The Journal of Infectious Diseases

MAJOR ARTICLE

The Negative Effect of Preexisting Immunity on Influenza Vaccine Responses Transcends the Impact of Vaccine Formulation Type and Vaccination History

Savannah A. Moritzky,[1] Katherine A. Richards,[1] Maryah A. Glover,[1] Florian Krammer,[2,3] Francisco A. Chaves,[1] David J. Topham,[1] Angela Branche,[4] Jennifer L. Nayak,[5] and Andrea J. Sant[1]

[1]David H. Smith Center for Vaccine Biology and Immunology, Department of Microbiology and Immunology, University of Rochester Medical Center, Rochester, New York, USA, [2]Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, New York, USA, [3]Department of Pathology, Molecular and Cell Based Medicine, Icahn School of Medicine at Mount Sinai, New York, New York, USA, [4]Department of Medicine, Division of Infectious Diseases, University of Rochester Medical Center, Rochester, New York, USA, and [5]Department of Pediatrics, Division of Pediatric Infectious Diseases, University of Rochester Medical Center, Rochester, New York, USA

The most effective measure to induce protection from influenza is vaccination. Thus, yearly vaccination is recommended, which, together with infections, establishes diverse repertoires of B cells, antibodies, and T cells. We examined the impact of this accumulated immunity on human responses in adults to split, subunit, and recombinant protein-based influenza vaccines. Enzyme-linked immunosorbent assay (ELISA) assays, to quantify serum antibodies, and peptide-stimulated CD4 T-cell cytokine ELISpots revealed that preexisting levels of hemagglutinin (HA)-specific antibodies were negatively associated with gains in antibody postvaccination, while preexisting levels of CD4 T cells were negatively correlated with vaccine-induced expansion of CD4 T cells. These patterns were seen independently of the vaccine formulation administered and the subjects' influenza vaccine history. Thus, although memory CD4 T cells and serum antibodies consist of components that can enhance vaccine responses, on balance, the accumulated immunity specific for influenza A H1 and H3 proteins is associated with diminished future responses.

Keywords. influenza; vaccines; CD4 T cells; immune memory; human immunity.

Sterilizing immunity to influenza virus primarily relies on neutralizing hemagglutinin (HA)-specific antibodies that block infection of host cells. The most common strategy to induce this protection for humans is intramuscular vaccination, typically involving inactivated influenza vaccines derived from viruses anticipated to match circulating strains (reviewed in [1]). Because of antigenic drift and waning of serum antibodies over time, many countries recommend annual influenza vaccination. These vaccines provide only incomplete protection, depending in part on their match with the viruses that emerge in the subsequent influenza season [2, 3]. Despite the benefits of yearly vaccination, it has long been recognized that responses to influenza vaccines can be highly variable. Publications have considered the mechanisms that underlie deficiencies in vaccine responses. These include a history of repeated vaccination, vaccine type, preexisting B cells, and circulating antibodies (reviewed in [4–7]). There are also genetic signatures in nonantigen-specific cells within peripheral blood prior to vaccination that are correlated with influenza vaccine response magnitude [8–12].

Accumulated studies have illustrated the range in responses to vaccination. Our laboratory [13–15] and others [16, 17] have demonstrated variable B-cell responses, measured by serum antibodies detected by hemagglutination inhibition assays, microneutralization assays, or enzyme-linked immunosorbent assays (ELISA). Measurements of plasmablasts emerging into peripheral blood also show the variability in response magnitude (reviewed in [18]). Our studies have mainly focused on CD4 T-cell responses, revealing that human subjects can elicit more than several hundred-fold differences in response magnitude, when measured by cytokine enzyme-linked immunospot (ELISpot) assays or intracellular cytokine staining [13–15, 19, 20], in agreement with others using these and other experimental strategies [21–24]. Because of the variability in responses to influenza vaccination in humans, we sought to identify the key parameters in the preexisting T-cell and B-cell repertoire that most robustly predict response magnitude, with the hope that such knowledge would provide insight into mechanisms and development of strategies to predict responses. If successful, individuals anticipated to mount relatively weak responses could be vaccinated with increased doses of vaccines or with vaccines that include adjuvants [25, 26].

In this study, we have comprehensively evaluated features of the preexisting immune repertoire in a cohort of over 230

Received 17 December 2021; editorial decision 19 February 2022; accepted 22 February 2022; published online 24 February 2022.

Correspondence: Andrea J. Sant, PhD, University of Rochester Medical Center, David H. Smith Center for Vaccine Biology and Immunology, 601 Elmwood Avenue, Box 609, Rochester, NY 14642 (andrea_sant@urmc.rochester.edu).

The Journal of Infectious Diseases® 2023;227:381–90

https://doi.org/10.1093/infdis/jiac068

human subjects who varied in vaccine history and were administered trivalent or quadrivalent influenza vaccines, collectively, over the course of 5 years. We have quantified total preexisting anti-HA antibodies and the levels of preexisting CD4 T cells specific for influenza A H1 and H3. The relationships between these variables in the preexisting immune repertoire and the elicited B- and T-cell responses to vaccination were quantified. These studies revealed that the variables of vaccine type and previous vaccine history do impact vaccine responsiveness but do not explain the range in response magnitude. Instead, several notable elements in the prevaccination immune repertoire were associated with alternative outcomes to vaccination. Most striking were our findings that revealed a negative relationship between the abundance of influenza-specific CD4 T cells and future expansion of CD4 T cells after vaccination, and an inverse relationship between preexisting circulating levels of anti-HA antibody and subsequent CD4 T-cell and B-cell responses to licensed influenza vaccines.

METHODS

Study Design

The study took place in 5 seasons between 2015 and 2020 and was approved by the Division of Microbiology and Infectious Diseases and the University of Rochester Research Subjects Review Board (protocol l5-0055). Healthy human subjects aged 18–49 years were enrolled in each year of the study after informed consent and received either Fluzone, Flublok, or Flucelvax. Blood was drawn at day 0, before vaccination, and at days postvaccination (Supplementary Figure 1). Day 14 postvaccination samples of plasma and peripheral blood mononuclear cells (PBMC) were used, as these samples exhibited peak responses (data not shown). Plasma was removed and PBMC were isolated and frozen until use. Self-reported vaccination history in the previous season was recorded for each subject, and approximately half of the subjects were vaccinated in the previous season. Eight subjects were reenrolled in sequential seasons.

