Elucidating allergic reaction mechanisms in response to SARS-CoV-2 mRNA vaccination in adults.

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Abstract:

Background: During the COVID-19 pandemic, novel nanoparticle-based mRNA vaccines were developed. A small number of individuals developed allergic reactions to these vaccines although the mechanisms remain undefined.

Methods: To understand COVID-19 vaccine-mediated allergic reactions, we enrolled 19 participants who developed allergic events within 2 hours of vaccination and 13 controls, non-reactors. Using standard hemolysis assays, we demonstrated that sera from allergic participants induced stronger complement activation compared to non-allergic subjects following *ex vivo* vaccine exposure.

Results: Vaccine-mediated complement activation correlated with anti-polyethelyne glycol (PEG) IgG (but not IgM) levels while anti-PEG IgE was undetectable in all subjects. Depletion of total IgG suppressed complement activation in select individuals. To investigate the effects of vaccine excipients on basophil function we employed a validated indirect basophil activation test that stratified the allergic populations into high and low responders. Complement C3a and C5a receptor blockade in this system suppressed basophil response, providing strong evidence for complement involvement in vaccine-mediated basophil activation. Single cell multiome analysis revealed differential expression of genes encoding the cytokine response and Toll-like receptor (TLR) pathways within the monocyte compartment. Differential chromatin accessibility for IL-13 and IL-1B genes was found in allergic and non-allergic participants, suggesting that *in vivo*, epigenetic modulation of mononuclear phagocyte immunophenotypes determines their subsequent functional responsiveness, contributing to the overall physiologic manifestation of vaccine reactions.

Conclusion: These findings provide insights into the mechanisms underlying allergic reactions to COVID-19 mRNA vaccines, which may be used for future vaccine strategies in individuals with prior history of allergies or reactions and reduce vaccine hesitancy.

MAIN TEXT

Introduction

The mRNA vaccines, BNT162b2 (Pfizer-BioNTech) and mRNA-1273 (Moderna), have been proven to be effective and safe, with approximately 95% efficacy in preventing COVID-19 in early studies against the original Sars-CoV-2 strains (*1-4*). However, a small portion of the population has experienced allergic reactions resulting from vaccination, specifically 11.1 cases of anaphylaxis per million doses for the BNT162b2 vaccine and 2.5 cases of anaphylaxis per million doses for the mRNA-1273 vaccine *(1-4)*. Despite these findings, the mechanisms underlying these allergic reactions have yet to be characterized.

The COVID-19 mRNA vaccines each utilize different liposomal delivery vehicles that contain PEG2000 (*5*). Polyethylene glycol (PEG) is used in many drug and vaccine formulations in addition to cosmetics and lotions, to improve water-solubility. Reports have shown that up to 70% of patients who received PEGylated formulations develop anti-PEG antibodies (*6*). It is hypothesized that allergic reactions to COVID-19 vaccines could be due to a pre-existing anti-PEG allergy (*5, 7*), although true IgE-mediated reactions to PEG in COVID-19 mRNA vaccines have not been definitively proven.

The complement system presents a rapid-acting, first-line defense of the intravascular space and other biological compartments from foreign invaders and facilitates the safe removal of apoptotic cells, immune complexes, and cellular debris (*8*). Early evidence suggesting that nanoparticles can activate complement came from the clinical use of Doxil®, a PEGylated liposomal formulation of doxorubicin for cancer treatment (*9, 10*). Patients typically exhibited signs of cardiopulmonary distress that developed immediately after the start of infusion, including dyspnea, tachypnea, tachycardia, hypotension, and chest and back pain. These

symptoms coincided with a rise in the plasma concentration of complement activation products and typically occurred within minutes upon first exposure to the drug (*11*). Subsequent animal studies demonstrated that reaction to Doxil® was triggered by anti-PEG IgM-induced complement activation and this phenomenon became known as complement activation-related pseudoallergy or CARPA (*9, 10, 12*). Since that time, the spectrum of reagents that cause pseudoallergic symptoms has been broadened to include micelle-solubilized drugs, certain antibodies, and contrast media, in addition to PEGylated formulations and other liposomal drugs (*13*).

Additionally, most studies on COVID-19 mRNA allergic reactions have focused mainly on the types of immune responses while genetic predisposition is so far limited to testing for alpha tryptasemia (*14*). For those that consented and were tested, no genetic predispositions to alpha tryptasemia were found, prompting us to further examine the genetic makeup of allergic individuals. Allergies and allergic reactions are complex processes involving both genetics and the environment factors that affect gene expression without altering the DNA sequence, resulting in epigenetic traits that modulate the immune response plasticity (*15, 16, 17*). In this study, we explored the role of epigenetics in COVID-19 mRNA reactions as contributing factors to the variable vaccine reaction predisposition.

METHODS

Study Design

From December 2020 to April 2021, individuals who called the Stanford University or Washington University allergy clinics to consult for possible allergic reactions to the COVID-19 mRNA vaccines and who met the definition of immediate (within 2 hours of vaccine receipt) allergic reaction as per our prior

publication (*18*) were invited to participate in our IRB approved studies. 32 participants (19 allergics and 13 controls) were enrolled for this study under IRBs approved by Stanford University Institutional Review Board (IRB 8269) and the Washington University in St. Louis Institutional Review Board (IRB 202101196). They were consented at different time points (0-86 days) after their allergic reactions and asked to provide up to 3 blood samples on separate days after their first dose of vaccination. Due to the nature of the COVID-19 pandemic at the time, there was a necessity to immediately vaccinate individuals; thus, it was not possible to collect blood samples prior to vaccination and allergic reaction. Individuals <18 yo were not included since the vaccines were not approved for that age group at the time of study. None of the individuals had tryptase levels and/or CH50 levels drawn. These blood samples were utilized to perform all the experiments outlined within the study. The experiments were performed without randomization or blinding. 10 out of 19 allergic reaction participants were tested and were negative for an alpha tryptasemia mutation, to ensure allergic symptoms post vaccination were not caused by an elevated copy number of *TPSAB1* encoding alpha tryptase (*47*). Individuals within the non-allergic group have ID numbers of N-X, where X is a number between 1 and 13 and individuals within the allergic group have ID numbers of A-X, where X is a number between 1 and 19. This entire study was conducted within full compliance of Good Clinical Practice.

Anti-PEG-IgG and IgE ELISAs

Maxisorp 96-well microplates (NUNC) were coated with 5ug/ml DSPE-PEG (2000) Biotin (Sigma Aldrich). After washing plates with 0.05% CHAPS (Sigma Aldrich) in PBS and blocking the wells with 2% BSA solution the obtained plasma samples were incubated at 4 different dilutions (1:20, 1:40, 1:80, 1:160). For the detection of specific PEG-IgG antibodies alkaline phosphatase conjugated goat anti-human IgG (Thermo Fisher) was added at 1:2000 dilution. Specific PEG-IgE antibodies were detected by incubating samples first with a 1:3000 dilution of a mouse anti-human IgE followed by adding an alkaline phosphatase conjugated goat-anti-mouse IgG (Thermo Fischer) antibody at 1:2000 dilution. After a final wash step,

substrate buffer containing 1.5 mg/ml Nitrophenylphosphate (NPP, Sigma Aldrich) was added, and plates were read at a wavelength of 405nm on microplate reader (Berthold Mithras LB940). Specific IgG and IgE Abs to PEG concentrations of each plasma were interpolated from a standard curve created with anti-PEG human-IgG and anti-PEG human-IgE, respectively (Academia Sinica, Taiwan).

Minimum detections cut offs were determined as OD405 0.2 and OD405 0.4 for PEG IgE and PEG IgG respectively; maximum detection cut offs were determined as OD405 1.0 and OD405 1.9 for PEG IgE and PEG IgG respectively. High PEG IgG was considered for levels > than OD405 1.5.

Anti-PEG-IgM ELISA

Maxisorp 96-well microplates (NUNC) were coated with 5ug/ml DSPE-PEG (2000) Biotin (Sigma Aldrich). After washing plates with 0.05% CHAPS (Sigma Aldrich) in PBS and blocking the wells with 2% BSA solution the obtained plasma samples were incubated at 3 different dilutions (1:20, 1:40, 1:80). Peroxidase conjugated goat-anti-human IgM (Jackson ImmunoResearch) was added at 1:5000 dilution to detect specific PEG-IgM antibodies. After a final wash step, substrate buffer containing 3,3',5,5'-Tetramethylbenzidine (TMB) was added, and plates were read at a wavelength of 450nm on microplate reader (Berthold Mithras LB940). Specific IgM to PEG concentrations of each plasma were interpolated from a standard curve created with anti-PEG human-IgM antibody (Academia Sinica, Taiwan).

Minimum detection cut off was determined as OD450 0.3 for PEG IgM, maximum detection cut off was determined as OD450 1.5.

Complement Buffers

Dextrose veronal-buffered saline with divalent cations (DGVB++) (72.7 mM NaCl, 2.47 mM Na-5'-5'' diethyl barbiturate, 1 mM $MgCl_2$, 0.15 mM $CaCl_2$, 2.5% (w/v) dextrose, 0.1% gelatin, pH 7.3–7.4) was prepared following the protocol in the supplement to reference 16.

Antibody-sensitized sheep cells (EA)

The cells were prepared as previously described in reference 13 with some modifications: Five mL of sheep erythrocytes shipped in Alsever's solution (Colorado Serum Company) were washed 3x with and resuspended in 50 mL of Dulbecco's PBS (DPBS; Sigma, St. Louis, MO) and then divided equally into 2 centrifuge tubes. 100 uL rabbit anti-sheep RBC/haemolysin (Cedar Lane Labs, catalog # CL9000, resuspended in 1 mL filter-sterilized purified water) was mixed in 50 mL of DPBS. Cells and antibody were separately pre-incubated at 37°C for 10 min. 25 mL of antibody solution was added to each tube of cells with gentle agitation. Cell:antibody mixtures were incubated with gentle rotation at 37°C for 30 min. Cells were washed 2x in 50 mL 10 mM EDTA buffer, 2x in DGVB++, and resuspended in 80 mL of DGVB++ for immediate use. Cell preparations were stored at 4°C.

Human Serum

Pooled human serum was purchased from CompTech (Tyler, TX), divided into 0.1 mL aliquots and stored at -80ºC until use.

Vaccines

BNT162b2 COVID-19 vaccine was obtained from the Barnes-Jewish Hospital/Washington University vaccine clinics and used the same day or stored at 4°C until use. Doxil Control nanoparticle, a PEGylated liposomal vehicle, was purchased from (Cat. #300113, Avanti Polar Lipids, AL) and stored at -80°C until use.

