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BRIEF REPORT

Differential Severe Acute Respiratory Syndrome Coronavirus 2 Antibody Profiles After Allergic Reactions to Messenger RNA Coronavirus Disease 2019 Vaccine

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Allergic symptoms after messenger RNA (mRNA) coronavirus disease 2019 (COVID-19) vaccines occur in up to 2% of recipients. Compared to nonallergic controls (n = 18), individuals with immediate allergic reactions to mRNA COVID-19 vaccines (n = 8) mounted lower immunoglobulin G_1 (IgG_1) to multiple antigenic targets in severe acute respiratory syndrome coronavirus 2 spike following vaccination, with significantly lower IgG_1 to full-length spike (P = .04). Individuals with immediate allergic reactions to mRNA COVID-19 vaccines bound Fcγ receptors similarly to nonallergic controls. Although there was a trend toward an overall reduction in opsonophagocytic function in individuals with immediate allergic reactions compared to nonallergic controls, allergic patients produced functional antibodies exhibiting a high ratio of opsonophagocytic function to IgG_1 titer.

Keywords. systems serology; SARS-CoV-2; anaphylaxis; hypersensitivity; Pfizer; Moderna; messenger RNA; COVID-19; vaccination; humoral immunity.

There have been >555 million coronavirus disease 2019 (COVID-19) vaccine doses administered in the United States to date, largely with the messenger RNA (mRNA) vaccines from Pfizer-BioNTech (BNT162b2, Comirnaty) or Moderna (mRNA-1273, Spikevax) [1]. Shortly after the initial vaccination rollout, reports of anaphylaxis and allergic reactions began [2]. Allergic reactions have now been reported in up to 2% of individuals after mRNA COVID-19 vaccination, with mRNA vaccine anaphylaxis incidence confirmed in 8 to 250 cases per million [3, 4].

Limited serologic studies in mRNA COVID-19 vaccine–allergic individuals have assessed for antibodies to the vaccine or its excipients in order to begin to elucidate the mechanism(s) of these reactions [5]. However, with allergic symptoms after vaccination resulting in incomplete COVID-19 vaccination [6, 7], we sought to assess SARS-CoV-2 antibody quantities, Fcγ receptor (FcγR) binding, and antibody functions in individuals with mRNA vaccine allergic reactions.

MATERIALS AND METHODS

Study Design

Massachusetts General Hospital (MGH) Allergy/Immunology patients with history of recent (<2 months), immediate-onset (<6 hours) allergic reactions after mRNA COVID-19 vaccine from Pfizer-BioNTech or Moderna were prospectively identified, consented, and enrolled in this study by allergy specialists (M. C., T. M., A. B., K. G. B.). We matched mRNA vaccine–allergic patients to nonallergic (ie, vaccine-tolerant) controls, enrolled through a separate MGH study [8], considering matching factors sex, age, vaccine manufacturer, vaccine dose, and time since vaccination. Study procedures were approved by the Mass General Brigham Human Research Committee.

Immunoglobulin G Subclass, Antibody Isotype Titer, and FcγR binding profiles

The relative titers of antigen-specific immunoglobulin G (IgG) subclasses, antibody isotypes, and FcγR binding in the human plasma samples were analyzed with a customized multiplexed Luminex assay, as previously described [9]. SARS-CoV-2 wild-type spike (S; purchased from Lake Pharma), receptor-binding domain (RBD; provided by Aaron Schmidt at the Ragon Institute), and N-terminal domain (NTD; provided by Erica Saphire at the La Jolla Institute for Immunology) were covalently coupled to Luminex bead regions by *N*-hydroxysuccinimide (NHS) ester linkages using Sulfo-NHS and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (Thermo Scientific). The antigen-coupled beads were incubated with phosphate-buffered saline (PBS)–diluted human serum samples (1:500 for IgG_1; 1:100 for IgG_2, IgG_3, IgG4, immunoglobulin A [IgA], and immunoglobulin M [IgM]; 1:1000 for FcγR2A, -2B, -3A, and -3B readouts) for 2 hours at 37°C in duplicate. Antigen-bound antibodies of interest were detected

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with R-phycoerythrin (PE; Agilent Technologies)–conjugated antibody for each subclass and isotype (IgG_1, IgG_2, IgG_3, IgG_4, IgA, and IgM; Southern Biotech). PE-streptavidin (Agilent Technologies) was conjugated to recombinant, biotinylated FcγRs (FcγR2A, FcγR2B, FcγR3A, and FcγR3B; Duke Protein Production Company). Each secondary antibody was incubated with the immune complexes for 1 hour, 800 rpm, at room temperature. The beads were resuspended in QSol buffer (Sartorius) for flow cytometric acquisition (iQue, Sartorius) and analyzed with ForeCyt 8.1 software. Median fluorescence intensity of PE is reported for relative antigen-specific antibody subclass or isotype titers and FcγR binding.

