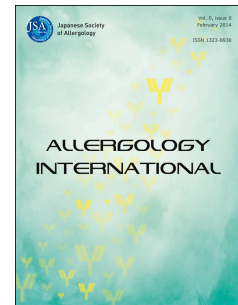


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Serum polyethylene glycol-specific IgE and IgG in patients with hypersensitivity to COVID-19 mRNA vaccines

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Original Article

Serum polyethylene glycol-specific IgE and IgG in patients with hypersensitivity to

COVID-19 mRNA vaccines

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Authors' contributions:

MMou, MI, and KK developed the research idea and wrote the manuscript. TS, HM, MMor, TO, MY, HK, and MK supervised the study. MMou, MI, SS, TK, YI, KS, HN, NM, MU, TA, SO, HK, and TO treated many patients and provided peripheral blood samples from the patients included in this study. MMou, SS, TK, and MI collected and processed blood samples. MMou and MI were responsible for most of the experiments and analyzed the data.

ABSTRACT

BACKGROUND

The mechanism of allergic reactions to COVID-19 mRNA vaccines has not been clarified. Polyethylene glycol (PEG) is a potential antigen in the components of vaccines. However, there is little evidence that allergy after COVID-19 mRNA vaccination is related to PEG. Furthermore, the role of polysorbate (PS) as an antigen has also not been clarified. The objective of this study was to investigate whether PEG and PS allergies are reasonable causes of allergic symptoms after vaccination by detecting PEG-specific and PS-specific antibodies.

METHODS

Fourteen patients who developed immediate allergic reactions to BNT162b2 (Pfizer-BioNTech) or mRNA-1273 (Moderna) vaccines and nineteen healthy controls who did not present allergic symptoms were recruited. Serum PEG-specific immunoglobulin E (IgE) and immunoglobulin G (IgG) and PS-specific IgE and IgG were measured by enzyme-linked immunosorbent assay. Skin tests using PEG-2000 and PS-80 were applied to five patients and three controls.

RESULTS

Serum levels of PEG-specific IgE and IgG in patients with immediate allergic reactions to the COVID-19 mRNA vaccine were higher than those in the control group. Serum levels of PS-specific IgE in patients with allergy to the vaccine were higher than those in patients of the control group. Intradermal tests using PEG verified the results for PEG-specific IgE and IgG.

CONCLUSIONS

The results suggest that PEG is one of the antigens in the allergy to COVID-19 mRNA vaccines. Cross-reactivity between PEG and PS might be crucial for allergy to the vaccines. PEG-specific IgE and IgG may be useful in diagnosing allergy to COVID-19 mRNA vaccines.

Key words: COVID-19 mRNA vaccines; PEG-specific IgE; PEG-specific IgG; Polyethylene glycol; Polysorbate

Abbreviations

PEG, Polyethylene glycol; PS, Polysorbate; COVID-19, Coronavirus disease 2019

83

84 **INTRODUCTION**

85 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in December
86 2019 and rapidly spread globally, thereby, causing a respiratory disease pandemic named
87 coronavirus disease 2019 (COVID-19).¹ As the global COVID-19 epidemic continued to
88 aggravate, the importance of vaccination increased rapidly.

89 In Japan, BNT162b2 (Pfizer-BioNTech) mRNA vaccination started in February 2021.
90 Subsequently, vaccination with the mRNA-1273 (Moderna) mRNA vaccine and
91 AstraZeneca viral vector vaccine were also initiated, and now three types of COVID-19
92 vaccines are being administered. The COVID-19 vaccines have been highly effective,²⁻⁴
93 but adverse reactions have also been reported. In particular, allergic reactions are one of
94 their most important side effects.⁵⁻⁷ The anaphylaxis frequency reported in the first month
95 of BNT162b2 vaccination was 8.1/1 million doses in Japan, corresponding to levels 1-3
96 of the Brighton Collaboration criteria.^{8,9} This frequency is similar to that reported by
97 Castells *et al.* for BNT162b2 in approximately 2 million healthcare workers in the United
98 States,¹⁰ where the anaphylaxis incidence was 1 case per 100 000 doses, which is higher
99 than the incidence reported for other vaccines developed prior to COVID-19 mRNA

vaccines.

Both BNT162b2 and mRNA-1273 contain polyethylene glycol (PEG)-2000 as an excipient to stabilize lipid nanoparticles that encapsulate mRNA.¹¹ Although PEG is considered a possible cause of hypersensitivity to these mRNA vaccines,¹² it is still not clear which vaccine component is responsible for the allergic reactions.¹⁰

Polysorbate (PS) is also an excipient used to increase the water solubility of drugs and vaccines. Before the widespread use of COVID-19 mRNA vaccines, there were few injectable drugs containing PEG. In contrast, PS has been widely used in other vaccines and biologics. The cross-reactivity of PEG with PS owing to their shared chemical moiety has been described in previous studies.¹³⁻¹⁶ Therefore, patients allergic to PS should be carefully monitored when receiving COVID-19 mRNA vaccines containing PEG.

Immediate allergies, including anaphylaxis, are often caused by immunoglobulin E (IgE)-mediated type I hypersensitivity. However, there are no established commercial PEG-specific IgE antibody tests. Stone *et al.* detected PEG-specific IgE and immunoglobulin G (IgG) antibodies in the plasma by enzyme-linked immunosorbent assay (ELISA) for patients with history of immediate hypersensitivity to PEG-containing medications.¹⁴ Nevertheless, there is little evidence that attributes these vaccine allergic

reactions to PEG. Some studies have shown that PEG-specific IgE has not been detected in the serum of patients allergic to COVID-19 mRNA vaccines.^{17,18} Furthermore, skin testing for PEG has shown limitation for the prediction of allergic reactions.¹⁹

In this study, we used an ELISA system for the detection of PEG-specific IgE and IgG antibodies to investigate if PEG allergy is a reasonable cause of allergic symptoms after COVID-19 mRNA vaccination. Healthy subjects were used as a control group.

METHODS

Study design and participants

This study was a single-center retrospective investigation. The allergic and control groups were recruited by health care workers and medical students who received the BNT162b2 vaccine at the St. Marianna University of Medicine between March 8 and April 30, 2021 (Fig. 1).

For the analysis of PEG- and PS-specific IgE and IgG based on ELISA, patients with allergic symptoms who received the BNT162b2 and mRNA-1273 in other institutions by August 15, 2021, were referred to the Department of Allergy at St. Marianna University School of Medicine and were included in the allergic group (Fig. 1). The allergic group included patients who presented allergic symptoms²⁰ (definition shown in Supplementary Table 1) after mRNA vaccination, and the control group included those who did not show any allergic symptoms. The former was further classified into immediate allergy group, which presented symptoms within 3 h of vaccination,¹⁷ and delayed allergy group, which presented symptoms 3 h to 7 d after the vaccination.

