WU 472: Age-Associated Differences in Germinal Center Responses to Influenza and Rabies Vaccination in Humans

STATEMENT OF COMPLIANCE

The study will be carried out in accordance with Good Clinical Practice (GCP) as required by the following:

- U.S. Code of Federal Regulations applicable to clinical studies (45 CFR 46)
- 21 CFR 312
- ICH GCP E6
- Completion of Human Subjects Protection Training
- NIH Clinical Terms of Award

Version 1 July 22, 2026

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments and provides the necessary assurances that this clinical study will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Signed: _____ Date_____

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WU 472: Age-Associated Differences in Germinal Center Responses to Influenza and Rabies Vaccination in Humans

PROTOCOL SUMMARY

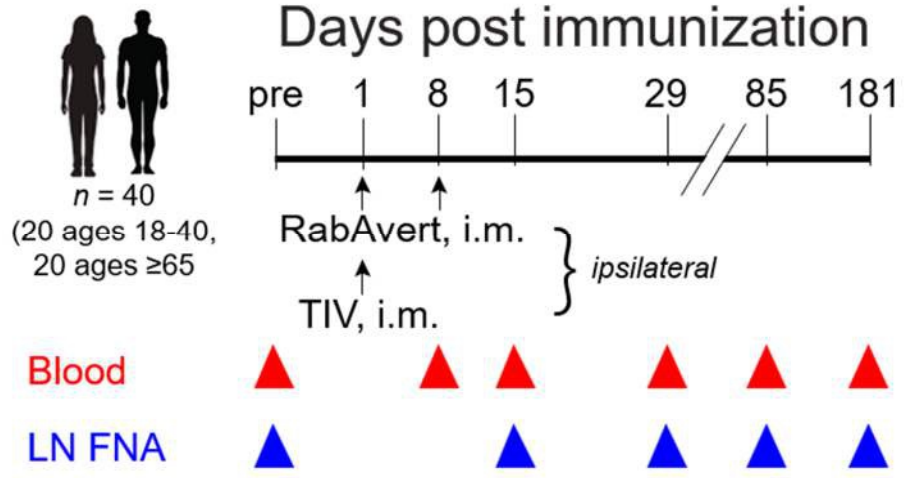
Participants: Healthy adults aged 18–40 years or ≥65 years with no history of rabies exposure or prior rabies vaccination, willing to receive influenza and rabies vaccinations and undergo biological sample collection including peripheral blood draws and optional lymph node fine needle aspirates.

Study Site: Infectious Disease Clinical Research Unit (ID CRU) Washington University School of Medicine 620 South Taylor, St. Louis MO 63110

Study Duration: 6 months

Number of participants: 40 total participants (20 aged 18–40 years and 20 aged ≥65 years). All participants will undergo peripheral blood draws. Lymph node fine needle aspirates (FNA) will be optional.
Participant Participation Duration: 6 months

Description of Intervention: Participants will receive two licensed vaccines administered intramuscularly in the same arm: seasonal trivalent influenza vaccine (TIV, standard dose for ages 18-40, high-dose or adjuvanted for ages 65 and older) and rabies vaccine (RabAvert). TIV will be administered as a single dose at day 1. RabAvert will be administered as two doses at days 1 and 8. No investigational products will be used. All participants will undergo peripheral blood draws at scheduled intervals over approximately 6 months to assess vaccine-induced immune responses. Optional procedures include ultrasound-guided axillary lymph node fine needle aspirates (FNA) to directly examine antigen-specific germinal center and memory B cell responses to vaccination.



OBJECTIVES AND HYPOTHESIS:

Objectives:

Primary
Define the antibody response to rabies vaccination by measuring rabies-specific antibody titers at day 29 compared with the pre-vaccination baseline.

Secondary
Evaluate the safety of RabAvert and TIV vaccination in study participants by assessing the frequency of serious adverse events during the study period.

