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Abbreviated Title: COVID vaccine immune response

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Title: Analysis of the immune response to COVID-19 vaccination and outcomes in individuals with and without immune deficiencies and dysregulations

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Table of Contents

STATEMENT OF COMPLIANCE

The protocol will be carried out in accordance with International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

NIH-funded investigators and study site staff who are responsible for the conduct, management, or oversight of NIH-funded studies have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent using a previously approved consent form.

1 PROTOCOL SUMMARY

1.1 Synopsis

Title:	Analysis of the immune response to COVID-19 vaccination and outcomes in individuals with and without immune deficiencies and dysregulations
Study Description:	This prospective cohort study will assess the pre- and post-vaccination immune responses in individuals with select immunodeficiencies and immune dysregulations compared to healthy volunteers who receive a coronavirus disease 2019 (COVID-19) vaccine, as well as any adverse events (AEs) experienced after vaccination. All required study visits for this protocol may be conducted remotely; in-person visits at the NIH are optional. Subjects who have not yet been vaccinated will undergo baseline blood sampling using finger stick microsampler kits and/or venous blood draw within 7 days prior to receiving the vaccine. Additional samples will be requested from participants approximately 14-21 days after dose 1 and 21-28 days after dose 2 (if applicable). Optional samples may be collected at 6, 12, and 24 months post-vaccination. If subsequent booster doses are received while a participant is still on study, blood samples will again be requested approximately 28 days after each booster dose, through the 5th Covid-19 vaccine dose received, and then participants may proceed with the optional 6-, 12-, and 24-month follow-up sample collection. Participants who are able to attend in-person visits at NIH will have optional on-site blood draws 1 and 3 days after doses 1 and 2 (as applicable). Research evaluations will include baseline severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) antibody titers to the spike (S), nucleocapsid (N), and receptor binding domain (RBD) proteins, to assess pre-vaccination SARS-CoV-2 exposure and evaluate responses to vaccination. Additional immune markers of interest may include presence of autoantibodies, transcriptomic profiling, T-cell receptor (TCR) repertoire, among others. Participants who only submit finger stick home microsampler kits at the timepoints listed above will be evaluated for SARS-CoV-2 antibody titers and autoantibodies only. All subjects will be asked at baseline about prior COVID-19 diagnosis, symptoms, and severity, and will be asked additional questions at follow-up timepoints (including after additional booster doses) about vaccine AEs using standardized questionnaires.

Primary Objective: To characterize the immune response to COVID-19 vaccination among immunodeficient and immune dysregulated individuals compared to healthy volunteers.

Secondary Objectives:

- To characterize the COVID-19 vaccine-associated AEs among immunodeficient and immune dysregulated individuals compared to healthy volunteers.

Exploratory Objectives:

- Assess the relationship between prior SARS-CoV-2 infection and vaccine-induced immune response.
- To characterize pre-vaccine COVID-19 disease prevalence and severity among immunodeficient and immune dysregulated individuals.
- To assess induction, strength, and durability of T- and B-cell-specific immune responses to SARS-CoV-2 (as measured by frequency and diversity of SARS-CoV-2 specific T and B cell clonotypes and titers of specific antibody responses), and their correlation to the underlying immune deficiency/dysregulation.
- To characterize autoantibodies present in immunodeficient and immune dysregulated individuals before and after COVID-19 vaccination.
- To assess the incidence of post-vaccination breakthrough SARS-CoV-2 infection and to determine the SARS-CoV-2 virus genomic sequence in such cases.

Primary Endpoint: Change in S and RBD immunoglobulin G (IgG) antibody titer from baseline to 14-21 days or 21-28 days (depending on vaccine manufacturer and platform) after vaccine dose 1, and 21-28 days after dose 2 and any subsequent doses (depending on vaccine manufacturer and platform).

Secondary Endpoints:

- Incidence of vaccine-associated AEs experienced by immunodeficient individuals compared to healthy volunteers.

Exploratory Endpoints:

- Characterization of post-vaccine immune response and AEs in patients with antibody evidence of prior SARS-CoV-2 infection
- Pre- and post-vaccination incidence of COVID-19-associated symptoms experienced in individuals with immune deficiency/dysregulation who are positive for SARS-CoV-2 by serology or diagnostic polymerase chain reaction (PCR).
- Characterize how various forms of immune deficiency or dysregulation impact generation, strength, and durability of T- and B-cell responses.

	• Incidence of autoantibodies pre- and post-COVID-19 vaccination comparing individuals with immune deficiency/dysregulation to healthy volunteers. • Incidence and genetic sequence of post-vaccination SARS-CoV-2 infection as reported by patient and confirmed by PCR and sequencing.
Sample Size:	N=500 (n=400 with immune deficiency/dysregulation, n=100 healthy volunteers)
Accrual Ceiling:	N=600
Study Population:	We will enroll individuals 3 years of age and older who are receiving COVID-19 vaccination outside of this study. Affected study participants must have evidence of a primary or secondary immune deficiency or dysregulation as documented on another NIAID protocol or by an outside physician. Control participants will be healthy volunteers, and may include unaffected relatives of immunodeficient/dysregulated participants.
Description of Sites/Facilities Enrolling Participants:	This is a single-site study being conducted at the NIH Clinical Center (CC) via in-person and remote visits. Subjects may be recruited from existing NIH protocols. They may also be referred from community practitioners who see this population. Healthy volunteers may also be recruited through the NIH Clinical Research Volunteer Program, the Office of Patient Recruitment, MMG Patient Recruitment, ResearchMatch, or BuildClinical. Individuals may be enrolled in person or remotely.
Study Duration:	4 years
Participant Duration:	Up to 24 months from participant's last vaccine dose. This may be extended for participants who receive additional booster doses and agree to additional extension visits, up to 2 years after the booster vaccination, through the 5th dose only.

1.2 Schedule of Activities

Evaluation	Study Day (window)							
	Screening and Baseline*	Vaccine dose 1 Follow-up			Vaccine dose 2 Follow-up‡			Optional Follow-up†
	-7 to 0	1 day after vaccination§	3 days after vaccination§	14-21 OR 21-28 days after vaccination**	1 day after vaccination§	3 days after vaccination§	21-28 days after vaccination**	6, 12, and 24, months (±2 weeks) after last vaccination
Informed consent	X							
Clinical Procedures and Evaluations								
COVID-19 history and symptom questionnaire	X							
Baseline medical history and screening questionnaire	X							
Changes in medical history questionnaire				X			X	X
Electronic AE questionnaire^				X			X	
Behavioral questionnaire							X	
Clinical Laboratory Evaluations^^	Blood Volume (mL)							
CBC with differential	3 mL	4 mL	4 mL	3 mL	4 mL	4 mL	3 mL	
TBNK lymphocyte phenotyping/ flow/cytek		[X]	[X]		[X]	[X]		

Research Laboratory Evaluations^^	Blood Volume (mL)							
Mitra microsampler¶ • SARS-CoV-2 antibody (S, RBD, N – IgG, IgM, IgA) • Autoantibodies	0.8 mL			0.8 mL			0.8 mL	0.8 mL
Serum (red cap) †† • Neutralizing SARS-CoV-2 antibodies	5 mL	5 mL	5 mL	5 mL	5 mL	5 mL	5 mL	5 mL
Plasma isolation (EDTA purple cap) ¶ • DNA isolation (for high throughput sequencing of TCR/BCR repertoire and for genetic studies)	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL
PAXgene tube†† • Whole blood RNA	2.5 mL	2.5 mL	2.5 mL	2.5 mL	2.5 mL	2.5 mL	2.5 mL	2.5 mL
PBMC isolation (sodium heparin green top OR EDTA purple cap) †† • Lymphocyte/plasmablast • Functional T cells	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL
Saliva‡‡ • SARS-CoV-2 PCR and sequencing	Bi-weekly through 6 months							
Daily Blood Volume	31.3 mL	31.5 mL	31.5 mL	31.3 mL	31.5 mL	31.5 mL	31.3 mL	31.3 mL
Cumulative Blood Volume	31.3 mL	62.8 mL	94.3 mL	125.6 mL	157.1 mL	188.6 mL	219.9 mL	251.2 mL (6 mos.) 282.5 mL (12 mos.) 313.8 mL (24 mos.)

[X] = will be performed with portion of sample collected for CBC with differential and does not require additional volume.

Abbreviations: AE, adverse event; BCR, B cell receptor; CBC, complete blood count; COVID-19, coronavirus disease 2019; DNA, deoxyribonucleic acid; EDTA, ethylenediaminetetraacetic acid; Ig, immunoglobulin; PBMC, peripheral blood mononuclear cell; N, nucleocapsid; RBD, receptor binding domain; RNA, ribonucleic acid; S, spike; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TBNK, T, B, and natural killer (cells); TCR, T cell receptor; NP, nasopharyngeal; OP, oropharyngeal; PCR, polymerase chain reaction.

