SPECIFIC AIMS

A persistent challenge to the design of effective influenza vaccines and forecasting of viral evolution and epidemiology is uncertainty about the immune responses underlying protection from infection. Antibody titers to the hemagglutinin (HA) surface protein were established as a correlate of protection 50 years ago, and more recent evidence shows many anti-HA antibodies directly and indirectly contribute to viral neutralization. However, HA titers remain only moderately predictive of an individual's risk of infection on exposure, and the contributions of other immune responses are less well understood. Understanding the causes in addition to correlates of protection could increase the accuracy of forecasts of viral fitness and provide reliable endpoints for vaccine development. But the impact of this knowledge might be limited unless a related challenge is solved: the high individual variability in immune responses to influenza vaccination and infection. Studies of seasonal influenza vaccines routinely find eight-fold differences in post-vaccination antibody titers between people, including apparent non-responders. This variation has been associated with age, infection and vaccination history, and immune status. After infection, some individuals appear to raise primarily non-neutralizing antibodies, and others do not. The ability to predict or even modulate immune responses, especially protective immune responses, could lead to more effective vaccination strategies that mitigate vaccine failure in different subpopulations.

We propose complementary approaches to identify the correlates and drivers underlying protection from infection and heterogeneity in vaccine responses. Because targeted experimental manipulation of immunity is difficult in humans, and animal models require careful vetting for relevance, we propose to leverage samples and observations from existing, mostly multiannual longitudinal studies of influenza virus infection and vaccination in humans. First, we will use computational and single-cell approaches to investigate how past vaccination and infection impact host immune status. Emerging evidence, including our recent work, suggests that vaccination and infection can establish antigen-agnostic immune set points that affect future vaccine responses. Next, we propose to integrate complementary computational approaches, spanning machine learning, causal mediation analysis, and mechanistic modeling, to predict and develop mechanistic insight into vaccine responsiveness and protection from severe and mild infection. Due to the complexity of influenza evolution and immune dynamics, fitting hierarchical models across different contexts (populations, strains, vaccines, infections) and leveraging conserved signals across cohorts will increase statistical power. Additionally, our diverse statistical approaches will increase the accuracy and insight of our conclusions.

Aim 1. Assess the impact of vaccination and infection history on antigen-agnostic baseline immune states. We hypothesize that in addition to impacting antigen-specific immune statuses, prior infection and vaccination can affect immune states in antigen-agnostic manners. This broad exposure history contributes to variation in baseline states between people with potential consequences for future responses to vaccination and infection.

Aim 2. Predict immune responses (including their magnitude, breadth, and durability) to vaccination based on intrinsic characteristics, baseline immune states, and influenza exposure history. We will integrate diverse variables, including infection and vaccination history; baseline antigen-specific and antigen-agnostic immune states; and intrinsic characteristics including age, sex, and body mass to predict responses to influenza vaccination and extract mechanistic insight.

Aim 3. Determine multidimensional correlates and mechanisms of protection against infection and disease. We hypothesize that innate immune states, memory B/T cell repertoires, and neutralizing antibody titers to the hemagglutinin and neuraminidase contribute to protection against infections of varying severity, leading to different mechanisms of protection in different individuals over time.

Our work will directly contribute to NIAID's strategic plan for the development of a universal influenza vaccine, but the impacts of this research are broader. We will create and distribute a suite of accompanying tools to ensure accessibility of the novel methods and approaches that we develop to less quantitatively trained scientists. Critically, we will also provide tutorials and supporting resources describing the appropriate use of different tools. Our proposed work is facilitated by data from a large number of cohorts from diverse populations and makes an excellent model for us to develop these approaches, most of which can provide foundational frameworks to dissect responses to other vaccines and pathogens in the future, and to understand individual heterogeneity in immune states over time.

SIGNIFICANCE

The induction of protective immune responses through vaccination is central to the management of many pathogens. For antigenically variable pathogens such as influenza, protective immune responses impose a major selective pressure on viral populations and indirectly influence vaccine strain selection and vaccine effectiveness. Our poor understanding of the generation and maintenance of protective immunity to influenza hinders vaccine development and the accuracy of evolutionary forecasts; improving understanding of influenza immunity is a key element of the NIAID strategic plan on universal influenza vaccines[1]. The low effectiveness of seasonal influenza vaccines stems at least partly from the puzzling fact that many people respond weakly to the vaccine. This has manifested as low post-vaccination antibody titers, including neutralizing antibody titers, to vaccine strains in studies spanning different seasons, vaccine platforms, and populations. Low post-vaccination antibody titers have been associated with individuals' "intrinsic" characteristics, including sex[2–4], obesity and body mass index (BMI)[5,6], and advanced age[6–9]. Low vaccine responsiveness has also been associated with "baseline" (pre-vaccination) immune statuses, such as temporally stable transcriptional signatures of reduced immune activation involving the plasmacytoid dendritic cell (pDC)-type I Interferon (IFN)-T/B lymphocyte network[10–14]. Finally, low post-vaccination antibody titers are more common in people vaccinated annually against influenza and who have had a longer time since documented influenza virus infection[15–17]. Clearly, there is a need to reconcile and better understand the relationships among these correlates of vaccine responsiveness with one another to quantify their individual abilities to predict who will respond well, and an additional need to identify the ultimate drivers or mechanisms of vaccine responsiveness[18,19]. This information could assist the design of vaccines or vaccination strategies that are more reliably immunogenic, given heterogeneity of vaccine recipients.

Good vaccines are not only reliably immunogenic but also induce protective immune responses. Despite the importance of immune protection to clinical outcomes, epidemiology, and viral evolution, the precise mechanisms of protection are often overlooked in favor of the identification of correlates. (Some vaccinologists define vaccine correlates also as causes, with non-causal correlates referred to as surrogates[20], but we will use the traditional statistical definition of a correlate as demonstrating association but not necessarily causation.) Serum hemagglutination inhibition (HI) titers, virus neutralization titers, neuraminidase inhibition titers, anti-NA enzyme-linked immunosorbant assays (ELISAs), hemagglutinin (HA) stalk antibody titers, and cross-reactive CD4+ and CD8+ T cells have been associated with protection from infection and disease in observational and challenge studies[21–26]. Their causal or mechanistic roles have also been established in animal experiments[27–29], but it remains difficult to draw quantitative conclusions about the required thresholds for protection in humans (from infection, disease, etc.) due to differences between human and animal physiology, especially in the context of a human-adapted virus. A further challenge is that observational studies have repeatedly shown that associations only moderately predict outcomes. Hobson et al. (1972) observed that some individuals with undetectable HI titers appeared less susceptible to infection than individuals with detectable titers[21]. It stands to reason that in addition to identifying mechanisms, not just correlates, of protection, it is necessary to quantify potentially interchangeable impacts of diverse components of the immune response. For example, the threshold for anti-HA antibody-mediated protection may be lower in individuals with especially strong T cell memory, and vice-versa. Recent observational studies suggest different mechanisms of protection affect infection risk by age[24]. In addition, antigen-agnostic mechanisms of protection, such as enhanced mucosal and innate immune capacity can potentially be shaped by vaccinations and past infections as well, and may help improve outcomes such as asymptomatic as opposed to symptomatic disease. However, these mechanisms and their pre-infection correlates remain poorly understood, but some may be shared with baseline predictors and potential determinants of vaccination outcomes[13].