Exploratory

1. Compare the magnitude and kinetics of antigen-specific germinal center B cell responses in draining lymph nodes following influenza and rabies vaccination between young (18–40 years) and older (≥65 years) adults.
2. Determine whether age-associated differences in draining lymph node GC B cell responses are specific to influenza or represent a general feature of immune aging by comparing primary (rabies) and secondary (influenza) responses within the same individual.
3. Characterize the frequency and phenotype of antigen-specific memory B cells in peripheral blood at days 29 and 181 between young and older adults.
4. Define transcriptional profiles of antigen-specific GC and memory B cells following influenza and rabies vaccination in young and older adults.
5. Characterize the clonal composition and somatic hypermutation of antigen-specific B cells across peripheral blood and draining lymph nodes between young and older adults.
6. Examine the relationship between draining lymph node GC responses and circulating antigen-specific memory B cells and serum antibody titers across age groups.

Hypothesis:

We hypothesize that the ability to mount robust germinal center responses in draining lymph nodes following vaccination is impaired in older adults, and that this impairment is not specific to influenza but represents a general feature of immune aging. We further hypothesize that defective GC responses in older adults will be reflected by reduced frequencies of antigen-specific memory B cells in the peripheral blood and lower serum antibody titers following both primary (rabies) and secondary (influenza) vaccination. Finally, we hypothesize that transcriptional and clonal analyses of antigen-specific B cells will reveal distinct molecular signatures underlying age-associated defects in humoral immunity, providing insight into the cellular and molecular mechanisms that limit vaccine efficacy in older adults.

BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

Influenza virus infections remain a major public health burden[1,2]. Induction of hemagglutinin (HA)-specific antibodies is essential and sufficient for protection against influenza viruses[3]. However, antibody-mediated protection is complicated by 1) the rapidly evolving nature of the virus and 2) the short duration of antibody responses induced by currently licensed seasonal vaccination[4]. Despite the extensive efforts deployed to combat the virus, a vaccine that induces durable and broadly protective immunity remains elusive. The proposed studies will help us better understand not only the factors controlling the durability of humoral immune responses to

influenza vaccinations but also the basic biology of human B cell responses, enhancing our capacity to design vaccines against influenza viruses and other rapidly evolving pathogens.

The immune response following vaccination consists of a series of highly orchestrated interactions between B cells, CD4+ follicular helper T (T_{FH}) cells, dendritic cells, and follicular dendritic cells (FDCs) in secondary lymphoid organs[5,6]. These interactions ultimately dictate the quality and longevity of the immune response, and each could be targeted to elicit longer-lasting humoral immune responses to vaccination. Effective vaccines elicit protective antibodies that are secreted by a robust, long-lived population of bone marrow plasma cells (BMPCs). Long-lived BMPCs provide the host with a persistent source of protective antibodies and are needed to maintain durable immune protection following vaccination[7,8]. BMPCs and memory B cells (MBCs) are produced by the germinal center (GC) reaction, a process critical for generating high-affinity antibodies. GC B cells do not circulate, so studies that examine antibody responses to vaccination in humans by only sampling the blood compartment miss key elements of the response.

The ability of the immune system to respond to new antigenic challenges erodes with increasing age[9]. Most influenza-related deaths in the U.S. occur in elderly individuals. Recent data showed that the MBC response to influenza vaccination is impaired in older persons, suggesting a defective GC response. While we have found that both naïve B cells and MBCs participate in GC responses in young participants, it is not understood whether this is the case in older adults, and more generally how primary and secondary immune responses differ across the lifespan[10]. Improving vaccine responses in the elderly has proven to be a challenge[9]. For influenza vaccines, attempts so far have focused on increasing the antigen dose or including an adjuvant. However, the overall vaccination efficacy in this vulnerable population remains unsatisfactory. To improve vaccine responses in the elderly, we need to better understand the cellular and molecular defects that occur with immune aging.

In the proposed studies, we will directly examine antigen-specific GC and MBC responses induced after seasonal influenza and rabies vaccination in humans with no history of rabies exposure to assess primary and secondary responses within the same individual. These responses will be examined in two cohorts of 18-40 year-old and 65 and older human adults. We will compare the magnitude, persistence, clonal composition, and transcriptional profiles of GC and MBC responses to influenza and rabies between the two cohorts to determine how aging alters primary and secondary B cell responses.