Antibody-Dependent Neutrophil Phagocytosis

The antibody-dependent neutrophil phagocytosis (ADNP) activity assay using isolated primary human neutrophils was performed as described previously [10]. In brief, immune complexes were formed by incubating biotinylated SARS-CoV-2 wild-type S antigen (purchased from Lake Pharma) coupled to 1.0 µm yellow-green, fluorescent neutravidin-labeled microspheres (Thermo Fisher Scientific) with human serum, diluted 1:50 in PBS, for 2 hours at 37°C. White blood cells were isolated from whole blood of 2 healthy donors, collected by the Ragon Institute, as experimental replicates. Red blood cells were lysed with ammonium-chloride-potassium lysis buffer (Thermo Fisher Scientific). White blood cells and neutrophils were isolated by centrifugation and diluted to 250 000 cells/mL in R10 media (RPMI-1640, Sigma) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 20 mM HEPES, and 100 U/mL penicillin/streptomycin. White blood cells were diluted in R10 (50 000 cells/well) and incubated with immune complexes for 1 hour at 37°C. The cells were stained with CD66b-PacBlue (BioLegend) for 20 minutes, fixed with 4% paraformaldehyde, washed with PBS, and then resuspended in PBS. Neutrophil phagocytosis of beads was assessed by flow cytometry (iQue, Sartorius). The reported phagocytic score (phagoscore), the product of the percentage of neutrophils that phagocytosed beads and the fluorescent signal of phagocytosed beads (geometric mean fluorescence intensity of bead-positive neutrophils), was calculated for each sample with ForeCyt 8.1 software.

Antibody-Dependent Cellular Phagocytosis

Monocyte THP-1 cell line–mediated phagocytosis assay was performed as described previously [11]. In brief, immune complexes were formed by incubating 1.0 µm yellow-green fluorescent, neutravidin-labeled microspheres (Thermo Fisher Scientific) coupled biotinylated SARS-CoV-2 wild-type S antigen (purchased from Lake Pharma) and human serum diluted 1:100 in PBS for 2 hours at 37°C in duplicate. THP-1 monocytes (ATCC TIB-202) were added to immune complexes at 250 000 cells/well in R10 media (RPMI-1640, Sigma) supplemented with 10% FBS, 2 mM L-glutamine, 100 U/mL penicillin/streptomycin, 20 mM HEPES, and 50 µM β-mercaptoethanol. Cells were incubated with immune complexes for 16 hours at 37°C, 5% carbon dioxide, fixed with 4% paraformaldehyde, and resuspended in PBS for flow cytometric acquisition (iQue, Sartorius). The phagoscore was calculated by dividing the product of percentage bead-positive cells and bead-positive median fluorescence intensity by 106 using ForeCyt 8.1 software.

Statistical Analysis

Data were analyzed with GraphPad Prism 9.2.0 software. Univariable comparisons between groups used nonparametric, 2-sided Mann–Whitney test with $P < .05$ considered significant.

RESULTS

Allergic individuals were all female with mean age 40 years (standard deviation, 16 years); 6 (75%) had reactions to Pfizer-BioNTech (BNT162b2) and 2 (25%) had reactions to Moderna (mRNA-1273) (Table 1). Patient 2 had prior SARS-CoV-2 infection history. With the exception of patient 8 whose reaction was after the second Pfizer-BioNTech dose, all allergy patients had first dose reactions. The mRNA vaccine–allergic patients had prominent allergy histories with 3 (38%) having a history of prior anaphylaxis. No allergic patients were on systemic immunosuppressants. Vaccine reactions were treated with corticosteroids in 2 patients (25%; patient 4 and patient 6). To better understand the antibody profiles in individuals with acute systemic allergic reactions to mRNA COVID-19 vaccination, we compared SARS-CoV-2 S-directed antibody profiles of 8 mRNA vaccine–allergic individuals to 18 matched nonallergic controls. While matching characteristics were largely balanced between groups, mRNA vaccine–allergic individuals had more drug allergy and atopic disease history (Supplementary Table 1).