Peptides

A/California/04/2009 (H1N1) HA (H1) and A/New York/384/2005 (H3N2) HA (H3) peptide libraries were obtained from BEI Research Repository, National Institute of Allergy and Infectious Diseases, National Institutes of Health. Peptides were 15–17mers with 11 amino acid overlap encompassing the entire translated sequence of HA proteins. Peptides were dissolved using dimethyl sulfoxide and phosphate-buffered saline (PBS) with empirically determined ratios of the 2 solvents and dithiothreitol for cysteine-containing peptides. Each peptide was used at a final concentration of 0.5–1 μM. The H1 peptide pool has a > 95% identity across each vaccine. The H3 peptide pool was supplemented with peptides, purchased from Genescript, chosen by aligning H3N2 strains from 2015 to 2020 vaccine formulations with A/New York/384/2005 HA. A negative pool of peptides from Sin Nombre virus glycoprotein precursor or a media-alone control was used to estimate and subtract background.

Cytokine Enzyme Linked ImmunoSpot Assay

Interferon-γ (IFN-γ) ELISpot assays were performed as previously described [27]. In brief, PBMC were thawed, rested overnight in culture, then washed. $CD8^+$ and $CD56^+$ cells were depleted from the PBMCs using magnetic-activated cell sorting microbeads (Miltenyi Biotec) and typically 50%–60% of the enriched population were CD4 T cells, containing less than 1% CD8 T cells. CD4 T-cell–enriched PBMC were cultured with peptide pools in 96-well plates coated with anti-human IFN-γ (clone 1-D1K, MabTech, 10 μg/mL) for 36 hours. Plates were washed, incubated with biotinylated anti-human IFN-γ (clone 7-B6-1, MabTech; 1 μg/mL) for 1 hour, then washed, incubated with alkaline phosphatase-strepavidin (Jackson Laboratories; 1:1000 dilution) for 30 minutes and then developed for detection of cytokine-producing cells using Vector Blue Substrate kit (Vector Labs) and scored using an Immunospot reader (series 5.2), using Immunospot software, version 5.1.

Enzyme-Linked Immunosorbent Assay

Plates were coated with 200 ng/well purified HA proteins from A/California/04/09 (H1) (BEI Resources) or produced in-house using a mammalian expression system [28], or with A/Switzerland/9715293/13 (H3) (Protein Sciences), or provided as described [29] in PBS overnight at 4°C. After blocking with 0.1% bovine serum albumin/PBS, plasma was added, beginning at a 1:50 dilution and then titrated by 10-fold dilutions, incubated for 2–3 hours at room temperature, washed, and incubated for 1 hour with 100 μL/well alkaline phosphatase-conjugated anti-human immunoglobulin G (1:1000 dilution, clone MT78; MabTech). 1-*p*-nitrophenyl phosphate substrate was added and 2N NaOH was used to stop the reaction.

Statistics

Mann-Whitney tests were used to test differences between 2 paired groups, and a Wilcoxon test to test differences between 2 unpaired groups and nonparametric Spearman correlation coefficient was used to calculate *R* values. We performed multiple regression analysis using a multiple logistic regression analysis to examine independent effects of baseline antibodies, baseline CD4 T cells, previous season vaccination, birth year, and vaccination received on the fold change (day 14/day 0) CD4 T-cell or antibody response after vaccination. For the multiple logistic regression analysis, a fold-change ≥ 3 was considered a response to vaccination. All tests were 2 sided and at the .05 significance level. Statistical analyses were performed using GraphPad Prism software version 9.2.

Table 1. Number of Subjects Included in Each Vaccine Group

Year	Flublok	Flucelvax	Fluzone	Total
1	11	12	9	32
2	19	18	18	55
3	20	22	22	64
4	16	19	20	55
5	11	6	14	31
Total	77	77	83	237

RESULTS

Analyses of Large Vaccine Cohort Reveals Broad Range in Levels of Preexisting Immunity and Response Magnitude

The subjects, adults aged 18–49 years, were assessed for their levels of serum antibody and CD4 T cells specific for H1 and H3 prior to vaccination, and their gains after vaccination with 1 of 3 licensed influenza vaccines. Table 1 shows the subjects in each vaccine group for the 5-year duration of the study, and Table 2 shows the demographics of the subjects. Supplementary Figure 1 shows the details of the study design. Serum antibodies specific for HA were quantified by ELISA to detect all specificities, using the best matched HA protein to that included in the vaccine, while circulating CD4 T cells were measured by cytokine ELISpot assays, using CD4 T cells enriched by magnetic bead technology and overlapping peptide libraries that can recall CD4 T cells specific for the viral HA antigens. The results, shown in Figure 1, depict the day 0, day 14, and fold-change for both H1 (left) and H3 (right) specific responses, with ELISA (Figure 1A) and CD4 T-cell responses (Figure 1B). When antibodies to H1 and H3 were quantified, a large range in titers was observed both pre- and postvaccination. For example, for H1 (Figure 1A, left), there was over a 200-fold difference in antibodies at day 0, and a similar range postvaccination. When antibody responses were calculated as fold-change from day 0, gains in serum antibodies to H1 and H3 exhibited high variability as well, at 340- and 570-fold, respectively. Similarly, for each CD4 T-cell specificity, the prevaccination levels varied considerably, as much as 1200-fold for CD4 T cells specific for H1 and over 370-fold variation for H3. The gains detected postvaccination were between 500 and 1500 for H1 and H3, respectively. These data emphasize the substantial diversity in responses that adults exhibit after administration of licensed influenza vaccines.

Impact of Preexisting Antibody in the Response to Influenza Vaccination

Because of the variability in responses detected, measures of preexisting immunity to influenza antigens that might predict response magnitude were tested. We expected that the large number of subjects assayed over this 5-year study would provide sufficient statistical power to detect these relationships. To evaluate how anti-HA antibodies prior to vaccination might influence future responses, we examined the correlation between serum antibodies, detectable by ELISA, and antibody response postvaccination (Figure 2). In Figure 2A and 2B are shown the relationships between preexisting antibodies and the elicited antibody response for H1 (Figure 2A) and H3 (Figure 2B). A striking negative correlation was observed between the prevaccination titers and the subsequent antibody response. The relationship between preexisting circulating antibodies and subsequent antibody response magnitude was also apparent when the upper and lower quartiles were directly compared (Figure 2A and 2B, right graphs).

