Modulation of the Immune Response to Influenza Vaccination by Host and Vaccine Characteristics

Dissertation proposal by

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# Project Summary

Current influenza vaccines fail to protect against novel strains. The development of a universal influenza vaccine is hindered by a current lack of understanding of factors driving variation in the immune response to influenza. We will use longitudinal cohort data from multiple sources to explore how characteristics of vaccines interact with characteristics of the vaccine recipient in order to clarify potential drivers and mechanisms of the immune response to vaccination. We aim to explore how antigenic differences between the vaccine strain and historical strains (a surrogate for potential future strains) interact with the preexisting immune repertoire of the recipient, and how vaccine dose and prior vaccination history can modulate this relationship. In addition, we will propose metrics for the fair evaluation of vaccine candidates to a wide variety of historical strains, which can allow the selection of an optimal broadly reactive vaccine. Our analyses will allow for improved development and evaluation of universal vaccine candidates.

# Project Narrative

Influenza is a respiratory disease that occurs in seasonal epidemics worldwide. The Centers for Disease Control and Prevention recommend annual vaccination against seasonal influenza with a modified vaccine for adults in the United States, but the effectiveness of these vaccines varies greatly across seasons and individuals. Improved understanding of the drivers of the immune response following vaccination is crucial for improving influenza vaccines.

# 1. Specific Aims

Recent concerns about H5N1 influenza spillover events highlight the need for a universal influenza vaccine with the ability to mitigate future pandemic events. However, a combination of rapid antigenic evolution and heterogeneity in individual host response makes developing such a universal vaccine difficult. Previous literature has characterized the importance of prior immunity, vaccine design, and antigenic distance between the vaccine and host strain. The goal of my thesis is to understand how pre-existing immunity affects individual response to the seasonal influenza vaccine, and how this effect is modified by other factors. A better understanding of the host immune response will inform the design and evaluation of vaccines which induce a broader, more robust response.

We will use Bayesian hierarchical models, causal inference, and machine learning methods to quantify these effects. Our models will account for interactions between predictors, nonlinear effects, and clustered measurements. We will analyze longitudinal influenza vaccination data from the UGAFluVac cohort study (PI: Ross), wherein individuals provided serum samples, potentially for multiple years, and these samples were tested against a panel of historical viruses. We will also combine the UGAFluVac data with other data sources provided by Ben Cowling, Andrea Sant, and potentially other investigators involved with the DIVERsity study (NIH project number 1R01AI170116-01) or the CIVR-HRP NIH CIVICs site (NIH project number 75N93019C00052) to answer questions about vaccine dose and pre-existing immunity.

**Aim 1. Develop metrics for the quantification of the total immune response to an influenza vaccine, incorporating both strength and breadth.** Using the UGAFluVac data, we will analyze the relationship between immune response and *antigenic distance*, a measurement of how different the assay strain and the vaccine strain are. We will develop metrics for quantifying the overall strength of the immune response to a panel of historical strains, and the breadth of the response – intuitively, how the immune response diminishes as antigenic distance increases. We will also use subsamples of the UGAFluVac data to analyze the robustness of these metrics across differing panels of historical viruses.

**Aim 2. Quantify the role of pre-vaccination titer, prior vaccinations, vaccine dose, and antigenic distance on individual vaccine response.** Influenza vaccinations provide diminishing boosts for recipients with high antibody titers, called the “antibody ceiling” effect. However, the threshold and rate of diminishing boosts depend on several other host and vaccine factors, including prior vaccination history, vaccine dose, and antigenic distance. We will use hierarchical statistical models and mechanistic models to disentangle the individual effects and interactions.

**Aim 3. Explore the interaction of age and vaccine dose on the impact of antibody response.** The UGAFluVac cohort allows participants over the age of 65 to choose whether they receive FluZone standard dose or FluZone high dose, and Andrea Sant’s cohort study administered FluZone HD to individuals aged 18 – 49. By combining the two datasets, we can use causal inference and hiearchical modeling techniques to understand the effect of dose, and how this relates to previous mechanistic modeling predictions.