*Baseline sampling and questionnaire can happen on the day of vaccination as long as it is prior to receiving the dose. Participants may enroll after vaccination was already performed. If these individuals do not have current plans for an additional booster dose, previous timepoint samples will not be obtained and they will proceed with the next relevant sampling timepoint. In this situation, if stored blood samples from studies in which subjects are concurrently enrolled are available, they will be used. If stored samples are not available, these samples will be considered waived. If these individuals plan to receive an additional booster vaccination(s), they will be enrolled prior to the booster dose and follow the normal schedule, including a baseline draw prior to the booster.

**The exact timing of these visits will depend upon vaccine manufacturer and platform (as detailed in the Manual of Procedures) but will fall within the ranges indicated. For subjects who decline participation in the optional long-term follow-up visits, study completion will end after the dose 1 or 2 follow-up visit(s), depending on whether the subject received a 1-dose or 2-dose vaccine. Each time a participant receives an additional booster dose while they are still on study, a 21-28–day visit will be performed, and optional long-term follow-up visits will be offered. Samples will be requested from participants as scheduled after each vaccination and booster (+/- 7 days) however samples received outside of the requested windows are acceptable and will be analyzed at the discretion of the PI.

‡ Not all vaccines require a second dose and therefore these follow-up timepoints will be not applicable for participants receiving those vaccines.

^AE questionnaire will be administered after any additional booster dose.

^^Due to the ongoing pandemic, venous blood draws may be conducted at the NIH or at off-site facilities (e.g., subjects' private physicians, Quest laboratories). Subjects will be provided microsampler kits for at-home fingerstick sample collection. Venous blood draw will be waived for participants who are unable to obtain one at any given timepoint. Microsamples will not be collected at on-site visits, as the small volume needed can be taken from another tube.

††May not be drawn at every visit, per PI discretion based on the participant's underlying immunodeficiency and study timepoint.

†If the subject opts in for the optional follow-up, then the final study visit will be the last visit that the participant chooses to have.

‡‡Participants will be sent optional saliva collection kits to return to the NIH bi-weekly for PCR and genetic sequencing of the virus. Participants whose samples are PCR positive will be asked to submit an additional COVID-19 symptom questionnaire and will be referred for care as needed. Each time a participant receives an additional booster dose while they are still on study, they may participate in optional bi-weekly saliva collections for an additional 6-month period.

§Optional additional visits for participants having specimens drawn at NIH only.

¶Only 1 sample type is required for off-site visits (either Mitra microsampler or plasma isolation). Plasma isolation is the preferred sample type.

2 INTRODUCTION

2.1 Study Rationale

COVID-19 is caused by the novel SARS-CoV-2 and is associated with a hyper-inflammatory immune response which leads to severe and potentially life-threatening symptoms. Little is known about how and the extent to which COVID-19 presents in people with immunodeficiencies, but because of the known severity of the illness in the general population, vaccination against SARS-CoV-2 is critical. Understanding whether immunodeficient individuals produce an adequate immune response to the COVID-19 vaccine, as well as the number and type of AEs experienced in these people is necessary.

2.2 Background

The novel human coronavirus SARS-CoV-2 is transmitted primarily via respiratory particles and infects the respiratory tract via the receptor angiotensin-converting enzyme 2 (ACE2), which is highly expressed in the respiratory tract as well as on various epithelial tissues throughout the body.[1] This receptor is also used by SARS-CoV, however SARS-CoV-2 has been found to bind to ACE2 with higher affinity than SARS-CoV, potentially increasing the infectivity seen with this virus.[2] Since the beginning of the pandemic, and as of the writing of this protocol (a period of just over 1 year), SARS-CoV-2 has resulted in over 93 million confirmed infections globally, with over 2 million deaths.[3]

The disease caused by this virus, COVID-19, has a wide range of clinical manifestations, from completely asymptomatic or mild disease, to severe illness requiring hospitalization, to long-term sequelae (dubbed "long COVID"). Much of the pathology associated with COVID-19 is due to an aggressive immune response which can result in airway damage and acute respiratory distress syndrome (ARDS), among other pathologies.[4] Unfortunately, there is currently little to no published data on the extent to which COVID-19 is experienced in individuals with immune deficiencies, especially those caused by inborn errors. It is important to know the incidence and clinical presentation of COVID-19 in these individuals to further our understanding of the role immune system activation plays in the development and progression of COVID-19. Additionally, with the recent emergency use authorizations (EUAs) granted by the US Food and Drug Administration (FDA) for two messenger RNA (mRNA)-based COVID-19 vaccines and subsequent approval of one of these, and with more vaccines in the pipeline, there is a need to understand the post-vaccine immune response in individuals after vaccination to ensure adequate induction and duration of protection. However, individuals with immunodeficiencies are typically excluded from vaccine trials measuring safety and immunogenicity, as was the case for the two COVID-19 vaccines currently authorized or approved. These groups of people must be evaluated to determine the efficacy and tolerability of vaccines, especially in light of concerning anaphylaxis episodes in a minority of people who were vaccinated against COVID-19.[8]

There is some literature regarding the immune response in individuals with inborn errors of immunity to vaccination, including inactivated or subcomponent vaccines like the influenza or *Haemophilus influenzae* type b vaccines, [5,6] and live-attenuated vaccines such as the measles-mumps-rubella and varicella vaccines.[7] Depending on the immunologic defect, vaccine immunogenicity can vary widely, leaving some immunodeficient individuals inadequately protected. For example, individuals with complement defects should produce B- and T-cells in levels relatively similar to that of healthy volunteers, while others, like those with Wiskott-

Aldrich Syndrome, produce antibodies but a weak or absent T-cell response, and those with common variable immunodeficiency are only capable of producing T-cells without antibody. Because of this complexity, it is necessary to obtain a comprehensive understanding of the immunologic response to vaccines to better understand both the level of cellular and humoral protection against COVID-19 and also their estimated contribution to herd immunity.

Unfortunately, individuals with immunodeficiencies are typically excluded from vaccine trials measuring immunogenicity and safety, as was the case for the COVID-19 vaccines currently authorized or approved for use in the United States. This creates a need to study AEs in immunodeficient people to determine the safety and tolerability of the COVID-19 vaccines, especially in light of concerning anaphylaxis episodes in a minority of people who were vaccinated against COVID-19,[8] even though AEs in this group, specifically in individuals with impaired T-cell responses, are usually limited to live vaccines.

The aims of this study are to characterize the immune response to COVID-19 vaccination, the number and type of vaccine-associated AEs, and pre- and post-vaccine COVID-19 disease prevalence and severity in individuals with different types of immune defects. Potential diseases of interest that are followed at NIAID include those with neutrophil dysfunction such as chronic granulomatous disease and leukocyte adhesion deficiency; combined immune deficiencies such as DOCK8 (dedicator of cytokinesis 8) deficiency, leaky severe combined immunodeficiencies (SCIDs; e.g. RAG (recombination-activating gene) deficiency), X-linked immunodeficiency with magnesium defect, Epstein-Barr virus infection, and neoplasia (XMEN) disease (T-cell deficiency), STAT3 (signal transducer and activator of transcription 3) deficiency (Hyper IgE syndrome), defects of the PI3K (phosphoinositide 3-kinase) pathway; predominantly B lymphocyte defects such as X-linked agammaglobulinemia and common variable immune deficiency (CVID); defects of the Interleukin 12 (IL-12)/Interferon gamma (IFNg)/STAT1 pathway; *GATA2* deficiency; and those with immune activation such as neonatal-onset multisystem inflammatory disease (increased inflammasome activity) and autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED).

2.3 Risk/Benefit Assessment

2.3.1 Known Potential Risks

Blood collection: The risks of venous blood draws include pain, bruising, bleeding, fainting, and, rarely, infection. The risk of finger sticks include minor, transient pain.

Saliva collection: There are no risks associated with collection of saliva by expectoration.

Questionnaire completion: There is a minimal risk of loss of confidentiality of information collected in the questionnaires.

2.3.2 Known Potential Benefits

Participants will not receive direct benefit by participating in this study. The information learned in this study may improve the investigators' understanding of the immune response and AE profile in individuals with immune deficiencies to COVID-19 vaccination, as well as the severity of COVID-19 in these individuals, which may have implications for individual care and further prevention of COVID-19. Such information may also be useful in designing more effective vaccines to prevent the spread of SARS-CoV-2, which may benefit others in the future.

