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Comparison of A(H3N2) Neutralizing Antibody Responses Elicited by 2018–2019 Season Quadrivalent Influenza Vaccines Derived from Eggs, Cells, and Recombinant Hemagglutinin

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Background. Low vaccine effectiveness against A(H3N2) influenza in seasons with little antigenic drift has been attributed to substitutions in hemagglutinin (HA) acquired during vaccine virus propagation in eggs. Clinical trials comparing recombinant HA vaccine (rHA) and cell-derived inactivated influenza vaccine (IIV) to egg-derived IIVs provide opportunities to assess how egg-adaptive substitutions influence HA immunogenicity.

Methods. Neutralization titers in pre- and postimmunization sera from 133 adults immunized with 1 of 3 types of influenza vaccines in a randomized, open-label trial during the 2018–2019 influenza season were measured against egg- and cell-derived A/Singapore/INFIMH-16-0019/2016-like and circulating A(H3N2) influenza viruses using HA pseudoviruses.

Results. All vaccines elicited neutralizing antibodies to all H3 vaccine antigens, but the rHA vaccine elicited the highest titers and seroconversion rates against all strains tested. Egg- and cell-derived IIVs elicited responses similar to each other. Preimmunization titers against H3 HA pseudoviruses containing egg-adaptive substitutions T160K and L194P were high, but lower against H3 HA pseudoviruses without those substitutions. All vaccines boosted neutralization titers against HA pseudoviruses with egg-adaptive substitutions, but poorly neutralized wild-type 2019–2020 A/Kansas/14/2017 (H3N2) HA pseudoviruses.

Conclusion. Egg- and cell-derived 2018–2019 season influenza vaccines elicited similar neutralization titers and response rates, indicating that the cell-derived vaccine did not improve immunogenicity against the A(H3N2) viruses. The higher responses after rHA vaccination may be due to its higher HA content. All vaccines boosted titers to HA with egg-adaptive substitutions, suggesting boosting from past antigens or better exposure of HA epitopes. Studies comparing immunogenicity and effectiveness of different influenza vaccines across many seasons are needed.

Keywords. influenza vaccine; neutralizing antibodies; egg-adaptive mutations; hemagglutinin; vaccine effectiveness.

Vaccination remains the most effective public health measure for preventing influenza virus infection. However, the effectiveness of licensed inactivated influenza vaccines (IIV) has been variable and sometimes suboptimal, especially for A(H3N2) viruses in recent years [1]. Antigenic differences between vaccine antigens and circulating A(H3N2) viruses as a result of viral antigenic drift or mutations that emerge during vaccine virus propagation in eggs may contribute to low vaccine effectiveness [2–8]. Influenza vaccination in the 2018–2019 influenza season did not result in measurable protection against A(H3N2) because of the emergence of an antigenically drifted A(H3N2) variant [9]. Egg-adaptive substitutions in hemagglutinin (HA), the principal vaccine antigen, often occur around the receptor binding site and frequently involve glycosylation changes that can alter exposure of neutralizing epitopes, resulting in lower vaccine effectiveness [6, 8, 10]. Newer influenza vaccine-manufacturing technologies with cell-derived vaccine viruses and recombinant HA (rHA) antigens can avoid introduction of egg-adaptive substitutions in HA antigens [2, 11, 12].

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Each season, influenza vaccines used in the United States are tested for appropriate antigenicity and adequate potency using US Food and Drug Administration (FDA)-approved reference reagents, but the vaccines are not identical. The HAs used in each vaccine may vary in a small number of amino acid residues, which sometimes derive from egg-adaptive mutations. In addition, a standard dose of the rHA vaccine contains at least 45 μg of each HA antigen, whereas standard dose IIVs contain at least 15 μg of each HA antigen. To date, data directly comparing the neutralizing antibody responses of cell-derived IIV (cIIV) and rHA vaccine to that of the more widely used egg-derived IIV (eIIV) using each of the vaccine-matched HA antigens are limited. Moreover, relative responses elicited by the vaccines may vary with influenza strain, as well as the host's age and prior immunity, making it necessary to study comparative effectiveness in many settings. Here, we compared neutralizing antibody responses to vaccine-matched and circulating virus antigens among adult military healthcare beneficiaries who were immunized with 1 of 3 types of influenza vaccines during the 2018–2019 influenza season and investigated whether egg-adaptive amino acid substitutions influenced neutralizing antibody responses.

METHODS

Ethics Statement

The FDA Research Involving Human Subjects Committee approved use of deidentified sera as exempt research (Protocol #09-043B), as described under 45 CFR 46.101(b)(4).