Previous work by our group on the first 3 years of this study characterized the impact of the previous years' vaccination [15, 30]. Here, we analyzed the 5-year cohort of over 230 subjects to determine if vaccination history explained the range seen in overall responses and whether the microheterogeneity in the negative correlations remained evident within the subjects distinguished by this parameter. Figure 2C and 2D, representing H1 and H3, show the subjects' history of influenza vaccination indicated by turquoise (not vaccinated in the previous season) and rust (vaccinated). While the patterns exhibited by these 2 types of subjects support the impact of this variable (eg, individuals

Table 2. Demographics of Study Participants

Characteristic	Flublok	Flucelvax	Fluzone	Total
No. of subjects	77	77	83	237
Year of birth, average (SD)	1987 (9)	1992 (6)	1988 (9)	1989 (8)
Sex, female, No. (%)	50 (65)	44 (57)	42 (51)	135 (55)
Previous season vaccination, No.				
Yes	43	49	49	141
No	31	27	29	87
Unknown	3	1	5	9
Race, No. (%)				
White	59 (77)	60 (78)	68 (82)	187 (79)
Asian	8 (10)	8 (10)	8 (10)	24 (10)
Black or African American	8 (10)	4 (5)	4 (5)	16 (7)
More than 1 race	2 (3)	3 (4)	3 (4)	8 (3)
Unknown	0 (0)	2 (2.5)	0 (0)	2 (1)

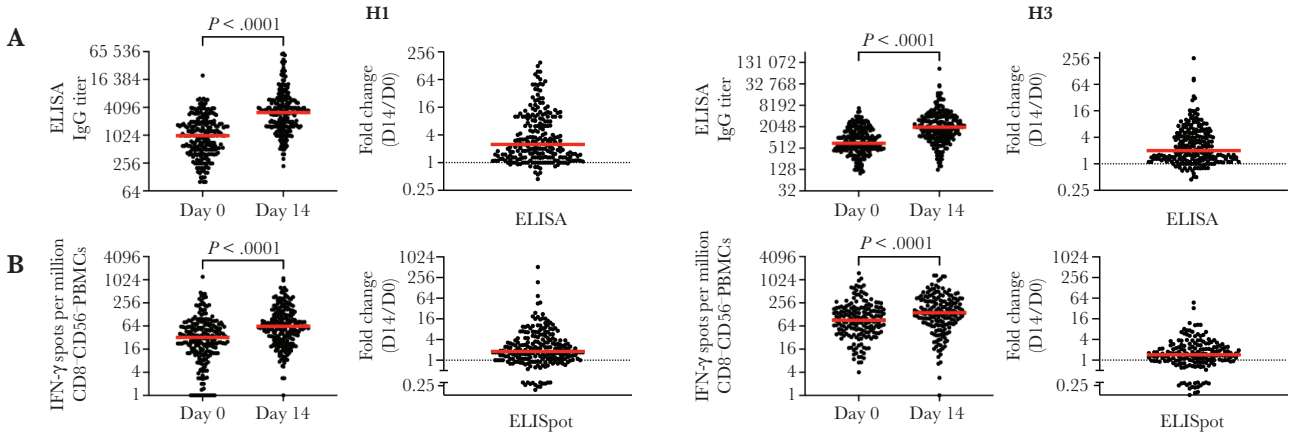

Figure 1. *A*, ELISAs were used to measure the IgG titers at day 0 and day 14 postvaccination using H1 (left) or H3 (right) proteins. Fold change was calculated as day 14/day 0 and plotted for each protein. *B*, IFN-γ production was measured using CD4-enriched PBMCs stimulated with overlapping pools of peptides from H1 (left) or H3 (right) HA in cytokine ELISpots at day 0 and day 14 postvaccination. Each point represents an individual subject and the red line is the median. *P* values were calculated by using a paired Wilcoxon test, where $P < .05$ was considered significant. H1 and H3 ELISpot assays contained samples from 223 and 182 subjects, respectfully. H1 and H3 ELISAs contained samples from 235 subjects. Abbreviations: D, day; ELISA, enzyme-linked immunosorbent assay; ELISpot, enzyme-linked immunospot; HA, hemagglutinin; IFN-γ, interferon-γ; IgG, immunoglobulin G; PBMC, peripheral blood mononuclear cell.

unvaccinated in the previous season exhibited more robust responses, shown as the composite in Figure 2C and 2D left-most graphs), there is a similar statistically significant influence of preexisting antibodies and response magnitude in both groups.

Vaccine formulation type (split, subunit, or recombinant protein) can also impact vaccine responsiveness [14, 31, 32]. When subjects receiving Fluzone (inactivated, egg-based split vaccine), Flucelvax (mammalian cells-based subunit vaccine),