2.3.3 Assessment of Potential Risks and Benefits

COVID-19 is a global pandemic with few approved treatments and a wide range of disease presentations and outcomes. Better characterization of disease presentation and immune response in individuals with immune dysfunction, which is the objective of this study, may contribute to the knowledge of how the immune system responds to COVID-19 vaccination and how it contributes to COVID-19 disease development. While study participants will be exposed to some risk from blood draws, those risks are outweighed by the benefits to generalizable knowledge.

3 OBJECTIVES AND ENDPOINTS

Objectives	Endpoints	Justification for endpoints
Primary		
To characterize the immune response to COVID-19 vaccination among immunodeficient and immune dysregulated individuals compared to healthy volunteers.	Change in S and RBD IgG antibody titer from baseline to 14-21 days or 21-28 days (depending on vaccine manufacturer and platform) after dose 1, and 21-28 days after dose 2 and any subsequent doses (depending on vaccine manufacturer and platform).	It is of interest to understand the changes in anti-SARS-CoV-2 antibody levels induced by vaccination in individuals with immune deficiency/dysregulation.
Secondary		
To characterize the COVID-19 vaccine-associated AEs among immunodeficient and immune dysregulated individuals compared to healthy volunteers.	Incidence of vaccine-associated AEs experienced by immunodeficient and immune dysregulated individuals compared to healthy volunteers.	It is of interest to understand the safety (by knowing the incidence of AEs) of COVID-19 vaccines in individuals with immune deficiency/dysregulation.
Exploratory		
Assess the relationship between prior SARS-CoV-2 infection and vaccine-induced immune response.	Characterization of post-vaccine immune response and AEs in patients with antibody evidence of prior SARS-CoV-2 infection compared to those without evidence of prior infection.	Because so many people have had SARS-CoV-2 infections prior to vaccination, it is of interest to understand whether this affects the immune response to vaccination.

To characterize pre-vaccine COVID-19 disease prevalence and severity among immunodeficient and immune dysregulated individuals.	Incidence of COVID-19-and associated symptoms experienced in individuals with immune deficiency/dysregulation who are positive for SARS-CoV-2 by serology or diagnostic PCR.	It is of interest to understand the incidence and clinical presentation of COVID-19 in individuals with immune deficiency/dysregulation, especially as COVID-19 is an inflammatory-mediated disease.
To assess induction, strength, and durability of T- and B-cell-specific immune responses to SARS-CoV-2 (as measured by frequency and diversity of SARS-CoV-2 specific T and B cell clonotypes and titers of specific antibody responses), and their correlation to the underlying immune deficiency/dysregulation.	Characterization how various forms of immunodeficiency impact generation, strength, and durability of T- and B-cell responses.	It is of interest to have a detailed understanding of the immune response to COVID-19 vaccination in individuals with immune deficiency/dysregulation.
To characterize autoantibodies present in immunodeficient and immune dysregulated individuals before and after COVID-19 vaccination.	Incidence of autoantibodies pre- and post-COVID-19 vaccination comparing individuals with immune deficiency/dysregulation to healthy volunteers.	Autoantibodies are effectors of immune response and can influence the efficacy of vaccination.
To assess the incidence and genetic sequence of post-vaccination SARS-CoV-2 infection.	Incidence and genetic sequence of post-vaccination SARS-CoV-2 infection as reported by patient and confirmed by PCR and sequencing.	With the rise in genetic variants of SARS-CoV-2, it is important to understand how many vaccinated individuals are still susceptible to infection.

4 STUDY DESIGN

4.1 Overall design

This is a prospective, exploratory, cohort study to evaluate the baseline and post-vaccination immune responses in individuals with select immune deficiencies compared to healthy volunteers who receive a COVID-19 vaccine, as well as any AEs experienced after vaccinations. Due to the ongoing pandemic and limited availability of vaccines at the time of preparation of this protocol, participants will receive vaccine externally (i.e., via private physicians or community resources, and not as study interventions). Study visits may be conducted in person or remotely. If participants cannot attend in-person visits at the NIH CC, they can choose to have blood drawn at a primary care provider, academic hospital laboratory, or Quest Diagnostics, as

necessary, and will be sent sampling kits containing all tubes and instructions for the drawing facility. All participants will also be provided with microsampler kits and mailing supplies to collect and send in fingerstick samples if they are unable to obtain venous blood samples at an off-site facility. Participants will be asked to provide samples at baseline (collected within 7 days prior to receiving the vaccine), 14-28 days (depending on vaccine manufacturer and platform) after dose 1 and 21-28 days after dose 2 (if applicable). Samples will be requested from participants as scheduled after each vaccination (+/- 7 days) however samples received outside of the requested windows are acceptable and will be analyzed at the discretion of the PI.

Participants may enroll after vaccination was already performed. If these individuals do not have current plans for an additional booster dose, previous timepoint samples will not be obtained and they will proceed with the next relevant sampling timepoint. In this situation, if stored blood samples from studies in which subjects are concurrently enrolled are available, they will be used, otherwise they will be waived. If these individuals plan to receive an additional booster vaccination(s), they will be enrolled prior to the booster dose and follow the normal schedule, including a baseline draw prior to the booster.

Participants who are able to come to the NIH will have the additional option to provide blood samples at days 1 and 3 after each vaccination in order to capture immediate vaccine-induced perturbations of immune responses. Samples will be used to study short-term immunological effects of immunization and boosting. If additional booster doses are received, participants will be asked to submit optional blood samples 21-28 days after the booster vaccination(s), through the 5th dose of a COVID-19 vaccine. Samples received outside of that window samples received outside of the requested windows are acceptable and will be analyzed at the discretion of the PI. Additional optional blood samples may be provided at approximately 6, 12, and 24 months after the final vaccination to assess long-term immunological persistence of vaccine-derived antibodies and T-cell responses. Research evaluations on these samples will include baseline and post-vaccine autoantibody levels as well as SARS-CoV-2 antibody titers to the S, N, and RBD proteins. Serology has been validated across fresh serum and dried blood samples collected using microsamplers (see Figure below)[8]. Venous blood draw may be waived if a participant is unable to obtain it at any given timepoint.

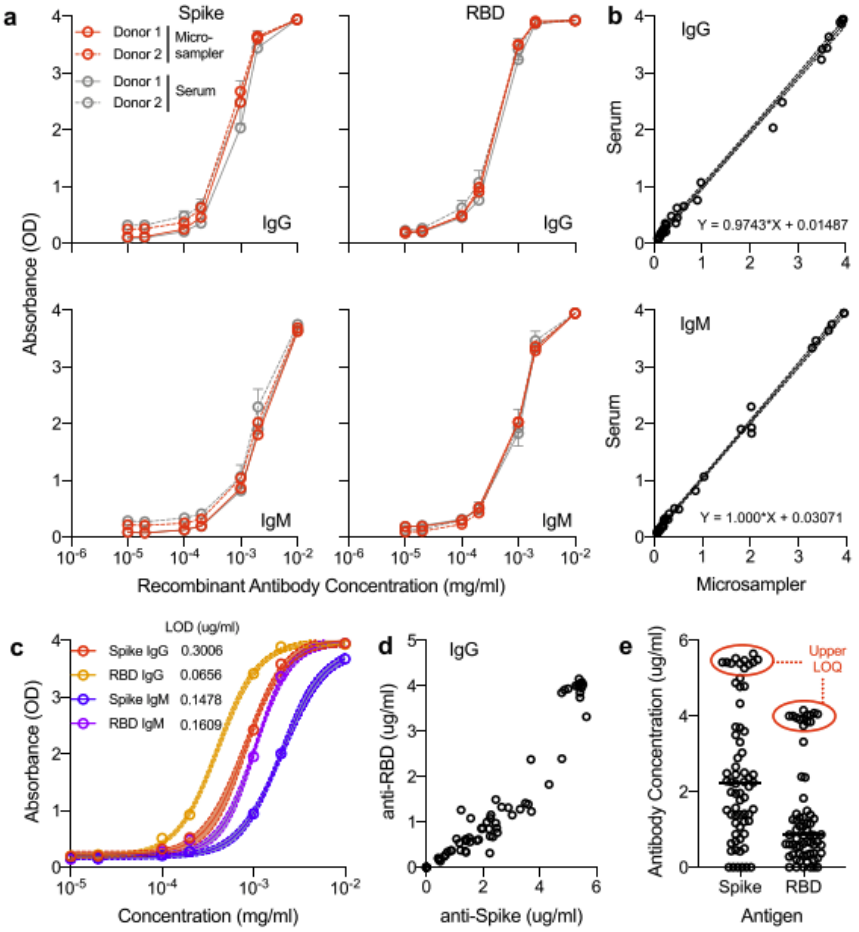

Figure 1. Quantification of antibody concentration utilizing 4PL sigmoidal model of recombinant antibody spiked into seronegative blood. A) Anti-RBD recombinant human antibody (IgG and IgM) was added to whole blood from two seronegative donors, then absorbed to microsamplers and remaining blood was spun down to isolate serum and analyzed on full spike ectodomain trimer (spike) or receptor-binding domain (RBD) ELISA. Data are mean ± SEM, n = 3, red = microsampler eluate, gray = matched serum. **B)** Direct comparison of absorbance of range of recombinant antibody concentration in serum (y axis) versus microsampler (x axis) blood samples. **C)** Sigmoidal four-parameter logistic (4PL) curve fitting to recombinant antibody dilution series, 95% confidence intervals shown shaded around fit curve, n = 12 replicates per data point, red = Spike IgG, orange = RBD IgG, blue = Spike IgM, purple = RBD IgM. **D)** Quantification of IgG levels in a sample high-incidence population. **E)** Upper limit of quantification at 1:400 dilution of serum into ELISA (1:10 dilution of microsampler eluate), n =68. From ref [8]