Participants and Serum Samples

Serum samples were obtained from a subset of volunteers in a randomized, open-label clinical trial, the Pragmatic Assessment of Influenza Vaccine Effectiveness in the Department of Defense study (Protocol IDCRP-120) performed at 5 military medical treatment facilities in the United States. Information about the vaccine strains and volunteer number are summarized in Table 1. Briefly, northern hemisphere 2018–2019 season quadrivalent IIV, including the eIIV (Fluarix, GlaxoSmithKline Biologicals), cIIV (Flucelvax, Seqirus, Inc.), and rHA vaccine

Table 1. Vaccine Groups With H3N2 Strains and Number of Volunteers in Each Group

Vaccine Group	H3N2 Vaccine Strain (A/Singapore/INFIMH-16-0019/2016-like)	No. of Volunteers
Egg-derived inactivated influenza vaccine (eIIV) Fluarix	A/Singapore/INFIMH-16-0019/2016 NIB-104 (egg passaged) (SGP/19/16 NIB-104)	46
Cell-derived inactivated influenza vaccine (cIIV) Flucelvax	A/North Carolina/04/2016 (cell passaged) (NC/04/16)	36
Recombinant hemagglutinin vaccine (rHA) Flublok	A/Singapore/INFIMH-16-0019/2016 (wild type) (SGP/19/16 WT)	51

Table 2. HA Residue Differences Among H3N2 Vaccine and Emerging Strains

H3N2 strain	GISAID	HA residue difference[a]																			
		62	91	92	121	128	135	138	142	144	159	160	171	193	194	225	311	326	406	478	479	484
SGP/19/16 (WT, rHA)[b]	780183	E	S	K	K	T	T	A	G	S	Y	**T**	K	F	**L**	**D**	H	K	V	I	E	E
SGP/19/16 NIB-104 (egg)	1151864	E	S	K	K	T	T	A	G	S	Y	**K**	K	F	**P**	**G**	H	K	V	I	E	E
NC/04/16 (cell)	1390880	E	S	K	**N**	T	T	A	**R**	S	Y	T	K	F	L	D	H	K	V	I	**G**	E
ADB/240/18 (WT)	1221483	**G**	S	**R**	K	**A**	**K**	A	G	S	Y	T	K	F	L	D	**Q**	K	V	I	**G**	E
KS/14/17 (WT)	1146345	E	**N**	K	**N**	**A**	T	**S**	G	**K**	**S**	**K**	**N**	**S**	L	D	**Q**	**R**	**I**	**M**	**G**	**G**
HK/4801/14 (cell)	539576	E	S	K	**N**	T	T	A	**R**	S	Y	T	**N**	F	L	D	H	K	**I**	I	**G**	**G**

HA residues that are different from SGP/19/16 WT HA are indicated in bold. SGP/19/16: A/Singapore/INFIMH-16-0019/2016; NC/04/16: A/North Carolina/04/2016; ADB/240/18: A/Abu Dhabi/240/2018; KS/14/17: A/Kansas/14/2017; HK/4801/14: A/Hong Kong/4801/2014.

[a]H3 HA number without signal peptide.

[b]WT clinical sample used for rHA (recombinant HA).

Abbreviations: cell, cell passaged; egg, egg passaged; GISAID: Global Initiative on Sharing All Influenza Data Accession EPI #; HA, hemagglutinin; WT, wild-type.

(Flublok, Protein Sciences Corporation) were used to immunize 133 adult military healthcare beneficiaries ages 18–83 years. Median ages were 48.3, 40.45, and 50.2 years for participants in the eIIV, cIIV, and rHA groups, respectively. This included active-duty personnel, military retirees, and beneficiaries. There were no statistically significant differences among the participants in the vaccine groups for ages, sex, military status, education status, race, and days between clinic visits for blood samples (Supplementary Table 1). Each vaccine type was administered to approximately one-third of the subjects. Pre- (day 0) and postimmunization (days 21–35) sera were collected for this study. Neutralizing antibody titers from day 21 sera were presumed to be due to the vaccination.