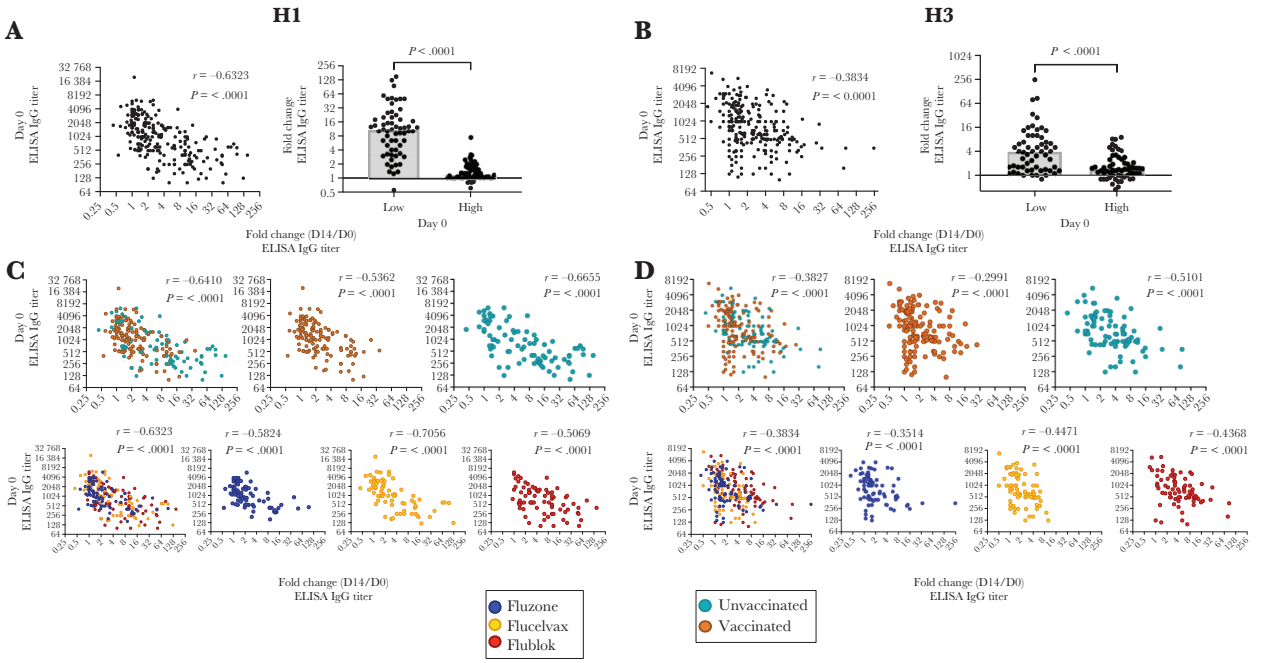

Figure 2. ELISAs were used to measure the IgG titers at day 0 and day 14 postvaccination using H1 (*A*) or H3 (*B*) HA proteins. Fold change was calculated as day 14/day 0. *A* and *B*, Left, the baseline (day 0) IgG responses were compared to the fold change (D14/D0) IgG response after vaccination. *A* and *B*, Right, the top (high) and bottom (low) quartile of day 0 IgG responses were selected, and the fold change response was compared. Mann-Whitney test was used to compare the 2 groups, and *P* values < .05 were considered significant. *C* and *D*, Upper row, the baseline (day 0) IgG responses were compared to the fold change (D14/D0) IgG response after vaccination with symbols colored according to the subjects' previous season vaccination status: turquoise = unvaccinated and rust = vaccinated. The combined graph is shown on the left, with the 2 right graphs showing the separated vaccinated and unvaccinated subjects. *C* and *D*, Lower row, the baseline (day 0) IgG responses were compared to the fold change (D14/D0) IgG response after vaccination with symbols colored according to which vaccine subjects had received: blue = Fluzone, gold = Flucelvax, and red = Flublok. The graph on the left shows the combined plots, with the right graphs showing results separated by vaccine type. Spearman correlation was used, and *P* values < .05 were considered significant. H1 and H3 ELISAs contained samples from 235 subjects. Abbreviations: D, day; ELISA, enzyme-linked immunosorbent assay; HA, hemagglutinin; IgG, immunoglobulin G.

or Flublok (recombinant HA vaccine) were compared, Flublok was the most efficacious [14]. When the patterns of responses were examined for the entire cohort, and subjects vaccinated with Fluzone, Flucelvax, or Flublok (shown in blue, gold, and red, respectively) were separately queried, although the more robust response to Flublok was again observed, each vaccine cohort exhibited a similar, negative correlation between their preexisting antibodies to H1 (Figure 2C, lower graphs) and H3 (Figure 2D, lower graphs) and their antibody response.

Impact of Preexisting HA-specific CD4 T-Cell Repertoire in the Response to Influenza Vaccination

In Figure 3, preexisting levels of CD4 T cells, quantified by cytokine ELISpots, were compared with the expansion of CD4 T cells postvaccination. Here, as was seen with the serum antibody responses, preexisting levels of cytokine-producing CD4 cells were negatively associated with future responsiveness to influenza vaccination for both H1 (Figure 3A) and H3 (Figure 3B) specific responses. As shown in the right graph of Figure 3A and 3B, when patterns of responses in the top and bottom quartiles were compared, the striking differences in response magnitude are readily observed, with those with the least preexisting CD4 T cells exhibiting the greatest expansion after vaccination, while those with the highest levels exhibiting considerably less expansion. Although previously vaccinated subjects (Figure 3C and 3D, upper graphs) exhibited lower CD4 T-cell expansion than unvaccinated subjects (Figure 3C and 3D, upper graphs), both groups exhibited the negative trends related to preexisting CD4 T cells. Similarly, when the type of administered vaccine the subjects received (Figure 3C and 3D, lower graphs) was compared, subjects who received the recombinant HA Flublok vaccine exhibited the most robust responses, relative to Flucelvax and Fluzone. This trend was detectable for both H1- and H3-specific CD4 T-cell responses and each of these vaccinated cohorts showed a negative correlation between their day 0 CD4 T-cell levels and their CD4 T-cell expansion.

Preexisting circulating antibodies have the potential to encounter the administered vaccine and impact its ability to elicit an immune response, either in a negative or positive way [5, 33, 34], depending on the handling of immune complexes, and elicitation of CD4 T cells. Therefore, we evaluated if and how the levels of circulating anti-HA antibodies present at day 0 were related to the ultimate CD4 T-cell response. Figure 4 shows that, overall, preexisting levels of H1 antibodies exhibited a weak but statistically significant negative association with H1-specific CD4 T-cell expansion (Figure 4A) ($P = .0002$, $r = -0.247$). H3 responses (Figure 4B) exhibited the same trends, although they did not reach statistical significance ($P = .1160$, $r = -0.1176$).