The goal of the proposed research is to explain heterogeneity in immune response dynamics and outcomes, especially after seasonal influenza vaccination, and to identify the mechanisms underlying immune protection from infection and disease. Our approach is motivated by the recurring observation, in our studies and others, that the history of exposure to influenza and other pathogens affects influenza vaccine responses and infection risk. Complex responses involving innate and adaptive immunity necessitate the inclusion of high-dimensional data from diverse populations. For these reasons, we will integrate data from longitudinal studies involving active respiratory pathogen surveillance and vaccination of carefully profiled individuals over short (acute response within days) and long (many years) time scales. The main deliverables will be predictive models that evaluate the relationship of past vaccination and infection to pre-vaccine, antigen-agnostic baseline immune status (Aim 1); that associate and attempt to explain antigen-specific and -agnostic baseline immune status and other host characteristics with protective responses to vaccination (including magnitude, breadth, and durability; Aim 2); and that quantify the multidimensional correlates and potential mechanisms of protection to more and less

symptomatic infection (Aim 3). As part of this work, we will produce well documented software tools and workflows demonstrating applications of our quantitative approaches—hierarchical machine learning (ML), mechanistic models, and causal mediation analyses (CMA)—and explaining their statistical and biological context.

The impacts of this work extend beyond improved approaches to study influenza vaccination and infection: our results will also deepen knowledge of basic immunology and influenza evolution and epidemiology. We have previously demonstrated that transcriptional signatures that predict post-vaccination antibody titers to influenza vaccination also predict responses to yellow fever vaccination and flares of autoimmune disorders[10]. Thus, these signatures most likely reflect antigen-agnostic immune states, and our data further indicate that some of these baseline signatures are affected by previous vaccination and infection. Our proposed work is likely to help establish a crucial connection between vaccination, exposure history, and future response potential—a link that goes beyond classic, antigen-specific immunity. Furthermore, the immunological drivers we identify will suggest hypothetical mechanisms of protection to other pathogens, improved endpoints for vaccine studies, and revisions to clinical guidelines on booster doses. Because the timing and sizes of epidemics as well as the predominant selective pressures on influenza are driven by the dynamics of protective immunity, our work will also provide fundamental knowledge for improving influenza forecasts. It will also inform hypotheses about how influenza's year-to-year evolution is driven by heterogeneity in immune responses[30–34].

INNOVATION

The proposed work demonstrates conceptual and technical innovations. First is the explicit consideration and modeling of causes and mechanisms beyond statistical correlates of protection[35]. We will attempt to identify causal pathways using mediation analysis, a new approach in immuno-epidemiology, accompanied by mechanistic modeling. The investigation of causes should deepen insight into underlying immune interactions.

Next, we are conceptualizing mechanisms of protection as a multidimensional surface representing contributions from multiple immune parameters: equivalent protection against infection of a given severity can be conferred by different immune states and parameter combinations. This is an immunologically justified hypothesis that should advance the field beyond simple cutoffs of immune measures, and it should reveal how individuals can be protected against influenza in more than one way.

Another innovative feature is the breadth of immune history considered. Original antigenic sin is a well-known feature of antibody responses to influenza, but this phenomenon largely explains the fine-scale specificity of antibodies. Here we seek to explain responsiveness and risk more broadly. We investigate how inferred early influenza exposures (e.g., the imprinting strain), recent influenza exposures (documented or serologically inferred infections or vaccination), and non-influenza exposures (which we have shown to impact baseline immune cell states) predict vaccine responses and infection risk. While vaccination and infection history are known to correlate with vaccine and infection outcomes, investigation of causal mediators has largely focused on influenza-specific immunity. Here we consider transcriptional and cellular mediators that are antigen-agnostic.

Finally, the proposed work is unusually thorough and rigorous. We will use data from 12 different studies involving >14,000 participants. We apply a comprehensive set of approaches: statistical and ML methods, CMA, and mechanistic modeling. This will allow us to extract conserved signals across cohorts and evaluate the strength of our conclusions from different directions. We will use hierarchical models and leave-samples-out cross-validation to derive insights from populations of diverse ages, races/ethnicities, and influenza exposures. This will increase the robustness of hypotheses that we can test. This innovation is also reflected in some of our tools, designed to integrate diverse data sets and fit hierarchical models.

APPROACH

Overview. We will generate and integrate select data across twelve studies to assess the impact of vaccination and infection history on antigen-agnostic baseline immune states (Aim 1), to predict the impact of these states and other characteristics on vaccine responses (Aim 2), and to characterize correlates and mechanisms of protection from infections with varying symptom severity (Aim 3). We will use statistical and ML models to infer the best predictors of strong and durable vaccine responses. We will also use causal mediation analysis, guided by statistical associations and immunological hypotheses, to evaluate whether strongly correlated variables show evidence of mediating effects on post-vaccination antibody responses and infections of varying severity. These

putative drivers will be further evaluated via mechanistic models to assess biological plausibility. Importantly, we will use traditional model selection criteria as well as cross-validation within the same cohorts, independent cohorts, and public data sets to validate our results. We will compare the performance of more mechanistic models with simple associational models (e.g., involving age, geographic location, year) to evaluate the strength of biological insight. The result will be predictors of baseline immune states (as a function of vaccination and infection history), influenza vaccine responses, and risk of infection and disease, with some insight into potential mechanisms. We will package and release supporting software and tutorials.

Study team. The team has expertise in influenza, systems immunology, diverse quantitative methods, and the development of computational tools. At NIH, PI Tsang served as the chief of the Multiscale Systems Biology Section and co-director of the Center for Immunology, and he will soon direct the Center for Systems and Engineering Immunology at Yale University. He discovered baseline transcriptional signatures that predict influenza vaccine responses and found these signatures generalize to other vaccines and immune conditions. His work integrates large-scale immune data generation from clinical studies with stochastic dynamical modeling, hierarchical modeling, Bayesian inference, and ML. PI Cowling brings expertise in statistical modeling of influenza transmission, vaccine effectiveness, and correlates of protection. In addition to running and leading analyses of six of the studies used here, he has pioneered the use of CMA to infer drivers of protection from clinical data. PI Cobey builds dynamic longitudinal models of antibody titers and protection, analyzes influenza vaccine effectiveness and infection risk accounting for past exposures, and models evolution of B cell repertoires. Investigator Handel develops and uses statistical and mechanistic models of within-host influenza virus infection dynamics, teaches courses on the subject, and has released relevant R packages. Investigator Leung is an epidemiologist with a background in immunology who bridges the two disciplines. Investigator Shen is a biostatistician with expertise in hierarchical models and longitudinal data analysis. Other team members have developed tools (e.g., investigators Thomas and Kleinstein), and they lead the dozen studies contributing data and specimens (investigators Leung, Martin, Ross, Schultz-Cherry, and Thomas as well as PIs Cowling, Cobey, and Tsang). The team's embedding in major networks in influenza research (CIVIC[36], CEIRR[37]), infectious disease modeling (MIDAS[38]), and immunology (HIPC[39], AIRR[40]) should accelerate scientific discovery and tool dissemination.