HA Pseudovirus Neutralization Assay

The codon optimized H3 HAs listed in Table 2 were used to construct HA pseudoviruses and tested for sera neutralization, as previously described [13, 14]. Clade 3C.2a1 HA vaccine antigens included A/Singapore/INFIMH-16-0019/2016 wild-type (SGP/19/16 WT), A/Singapore/INFIMH-16-0019/2016 NIB-104 egg-derived (SP/19/16 NIB-104), and North Carolina/04/2016 cell-derived (NC/04/16) HAs. Other HAs based on wild-type sequences included clade 3C.2a1b A/Abu Dhabi/240/2018 (ADB/240/18) and clade 3C.3a A/Kansas/14/2017 (KS/14/17), both representing emerging strains and clade 3C.2a/Hong Kong/4801/2014 (HK/4801/14) representing a past circulating strain. SGP/19/16 WT HA with T160K or L194P or D225G substitutions were created using standard molecular biology methods for HA pseudovirus production and neutralization assays. To align with the recent change in reporting microneutralization titers by World Health Organization reference laboratories, our serum neutralization titers here are reported as the serum dilution before mixing serum with virus rather than the final dilution after mixing sera with virus. This change in reporting results in 2-fold lower titers compared with the way that we reported titers previously [13, 15, 16].

Statistical Analysis

Two sample comparisons, geometric mean titers (GMTs) with 95% confidence intervals were analyzed using GraphPad Prism software. Mann-Whitney test for the comparison of 2 groups (unpaired) and Wilcoxon test for the comparison of 2 groups with matched pairs (pre vs post) were applied. All neutralization titers were log2 transformed for analyses.

RESULTS

Recombinant HA Vaccine Elicited the Highest Neutralizing Antibody Responses

SGP/19/16-like virus was recommended as the northern hemisphere 2018–2019 A(H3N2) vaccine antigen. The H3 HAs from A(H3N2) viruses SGP/19/16 NIB-104 (egg passaged), NC/04/16 (cell passaged), and SGP/19/16 WT were used to make Fluarix quadrivalent eIIV, Flucelvax quadrivalent cIIV, and Flublok rHA quadrivalent vaccine, respectively (Table 1). We measured HA pseudovirus neutralization titers against the H3 HA antigen used in each vaccine.

Individuals who received the eIIV, cIIV, or rHA vaccine had similar preimmunization neutralization titers against each of the H3 vaccine antigens, and all vaccines induced neutralizing antibody responses (Figure 1A and Supplementary Table 2). However, rHA vaccine induced the highest neutralization titers, resulting in the greatest percentage of persons who had a 4-fold rise in neutralization titers (seroconversion rates) against all 3 SGP/19/16-like A(H3N2) vaccine antigens. Seroconversion rates (SCRs) for the rHA group were 47.1%, 43.1%, and 56.9% against SGP/19/16 NIB-104, NC/04/16, and SGP/19/16 HA pseudoviruses, respectively. Although eIIV and cIIV elicited lower neutralization responses compared with rHA vaccine, they were similar to each other against each of the H3 antigens, suggesting that the cIIV did not improve overall immunogenicity in the 2018–2019 season compared with eIIV. Against SGP/19/16 NIB-104, NC/04/16, and SGP/19/16 HA pseudoviruses respectively, SCRs were 8.7%, 8.7%, and 4.3% for the eIIV group and 13.9%, 8.3%, and 16.7% for the cIIV group. Differences in postimmunization neutralization titers against SGP/19/16 WT HA antigen between the eIIV and cIIV groups were not statistically significant (Figure 1A). Notably, all pre- and postimmunization sera had higher neutralization titers against HA pseudovirus representing SGP/19/16 NIB-104 egg-adapted H3 virus compared with HA pseudovirus representing cell-derived or wild-type H3 viruses, NC/04/16 and SGP/19/16, respectively (Figure 1A left panel, and Supplementary Table 2). Based on our prior studies measuring HA pseudovirus neutralization titers in military recruits during an A(H3N2) outbreak, we consider a titer reported as a serum dilution ≥80 in our assay (previously reported as final sera and virus dilution ≥160) to be an approximate benchmark for conferring significant protection [16].

Next, we evaluated the breadth of neutralizing antibodies against HA pseudoviruses representing the prior year circulating strain A(H3N2) HK/4801/14 (cell, wild-type) that was recommend for inclusion in 2016–2018 seasonal influenza vaccines, the recently circulating strain A(H3N2) ADB/240/18 (wild-type), or the emerging influenza strain KS/14/17 (wild-type), which was recommended for inclusion in 2019–2020 seasonal influenza vaccines. KS/14/17 is a representative of genetic group 3C.3a viruses, which increased in frequency early in 2018. All 3 vaccines elicited neutralizing antibodies against these HA pseudoviruses (Figure 1B and Supplementary Table 2). The postimmunization neutralization titers between the eIIV and cIIV groups were not statistically different, but the rHA vaccine again elicited significantly higher titers than eIIV and cIIV.