Vaccine/NP/serum incubation and hemolytic titration

To investigate whether complement might contribute to the adverse vaccine reactions, we measured the depletion of complement when patient-derived sera are incubated with vaccine. We used this RHA method (Residual Hemolytic Activity) in a previous study of lipid encapsulated nanoparticles (*22*) and found it more sensitive than ELISA-based measures of complement activation protein fragments. Activation of the complement alternative pathway during serum incubation in the absence of nanoparticles produces a very high background that confounds C3a ELISA methodology while complement C3 convertase activity on some nanoparticles does not appear to transition well to C5 convertase to produce C5a. In addition, with respect to the current study, the RHA metric measures vaccine-mediated complement activation, independent of any pre-exiting activation products. The following protocol was adapted from reference 13: vaccine or Doxil Control (0.25% v/v) was incubated in 10% serum in DGVB++ buffer (170 uL total) for 30 min at 37°C. In some experiments, reactions also included rabbit monoclonal anti-PEG IgG (Abcam, MA). Control reactions lacking vaccine were also performed with each serum. Additional controls included pooled normal human serum (CompTech, Tyler, TX) incubated with and without vaccine and with pre-activated zymosan ($1.6x10^7$ particles/reaction; CompTech, Tyler TX) or PAP (50 ug/mL, MP Biomedicals), both complement activators. Reaction mixtures were then chilled to 4°C and cold DGVB++ buffer was added to a total of 900 uL. For each reaction, a titration series was constructed consisting of 10 tubes containing ~17 uL increments of diluted reaction mixture from 0 uL (the buffer control) to 150 uL, 100 uL of EA, and DGVB++ buffer to a total of 250 uL. Titration reactions were incubated at 37°C for 1 h with shaking, then supplemented with 665 uL of DGVB++ buffer and subjected to centrifugation @1800 RPM (671 rcf) at 10ºC for 5 min. 150 uL supernatant was transferred to a microtiter well and optical density determined at 405 nm by spectrophotometry. A value for total lysis was provided in each titration series with a point consisting of 100 uL EA mixed with water instead of buffer. The fraction of cells lysed (Y) is calculated for each member of the titration series and the area under each titration curve (AUC) is determined:

$$Y = \frac{\mathrm{OD(sample)} - \mathrm{OD(buffer\ control)}}{\mathrm{average\ OD(100\%\ lysis\ control)} - \mathrm{OD\ (buffer\ control)}}$$

AUC=SUM(Y) for each titration curve

The response of each serum to vaccine was then quantified using the Residual Hemolytic Activity (RHA) metric[16]. RHA ratio compares the area under the titration curve obtained from serum incubated with vaccine to the area under the titration curve obtained from serum incubated in the reaction without vaccine.

RHA ratio = AUC serum with vaccine/AUC serum without vaccine

RHA ratios for individual sera are averages of determinations obtained on 3-5 separate days. A value of 1 indicates no detectable vaccine-mediated complement activation while a value near 0 indicates extensive vaccine-mediated complement activation.

Depletion of IgG from human sera

Sepharose-conjugated Protein A/G (cat# ab193262, Abcam, Cambridge, MA) were used to deplete IgG antibodies from human sera. Protein A/G-Sepharose (150 µL) slurries were washed extensively with PBS 3x, supernatant removed, human serum was added (150 µL) to pelleted beads. Sera were incubated for 1 hour at room temperature with end-over-end mixing to allow IgG from serum to bind to the resins. Control sera were processed the same way but without the Sepharose/agarose beads. Following incubation, the

beads were pelleted by centrifugation; serum was carefully removed to avoid contamination with beads and used the same day or frozen at -80C until use. IgG depletion was confirmed by Western blotting.

PBMC isolation and basophil enrichment

Non-atopic healthy volunteers were recruited to obtain donor basophils and PBMCs were isolated from fresh heparinized venous blood through centrifugation over Ficoll gradients. Heparinized blood was diluted 1:1 with RPMI-1640 media (Invitrogen, Paisley, United Kingdom), layered on 30% Ficoll-Paque™ PLUS density gradient (GE Healthcare, Uppsala, Sweden) and centrifuged for 25 minutes at 1,136 *g* at room temperature. PBMC layer was collected, washed, and resuspended in RPMI-1640. Cell viability was determined using trypan blue exclusion.

In experiments where enriched basophils were used, leukocyte-rich plasma was prepared from fresh peripheral blood sample by sedimentation of red blood cells (RBC) through HetaSep™ (StemCell Technologies, Cambridge, UK) using centrifugation. In brief, 1-part HetaSep™ solution was added to 5-parts of fresh whole blood and centrifuged at 90 *g* for 4 minutes at room temperature with breaks off. Leukocyte-rich supernatant was harvested and washed to remove platelets. Enrichment of basophil was performed using EasySep™ Human Basophil Isolation kit following the manufacturer's instructions (StemCell Technologies, Cambridge, UK). All purified cells were counted with trypan blue exclusion and checked for purity before processing utilized for downstream processes.

Stripping and resensitization of basophils

Peripheral blood mononuclear cells (PBMCs) or enriched basophils were isolated from whole blood collected from non-atopic healthy subjects. To strip basophils from their native IgE, cells were treated with 0.01 M (4%) lactic acid-containing buffer (pH 3.9) for 2 minutes at 4°C. Cells were washed with Ca^{2+} and Mg^{2+}-free buffer and resensitized with IgE-containing serum for 20 minutes at 37°C, followed by incubation at 4°C for 30 minutes.

Basophil activation test

Basophils resensitized with various serum samples were assessed for their reactivity towards anti-IgE (5 µg/mL), various vaccine components and varying doses of either BNT162b2 or mRNA-1273 vaccine (0.7, 7 and 15 µg/mL) in a 37°C water bath for 30 minutes. In conditions where C5aRA (75 nM) or C3aRA (1 µM) were tested, cells were pre-treated with these antagonist for 30 minutes at 37°C and washed twice with PBS before stimulated with vaccine. Cells were immunostained with anti-human CD3 (BD Biosciences), CD303, CD294 (CRTh2) (both, Miltenyi Biotech), CD63, C5aR and C3aR (all from Biolegend unless indicated otherwise). Samples were washed with 2 mL PBS (without Ca^{2+} and Mg^{2+}) and centrifuged (for 5 minutes, 400g) before incubation with Fixable viability stain 780 (FVS780) (Thermo Fisher) for 10 minutes in the dark. Samples were washed with 2 mL PBS and cell pellet was resuspended in 150 µL of ice-cold fixative solution (CellFix, BD Biosciences). All samples were acquired on the BD LSR Fortessa X20 and activated cells are phenotyped as those that are $CD63^{+}CRTh2^{+}CD303^{-}$. Analyses were performed on FlowJo v10.6.1 and validated using unbiased clustering tools viSNE and FlowSOM (Cytobank).

Single cell RNA-seq and ATAC-seq

Cryopreserved PBMCs were recovered and counted using trypan blue exclusion and left to incubate at 37^{o}C for 20 minutes on its own or with autologous sera at a 1:1 ratio. Cells were prepared for nuclei isolation for single cell multiome ATAC + GEX Sequencing using the manufacturer's instructions (Rev E). Briefly, cells were washed with wash buffer before being exposed to lysis buffer for 1 minute at 4^{o}C. Following a final wash, lysed nuclei suspension was diluted in nuclei buffer and counted using trypan blue to determine cell concentration and viability. Single cell nuclei were then loaded onto a Chromium Single Cell Chip and prepared using the Chromium Next GEM Single Cell Multiome ATAC + Gene expression Reagent Kit (10x Genomics) according to the manufacturer's instructions to allow encapsulation with barcoded Gel Beads at a target capture rate of approximately 10,000 individual nuclei per sample. Single cell RNA-seq and ATAC-seq libraries were prepared for Illumina sequencing according to the manufacturer's instructions. All samples for a given donor were processed simultaneously with the Chromium controller and the resulting libraries were prepared in parallel in a single batch. The RNA and protein libraries were sequenced on a NextSeq 2000. A minimum of 25,000 reads per cell or nuclei was sequenced from the RNA and ATAC libraries.

Pre-processing and analysis of single-cell RNA and ATAC sequencing data

Raw sequencing reads were processed with the Cell Ranger ARC pipeline (10X genomics, v2.0.2) using the GRCh38 reference genome. The resulting RNA counts and ATAC peaks matrices were further processed in R v4.2.2 (*48*) with the packages Seurat v4.3.0 (*49*) and Signac v1.9.0 (*50*) using default parameters (unless otherwise stated or indicated on **Fig. 6**). A more detailed method can be found on the supplementary material.

Unbiased clustering analyses

Machine learning-driven unbiased analyses (FlowSOM) were performed on flow cytometry dataset using Cytobank. Analysis using viSNE and FlowSOM was performed on the basophil population and cluster setting was set to surface markers CD63, CD203c, C5aR and C3aR. FCS files from six serum samples for each condition were concatenated to generate a representative dataset (FCSConcat2). All FlowSOM analysis was performed on a pre-determined metacluster setting of 12. Star plots generated from FlowSOM allows the identification of two pieces of information: 1) size of the cluster nodes representing population abundance; 2) proportion of pie chart within each cluster node representing the expression of markers. The distance between the nodes is proportional to the dissimilarity of expression patterns of nodes or clusters.

Correlation Analyses

Simple linear regressions were performed to test for correlations within the dataset (RHA, anti-PEG Ig Levels, and basophil activity) for each group of patients using Prism 9. The effect of the varying number of days or weeks between blood draws for each patient does not have a significant impact on the results of the dataset as determined based on multiple linear regression performed in Prism 9. Thus, the varying number of days or weeks was not controlled for within the simple linear regression performed on the dataset. To examine the strength of the relationship, Pearson correlation coefficient *r* was used to calculate *P* value. Correlations are considered statistically significant if the *P* value is < 0.05.

RESULTS

Subhead 1: *Characteristics of study participants*

A total of 32 adults who received either the BNT162b2 or mRNA-1273 vaccines were enrolled for these studies. Of the individuals who reported allergic reactions to the COVID-19 mRNA vaccines, 19 met the definition of having experienced an immediate allergic reaction as per our prior publication, classified as the allergic group (*18*). Median interval between vaccination and allergic reaction was 5 minutes (range 1-120 minutes); 12/19 participants (63%) had onset within 5 minutes; 15/19 participants (80%) had onset within 20 minutes. We also enrolled 13 age-matched individuals who had received COVID-19 mRNA vaccines without allergic reactions, classified as the non-allergic, control group. The demographics of all participants are shown in **Tables 1 and 2.** Four subjects in the allergic group reacted within 120 minutes of receiving the second dose of the vaccine (but did not react after the first dose), whereas the remaining 15 subjects reacted within 20 minutes of receiving the first dose of the vaccine.