POTENTIAL RISKS AND BENEFITS

Known Potential Benefits
There is potential benefit to participants. Participants will receive the annual trivalent influenza vaccine licensed for their age cohort, as recommended by the CDC[11]. Vaccination will also likely generate an immune response against rabies. This infection carries a significant risk of mortality. However, it is unclear if this vaccination will provide protection outside of the FDA indicated populations. Participants will be given information about whether further vaccines (i.e. a third dose of RabAvert) would be required for full vaccination in the future. Indirect benefits include contribution to knowledge that may improve future vaccine development and understanding of immune responses.

Known Risks
Trivalent Influenza Vaccine (TIV)

The influenza vaccine, including trivalent and quadrivalent inactivated formulations, is widely used and has a well-established safety profile. Common side effects include soreness, redness, or swelling at the injection site, along with mild systemic symptoms such as low-grade fever, headache, or muscle aches[12]. Rare adverse events include allergic reactions (e.g., urticaria, angioedema, or anaphylaxis), particularly in individuals with severe allergies to vaccine components such as egg protein[13]. Neurologic complications like Guillain-Barré syndrome have been reported rarely and may occur at a rate of approximately one to two additional cases per million doses administered[14]. The benefits of influenza vaccination in preventing severe illness and hospitalization significantly outweigh these minimal risks.

Rabies Vaccine (RabAvert)
The side effects from rabies vaccines can include soreness, redness, swelling, or itching at the site of the injection, and headache, nausea, abdominal pain, muscle aches, or dizziness[15]. Rare cases of neurologic illness resembling Guillain-Barré syndrome temporally associated with rabies vaccination, have been reported[16].

Blood collection
The blood collection procedure carries a risk of pain (typically mild pain and very temporary), vasovagal symptoms (e.g. temporary lightheadedness or low blood pressure), bleeding, bruising, redness or swelling and, very rarely, infection. These risks are very small, and phlebotomy will be performed by registered physicians, experienced clinical research nurses or accredited phlebotomists to minimize any potential risk or discomfort.

Axillary lymph node fine needle aspiration (FNA)
The procedural risks of FNA in the axillary lymph nodes are negligible. Lymph nodes are not prone to significant bleeding, and the FNA needles used are so small that they pose minimal risk of injury to sensitive structures. FNA will only be performed when lymph nodes are of appropriate size and position as confirmed by ultrasound, and will be performed by trained interventional radiologists or clinicians using ultrasound guidance. After the procedure, the puncture site on the skin is usually so small that it is difficult to identify and is covered with a Band-Aid. At most, participants may feel mild soreness in the armpit on the side of biopsy and mild swelling, redness, or bruising. Application of ice and oral, over-the-counter Tylenol or other analgesics may be helpful in alleviating these symptoms. There is a theoretical risk of infection at the skin puncture site. Participants should keep the area clean and dry and should monitor their skin at the puncture site for inflammation (redness, tenderness, swelling) lasting beyond 24 hours or pus/discharge. If they notice such symptoms, they should contact their physician.

Lidocaine administration
Risk from administration of lidocaine can occur rarely and include cutaneous lesions, urticarial, edema or anaphylactoid reactions. Pruritus, burning, edema, erythema, purpura and bleeding may occur at the local injection site.

Financial risks
Financial risks are largely related to time for clinic visits and travel costs. Participants will not be charged for any of the services or procedures provided as a part of the study, although they may have charges for standard of care medical visits which may occur during the study or as a result of information obtained as part of the study.

Participants will be fully consented and made aware of the potential risks associated with their participation in the study and have the knowledge that they can discontinue the study at any time. Each participant will be monitored carefully and appropriately and will be instructed to

contact the study team promptly upon noticing any complication or health issues. Should this occur, participants' health history will be reviewed by the study team

All participants asked to participate in the clinical study will be advised that their participation in the study is entirely voluntary, that they can choose discontinue participation at any time and that this will not negatively impact their access to routine health care.