Main data and sample sources. We will develop models incorporating high-quality observations of vaccine responses and infection over time in diverse populations. Critically, it is not necessary for our approaches that all studies measure the same facets of immunity, ascertain infections the same way, or obtain specimens at the same times: unmeasured variables can be treated as latent or unobserved states, and we can integrate over uncertainty in what was not observed. We will synthesize data from observational studies and clinical trials conducted in the United States (US), Hong Kong (HK), and Colombia (Figs. 1 and 2):

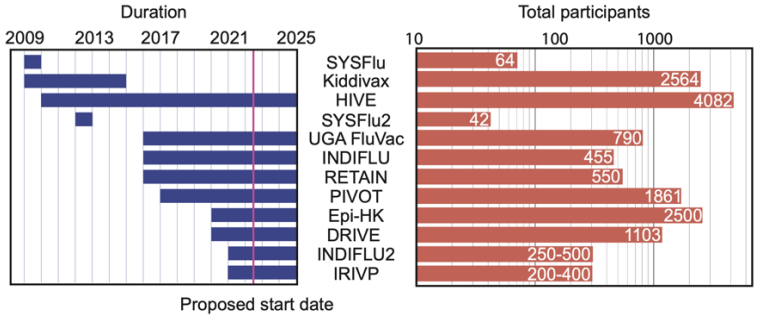


Figure 1. The primary analyses use data from 12 studies conducted since 2009 and comprising observations from ~14,700 people.

1. **SYSFlu** (Systems immunology of seasonal + pandemic H1N1 vaccination) is a trial of NIH employees who received both the seasonal trivalent and H1N1pdm09 vaccines (ClinicalTrials.gov: NCT01191853).
2. **Kiddivax** (A randomised controlled trial of the effectiveness of vaccinating children to reduce household transmission of influenza) is a cohort study involving 796 children and all members of their households (NCT00792051)[24,41,42]. In the first year only, children were randomly assigned to receive vaccine or placebo.
3. **HIVE** (Household Influenza Vaccine Evaluation) is a household cohort that since 2010 has been used to estimate vaccine effectiveness in the US and correlates of protection.
4. **SYSFlu2** (Systems immunology of H5N1+/- AS03 vaccination, 2012-13) randomized NIH employees to receive the adjuvanted or non-adjuvanted two-dose H5N1 (+/-AS03) vaccine (NCT01578317).
5. **UGAFluVac** is an open observational cohort that investigates responses to different seasonal influenza vaccines and vaccine platforms. Many participants have enrolled in multiple years.
6. **INDIFLU** (Influenza in Indigenous Populations of Colombia) is a cohort study of indigenous Colombians.
7. **RETAIN** (Immunogenicity of twice-annual vaccination against seasonal influenza for two hemispheres in older adults in HK—a randomized controlled trial) assigned older adults to receive annual or semi-annual vaccination (NCT02957890).

Figure 2. Study characteristics. *Includes 0 h, 2 h, 4 h, 12 h and 24 h after vaccination. †Before 3/2020 and after influenza resumes circulating. The protocol for INDIFLU included active surveillance but is shown as passive due to low engagement.

8. **PIVOT** (Immunogenicity of alternative annual influenza vaccination strategies in older adults in Hong Kong –a randomized controlled trial)[43] randomized participants to receive Northern hemisphere formulations of a standard-dose quadrivalent vaccine, MF59-adjuvanted trivalent vaccine, high-dose trivalent vaccine, or

Flublok. In years 2-4 participants were re-randomized to receive the same or a different vaccine, resulting in alternative combinations of vaccines. In years 5-8 all participants receive Flublok annually (NCT03330132).

9. **Epi-HK** (Evaluating Population Immunity in Hong Kong) is a community-based cohort study of individuals from the general community. They are followed prospectively to assess immunity and infections.
10. **DRIVE** (Dynamics of Immune Response to Repeat Vaccination) is a randomized, placebo-controlled trial of adults in HK (NCT04576377). Individuals receive either Flublok or a placebo once annually.
11. **INDIFLU2** (Influenza Viral Infection and Immunity in Indigenous Populations) is a new case-ascertained household transmission study. Households will be longitudinally followed up for 180 days.
12. **IRIVP** (Immune Responses in Indigenous Vaccine-Naïve Populations) is a cohort of rural indigenous residents in Colombia, who will be receiving annual TIV/QIV. The study will start at the end of 2021.

Additional data sources for testing. The studies above provide the main data to fit and evaluate models. We will perform cross-validation within and across those studies. We will also assess generalizability by measuring the performance of our models in new contexts:

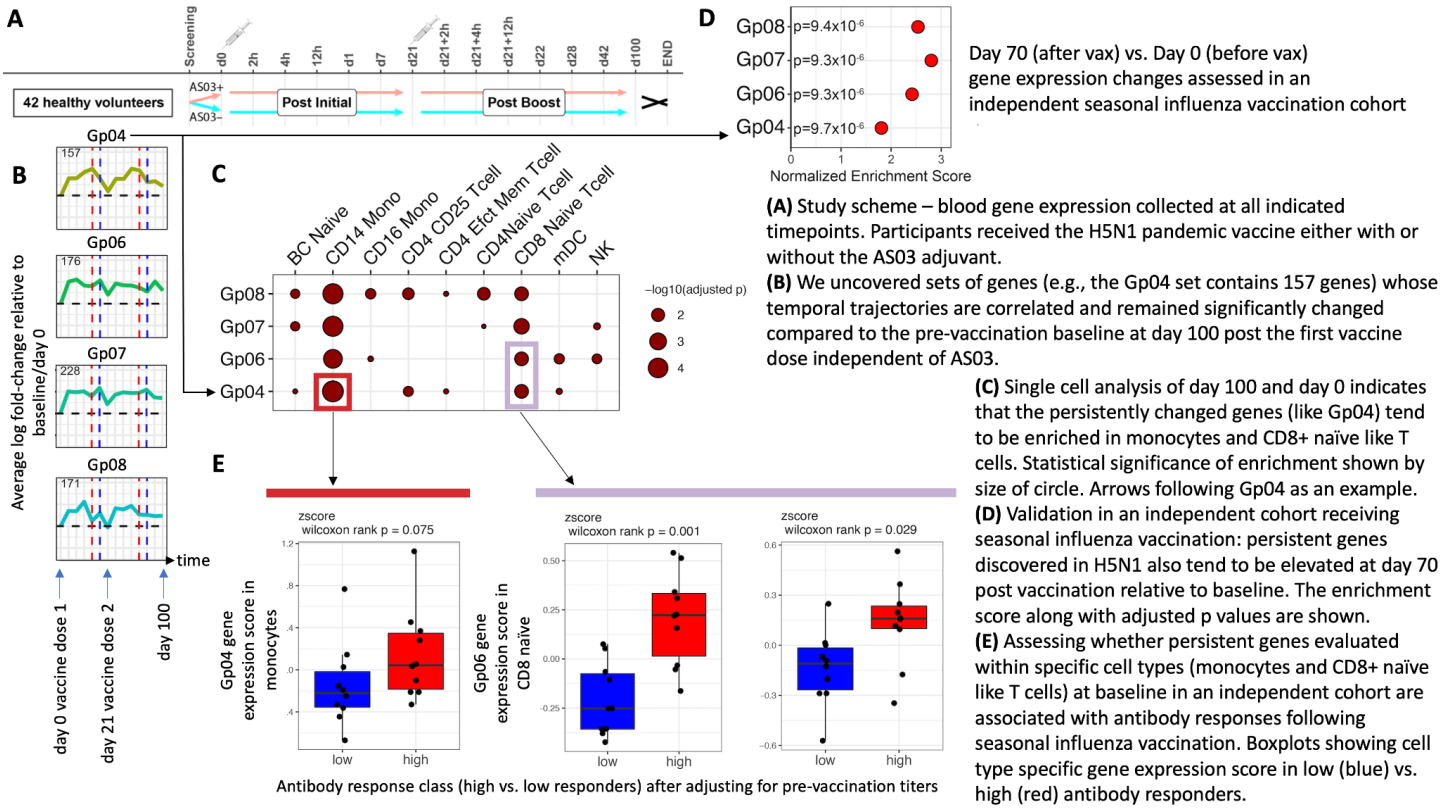
1. Our colleagues Richard Webby and Sue Huang will share data from their studies in New Zealand (see Letter of Support). The original **SHIVERS** (Southern Hemisphere Influenza and Vaccine Effectiveness Research Surveillance, 2012-2017) surveilled for respiratory illness, conducted serology, and recorded vaccination history.[44] **SHIVERS 2** (WellKiwis Adult, 2018-2025) studies the impact of repeat vaccination on influenza infection and has enrolled >1500 participants.
2. Investigator Thomas will share data from his **FLU2009** study. A total of 73 index cases with respiratory illness and 126 asymptomatic household contacts were enrolled from 2009-2011 with vaccination history recorded since 2006. In total, 84 participants (including households) tested positive for influenza; 19 were hospitalized.
3. The **HIPC Signatures Project** (co-led by investigator Kleinstein) curated 16 public influenza vaccination datasets including HI/MN and blood transcriptomics (~700 subjects covering different seasons and geographic locations). We plan to use these measures in Aims 1 and 2.
4. We will also use publicly available data from other studies investigating immune states and influenza exposures. As of 8/25/21, we found 81 relevant **ImmPort** entries, with 73 focusing on influenza vaccine responses and most reporting blood transcriptional and HI titers.

	HAI	NAI	HA neut. ELISA		AD**	FcR	Cyto/chemokine	Transcriptomics: Single cell	Transcriptomics: Bulk	Flow cytometry
SYSFlu			MN H1, B, H3					CITE-seq	micro-array	clinical flow (T, B, NK) 10 color flow (HIPC)
Kiddi-vax	●									
HIVE	●	●	Lucifer-ase							T cell/mono -cyte ICS
SYSFlu2	●		MN, H1, H5				●	CITE-seq	micro-array	Th17,Treg, DC,Th1/2, B naive/ memory sorted
UGA FluVac	●	●	FRA	HA, IgG, IgA				●		T cell/mono-cyte ICS,hi-dim phenotype
INDIFLU	●		MN H3, H1, B	stem, NS, NA, IgG†, IgA†	●	●	●†	●		T cell/mono-cyte ICS, hi-dim phenotype
RETAIN	●	●		stem, NA	●*					T cell ICS
PIVOT	●	●	MN H3N2	stem, NA						T cell ICS
Epi-HK	●		MN, FRNT	stem, NA, NP, IgG subclass isotype						T cell ICS
DRIVE	●		MN H3N2	H3N2		●†	●	B cells: rep-seq, expres-sion	immune cell freq., epi-gen	T cell ICS, B cell w/ recomb. probe
INDI-FLU2	●		MN H3, H1, B	stem, NS, NA, IgG†, IgA†	●		●†	●		T cell/mono-cyte ICS, hi-dim phenotype
IRIVP	●		MN H3, H1, B	stem, NS, NA, IgG†, IgA†	●		●†			T cell/mono-cyte ICS, hi-dim phenotype

Table 1. Select assays for comparison across studies. AD**: ADCC, ADCP, ADNK. *ADCC only. † Nasal specimens obtained.

Aim 1. Assess the impact of vaccination/infection history on antigen-agnostic baseline immune states

Background and preliminary data. The impact of vaccination and infection history on variable vaccine and infection responses, especially adaptive immune responses, has long been recognized. What is less explored is whether and how prior exposures impact antigen-agnostic immune states, i.e., the possibility that an exposure can establish new, stable baseline immune states, e.g., altering the transcriptional and epigenetic states of cells,



(A) Study scheme – blood gene expression collected at all indicated timepoints. Participants received the H5N1 pandemic vaccine either with or without the AS03 adjuvant.
(B) We uncovered sets of genes (e.g., the Gp04 set contains 157 genes) whose temporal trajectories are correlated and remained significantly changed compared to the pre-vaccination baseline at day 100 post the first vaccine dose independent of AS03.
(C) Single cell analysis of day 100 and day 0 indicates that the persistently changed genes (like Gp04) tend to be enriched in monocytes and CD8+ naïve like T cells. Statistical significance of enrichment shown by size of circle. Arrows following Gp04 as an example.
(D) Validation in an independent cohort receiving seasonal influenza vaccination: persistent genes discovered in H5N1 also tend to be elevated at day 70 post vaccination relative to baseline. The enrichment score along with adjusted p values are shown.
(E) Assessing whether persistent genes evaluated within specific cell types (monocytes and CD8+ naïve like T cells) at baseline in an independent cohort are associated with antibody responses following seasonal influenza vaccination. Boxplots showing cell type specific gene expression score in low (blue) vs. high (red) antibody responders.

Figure 3. Persistently elevated transcriptional signatures after vaccination.

including longer-lived lymphocytes specific not only to influenza. We and others have shown that the statuses of certain immune cells before vaccination (pre-vaccination baseline), such as non-antigen-specific cell frequencies or cell-type-specific transcriptional signatures, can predict and therefore potentially impact antibody responses to vaccines[12,45]. Our recent study[6] revealed that such baseline antigen-agnostic "set points" can be shared by different vaccines (influenza and yellow fever) in naïve and previously exposed populations and implicated a cellular circuit involving myeloid and lymphoid cells in the set points. Our hypothesis for this aim causally links vaccination history to baseline immune states that impact later vaccination responses; it is also consistent with the concept of "trained immunity"[46], which posits that prior inflammatory encounters can induce long-lasting, antigen-non-specific memory, which in turn can influence future perturbations. This hypothesis was originally motivated by the epidemiological observation that BCG vaccination increases protection against multiple pathogens and reduces all-cause mortality[46]; there are also recent reports of potential protection from SARS-CoV-2 infection associated with prior seasonal influenza vaccination[47–50]. However, the molecular and cellular mechanisms mediating these effects remain largely unknown, although chromatin modifications in myeloid cells and their progenitors in the bone marrow are potential mechanisms[51,52].