The mRNA vaccine–allergic individuals mounted significantly lower IgG_1 titers against full-length SARS-CoV-2 S antigen ($P = .04$) with lower trends in IgG_1 against the RBD and NTD subdomains following vaccination that did not reach statistical significance (Figure 1A). In contrast, similar median IgG_3 (Figure 1A), IgM, and IgA titers and median FcγR binding to SARS-CoV-2 spike were observed between the allergic and nonallergic groups (Supplementary Figure 1). There was a trend toward a reduced median phagocytic function in SARS-CoV-2 S-directed THP-1 monocyte-mediated cellular or neutrophil opsonophagocytic functions in the allergic group that did not reach statistical significance (Figure 1B). However, allergic individuals had higher median opsonophagocytic effector functions per IgG_1 than nonallergic controls (Figure 1C). This suggests that the S-specific antibodies produced by vaccine-allergic individuals are capable of inducing antibody-dependent cellular phagocytosis

Table 1. Clinical Characteristics of Patients With Allergic Reactions After Messenger RNA Coronavirus Disease 2019 Vaccination (N = 8)

	Patient Number							
Characteristic	1	2	3	4	5	6	7	8
Sex	Female	Female	Female	Female	Female	Female	Female	Female
Age, y	47	30	72	38	27	23	46	38
SARS-CoV-2 infection history	No	Yes	No	No	No	No	No	No
Allergic history	Asthma, food allergy (shellfish[a], peach), environmental allergy (cat)	Drug allergy (penicillin)	Drug allergy (penicillin, sulfonamide antibiotics)	Drug allergy (doxycycline)	Food allergy (beef, goat, pork), environmental allergy (cat, dust mite)	Allergic asthma[b]	Allergic rhinitis, drug allergy (penicillin, sulfonamide antibiotics[a], azithromycin, cephalosporins, vancomycin, proton pump inhibitors, ciprofloxacin, clindamycin, vaccine allergy (influenza), oxycodone)	Allergic asthma[b], allergic rhinitis, food allergy (apple[a], melon[a]), drug allergy (penicillin[a])
mRNA vaccine	Pfizer-BioNTech	Moderna	Pfizer-BioNTech	Moderna	Pfizer-BioNTech	Pfizer-BioNTech	Pfizer-BioNTech	Pfizer-BioNTech
Dose number	1	1	1	1	1	1	1	2
Onset of reaction symptoms, minutes	35	15	5	15–20	360	30	30	60
Symptoms and signs of reaction	Dizziness, nausea, wheezing, facial flushing, swelling, headache	Swelling of tongue, flushing, tightness of the arm, poor range of motion in her head	Flushing, facial swelling, hypertension	Hypertension, tachycardia, uvular swelling, face swelling, urticaria	Shortness of breath, fatigue, chest tightness, wheezing	Cough, shortness of breath, stridor, chest pressure, tingling	Tingling, urticaria	Flushing, face/lip/extremity swelling, urticaria, chest tightness
Reaction treatment	Albuterol	Epinephrine	None	Diphenhydramine, prednisone, epinephrine	None	Albuterol, cetirizine, corticosteroids, epinephrine	Diphenhydramine	Diphenhydramine, famotidine
Time to resolution	1 hour	20 minutes	12 hours	12 hours[c]	2 days	3–5 hours	48 hours	5–6 hours
Anaphylaxis[d]	No	Yes	No	No	No	No	No	Yes

Abbreviations: mRNA, messenger RNA; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

[a]History of anaphylaxis.

[b]Prescribed inhaled corticosteroids.

[c]Hypertension persisted for approximately 2 weeks.

[d]National Institute of Allergy and Infectious Diseases/Food Allergy and Anaphylaxis Network criteria.