# 2. SIGNIFICANCE

Influenza is a rapidly evolving pathogen that evolves to escape current vaccines (1,2). All seasonal influenza vaccines currently available to the public focus on four lineages of influenza, which are predicted to be the dominant circulating strains for the upcoming season. The seasonal vaccine is thus not effective at preventing circulation of new antigen phenotypes that arise spontaneously from zoonotic spillover events, leading to pandemics like the 2009 H1N1 outbreak (3). The highly pathogenic H5N1 spillover cases in early 2023 (4) acutely demonstrate the need for a “universal” influenza vaccine that is broadly protective, and provides protection against emergent strains.

Designing a universal influenza vaccine has proven to be challenging (5–7). Understanding the immune response to influenza is complicated by rapid pathogen evolution and the accompanying change in vaccine formulation. Since the immune response depends on an individual’s history of influenza infection and vaccination events, the susceptible population displays an incredible diversity in immune repertoires even after controlling for effects like imprinting and prior vaccination. Decomposing the immune response to novel influenza strains into a set of mechanisms, and quantifying the relative contribution of each of these mechanisms is an open problem. Dissecting the components of the response would yield key insights into the design of vaccines which are reliably broadly-protective for individuals regardless of their immune state at time of vaccination.

**Aim 1. Develop metrics for the quantification of the total immune response to an influenza vaccine, incorporating both strength and breadth.**

There is no universally accepted way to quantify the breadth of the immune response to a vaccine. Since direct trials of vaccine efficacy are expensive, correlates of protection (CoP) are typically used to measure the immune response to a given influenza vaccine. Several CoPs are currently in use with no clear consensus on which is best (8), but the most common CoP used in practice is hemagglutination inhibition (HAI) titer. We will focus on HAI titer in this project, but the methods we propose could be applied to any quantitative CoP.

HAI is correlated with protection from influenza with a 50% protection titer of 1:40 (9,10). If an invidual’s reciprocal titer is 40 or greater for a particular strain of influenza, the individual is said to be *seroprotected* against that strain. If a vaccine induces a 4-fold or greater increase in HAI titer, with the final post-vaccination measurement above 40, the individual is said to have *seroconverted* against that strain. Seroconversion and seroprotection are commonly used dichotomous clinical endpoints for assessing the immunogenecity of an influenza vaccine candidate.

The traditional method for evaluating the breadth of an individual’s immune response following vaccination is to perform CoP assays against a variety of historical strains of influenza. The breadth of the response is then taken as either the count or proportion of strains to which the individual seroconverted (11,12). While this method is easy to quantify in a laboratory setting, the estimates of breadth are biased by the selection of the panel of historical strains, and variation in panels between laboratories makes comparing these estimates across studies difficult (13).

Modern methods for assessing the antigenic distance between strains of influenza allow the development of a consistent framework for assessing the breadth of a vaccine candidate. The simplest method for computing antigenic distance is the *time-based* method, where the antigenic distance is taken as the difference in isolation year between strains (14,15). Other methods include sequence-based methods, which assess the similarity of the genetic or protein sequence of the two strains (16–20); and antigen-based methods, which use immunogenecity data to inform distance between strains (21–25). There is no clear consensus on which measures of distance are most useful for informing vaccine evaluation. While previous work has explored the quantitative comparison of these so-called “antibody landscapes” (referring to the CoP measurement as a function of antigenic distance) (14), such approaches have not been widely utilized.