We have assessed H5N1 influenza vaccination with or without the AS03 adjuvant after prime and boost. Elevated transcriptional signatures induced by the vaccine persisted 100 days after the first dose, independent of adjuvant (Fig. 3). Single-cell CITE-seq profiling revealed classical monocytes and CD8+ naïve-like T cells in these persistent signatures. Furthermore, these signatures evaluated in an independent cohort via single-cell data predicted antibody responses to vaccination, independent of pre-vaccination titers, suggesting that baseline immune states can be tuned by prior vaccinations and impact future responses in an antigen-agnostic manner. Cell-type-specific differences at baseline between high and low antibody responders also correlated with the extent of activation of the same cell types in the 24 hours after vaccination (data not shown). Linking baseline to innate response variability—the earliest vaccine responses—is important for understanding heterogeneous vaccine outcomes.

Approach. We have developed an experimental and computational toolkit that integrates MSC analysis (covering ~200 surface proteins, transcriptomes, BCR/TCR V(D)J sequences, and chromatin accessibility) and blood/PBMC transcriptomics (and other omics) to analyze vaccine and infection responses in human cohorts[10,53–55]. This involves multiplexed (i.e., host genetic variation to resolve individuals and barcodes to resolve time: ~40 samples/batch) PBMC and analysis of enriched rare cell subsets (e.g., pDCs, Tscm); computationally, we utilize

custom protein denoising and normalization with hierarchical statistical models to uncover the effects of biological variables (e.g., vaccination, time, and age) on cell-type-specific transcriptional and epigenetic states. Due to cost, MSC cannot be applied to many samples. Thus, our approach is to integrate blood transcriptomics (BTX) and MSC. The former is inexpensive for larger sample sizes, while the latter offers the resolution to deconvolve cell-type-specific differences. As shown in our preliminary (Fig. 2) and published data[10,54,56], cell-type-specific signatures, once discovered via MSC, can be evaluated using BTX; conversely, BTX signatures can be evaluated to identify cell-type-specific contributions. The sample sizes for the proposed BTX and MSC analyses derive from our existing data and experience analyzing responses to influenza vaccines and SARS-CoV-2 infections.

Assess and define long-lasting antigen-agnostic transcriptional signatures induced by vaccination via MSC and BTX analyses. Our first goal is to further assess the effect of vaccination on long-lasting, cell-type-specific signatures that might affect later vaccination responses. Using existing resources, we will expand our MSC analysis to days 100 and 70 samples from our SYSFlu2 and SYSFlu cohorts, respectively, to examine the cell-type-specific signatures (SC5 and SC6; Table 2). Next, we will take advantage of the randomized design of DRIVE for assessing repeated vaccination effects (Table 2). (Note that BTX data will be generated outside this proposal; BTX4, Table 2.) By using pre- and day 91 post-vaccination samples from two arms (vaccination vs. placebo first year followed by vaccination of both arms in the second year), we will integrate MSC and BTX data to (1) further define signatures induced by vaccination, taking advantage of the matched placebo arm to assess whether signatures 91 days after vaccination are distinct and consistent with preliminary data. (2) Assess the temporal stability of the induced signatures: we will follow the same individuals over two years and use statistical approaches we developed previously[10,11,14] to evaluate temporal stability. We have often found that immune states are stable within individuals but markedly differ person-to-person. (3) We will follow the same subjects up to and after the second vaccination to evaluate the impact of the baseline, potentially altered by the first vaccine, on responses to the second vaccine. Analyzing both baseline and day 1 could reveal how the altered cellular states impact early responses. (4) Using data from 91 days after the second vaccination, we will assess the impact of the second vaccination on antigen-agnostic immune states, e.g., we will test for ceiling effects and whether

Dataset	Type	Source	Data generation/description	Status	Aims	Additional notes
SC1	MSC	DRIVE	First two years of DRIVE; N=20 (10 per arm) days 0 and 91 & days 0, 1, and 91 year 2	P	1 & 2	vaccine induced cell type specific states
SC2	MSC	DRIVE	Day 0 before final year vaccination in DRIVE for groups with controlled vaccination histories; N=5 per arm	P	1 & 2	helps to deconvolve cell type signatures in BTX3
SC3	MSC	PIVOT	Two arms: High-dose QIV and MF59+TIV, days 0, 30, and ~300; N=5 per arm	P	1 & 2	vaccine induced cell type speciifc states in elderly
SC4	MSC	HIVE	Two groups: with and without infection 2017-18 season pre- and post-season; N=5 per group	P	1 & 3	infection induced cell type specific states
SC5	MSC	SYSFlu	days 0 and 70 post QIV; N=20	O/A	1 & 2	like SC1
SC6	MSC	SYSFlu2	days 0 and 100 H5N1+/- AS03; N=10 per arm	O/A	1 & 2	like SC1
BTX1	WB Tx	PIVOT	A superset of SC3: 20 subjects per arm	P	1 & 2	see SC3
BTX2	WB Tx	HIVE	A superset of SC4: 30 subjects per arm	P	1 & 3	see SC4
BTX3	WB Tx	DRIVE	A superset of SC1: all 5 years, baseline and post	O	1 & 2	association of vaccination history vs. baseline states
BTX4	WB Tx	SYSFlu and SYSFlu2	A superset of SC5/6: all time-points subjects	A	1 & 2	baseline prediction model
BTX5	WB Tx	UGAFlu	Multi-year repeated vaccinees since 2017	O/A	1 & 2	like BTX3 but US population
BTX6	WB Tx	NCBI GEO	Natural infection or live influenza challenge studies with pre-infection baseline data (GEO: GSE68310; GSE17156; GSE30550; GSE52428; GSE73072)	A	1 & 3	Baseline predicts infection outcomes (symptomatic vs. not) validation cohorts
BTX7	WB or PBMC Tx	HIPC Signatures	16 influenza vaccination cohorts with day 0 (often also 1/3 and 7) and HAI/MN; N~=700	A	2	Baseline predicts vaccination outcomes validation cohorts

Table 2. Multimodal single cell and bulk transcriptomic data sets. P: this proposal; O: already funded to be generated/being generated; A: already available and ready for use. MSC refers to (proteins, transcriptome, BCR/TCR) and multiome/scATAC PBMC and select subsets (DCs, Tscm). WB or PBMC Tx: whole blood or PBMC RNA-seq or microarray.

participants receiving their first study vaccine mount similar responses in both years. (5) We will evaluate whether the signature induced by the first vaccination predicts the antibody response to the second vaccination.

Assess and define vaccine-induced, long-lasting, antigen-agnostic transcriptional signatures in older adults. The analyses above focus on younger adults. We will next generate BTX and MSC data from PIVOT (BTX1 and SC3; Fig. 2 and Table 2). We will focus on the high dose quadrivalent (QIV) without adjuvants and the MF59-adjuvanted trivalent (TIV). BTX data will be generated from baseline, d30, and ~d300 (baseline sample from the next season, i.e., the earliest long-term PBMCs available after d30) after vaccination from the 2017-18 season in 20 subjects of each vaccine arm; MSC data will be generated from a subset of 5 subjects per arm to computationally deconvolve cellular origin and epigenetic underpinning of transcriptional signatures.