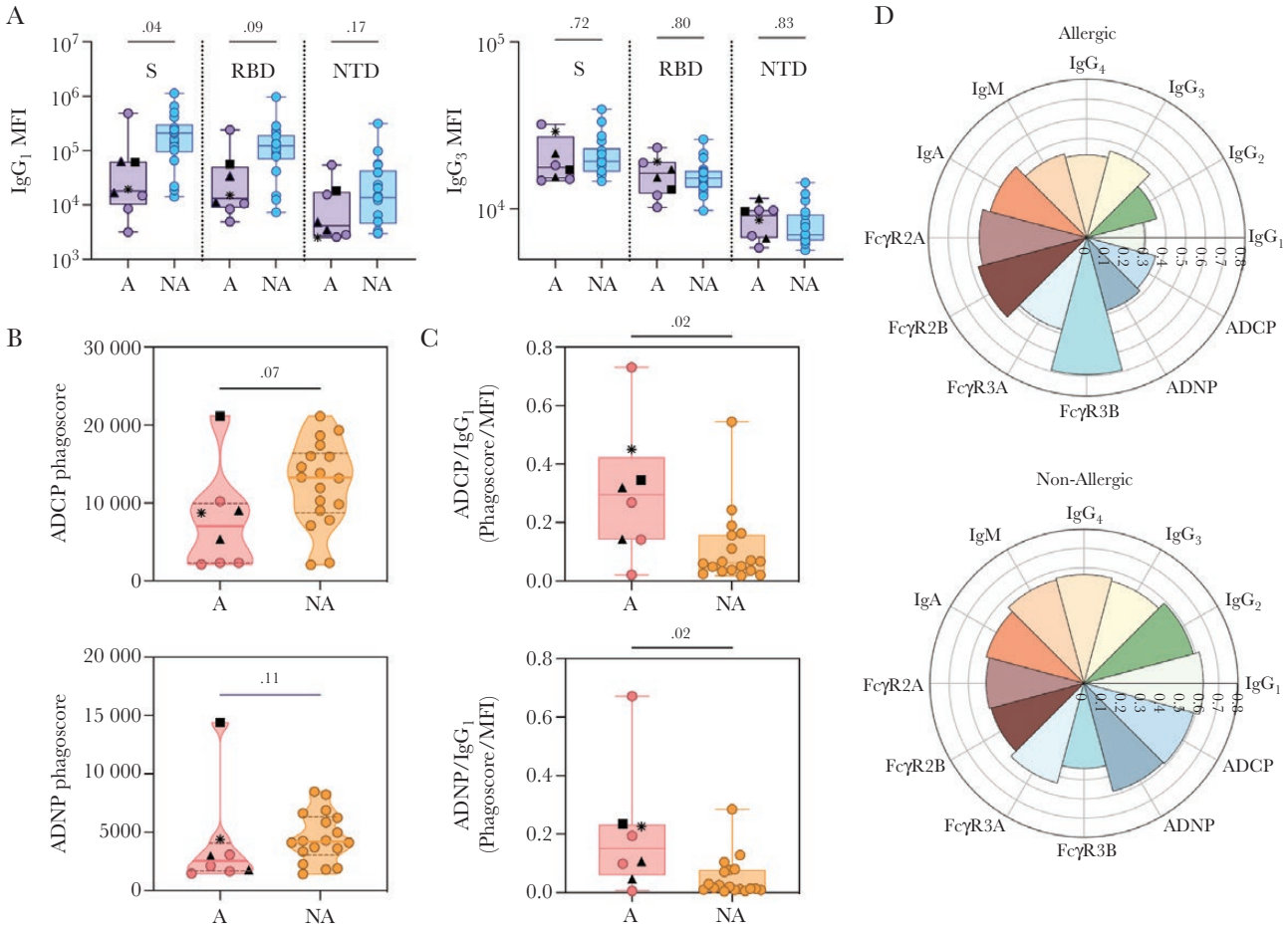

Figure 1. Vaccine-allergic and -nonallergic antibody profiles to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike (S) protein. *A*, Box plots of vaccine-allergic (A; n = 8) and nonallergic (NA; n = 18) anti-S, anti–receptor-binding domain (RBD), or anti–N-terminal domain (NTD) IgG_1 and IgG_3 binding in log median fluorescence intensity (MFI). Lines in each box represent minimum, lower quartile, median, upper quartile, and maximum values of each group. *B*, Violin plots of S-specific antibody-dependent cellular phagocytosis (ADCP) or antibody-dependent neutrophil phagocytosis (ADNP) reported as phagocytic score. Dashed lines represent the lower and upper quartiles. Solid line indicates the median. *C*, Box plots showing the ratio of S-specific ADCP or ADNP to IgG_1 binding levels. Lines indicate minimum, lower quartile, median, upper quartile, and maximum values. *A–C*, Statistical significance was tested by a nonparametric, 2-sided Mann–Whitney test with *P* values as indicated. Patients 4 and 6, who received corticosteroids for allergic reaction treatment, are represented with triangles. Patient 2, with prior history of SARS-CoV-2 infection, is represented with a square. Patient 8, who received 2 vaccine doses, is represented with a star. *D*, Flower plots summarizing SARS-CoV-2 S-specific antibody profiles of allergic and nonallergic groups. Each petal corresponds to the normalized average, *z*-scored value for each antibody feature.