Additional research evaluations include analysis of the nature, diversity, and frequency of SARS-CoV-2 specific T- and B-cell receptor clonotypes. These evaluations will be used to assess pre-vaccination SARS-CoV-2 exposure and expected response to vaccination. Additional research evaluations may include virus neutralization assays to assess antibody functional response, flow cytometry, and transcriptome and genetic analyses. Comparisons of the immune response will be made among immunodeficiency/dysregulation subtypes and against healthy controls. This will allow characterization of the differences in post-vaccination immune responses and AE presentation.

Subjects will be asked at baseline about prior COVID-19 diagnosis, symptoms, and severity using standardized questionnaires. This will allow us to characterize the different manifestations of COVID-19, potentially including a lack of hyper-inflammatory symptoms associated with severe disease due to dysregulation of the immune response that will be present in many of the study participants. To conduct active screening for post-vaccination COVID-19 infections, participants will be sent optional saliva collection kits to return to the NIH bi-weekly for 6 months, for PCR and genetic sequencing of the virus. Participants whose samples are PCR positive will be asked to submit an additional COVID-19 symptom questionnaire and will be referred for care as needed. Each time a participant receives an additional booster dose while they are still on study (through the 5th COVID-19 vaccine a person receives), they may participate in optional bi-weekly saliva collections for an additional 6-month period. Since this is optional, saliva samples received outside of the requested windows are acceptable and will be analyzed at the discretion of the PI. At follow-up timepoints, participants will be asked about all possible vaccine-related AEs, which will be particularly important given recent information about possible vaccine-induced anaphylaxis.

5 STUDY POPULATION

5.1 Inclusion Criteria

In order to be eligible to participate in this study, an individual must meet the following criteria:

1. Aged 3 years and older.
2. Must be eligible to receive (based on official FDA authorization or approval) and scheduled to receive or have already received a COVID-19 vaccine outside of this study.
3. Must meet the definition of affected participant or control participant:
    a. Affected participants must have evidence of a primary or secondary immune deficiency or dysregulation under another NIAID protocol or as documented by an outside physician.
    b. Control participants are healthy volunteers that do not have evidence of a primary or secondary immune deficiency or dysregulation and may include unaffected relatives of affected participants.
4. Ability to provide informed consent.
5. Willing to have blood samples stored for future research.
6. Able to proficiently speak, read, and write English.

5.2 Exclusion Criteria

Individuals meeting any of the following criteria will be excluded from study participation:

1. Receipt of any other vaccine within 14 days prior to screening.
2. Planned non-COVID-19 vaccination within 28 days after COVID-19 vaccination(s).
3. Any condition that, in the opinion of the investigator, contraindicates participation in this study (e.g. specific autoinflammatory diseases, interferonopathies).
4. Self-reported history of HIV.

5.2.1 Exclusion of Special Populations

Children: Children <3 years old will be excluded from enrollment in this study because this population is not typically seen at NIAID.

Non-English speakers: Because this study will use questionnaires that are not validated in languages other than English, individuals who are not fluent in English will be excluded from enrollment.

5.3 Inclusion of Vulnerable Participants

Children: As the immune deficiencies and dysregulations being studied under this protocol affect all age ranges, information gathered from all eligible children (both affected and control participants) will be important for providing a full understanding of any potential differential effects across age ranges. Children will only be enrolled if there is FDA authorization or approval for use of a COVID-19 vaccine in that age group.

Adult subjects who lack capacity to consent to research participation: Individuals who are unable to provide initial informed consent are excluded from the study. If participants lose the ability to provide ongoing consent subsequent to giving initial consent, they will be withdrawn from the study.

NIH staff or family members of study team members: NIH staff and family members of study team members may be enrolled in this study if they meet the study entry criteria. Neither participation nor refusal to participate in the research will have an effect, either beneficial or adverse, on an individual's employment or position at NIH.

Every effort will be made to protect subject information, but such information may be available in medical records and may be available to authorized users outside of the study team in both an identifiable and unidentifiable manner.

The NIH investigator will provide and request that the NIH staff member review the Frequently Asked Questions (FAQs) for Staff Who are Considering Participation in NIH Research and the Leave Policy for NIH Employees Participating in NIH Medical Research Studies (NIH Policy Manual 2300-630-3). Please see section 9.1.2 for consent of staff members.

5.4 Inclusion of Pregnant Women, Fetuses, or Neonates

As accumulating data indicate that the vaccines are safe and effective in pregnant people, this protocol will allow the enrollment of this population to allow evaluation of the immune response to COVID-19 vaccines in pregnant people with immune disorders, which may differ from the response in non-pregnant people.

5.5 Lifestyle Considerations

Not applicable.

5.6 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently entered in the study. Individuals who do not meet the criteria for participation in this study (screen failure) may be rescreened if they turn 12 before the study enrollment closes and have not yet received a COVID-19 vaccination, if the participant's time since non-COVID-

19 vaccination has passed 14 days and they remain unvaccinated for COVID-19, or their medical status changes such that the PI feels exclusion is no longer necessary.

5.7 Strategies for Recruitment and Retention

Immunodeficient individuals will be recruited from NIAID protocols that evaluate primary or secondary immune deficiencies. Potential subjects will be identified by discussion between the study teams and review of medical and research records if necessary. They may also be referred from community practitioners who see this population.

Healthy adult volunteers will be recruited through the NIH Clinical Research Volunteer Program, the Office of Patient Recruitment, MMG Patient Recruitment, ResearchMatch, or BuildClinical. Volunteers will also be recruited from existing NIH protocols, including but not limited to protocol 18-I-0101 ("Sample Collection From Healthy Volunteers For Assay Optimization"), 93-I-0119 ("Detection and Characterization of Host Defense Defects"), 18-I-0041 ("Investigating the Mechanistic Biology of Primary Immunodeficiency Disorders"), 11-I-0187 ("Natural History of Individuals with Immune System Problems that Lead to Fungal Infections"), and 17-I-0122 ("NIAID Centralized Sequencing Protocol"). Healthy relatives of immunodeficient participants can be recruited for participation as controls if they meet enrollment criteria.

An IRB-approved recruitment email will be distributed to potential participants, and flyers with study team contact information will be posted throughout the CC and provided to community practitioners to share with potentially eligible patients. We may also conduct outreach activities with local groups involving patients with immune deficiencies (eg, support groups and patient advocacy groups). Interested individuals may also contact the study team directly via email.

A pre-screening phone call will be conducted with individuals who express interest, for an initial discussion of eligibility (including any past SARS-CoV-2 testing) and scheduling prior to the screening visit.

5.7.1 Costs

There will be no costs to participants for procedures performed at the NIH CC. Similarly, participants will not incur costs for blood drawn at Quest laboratories, as Quest will be paid directly through a contract set up for this purpose. Participants may incur costs of blood draws performed at off-site facilities other than Quest laboratories; in this case, they will be reimbursed up to $85 for each blood draw.

5.7.2 Compensation

Compensation will be dependent on the sample collections and study visits that a subject participates in. Participants will be compensated maximum amounts as follows:

- $10/off-site microsampler or $20/off-site venous blood x up to 6 timepoints
- $50/on-site venous blood x up to 10 timepoints
- $10/on time x up to 2 timepoints (for on-time completion of follow-up at 14-21 or 21-28 days after each vaccination)
- $10 bonus (for completing all sample collections)
- $5/saliva x 13 timepoints

Participants may receive a total maximum compensation of $595 for participating in all possible on-site sample collections and visits. If participants participate in additional visits following booster vaccination(s), they will be compensated for each additional visit/sample collection according to the amounts listed above.