Overall Risk/Benefit Assessment

The overall benefit/risk assessment for this clinical study is considered acceptable for the following reasons:

- These are FDA-approved vaccines.
- Only study participants who meet all inclusion criteria and none of the exclusion criteria will be allowed to participate in this study.
- The selection criteria include adequate provisions to minimize the risk and protect the well-being of study participants in the study.
- Safety will be closely monitored throughout the study:
- After study product administration, study participants will remain in the clinic and be closely observed by study staff for at least total 30 minutes post-vaccination or longer if deemed necessary by the Investigator, to monitor the development of any acute reactions. Any unsolicited, solicited local or systemic AEs will be documented during this period.
- The Investigator or the designee will document all AEs from the time of informed consent through Day 29. All SAEs or AEs leading to study discontinuation will be documented throughout the duration of the study.
- Any clinically significant abnormalities (including those persisting at the end of the study/early withdrawal) will be followed by the Investigator until resolution or until a clinically stable endpoint is reached.

Outcome Measures

Primary
Rabies-specific antibody titers at day 29 compared to pre-vaccination baseline.

Secondary
Frequency of serious adverse events.

Exploratory

- Compare the magnitude and kinetics of antigen-specific germinal center B cell responses in draining lymph nodes following influenza and rabies vaccination between young (18–40 years) and older (≥65 years) adults.
- Determine whether age-associated differences in draining lymph node GC B cell responses are specific to influenza or represent a general feature of immune aging by comparing primary (rabies) and secondary (influenza) responses within the same individual.

- Characterize the frequency and phenotype of antigen-specific memory B cells in peripheral blood at days 29 and 181 between young and older adults.
- Define transcriptional profiles of antigen-specific GC and memory B cells following influenza and rabies vaccination in young and older adults.
- Characterize the clonal composition and somatic hypermutation of antigen-specific B cells across peripheral blood and draining lymph nodes between young and older adults.
- Examine the relationship between draining lymph node GC responses and circulating antigen-specific memory B cells and serum antibody titers across age groups.

STUDY DESIGN

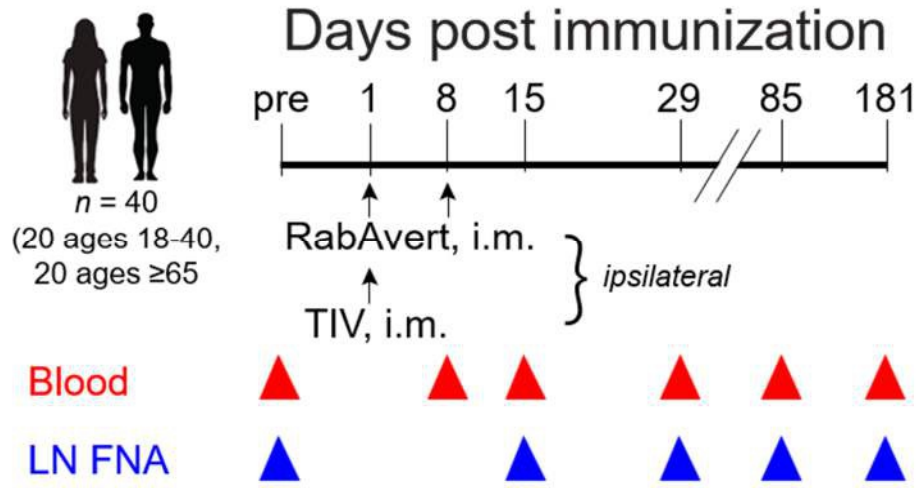
This is a single-center, two-cohort mechanistic study designed to characterize germinal center B cell responses in draining lymph nodes following simultaneous influenza and rabies vaccination and to determine how aging alters primary and secondary humoral immune responses to vaccination.

A total of 40 healthy adult participants will be enrolled across two age cohorts: 20 participants aged 18–40 years and 20 participants aged ≥65 years. Both vaccines will be administered intramuscularly in the same arm to allow sampling of a single draining lymph node chain. Immune responses will be evaluated in peripheral blood and draining lymph nodes. Peripheral blood will be used to assess circulating immune cell populations and antibody responses. Draining lymph nodes will be sampled to directly characterize germinal center responses and local immune activation following vaccination.