This study was approved by the ethics committee of the St. Marianna University School of Medicine (approval number 5319). Serum was collected from those who provided

written consent. The time of the first vaccination was set as day 0, and serum samples were collected after day 21.²¹

Detection of PEG-specific IgE and IgG by ELISA

ELISA was used to detect the PEG and PS-specific antibodies. For PEG-specific IgE and IgG, 96-well microplates were coated with diamino-polyethylene glycol (molecular weight 3000, Sigma-Aldrich Cat#14502) at 5 µg/ml.¹⁴ The serum was obtained 21 or more days after the first injection of BNT162b2 or mRNA-1273 mRNA vaccines. The serum samples were diluted 4-fold in phosphate-buffered saline (PBS) and then incubated. Subsequently, biotin-conjugated goat anti-human IgE antibody (Bio-Rad STAR147B) was added at a 1:10 000 dilution. Horseradish peroxidase (HRP)-conjugated streptavidin (BD Bioscience 554066) was then added at a 1:3000 dilution, and a 3,3',5,5'-tetramethylbenzidine (TMB) substrate (KPL 5120-0053) was used as the detection reagent. Phosphoric acid (1 M) was used as the stop solution, and the plates were read at dual wavelengths of 450 and 600 nm in a microplate reader (iMark, Bio-Rad). For PEG-specific IgG detection, coating was the same as that for PEG-specific IgE, but serum samples were diluted 32-fold. Subsequently, HRP-conjugated goat anti-human IgG antibody (Millipore AP309P) was incubated at 1:100 000 dilution, and the TMB substrate was used for detection.

To prepare a standard curve for PEG-specific IgE and IgG, serum at day 84 (12 weeks after vaccination) was collected from patient #3, whose absorbance of PEG-specific IgE and IgG observed in the preliminary study was relatively high. The serum of #3 was aliquoted and stored at -80°C , and thawed at the time of use. The standard serum sample was diluted with PBS. A standard curve for PEG-specific IgE was created with a 2-fold dilution set to 20 000 units and a 256-fold dilution set to 156.25 units. The standard curve for PEG-specific IgG was created with a 4-fold dilution set to 10 000 units and a 512-fold dilution set to 78.13 units.

Detection of PS-specific IgE and IgG by ELISA

PS-specific IgE and IgG were also measured using ELISA. The 96-well microplates were coated with PS-80 (Wako-Fujifilm) at a 1000-fold dilution. The protocol for diluting serum samples was the same as that for the detection of PEG-specific IgE and IgG. The standard curves for PS-specific IgE and IgG ELISA were prepared using the serum of #3 and the same method as that for the preparation of the standard curve for PEG-specific IgE.

Skin testing with PEG and PS

Skin tests were performed on the forearm of healthy controls and patients suspected of

having immediate hypersensitivity to the COVID-19 mRNA vaccine and wished to receive the respective second dose. In addition, skin testing was performed for one patient with delayed hypersensitivity to COVID-19 mRNA vaccine upon request. The PEG-2000 and PS-80 concentrations were determined by referring to the reports of Stone *et al.*¹⁴ and Banerji *et al.*⁵ (Supplementary Table 2). The reagents used in the skin tests were PEG-2000 (Wako-Fujifilm), which has the same molecular weight as the PEG used in COVID-19 vaccines, and PS-80 (Wako-Fujifilm), which is used in viral vector vaccines from AstraZeneca and others. The negative control was saline for both the prick and intradermal tests. In the prick test, the PEG-2000 concentration was increased from 0.1 to 1, 10, and 100 mg/ml, and PS-80 was 0.25%. If the prick test was negative, we performed an intradermal test with PEG-2000 at concentrations of 0.1, 1, 10, and 30 mg/ml and PS-80 at 0.025%. The final concentration of PEG-2000 (30 mg/ml) in the current study was the same as that in Depo-Medrol, and was 10-times higher than that reported by Banerji *et al.* We confirmed that intradermal tests with PEG-2000 (30 mg/ml) did not cause skin rash or allergic symptoms in three healthy controls. Therefore, we decided the maximum concentration of PEG-2000 to be 30 mg/ml. If all results were negative, we scheduled a challenge test with BNT162b2. The prick, intradermal, and challenge tests were all judged at 15 min. The skin prick test was judged positive if a

wheal of 3 mm or greater was observed. If a wheal greater than 9 mm or erythema greater than 20 mm was observed, which was not observed in the case of saline, the intradermal test was considered positive. If the wheal or erythema was not seen in the skin test area, but the symptoms appeared at the time of vaccination, we judged the test to be positive.

Statistical analysis

The difference between the two groups for serum PEG-specific and PS-specific IgE and IgG levels were examined using the Mann–Whitney U test. In all analyses, $p < 0.05$ was considered statistically significant. All statistical analyses were performed using the GraphPad Prism software (version 5.01).

RESULTS

Characteristics of patients with allergy to mRNA vaccines

At the St. Marianna University Hospital, approximately 7809 doses of BNT162b2 mRNA vaccine were administered from March 8 to April 30, 2021. Almost all cases of suspected immediate hypersensitivity after vaccination were identified by the Health Management Center of St. Marianna University School of Medicine Hospital and referred to the outpatient clinic of the Department of Allergy. Nine patients (0.12%) showed immediate-type allergic reactions, and three patients (0.04%) were diagnosed with anaphylaxis according to the Brighton classification,⁹ at the St. Marianna University School Hospital (Fig. 1). Twenty patients were diagnosed with immediate or delayed allergy after mRNA vaccination, and all patients in the allergy group developed allergic symptoms after the first vaccination. Table 1 presents a summary of the cases. Fourteen patients, all female, developed symptoms of immediate allergy, such as skin rash and cough, within 3 h of the first vaccination shot (Fig. 1), and four of them were immediately hospitalized after epinephrine administration. Serum samples collected after day 21 were available for 18 patients from the allergy group (immediate-type: 14, delayed-type: 4) and 19 controls (Fig. 1).

The share of female patients was 100%, 50%, and 79% for immediate-type, delayed-type, and control groups, respectively, and the respective median ages in these groups were 34, 34, and 33 y (Table 2).