Subhead 2: Serum and vaccine-mediated basophil responsiveness

Basophils are key effector cells that play a crucial role in both IgE-dependent and independent allergic reactions. To investigate the underlying mechanism of possible IgE-mediated allergic reactions following BNT162b2 or mRNA-1273 vaccination, we measured the effect of the whole vaccines, along with their 'inactive' components (excipients PEG2000 and P80) on basophils using an in-house validated indirect basophil activation assay (iBAT) (*19, 20*). PBMCs or enriched basophils from non-atopic donors were stripped of their native cell-bound surface IgE using 4% lactic acid followed by resensitization with a control serum collected from an individual with an allergy to the grass pollen (GP) antigen Phleum pratense (Phl p) or serum collected from non-allergic and allergic groups.

We tested our iBAT system by testing the effect of stripping and resensitization of basophils with the control GP serum using enriched basophils or PBMCs. Upon stripping of cell-bound surface IgE, we observed a reduction in anti-IgE-mediated basophil activation. This effect was reversed following

resensitization with the GP serum in both cell systems (**Fig. 1A**). Furthermore, resensitization of IgE-stripped cells with GP serum increased in the proportion of $CD63^+$ basophils in response to grass pollen allergen Phl p purified protein, confirming the binding of serum grass pollen-specific IgE onto the surface of donor basophils. In the same comparative model of enriched basophils or PBMCs, we investigated the effect of BNT162b2 vaccine, PEG2000, and P80 on basophil activation following resensitization of cells with serum collected from individuals who have allergies to the vaccine. Vaccine at 15 μg/mL resulted in basophil activation (increase in percentage of $CD63^+$ cells), which was comparable when using either enriched basophils or PBMCs (**Fig. 1B**). In this validation experiment, we performed this side-by-side basophil vs. PBMC comparison in 5 allergic serum samples using 2 donor basophils. This concentration is similar to that used in previously published BAT assays (*18*). No basophil activation was observed following stimulation with PEG2000 or P80 alone, indicating serum or cell components were needed for basophil reactivity to occur.

To further investigate the effects of different sera from non-allergic and allergic individuals on basophil activation, we first examined varying concentrations of the COVID-19 mRNA vaccines (BNT162b2 or mRNA-1273) on basophils upon resensitization with PBMC incubation. Overall, we found basophil activation to be generally higher when using sera from allergic compared to the non-allergic group. However, a significant difference in the percentage of $CD63^+$ basophils was only observed at 0.7 μg/mL of the vaccine ($P < 0.05$, **Fig. 1C**) while a significant increase in the percentage of $CD203c^{bright}$ basophils was observed across all vaccine concentrations (all, $P < 0.05$, **Fig. S1A**). To identify whether the time of onset was associated with basophil activation, we stratified the allergic group into those who developed reaction within 5, 20, or 120 minutes (**Fig. 1D**). We found that most individuals whose serum induced the highest level of basophil activation ('high basophil responder') had a shorter time to allergic reaction development (mostly within 5 minutes of receiving the vaccine). A significant difference in the percentage of $CD63^+$ and $CD203c^{bright}$ basophils was found in serum-induced basophil activation in the 'high basophil responders'

compared to ‘low basophil responders' (**Fig. 1E and Fig. S1B**; all, $P < 0.001$). There were no differences in BAT activation assays detected between the mRNA vaccines (BNT162b2 or mRNA-1273).

To substantiate and confirm our flow cytometry observations, we used an unbiased machine learning algorithm, FlowSOM, to better characterize cell subsets that may be targeted by the serum, the vaccine, or the vaccine components. FlowSOM analysis allows the unbiased subclassification of cells into metaclusters (MC) of similar phenotypes. In summary, our analysis revealed three specific metaclusters (MC9, MC10, MC11) that were present in different proportions in ‘low’ and ‘high’ basophil responder individuals (**Fig. 1F**). Heatmap analysis used to illustrate the expression of CD63 and CD203c in each MC demonstrated that MC9 and 11 corresponded to activated basophils that expressed both CD63 and CD203c (**Fig. 1G**). MC10, on the other hand, comprised of activated basophils expressing the $CD63^{lo}$ and $CD203c^{bright}$ phenotypes. Quantification of each MC showed higher abundance in the MC11 following resensitization with serum alone in the ‘high basophil responders’ compared to the ‘low basophil responders’ (**Fig. 1H**). These results suggest that components in certain sera of the ‘high basophil responders’ cohort can elicit MC11, independent of COVID-19 vaccine.

Subhead 3: ***Anti-PEG IgE, IgG, and IgM***

Specific anti-PEG IgE, IgG, and IgM antibody concentrations in non-allergic and allergic groups were measured by direct ELISA using 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[biotinyl(polyethylene glycol)-2000] (DSPE-PEG(2000)-Biotin) as the antigen source. Absolute concentrations of specific PEG IgE, IgG, and IgM were interpolated from a standard curve. Anti-PEG IgE was undetectable in all subjects (**Fig. 2A**). Thirty-seven percent of the non-allergic group were positive for specific anti-PEG IgG (range 157-405 ng/ml) while 63.2% of allergic individuals harbored specific anti-PEG

IgG (range 156-6903 ng/ml) (**Fig. 2B**). The range of anti-PEG IgG antibodies tended to decrease over time (**Fig. 2B**) in allergic individuals, even for those who received a second dose of vaccine. Of note, we also evaluated anti-spike protein IgG antibody responses and found no differences between allergic and non-allergic individuals (*21*).

The two groups were stratified according to the timing (i.e. days) of blood collection after the first administration of the vaccine. Significant differences between the non-allergic and allergic groups were noted for specific anti-PEG IgG at both early (Draw 1, $P = 0.0377$) and late (Draw 2, $P < 0.0001$) blood collection time points (**Fig. 2B**). The percentage of participants who had specific anti-PEG IgM antibodies was similar in non-allergic and allergic groups, 85.7% and 78.9%, respectively. In addition, no significant differences in the median of specific PEG IgM titers could be observed between the groups (**Fig. 2C**).

Subhead 4: ***COVID-19 mRNA vaccine-mediated hemolytic activity***

To investigate whether CARPA contributes to the COVID-19 mRNA vaccine reactions, we examined the capacity for the vaccines to promote serum complement activation. We used a validated, standard hemolysis assay that measures the serum dilutions that promote complement-dependent lysis of antibody (Ab)-sensitized sheep red blood cells (*22*). We previously established that pre-incubation of a serum sample with complement-reactive nanoparticles depleted critical complement components and diminished the subsequent hemolytic activity of that serum, indicating that a complement-mediated reaction occurred (*22*). Herein, the BNT162b2 mRNA vaccine caused little loss of hemolytic activity in this assay when pre-incubated with pooled normal human serum (NHS) (**Fig. S2A**). In contrast, as a positive control, NHS pre-incubated with zymosan or with peroxidase/anti-peroxidase complex (PAP), two strong complement activators, led to significantly diminished residual hemolytic activity (**Fig. S2A**).

Both COVID-19 mRNA vaccines are lipid nanoparticles formulated with different components including PEG-lipids, specifically PEG2000 that could trigger complement activation in those individuals harboring anti-PEG IgG and/or IgM. To test this possibility, we studied the ability of a well characterized rabbit anti-PEG IgG to mediate complement activation in NHS exposed to the BNT162b2 vaccine. We quantified vaccine-mediated complement depletion using the residual hemolytic activity (RHA) metric, which compared the areas under the titration curve obtained with NHS pre-incubated with the vaccine to that of NHS pre-incubated in buffer alone. RHA measures vaccine-mediated complement activation in vitro and is independent of any pre-existing complement activation products. RHA ratios near 1 indicate little or no vaccine-dependent complement activation while RHAs near 0 indicate complete vaccine-mediated depletion of serum complement activity. There was little loss of hemolytic activity occurred when NHS was pre-incubated with either vaccine or anti-PEG IgG (**Fig. S2B)**. In contrast, hemolytic activity was depleted when serum was pre-incubated with vaccine and anti-PEG IgG together. As seen in **Fig. S2B**, we found a high degree of correlation between RHAs and exogenously added anti-PEG IgG concentrations (R^2 = 0.9414, P = 0.0013). Similar correlation was obtained with a Doxil® control nanoparticle, a liposomal formulation that also contains PEG2000 (R^2 = 0.9089, P = 0.0120, **Fig. S2C**), strongly suggesting that PEG-anti-PEG antibody complex drives this in vitro complement activation, in a concentration-dependent manner.

Next, we assayed sera obtained from the two populations, non-allergic and allergic. Sera were incubated with the BNT162b2 vaccine and subjected to the hemolysis assay. We noted significantly higher complement-activation activity (i.e., lower average RHAs) in Draw 1 sera of the allergic population compared with Draw 1 sera of the non-allergic population (P = 0.0221) (**Fig. 2D**). On the other hand, we

noted no significant differences in Draw 2 sera (i.e., similar average RHAs, **Fig. 2D**) between the groups. While the average RHAs of non-allergic sera remained relatively stable between day 0 and day 50 following the first vaccination (**Fig. S2D**), the average RHAs of allergic sera tended to be lower at day 50 (**Fig. S2E**). Although there were striking differences in the anti-PEG IgG levels of non-allergic and allergic individuals (**Fig. 2B**), we found no such correlation between RHAs and anti-PEG IgG (**Fig. S2F**) or anti-PEG IgM levels when considered as a group (**Fig. S2G**). Nonetheless, depletion of total IgG (confirmed by the absence of IgG heavy and light chains on Western blot) from a subgroup of allergic individuals led to higher RHAs (**Fig. 2E**), suggesting that IgG antibody partially contributed to the observed vaccine-mediated complement-depleting activity.

Subhead 5: Inhibition of C5a and C3a receptors suppressed serum+vaccine-induced basophil responsiveness

Our analyses thus far showed that sera obtained from individuals who had received the BNT162b2 or mRNA-1273 vaccine had varying capacity to activate complement when exposed to BNT162b2 vaccine *ex vivo* (**Fig. 2D**). Complement activation leads to the generation of anaphylatoxins (i.e., C3a and C5a), which bind to their respective receptors and potently activate innate immune cells such as basophils. First, we quantified the proportion of C3a and C5a receptors (C3aR and C5aR) on the surface of enriched basophils and PBMCs from non-atopic healthy donors and confirmed that C5aR and C3aR were highly expressed, approximately 90% and 80%, respectively, in both of enriched basophils and PBMCs (**Fig. 1F and Fig. 3A**). Thus, all analyses involving complement antagonists, hereafter, were performed on PBMCs.