Vaccination will occur at Day 1 and Day 8. A single dose of TIV and RabAvert will be given in the same arm of Day 1. A second, single dose of RabAvert will be given in the ipsilateral arm on Day 8.

Blood samples will be collected at Day 0 (pre-vaccination) and on Day 8 (±3 days), Day 15 (±3 days), Day 29 (±7 days), Day 85 (±14 days), and Day 181 (±14 days).

Lymph node sampling by FNA will be optionally performed at Day 0 (pre-vaccination) and on Day 15 (±3 days), Day 29 (±7 days), Day 85 (±14 days), and Day 181 (±14 days). FNAs will be performed unilaterally from the lateral axillary lymph nodes ipsilateral to the vaccination site.



STUDY DRUGS
The vaccines used in this study are FDA approved.

1. The investigation is not intended to be reported to the FDA as a well-controlled study in support of a new indication for use nor intended to be used to support any other significant change in the labeling.
2. The investigation is not intended to support a significant change in the advertising for the product.
3. The investigation does not involve a route of administration or dosage level or use in a patient population or other factors that significantly increase the risks (or decreases the acceptability of the risks) associated with the use of the product.

Study Population
Up to 40 males and non-pregnant females will be enrolled from the existing population of adults residing in the St. Louis metropolitan area. We expect that many of these participants will be employees of Barnes Jewish Hospital or Washington University Medical School. Persons for whom the study team has a supervisory role will not be enrolled to avoid any potential for coercion. Study team members will not be allowed to participate. We plan to enroll 20 evaluable participants aged 18–40 years and 20 evaluable participants aged ≥65 years in the coming year.

PARTICIPANT RECRUITMENT PLANS AND CONSENT PROCESS

Participants will be recruited by inviting prior participants, word of mouth, referrals, advertising through flyers, and Volunteers for Health. Outreach may include the following:
Fliers/ Posters/ Social media
1. Recruitment flier will be designed by study staff for use as a flier, poster, and email attachment for distribution to potential participants.
2. Washington University Volunteers for Health will use the Washington University Study Search website, email, and social media to disseminate approved recruitment materials to potential participants who have previously expressed interest in participating in research studies.

Once a potential participant is identified, the following will take place:
1. Potential participants will be invited to participate by screener
2. Screener will provide ICF, review ICF, and review study procedures with subject. If interested subject will be appointed for screening/Day 0 visit.
3. Study Team which will usually include the PI or sub-investigator meets subject, reviews consent with subject and any significant others, discussing in detail the nature, aims, duration, potential hazards of the study, and procedures to be performed during the study. All aspects of the study will be explained in lay language and questions answered. The study team will also explain that the participants are completely free to refuse to enter the study or to withdraw from it at any time. If acceptable to subject and team is confident that subject can offer informed consent, ICF is signed and a copy is given to the subject.
The structure of the ID CRU helps minimize the possibility of coercion or undue influence during the consent process. Participants will have as much time as they desire to consider enrolling in the study, including:
1. An opportunity to thoroughly review the consent materials with knowledgeable members of the research team, and with family and/or friends as appropriate.
2. Sufficient time to have all of their questions answered.
3. Any subsequent consent revisions will be handled in a similar fashion.

PARTICIPANT INCLUSION CRITERIA

1. Healthy participants 18 to 40 years of age or ≥65 years of age.
2. Able to understand and give informed consent.
3. Willing to receive RabAvert vaccine.
4. Willing to receive TIV vaccine.
5. In stable health, as determined by medical history and targeted physical exam related to this history.
6. Willing to give blood and optional lymph node FNA samples.

PARTICIPANT EXCLUSION CRITERIA

1. Has a history of severe local or systemic reactions to any vaccination.

2. Has a history of severe allergic reaction to any component of the rabies or influenza vaccines.

3. Has a history of prior rabies vaccination, receipt of rabies post-exposure prophylaxis or rabies immunoglobulin, or known or suspected prior exposure to rabies virus.

4. Has received any influenza vaccines ≤ 180 days prior to the day of injection (Day 1).

5. Has a current or previous diagnosis of immunocompromising condition to include human immunodeficiency virus, immune-mediated disease requiring immunosuppressive treatment, or other immunosuppressive condition.