We will explore metrics for evaluating vaccine candidates that explain the **strength** of the response to the homologous strain; the **breadth** of the response explaining how the immune response is related to antigenic distance; and the **overall** response, which will weight the strength and breadth into an overall metric for the immune response induced by the vaccine. Using our framework for vaccine evaluation, we will compare measures of antigenic distance. We also plan to test the robustness of our metric to the selection of the virus panel in order to compare our framework to the traditional method. We will explore simple regression models, flexible spline models, and functional data analytic techniques for their potential to characterize the overall strength and breadth of the immune response. For this aim, we will use longitudinal cohort data with a wide panel of heterologous responses for each individual collected by Ted Ross (26–28).

**Aim 2. Quantify the role of pre-vaccination titer, prior vaccinations, vaccine dose, and antigenic distance on individual vaccine response.**

Several characteristics of both the vaccine and the recipient are known to be associated with the immune response to the vaccine. In addition to antigenic distance between the vaccine strain and the strain of interest, several details of the vaccine formulation are associated with immunogeneicity including dose (29–31), route of administration (32–34), and vaccine type (35–37). Promising vaccine candidates have been developed using intranasal, intramuscular, and subdermal routes of administration, and recombinant protein or mRNA vaccines may prove to be superior to the traditional split-inactivated or live attenuated vaccine types.

In addition to vaccine design choices, “intrinsic” characteristics – baseline characteristics of the vaccine recipient – are also known to potentially alter the vaccine response. These characteristics fall into two large categories: multifaceted characteristics that vary with every member of the susceptible population, and factors which can be aggregated at the population level. In the former category are genetic differences (38–40), epigenetic modifications, and differential gene expression (27,41–45), all of which play a significant role in the immune response to influenza. In the latter category are individual characteristics like sex, obesity, and age, which are easier to measure and understand.

Birth sex and circulating hormones may influence the immune response to flu through both sex-associated genetic differences or through sex-differentiated hormonal signaling, although results are ambiguous with no mechanism yet discovered (46–50). Obesity, typically measured through BMI, is associated with a decreased response or with more rapid waning of antibodies (51). As individuals age, they undergo immunosenescence and a gradual decline in immune protection (12,52).

In addition to the immunosenscence effect of age, an individual’s birth year is predictive of the immune response to specific strains of influenza, because birth year is strongly associated with the first strain of influenza to which an individual is exposed (53–55). The theory of imprinting (also called original antigenic sin) predicts that an individual’s first influenza exposure leads to the development of a memory response to that strain. Future exposures can then activate the memory response to the original strain (assuming the strains are somewhat similar), which dominates the novel immune response to the new strain, as influenza antibodies reach a saturation level called the “antibody ceiling”, which can vary between individuals (56–58)

One consequence of the imprinting effect and of the antibody ceiling effect is a strong negative relationship between pre-vaccination immunity and the response to a vaccine (36,59). Prior and repeat vaccination also has a strong effect on vaccine response (12,60), potentially independent of the antibody ceiling effect. In fact, the reactivation of the memory response at every exposure to a somewhat similar strain makes the response to vaccination dependent on an individual’s entire history of influenza infection and vaccination (15,23,56,61). In addition, the immune response to influenza may be affected by prior exposure to other pathogeons, including herpesviruses like Epsteinn-Barr (62) or cytomegalovirus (63), or through antigen-independent effects which modify the baseline immune state and induce a differential response (64).

While measuring all of these effects simultaneously is impossible, we intend to model the effects of vaccine dose, pre-vaccination titer, prior vaccination, and antigenic distance using data from the cohort studies conducted by Ted Ross. While our analysis will be limited to a single vaccine (Sanofi Pasteur’s FluZone, with standard and high dose formulations), we have data on an extensive panel of historical viruses along with reported vaccination history for each patient. We will incorporate predictive machine learning approaches and hierarchical bayesian modeling to understand the individual contributions of these factors to the overall immune response. We also plan to modify previously-developed mechanistic models for multiple influenza epitope responses (65–67) to include a degree of similarity between epitopes (conceptually representing antigenic distance), and compare the results from the updated model to our data.