6 PARTICIPANT DISCONTINUATION/WITHDRAWAL

6.1 Participant discontinuation/withdrawal from the study

Participants are free to withdraw from participation in the study at any time upon request. An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Refusal to submit blood samples for testing
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation
- Screen failure

The reason for participant discontinuation or withdrawal from the study will be recorded.

6.2 Lost to follow-up

A participant will be considered lost to follow-up if they fail to provide required post-vaccine blood samples and are unable to be contacted by the study site staff.

The following actions will be taken if a participant fails to return required study samples:

- A study staff member will attempt to contact the participant and reschedule the missed sample within the allowable window if possible, counsel the participant on the importance of maintaining the assigned sample schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make a reasonable effort to regain contact with the participant (where possible, 3 telephone calls). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

All samples and data received prior to a subject dropping out or being lost to follow-up will be analyzed unless the participant explicitly requests their data not be included.

7 STUDY ASSESSMENTS AND PROCEDURES

The following study assessments and procedures will be performed according to the schedule provided in section 1.2.

7.1 Screening procedures

7.1.1 Screening activities performed prior to obtaining informed consent

Minimal risk activities that may be performed before the subject has signed a consent include the following:

- Email, written, in-person, or telephone communications with prospective subjects.

- Review of the NIH CC patient medical records (if applicable).

7.1.2 Screening activities performed after obtaining informed consent

After the subject signs the consent form, eligibility will be confirmed and the baseline study procedures will continue.

7.2 Clinical Evaluations

Medical and medication history: Subjects enrolled on a protocol at NIAID will have their clinical history extracted from the NIH Clinical Research Information System (CRIS) for complete review of medical and medication history, including review of vaccination history and SARS-CoV-2 testing and symptom history. The questionnaire (described below) will also elicit information about relevant medical history, including history of SARS-CoV-2 infection. Subjects not enrolled on another NIAID protocol will undergo standard medical and medication history review by questioning.

Venous blood draw: Blood will be collected by venipuncture for laboratory testing (section 1.2) and storage. If participants cannot be brought to the NIH CC due to the ongoing COVID-19 pandemic, individuals can choose to have blood drawn at a primary care provider, academic hospital laboratory, Quest Diagnostics, or through collaboration with the Immune Deficiency Foundation, as necessary. Samples collected at clinics or at Quest may be labeled with a coded identifier, gender, and date of birth, as required by the local facility. The amount of blood drawn for research purposes in pediatric subjects will not exceed 5 mL/kg in a single day or 9.5 mL/kg over any eight-week period, in accordance with the CC Medical Administrative Series Policy M95-9 Guidelines for Limits of Blood Drawn for Research Purposes in the Clinical Center. The amount of blood drawn from pregnant participants will not exceed 50 mL over any eight-week period.

Fingerstick blood collection: All participants will be provided with a microsampler kit and mailing supplies to collect an at-home blood sample by fingerstick and send it to the CC. The sample will be used for laboratory testing (section 1.2) and storage.

Saliva collection: All participants will be provided with saliva collection kits and mailing supplies to collect post-vaccination saliva samples to return to the CC.

Electronic questionnaires: Five separate Research Electronic Data Capture system (REDCap) questionnaires will be completed electronically in REDCap by subjects and/or their parents/guardians throughout the study. A link to the questionnaires will be sent to the personal email address on file. The baseline questionnaire will capture medical history, and any COVID-19 symptoms, severity, and treatment the participant may have experienced. The follow-up questionnaires will assess changes in health history or medications and AEs following study vaccination. An optional survey will ask participants about their beliefs and behaviors around COVID-19 vaccinations, and whether they have changed since before the primary vaccine series and/or booster doses. The questionnaires and their timing are listed below:

1. Baseline medical history and screening questionnaire (Baseline)
2. COVID-19 baseline/post-vaccine infection symptom questionnaire (Baseline and following COVID-19 infection)
3. Changes in medical history questionnaire (Following sample collection)
4. Post-vaccine adverse events questionnaire (Following vaccination)

5. COVID-19 Attitudes and Behaviors Survey (Following primary series and/or booster)

AE assessment: Only AEs related to study procedures (blood draw, fingerstick, and saliva collection) will be followed. COVID-19 vaccines are not administered as study procedures under this protocol; therefore, while we are interested in collecting AEs related to vaccination in our study population, these are not considered study-related events and will not be followed as such. Subjects will be assessed for AEs at each study visit. Information on AEs related to the study procedures that develop within 2 days after the procedure will be collected by participant report and followed through resolution or until the site investigator judges that the event has stabilized and no additional follow-up is required.

7.3 Biospecimen Evaluations

Pre- and post-vaccination whole blood samples will be processed to isolate serum, peripheral blood mononuclear cells (PBMCs), RNA, and DNA. Specimens will be processed at the Neutrophil Monitoring Laboratory (NML), LCIM, and the Laboratory of Virology at the Rocky Mountain Laboratories at NIAID, the Department of Laboratory Medicine (DLM) at NIH, and the Section on Immunoengineering (IE) at the National Institute of Biomedical Imaging and Bioengineering (NIBIB). The saliva testing for SARS-CoV-2 using PCR will be performed in the DLM under an EUA.

Sample types, volumes, and laboratory evaluations are provided in section 1.2. For pediatric subjects, blood volume limitations may preclude execution of all the tests required. In such cases, the PI will prioritize which tests will be performed.

7.3.1 Samples for Genetic/Genomic Analysis

Targeted genetic analyses may include high throughput sequencing of T- and B-cell receptor repertoires, human leukocyte antigen (HLA) typing, and single nucleotide polymorphism (SNP) chips. This study will not involve genetic tests intended to discover disease-determining genes, but will only involve amplification of rearranged TCR immunoglobulin heavy chain (IGHC) gene segments. It is possible that the investigators might refer interested participants and their families to the Centralized Sequencing Initiative protocol (17-I-0122). If a participant chooses to co-enroll on this protocol and 17-I-0122, we will have access to the identified findings from whole genome/exome sequencing via the Genomic Research Information System platform for use in this study to confirm a known genetic mutation or update information about a participant's immune status if an unknown but clinically significant mutation is discovered. The informed consent document will address the sharing of data between studies, and no additional samples outside of those required of each protocol will be obtained.

If a saliva sample is found to be positive for SARS-CoV-2, the virus will be isolated from the sample for genomic profiling. The viral genetic sequences will be deposited into GenBank, along with donor participant information that includes age category, sex, and whether the donor is immune compromised. No human genetic information will be shared in this process, as the goal is sequencing of the virus and any human data will be filtered out.

7.4 Plan for the Return of Results

Analyses under this protocol may identify results or incidental findings that are relevant to the health or medical care of subjects (either affected participants or healthy controls). Results of clinical laboratory assessments performed on this study (ie, CBC w/differential) will be returned

to participants, as will results of the SARS-CoV-2 PCR tests. All other laboratory assays, including the SARS-CoV-2 antibody tests, are for research only, and a result would only be returned if confirmatory testing in a laboratory certified by the Clinical Laboratory Improvement Amendments (CLIA) is available. If needed, the study team will contact the subject and request a sample for confirmatory testing or recommend other laboratories where they can go for testing. After confirmation, the principal investigator or designee will contact the subject to inform them of the finding and counsel them on the result. The study team may also provide consultation with a subject's healthcare provider based on the result.

8 STATISTICAL CONSIDERATIONS

8.1 Study Hypotheses

This is an exploratory study to characterize the immune response and vaccine-associated AEs in individuals with a variety of immune disorders who receive COVID-19 vaccination, in addition to describing disease prevalence and severity among subjects previously infected with SARS-CoV-2 and whether this affects vaccine immune response. We therefore hope to generate and test a number of hypotheses, including but not limited to:

1. Assessing whether and which of the immune correlates measured for the primary, secondary and exploratory endpoints change significantly after vaccination from baseline, comparing the response among various immune deficiency/dysregulation conditions and healthy volunteers (longitudinal change in immune response).
2. Assessing whether and which of the immune correlates measured for the primary, secondary, and exploratory end-points are significantly different among various immune disorder subgroups and healthy volunteers (cross-sectional immune response).
3. Assessing whether the dose 1 antibody response is correlated with the dose 2 antibody response among various immune disorder subgroups and healthy volunteers.
4. Assessing the incidence of vaccine-associated AEs among various immune disorder subgroups and healthy volunteers.
5. Assessing the prevalence and severity of COVID-19 symptoms among various immune disorder subgroups and healthy volunteers.