Assess association between vaccination history and baseline immune states. An implication of our hypothesis is that signatures vary between vaccinees depending on vaccination history. We will test this expectation with DRIVE transcriptomic data (BTX1 and SC2; Table 2) by comparing subjects from different arms. Data from the UGAFlu and HIPC cohorts (with exposure history inferred from baseline antibody titers[24,57]) provide further tests.

Assess and define long-lasting antigen-agnostic transcriptional signatures induced by infection. We hypothesize that natural infections could also induce long-lasting, antigen-agnostic, cell-type-specific states that impact later responses to vaccines and infections. In addition to some viral and bacterial infections in animals[58–60], infections in humans are known to have long-lasting, non-antigen-specific effects, including unresolved immune cell activation and dramatic shifts in the B cell repertoire[61–64]. We will generate and analyze BTX and MSC data by comparing two matching groups from the HIVE study (Table 2, BTX2 and SC4): subjects who had symptomatic influenza infection in the 2017-2019 seasons vs. matched control participants without reported infections. Data will be generated from samples from the start and end of the season (pre- and post-infection). We will validate with public data from natural influenza infection studies (BTX6; e.g., GEO: GSE73072).

Leverage time-resolved single cell data to quantitatively model on how baseline immune states impact innate responses to vaccination. Our preliminary data suggest that altered innate responses after vaccination are a pathway through which variable baseline immune states can impact vaccination outcomes. Using DRIVE data from days 0 and 1 (second-year vaccine; SC1 and BTX3; Table 2), we will assess whether the baseline signatures induced by the first-year vaccine are associated with the innate response to the second. We will leverage this and other single-cell vaccine data (e.g., SC5 and SC6) to develop trajectory inference methods that integrate single-cell pseudotime and real time[65–67] to evaluate how altered baseline immune states change innate response dynamics. Waddington OT[66] infers “couplings” between ancestral and descendant cells at different timepoints, which we will use to connect cells from the baseline and day 1 after vaccination. We will extend the method to infer the rate at which coupled states change within hosts by comparing their gene expression differences. This analysis will also provide dynamic parameters (e.g., activation rate in DCs) for quantitative modeling of the cellular interactions, like our recent temporal-spatial cellular interaction model[68].

Expected outcome and significance. The first main outcome will be a set of cell-type-specific, antigen-agnostic transcriptional signatures induced by vaccination and infection, providing systematic analyses and information on their temporal stability and behavior (months to several years), and measures of these signatures’ associations with age and prior vaccinations. This set of baseline signatures will be tested for predictive capacity in Aims 2 and 3 to test the hypothesis that in addition to antigen-specific effects, prior vaccination and infection leave antigen-agnostic marks that predict and determine future responsiveness. Another significant outcome is a better understanding of how baseline immune states impact innate responses to vaccination.

Potential problems and alternative strategies. The sample sizes we proposed for BSC analysis may not fully resolve cell-type-specific signatures. However, our experience indicates that these numbers are sufficient, especially because we plan to use larger sample sizes from BTX analysis. We can perform cell-sorting followed by RNA-seq to further test suggestive signals emerging from BSC analysis. Given that we are leveraging diverse cohorts and samples, another caveat is that it is unclear how variables such as geographic region, demography, and vaccine type impact the signatures we aim to uncover. Our preliminary data suggest that these vaccine-induced effects are at least partly independent of the vaccine, since we detected similar changes by d100 with and without adjuvant and after the seasonal vaccine from a different year (Fig. 2). In addition, we will pool signals across datasets as we did in our meta-analysis of influenza vaccination cohorts[12]. Finally, seasonal variation in

gene expression may confound our results, but DRIVE's placebo and vaccine arms are well matched in timing.

Aim 2. Predict immune responses (including their magnitude, breadth and durability) to vaccination based on intrinsic characteristics, baseline immune states, and influenza exposure history.

Background and preliminary data. Many variables have been associated with individual variability in influenza vaccine responses. They include "intrinsic" characteristics such as sex[2–4], BMI[5,6], age[6–9], CMV serostatus[69], and high-risk medical conditions[70]. As discussed, individual variability is also associated with baseline immune states. These states can be antigen-specific, e.g., pre-existing HI titers and influenza-specific cellular immunity, and antigen-agnostic e.g., non-antigen-specific transcriptional signatures of immune cells[10,11]. It is also well known that individuals with higher initial antibody titers to the vaccine strain mount lower fold-changes due to the "antibody ceiling effect," potentially due to sequestration of antigen. Influenza exposure history also often contains additional information on vaccination outcomes. For example, we have found that even after adjusting for vaccine-specific antibody levels, repeat vaccinees boost their titers less[15,16]: two prior years of vaccination is enough to offset the benefits of increasing antigen dose three- or four-fold[16]. Data from the HIVE cohort suggest that in repeat vaccinees, boosted antibody titers also decay faster[71], suggesting poor responses of influenza-specific long-lived memory B or plasma cells. A longitudinal analysis has shown that individuals with confirmed influenza virus infections in the past ten years have higher antibody titers and higher titer boosts after influenza vaccination compared to people without such infections[17], demonstrating the need to track not only recent influenza vaccination but also infection history. Seminal work on original antigenic sin and more recent studies of vaccine effectiveness shows that vaccine responses can be affected by influenza virus infections even decades earlier[72–74].

The goal of Aim 2 is to develop predictive and biologically insightful models of vaccine responses by integrating data on intrinsic characteristics, baseline states, and influenza exposure history. Drawing on results from Aim 1, we will apply causal inference techniques and mechanistic modeling to learn the drivers of individual variability in vaccine responses. CMA is a set of statistical tools that can be used to estimate the causal links between variables based on a mechanistic understanding of the underlying processes. We have previously used CMA and related approaches to study risk of influenza virus infection[26,35] and other topics[75]. We also have a long track record of using mechanistic, dynamical models to extract insight about individual immune responses to influenza[76–82]. We have also modeled intracellular and intercellular interactions underlying immune response[83], including how regulatory T cells, conventional T cells, and dendritic cells can limit the peripheral expansion of autoreactive T cells and risk of autoimmunity[68].

***Approach*.** The goal of this aim is to predict immune responses to vaccination. We aim to develop and apply reusable conceptual frameworks that first transform population-level data into correlative insights via ML, then predict causal variables and parameters via CMA, and finally iteratively assess the mechanistic plausibility of proposed mechanisms via dynamical modeling. To facilitate comparison across studies, we focus mainly on antibody responses to HA, an established mechanism of protection. We will determine the factors that best explain variability in the magnitude, durability, and breadth of HA responses. *Magnitude* will be quantified as both the titer rise after vaccination and the post-vaccination titer to the vaccine strain; the former sheds light on immune activation and the latter on epidemiology. *Breadth* will be measured as the response to heterologous strains which are not part of the vaccine. *Durability* will be measured as the rate of waning after vaccination and peak titer (whose timing can be estimated, or which can be assumed to be 30 days post-vaccination with sensitivity analysis for uncertainty); to measure this waning rate, we will account for the probability of infection (by, e.g., incorporating confirmed infections, boosts to NA and HA titers, and information on circulating subtypes). We will examine support for bi-phasic waning or waning that follows a power law[35,84,85].