We next investigated the role of complement in basophil activation using two different antagonists: C3aR antagonist (C3aRA) and C5aR antagonist (C5aRA) (*23, 24*). First, we used serum from 6 allergic individuals

to evaluate the effects of C3aRA and C5aRA on vaccine-induced basophil activation. We found that C3aRA and C5aRA, at the concentrations used, both profoundly inhibited vaccine-mediated basophil activation in these test sera (**Fig. 3B**). Next, we examined the effects of C3aRA and C5aRA on basophil activation induced by sera from non-allergic and allergic individuals. When considered as a group, we observed equivalent suppression in serum+vaccine-induced basophil activation following C3aRA and C5aRA pre-treatment of sera from both non-allergic and allergic individuals (**Fig. 3C and Fig. S1C-D**). When the sera were examined individually, we found a range of suppression by either C3aRA or C5aRA (**Fig. S1C-D)**. However, pre-treatment with C3aRA and C5aRA combined resulted in no further suppression of vaccine-induced basophil activation (**Fig. S1E**). The fact that we observed suppression of activation in this test system with C3aRA and C5aRA provides strong evidence for complement involvement in serum+vaccine-induced basophil activation. Unbiased clustering analysis using FlowSOM highlighted the same 3 MCs (MC9-11) targeted by C5aRA and C3aRA (**Fig. 3D**). Quantification of population abundance showed that the MCs predominantly inhibited by complement receptor antagonists in non-allergics and low basophil responders were MC9 and MC9/MC10, respectively. Meanwhile, complement receptor antagonists suppressed MC9, MC10 and MC11 in the allergic, high basophil responders (**Fig. 3E**). Interestingly, one particular MC that appeared to be suppressed across all study subjects was MC9, which displayed high expression of CD63 and CD203c, denoting a highly activated state (**Fig. S3**). The high expression of basophil activation markers in MC9 was accompanied by low expression of C5aR and C3aR, which could be due to receptor internalisation, further highlighting their potential role in serum-mediated basophil activation.

Subhead 6: ***Correlations between RHA:PEG Ig levels and RHA: basophil response***

Although we observed low correlation between RHA and anti-PEG Ig levels when all the allergic participants were considered as a group (**Fig. S2F**), we found moderate correlation between RHA and anti-

PEG IgG levels when the 'high basophil responders' group was examined separately (R^2 = 0.5813, P = 0.028, **Fig. 4A**). These results suggest that anti-PEG IgG was at least partially responsible for in vitro vaccine-induced complement activation in some of the high basophil responders. On the other hand, there was no correlation between RHA and anti-PEG IgM levels in the 'high basophil responders' (R^2 = 0.056, P = 0.573, **Fig. 4B**). Moreover, we found no or weak correlations between RHA and anti-PEG IgG/IgM in the low basophil responders (**Fig. S4A-B**) and the 'non-allergics' (**Fig. S4E-F**). And while RHA, which measures the serum's potential to activate complement *ex vivo* in the presence of BNT162b2 vaccine, correlates with anti-PEG IgG levels in high basophil responders (**Fig. 4A**), we found no correlation between serum C3a and anti-PEG IgG levels in allergic participants (**Fig. S4G-H**). Moreover, we found no differences in C3a levels between non-allergics and allergics (**Fig. S4I)**.

Lastly, we examined the relationship between RHA and basophil response by iBAT. In the 'high basophil responders' group, we found that RHA correlated with the degree of basophil suppression by C3aRA (R^2 = 0.5680, P = 0.031, **Fig. 4C**); the sera with stronger complement-activating activity (i.e. lower RHAs) presaged a more complete suppression of basophil response by C3aRA (lower residual basophil activity). Conversely, the sera with weak complement-activating activity (i.e. higher RHAs) correlated with less suppression of basophil response by C3aRA. Moreover, there was weak correlation between RHA and basophil response in the presence of C5aRA (R^2 = 0.2182, P = 0.243, **Fig. 4D**), suggesting that the signaling downstream of C3aR and C5aR is non-overlapping or that the surface structure of the vaccines may not support efficient assembly of the C5 convertase (and C5a release). Additionally, there was no correlation between RHA and basophil response in the presence of C3aRA or C5aRA in the low basophil responders (**Fig. S4C-D**) and the 'non-allergics' (**Fig. S4J-K**).

Subhead 7: ***Single cell transcriptomic and ATAC-seq revealed no differential molecular changes pre- and post-serum incubation on PBMCs of different patient groups***

The fact that C3aRA and C5aRA, individually or in combination, were unable to completely suppress vaccine-mediated complement activation suggests that there are other factors contributing to the observed vaccine reactions. Cellular assessments on basophils have highlighted factors within the serum of these study participants that resulted in basophil activation, allowing us to stratify them as 'high basophil responders' and 'low basophil responders'. Here, using single cell multiome analysis, which simultaneously measure gene expression and chromatin accessibility at a single cell level, we sought to investigate the differential transcriptomic profile of the high basophil responders, low basophil responders, and non-allergics. A total of 7 study participants were investigated at a single cell level, comprising of 3 high basophil responders (Hi-BAT reactors), 3 low basophil responders (low-BAT reactors) and 1 non-allergic (non-reactor) (**Fig. 5A**). Weighted nearest neigbour (WNN) UMAPs of predicted cell types based on Azimuth classification revealed 8 clusters of cells (B cells, $CD4^+$ T cells, $CD8^+$ T cells, dendritic cells, monocytes, natural killer cells, non-CD4 and CD8 T cells, and 'other' cells) that were identified within the PBMC population of all study participants included in the single cell analysis (**Fig. 5B, left and middle panel**). Classifications of these clusters of cells based on the three patient groups demonstrated overlapping pattern and no clear distinction (**Fig. 5B, right panel)**. As we were seeing serum-induced basophil activation in the allergic groups, we wanted to understand the molecular mechanisms underlying this. We stimulated PBMCs from study participants with their autologous serum and studied their effect on the transcriptomic and ATAC profile. No transcriptome-wide differences was observed on correlation plots pre- and post-serum incubations (**Fig. 5C and 5D**). Correlation analysis of cell subsets against their serum treated counterparts did not induce any significant changes both in the RNA expression, as well as chromatin accessibility. Proportion analysis of Azimuth predicted cell types did not identify any changes in the general cell population pre- and post-serum incubation.

Subhead 8: ***Single cell multiome analysis on PBMCs identified contribution of the myeloid compartment on high basophil responders and low basophil responders***

To further understand the molecular mechanisms underlying the response observed in high basophil responders and low basophil responders, we focused our analysis specifically within the innate immune compartment comprising of monocytes and dendritic cells (DCs). A total of 14,846 cells were investigated, of which 13,670 and 1,176 were monocytes and DCs, respectively (**Fig. 6A**). Using myeloid cell phenotype annotation, two different clusters of monocytes ($CD14^+$ classical and $CD16^+$ non-classical monocyte) and two different clusters of DCs (cDC2s and plasmacytoid DCs or pDCs) were identified, with each phenotype of these cell subsets annotated (**Fig. 6B**). Upregulation of common monocyte genes were observed in both the classical (i.e. *VCAN, CD14, PLCB1, CXCL8* and *DPYD*) and non-classical (i.e. *PAPSS2, TCF7L2, FCGR3A, MTSS1* and *CX3CR1)* subsets. On the other hand, DC subsets express distinct genes such as *CCDC50, COBLL1, TCF4* and *RGS7* for pDC and *NDRG2, CLEC10A, FCER1A, CD1C* and *ENHO* for cDC2 (**Fig. 6B**). Hierarchical analysis was performed to generate a heat map and volcano plot in which a total of 145, 338, and 112 genes were differentialy expressed in high basophil responders *vs.* low basophil responders, high basophil responders *vs.* non-allergics, and low basophil responders *vs.* non-allergics, respectively (**Fig. 6C**). A few notable genes were differentially expressed between the three individual groups, indicating differential monocyte activation programs, and include *TGFB1, NFKB1, IL1B and TLR2* in high basophil responders, *CD163* in low basophil responders and *TLR4, IL-10, IRF3/4/5, STAT3 and JAK2* in non-allergics (**Fig. 6D**). In addition, we observed increased expression of complement receptor *C5AR1* in high basophil responders (**Fig. 6D**). Furthermore, pathway enrichment analysis demonstrated vast amount of gene modulation that were associated with signaling by interleukins, SARS-CoV infections, toll-like receptor (TLR) cascade, MyD88 cascade and MAP kinase activation (**Fig. 6E**). To complement the single cell transcriptomic analysis, chromatin accessibility was assessed for various genes using single cell ATAC-Seq

simultaneously. ATAC-Seq analysis revealed a region on the IL-13 locus that is more accessible in low basophil responders and non-allergics compared to high basophil responders while little differential accessibility of IL-4 (**Fig. 6F**) or IL-10 (**Fig. S5**) was observed in the three different study cohorts. On the other hand, analysis of IL-1B locus shows significantly higher accessibility in high basophil responders and low basophil responders compared with non-allergics (**Fig. 6F**). Taken together, our data indicates that monocytes in high basophil responders are programmed toward M1-like phenotype while non-allergics are polarized toward M2-like phenotype. Low basophil responders, on the other hand, have mixed phenotypes with high CD163 expression (M2) (*25*), high IL-13 chromatin accessibility (M2) combined with high IL-1B chromatin accessibility as well as gene expression (M1). The results suggest that monocytes *in vivo* are heterogeneous populations of cells that are epigenetically imprinted with different functional programs. In turn, the differential monocyte activation states of the different individuals likely shape the subsequent observed COVID-19 vaccine reactions, especially when considered in the context of basophil activation by vaccine-induced CARPA.

DISCUSSION

Allergic reactions to vaccines were initially reported to occur typically at a rate of 1.31 cases per million vaccine doses in large population studies (*26*). Among those who had immediate allergic reactions to the first vaccination, approximately 0.16% developed severe reactions upon revaccination (*27*). Allergic reactions are often attributed to inactive ingredients or excipients (*5*). Both COVID-19 mRNA vaccines contain the excipient PEG2000, which stabilizes the lipid nanoparticle that envelops the mRNA coding for SARS-CoV-2 spike protein. Although the PEG2000 in COVID-19 mRNA vaccines is different from the PEG3350 that is most commonly used in cosmetic and healthcare products, there is suspected pre-existing anti-PEG3350 antibody cross-reactivity to COVID-19 vaccines in reported allergic reactions (*5*). However, given that only 0.1% individuals in the general population likely harbor anti-PEG IgE antibody (*28*) and none of the individuals who developed allergic reactions to COVID-19 mRNA vaccines in our cohorts

565 exhibited detectable levels of anti-PEG IgE, alternative non-IgE pathways for activating mast
566 cells/basophils were considered in the present study and were investigated at the cellular and molecular
567 level.