8.2 Patient Populations for Analysis

The numbers provided for each group are an estimated number to be enrolled for each immune deficiency/dysregulation sub-group:

1. Combined immunodeficiencies (n=50)
2. Predominantly antibody deficiencies (n=40)
3. Disorders of immune regulation (n=40-60)
4. Congenital defects of phagocyte number or function (n=40-60)
5. Defects of intrinsic and innate immunity (n=25-30)
6. Autoinflammatory disorders (n=20)
7. Complement deficiencies (n=5-10)
8. Bone marrow failure syndrome (n=5-10)

9. Subjects with inborn errors of immunity who have received hematopoietic stem cell transplantation or gene therapy (n=20-30)
10. Subjects with secondary immunodeficiencies, to include those who have received hematopoietic stem cell transplantation or gene therapy (n=30-50)
11. Healthy volunteers (n=120 [2:1 healthy to largest immunodeficiency group])

Healthy volunteers will be enrolled to be representative of binned-age groups and sex distribution of the immune deficient study population. Age bins will be (3-5], (5-12], (12-16], (16-25], (25-45], (45-65], and 65+.

8.3 Sample Size Justification

The sample sizes proposed in this protocol were set based on the primary objectives of this study, which are to characterize the immune response to COVID-19 vaccination as measured by enzyme-linked immunosorbent assay (ELISA) anti-S-2P and anti-RBD IgG titers at approximately 3-4 weeks following each vaccination and in long-term follow-up. The following sample size determination is based on results of the phase 1 dose escalation study of the Moderna mRNA vaccine.[9] Fifteen participants received a dose of 100 ug of vaccine, and the means and standard errors of the log10 antibody titers at the nearest timepoints to the times at which we propose to collect samples are summarized in the table below.

Target	Study day	Mean $\log_{10}$-titer (Standard error)
S-2P	29	5.04 (0.28)
	56	5.89 (0.19)
RBD	29	4.97 (0.38)
	56	5.57 (0.27)

We assume that healthy volunteers in the upcoming trial have similar immune responses to those reported in the Moderna Phase I trial, and that immune deficiency/dysregulation shifts the log10-titer distribution among individuals with immune deficiency/dysregulation but does not change the standard deviation of the responses. We also suppose that healthy volunteers and immune deficient/dysregulated individuals have similar baseline distributions, which is reasonable as both groups are naïve at baseline. As we were unable to obtain specific estimates of antibody responses to the Pfizer vaccine that could be used to estimate the sample size for this study, we are left to assume that responses to the Pfizer vaccine are similar to the Moderna vaccine.

Using the estimates from the Moderna study, we calculate, and plot below, the smallest differences in mean log-titers that we would be able to detect using a two-sided two-sample t-test with either 80% or 90% power while controlling the type I error rate at α=0.05 as a function of sample size. We also display the widths of 95% confidence intervals for a difference in mean log-titers. As an example, a sample size of 20 immune deficient/dysregulated individuals and 20

healthy volunteers should be sufficient to detect a true difference in mean anti-RBD log-titer of 0.4 at day 29 with 90% power, or a true difference of 0.35 with 80% power, while controlling the type I error rate at α=0.05. We would also expect the width of a 95% confidence interval for the difference in mean log- titers to be approximately 0.35 units. It is also useful to consider the minimum detectable standardized effect size, also shown below, as antibody responses to other vaccines may differ from responses to the Moderna vaccine. Continuing with our example, a sample size of 20 participants per arm would be sufficient to detect a 1.05 standard deviation difference with 90% power using a two-sided two-sample t-test while controlling the type I error rate at α=0.05.

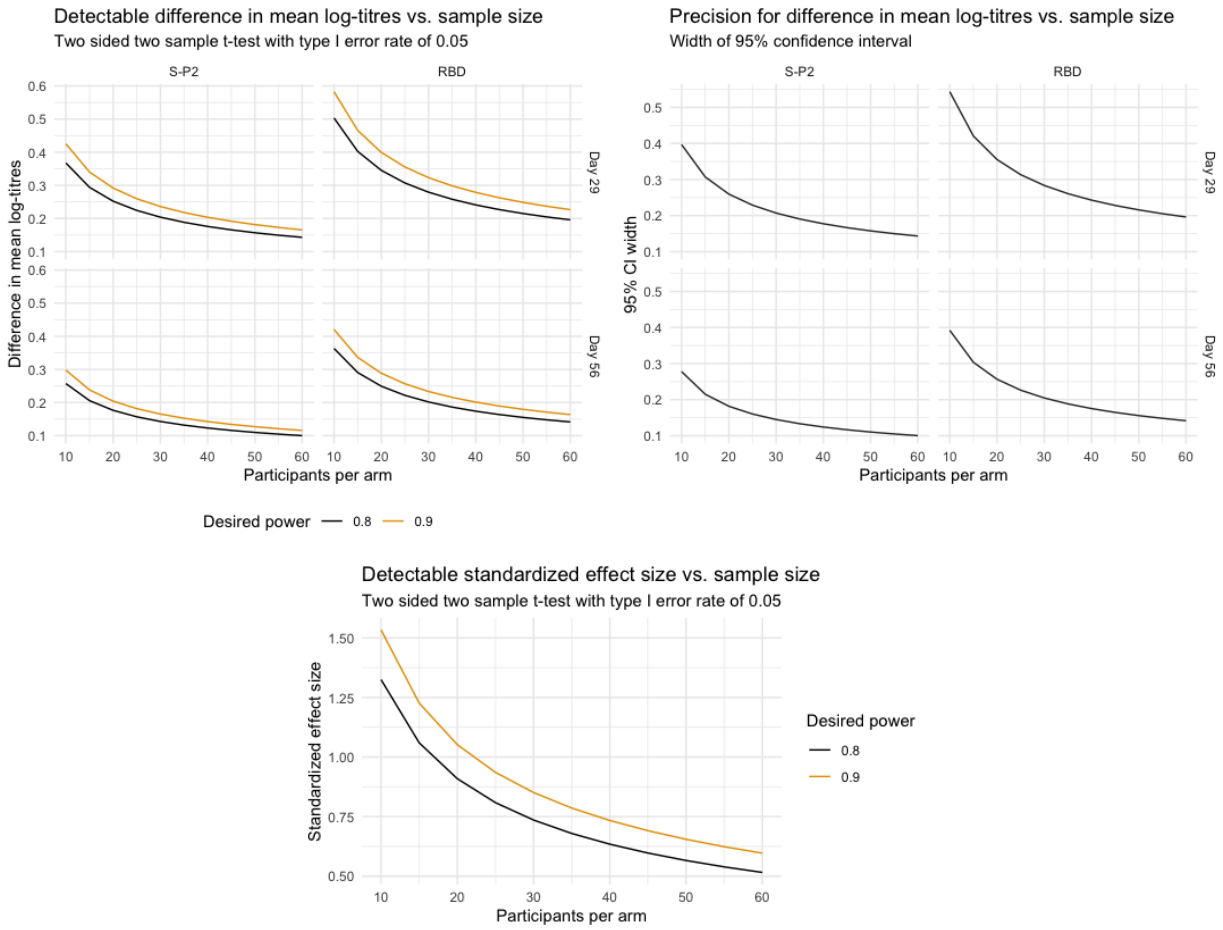

Figure 2. Sample size estimates for primary endpoint. A) Lines represent number of participations required in each group and the smallest detectable differences in mean log-titers using a two-sided two-sample t-test with either 80% or 90% power while controlling the type I error rate at α=0.05. **B)** Widths of 95% confidence intervals for a difference in mean log-titers. **C)** Number of participants required in each group to detect differences in standardized antibody effect size using a two-sided two-sample t-test with either 80% or 90% power while controlling the type I error rate at α=0.05.

While it is hard to generalize antibody responses across the many immune conditions in this study given the phenotypic heterogeneity between and within diseases, patients in the following groups would generally be expected to have poor antibody responses to vaccination:

- Defects of cellular and humoral immunity (n=20) (e.g. RAG, DOCK8, adenosine deaminase [ADA], nuclear factor-kappa B essential modulator [NEMO])
- Predominantly antibody deficiencies (n=40) (e.g. X-linked agammaglobulinemia [XLA], CVID, activated PI3K delta syndrome 1/2 [APDS1/2], Ras-related C3 botulinum toxin substrate 2 [RAC2], caspase recruitment domain-containing protein 11 [CARD11], gain-of-function [GOF])

Hence, we expect to have adequate power to resolve differences in mean antibody responses between patients in these immune deficiency groups and healthy controls. Vaccine responses from other disease groups can be variable,[10–12] but most would be expected to respond to vaccination. We would like to be able to detect at least a one standard deviation difference in mean log-titer for those immune deficient/dysregulated groups where we do not have prior information to inform our expectations about the magnitude of the difference vis-a-vis healthy controls. Therefore, the proposed sample sizes should suffice.