**Aim 3. Estimate the effect of vaccine dose and age on vaccine response.**

The role of vaccine dose is so important in vaccine response that determining a dose that balances efficacy and side effects is a crucial part of drug approval in the United States (5,68,69). High-dose influenza vaccines are approved for use in older adults and can substantially improve the immune response for older and otherwise immunocompromised individuals (31,70,71). While otherwise healthy recipients generate substantive immune responses even to fractional doses of influenza vaccine (72,73), mechanistic models predict that increased dose may be useful in overcoming the negative effects of prior immunity and original antigenic sin (65,66).

These mechanistic models allow for simulation of the immune response following vaccination for a wide range of inoculum doses, and predict that as the inoculum dose is increased, the effect of prevaccination titer is mitigated. That is, an individual with a higher prevaccination titer could potentially receive a higher dose than an individual with a low prevaccination titer, and observe the same fold-change in titer as a result of vaccination. To date, a randomized clinical trial comparing standard and high dose formulations of available vaccines has not been conducted in otherwise healthy adults 18 - 49, so these claims have not yet been evaluated in groups that are not elderly or immunocompromised.

We will combine observational data from multiple sources to estimate the effect of dose on influenza vaccine response while controlling for the effects of prevaccination titer, age, and other relevant effects as enumerated previously. In this analysis, we specifically plan to focus on the effect of dose on homologous vaccine response by combining the UGAFluVac data, where high dose vaccines were only administered to individuals aged 65 or older, with data collected by Angela Branche and Andrea Sant (36,74). We will obtain the average causal effect of dose, after controlling for age and other confounders, and compare the predictions to those of the mechanistic model, which does not currently account for age. Together, these analyses will provide a better substantive understanding of the effect of dose on the immunogenicity of a standard influenza vaccine.

# 3. APPROACH

## 3.1 Data description

We will combine data from multiple influenza vaccination cohort studies for our analyses. At the time of writing, we have data from two cohort studies conducted by Ted Ross, one study conducted by Andrea Sant and Angela Branche (see (36)), and a collaboration with Ben Cowling, who is the PI of several similar studies and has offered to share data with us. The two studies conducted by Ted Ross provide a unique opportunity to study heterologous antibody responses to influenza vaccination, as a wide historical panel of assays was conducted for each individual for several years of the study. Combining Ross’ data with data from Sant and Cowling will allow for us to compare the immunogenicity of multiple vaccines, and increase the power of our analyses of host factors.

We will refer to both of Ross’ cohort studies conjointly as the **UGAFluVac** study. This data set consists of longitudinal HAI measurements taken at three different study sites. From fall 2013 to spring of 2016, participants were recruited at one of two study sites in either Pittsburg, PA or Stuart, FL. Sample collection is further detailed in (26), among other references. Briefly, the study is a prospective open cohort design. Participants were adults aged 18 and up who were allowed to repeat each year, and the data includes a unique ID per participant that allows for the identification of longitudinal measurements. Each participant recieved a pre-vaccination blood draw, and was then administered a split-inactivated Fluzone seasonal influenza vaccine (Sanofi Pasteur). Patients aged 65 or older could opt to receive Fluzone High Dose instead. At the PA study site in 2013, some patients were administered an intradermal vaccine rather than the standard intramuscular Fluzone. Followup whole blood draws were targeted for 21 days post-vaccination.

Processed sera were used for HAI assays following standard protocols against the homologous vaccine strains as well as a heterologous panel of historical strains. The starting dilution was 1:10 and assays which did not agglutinate at the starting dilution were coded as 1:5. For all further analyses, we will use the reciprocal titer transformed as

This transformation serves to set 0 as the limit of detection on the log scale.

Additionally, the following data were collected from patients by a survey prior to vaccination: year of birth, age, gender (the covariate is listed as gender but is coded as male/female only), and race/ethnicity.