6. Has received systemic immunosuppressants or immune-modifying drugs for > 14 days in total within 6 months prior to Screening (for corticosteroids ≥ 10 mg/day of prednisone equivalent) or is anticipating the need for immunosuppressive treatment at any time during participation in the study.

7. Is acutely ill or febrile (temperature >38.0 C [100.4F] less than 72 hours prior to or at the Day 1 visit. Participants who meet this criterion may be rescheduled.

8. Currently has symptomatic acute or unstable chronic disease requiring medical or surgical care, to include significant change in therapy or hospitalization, at the discretion of the investigator.

9. History of excessive alcohol consumption, drug abuse, psychiatric conditions, social conditions or occupational conditions that in the opinion of the investigator would preclude compliance with the study.

10. Has received any vaccine ≤ 28 days prior to the injection (Day 1) or plans to receive a vaccine within 28 days before or after the study injection. These participants may be rescheduled.

11. Pregnant women and nursing mothers or women who are planning to become pregnant for the study duration.

12. Have donated blood, blood products or bone marrow within 30 days before study vaccination, plan to donate blood at any time during the duration of participant study participation, or plan to donate blood within 30 days after the last blood draw.

13. Any condition in the opinion of the investigator that would interfere with the proper conduct of the trial.

14. Unwilling to use an effective method of contraception if you are of childbearing potential.

Handling of Withdrawals

Participants are free to withdraw from the study at any time.

Participants may be taken off study without their consent if the study doctor determines that it is in the participant's best interest not to continue to participate in the study. In addition, the study doctor may remove a participant from study participation if the participant is unable to complete the required study procedures, or if the study is stopped by the institution, the sponsor, or the Food and Drug Administration (FDA) or other health authorities. If the participant is removed from the study, the principal Investigator or designee will contact the participant to discuss the study stopping procedures.

Participants who withdraw, or are withdrawn or terminated from the study, or are lost to follow-up after study enrollment visit will not be replaced. Participants who consented to the study but did not receive the vaccine will be replaced. These participants will be considered as screen failures.

Concomitant Medications/Treatments

All concomitant medications and treatments will be recorded in the source document at Day 0 – Day 29 and for any AEs leading to study withdrawal.

STUDY PROCEDURES

Procedures include vaccine administration, draining lymph node fine needle aspirate, targeted physical examination, phlebotomy, signs/symptom assessment (local and systemic) and vaccination site evaluation.

We do not anticipate serious adverse events but will review each participant's study progress at study visits and request that participants inform study site of adverse events between visits. Any serious adverse events thought to be possibly related to the study will result in the formation of an ad hoc data safety committee consisting of a statistician and 2 physicians (at least one from infectious disease), all playing no part in the study to review and determine whether the study should continue in consultation with the IRB.

For all participants:
Prior to vaccination (Date of administration of the vaccination is considered Day 1)
Screening can be performed on the same day or up to 28 days prior to vaccination. Participants will be consented. Collection of vital signs and targeted physical exam will be completed. Medical history and current medications will be collected and reviewed.

Day 0 (pre-vaccination) procedures will include blood collection and optional lymph node fine needle aspiration.

Vaccinations (Day 1 and 8)
All participants will receive a single IM dose of RabAvert and a single IM dose of TIV in the same arm on day 1.

All participants will receive a second single IM dose of RabAvert in the ipsilateral arm on day 8.

Post vaccination
Post vaccination visits will occur at days 8, 15, 29, 85, and 181. Vitals and targeted physical exam will be further collected at follow up visits if clinically indicated. A daily memory aid will be completed by participants days 1-7 after each vaccination to collect solicited adverse reactions. All visits involve collection of any inter-current illnesses, adverse event solicitation and blood collection.

Fine needle aspiration from ipsilateral axillary lymph nodes will be optional and will occur at days 15, 29, 85, and 181.