8.4 Statistical Analysis

Multiple sets of analyses will be done to characterize the immune response in each of the groups of participants with immune deficiency/dysregulation:

1. Within-group: Estimate the vaccine response at each timepoint relative to baseline within an immune deficiency/dysregulation group.
2. Longitudinal between-groups: Compare the vaccine response profiles of different groups at sampling times.
3. Adjusted analyses controlling for SARS-CoV-2 infection.

For symptoms: focus will be on estimation of the proportion of symptoms that are present or absent in these individuals. Variables will be presented by frequency distribution (e.g. frequency counts, percentages, and 95% confidence intervals). In addition, this study will be used as the basis for hypothesis generation to explore disease manifestations in individuals with different types of immune deficiency/dysregulation if the number of enrolled individuals allow.

8.4.1 Analysis of the Primary Endpoint

Log transformed antibody measurements will be modeled within a mixed model repeated measures (MMRM) framework, which is a longitudinal mixed model with fixed effects for (categorical) timepoint, immune deficiency/dysregulation, and the interactions of timepoint with immune deficiency/dysregulation and timepoint with baseline measurement, along with possible adjustment other covariates, such as age and sex. In this framework, we also include correlated subject-level random effects for each timepoint to account for clustered data. This also allows us to estimate the correlation between antibody responses to the first and second doses of vaccine. MMRM models are an appropriate tool for assessing response profiles in longitudinal studies where missing data is expected and it is appropriate to assume that data are missing at random conditional on observable covariates.[13,14] The previous sample size justifications based on two-sample t-tests are likely to be slightly conservative as we expect the MMRM framework to make more efficient use of the data. Models will either be fit using the `nlme` package in R, or, if a Bayesian formulation is deemed to be preferable for the purpose of incorporating prior information, can be fit using the ‘brms’ and `rstanarm` packages.

8.4.2 Analysis of the Secondary Endpoints

We will calculate descriptive summaries, stratified by immune deficiency/dysregulation group, for incidence of vaccine-associated AEs listed in the Post-Vaccine Adverse Events questionnaire. Incidence of AEs among immune deficient/dysregulated patients will be compared with incidence among healthy volunteers using regression analyses, possibly adjusting for covariates, such as age and sex, as deemed appropriate.

9 REGULATORY AND OPERATIONAL CONSIDERATIONS

9.1 Informed Consent Process

9.1.1 Consent/Assent Procedures and Documentation

Informed consent is a process where information is presented to enable persons to voluntarily decide whether or not to participate as a research subject. It is an ongoing conversation between the human research participant and the researchers that begins before consent is given and continues until the end of the participant's involvement in the research. Discussions about the research will provide essential information about the study and include purpose, duration, experimental procedures, alternatives, risks, and benefits. Coercion and undue influence will be minimized by informing participants that their decision to join the study will not affect any medical care they are currently receiving, or their eligibility to participate in other research studies at the NIH. Participants will be given the opportunity to ask questions and have them answered.

Informed consent will be obtained via telephone or video conference for participants unable to come to the NIH CC in person or will be obtained in person at the NIH by a study team member authorized to obtain consent. Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant, when in person) will be located in a private area (eg, clinic consult room). When consent is conducted remotely, the participant will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed. The participants will sign the informed consent document prior to undergoing any research procedures. The participants may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the participants for their records. The researcher will document the signing of the consent form in the participant's study record. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

Assent Process: Minor participants will be included in all discussions about the study, and age-appropriate language will be used to describe the procedures and tests involved in this study, along with the risks, discomforts and benefits of participation. Minor participants aged 16 or 17 years will provide assent by signing the informed consent document. Those aged 7 to 15 years will provide assent by signing the assent document. Children under the age of 7 will not be required to provide assent as they typically do not have the ability to fully understand the nature of research. The parent(s)/legal guardian(s) will provide permission for the minor participant to participate by signing the consent form. The consent/assent process will be documented in the child's medical record, including the assessment of the child's ability to provide written assent as applicable.

Remote Consent Process: The informed consent/assent document(s) will be sent via secure email or file transfer to the potential participant and their parents (as applicable) prior to the consent discussion. An explanation of the study will be provided over the telephone or video conference (e.g., Microsoft Teams) after the participant and parents (as applicable) has had the opportunity to read the consent/assent form(s). During the consent process, participants and investigators will view individual copies of the approved consent document on screens at their respective locations. The participant and parents (as applicable) can print the form(s) to sign and date in ink, or they can sign and date digitally with a finger, stylus, or mouse in iMed or Adobe.

The participant will return the signed and dated consent/assent form(s) to the consenting investigator, who will sign and date it with the date it was received. The consent/assent form(s) can either be printed and signed and dated in ink, or signed and dated digitally as described above. A fully executed copy will be sent to the participant for their records.

The informed consent process will be documented on a progress note by the consenting investigator. The investigator will confirm that written consent has been obtained prior to initiating any study interventions.

9.1.2 Considerations for Consent of NIH Staff

Consent for NIH staff will be obtained as detailed above and will comply with the requirements of NIH Human Research Protections Program (HRPP) Policy 404 *Research Involving NIH Staff as Subjects.*

Consent from NIH staff for whom this research is taking place within their own work unit or is conducted by any of their supervisors will, when possible, be obtained by an individual in a non-supervisory relationship with that staff member. When consent of that staff member is conducted, a third party will be present to observe the consent process in order to minimize the risk of undue pressure on the staff member.

9.1.3 Consent for minors when they reach the age of majority

When a pediatric subject reaches age 18, continued participation (including ongoing interactions with the subject or continued analysis of identifiable data) will require that consent be obtained from the now adult with the standard protocol consent document to ensure legally effective informed consent has been obtained.

If reconsent is not feasible, we request waiver of informed consent to continue to use data and/or specimens for those individuals who become lost to follow up or who have been taken off study prior to reaching the age of majority.

Requirements for Waiver of Consent consistent with 45 CFR 46.116 (d):

(1) The research involves no more than minimal risk to the subjects.

   a. Analysis of samples and data from this study involves no additional risks to subjects.

(2) The research could not practicably be carried out without the waiver or alteration.

   a. Considering the length of time between the minor's last contact with the research team and their age of majority, it will likely be very difficult to locate

them again. A significant reduction in the number of samples analyzed is likely to impact the quality of the research.

(3) As the research involves using identifiable private information or identifiable biospecimens, the research could not practicably be carried out without using such information or biospecimens in an identifiable format.

a. Though the purpose of future studies cannot yet be known, they often involve the correlation of clinical outcomes and clinical interventions with laboratory studies. Such information would be unavailable if access to medical record numbers was unavailable.

(4) The waiver or alteration will not adversely affect the rights and welfare of the subjects.

a. Retention of these samples or data does not affect the welfare of subjects.

(5) Whenever appropriate, the subjects will be provided with additional pertinent information after participation.

a. We only request a waiver of consent for those subjects who have been lost to follow-up or who have been taken off study prior to reaching the age of majority.

The informed consent process will be documented in the medical record.

The investigator will confirm that, when required, written legally effective consent has been obtained prior to initiating any study interventions.

9.1.4 Consent of Subjects who are/become Decisionally Impaired

Not applicable. No such subjects will be enrolled.

9.2 Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to the principal investigator. If the study is prematurely terminated or suspended, the principal investigator will promptly inform the study participants, IRB, and sponsor, as applicable, and will provide the reason(s) for the termination or suspension. Study participants will be informed of changes to study visit schedule, if applicable.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants.
- Insufficient compliance to protocol requirements.
- Data that are not sufficiently complete and/or evaluable.

In the case of a temporary suspension, the study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the IRB.

9.3 Confidentiality and Privacy

All records will be kept confidential to the extent provided by federal, state, and local law. Authorized representatives of the NIAID may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records. Records will be kept locked and data will be coded. Any personally identifiable information maintained for this study will be kept on restricted-access computers and networks. Personally identifiable information will only be shared with individuals authorized to receive it under this protocol. Individuals not authorized to receive personally identifiable information will be provided with coded information only, as needed. Clinical information will not be released without written permission of the participant, except as necessary for monitoring by the IRB, NIAID, and the Office for Human Research Protections (OHRP).