568 Numerous studies have examined the ability of PEGylated lipid nanoparticles to activate the immune
569 system, including complement. The interaction of IgM and IgG with a target surface initiates the
570 complement classical pathway activity, which is then amplified by the complement alternative pathway, a
571 cascade that generates the bioactive proteolytic products C3a and C5a. Our *in vitro* experiments
572 demonstrated this effect: anti-PEG IgG promoted a robust serum complement response to the BNT162b2
573 vaccine as well as to a PEGylated Doxil® control lipid nanoparticle. Consistent with a prior literature review,
574 which reported anti-PEG antibody levels in healthy participants ranging from 0.2% to 72% (*29*), we found
575 that the majority of our participants harbor anti-PEG IgM, IgG or both except for two individuals, N-11 and
576 A-14. Within the allergic group, 17 out of 19 individuals were women, a result that may be partially
577 explained by prior exposure to common over the counter products that contain PEG such as lotions and
578 cosmetics (*18, 30*). Previous studies have also shown that the binding of anti-PEG IgM antibodies to
579 PEGylated liposomes leads to complement activation *via* the classical pathway and hypersensitivity
580 reactions in animal models (*31, 32*). However, our results suggest that the presence of anti-PEG IgM alone
581 does not necessarily predict an allergic reaction to the COVID mRNA vaccines, even at high titers. On the
582 other hand, our cohorts exhibited significant elevations of anti-PEG IgG titers in the group that developed
583 allergic reactions while the non-allergic participants harbored no or low titers of anti-PEG IgG. Moreover,
584 we found significant correlation (R^2 = 0.5813, P = 0.028) between serum hemolytic activity (RHA) and anti-
585 PEG IgG levels in the 'high basophil responders' but no or weak correlation between RHA and anti-PEG
586 IgM in the low basophil responders and non-allergic groups. There were no observed differences between
587 high basophil responders and low basophil responders concerning RHA and anti-PEG IgG.

We confirmed basophil activation through the presence of $CD63^{+}$ and $CD203c^{bright}$ surface markers. Although we did observe differences between the percentage of $CD63^{+}$ basophils and the percentage of $CD203c^{bright}$ basophils across vaccine concentrations, it has previously been shown in the literature that both are markers for basophil activation *(33)*. In addition, the fact that some allergics harbored no anti-PEG antibody and vaccine-induced basophil activation was not completely blocked by C3aRA/C5aRA in some individuals suggests that additional mechanisms likely contribute to the observed allergic reactions to COVID-19 mRNA vaccines. Single cell RNAseq within the monocyte compartment demonstrated differential gene expression that are associated with downstream TLR signaling pathways, with indication of upregulation of these genes and enrichment of these pathways in the high basophil responders compared to the low basophil responders and non-allergics. We found significant upregulation of M1 activation programs (*IL-1B, NF-KB, IL-1A* and *TNF*) in the high basophil responders and upregulation of M2 activation programs (*FOS, JAK2, IL-10* and *IL-13*) in non-allergics while low basophil responders have mixed M1/M2 activation programs (*IL-1B, IL-13* and *CD163*). These results suggest that the high basophil responders and low basophil responders differ in their functional properties, as reflected in the differential expression of pro- and anti-inflammatory gene expression that is likely modulated by epigenetic mechanisms, as evidenced by differential chromatin accessibility. Additionally, differential expression of CD163, a scavenger receptor that can act as an innate immune sensor to promote inflammation (*34, 35*), and TLR2 in low basophil responders suggests that these polarized monocytes may release a distinct set of cytokines from high basophil responders upon stimulation, depending on whether the signals are propagated *via* CD163 or TLR2 or both.

Studies have shown that the ionizable cationic lipids in nanocarriers can directly activate TLR2 and TLR4 on the cell surface (*36-38*), leading to NF-κB activation and inflammatory mediator release. Whether the COVID-19 mRNA vaccines can directly stimulate CD163 remains to be determined. Complement and TLRs are rapidly activated as a frontline defense and provide a link between innate and adaptive immunity

following an infection (*39*). Complement and TLR crosstalk synergy has been reported at mucosal sites (*39*), with a study in murine ginginal tissue demonstrating that concomitant activation of C5aR and TLR2 through local injection of their agonists can result in the induction of TNF, IL-1B, IL-6 and IL-17A mRNA and protein (*40*). In another study, C5a produced during complement activation was shown to contribute towards inflammation through NLRP3 inflammasome, IL-1β and TNFα release, as well as induction of IL-6 and IL-17 (*41*). These corroborate with our findings in which upregulation of C5aR1 and enrichment of the NLRP3 inflammasome, IL-1, IL-17 and IL-6 signaling was observed in the macrophage compartment of high basophil responders. In addition, the basophil activation and release of cytokines and mediators can further induce the regulation of monocyte polarization and vice versa, contributing to the observed allergic reaction to COVID-19 mRNA vaccines. It has previously been shown by Egawa *et al.* that basophils can drive the differentiation of inflammatory monocytes into M2 macrophages, thereby regulating allergic skin inflammation though not many studies have shown these interaction (*42*). In our study, we observed dominance of M1 activation state within the high basophil responders cohort despite a higher level of basophil activation. Whether this discrepancy could be explained by the differential temporal roles displayed by basophils in allergic inflammation, i.e. initiatiation of M1 activation state at early stage of allergy followed by attenuation through M2 activation state in the later stages, is yet to be fully elucidated.

In summary, our studies suggest that anti-PEG IgG in the sera of 'high basophil responders' may trigger variable degrees of complement activation in the presence of the BNT162b2 vaccine, leading to the release of complement split products that bind to C3aR/C5aR, activating innate immune cells such as basophils to release inflammatory/allergic mediators. In the absence of the vaccine and elevated basophil activation response, our data suggests serum factors from allergic individuals who develop reactions in less than 5 minutes contribute towards the spontaneous activation. Furthermore, our multiome ATAC-seq analysis revealed differentially polarized monocytes, with M1 phenotype being predominantly observed in the high basophil responders while monocytes from low basophil responders

exhibit a mixed M1/M2 phenotype. The monocyte polarization is likely modulated by epigenetic mechanisms (*43*) and determines the subsequent macrophage response upon exposure to COVID-19 vaccine that when combined with CARPA determines the observed allergic phenotype (**Fig. 7**). Of note, BNT162b2 mRNA vaccination has been shown to induce short-term epigenetic changes in innate immune cells (*44*). However, these changes are short-lived, observed mostly after consecutive vaccination and affecting IFN-stimulated gene expression in monocytes. All subjects in our study received the COVID-19 mRNA vaccines, with all the non-reactors receiving two doses while the majority of the allergics only received one. Moreover, blood samples in the allergics were obtained an average of 21 days after the first vaccination. Thus, we do not believe that monocyte polarization and epigenetic changes in allergics are due solely to COVID-19 mRNA vaccination.

There are likely additional mechanisms. For example, the COVID-19 mRNA vaccines may absorb proteins on its surface once injected *in vivo*, forming an aggregate or a 'corona'. This corona comprises proteins such as immunoglobulins, lipoproteins, coagulation factors, complement proteins (*45*), which may be directly recognized by basophils/mast cells *via* the Mas-related G protein-coupled receptor-X2 (46) or different innate immune cells such as neutrophils, leading to histamine and cytokine release. In addition, there still exists the possibility for certain individuals in the population to experience anti-PEG IgE-mediated allergic reactions. Furthermore, it is important to note that healthy donors included in this study possessed detectable IgG, but did not experience allergic reactions. This could be explained by differences in the specific IgG subclasses, which we believe should be explored in future studies. Nonetheless, our findings shed new insight into mechanisms of vaccine reactions that may influence ongoing vaccine development, especially as more mRNA vaccines are being developed against novel variants of SARS-CoV-2 and other viruses. The results may also contribute to the development of strategies and guidance to manage subsequent vaccination in individuals with prior allergic responses and help reduce vaccine hesitancy.

Limitations:

There are several limitations to the interpretation of these studies. First, the sera and PBMCs were not collected during the reactions and thus complement activation and its potential effects on immune cells can only be inferred. Second, the fact that we did not find correlation between IgM levels and complement activating capacity of sera from allergic individuals does not necessarily rule out the contribution of IgM during the reactions since we performed studies using blood collected days to weeks after the reactions. Third, we focused mainly on CARPA and monocyte activation programs as the main mechanisms underlying COVID-19 mRNA vaccine reactions but acknowledge that several alternative mechanisms may be responsible for the observed effects. Fourth, our studies focused on basophils, whereas, *in vivo*, tissue mast cells may be the main cell type responsible for vaccine reaction and have functions that are partially distinct from basophils. Also, other immune cells such as neutrophils may play a role in these vaccine reactions. The indirect BAT assay uses donor basophils which are sensitised with IgE to the tested patient. In this study, due to logistical constraint associated with the nature of the COVID-19 pandemic, we were unable to process fresh blood samples on the BAT assay and thus, iBAT assay offered the best option for us to test their ability to induce basophil activation.

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Author Contributions:

Concept and design: MMS, JAL, DEH, MHS, CTNP, KCN

Acquisition, analysis, or interpretation of data: MMS, JAL, DEH, MHS, CTNP, KCN, AP, MRJ, JMM, ZR, PLK, ASL, JL, DD, IC, MV, AF, PSA, SJG, SDB, BP, MMD, RO, HP, BFG, SRD, OF, IA, AK, MC, LMM, AH, CAA

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Competing Interests:

All authors declare no competing financial interests.

References

1. Polack, F. P. *et al.* Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *New England Journal of Medicine* 383, 2603–2615 (2020). doi:10.1056/NEJMoa2034577
2. Baden, L. R. *et al.* Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. *New England Journal of Medicine* 384, 403–416 (2021). doi: 10.1056/NEJMoa2113017
3. Allergic Reactions Including Anaphylaxis After Receipt of the First Dose of Pfizer-BioNTech COVID-19 Vaccine — United States, December 14–23, 2020. *Centers for Disease Control and Prevention* (2021). Available at:

https://www.cdc.gov/mmwr/volumes/70/wr/mm7002e1.htm. (Accessed: 1st September 2021)

4. Allergic Reactions Including Anaphylaxis After Receipt of the First Dose of Moderna COVID-19 Vaccine — United States, December 21, 2020–January 10, 2021. *Centers for Disease Control and Prevention* (2021). Available at: https://www.cdc.gov/mmwr/volumes/70/wr/mm7004e1.htm. (Accessed: 1st September 2021).