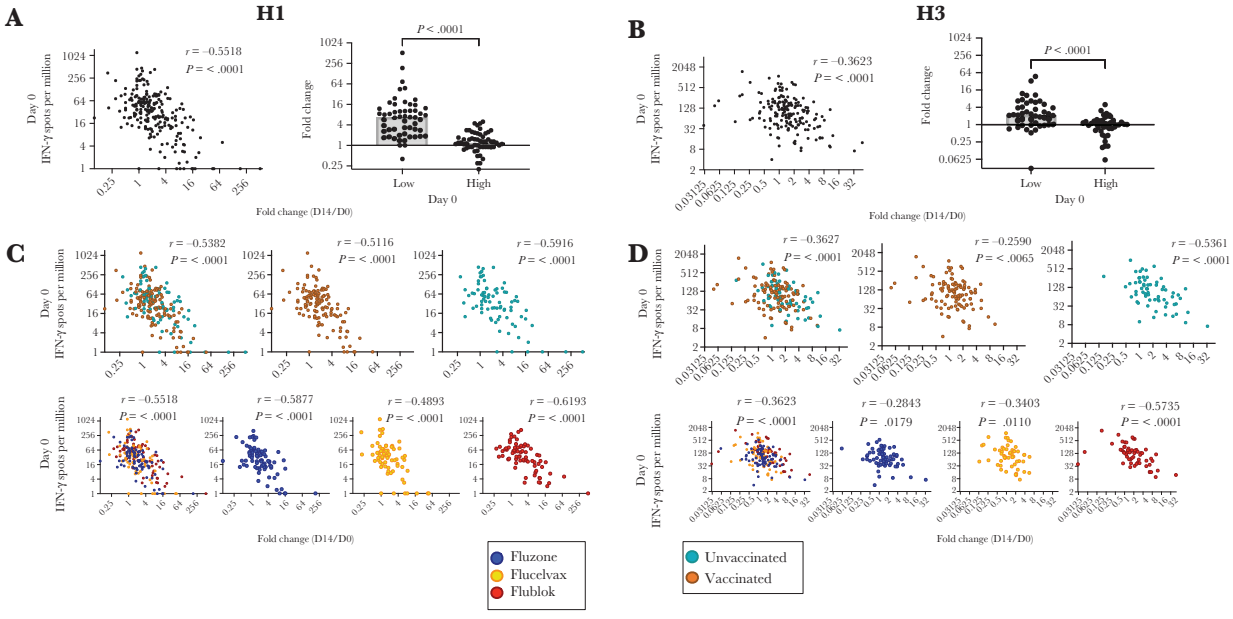

Figure 3. IFN-γ ELISpot assays were used to measure the CD4 T-cell response at day 0 and day 14 postvaccination using H1 (*A*) or H3 (*B*) overlapping peptide pools. Fold change was calculated as day 14/day 0. *A* and *B*, Left, the baseline (day 0) CD4 responses were compared to the fold change (D14/D0) CD4 response after vaccination. *A* and *B*, Right, the top (high) and bottom (low) quartile of day 0 CD4 responses were selected and the fold change response was compared. Mann-Whitney test was used to compare the 2 groups and *P* values < .05 were considered significant. *C* and *D*, Upper row, the baseline (day 0) CD4 responses were compared to the fold change (D14/D0) CD4 response after vaccination with symbols colored according to subjects' previous season vaccination status: turquoise = unvaccinated and rust = vaccinated. The combined graph is shown on the left, with the 2 right graphs showing the separated vaccinated and unvaccinated subjects. *C* and *D*, Lower row, the baseline (day 0) CD4 responses were compared to the fold change (D14/D0) CD4 response after vaccination with symbols colored according to which vaccine subjects had received: blue = Fluzone, gold = Flucelvax, and red = Flublok. The graph on the left shows the combined plots, with the right graphs showing results separated by vaccine type. Spearman correlation was used, and *P* values < .05 were considered significant. H1 and H3 ELISpot assays contained samples from 223 and 182 subjects, respectfully. Abbreviations: D, day; ELISpot, enzyme-linked immunospot; IFN-γ, interferon-γ.

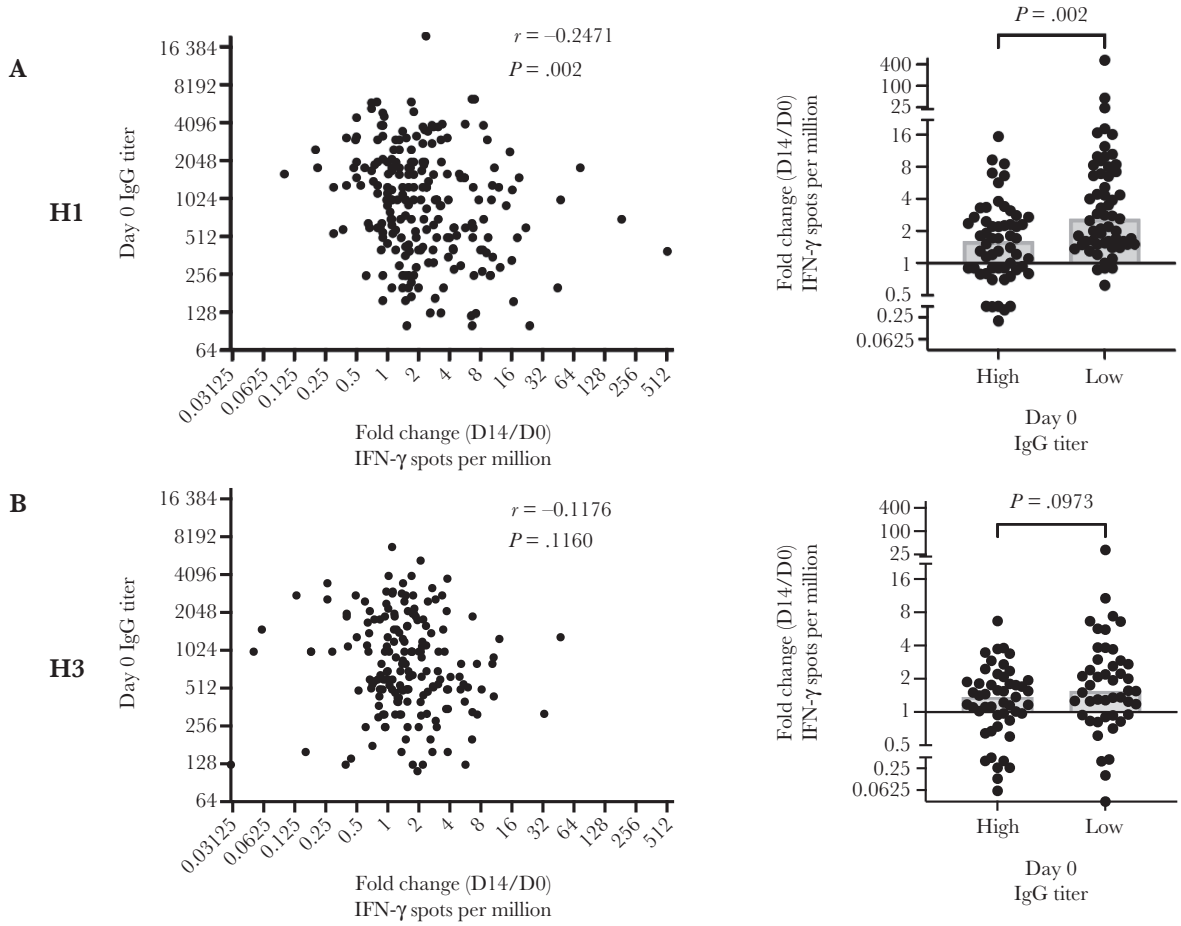

Figure 4. *A*, H1-specific antibodies were measured using ELISA at day 0 and compared to the H1 CD4 T-cell response measured as fold change (day 14/day 0). *B*, H3-specific antibodies measured using ELISA at day 0 were compared to the H3 CD4 T-cell response measured as fold change (D14/D0) using IFN-γ ELISpot. *A* and *B*, Right, the top (high) and bottom (low) quartile of day 0 CD4 responses were selected and the fold change response was compared. Spearman correlation was used (left graphs) and Mann-Whitney test was used to compare the high and low groups (right graphs). *P* values < .05 were considered significant. H1 contained samples from 222 subjects and H3 contained samples from 180 subjects. Abbreviations: D, day; ELISA, enzyme-linked immunosorbent assay; ELISpot, enzyme-linked immunospot; IFN-γ, interferon-γ.

These studies indicate that, on balance, circulating HA-specific antibodies are generally associated with less robust CD4 T-cell expansion after vaccination.