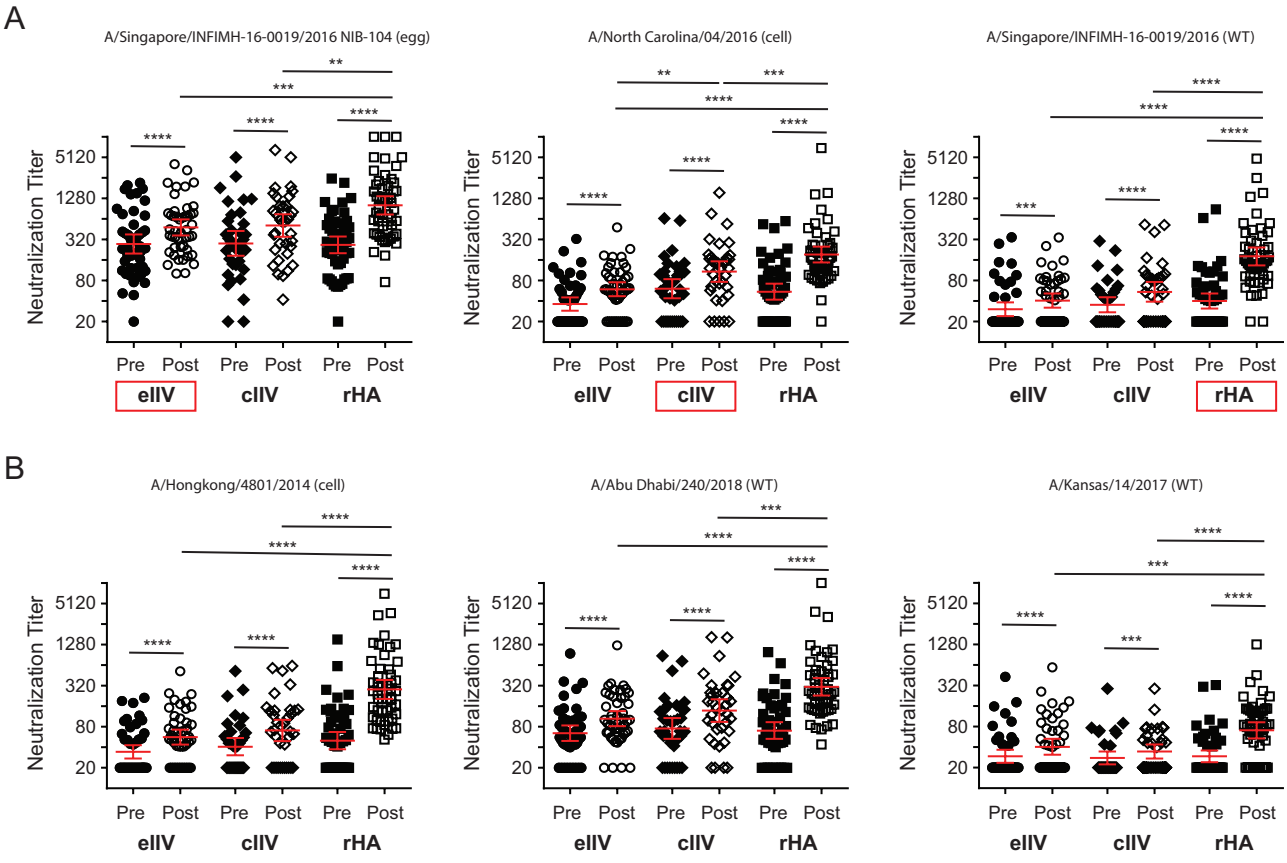

Figure 1. Neutralizing antibody titers against H3 hemagglutinin-pseudoviruses before and after immunization with egg- or cell-derived inactivated influenza vaccines or recombinant hemagglutinin vaccine. *A,* Pre- and postimmunization neutralization titers against hemagglutinin-pseudoviruses corresponding to the vaccine strain-matched viruses A/Singapore/INFIMH-16–0019/2016 NIB-104 (SGP/19/16 NIB-104, eIIV, Fluarix), A/North Carolina/04/2016 (NC/04/16, cIIV, Flucelvax), and A/Singapore/INFIMH-16–0019/2016 (SGP/19/16 WT, rHA, Flublok). *B,* Pre- and postimmunization neutralization titers against HA pseudoviruses corresponding to prior season vaccine strain A/Hong Kong/4801/2014 (HK/4801/14, cell passaged), recently circulating virus A/Abu Dhabi/240/2018 (ADB/240/18, wild-type), and emerging influenza strain A/Kansas/14/2017 (KS/14/17, wild-type). Red boxes indicate vaccine-matched antigen. Bars: geometric means of titers (GMT) with 95% CI. *P* values were calculated by Mann-Whitney test for the comparison of 2 groups (unpaired) and Wilcoxon test for the comparison of 2 groups with matched pairs (pre vs post). All neutralization titers were log2 transformed before test. **P* < .05; ***P* < .01; ****P* < .001; *****P* < .0001. Abbreviations: CI, confidence interval; cIIV, cell-derived inactivated influenza vaccine; eIIV, egg-derived inactivated influenza vaccine; rHA, Recombinant hemagglutinin vaccine (Flublok); WT, wild type.