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the NIH. This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

9.4 Future Use of Stored Specimens and Data

Coded specimens and data will be stored at the NIH indefinitely for future research after the study is complete. Human genetic testing may be performed. Plans for future use of specimens and data will be described in the informed consent document. Samples are stored in NML laboratories at the NIH campus in Frederick, MD or in the DLM, LCIM, or IE laboratories on the NIH main campus in Bethesda, MD located in secure buildings with limited access. Data will be kept in password-protected computers. Samples and data will be tracked using Biological Specimen Inventory (BSI) software and Clinical Research Information Management System of NIAID (CRIMSON). Only investigators or their designees will have access to the samples and data.

Other investigators (at NIH and elsewhere) may wish to study these specimens and data. If the planned research falls within the category of “human subjects research” on the part of the investigators, NIH IRB review and approval will be obtained. This includes the investigators sending out coded and linked specimens or data and getting results that they can link back to their participants.

9.5 Safety Oversight

All data will be collected in a timely manner and reviewed by the principal investigator and/or a designee on a regular basis (at least weekly). Events meeting requirements for expedited reporting as described in HRPP Policy 801 will be submitted within the required timelines.

The principal investigator will review all data on each subject to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

9.6 Clinical Monitoring

Monitors under contract to the NIAID Office of Clinical Research Policy and Regulatory Oversight (OCRPRO) will visit the clinical research site to monitor several aspects of the study in accordance with the appropriate regulations and the approved protocol. Only pediatric subjects will be monitored and the objectives of a monitoring visit will be: 1) to verify the existence of signed informed consent documents and documentation of the consent process for each monitored pediatric subject; 2) to verify AEs and serious adverse events (SAEs), including the prompt reporting of all SAEs; 3) to compare applicable CRIMSON data abstracts with individual subjects' records and source documents (subjects' charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original subject information); and 4) to help ensure investigators are in compliance with the protocol.

The investigator (and/or designee) will make study documents (eg, consent forms, CRIMSON data abstracts) and pertinent hospital or clinical records, including CRIMSON, readily available for inspection by the local IRB, the site monitors, and the NIAID staff for confirmation of the study data.

A specific protocol monitoring plan will be discussed with the principal investigator and study staff prior to enrollment. The plan will outline the frequency of monitoring visits based on such factors as study enrollment, data collection status, and regulatory obligations.

9.7 Quality Assurance and Quality Control

To help ensure that NIH Office of Research Support and Compliance procedures and GCP are being carried out, a Clinical Trials Management designee within the OCRPRO Regulatory Compliance and Human Subjects Protection Program will conduct a study initiation visit before study enrollment begins. The purpose of this meeting is to review with the principal investigator and study team designees the roles and responsibilities concerning their commitment to adhere to the requirements of the protocol, especially in terms of NIH Office of Human Subjects Research Protections (OHSRP) reporting requirements for reportable events. In addition, the quality management and data management plan for the study will be reviewed.

9.8 Data Handling and Record Keeping

9.8.1 Data Collection and Management Responsibilities

Study data will be maintained in REDCap and CRIMSON and collected directly from participants during study visits and telephone calls or via REDCap online questionnaire or will be abstracted from participants' medical records. Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary to confirm the data abstracted for this study. Data entry into REDCap and CRIMSON will be performed by authorized individuals. The investigator is responsible for assuring that the data collected are complete, accurate, and recorded in a timely manner. Study data, including cumulative subject accrual numbers, should be generated via the chosen data capture method and submitted to study oversight bodies as needed.

9.8.2 Study Records Retention

Study documents will be retained in accordance with regulatory and institutional requirements, ICH GCP guidelines, and the NIH Intramural Records Retention Schedule. No records will be destroyed without the written consent of the principal investigator and sponsor, as applicable.

Should the investigator wish to assign the study records to another party and/or move them to another location, the investigator will provide written notification of such intent to OCRPRO/NIAID with the name of the person who will accept responsibility for the transferred records and/or their new location. Relocation of research records will not proceed without written permission from OCRPRO/NIAID.

9.9 Unanticipated Problems

9.9.1 Definition of Unanticipated Problems (UP)

Any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied; and
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others (which may include research staff, family members or other individuals not directly participating in the research) at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or expected.

9.9.2 Unanticipated Problem Reporting

The investigator will report UPs to the NIH IRB according to Policy 801.

9.10 Protocol Deviations and Non-Compliance

It is the responsibility of the investigator to use continuous vigilance to identify and report deviations and/or non-compliance to the NIH IRB as per Policy 801. All deviations must be addressed in study source documents and reported as specified in the protocol quality management plan and/or monitoring plan. The investigator is responsible for knowing and adhering to the reviewing IRB requirements.

9.10.1 NIH Definition of Protocol Deviation

A protocol deviation is any change, divergence, or departure from the IRB-approved research protocol.

- Major deviations: Deviations from the IRB approved protocol that have, or may have the potential to, negatively impact the rights, welfare or safety of the subject, or to substantially negatively impact the scientific integrity or validity of the study.

- Minor deviations: Deviations that do not have the potential to negatively impact the rights, safety or welfare of subjects or others, or the scientific integrity or validity of the study.

9.11 Reporting to the NIAID Clinical Director

The principal investigator will report UPs, major protocol deviations, and deaths to the NIAID clinical director according to institutional timelines.

9.12 Publication and Data Sharing Policy

9.12.1 Human Data Sharing Plan

We will comply with NIH policies on data access, sharing, and dissemination, and clinical trials registration, as applicable. Human data generated in this study may be made available for future research as follows:

- De-identified data in an NIH-funded or approved public repository.
- De-identified data in another public repository.
- De-identified data with approved outside collaborators under appropriate agreements.
- De-identified data in publications and/or public presentations.

Data will be shared at the time of or shortly after publication.

9.12.2 Genomic Data Sharing Plan

This study is not expected to generate the amount of genetic data that triggers the NIH Genomic Data Sharing Policy, which applies to all NIH-funded research that generates large-scale human or non-human genomic data, as well as the use of these data for subsequent research. However, viral genomic data will be shared in GenBank, as described above (section 7.3.1).

9.13 Conflict of Interest Policy

The independence of this study from any actual or perceived influence is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with NIAID has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

ABBREVIATIONS

ACE2	Angiotensin-converting enzyme 2
ADA	Adenosine deaminase
AE	Adverse event

APDS1/2	Activated PI3K delta syndrome 1/2
APECED	Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy
ARDS	Acute respiratory distress syndrome
BSI	Biological Specimen Inventory
CARD11	Caspase recruitment domain-containing protein 11
CBC	Complete blood count
CC	Clinical Center
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
COVID-19	Coronavirus disease 2019
CRIMSON	Clinical Research Information Management System of NIAID
CRIS	Clinical Research Information System
CVID	Common variable immune deficiency
DLM	Department of Laboratory Medicine
DNA	Deoxyribonucleic acid
DOCK8	Dedicator of cytokinesis 8 (protein)
ELISA	Enzyme-linked immunosorbent assay
EUA	Emergency use authorization
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GOF	Gain-of-function
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HRPP	Human Research Protections Program

ICH	International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use
IE	Section on Immunoengineering
IFNg	Interferon gamma
Ig (IgG, IgE, etc.)	Immunoglobulin (immunoglobulin G, immunoglobulin E, etc.)
IGHC	Immunoglobulin heavy chain
IL-12	Interleukin 12
IRB	Institutional Review Board
LCIM	Laboratory of Clinical Immunology and Microbiology
MMRM	Mixed model repeated measures
mRNA	Messenger ribonucleic acid
N	Nucleocapsid (protein)
NEMO	Nuclear factor-kappa B essential modulator
NIH	National Institutes of Health
NIAID	National Institute of Allergy and Infectious Diseases
NIBIB	National Institute of Biomedical Imaging and Bioengineering
NML	Neutrophil Monitoring Laboratory
OCRPRO	Office of Clinical Research Policy and Regulatory Oversight
OHRP	Office for Human Research Protections
OHSRP	Office of Human Subjects Research Protections
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
PI3K	Phosphoinositide 3-kinase
RAC2	Ras-related C3 botulinum toxin substrate 2

RAG	Recombination-activating gene
RBD	Receptor binding domain (protein)
REDCap	Research Electronic Data Capture (system)
RNA	Ribonucleic acid
S	Spike (protein)
SAE	Serious adverse event
SARS-CoV-2	Severe acute respiratory syndrome coronavirus-2
SCID	Severe combined immunodeficiency
SNP	Single nucleotide polymorphism
STAT3	Signal transducer and activator of transcription 3 (protein)
TCR	T-cell receptor
UP	Unanticipated problem
US	United States
XLA	X-linked agammaglobulinemia
XMEN	X-linked immunodeficiency with magnesium defect, Epstein-Barr virus infection, and neoplasia

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