To determine the combined effects of demographics, preexisting immunity, vaccination status, and vaccine received on the antibody and CD4 T-cell response, we performed a multivariable logistic regression analysis (Figure 5). For both CD4 T-cell and antibody responses, fold change (day 14/day 0) ≥ 3 was considered a response to vaccination. Our results show that biological sex does not detectably impact the CD4 T-cell or antibody responses to vaccination. The effects of age at vaccination were also not apparent, with the odds ratios ranging from 1.002 to 1.005 likely reflecting the relatively narrow age range of subjects (ages 18–49 years). Our multivariable analysis has confirmed that for this large cohort, there is a negative correlation between the previous season vaccination history and both antibody (Figure 5A) and CD4 T-cell (Figure 5B) responses to vaccination for both H1 and H3. For antibody responses, shown in Figure 5A, Flublok recipients were more likely than Fluzone vaccinees to respond to vaccination. For H1 (Figure 5A, left) Flucelvax recipients were also more likely to respond to vaccination than those who received Fluzone (odds ratio [OR], 3.421; 95% confidence interval [CI], 1.386–8.890). Figure 5B similarly shows that CD4 T-cell responses are greater in Flublok recipients than Fluzone. A multivariable analysis was used to determine if preexisting CD4 T cells or antibodies present in the host have a detectable impact on the responses to vaccination. For our analysis, we found that baseline immunoglobulin G (IgG) titer was a factor that associated with the antibody response after vaccination, shown in Figure 5A. For CD4 T-cell responses, although baseline IgG titers had ORs that indicated a negative association, they were not statistically significant for either H1 (OR, 0.8966; 95% CI, .6884–1.163) or H3 (OR, 0.9395; 95% CI, .6393–1.378), shown in Figure 5B. In support of findings from Figure 2, Figure 5B demonstrates that baseline CD4 T cells negatively impact the CD4 T-cell response to vaccination. As shown in Figure 5A, we found that baseline CD4 T cells did not have a detectable impact on IgG responses

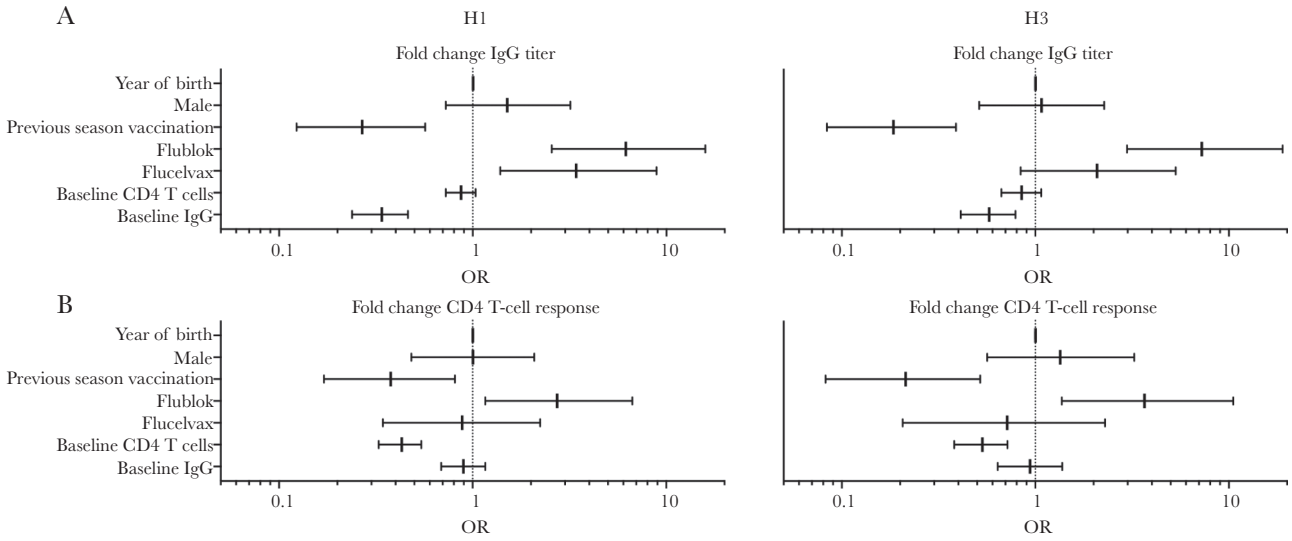

Figure 5. Multivariable logistic regression analysis was performed to identify factors affecting (*A*) the antibody responses or (*B*) CD4 T-cell response after vaccination. A response to vaccination was defined as subjects who had a ≥ 3-fold change (day 14/day 0) response. Previous season vaccination status, year of birth, sex, and type of vaccination received were all included as variables, as well as the preexisting antibody and CD4 T cells. The adjusted odds ratios (OR) are shown with 95% confidence intervals shown by bars. A *P* value of < .05 was considered statistically significant.

in H1 (left) or H3 (right). All OR values and statistics are shown in Supplementary Table 1.

DISCUSSION

Humans accumulate influenza virus-specific CD4 T cells and B cells through both infection and vaccination. To evaluate the impact of this accumulated immunity in a large cohort of vaccine recipients studied, we examined the relationships between preexisting antibodies to H1 and H3, measured by ELISA, and CD4 T cells specific for H1 and H3, quantified by cytokine ELISpots. These prevaccination levels were than analyzed in the context of the vaccine responses, measured by gains in serum H1 and H3 antibodies and expansion of H1- and H3-specific CD4 T cells. Both parameters at day 0 were found to be a negative predictor of future responses. Preexisting levels of serum antibody were negatively associated with both gains in serum antibody and expansion of CD4 T cells, while preexisting levels of influenza-specific CD4 T cells were negatively correlated with vaccine-induced expansion of CD4 T cells. Also notable was the significant microheterogeneity in these effects, even when 2 sources of variability were separately queried. This heterogeneity is evident by the moderate *r* values shown in Figure 1, Figure 2, and Figure 3. Both previously vaccinated and unvaccinated subjects exhibited these negative relationships, even though their overall response magnitude was impacted by their vaccine history. Similarly, independently of type of vaccine administered, all subjects displayed variability in the relative impact of preexisting immunity. Although we have only demonstrated correlations, which may not be causative, it seems reasonable to speculate that the accumulated immunity specific for influenza A virus H1 and H3 proteins in the CD4 T-cell and antibody repertoires can inhibit future responses.

The precise relationships and sources between the negative components studied here and future vaccine responses is important to consider. Unlike the immunity to many pathogens, T-cell and B-cell memory to influenza is established in most individuals by 2 distinct types of intermittent confrontations via influenza infections, often undiagnosed, and variable vaccinations with licensed vaccines (reviewed in [35]). These 2 types of confrontations with influenza antigens vary by involvement of toll-like receptor ligands, viral antigen abundance, persistence, location, and diversity. Thus, infection and vaccination likely seed distinct types of memory CD4 T cells, including follicular helper cells (Tfh), T helper 1 (Th1) cells, and cytotoxic CD4 T cells (reviewed in [36, 37]). The B-cell memory compartment is also complex [17, 38], as are the circulating levels, affinity, posttranslational modifications, and isotypes of antibody to influenza antigens (reviewed in [1]). Additionally, heterogeneity in nonantigen specific cells in different subjects' baseline lymphoid cell composition and transcriptional signatures [11] are likely to influence set points for vaccine responses. These variables may each contribute collectively to the microheterogeneity in the correlations observed.

