the number of days from the second vaccination was used as a covariate ($p = 0.010$) (Supple. Fig. 4 (d)).

Table 2
Multiple regression analysis of the correlation between antibody titers obtained by vaccination and associated factors in hematological and autoimmune diseases.

	Hematological disease			Autoimmune disease		
variables	coefficient	p value	VIF	coefficient	p value	VIF
Male	714.7	0.465	1.057	−2704.0	0.219	1.147
Age	−145.4	<0.001	1.053	−177.5	0.024	1.185
BMI	−94.5	0.468	1.046	−4.5	0.984	1.054
Lym	0.8	0.262	1.056	1.4	0.253	1.086
CRP	143.1	0.769	1.039	2919.0	0.035	1.182
Days from second vaccination	−57.0	0.001	1.030	−151.8	0.004	1.166
Anti-CD20 antibody monotherapy or in combination with cytotoxic agents	−4217.0	0.013	1.076	—	—	—
MTX	—	—	—	−1885.0	0.352	1.227

Abbreviation: MTX: Methotrexate.

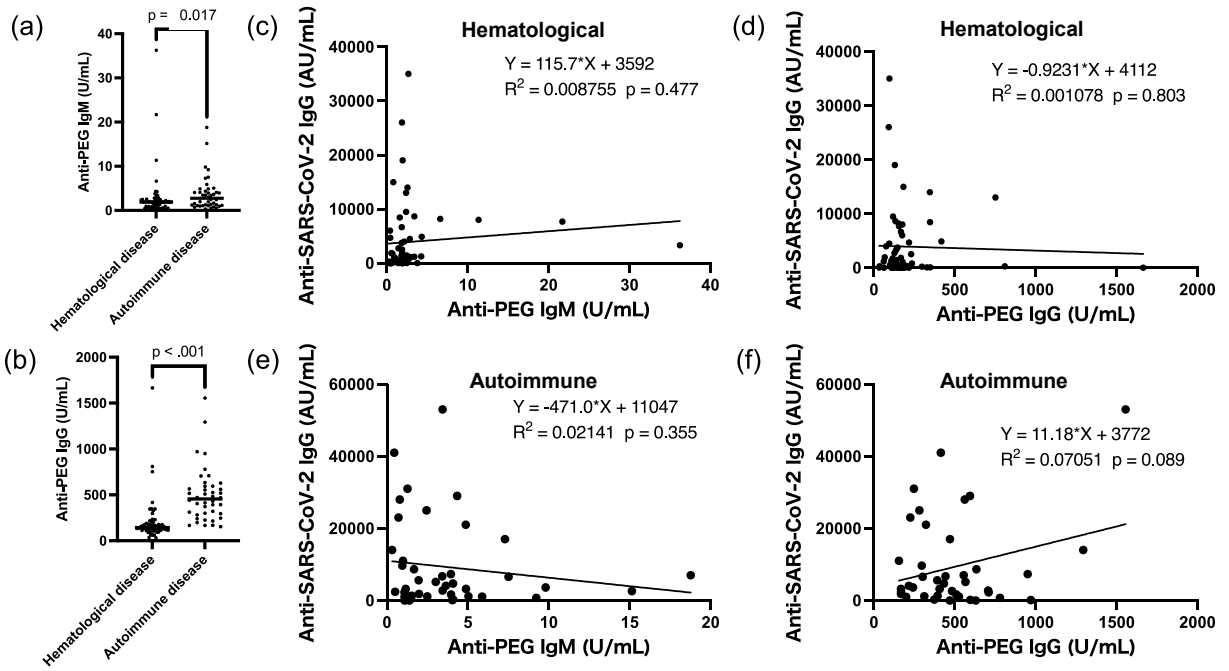

Fig. 2. (a) Anti-PEG IgM antibody titers before vaccination in hematological and autoimmune diseases. (b) Anti-PEG IgG antibody titers before vaccination in hematological and autoimmune diseases. (c) The association between anti-PEG IgM before vaccination and anti-SARS-CoV-2 IgG titers obtained after vaccination in hematological disease. (d) The association between anti-PEG IgG before vaccination and anti-SARS-CoV-2 IgG titers obtained after vaccination in hematological disease. (e) The association between anti-PEG IgM before vaccination and anti-SARS-CoV-2 IgG titers obtained after vaccination in autoimmune disease. (f) The association between anti-PEG IgG before vaccination and anti-SARS-CoV-2 IgG titers obtained after vaccination in autoimmune disease.

3.7. Association between baseline anti-PEG antibody titers and anaphylaxis by vaccination

None of the patients in our study developed anaphylaxis due to vaccination. Therefore, this study did not evaluate the association between baseline anti-PEG levels and anaphylaxis.