5. Banerji, A. *et al.* mRNA Vaccines to Prevent COVID-19 Disease and Reported Allergic Reactions: Current Evidence and Suggested Approach. *The Journal of Allergy and Clinical Immunology: In Practice* 9, 1423–1437 (2021). doi:10.1016/j.jaip.2020.12.047
6. Yang, Q. & Lai, S. K. Anti-PEG immunity: emergence, characteristics, and unaddressed questions. *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology* 7, 655–677 (2015). doi:10.1002/wnan.1339
7. Sellaturay, P., Nasser, S., Islam, S., Gurugama, P. & Ewan, P. W. Polyethylene glycol (PEG) is a cause of anaphylaxis to the Pfizer/BioNTech mRNA COVID-19 vaccine. *Clinical & Experimental Allergy* 51, 861–863 (2021). doi:10.1111/cea.13874
8. Ricklin, D., Hajishengallis, G., Yang, K. & Lambris, J. D. Complement: A key system for immune surveillance and homeostasis. *Nature Immunology* 11, 785–797 (2010). doi:10.1038/ni.1923
9. Szebeni, J. Complement activation-related pseudoallergy: A new class of drug-induced acute immune toxicity. *Toxicology* 216, 106–121 (2005). doi:10.1016/j.tox.2005.07.023
10. Szebeni, J., Wassef, N. M., Spielberg, H., Rudolph, A. S. & Alving, C. R. Complement Activation in Rats by Liposomes and Liposome-Encapsulated Hemoglobin: Evidence for Anti-lipid Antibodies

and Alternative Pathway Activation. *Biochemical and Biophysical Research Communications* 205, 255–263 (1994). doi:10.1006/bbrc.1994.2658

11. Chanan-Khan, A. *et al.* Complement activation following first exposure to pegylated liposomal doxorubicin (Doxil®): possible role in hypersensitivity reactions. *Annals of Oncology* 14, 1430–1437 (2003). doi:10.1093/annonc/mdg374
12. Szebeni, J., Simberg, D., González-Fernández, Á., Barenholz, Y. & Dobrovolskaia, M. A. Roadmap and strategy for overcoming infusion reactions to nanomedicines. *Nature Nanotechnology* 13, 1100–1108 (2018). doi:10.1038/s41565-018-0273-1
13. Szebeni, J. Complement activation-related pseudoallergy: A stress reaction in blood triggered by nanomedicines and biologicals. *Molecular Immunology* 61, 163–173 (2014). doi:10.1016/j.molimm.2014.06.038
14. Lyons, J. J. Hereditary alpha tryptasemia: genotyping and associated clinical features. *Immunology and Allergy Clinics of North America* 38, 483–495 (2018). doi:10.1016/j.iac.2018.04.003
15. DeVries, A., & Vercelli, D. Epigenetic Mechanisms in Asthma. *Annals of the American Thoracic Society*, 13(Supplement 1), S48-50 (2016). doi:10.1513/annalsats.201507-420mg
16. Fiuza, B. S. *et al.* Understanding Asthma and Allergies by the Lens of Biodiversity and Epigenetic Changes. *Frontiers in Immunology* 12, (2021). doi:10.3389/fimmu.2021.623737
17. Wang, J. *et al.* Pathogenesis of Allergic Diseases and Implications for Therapeutic Interventions. *Signal Transduction and Targeted Therapy* 8(1), (2023). doi:10.1038/s41392-023-01344-4
18. Warren, C. M. *et al.* Assessment of Allergic and Anaphylactic Reactions to mRNA COVID-19 Vaccines With Confirmatory Testing in a US Regional Health System. *JAMA Network Open* 4, (2021). doi:10.1001/jamanetworkopen.2021.25524
19. Gibbs, B. F. *Basophils and Mast Cells: Methods and Protocols*. (Springer, 2014).

20. Pruzansky, J. J., Grammer, L. C., Patterson, R., & Roberts, M. Dissociation of IgE from receptors on human basophils. I. Enhanced passive sensitization for histamine release. *J Immunol.* 131, 1949-1953 (1983).
21. Röltgen, K. *et al.* Immune imprinting, breadth of variant recognition and germinal center formation in human SARS-CoV-2 infection and vaccination. *Cell*, (2021). doi:10.1016/j.cell.2022.01.018
22. Pham, C. T. N. *et al.* Application of a hemolysis assay for analysis of complement activation by perfluorocarbon nanoparticles. *Nanomedicine: Nanotechnology, Biology and Medicine* 10, 651–660 (2014). doi:10.1016/j.nano.2013.10.012
23. Holland, M. C., Morikis, D., & Lambris, J. D. Synthetic small-molecule complement inhibitors. *Current Opinion in Investigational Drugs* 5, 1164-1173 (2004).
24. Reis, E. S. *et al.* C5A receptor-dependent cell activation by physiological concentrations of desarginated C5A: Insights from a novel label-free cellular assay. *The Journal of Immunology* 189, 4797–4805 (2012). doi:10.4049/jimmunol.1200834
25. Strizova, Z. *et al.* M1/M2 Macrophages and Their Overlaps – Myth or Reality? *Clinical Science* 137(15), 1067–1093 (2023). doi:10.1042/cs20220531.
26. McNeil, M. M. *et al.* Risk of anaphylaxis after vaccination in children and adults. *Journal of Allergy and Clinical Immunology* 137, 868–878 (2016). doi:10.1016/j.jaci.2015.07.048
27. Chu, D. K. *et al.* Risk of Second Allergic Reaction to SARS-CoV-2 Vaccines: A Systematic Review and Meta-analysis. *JAMA Internal Medicine* (2022). doi:10.1001/jamainternmed.2021.8515
28. Zhou, Z.-H. *et al.* Anti-PEG IgE in anaphylaxis associated with polyethylene glycol. *The Journal of Allergy and Clinical Immunology: In Practice* 9, (2021). doi: 10.1016/j.jaip.2020.11.011
29. Hong, L., Wang, Z., Wei, X., Shi, J. & Li, C. Antibodies against polyethylene glycol in human blood: A literature review. *Journal of Pharmacological and Toxicological Methods* 102, 106678 (2020). doi: 10.1016/j.vascn.2020.106678

30. Wenande, E. & Garvey, L. H. Immediate-type hypersensitivity to polyethylene glycols: a review. *Clinical & Experimental Allergy* 46, 907–922 (2016). doi:10.1111/cea.12760
31. Kozma, G. T., Shimizu, T., Ishida, T. & Szebeni, J. Anti-PEG antibodies: Properties, formation, testing and role in allergic immune reactions to pegylated nano-biopharmaceuticals. *Advanced Drug Delivery Reviews* 154-155, 163–175 (2020). doi:10.1016/j.addr.2020.07.024
32. Kozma, G. T. *et al.* Pseudo-anaphylaxis to Polyethylene Glycol (PEG)-Coated Liposomes: Roles of Anti-PEG IgM and Complement Activation in a Porcine Model of Human Infusion Reactions. *ACS Nano* 13, 9315–9324 (2019). doi:10.1021/acsnano.9b03942
33. Mukai, K. *et al.* Assessing basophil activation by using flow cytometry and mass cytometry in blood stored 24 hours before analysis. *Journal of Allergy and Clinical Immunology* 139, 889-899 (2017). doi:10.1016/j.jaci.2016.04.060
34. Polfliet, M. M. J. *et al.* The Rat Macrophage Scavenger Receptor CD163: Expression, Regulation and Role in Inflammatory Mediator Production. *Immunobiology* 211(6–8), 419–425 (2006). doi:10.1016/j.imbio.2006.05.015
35. Fabriek, B. O. *et al.* The Macrophage Scavenger Receptor CD163 Functions as an Innate Immune Sensor for Bacteria. *Blood* 113(4), 887–892 (2009). doi:10.1182/blood-2008-07-167064
36. Lonez, C. *et al.* Cationic lipid nanocarriers activate Toll-like receptor 2 and NLRP3 inflammasome pathways. *Nanomedicine: Nanotechnology, Biology and Medicine* 10, 775–782 (2014). doi:10.1016/j.nano.2013.12.003
37. Lonez, C. *et al.* Critical residues involved in Toll-like receptor 4 activation by cationic lipid nanocarriers are not located at the lipopolysaccharide-binding interface. *Cellular and Molecular Life Sciences* 72, 3971–3982 (2015). doi:10.1007/s00018-015-1915-1

38. Kedmi, R., Ben-Arie, N. & Peer, D. The systemic toxicity of positively charged lipid nanoparticles and the role of toll-like receptor 4 in immune activation. *Biomaterials* 31, 6867–6875 (2010). doi:10.1016/j.biomaterials.2010.05.027

39. Hajishengallis, G. & Lambris, J. D. More than complementing Tolls: complement-Toll-like receptor synergy and crosstalk in innate immunity and inflammation. *Immunological Reviews* 274, 233-244 (2016). doi:10.1111/imr.12467

40. Abe, T. *et al.* Local complement-targeted intervention in periodontitis: proof-of-concept using a C5a receptor (CD88) antagonist. *The Journal of Immunology* 189, 5442-5448 (2012). doi:10.4049/jimmunol.1202339

41. Samstad, E. O. *et al.* Cholesterol crystals induce complement-dependent inflammasome activation and cytokine release. *The Journal of Immunology* 192, 2837-2845 (2014). doi:10.4049/jimmunol.1302484

42. Kang, Y. H. & Biswas, S. K. Basophil-Macrophage Dialog in Allergic Inflammation. *Immunity* 38, 408-410 (2013). doi:10.1016/j.immuni.2013.02.015

43. Ivashkiv, L. B. Epigenetic Regulation of Macrophage Polarization and Function. *Trends in Immunology* 34(5), 216–223 (2013). doi:10.1016/j.it.2012.11.001

44. Yamaguchi, Y. *et al.* Consecutive BNT162b2 mRNA Vaccination Induces Short-Term Epigenetic Memory in Innate Immune Cells. *JCI Insight* 7(22), e163347 (2022). doi:10.1172/jci.insight.163347

45. Saha, K. *et al.* Regulation of Macrophage Recognition through the Interplay of Nanoparticle Surface Functionality and Protein Corona. *ACS Nano* 10, 4421–4430 (2016). doi:10.1021/acsnano.6b00053

46. Laisuan, W. COVID-19 Vaccine Anaphylaxis: Current Evidence and Future Approaches. *Frontiers in Allergy* 2, (2021). doi:10.3389/falgy.2021.801322

47. Lyons, J. J. Hereditary alpha tryptasemia: genotyping and associated clinical features. *Immunology and Allergy Clinics of North America* 38, 483–495 (2018). doi:10.1016/j.iac.2018.04.003
48. R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL:https://www.R-project.org/.
49. Hao, Y. *et al.* Integrated analysis of multimodal single-cell data. *Cell* 184, 3573-3587 (2021). doi:10.1016/j.cell.2021.04.048
50. Stuart, T. *et al.* Single-cell chromatin state analysis with Signac. *Nature Methods* 18, 1333-1341 (2021). doi:10.1038/s41592-021-01282-5
51. Germain, PL *et al.* Doublet identification in single-cell sequencing data using *scDblFinder* [version 2; peer review: 2 approved]. *F1000Research* 10, 979 (2022). doi:10.12688/f1000research.73600.2
52. Rainer J. EnsDb.Hsapiens.v86: Ensembl based annotation package. *R package version 2.99.0.* (2017). doi:10.18129/B9.bioc.EnsDb.Hsapiens.v86
53. Yu, G. & He, Q.Y. ReactomePA: an R/Bioconductor package for reactome pathway analysis and visualization. *Molecular BioSystems* 12, 477-479 (2016). doi:10.1039/c5mb00663e