Serum PEG-specific and PS-specific antibodies

PEG-specific IgE and IgG values were not normally distributed and were analyzed using the Mann–Whitney U test. The median serum levels of PEG-specific IgE in the immediate allergy and control groups were 4897.9 and 848.3 units, respectively. The serum levels of PEG-specific IgE in the immediate allergy group were significantly higher than those in the control group ($p = 0.009$; Fig. 2A). Furthermore, serum levels of PEG-specific IgE for the entire allergy group were significantly higher than those in the control group ($p = 0.001$; Supplementary Fig. 1A). The serum levels of PEG-specific IgG were also significantly higher in both immediate ($p = 0.030$) and entire ($p = 0.007$) allergy groups than in the control group (Fig. 2B; Supplementary Fig. 1B). PEG-specific IgE and IgG were significantly higher in the delayed allergy group than in the control group (PEG-specific IgE: $p = 0.011$, PEG-specific IgG: $p = 0.026$, Supplementary Fig. 2A, B). These results suggest that PEG is one of the antigens in the allergy to COVID-19 mRNA vaccines, and not only PEG-specific IgE, but also PEG-specific IgG might be crucial for the pathogenesis of allergic reactions.

PS-specific IgE and IgG values were also not normally distributed and were analyzed using the Mann–Whitney U test. There were no significant differences in the serum levels of PS-specific IgE between the immediate allergy and control groups ($p = 0.08$; Fig. 2C). However, the serum levels of PS-specific IgE tended to be higher in the immediate allergy group. Serum PS-specific IgE was significantly higher in entire allergy groups and delayed allergy group than in the control group (entire: $p = 0.015$; Supplementary Fig. 1C; delayed: $p = 0.009$; Supplementary Fig. 2C). In contrast, serum levels of PS-specific IgG were not different between the allergy and control groups (immediate: $p = 0.44$; Fig. 2D; entire: $p = 0.19$; Supplementary Fig. 1D; delayed: $p = 0.07$; Supplementary Fig. 2D). These data suggest that pre-sensitization with PS might induce an allergic reaction to COVID-19 mRNA vaccines.

Correlation between PEG-specific IgE and PEG-specific IgG or PS-specific IgE

We examined whether serum levels of PEG-specific IgE were associated with PEG-specific IgG or PS-specific IgE. There was a correlation between PEG-specific IgE and PEG-specific IgG levels (Fig. 3A) (Spearman's rank correlation coefficient = 0.855, $p < 0.001$). Some patients, including patients #2, #3, and #10, were predominantly PEG-specific IgE, whereas others, such as patient #14, were predominantly PEG-specific IgG. In addition, there was a correlation between PEG-specific IgE and PS-specific IgE levels

(Spearman's rank correlation coefficient 0.795, $p < 0.001$), which suggested a cross-reaction between PS and PEG-specific IgE (Fig. 3B).

Skin tests using PEG and PS

Skin tests were performed on six patients of the allergy group (five immediate allergic cases: patients #2, #3, #5, #8, and #15, one delayed allergic case, patient #20) and three patients in the healthy control group (HC #1, #2, #3). The results for all three control cases were negative for both skin prick and intradermal tests. In the intradermal test with PEG-2000 and PS-80, symptoms similar to those seen with BNT162b2 vaccination appeared in three of the five immediate allergic patients (#2, #3, and #8) (Supplementary Fig. 3, Table 3). The symptoms during the allergy onset and details on the results of the skin tests and PEG-specific IgE of five immediate allergic cases are shown in Table 3 and summarized in the Supplementary Methods. The results of the skin tests and PEG-specific IgE for one delayed allergic case are summarized in the Supplementary Methods.

The serum levels of PEG- and PS-specific IgE of the positive and negative skin test groups were compared. The serum levels of PEG-specific IgE in the positive skin test group ($n = 3$) were significantly higher than those in the negative skin test group ($n = 5$; $p = 0.036$) (Fig. 4A). The serum levels of PS-specific IgE were also significantly higher

272 in the positive skin test group than in the negative one ($p = 0.033$) (Fig. 4B). Of the
273 patients for whom a skin test was performed, only one patient, #15, received a second
274 dose of BNT162b2. She presented with low PEG-specific IgE, and no allergic reaction
275 upon receiving the second COVID-19 mRNA vaccine. These data suggest that both
276 PEG-specific and PS-specific IgE might be useful for the diagnosis of immediate-type
277 allergy to mRNA vaccines.

DISCUSSION

Despite several studies on this topic, the mechanism of allergic reactions to COVID-19 mRNA vaccines is still unclear.^{11,17,18,22-24} To the best of our knowledge, this is the first study to report elevated PEG-specific IgE and IgG levels in patients with suspected hypersensitivity to COVID-19 mRNA vaccines. In cases with borderline to high PEG-specific IgE levels, in the intradermal skin tests with PEG-2000 and PS-80, symptoms similar to those seen with mRNA vaccination appeared. Therefore, the results of this study suggest that PEG is one of the antigens in the allergy to COVID-19 mRNA vaccines, and both PEG-specific IgE and PEG-specific IgG might be useful in diagnosing immediate-type allergy to PEG. Furthermore, there was a tendency for higher levels of PS-specific IgE in the immediate allergy group. Although further investigation on PS-specific IgE requires the investigation of more cases, the present results are potentially useful as a diagnostic method for both PS and PEG allergy.

Previous studies suggest that PEG might be the target of hypersensitivity to COVID-19 mRNA vaccines,²⁵ but PEG-specific IgE has not been demonstrated. There is no consensus as to whether the allergic reactions to COVID-19 mRNA vaccines are caused by IgE-mediated hypersensitivity to PEG. Krantz *et al.* reported cases of anaphylaxis to the first dose of BNT162b2, and there was no elevation of tryptase, and all patients

tolerated the second dose with pre-medication, so they considered the mechanism to be non-IgE mediated.²² Warren *et al.* reported that PEG-specific IgE was not involved in post-vaccine allergic reaction after COVID-19 mRNA vaccine because PEG-specific IgE was not detected by ELISA and the skin prick test was negative.^{17,18} In our study, the skin prick results were negative for all cases, but some of intradermal results were positive. Troelnikov *et al.* reported that BNT162b2, but not PEG alone, induced the activation of basophils in patients, which suggested that PEGylated lipids within nanoparticles can induce degranulation.²⁵ It is not clear why PEG-specific IgE was not detected in patients with hypersensitivity to COVID-19 mRNA vaccines in previous studies. However, there were differences between theirs and ours ELISA protocols. Furthermore, the Japanese population in this study might have more PEG allergies than the cohort analyzed in other studies.