In January 2017, the study moved to the University of Georgia, Athens, GA. The paper (28) contains a description of the study, but at the time of writing, no published paper contains a complete description of the study cohort. The study design was similar, with additional covariate information collected: complete date of birth, sex assigned at birth, race/ethnicity, BMI, height, weight, and questions about smoking and comorbidities. Beginning in the 2017-2018 flu season, participants aged 10 and older were additionally recruited for the study. The HAI assays were conducted in the same way. The postvaccination time point target was changed to Day 28 beginning in fall 2018. Finally, additional subcohorts were administered other vaccines at certain points during the study. See [Figure 3.1](#fig-michael) for details.

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| Figure 3.1: Study diagram showing the subcohorts and associated timepoints collected during the ongoing UGA study. The diagram was created by Michael Carlock, the CIVR-HRP research director. |

We will refer to the data provided by Andrea Sant and Angela Branche as the **RocFluVac** study. We have not yet finished cleaning and processing the data, but it contains similar HAI measurements for homologous strains only, participants were administered a variety of vaccines (including Fluzone HD administered to participants aged 18 - 49), and also contains data on T cell responses to the vaccine. See (36) for one brief explanation of the data.

## 3.2 Aim 1

For this aim, we will use the cohort data collected by Ted Ross.

### 3.2.1 Preliminary results

We have calculated several metrics of antigenic distance, and will compare the year-based method (15), the dominant *p*-epitope sequence based method (17), and a distance based on antigenic cartography (21,75). To compute the dominane *p*-epitope distance, the pairwise Hamming distances are computed between the amino acid sequences of each of the five hemagglutinin head epitopes, and these are divided by the length of their respective sequences. The dominant *p*-epitope measurement is the maximum of each of these probabilities, representing the different between the two strains at their most different epitope.

Antigenic cartography analyses were conducted by Amanda Skarlupka, PhD, who continues to work with us on this project. In short, antigenic cartography uses a matrix where each row represents an individual in the study and each column represents an influenza strain. The cells of the matrix are populated with individual ’s titer to strain . Multidimensional scaling is used to reduce the matrix to a specified column dimension, while minimizing the change in the Euclidean distances between measurements. After performing iterative MDS on a variety of target dimensions, we found that two dimensions was satisfactory, and our maps were similar to those in (21). After calculating the MDS maps, we then obtained antigenic distances as the Euclidean distance between map coordinates of the vaccine strain and the other strains in the panel following the method of (75). For the purposes of this analysis, we ignored longitudinal measurements between individuals, and treated each observation of an individual as a unique measurement. Finally, all of the antigenic distance were normalized *for each vaccine strain*. Therefore, each vaccine strain had a distance of 0 with itself, and the most distant historical strain had a distance of 1 with that strain. (We normalized all three of the distance measurements in this way.)

After computing the normalized distance measurements, we fit simple linear regression models with either postvaccination titer (measuring the absolute immune response postvaccination) or fold-change in titer (measuring the relative boost postvaccination) against antigenic distance. [Figure 3.2](#fig-amandafits) shows sample linear regression models following this protocol for two vaccine strains.

Our proposed metrics for evaluating the **strength**, **breadth**, and **overall response** are the intercept of the regression line, slope of the regression line, and AUC respectively. The intercept measures how strong the response is to the homologous strain, the slope describes how the response weakens linearly as antigenic distance increases, and the AUC provides a framework for combining the strength and breadth measurements into one measurement of overall response.

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| Figure 3.2: Fitted models with titer outcomes and antigenic distance as the only predictor. The metrics reported are for titer increase for simplicity. |

As a case study to motivate our framework, we plan to analyze differences in these three metrics between the Fluzone SD and Fluzone HD vaccines, to determine if the high dose vaccine elicits a stronger or broader response in our cohort, as shown in [Figure 3.3](#fig-distdose). Our preliminary results suggests that this effect differs qualitatively across vaccines, and we plan to analyze which vaccines show the most notable differences between strains.