Optional assays:

Ultrasound-Guided Fine Needle Aspiration (FNA)
FNAs will be taken from unilateral axillary lymph nodes ipsilateral to the site of vaccination. Procedures will be performed at Barnes Jewish Hospital or Washington University School of Medicine. Up to two lymph nodes will be identified by the radiologist and measured according to the study protocol. The skin is marked. A timeout is performed. The skin is sterilized with antiseptic solution and draped. Using ultrasound guidance, up to 10 cc Lidocaine is administered via a 25-gauge needle for numbing from the skin surface to the lymph node. The participant may experience a mild stinging sensation with Lidocaine administration that wears off in a few seconds. An FNA pass is then made with a new 25-22 gauge needle. A pass involves advancement of the FNA needle into the cortex of the lymph node, after which it is pushed and pulled back and forth through the lymph node around 60 times and lasts approximately 15 seconds. Cells and fluid from the lymph node are collected inside of the needle by capillary action. This process is repeated for a total of 6 passes, using 6 separate FNA needles. After each pass, the needle is removed and handed to a member of the research team who processes the sample. Additional passes beyond the routine 6 are sometimes but rarely necessary if the withdrawn material is scant. None of the passes are usually felt by the participant. The most uncomfortable aspect of the procedure is typically positioning, which involves the participant either laying on their back or on their side with their arm raised overhead. This position can be straining to the shoulder, and the raised arm often “falls asleep” during the procedure. The team can assist with repositioning and arm support if necessary for participant comfort. If multiple reactive, enlarged lymph nodes are visualized by the radiologist, one additional ipsilateral axillary lymph node may undergo additional FNA. The entire procedure, from timeout to removal of the final needle, will last approximately 30 to 60 minutes depending on the size and visibility of the node and ease of approach. Additional time is required to identify and measure the most appropriate lymph node. This time varies widely according to how large the lymph nodes are and how easy they are to find, but may take up to 30 minutes (the complete procedure, including node identification, lasting approximately 30 to 80 min). After the procedure, the skin is cleaned and a Band-Aid is applied.

SAFETY ASSESSMENTS

All study participants will complete a 7 day solicited severe adverse reaction diary following each vaccination. They will be instructed to report any moderate to severe vaccine related side

effects that occur within 28 days post-vaccination to the study coordinator. These include fever, headache, fatigue and muscle pain (myalgia). They will be instructed to call the study coordinator or clinician for any serious vaccine related side effects.

A serious adverse event (SAE) is defined as fatal or life-threatening experience resulting in death, hospitalization, significant disability, or congenital anomaly/birth defect.

All adverse events after vaccination will be graded according to the Vaccine Adverse Event Reporting System criteria. Study investigators will assess the adverse events as being definitely related, probably related, possibly related or unrelated to the vaccination. Any serious adverse events or unexpected events related to vaccination or study conduct will be reported to Washington University HRPO following the IRB guidelines. Adverse events will be collected via telephone contact or direct evaluation and through participant interviews at each study visit.

FINAL STUDY VISIT

All participants will be asked about their interest in donating blood in the future for vaccine related immunological studies.

LAB ASSAYS OR PROCEDURES

Research Labs

The blood will be processed to isolate peripheral blood mononuclear cells (PBMCs) and plasma collected in evacuated phlebotomy tubes.
Plasma from all time points will be separated from whole blood and will be frozen at -80^{o}C.
Plasma and PBMCs will be isolated on all study days.

Specimen Preparation, Handling, and Shipping

Blood samples for research testing will be labeled at the ID CRU with a unique identifier and will be transported to Dr. Ali Ellebedy's lab BJC-IH 8th floor - Rm. 8020 via courier service in appropriate transport containers. FNA materials will be labeled with a unique identifier and will be transported to Dr. Ali Ellebedy's lab BJC-IH 8th floor - Rm. 8020 via courier service in appropriate transport containers.

Future use of data and specimens

We may use the blood, lymph nodes, and data we are obtaining in this study for studies going on right now as well as studies that are conducted in the future. These studies may provide additional information that will be helpful in understanding the immune response or other diseases or conditions, including research to develop investigational tests, treatments, drugs or devices that are not yet approved by the U.S. Food and Drug Administration. It is unlikely that what we learn from these studies will have a direct benefit to participants. There are no plans to provide financial compensation to participants. Participants cede property rights to the use of blood, lymph nodes, and data.