In the current study, an intradermal test using PEG validated the results of PEG-specific IgE, although the number of patients tested was limited. All skin prick results were negative for both PEG and PS, so intradermal testing was necessary to confirm hypersensitivity. For positive results, the values of PEG-specific IgE tended to be higher than those of the controls (Table 3). However, two patients who showed a negative skin test also presented low levels of PEG-specific IgE (Table 3).

Prior to the appearance of COVID-19 vaccines, reports of hypersensitivity to PEGs were rare, and most of them were attributed to the oral administration of PEGs.²⁶⁻³⁰ In Canada, 88% of the allergic cases to macrogol and common injectable medications containing PEG occurred for female patients.³¹ Prior to the distribution of the COVID-19 vaccine, few injectables contained PEG, except for PEGylated drugs. People who have been sensitized to PEG might have developed allergic symptoms when they were injected with PEG for the first time. PEG is described as a high-risk hidden allergen in drug and food items that can induce allergic reactions that are difficult to detect by healthcare providers and might, therefore, be underdiagnosed.^{13,15} Patients allergic to the COVID-19 mRNA vaccine did not complain of allergic reactions upon oral intake of PEG or PS containing food or tablets in subsequent outpatient visits. This suggests that patients who are allergic to injection with PEG may have tolerance to oral intake of PEG and PS. However, they should be carefully monitored when using laxatives containing PEG because the amount of oral intake of PEG through laxatives will be higher than that through food or tablets, which may cause allergic reactions.

In contrast, PS, which has structural similarities with PEG, is widely used in other vaccines and biologics.¹³⁻¹⁶ Nevertheless, the number of reports on PS allergy is remarkably lower than that for PEG.^{14,32-35} Although no study has reported elevated PS-

specific IgE for PS hypersensitivity,³⁴ we observed several cases of high serum PS-specific IgE levels in patients with hypersensitivity to drugs containing PS (data not shown). In the current study, for high PEG-specific IgE levels, there were cases with high PS-specific IgE levels. Three patients who underwent skin testing also presented hypersensitivity reactions to both PEG and PS, which suggests that PS allergy may also participate in drug allergy of unknown origin. Therefore, patients with PS allergy may have developed an allergic reaction to COVID-19 mRNA vaccines.

Although the IgE function in classical anaphylaxis has been established, the complementary activation of IgG or IgM could also lead to degranulation of mast cells and pseudo-allergic reactions or nonclassical anaphylaxis.¹¹ PEG-specific antibodies can be detected in the serum of healthy individuals.^{36,37} In this study, PEG-specific IgG was significantly higher in the immediate allergy group than in the control group, and there was a case where PEG-specific IgG was predominant. Therefore, in addition to PEG-specific IgE, PEG-specific IgG might also be involved in the pathogenesis of allergic reactions to COVID-19 mRNA vaccines. On the other hand, PEG-specific IgE was significantly higher in the delayed allergy group. The reason as to why PEG-specific IgE was detected in the delayed allergy group is not clear. In general, IgE-mediated reaction induces immediate symptoms, whereas delayed-type allergy is typically due to T cell-

mediated immunity.³⁸ However, chronic inflammation, such as atopic asthma and atopic dermatitis, shows Th2-type cellular immune responses with high amount of antigen-specific IgE. Serum samples from only a few patients with delayed allergy were able in the current study. Further study with more patients is needed to clarify the role of PEG-specific IgE in delayed allergy.

In the current study, the anaphylaxis frequency is higher than previously reported values.²⁴ One of the reasons may be that the vaccines were mainly given to younger age groups. Also, we established a flow to identify nearly all cases of suspected hypersensitivity after vaccination. Furthermore, there might be other possible causes, such as race and bias in vaccine serial numbers.

In this study, all cases of immediate hypersensitivity reactions occurred for female patients and were observed for the first dose of the vaccine, which is similar to the results of previous studies.^{7,8,10} These findings suggest that some women were sensitized to the components of the vaccine prior to the first vaccination.

PEG-specific IgE assays can be useful in cases of suspected hypersensitivity to the first dose vaccine or anaphylaxis caused by PEG or PS-containing drugs. For example, in patient #3, there were only respiratory symptoms, so that Brighton levels 1–3 were not

met, but hypersensitive reactions to both PEG and PS were suspected. Although these cases may not be recognized as allergies, the PEG-specific IgE ELISA might prevent them from being missed.

This study presented some limitations. First, the number of participants was small, especially for the skin test or delayed-type allergy, and there was insufficient matching of age and gender. PEG allergy to COVID-19 mRNA vaccines has been mainly diagnosed by skin tests,³⁹⁻⁴¹ and there have been no previous reports on the combined use of IgE measurement and skin tests. The number of patients in the allergic group with skin test or delayed type allergy and in the matching control group should be increased in future studies. Second, the timing of serum sampling for IgE measurement should also be further investigated. Serum samples were collected more than 3 weeks after the vaccination. Even if PEG-specific IgE is not observed immediately after anaphylaxis, it might be observed later because anaphylaxis might consume the serum levels of antigen specific IgE.²¹ Third, we could not evaluate if the values of serum PEG-specific IgE in patients with hypersensitivity to the first COVID-19 mRNA vaccination could predict anaphylaxis after the second dose. It has been reported that patients who had allergic reactions including anaphylaxis at the time of the first vaccination were able to receive the second vaccination under the administration of antihistamines.^{19,22} However, there were only a

few patients who challenged a second dose in the current study, because Japanese Ministry of Health, Labor and Welfare prohibited second vaccination using the same vaccine as the first to the patients who have experienced severe allergic reactions to any of the COVID-19 vaccines. Finally, it was difficult to determine the cutoff value for PEG-specific IgE based on the results of this study alone because some controls also showed high values. Further improvements and standardization of ELISA should be developed and applied in future studies.

In conclusion, the results of this study suggest that PEG is one of the antigens for allergic responses to COVID-19 mRNA vaccines, and both IgE- and IgG-mediated reactions are potential pathways. Therefore, the measurement of PEG-specific IgE and IgG might be useful to diagnose allergy to COVID-19 mRNA vaccines. Allergy to PS might also induce hypersensitivity to COVID-19 mRNA vaccines. Further studies with larger number of cases are needed to confirm the usefulness of the detection of PEG- and PS-specific antibodies.

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Figure Legends

Fig. 1. Flow chart of patient inclusion (disposition and population) in this study.

Fig. 2. Serum PEG-specific and PS-specific antibodies of immediate-type allergic patients (n = 14) and healthy controls (HC; n = 19). **A:** PEG-specific IgE; **B:** PEG-specific IgG; **C:** PS-specific IgE; **D:** PS-specific IgG. PEG-specific (**A**) and PS-specific (**C**) IgE are shown as natural logarithms. Bars represent median.