4. Discussion

Here, we report the humoral immune response following SARS-CoV-2 mRNA vaccination in patients with hematological and autoimmune diseases. We focused on two points: anti-SARS-CoV-2 antibody titers obtained by SARS-CoV-2 mRNA vaccination and anti-PEG antibodies before and after vaccination.

Factors that reduce anti-SARS-CoV-2 antibody titer are the male sex, old age, smoking, and time elapsed since vaccination in healthy people [19,20]. In healthcare workers at the age of 44.12 ± 12.65 years, the mean anti-SARS-CoV-2 antibody titers after 7–21 days of second BNT162b2 assayed with the same measurement system as ours was 24,084.06 AU/mL[22]. Our population seemed to have relatively lower humoral responses to vaccination than healthy individuals, although we could not directly compare them. When applying a threshold of >4160 AU/mL [21], which correlates with a 95 % probability of high neutralizing antibody titer, the percentage of patients with antibody titers above the threshold was low in our study: 34 % in hematological disease and 48 % in autoimmune disease.

A lower proportion of patients with hematological malignancies were reported to be seropositive following the BNT162b2 vaccine than in the comparison group (75 % vs. 99 %, $p < 0.001$) [22]. In patients with hematological disease, the seropositive (≧50 AU/mL) rate was 81 %, and the mean antibody titer was 4650 AU/mL after 2nd vaccination in our study. Patients recently treated with anti-CD20 antibodies, BCL2 inhibitors, BTK inhibitors, and JAK2 inhibitors have significantly fewer seropositive responses and lower antibody titers [22,23]. Patients treated with anti-CD20 antibodies within 6 months had the lowest seropositivity rate and lower antibody titers in our study (0 %, 0 AU/mL [0–0]). Vegivinti et al. reported that rituximab significantly increased mortality in patients with hematological malignancies [24], and it is speculated that they could not obtain anti-SARS-CoV-2 IgG enough to prevent COVID-19. The patients treated with BTK inhibitors or JAK2 inhibitors had lower median antibody titers in our study (50 %, 101 AU/mL [0–202], 80 %, 525 AU/mL [183−2798], respectively). However, the antibody titers obtained in patients treated with these drugs seemed low. BTK or JAK inhibitors suppress cytokine storms when patients develop COVID-19, which may lead to a good prognosis in patients with severe COVID-19 [25,26].

In patients with autoimmune disease, the seropositivity rate was 96 %, and the mean antibody titer was 7057 AU/mL after 2nd

vaccination in our study. Although the seropositivity rate and antibody titer were lower than those in healthy individuals (100 %) [27], they were higher than those in patients with hematological disease (81 %). Seroconversion after the first vaccination is reported to be significantly lower in patients with autoimmune disease treated with anti-CD20 antibodies or methotrexate (MTX) [28]. None of the patients with autoimmune disease used anti-CD20 antibodies in our study. However, in 56 patients treated with MTX, the seropositive rate was 98 %, and the median antibody titer was 3498 AU/mL after 2nd vaccination. Multiple regression analysis showed that MTX treatment was not a statistically significant factor for anti-SARS-CoV-2 antibody titers. This result suggests that age and days since the second vaccination are greater factors than MTX use in obtaining anti-SARS-CoV-2 IgG by vaccination. In turn, CRP was positively correlated with antibody titers in autoimmune disease but not in hematological disease. The correlation between CRP and anti-SARS-CoV-2 IgG in healthcare workers who received a BNT162b2 vaccine booster [29] and higher CRP levels may be associated with the humoral response against vaccination. In our study, patients with autoimmune disease had relatively low CRP levels (0.14 mg/dL [0.06–0.32]). Therefore, clinical significance is unclear. The administration of neutralizing antibodies, such as tixagevimab-cilgavimab, should be considered in patients who are expected to have difficulty obtaining sufficient antibody titers with vaccines [30].

A clinical question is whether anti-PEG antibodies are induced following vaccination because anti-SARS-CoV-2 mRNA vaccines contain PEG on the surface of the LNP. Yi Ju et al. and Carreño et al. reported that mRNA-1273 induced more anti-PEG antibodies than BNT162b2 in healthcare workers or volunteers [31,32]. Our study further targeted patients with immunodeficiency caused by the disease itself or treatment for the disease who were administered BNT162b2. Regarding the increase in anti-PEG IgG, patients whose anti-PEG IgG increased had higher anti-SARS-CoV-2 antibodies than those whose anti-PEG IgG did not increase in autoimmune disease. This phenomenon was not seen in patients with hematological disease; the humoral response in hematological disease may have been inferior to that in autoimmune disease. These data suggest that anti-PEG antibodies are induced by BNT162b2 in some patients, largely depending on the individual's ability to produce antibodies.