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| Figure 3.3: Linear models for titer increase vs. antigenic distance, stratified by vaccine dose. The same metrics are reported for both doses. |

### 3.2.2 Proposed studies

Going forward, we plan to consider more flexible models than the simple linear regression models we used in this step of the analysis. We will consider restricted spline GAMs and LOESS to fit a potentially nonlinear effect of antigenic distance, which we would expect in the presence of original antigenic sin if our panel contains strains which are distant enough to confer no cross-protection. We will also implement Bayesian multilevel generalizations of both the linear and spline models, in order to incorporate between-subject variability into the overall model fit.

We will then compare our metrics to traditional metrics, notably the mean titer increase, HAI composite score (76), and proportion of seroconverted strains, all calculated for each individual. In order to determine whether our proposed metrics are more robust than traditional metrics, we will subsample measurements from our cohort in order to mimic the use of different virus panels across labs. For labs, we will subsample strains from our panel, plus the homologous strain. For each of these panels, we will calculate the metrics for each individual, and then analyze the variability of mean metrics across labs.

Finally, we will explore weighting schema for our metrics. The unweighted AUC assigns equal weight to strains of all antigenic distances, but perhaps we would prefer to weight the response to distant strains higher or lower than similar strains–for example, if we are primarily considered with boosting the response to a specific pandemic strain, we could assign less weight to distant strains. But if we are considering candidates for a broadly reactive vaccine, we could weight distant strains higher in order to favor vaccine candidates that induce responses to the most distant strains. Examples of weighting schema are shown in [Figure 3.4](#fig-weights).

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| Figure 3.4: Example weighting schema for AUC measurements. In the linear scheme, weights decrease linearly with antigenic distance. In the 2 antigenic unit scheme, all strains which are within two antigenic units on the cartography map (or equivalent with the other distances) are weighted equally, while strains that are further away are not included in calculating the AUC. |

## 3.3 Aim 2

For this aim, we will use the UGAFluVac data.

### 3.3.1 Preliminary methods and results

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paragraph about slopes and prevaccination titers to show we know they interact

### 3.3.2 Proposed future methods

First, we will expand and refine our preliminary machine learning approach to include all predictors of interest. We use random forest and other similar models to quantify the predictive power of each variable using permutation importance. These models can learn nonlinear and interaction effects without prior specification and thus will allow us to understand how well all we can expect inferential models to capture trends in the outcome.

After we understand how much of the post-vaccination titer can be explained by the data we have, we will build Bayesian hierarchical models to take advantage of the clustered structure of the data. These hierarchical models will be allow us to make inferences about the relationships between variables, rather than only quantify the predictive power. Furthermore, we can implement random effects in these models to partially absorb unmeasured confounding, which is not possible within a predictive machine learning framework. However, we can compare the overall predictive power of our inferential models to the best predictive models.

Finally, we will modify previously developed mechanistic models (65,66) which model steric hindrance between multiple epitopes of the same antigen. The model for steric hindrance is mathematically similar to how we would model differences in binding avidity between antigenically distance strains, so by incorporating a notion of antigenic similarity, we can use these models to predict how the relationship between dose, prior immunity, and vaccine response varies with antigenic distance. We will compare the model predictions with the predictions made by our machine learning and inferential models. A notable limitation of the mechanistic models in this case is that we will not be able to make predictions about the effect of serial repeated vaccinations as we hope to do for the hierarchical models.

## 3.4 Aim 3

For this aim, we will combine the UGAFluVac data, RocFluVac data, and any applicable data provided by Ben Cowling.

### 3.4.1 Preliminary methods and results

Not sure we have any preliminary data here – maybe I can find the contrast plot that I made in Amanda’s project? That would be the ATE assuming no confounding which is not ideal but is preliminary

Include a DAG here limited to only show the variables we can include

Include the previous plot where we compared Ted’s and Andrea’s data?

### 3.4.2 Proposed future methods

summary of causal inference methods to determine the effect of dose

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