Regarding mechanism, we speculate that circulating antibodies specific for vaccine components encounter the vaccine and form immune complexes. The handling of these complexes in vivo can vary depending on Fc receptor interactions and Ig isotype. Studies have provided remarkable evidence for the impact of N-linked glycosylation on IgG Fc receptor interactions (reviewed in [39, 40]). As they engage cell surface receptors on different cell types [41–43], immune complexes could either

potentiate responses, via enhanced uptake by antigen presenting cells [34, 39] or could inhibit responses, by direct negative signaling, blocking specific epitopes, or through targeting the complexes for degradation. Antibody-mediated vaccine clearance would diminish the dose of vaccine available to initiate the CD4 T-cell and B-cell response [44, 45]. CD4 T cells can also have alternative impacts on B-cell responses. Tfh cells may facilitate B-cell responses while T follicular regulatory cells curtail the germinal center response (reviewed in [46–48]), Interestingly, a recent publication, tracking CD4 T cells to a single HA peptide epitope, suggests that CD127 (IL-7 receptor) expression identifies CD4 T cells at baseline that correlate with future responses [49]. More sophisticated and detailed quantification of alternative subsets of CD4 T cells available to be recruited to the sites of vaccine responses will likely enable more accurate predictions of future vaccine responses and offer potential strategies for populating the human host with influenza-specific CD4 T cells that contribute most to vaccine responses and protection from infection.

Collectively, our and others' studies suggest that the fine composition of the preimmune B cell, CD4 T cell, and serum antibodies, as well as the overall lymphoid cell composition, will ultimately determine the precise magnitude of the protective response induced by influenza vaccination. The dramatic negative relationships we have observed here suggest that, on balance, the influenza virus-specific immune repertoire that accumulates in adults is associated with negative consequences. It is not clear how the sequence of encounters experienced by different individuals with influenza virus antigens impacts the subsets of CD4 T cells or antibodies that exist prior to vaccination. Future experiments could explore whether very young vaccine recipients, who have been vaccinated since infanthood, will have a distinct composition of CD4 T cells and B cells compared to many of the adult subjects studied here, due to repeated confrontations with multivalent influenza vaccines introduced in the absence of adjuvants at peripheral sites, or if individuals in countries with very low vaccination rates populate the host with cells poised to better respond to infection relative to vaccination. Analyses of these types of subjects, as well as further dissection of subsets of antibodies and CD4 T cells in the prevaccination repertoire, are likely to provide new insights into how to best predict and ultimately manipulate responses to influenza vaccination.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. We acknowledge the New York Influenza Center of Excellence clinical core for their contribution of enrolling study subjects and obtaining and processing all clinical samples. We thank Hongmei Yang and Matthew Sant-Miller for their contribution to the oversight of study statistics. We are very grateful to our study participants, whose participation made this study possible.

Financial support. This work was supported by the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services (Centers of Excellence for Influenza Research and Surveillance [CEIRS] grant number HHSN272201400005C and Dissection of Influenza Vaccination and Infection for Childhood Immunity [DIVINCI] grant number U01 AI14461602S1 to A. J. S.; and Centers of Excellence for Influenza Research and Response [CEIRR] grant number 75N93019R00028 and Collaborative Influenza Vaccine Innovation Centers [CIVIC] grant number 75N93019C00051 to F. K.); and the Burroughs Wellcome Fund Institutional Program Unifying Population and Laboratory Based Sciences Award (grant number 1014095 to S. A. M.).

Potential conflicts of interest. F. K. has consulted for Merck and Pfizer (before 2020); is currently consulting for Pfizer, Seqirus, and Avimex; is collaborating with Pfizer on animal models of SARS-CoV-2; has an active research program with Dynavax; and has received funding from GSK in the influenza virus vaccine space. A. B. has grant funding as an investigator from Pfizer, Janssen, Cyanvac, and Merck; and consults for GSK and Janssen. J. N. has grant funding as an investigator from Pfizer, Merck, and Moderna. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Data availability. The full complement of data accumulated for these studies is available upon request.

References

1. Krammer F. The human antibody response to influenza A virus infection and vaccination. Nat Rev Immunol **2019**; 19:383–97.
2. Poland GA. Influenza vaccine failure: failure to protect or failure to understand? Expert Rev Vaccines **2018**; 17:495–502.
3. Vemula SV, Sayedahmed EE, Sambhara S, Mittal SK. Vaccine approaches conferring cross-protection against influenza viruses. Expert Rev Vaccines **2017**; 16:1141–54.
4. Belongia EA, Skowronski DM, McLean HQ, Chambers C, Sundaram ME, Serres GD. Repeated annual influenza vaccination and vaccine effectiveness: review of evidence. Expert Rev Vaccines **2017**; 16:1–14.