Figures and Tables

Tables

Table 1: Participant demographics

Variable	Non-Allergic N=13 N (%)	Allergic Reaction N=19 N (%)
Age at Enrollment with Median (Range)	36 (27-79)	42 (28-70)

Sex		
Female	8 (62)	17 (89)
Male	5 (38)	2 (11)
Race		
White	4 (31)	10 (53)
Asian	7 (54)	3 (16)
Black	1 (8)	2 (11)
Native American	1 (8)	0 (0)
Other/Mixed	0 (0)	4 (21)
Ethnicity		
Not of Hispanic, Latinx, or Spanish origin	13 (100)	18 (95)
Hispanic, Latinx, Spanish origin	0 (0)	1 (5)
History of Allergies		
Drug Allergic History	2 (15)	10 (53)
Food Allergic History	0 (0)	3 (16)
Latex Allergic History	1 (8)	0 (0)
Environmental Allergic History	2 (15)	2 (11)
Unkown Etiology Allergic History	0 (0)	1 (5)
No Allergic History	10 (77)	8 (42)
Days Between Vaccine and First Blood Draw, days (Median [Range])	0 (0-45)	21 (0-86)
IgG to PEG, ng/mL-Median (Range) in first sample	0 (0-405.21)	314.8 (0-6903.24)
IgM to PEG-Median (Range) in first sample	356.35 (0-2716.63)	203.22 (0-2107.27)
IgE to PEG, ng/mL (Median (Range) in first sample	0 (0-0)	0 (0-0)

837

838

Table 2:

PID	Age	Sex	Race/ Ethnicity	Alpha Tryptasemia Testing	Type of Vaccine	Hx of allergies	Reaction After Dose 1 or 2	Onset after receipt (min)	Signs and Symptoms during the initial reaction	Days Between First Dose and Blood Draw One	IgE Levels (ng/mL) At Draw One	IgG Levels (ng/mL) at Draw One	IgM Levels (ng/mL) at Draw One	Days Between First Dose and Blood Draw Two	IgE Levels (ng/mL) At Draw Two	IgG Levels (ng/mL) at Draw Two	IgM Levels (ng/mL) at Draw Two
A-1	20-29	F	White / Non-Hispanic/Latino	Negative	BNT162b2	N	Dose 2	Within 120 Minutes	Redness At Site, Urticaria, Pruritis on leg	0 Days	0	888.44	205.97	44 Days	0	987.29	190.23
A-2	20-29	F	Black / Non-Hispanic/Latino	Negative	BNT162b2	N	Dose 2	Within 120 Minutes	Difficulty Breathing	0 Days	0	156.62	207.64	45 days	0	514.86	5794.06
A-3	30-39	M	Asian / Non-Hispanic/Latino	Negative	BNT162b2	N	Dose 2	Within 120 Minutes	Swelling At Site, Urticaria, Lip Edema	0 Days	0	626.01	203.22	42 days	0	901.61	261.45
A-4	70-79	M	Asian / Non-Hispanic/Latino	Negative	BNT162b2	N	Dose 2	Within 120 Minutes	Redness at Site, Swelling At Site, Urticaria on Tongue and Philthrum	0 Days	0	491.41	0	42 days	0	317.11	0
A-5	50-59	F	White / Non-Hispanic/Latino	Negative	mRNA-1273	Drug and Food	Dose 1	1 Minute	Itchiness on Lip, Tachycardia, Dizziness	20 Days	0	586.02	380.71	57 days	0	679.07	313.25
A-6	50-59	F	White / Non-Hispanic/Latino	Negative	BNT162b2	Drug	Dose 1	2 Minutes	Hypertension, Dizziness	68 Days	0	1518.63	2107.27	Only One Draw	NA	NA	NA
A-7	30-39	F	Mixed / Non-Hispanic/Latino	Negative	mRNA-1273	Drug	Dose 1	1 Minute	Dizziness, Throat Swelling	34 Days	0	6903.24	1349.01	Only One Draw	NA	NA	NA
A-8	30-39	F	Mixed / Hispanic/Latino	Negative	BNT162b2	Drug and Food	Dose 1	1 Minute	Rash, Urticaria on Opposite Arm	38 Days	0	679.9	0	74 Days	0	390.32	0
A-9	30-39	F	Asian / Non-Hispanic/Latino	Negative	BNT162b2	Drug	Dose 1	1 Minute	Urticaria on Trunk	47 Days	0	0	359.09	78 Days	0	667.56	364.23
A-10	40-49	F	White / Non-Hispanic/Latino	Negative	mRNA-1273	N	Dose 1	14 Minutes	Urticaria, Edema Of Tounge	48 Days	0	198.88	181.86	Only One Draw	NA	NA	NA
A-11	50-59	F	White / Non-Hispanic/Latino	Not Tested	BNT162b2	N	Dose 1	1 Minute	Edema, Erythema, Throat Swelling, Tachycardia	19 Days	0	0	109.27	Only One Draw	NA	NA	NA

A-12	50-59	F	White / Non-Hispanic/Latino	Not Tested	BNT162b2	Drug	Dose 1	1 Minute	Shortness of Breath, Tachycardia	17 Days	0	0	1658.88	Only One Draw	NA	NA	NA
A-13	40-49	F	Mixed / Non-Hispanic/Latino	Not Tested	BNT162b2	N	Dose 1	5 Minutes	Dizziness, Hypertension, Chllls, Headache	21 Days	0	314.8	1223.42	Only One Draw	NA	NA	NA
A-14	40-49	F	White / Non-Hispanic/Latino	Not Tested	mRNA-1273	Drug	Dose 1	20 Minutes	Urticaria, Edema, Lip Swelling, Hives, Shortness of Breath	12 Days	0	0	0	Only One Draw	NA	NA	NA
A-15	30-39	F	White / Non-Hispanic/Latino	Not Tested	BNT162b2	Drug	Dose 1	5 Minutes	Urticaria, Edema, Throat Swelling, Stridor, Shortness of Breath	86 Days	0	0	466.16	Only One Draw	NA	NA	NA
A-16	30-39	F	White / Non-Hispanic/Latino	Not Tested	BNT162b2	Drug and Environmental	Dose 1	20 Minutes	Facial Edema, Erythema, Hypotension	33 Days	0	1181.77	113.6	Only One Draw	NA	NA	NA
A-17	50-59	F	White / Non-Hispanic/Latino	Not Tested	BNT162b2	N	Dose 1	3 Minutes	Edema, Tachycardia, Lip edema	38 Days	0	0	131.91	Only One Draw	NA	NA	NA
A-18	20-29	F	Mixed / Non-Hispanic/Latino	Not Tested	BNT162b2	Unknown Etiology	Dose 1	5 Minutes	Urticaria, Pruritis on Chest and Back	34 Days	0	986.89	0	Only One Draw	NA	NA	NA
A-19	50-59	F	Black / Non-Hispanic/Latino	Not Tested	BNT162b2	Drug, Food, and Environmental	Dose 1	5 Minutes	Edema, Tongue Swelling, Dizziness	30 Day	0	0	140.19	Only One Draw	NA	NA	NA

NA=Not available

Figure Legends

Figure 1. Allergic subjects can be further sub-divided into those with low or high serum-induced basophil responses ('low basophil responders' vs 'high basophil responders'). (A) Proportion of $CD63^{+}CRTh2^{+}$ basophils in response to anti-IgE (5 µg/mL) or grass pollen allergen (Phleum pratense; Phl p) in enriched basophils (left panel, n=3) or PBMCs (right panel; n=6). Basophil activation was evaluated in cells that were

left untreated, IgE-stripped with 4% lactic acid or IgE-stripped and resensitized with indicator serum from a grass pollen highly allergic individual (GP IS, grass pollen-specific IgE >100kU$_A$/L). **(B)** Concentration-ranging effect of COVID-19 vaccine (BNT162b2), PEG (1 µg/mL) and P80 (1 µg/mL), on CD63$^+$CRTh2$^+$ basophil activation in enriched basophil or PBMCs. In this validation experiment, we performed this side-by-side basophil vs. PBMC comparison in 5 allergic serum samples using 2 donor basophils. **(C)** Concentration-ranging effect of COVID-19 vaccine (BNT162b2 or mRNA-1273), PEG and P80 on non-allergics (n=13) and allergics (n=19). **(D)** Proportion of individuals in the allergic groups who had a reaction within $\leq$ 5 min, $\leq$ 20 min or $\leq$ 120 min. **(E)** Allergic individuals can be sub-divided into those with low (n=11) or high (n=8) basophil response upon resensitization with serum alone. **(F)** Unbiased clustering analysis FlowSOM showed differences in metaclusters (MC) 9, 10 and 11 in allergics with low or high basophil response following stimulation with serum alone. FlowSOM was performed on concatenated files. **(G)** Heatmap representing the expression of CD63, CD203c, C5aR and C3aR in all MC. Blue and red denotes low and high expression, respectively. **(H)** Population abundance for MC 9, 10 and 11 in allergics with low or high basophil response following stimulation with serum alone, vaccine (15 ug/mL), PEG2000 or P80. Data are presented as mean $\pm$ SEM. Violin plots are presented with its media represented in black line. Mann-Whitney U Test, * P<0.05, ** P<0.01.

Figure 2. Anti-PEG antibody levels and COVID-19 mRNA vaccine-mediated hemolytic activity. (A) Concentration of anti-PEG-IgE in serum of non-allergic (n=13, blue) and allergic individuals (n=19, orange) from the earliest timepoint of blood collection shortly after receiving the first dose of vaccine (Median: 11.5 days, Range 0 – 86 days). **(B)** Dot plot depicting concentration of anti-PEG-IgG in serum of non-allergic (n=13, blue) and allergic individuals (n=19), split into high (n=8, green) and low (n=11, pink) basophil responses, as characterized in **Fig. 1**. **(C)** Dot plot depicting concentration of anti-PEG-IgM in serum of non-allergic (n=13, blue) and allergic individuals (n=19), split into high (n=8, green) and low (n=11, pink) basophil responses, as characterized in **Fig. 1**. Values were compared between the two groups at the same

relative times using two-tailed Mann-Whitney test. **(D)** Serum was incubated with BNT162b2 vaccine for 30 min to trigger complement activation. Residual complement activity in the serum following incubation with BNT162b2 vaccine is expressed as average residual hemolytic activity (Avg RHA). Dot plots showing Avg RHA in non-allergic (n = 13, blue) and allergic individuals (n = 19), split into high (n=8, green) and low (n=11, pink) basophil responses, as characterized in **Fig. 1**. Values were compared between the two groups at the same relative times using two-tailed Mann-Whitney test. **(E)** A subgroup of sera from allergic individuals were depleted of IgG (Dpl) and subjected to the hemolysis assay. Depletion was confirmed by the absence of IgG heavy and light chains by Western blotting (data not shown). Antibody depletion diminished complement activation, resulting in higher average RHAs in the majority of sera tested. Wilcoxon signed rank test, * P<0.05.