Recently, anti-PEG antibodies have been detected in high percentages of humans who have not knowingly received PEGylated therapeutics. Chen et al. reported that 44.3 % of individuals had "pre-existing" anti-PEG IgG and/or IgM antibodies in their serum, and its prevalence was markedly higher in females than in males (32.0 % vs. 22.2 % for anti-PEG IgM, 28.3 % vs. 23.0 % for anti-PEG IgG) among 1504 healthy Chinese donors [33]. In our study, in terms of pre-existing anti-PEG antibodies, the percentage of women with autoimmune disease was predominant. We recently indicated that the daily application of cosmetics containing PEG derivatives primed the immune system, inducing anti-PEG IgM production in mice [34]. PEG derivatives in cosmetic products may be the source of the "pre-existing" anti-PEG antibodies detected in healthy individuals.

It is well known that injection of PEGylated liposomes attenuates the long-circulating characteristics of a second dose of PEGylated liposomes, which is referred to as the "ABC phenomenon" [35]. The ABC phenomenon is thought to be mediated by anti-PEG IgM produced in the spleen in response to the first dose of PEGylated substances via anti-PEG IgM-mediated complement activation [36]. Therefore, as stated above, anti-PEG antibodies, pre-existing or induced by anti-SARS-CoV-2 mRNA vaccination, may cause the ABC phenomenon and hamper the acquisition of anti-SARS-CoV-2 antibodies via SARS-CoV-2 mRNA vaccination. Contrary to expectations, the baseline anti-PEG IgM and anti-PEG IgG titers did not correlate with those of anti-SARS-CoV-2 antibodies obtained via vaccination. Therefore, our data suggest that the pre-existing and/or induced anti-PEG antibodies observed in the patients do not attenuate the specific antibody production induced by BNT162b2 in the patients with immunodeficiency caused by the disease itself or treatment.

There are no studies comparing patients with hematological disease in which antibody production is less likely to occur and autoimmune disease in which antibody production is thought to be more likely. This is the first paper to conclude that pre-existing anti-PEG antibodies do not contribute to vaccine efficacy in these patients. It is important to show the effectiveness of PEG-containing vaccines in patients with hematological disease who are often exposed to PEG products such as PEGylated recombinant factor VIII products and pegylated granulocyte colony-stimulating factor. In future studies, it is necessary to monitor adverse events other than anaphylaxis caused by PEG-containing vaccines in patients with anti-PEG antibodies. The factors associated with having pre-existing anti-PEG antibodies are also an important research topic.

This study had some limitations. Because this study was conducted within the scope of routine medical care, and there was a tendency to refrain from hospital visits due to the COVID-19 outbreak, the timing of serum harvest after vaccination was inconsistent among patients. It was not possible to have the patient visit the hospital after 3 weeks and collect the serum due to concerns about the slow response caused by the second vaccination. Only a limited number of patients have been able to measure anti-PEG antibodies, and it may not be generalizable despite this study focused on patients with hematological and autoimmune diseases because the disease conditions and treatments vary among individuals. Comparisons among the effects of diseases and drugs are not possible because it was difficult to prepare healthy participants that matched to the population. In addition, many assay methods are available for detecting anti-PEG antibodies, such as Western blotting, acoustic membrane microparticle technology, ELISA, flow cytometry, and surface plasmon resonance technology [32,37]. We selected ELISA to estimate anti-PEG antibody titers, but this is not enough to be standardized among laboratories until now, which may be a gap in the experimental conditions. There may be deviations due to differences in serum storage methods and measurement kits when measuring anti-PEG antibodies and when measuring anti-SARS-CoV-2 IgG.

5. Conclusion

It is difficult to acquire anti-SARS-CoV-2 antibodies due to factors such as age and the number of days after the second vaccination, as well as anti-CD20 antibodies in patients with hematological disease. Increases in anti-PEG IgG and anti-SARS-CoV-2 antibodies are associated, reflecting the ease with which humoral responses can be obtained in patients with autoimmune disease. Pre-existing anti-

PEG antibody had no effect on anti-SARS-CoV-2 antibody acquisition by the vaccine.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Taiki Hori: Writing – original draft, Data curation, Conceptualization. **Taro Shimizu:** Investigation, Data curation. **Hidenori Ando:** Investigation, Data curation. **Naoto Okada:** Formal analysis, Data curation, Conceptualization. **Hiroki Yamagami:** Investigation. **Saya Yasui:** Investigation. **Minae Hosoki:** Investigation. **Akihiro Tojima:** Investigation, Data curation. **Toshiki Otoda:** Investigation. **Tomoyuki Yuasa:** Investigation. **Ken-ichi Aihara:** Writing – review & editing, Supervision. **Makoto Takishita:** Investigation. **Sumiko Yoshida:** Investigation, Conceptualization. **Masahiro Abe:** Supervision. **Tatsuhiro Ishida:** Methodology, Formal analysis, Data curation. **Shingen Nakamura:** Writing – review & editing, Writing – original draft, Project administration, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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