Fig. 3. Correlation between (A) PEG-specific IgE and PEG-specific IgG levels, and (B) PEG-specific IgE and PS-specific IgE levels. Data (n = 37) were obtained from patients with immediate or delayed allergies (n = 18) and healthy controls (n = 19), and Spearman's correlation coefficient was adopted.

Fig. 4. (A) PEG-specific and (B) PS-specific IgE of positive (n = 3) and negative (n = 5) skin test groups. PEG-specific and PS-specific IgE are displayed as natural logarithms. Bars represent median.

Table 1. Patients characteristics

Type of allergy	Patient No.	Age	Sex	Past history of allergies	Vaccine	Onset after vaccination (1 st or 2 nd dose of vaccination [†])	Signs and symptoms	Received epinephrine/ Emergency admission	Brighton level	Subsequent vaccination status [‡]	PEG-specific PS-specific IgE (U) [§]
Immediate	1	37	F	BA	BNT162b2	15 min (1 st)	Diffuse erythematous rash, difficulty breathing	Yes/Yes	3	No	9.12 8.98
	2	25	F	(-)	BNT162b2	55 min (1 st)	Urticaria, pharyngeal itching	No/No	4	No	11.08 11.48
	3	25	F	AD, AR	BNT162b2	30 min (1 st)	Stridor, persistent dry cough	Yes/Yes	4	No	11.2 10.67
	4	37	F	Metallic allergy	BNT162b2	5 min (1 st)	Urticaria, pharyngeal itching	No/No	4	No	6.14 0
	5	28	F	(-)	BNT162b2	2 h (1 st)	Erythematous rash, pharyngeal itching	No/No	4	2 nd dose, safety	6.14 4.69
	6	33	F	NSAIDs allergy	BNT162b2	10 min (1 st)	Diffuse erythematous rash, difficulty breathing, desaturation	Yes/Yes	1	No	¶ ¶
	7	31	F	AR	BNT162b2	5 min (1 st)	Coldness of limbs, shivering	No/No	4	No	9.87 10.05
	8	18	F	BA, AR	BNT162b2	10 min (1 st)	Hypotension, vomiting, abdominal pain	Yes/Yes	3	No	8.33 8.40
	9	20	F	AR	BNT162b2	2 h (1 st)	Headache	No/No	4	No	8.64 7.65
	10	48	F	AD, BA, AR, FA	BNT162b2	30 min (1 st)	Pharyngeal itching	No/No	4	No	11.28 11.42
	11	44	F	(-)	BNT162b2	2 h (1 st)	Erythematous rash	No/No	-	No	7.80 7.50
	12	49	F	LVFX allergy, eczema caused by cosmetics	BNT162b2	10 min (1 st)	Vomiting, Erythematous rash	No/Yes	4	No	6.31 4.33
	13	18	F	(-)	BNT162b2	30 min (1 st)	Nausea, diarrhea	No/No	4	No	7.8 7.31

	14	51	F	ST allergy	mRNA-1273	20 min (1 st)	Erythematous rash	No/No	4	No	9.27 9.09
	15	58	F	BA, FA	BNT162b2	3 min (1 st)	Cough	Yes/No	4	2 nd dose, safety	6.51 0
Delayed	16	49	M	AR	BNT162b2	3 d (1 st)	Diffuse erythematous rash	No/No	-	No	¶ ¶
	17	55	F	AD	BNT162b2	24 h (1 st)	Headache	No/No	-	No	7.95 7.80
	18	24	M	BA	BNT162b2	24 h (1 st)	Cough	No/No	-	No	8.98 8.61
	19	48	F	BA	BNT162b2	>24 h (1 st)	Erythematous rash	No/No	-	2 nd dose, safety	9.95 10.35
	20	19	M	BA, FA	mRNA-1273	18 h (1 st)	Erythematous rash	No/No	-	2 nd dose, safety	8.53 9.03

F, female; M, male; BA, bronchial asthma; AD, atopic dermatitis; AR, allergic rhinitis; FA, food allergy; NSAIDs, non-steroidal anti-inflammatory drugs; LVFX, levofloxacin; ST, sulfamethoxazole-trimethoprim; BNT162b2, Pfizer-BioNTech mRNA vaccine; mRNA-1273, Moderna mRNA vaccine.

† Allergic symptoms appeared at the time of the first dose or after the second dose. 1st, first dose; 2nd, second dose.

‡ No, No subsequent vaccinations were given; 2nd dose, safety, The second vaccinations were safely administered, but patient #19 and #20 had the same erythema as in the case of the first dose.

§ PEG-specific IgE and PS-specific IgE are expressed in natural logarithm. Top, PEG-specific IgE; bottom, PS-specific IgE.

¶ No serum samples were obtained.

Table 2. Characteristics of the immediate and delayed allergy groups and HC group

Characteristics	Immediate type (n = 14) [†]	Delayed type (n = 4) [‡]	HC (n = 19)
Female, n (%)	14 (100%)	2 (50%)	15 (79%)
Age, median (IQR)	34 (25–47)	34 (18–48)	33 (27–42)
Allergic history, n (%)	10 (71%)	4 (100%)	8 (42%)
BNT162b2, n (%)	13 (93%)	3 (75%)	19 (100%)
Anaphylaxis, n (%)	2 (14%)	0 (0%)	0 (0%)

IQR, interquartile range; HC, healthy control.

Two patients ([†] Patient #6, [‡] Patient #16) who did not give their consent were excluded from the study.

Table 3. Skin test and PEG-specific IgE test results

Patient No.	SPT	IDT [†]					Challenge test	PEG-specific IgE (U) [‡]	PS-specific IgE (U) [‡]
		Saline (negative control)	PEG-2000 0.1 mg/ml	PEG-2000 1 mg/ml	PEG-2000 30 mg/ml	PS-80 0.025%	BNT162b2		
#2	(-)	(-)	(-)	(-)	positive [§] 4×4/56×41	positive [§] 4×4/27×27	N/A	11.08	11.5
#3	(-)	(-)	(-)	(-)	positive [§] 0×0/0×0 [¶]	positive [§] 0×0/15×15 [¶]	N/A	11.2	10.7
#5	(-)	(-)	(-)	(-)	(-)	(-)	(-)	6.14	4.69
#8	(-)	(-)	(-)	(-)	positive [§] 5×7/45×24	positive [§] 6×6/44×27	N/A	8.33	8.40
#15	(-)	(-)	(-)	(-)	(-)	(-)	(-)	6.51	0
HC #1	(-)	(-)	(-)	(-)	(-)	(-)	N/A	7.70	6.62
HC #2	(-)	(-)	(-)	(-)	(-)	(-)	N/A	6.04	0
HC #3	(-)	(-)	(-)	(-)	(-)	(-)	N/A	6.23	0

SPT, skin prick test; IDT, intradermal test; HC, healthy control; BNT162b2, Pfizer-BioNTech mRNA vaccine; N/A, not available; (-), negative.