and antibody-dependent neutrophil phagocytosis and that their antibody response may compensate for reduced IgG_1 titer with higher phagocytic function per IgG (Figure 1C). The relatively higher magnitude of S-specific FcγR3B binding in allergic individuals normalized across all spike-specific antibody features (Figure 1D) may contribute to the high ratio of phagocytosis to IgG_1 as FcγR3B is capable of neutrophil activation [12]. Overall, allergic individuals have differential S- and RBD-specific antibody profiles compared to the uniform magnitude of the nonallergic vaccine recipient group (Figure 1D). In Figure 1D, the normalized anti-S antibody features across allergic and nonallergic groups show lower IgG subclass titers in the allergic group, indicated by the size of the petal corresponding to IgG_1, IgG_2, IgG_3, and IgG_4, with statistically significant differences only in anti-S IgG_1 at the univariate level. Repeating the experiments without patient 2 (prior SARS-CoV-2 infection) or patient 8 (received 2 vaccine doses) did not alter these findings (Supplementary Figure 2).

DISCUSSION

We observed a significant reduction of anti-S IgG_1 antibodies in mRNA COVID-19 vaccine–allergic individuals vs mRNA COVID-19 vaccine–nonallergic individuals. We also identified an overall reduction in antibody-mediated opsonophagocytic functions in allergic vaccine recipients compared to nonallergic. Prior data suggest that the functional quality of the humoral immune response is a correlate of vaccine-induced immunity, with S-specific antibodies as immune correlates of mRNA-1273 vaccine–induced immunity and Fc-mediated functions in protection against SARS-CoV-2 [13, 14].

The 2 mRNA COVID-19 vaccines authorized for use require 2 doses with booster vaccinations recommended [7]. Although only severe and immediate-onset mRNA COVID-19 vaccine allergic reactions contraindicates additional doses [7, 15], any allergic symptoms after vaccination may result in incomplete vaccination [6], jeopardizing individual protection and population immunity. Our finding that individuals with allergic reactions to mRNA COVID-19 vaccine exhibit differential SARS-CoV-2 S- and RBD-directed antibody profiles with lower IgG_1 and an overall reduced trend in antibody-mediated opsonophagocytic function directed against SARS-CoV-2 S therefore may have important implications for vaccine efficacy and/or durability in the allergic population.

Our study was a small, single-center pilot study. While we matched on key demographic and vaccine factors likely to influence antibody response, residual confounding may be present. Comprehensive clinical data, such as medical comorbidities and detailed medication exposures, were not collected similarly in cases and controls. However, allergic cases were not prescribed systemic immunosuppressive medications and regularly prescribed corticosteroids included inhaled corticosteroids for 2 allergic patients. Additionally, only 2 allergic cases received corticosteroids as part of their vaccine reaction treatment. Given that all individuals with mRNA COVID-19 vaccine allergy also had strong allergic histories, but control patients infrequently had allergic histories, future studies must distinguish whether this humoral immune pattern is associated with mRNA vaccine–allergic individuals or is associated with the allergic host more generally. Although all of the vaccine allergy cases included in this study were women, women comprise the majority of the mRNA vaccine allergy cases to date [4, 5, 15]. Although allergy is a clinical diagnosis, all cases were diagnosed by allergy specialists.

These findings suggest potential quantitative deficits in COVID-19 protection in individuals with mRNA COVID-19 vaccine allergy. While larger confirmatory studies are needed, these initial data support the need for additional immunologic investigations in individuals with allergic responses and clinical and population efforts to assist mRNA vaccine–allergic individuals in completing and optimizing their COVID-19 vaccination protection.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Disclaimer. The funders had no role in the design of this study, nor in its execution, analyses, interpretation of the data, or decision to submit results for publication.

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