Figure 3. C5a and C3a receptors play a role in serum+vaccine-induced basophil activation. (A) Proportion of C5aR and C3aR on the surface of enriched basophil (black bar) and PBMC (white bar) presented as a percentage or geo mean fluorescence intensity (MFI) value. **(B)** Effect of C5aR (75 nM) and C3aR (1 μM) antagonist on vaccine induced $CD63^{+}CRTh2^{+}$ basophil activation in enriched basophil (black bar) and PBMC (white bar). **(C)** Effect of complement antagonist (C5aRA and C3aRA) on serum+vaccine-induced basophil activation in non-allergics (n=13; blue bar), allergics with low basophil response (n=11; magenta bar) or high basophil response (n=8; green bar). **(D)** Unbiased clustering analysis FlowSOM showed differences in metaclusters (MC) 9, 10, 11 and 12 in non-allergics and allergics (low or high basophil response) targeted by complement receptor antagonists C5aRA and C3aRA. FlowSOM was performed on concatenated files. **(E)** Population abundance for MC 9, 10, 11 and 12 in non-allergics or allergics with low or high basophil response following stimulation with serum+vaccine, or in the presence of C5aRA and C3aRA. Data are presented as mean ± SEM. Violin plots are presented with its media represented in black line. Kruskal-Wallis Test, * P<0.05, ** P<0.01, *** P<0.001.

Figure 4. Hemolysis assay and correlation with anti-PEG antibody. (A) Higher anti-PEG IgG levels correlated with higher complement activation, and reflected in lower Avg RHA in allergic, high basophil responders (R^2 = 0.5813, P = 0.028). **(B)** No correlation between anti-PEG IgM levels and Avg RHA (R^2 = 0.056, P = 0.573) in allergic, high basophil responders. **(C)** Complement activation generated complement anaphylatoxin C3a following incubation of serum with COVID-19 mRNA vaccine leading to basophil activation, which was partially blocked by complement C3a receptor antagonist (C3aRA) (R^2 = 0.5680, P = 0.031). **(D)** There is low correlation (R^2= 0.2182, P = 0.243) between basophil response in the presence of C5aRA and Avg RHA in the allergic, high basophil responders.

Figure 5. Singe cell multiome analyses of individuals with differing basophil responses to COVID vaccines. **(A)** A total of 1 non-reactor, 3 low-(basophil) BAT reactors and 3 high-(basophil) BAT reactors were chosen for single cell ATAC sequencing based on results from the basophil activation tests. The PBMCs were collected from blood and stimulated with autologous patient serum collected after first dose of the vaccine for 20 minutes at 37C before single cell multiome sequencine. **(B)** Weighted nearest neighbor (WNN) UMAPs of predicted cell types based on azimuth classifications (level 1). UMAP of cells classified from different patient responses. Proportions of predicted cell types for each patient group. **(C)** RNA transcriptome correlation analysis based on sctransformed normalized data recapitulated across different cell types of stimulation condition. **(D)** Inferred gene activity correlation analysis based on scATAC-seq data captures similar profiles across treatment conditions.

Figure 6. Single cell multiome analyses on PBMCs identified contribution of the myeloid compartment on high basophil responders and low basophil responders. (A) Weighted nearest neighbor (WNN) UMAPs

of predicted cell types based on azimuth classifications. Four subsets of cells within the myeloid compartment were identified (classical and non-classical monocytes, cDC2s and pDCs). **(B)** Top genes highly expressed within each clusters of cells identified. **(C)** Volcano plot to illustrate differentially expressed genes between high basophil responders versus low basophil responders versus non-allergics. **(D)** Heatmap and violin plots denoting a selection of genes differentially expressed within the myeloid compartment of differen study subjects. **(E)** Pathway enrichment analysis in high basophil responders vs. low basophil responders, high basophil responders vs non-allergics and low basophil responders vs non-allergics. **(F)** Peak callings to illustrate accessible regions within the *IL-13*, *IL-4*, and *IL-1B* loci of high basophil responders, low basophil responders and non-allergics.

Figure 7. Schematic representing vaccine-induced molecular pathway in high basophil responders and low basophil responders.

Figure S1. C5a and C3a receptors play a role in serum-induced basophil activation. Effect of **(A)** varying concentration of COVID-19 vaccine (BNT162b2 or mRNA-1273), **(B)** PEG and P80 on CD203c^{bright}CRTh2^{+} basophils of non-allergics (n=13) and allergics (n=19). **(C)** Flow cytometry representative plot for the effect of serum alone or in the presence of C5aRA or C3aRA on CD63^{+} (left panel) or CD203c^{bright} (right panel) basophils. Plots are shown for three groups of subjects: non-allergics (top panel), allergics with low basophil response (middle panel) and allergics with high basophil response (bottom panel). **(D)** Effect of complement antagonist (C5aRA; 75nM and C3aRA; 1µM) on serum- and vaccine-induced basophil activation in non-allergics (n=13; blue bar), allergics with low basophil response (n=11; magenta bar) or high basophil response (n=8; green bar). **(E)** Effect of complement antagonist (C5aRA, C3aRA or C5aRA+C3aRA) on serum- and vaccine-induced basophil activation (CD63^{+} basophils) in allergics with low

basophil response (n=5; magenta bar) or high basophil response (n=4; green bar). Data are presented as mean ± SEM. Violin plots are presented with its media represented in black line. Kruskal-Wallis Test, * P<0.05, ** P<0.01, *** P<0.001.

Figure S2. Hemolysis assay and correlation with anti-PEG antibody. Modified hemolysis assay was performed as detailed in the Materials and Methods section and as previously described in (*22*). Serum was incubated with BNT162b2 vaccine for 30 min to trigger complement activation (CA). The reaction mixture was then titrated to determine residual complement activity with the addition of antibody-sensitized sheep red blood cells (sRBCs). Lysis of sRBCs by residual complement activity in the serum is expressed as residual hemolytic activity (RHA). **(A)** Modified hemolysis assay of vaccine-mediated complement activation (CA). Each curve in a titration series is derived by plotting the volume of reaction mixture (V) on the x-axis against the fraction of cells lysed (Y) on the y-axis. Complete lysis is defined as Y = 1. Incubation of normal human serum (NHS) with zymosan or peroxidase/anti-peroxidase (PAP), both strong complement activators, diminished the hemolytic activity, resulting in a downward shift of the titration curve. Vaccine (2% v/v) added to NHS did not change the hemolytic activity significantly. **(B)** Impact of anti-PEG antibody on BNT162b2 vaccine-induced CA, (R^2 = 0.9414, P = 0.0013). NHS was incubated with BNT162b2 vaccine (2% v/v) in the absence or presence of increasing concentrations of rabbit anti-PEG IgG. Average RHA compares the area under the titration curve (AUC) obtained from NHS incubated with BNT162b2 to the AUC of the control NHS. The purple dot represents the RHA of serum + highest concentration of rabbit anti-PEG IgG without added BNT162b2 to show that the antibody by itself does not activate complement. **(C)** Impact of anti-PEG antibody on Doxil C-induced CA, (R^2 = 0.9089, P = 0.0120). NHS was incubated with Doxil Control (0.25% v/v) in the absence or presence of increasing concentrations of rabbit anti-PEG IgG. Average RHA compares the area under the titration curve (AUC) obtained from NHS incubated with Doxil Control to the AUC of the control NHS. The purple

dot represents the RHA of serum + highest concentration of rabbit anti-PEG IgG without added Doxil Control to show that the antibody by itself does not activate complement**. (D)** There are no changes in Avg RHA in the non-allergic group between the time of vaccination and blood draws over the first 50 days. **(E)** There is a trend toward lower Avg RHA in the allergic group between the time of vaccination and blood draw over the first 50 days. **(F)** The majority of non-allergic individuals have no anti-PEG IgG or low titer of anti-PEG IgG ($\leq$ 400 ng/ml) although there is no clear correlation between anti-PEG IgG levels and Avg RHA in either group. **(G)** There is no clear correlation between anti-PEG IgM levels and Avg RHA in non-allergic and allergic individuals.

Figure S3. Expression of CD63, CD203c, C5aR and C3aR on MC9, 10 and 11.

Figure S4. Correlation between RHA:anti-PEG Ig's and RHA:basophil activation

(A) Higher anti-PEG IgG levels had a weak correlation with higher complement activation, and reflected in lower Avg RHA in allergic, low basophil responders (R^2 = 0.23, P = 0.135). **(B)** Higher anti-PEG IgM levels correlated with weak correlation activation, and reflected in lower Avg RHA in allergic, low basophil responders (R^2 = 0.1787, P = 0.195). **(C)** No correlation between complement C3a receptor antagonist (C3aRA) and complement activation in allergic, low basophil responders (R^2 = 0.017, P = 0.719). **(D)** No correlation between complement C5a receptor antagonist (C5aRA) and complement activation in allergic, low basophil responders (R^2 = 0.0158, P = 0.729). **(E)** No correlation between anti-PEG IgG levels and Avg RHA (R^2 = 0.0056, P = 0.818) in non-allergic individuals. **(F)** Higher anti-PEG IgM levels had a weak correlation with higher complement activation and reflected in lower Avg RHA in non-allergic individuals (R^2 = 0.1734, P = 0.178). **(G)** Higher C3a levels had a weak correlation with anti-PEG IgG levels in allergic, low basophil responders (R^2 = 0.1250, P = 0.390). **(H)** Higher C3a levels no correlation with anti-PEG IgG

levels in allergic, high basophil responders (R^2 = 0.0051, P = 0.893). **(I)** No significant differences in C3a levels between non-allergic (blue), low basophil responders (pink), and high basophil responders (green). **(J)** No correlation between complement C3a receptor antagonist (C3aRA) and complement activation in non-allergic individuals (R^2 = 0.0176, P = 0.698). **(K)** No correlation between complement C5a receptor antagonist (C5aRA) and complement activation in non-allergic individuals (R^2 = 0.0419, P = 0.546).

Figure S5. Peak callings to illustrate accessible regions within the IL-10 locus of high basophil responders, low basophil responders, and non-allergics.