Future research may also include genetic research. We may share participant's blood, lymph node biopsies, and data with other researchers. They may be doing research in areas similar to this research or in other unrelated areas. These researchers may be at Washington University, at other research centers and institutions, or industry sponsors of research. We may also share

research data with large data repositories (a repository is a database of information) for broad sharing with the research community. Participants' research data placed in one of these repositories are only available to qualified researchers, who have received prior approval from individuals that monitor the use of the data.

Participants' blood and lymph nodes aspirates will be stored without name or any other kind of link that would enable researchers to identify which sample(s) or data are from a particular individual. Therefore, it will be available for use in future research studies indefinitely and cannot be removed.

STATISTICAL CONSIDERATIONS

The data will be analyzed in consultation with biostatisticians in the Research Design and Biostatistics Group, Washington University ICTS. Our hypotheses are exploratory, and effect sizes are unknown; thus, adequate power analyses cannot be performed at present. The proposed enrollment numbers are designed to maximize use of the current infrastructure for lymph node biopsy to provide the most power to detect potentially relevant differences in immune responses between cohorts.

SOURCE DOCUMENTATION

All source documents will be kept at the ID CRU clinic in a locked high density filing system in a locked office. The building access is secured security officers during working hours and secure badge access at all other times. Offices with the ID CRU are all locked.

QUALITY CONTROL

All ICFs and a portion of the study charts (approximately 10%) will be audited annually by the ID CRU clinic quality management personnel per the ID CRU clinic SOPs.

ETHICS AND PROTECTION OF HUMAN SUBJECTS

Once the study is approved by the Washington University Human Research Protection Office (HRPO), recruiting and screening will begin for the study. All participants are required to read English, understand and sign the informed consent document.

DATA HANDLING AND RECORD KEEPING

The clinical data on the participants will be maintained at the ID CRU clinic in a locked file cabinet. The data on immunological assays will be de-identified and will only be identified with a unique code number and will be maintained in the laboratory of Dr. Ali Ellebedy. The links to the unique codes will be stored at the ID CRU clinic and will not be available to the laboratory personnel.

The study records will be maintained up to ten years after the completion of the study.

PUBLICATION POLICY

The PI and co-investigators will participate in manuscript preparation and submission to journals.

Schedule of Events

	Day 0 Screening (up to 28 days prior to Day 1)	*Day 1 (date of vaccine administration)[1]*	*Day 8 +/- 3 days*	*Day 15 +/- 3 days*	*Day 29 +/- 7 days*	*Day 85 +/- 14 days*	*Day 181 +/- 14 days*
Visit Number	***1***	***2***	***3***	***4***	***5***	***6***	***7***
Informed consent	X						
Review eligibility	X	X[1]					
Demographics	X						
Collect/Update Med HX, Meds	X	X[1]	X	X	X	X[5]	X[5]
Vital Signs	X	X[1]	X[2]	X[2]	X[2]	X[2]	X[2]
Targeted Physical Exam	X	X[1,2]	X[2]	X[2]	X[2]	X[2]	X[2]
Fine needle aspiration (FNA)	X	X[1,5,6,7]		X[6]	X[6]	X[6]	X[6]
Blood Draw	X	X[1,5,7]	X[7]	X	X	X	X
Research Lab 10 ml CPT – EDTA	*80ml*	*80ml*	*80ml*	*80ml*	*80ml*	*80ml*	*80ml*
Adverse Events		X[1]	X[3,4]	X	X	X[4]	X[4]
Study Vaccine: RabAvert		X[1]	X[1]				
Study Vaccine: TIV		X[1]					
Review participant 7 day solicited adverse reactions diary			X	X			

- [1]***Assessments should be performed prior to administration of vaccine***
- [2] ***If clinically indicated***
- [3] ***Solicited Adverse Reactions***
- [4]***Collection of SAEs and AEs leading to study withdraw***
- [5] ***If not done at Day 0***
- [6] ***Optional for all participants***
- [7] ***Blood and FNA will be collected before RabAvert vaccination***

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