5. Wang TT, Bournazos S, Ravetch JV. Immunological responses to influenza vaccination: lessons for improving vaccine efficacy. Curr Opin Immunol **2018**; 53:124–9.
6. Kim H, Webster RG, Webby RJ. Influenza virus: dealing with a drifting and shifting pathogen. Viral Immunol **2018**; 31:174–83.
7. Castrucci MR. Factors affecting immune responses to the influenza vaccine. Hum Vaccin Immunother **2018**; 14:637–46.
8. Furman D, Jojic V, Kidd B, et al. Apoptosis and other immune biomarkers predict influenza vaccine responsiveness. Mol Syst Biol **2013**; 9:659.
9. Kotliarov Y, Sparks R, Martins AJ, et al. Broad immune activation underlies shared set point signatures for vaccine responsiveness in healthy individuals and disease activity in patients with lupus. Nat Med **2020**; 26:618–29.
10. Avey S, Mohanty S, Chawla DG, et al. Seasonal variability and shared molecular signatures of inactivated influenza vaccination in young and older adults. J Immunol **2020**; 204:1661–73.
11. Tsang JS, Schwartzberg PL, Kotliarov Y, et al. Global analyses of human immune variation reveal baseline predictors of postvaccination responses. Cell **2014**; 157:499–513.
12. Avey S, Cheung F, Fermin D, et al. Multicohort analysis reveals baseline transcriptional predictors of influenza vaccination responses. Sci Immunol **2017**; 2:eaal4656.
13. Nayak JL, Richards KA, Yang H, Treanor JJ, Sant AJ. Effect of influenza A(H5N1) vaccine prepandemic priming on $CD4^+$ T-cell responses. J Infect Dis **2015**; 211:1408–17.
14. Richards KA, Moritzky S, Shannon I, et al. Recombinant HA-based vaccine outperforms split and subunit vaccines in elicitation of influenza-specific CD4 T cells and CD4 T cell-dependent antibody responses in humans. NPJ Vaccines **2020**; 5:77.
15. Richards KA, Shannon I, Treanor JJ, Yang H, Nayak JL, Sant AJ. Evidence that blunted CD4 T-cell responses underlie deficient protective antibody responses to influenza vaccines in repeatedly vaccinated human subjects. J Infect Dis **2020**; 222:273–7.
16. Guthmiller JJ, Wilson PC. Harnessing immune history to combat influenza viruses. Curr Opin Immunol **2018**; 53:187–95.
17. Guthmiller JJ, Utset HA, Wilson PC. B cell responses against influenza viruses: short-lived humoral immunity against a life-long threat. Viruses **2021**; 13:965.
18. Knight M, Changrob S, Li L, Wilson PC. Imprinting, immunodominance, and other impediments to generating broad influenza immunity. Immunol Rev **2020**; 296:191–204.
19. Nayak JL, Fitzgerald TF, Richards KA, Yang H, Treanor JJ, Sant AJ. $CD4^+$ T-cell expansion predicts neutralizing antibody responses to monovalent, inactivated 2009 pandemic influenza A(H1N1) virus subtype H1N1 vaccine. J Infect Dis **2013**; 207:297–305.
20. Richards KA, Nayak J, Chaves FA, et al. Seasonal influenza can poise hosts for CD4 T-cell immunity to H7N9 avian influenza. J Infect Dis **2015**; 212:86–94.
21. Li X, Miao H, Henn A, et al. Ki-67 expression reveals strong, transient influenza specific CD4 T cell responses after adult vaccination. Vaccine **2012**; 30:4581–4.
22. He X-S, Holmes TH, Zhang C, et al. Cellular immune responses in children and adults receiving inactivated or live attenuated influenza vaccines. J Virol **2006**; 80:11756–66.
23. Tan S, Zhang S, Wu B, et al. Hemagglutinin-specific $CD4^+$ T-cell responses following 2009-pH1N1 inactivated split-vaccine inoculation in humans. Vaccine **2017**; 35:5644–52.
24. Dawen Yu E, Grifoni A, Sutherland A, et al. Balanced cellular and humoral immune responses targeting multiple antigens in adults receiving a quadrivalent inactivated influenza vaccine. Vaccines **2021**; 9:426.
25. Li APY, Cohen CA, Leung NHL, et al. Immunogenicity of standard, high-dose, MF59-adjuvanted, and recombinant-HA seasonal influenza vaccination in older adults. NPJ Vaccines **2021**; 6:25.
26. Zhu W, Dong C, Wei L, Wang BZ. Promising adjuvants and platforms for influenza vaccine development. Pharmaceutics **2021**; 13:68.
27. Richards KA, Treanor JJ, Nayak JL, Sant AJ. Overarching immunodominance patterns and substantial diversity in specificity and functionality in the circulating human influenza A and B virus-specific $CD4^+$ T-cell repertoire. J Infect Dis **2018**; 218:1169–74.
28. DiPiazza AT, Fan S, Rattan A, et al. A novel vaccine strategy to overcome poor immunogenicity of avian influenza vaccines through mobilization of memory CD4 T cells established by seasonal influenza. J Immunol **2019**; 203:1502–8.
29. Margine I, Palese P, Krammer F. Expression of functional recombinant hemagglutinin and neuraminidase proteins from the novel H7N9 influenza virus using the baculovirus expression system. J Vis Exp **2013**; (81):e51112.
30. Khurana S, Hahn M, Coyle EM, et al. Repeat vaccination reduces antibody affinity maturation across different influenza vaccine platforms in humans. Nat Commun **2019**; 10:3338.
31. Dawood FS, Naleway AL, Flannery B, et al. Comparison of the immunogenicity of cell culture-based and recombinant quadrivalent influenza vaccines to conventional egg-based quadrivalent influenza vaccines among healthcare personnel aged 18-64 years: a randomized open-label trial. Clin Infect Dis **2021**; 73:1973–81.
32. Gouma S, Zost SJ, Parkhouse K, et al. Comparison of human H3N2 antibody responses elicited by egg-based, cell-based, and recombinant protein-based influenza vaccines during the 2017–2018 season. Clin Infect Dis **2020**; 71:1447–53.

33. Bournazos S, Wang TT, Ravetch JV. The role and function of Fcγ receptors on myeloid cells. Microbiol Spectr **2016**; 4:10.1128/microbiolspec.MCHD-0045-2016.
34. Wen YM, Shi Y. Immune complex vaccination. Curr Top Microbiol Immunol **2019**; 423:95–118.
35. Francis ME, King ML, Kelvin AA. Back to the future for influenza preimmunity—looking back at influenza virus history to infer the outcome of future infections. Viruses **2019**; 11:122.
36. Koutsakos M, Nguyen THO, Kedzierska K. With a little help from T follicular helper friends: humoral immunity to influenza vaccination. J Immunol **2019**; 202:360–7.
37. Ueno H. Tfh cell response in influenza vaccines in humans: what is visible and what is invisible. Curr Opin Immunol **2019**; 59:9–14.
38. Palm AE, Henry C. Remembrance of things past: long-term B cell memory after infection and vaccination. Front Immunol **2019**; 10:1–13.
39. Wang TT, Ravetch JV. Functional diversification of IgGs through Fc glycosylation. J Clin Invest **2019**; 129:3492–8.
40. Wang TT. IgG Fc glycosylation in human immunity. Curr Top Microbiol Immunol **2019**; 423:63–75.
41. Guilliams M, Bruhns P, Saeys Y, Hammad H, Lambrecht BN. The function of Fcγ receptors in dendritic cells and macrophages. Nat Rev Immunol **2014**; 14:94–108.
42. Nimmerjahn F, Ravetch JV. Fc-receptors as regulators of immunity. Adv Immunol **2007**; 96:179–204.
43. Patel KR, Roberts JT, Barb AW. Multiple variables at the leukocyte cell surface impact Fcγ receptor-dependent mechanisms. Front Immunol **2019**; 10:223.
44. Ellebedy AH. Immunizing the immune: can we overcome influenza's most formidable challenge? Vaccines **2018**; 6:68.
45. Lu LL, Suscovich TJ, Fortune SM, Alter G. Beyond binding: antibody effector functions in infectious diseases. Nat Rev Immunol **2018**; 18:46–61.
46. Stebegg M, Kumar SD, Silva-Cayetano A, Fonseca VR, Linterman MA, Graca L. Regulation of the germinal center response. Front Immunol **2018**; 9:2469.
47. Wing JB, Lim EL, Sakaguchi S. Control of foreign Ag-specific Ab responses by Treg and Tfr. Immunol Rev **2020**; 296:104–19.
48. Aloulou M, Fazilleau N. Regulation of B cell responses by distinct populations of CD4 T cells. Biomed J **2019**; 42:243–51.
49. Wild K, Smits M, Killmer S, et al. Pre-existing immunity and vaccine history determine hemagglutinin-specific CD4 T cell and IgG response following seasonal influenza vaccination. Nat Commun **2021**; 12:6720.