[†] Positive criteria: Wheal size greater than 9 mm or erythema size greater than 20 mm.

[‡] PEG-specific IgE and PS-specific IgE are expressed in natural logarithm.

[§] Wheal / erythema size (mm) were noted below.

[¶] Patient #3 presented a dry cough similar to that after vaccination.

Supplementary Methods

Details of patients who underwent skin testing

Patient #2

A 25-year-old woman with allergic rhinitis arrived at the emergency room complaining of urticaria and itchy throat, which started 55 min after the first vaccination shot. She was treated with methylprednisolone (mPSL), an antihistamine, which resolved the symptoms, and hospitalization was not required. Two months after the vaccination, urticaria appeared after the use of an emulsion containing PEG-32 and PEG-60. Allergy to PEG was immediately suspected and a skin test was performed; skin prick test (SPT) at 100 mg/ml PEG caused itching on the forearm on the side of the puncture, but no obvious erythema was observed. The SPT of PS was negative. Immediately after the intradermal injection of PEG 30 mg/ml, a pruritic erythema of 56 mm × 41 mm appeared, and immediately after the intradermal injection of PS-80, a 27 mm erythema also appeared in the injection site.

Patient #3

A 33-year-old woman with history of atopic dermatitis and seasonal allergic rhinitis

presented dry cough and stridor 30 min after the vaccination. After epinephrine was administered, blood oxygen saturation was 96%, and symptoms improved. She was admitted to the hospital as an emergency case and was treated with antihistamines and mPSL. She was discharged from the hospital and continued steroid inhalation for one month, which resolved the symptoms. A skin test was performed, and the SPT was negative for both PEG and PS. After 5 min of an intradermal injection of 30 mg/ml of PEG, the patient presented a dry cough similar to that after vaccination. Thirty minutes later, the symptoms had almost disappeared, and no medication was required. One hour after the symptoms disappeared, the intradermal PS injection was applied. Four minutes after the injection, the patient presented dry cough, similar to that for the intradermal PEG injection, and an erythema of 15 mm appeared around the intradermal injection site. The dry cough resolved within 30 min without medication. After discharge from the hospital, dry cough reappeared, and steroid inhalers were restarted.

Patient #5

A 29-year-old woman with no history of allergies presented erythema on her face and extremities and pharyngeal itching 2 h after the BNT162b2 injection. The symptoms improved with oral fexofenadine and topical glucocorticoids. A skin test was performed,

and all results, including that for intradermal injection of BNT162b2, were negative.

Patient #8

An 18-year-old woman with history of asthma, vasovagal reflex, and seasonal allergic rhinitis presented nausea, abdominal pain, and hypotension (systolic blood pressure [SBP]: 88 mmHg; diastolic blood pressure [DBP]: 59 mmHg) 10 min after the vaccination.

She received an intramuscular injection of adrenaline twice and intravenous injections of antihistamines and mPSL. The patient was admitted to the hospital as an emergency case. Although the reaction onset did not occur immediately after vaccination, a skin test was performed because the patient had a history of vasovagal reflex, and the diagnosis of anaphylaxis was not clear. Vasovagal reflex was observed when securing the venous route, which improved with lower extremity elevation. SBP improved from 61 to 105 mmHg, and a skin test was conducted. The intradermal PEG 30 mg/ml test resulted in a 45 mm × 24 mm erythema and a 4 mm swelling, whereas the intradermal PS test led to a 44 mm × 27 mm erythema centered on the injection site immediately after administration and remained after 15 min.

Patient #15

A 49-year-old woman with history of asthma started coughing 5 min after vaccination. She received an intramuscular injection of adrenaline and intravenous injection of hydrocortisone. The symptoms were relieved, and hospitalization was not required. A skin test was performed, and all results, including allergy to the intradermal injection of BNT162b2, were negative. She received the second dose of BNT162b2 and did not present an allergic reaction.

Patient #20

A 19-year-old man with history of asthma and food allergies presented erythematous rash 18 hours after the vaccination (mRNA-1273). We included him as a delayed allergy group. He and his family strongly wished to undergo the skin test.

Both the skin prick test and the intradermal test with PEG-2000 were negative; the intradermal test with BNT162b2 was negative at 15 minutes, but erythema (size: 8×4 cm) was observed at the injection site after 8 hours and persisted for about 3 days. The patient was able to safely receive the second dose of mRNA vaccine without immediate allergic reaction, but presented erythematous rash on his legs 6 hours after the second

vaccination (BNT162b2).

Supplementary Table 1. Inclusion criteria

Organ system	Symptoms
Skin and mucous membrane	Erythema, urticaria, angioedema, pruritus, burning sensation, eczema, conjunctival hyperemia and edema, pruritus, lacrimation, blepharedema, discomfort and/or swelling of the oral cavity/pharynx/lips/tongue
Cardiovascular	Decreased blood pressure, tachycardia, bradycardia, arrhythmia, coldness of limbs, pallor
Respiratory	Discomfort/itching/tightness in the pharyngo-larynx, hoarseness, dysphagia, coughing, wheezing, retractive breathing, feeling of chest tightness, dyspnea, cyanosis, rhinorrhea, nasal congestion, sneezing
Gastrointestinal	Nausea, vomiting, abdominal pain, diarrhea, hematochezia
Nervous system	Headache, lowered vigor, unrest, impaired consciousness, incontinence

Supplementary Table 2. Skin test protocol

		PEG-2000	PS-80	BNT162b2
Step 1	Skin prick test	0.1 mg/ml	0.25%	
		1 mg/ml		
		10 mg/ml		
		100 mg/ml		
Step 2	Intradermal test	0.1 mg/ml	0.025%	
		1 mg/ml		
		10 mg/ml		
		30 mg/ml		
Step 3	Challenge test			