Statistical and ML analysis. We favor using so-called "explainable" ML approaches that quantify the contribution of individual variables, as in the cross-validation framework/R package we developed for using the elastic net family of models to uncover predictors[86]. First, we will use information on intrinsic characteristics, baseline immune states (including signatures from Aim 1), and influenza exposure history, and build Bayesian hierarchical multivariable models to investigate associations. We are currently using similar approaches, including boosted regression and classification trees, random forest models, support vector machines, and regularization methods such as elastic nets[87–89]. We will test not only hypothesized and established drivers but also more complex covariates, such as general effects attributable to the study, year, and individual. If these variables are highly predictive, they imply studies are not measuring the appropriate immunological variables, or their complex relationships are not represented accurately. As in our previous work, to minimize the risk of overfitting, we will

perform within-cohort cross-validation and across-cohort train/test approaches with importance sampling. We will additionally use analyses such as leave-some-(individual)-out analyses across different studies. From this approach, we will be better able to determine which inputs show a robust correlation with the different outcomes.

Causal modeling. The second stage is to focus on predictors from above and explore potential causal pathways. It is possible for causal variables to not show strong statistical association. Thus, we will use biological expert knowledge and results from the previous analyses to inform the models. Consider the directed acyclic graph (DAG; Fig. 4), which shows postulated pathways from input variables (identified in the previous stage) to the outcome through putative biological processes, indicated by arrows. By exploring variations of this DAG with different causal paths turned off, and assessing the fit of such sub-DAGs with the data, we can hone in on the likely causal processes.

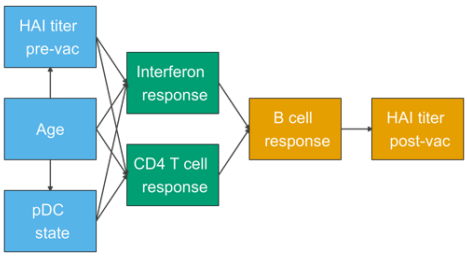


Figure 4. Example causal diagram (DAG) of mediation model between pre-vaccination HI titers, age, pDC activation state, interferon and CD4 T cell response and subsequent B cell response and HAI (HI) titers following vaccination.

Mechanistic modeling. CMA indicates how variables causally influence others. Next, we will implement models that explicitly represent putative dynamical interactions between variables. Such models are often formulated as sets of differential equations. They allow us to explore putative mechanisms and discriminate hypotheses through comparison with data. Consider a simplified version of the DAG in Fig. 4, where we ignore the possible impacts of age and immune baseline status. We can formulate a mechanistic model that shows how, after vaccination, antigen interacts with interferon and CD4+ T cells to influence B-cell response and thus vaccine-induced antibody titers (Fig. 5). In one version, induction and proliferation is assumed to be proportional to antigen and interferon (red). An alternative has induction proportional to CD-4 T-cells and interferon (blue), with antigen only acting indirectly through CD4+ T cells. More immunological complexity, negative feedbacks, and separation of timescales can be incorporated based on existing knowledge and emerging data.

$$
\begin{aligned}
\text{Antigen} \quad & \dot{H} = -kAH - cH \\
\text{Interferon} \quad & \dot{F} = p - mF + q(F_{max} - F)\frac{H}{H+n} \\
\text{CD4+ T cells} \quad & \dot{T} = \frac{FHT}{FH + h_T} + g_T T \\
\text{B cells} \quad & \dot{B} = gB\left(\frac{r_H HF}{s_1 + HF} + \frac{r_T TF}{s_2 + TF}\right) \\
\text{Antibodies} \quad & \dot{A} = rB - kAH - dA
\end{aligned}
$$

Figure 5. Example mechanistic dynamical model formulated as ordinary differential equations. Vaccine antigen induces interferon and CD4 T-cell response, which in turn trigger B cell and subsequent antibody responses.

The proposed mechanistic modeling component serves two purposes: Mechanistic models can evaluate the strength of support for different hypothesized interactions, and the models can make predictions about the behavior of the system, e.g., how a change in interferon might impact antibodies. Due to uncertainty in molecular and cellular parameters, we have also developed approaches to use ML to learn from simulations of the entire plausible parameter space of such models to uncover parameter combinations or interactions most important for determining the immune response outcomes[68,90].

To fit mechanistic models, we will use frequentist approaches, which have the advantage of being fast, and Bayesian approaches, which have the advantage of incorporating informative priors. Frequentist fitting of dynamical models will use the *pomp* R package[91–93], which performs likelihood-based estimation of stochastic dynamical systems using iterated particle filtering, and with which we have experience[24,94]. For Bayesian inference, we will use pMCMC for stochastic dynamical models involving discrete parameters and Stan (e.g., *brms* package[95]) for models without discrete parameters. Model performance will be judged using WAIC and LOOIC[96] with omitted participants and time points. Cross-validation will later be extended to independent cohorts and data sets.

Expected outcome and significance. The first outcome will be an evaluation of the strongest predictors, in terms of variance explained, of the three metrics of vaccine responsiveness (magnitude, duration, and breadth). This will be coupled with a predictive statistical model of individual vaccine responsiveness. Currently there are no models to predict how well someone will respond to influenza vaccination: this work will thus pose an advance for influenza and a starting point for other vaccines. The second outcome will be insight into the causal relations underlying vaccine responses, including insight into how antigen-agnostic signatures impact outcomes.

Potential problems and alternative strategies. As mentioned above, if naive variables such as study location perform well, we can conclude the studies are not measuring the right biological variables, or the interactions are sufficiently nonlinear and complex that we have not measured the right variables with sufficient sample size. Discovering that predictors of vaccine response in one study provide little insight into the next will be informative and implies traditional studies need to be designed differently, or potentially that measurement error (e.g., batch effects) needs to be reduced. It would also be interesting to learn that a plausible but vague immune perturbation (e.g., a confirmed influenza infection) is predictive while precise immune measurements are not; this again would imply the wrong variables are being measured. Most likely, we expect a combination of more and less mechanistic variables to be predictive. Such a result naturally leads to pathways for further investigation.

Aim 3. Predict multidimensional correlates and mechanisms of protection against infection and disease.

Background and preliminary data. It is valuable to identify which immune markers play a causal role in protection against influenza virus infection, so that the marker can be used as a proxy outcome to judge candidate vaccines and to improve understanding of the mechanisms of protection. In a previous analysis of the Kiddivax data, co-PI Cowling used a CMA framework to estimate that the HI titer mediates more than half of the protective efficacy of inactivated influenza vaccination in children[26]. Other studies, including previous analysis of the HIVE cohort, have estimated that anti-stem and anti-NA antibodies separately contribute to protection[22,23], and have linked influenza disease severity to coinfection with herpesvirus[97]. Baseline innate immune status may also play a role in protection and disease severity[98,99]. We have used longitudinal mechanistic modeling to demonstrate in the Kiddivax cohort that HI predicts infection risk over time in children, whereas time since last infection better predicts infection risk in adults[24]. These correlates have largely not been integrated, especially into predicrive models that recognize that different immune mechanisms might lead to protection in different individuals.