Fig.1

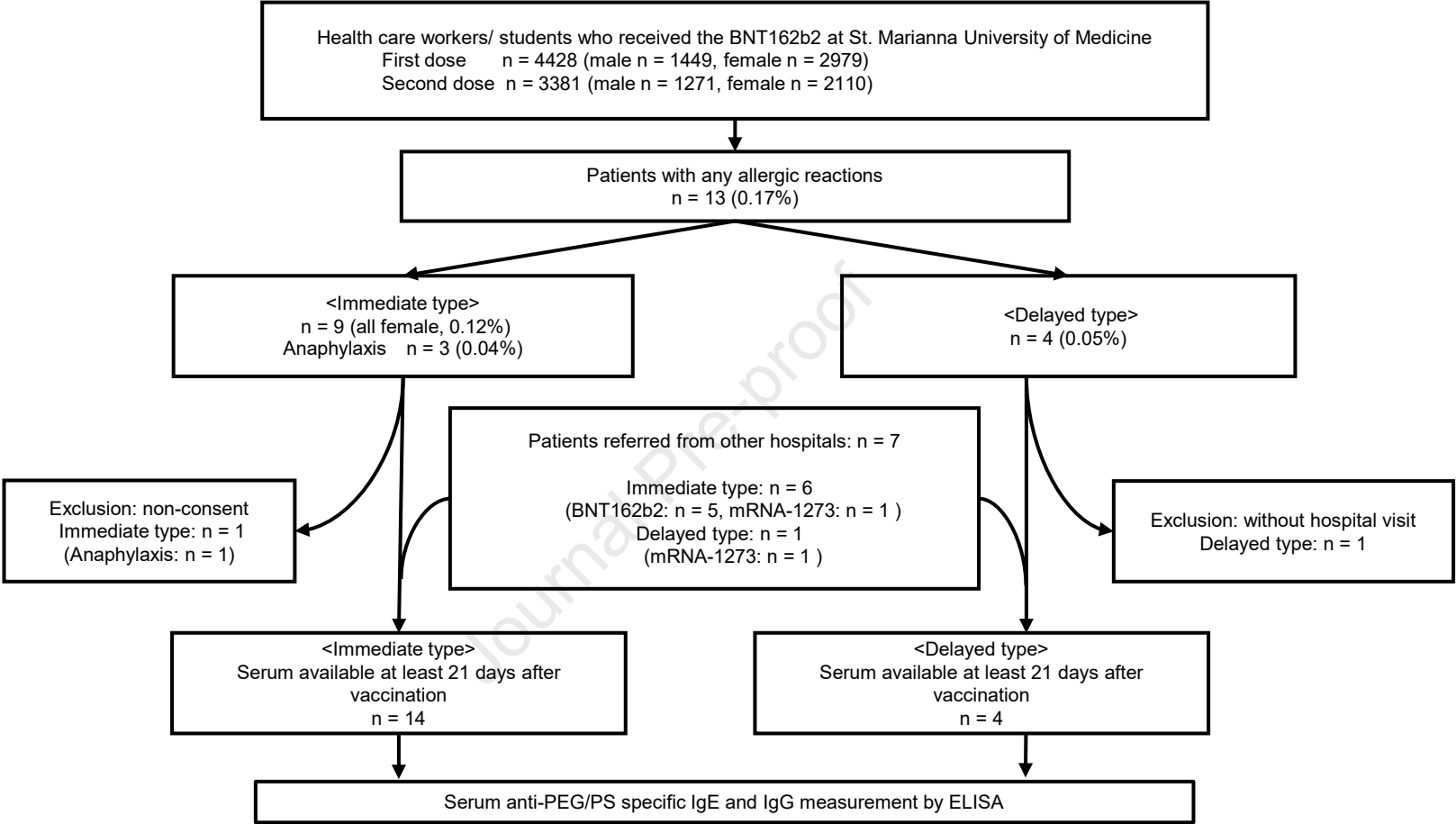


Fig. 2

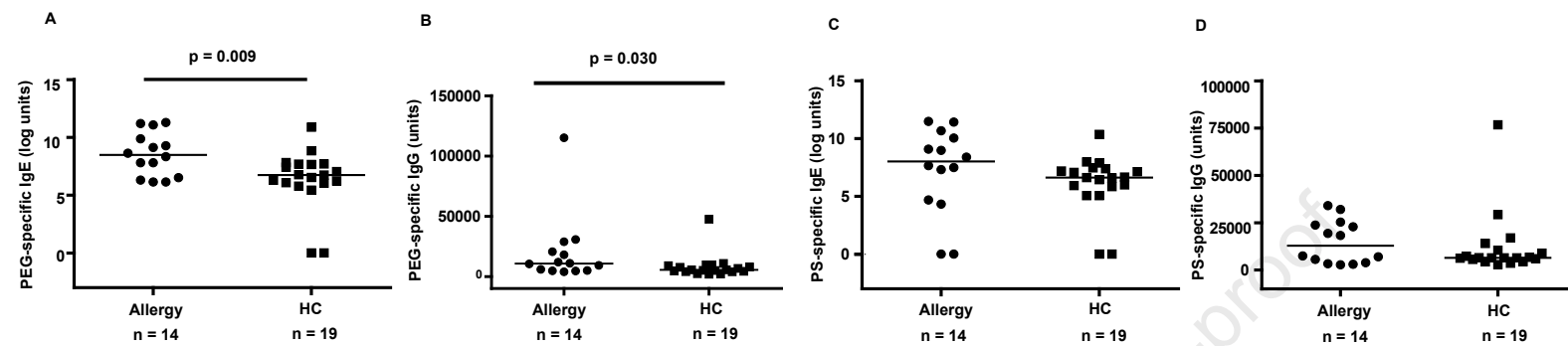


Fig. 3

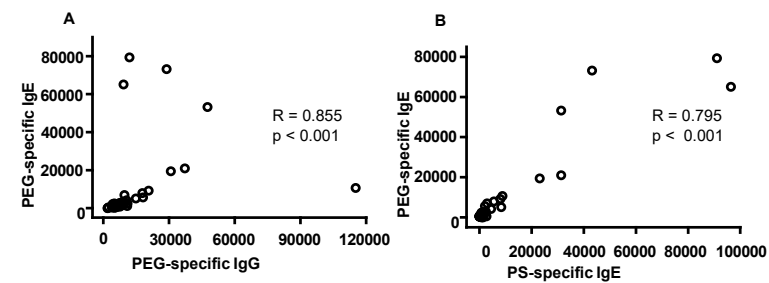
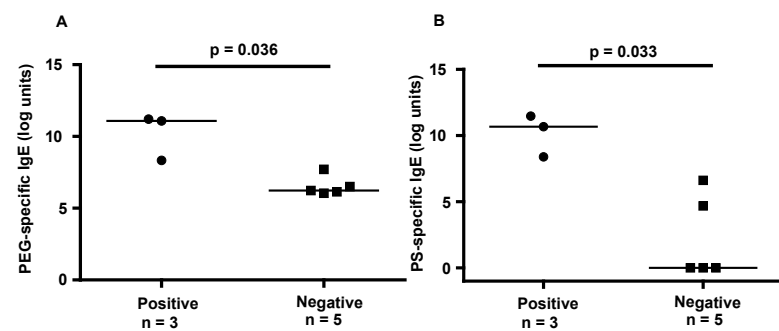
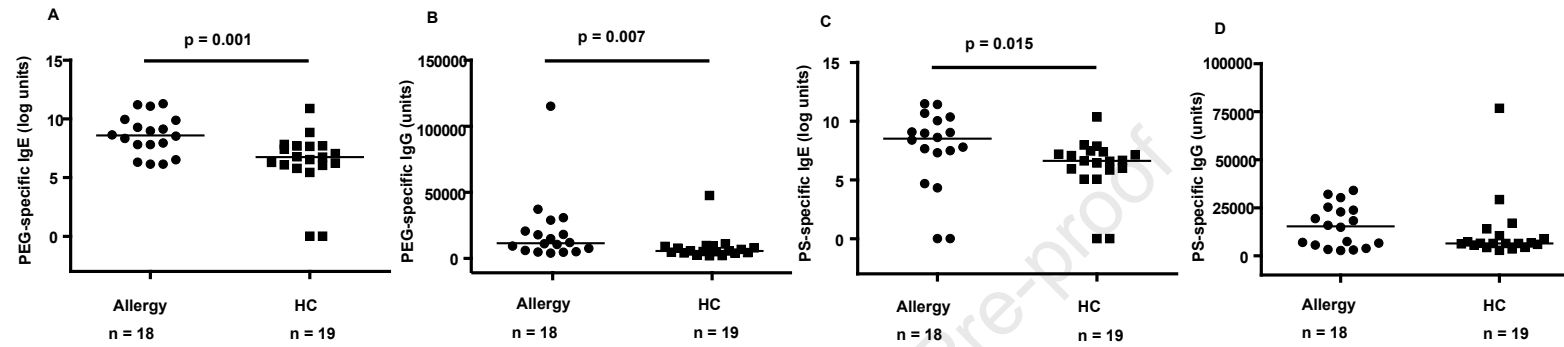
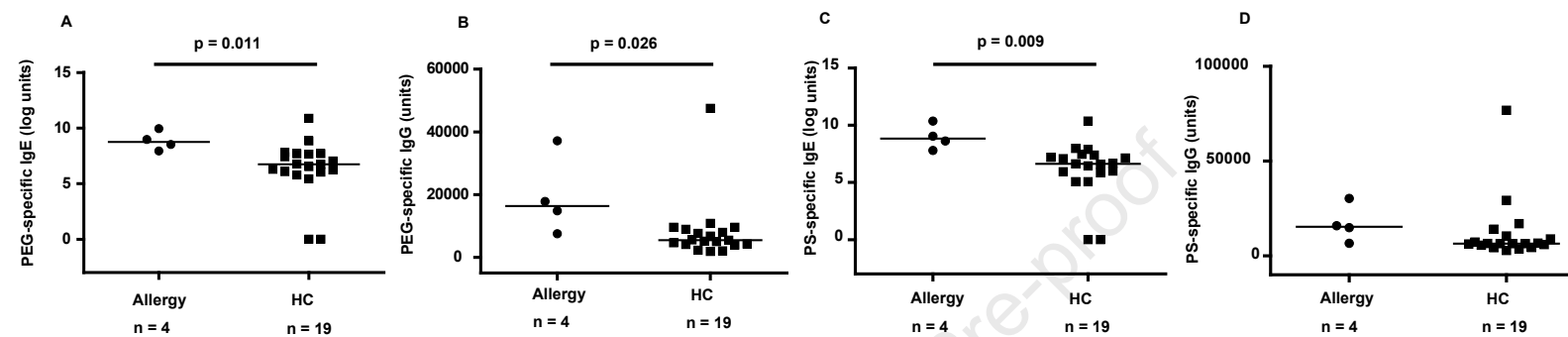


Fig. 4

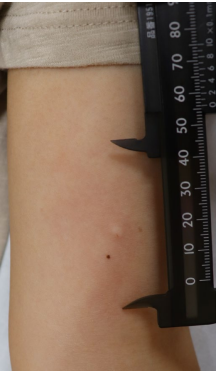
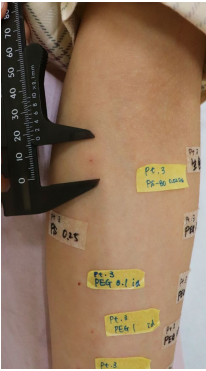
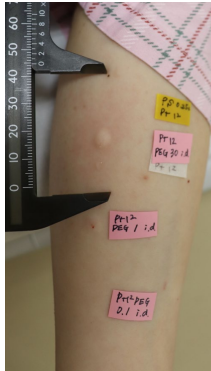
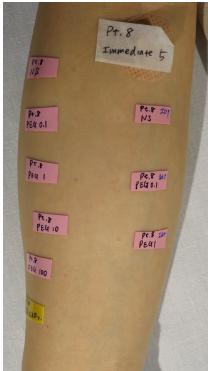
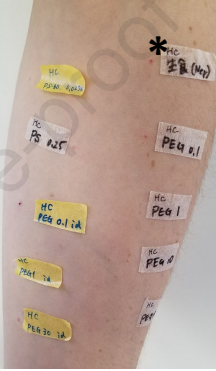




Supplementary Figure 1. Serum (A) PEG-specific IgE, (B) PEG-specific IgG, (C) PS-specific IgE, and (D) PS-specific IgG levels of entire allergic patients (n = 18) and healthy controls (HC; n = 19). (A) and (C) are shown as natural logarithms. Bars represent median.



Supplementary Figure 2. Serum (A) PEG-specific IgE, (B) PEG-specific IgG, (C) PS-specific IgE, and (D) PS-specific IgG levels of delayed allergic patients (n = 4) and healthy controls (HC; n = 19). (A) and (C) are shown as natural logarithms. Bars represent median.

A	B	C	D	E	F	G
#2	#3	#8	#5	#15	HC #1	#2
						

Supplementary Figure 3. Pictures of intradermal skin tests by PEG-2000 and PS-80: (A-C) erythema, and (D-F) negative results 15 min after the intradermal skin test using PEG-2000 (30 mg/ml). A–E: Immediate allergic reaction to the Pfizer-BioNTech mRNA vaccine. F: Healthy control (HC) #1. G: Facial erythema in patient #2, 2 h after intradermal skin test by PEG-2000 (30 mg/ml). * Saline (negative control).