Approach. The goal of this aim is to determine correlates and mechanisms of protection against infection and disease. Infections are identified by PCR in studies with active surveillance, but even active surveillance will miss mild infections that can result in seroconversion. We will infer these subclinical infections probabilistically from longitudinal serology as before[24], but also considering seroconversion by NAI. Disease will be determined on a severity scale, since symptom data are available for all studies conducting surveillance.

Statistical and ML analysis. The approach will follow that of Aim 2. The only changes are that the outcomes of interest are different, and the model inputs include time of vaccination and post-vaccination immune states. When many transcriptional immune state variables are included, we will apply an approach we developed in recent work that uses conditional independence inference to distill direct correlates of disease severity[53].

Causal analysis. We will again use causal mediation analysis to explore putative mechanisms of protection. The strongest associations identified through the statistical/ML models and other leading hypotheses will be evaluated. In Fig. 6, vaccination is hypothesized to increase HI titers, and higher HI titers reduce the risk of clinical infection. We hypothesize that influenza vaccination also confers protection against infection through other mechanisms, including the innate antigen-agnostic mechanisms from Aim 1. By comparing different DAGs to data, we can determine which components likely form causal pathways and which do not. Many mediators (NAI, stem, immune states such as those from Aim 1) will be tested. Using DAGs for causal modeling also allows for the detection of confounders, colliders, and other types of bias to inform the other modeling approaches we will use.

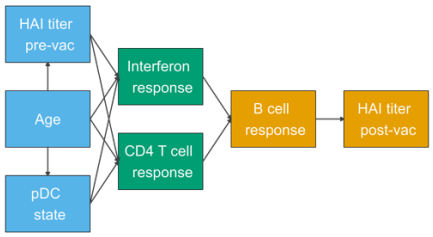


Figure 6. Example causal diagram (DAG) of mediation model between vaccination, intrinsic characteristics, post-vaccination HI titer, and infection.

Mechanistic modeling. In tandem, we will fit mechanistic models to test competing explanations of response variability and mechanisms of protection. The CMA might suggest within-host components that drive protection. We can run simulation models like the one shown in the Aim 2 to explore how different levels of those immune components might lead to rapid viral clearance and thus subclinical infection. This also allows us to quantify how different immune states impact the outcome. Since this is an inherently probabilistic question, we will use stochastic mechanistic models and sampling of parameters and initial conditions to determine the probability of clinical infection for different initial conditions[90]. We will also extend our individual-level dynamical, longitudinal mechanistic models that predict infection risk given time-changing, partially observed immune states[24]: rather

than a simple function of HI titer or time since last infection, we can test models where individual risk is a function of several latent immune (and other) states. This extends the more naive ML approaches to incorporate both latent and cell-specific state dynamics and again helps evaluate plausibility of different protective mechanisms.

Expected outcome and significance. The outcomes of Aim 3 will be multivariate statistical models that predict individuals' protection against influenza virus infection of varying severity. These correlates of protection will include combinations of parameters and covariates that together may capture equivalent risk. Additionally we will have identified the likeliest mechanisms of protection using CMA, whose plausibility we will test through mechanistic models. We will evaluate how results vary with age, race/ethnicity, and other variables.

Potential problems and alternative strategies. As in Aim 2, the best-fitting models might be vague or inconsistent with plausible biology. This is still a scientific insight and a necessary starting point for iterative model refinement and data collection, in that it implies the measurements are poor or the biology oversimplified. As an alternative to mediation analysis, we will investigate the controlled risk curve over different biomarker levels[100]. This approach (controlled effects causal inference) can help us adjust for unmeasured confounding.

OUTREACH AND TOOL DEVELOPMENT

We aim not only to develop accessible statistical and modeling tools but also to educate other scientists on the logic of their use. Thus, our tool dissemination is grounded on teaching:

- **Tutorials and online learning materials.** Our review for *Nature Reviews Immunology*[101] and course website[102] describe the why and how of mechanistic simulation modeling in immunology. For this project, we will write a "roadmap" paper covering the modeling approaches described here. We will develop supplementary online materials (tutorials, exercises, etc.). Our aim is to show when different approaches are appropriate.
- **Workshops.** Several of the investigators (Cobey, Handel, Thomas) have extensive experience teaching workshops on longitudinal, within-host data analysis and modeling, e.g. as part of the annual Summer Institute in Statistics and Modeling in Infectious Diseases (SISMID)[103]. We will conduct at least two workshops on modeling and data analysis in immunology. The target audience will be bench scientists and interested quantitative scientists. To increase equity, we will offer these workshops online and free of charge.
- **Software to learn dynamical modeling.** We previously developed the software package *Dynamical Systems Approaches to Immune Response Modeling* (DSAIRM)[104,105]. DSAIRM teaches the basics of modeling immune responses to scientists with little coding experience. We will build more teaching units in DSAIRM featuring influenza responses, as well as units that introduce the other modeling approaches here.

We will also produce software resources that are fully documented and easy to use (e.g., containerized):

- **Flow cytometry software.** Analysis of flow cytometry data has become a staple of immunological research. While tools for data preparation and limited analysis exist (e.g., SPICE[106]), there are no user-friendly, comprehensive tools that allow investigators to explore data (e.g. to check dynamic range) and perform preliminary analyses (e.g., clustering analysis). Dr. Thomas and his team have written in-house code for some of these common tasks, and Dr. Tsang has developed other methods for flow analysis[107,108]. Dr. Handel and his team will use their experience developing R packages and interfaces[104,105,109–112] to integrate these tools.
- **OMiCC** is a free, community-based, biologist-friendly web platform we developed for annotating, creating, and meta-analyzing signatures from gene-expression data[115–117]. The platform currently hosts community-created signatures and data from >40,000 human and mouse studies from Gene Expression Omnibus (GEO) and recount2. We will deposit the expression signatures we identify here to facilitate analysis by others.
- ***modelbuilder.*** This R package[118] allows users to build dynamical simulation models without having to write code. While it is currently possible to build and analyze models, the ability to fit models to data is not yet available in the package and remains a hurdle. We will add this functionality by integrating several powerful R packages (*nloptr, pomp, brms)* that allow fitting of compartmental models in different statistical frameworks (frequentist and Bayesian, deterministic and stochastic).
- **Analysis of time-resolved, MSC data in human cohorts.** Multimodal single-cell data (e.g., proteins, mRNA, TCR/BCR, chromatin accessibility) from multiple individuals over time are challenging to analyze. We have been developing a suite of approaches[53,55,119] involving noise removal as well as hierarchical modeling and ML to integrate single-cell, temporal, and human population variation to assess various biological (e.g., age, individual) and temporal effects. As a part of this project, we will add more features to infer cellular dynamics to build mechanistic cellular interactions models.

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