Postimmunization neutralization titers against KS/14/17 HA pseudoviruses were the lowest among the tested strains for all vaccines, consistent with significant antigenic drift (Figure 1B right panel, and Supplementary Table 2). Against KS/14/17 HA pseudoviruses, the GMT was 70 for the rHA vaccine group, and the GMTs were 40 and 35 for the eIIV and cIIV groups, respectively.

Interestingly, preimmunization titers against HK/4801/14 (cell, wild-type) HA pseudoviruses, which represented the H3 antigen component in 2 prior season IIVs, were also low for all 3 vaccine groups. These low preimmunization neutralization titers are consistent with the low vaccine effectiveness reported for the 2017–2018 influenza season, perhaps reflecting prior immunizations with egg-adaptive substitutions in HA [17, 18]. Titers against ADB/240/18 HA pseudoviruses were slightly higher for each vaccine group. Importantly, titers against HA pseudoviruses correlated well with titers against replicating influenza virus in standard microneutralization assays using the same cIIV pre- and postimmunization sera (Table 3).

T160K and L194P Substitutions are Responsible for High Neutralization Titers Against Egg-adapted SGP/19/16 HA Pseudovirus

Differences in neutralization titers against HA pseudoviruses representing SGP/19/16-like, prior year, and advanced year vaccine antigens led us to compare HA sequences among these viruses (Table 2). Because neutralization titers against SGP/19/16 WT, NC/04/16, and HK/4801/14 HA pseudoviruses were generally similar for each vaccine (Supplementary Table 2), we surmised that the differences in residues 121, 142, and 479 did not have a major effect on HA antigenicity. Likewise, although T128A and T135K changes led to the loss of potential glycosylation of residues N126 and N133, respectively, in ADB/240/18 HA, these residue differences together with other

Table 3. Comparison of Microneutralization and HA Pseudovirus Neutralization Titers in Sera from the Cell-derived Inactivation Influenza Vaccine Group

HA Pseudovirus Tested	SCR (% 4-Fold Rise)	Pre-GMT	Post-GMT	% Pre ≥80	% Post ≥80	% Pre ≥160	% Post ≥160
NC/04/16 (MN)	14	28	58	22	56	8	19
NC/04/16 (PVN)	8	61	108	42	61	11	33
KS/14/17 (MN)	14	19	34	14	36	0	11
KS/14/17 (PVN)	0	28	35	6	6	3	3
ADB/240/18 (MN)	22	44	90	39	67	22	42
ADB/240/18 (PVN)	11	75	138	47	70	19	47

% indicates percent persons with the indicated titer.
Abbreviations: ADB/240/18: A/Abu Dhabi/240/2018 (wild-type) (H3N2); GMT, geometric mean titer; HA, hemagglutinin; KS/14/17: A/Kansas/14/2017 (wild-type) (H3N2); MN, microneutralization involving replicating virus; NC/04/16: A/North Carolina/04/2016 (cell passaged) (H3N2); post, postimmunization; pre, preimmunization; PVN, HA pseudovirus neutralization; SCR, seroconversion rate.

residue differences in ADB/240/18 also likely have little effect on HA antigenicity because of similar titers and SCRs against ADB/240/18 and SGP/19/16 WT HA pseudoviruses within each vaccine group. In contrast, differences in residues 91, 138, 144, 159, 193, 326, and 478 between SGP/19/16 WT and KS/14/17 HA pseudoviruses may contribute to the lower titers elicited by SGP/19/16-like HA antigens.

Significantly, the higher pre- and postimmunization neutralization titers in all vaccine groups against SGP/19/16 NIB-104 compared to SGP/19/16 WT HA pseudoviruses clearly pointed to the 3 residue differences at position 160, 194, and 225 as key residues that accounted for the high titers. Moreover, the T160K, L194P, and D225G substitutions in SGP/19/16 NIB-104 HA are localized in the receptor-binding region (Figure 2) and have previously been reported to alter HA antigenicity in other influenza strains [8, 19].

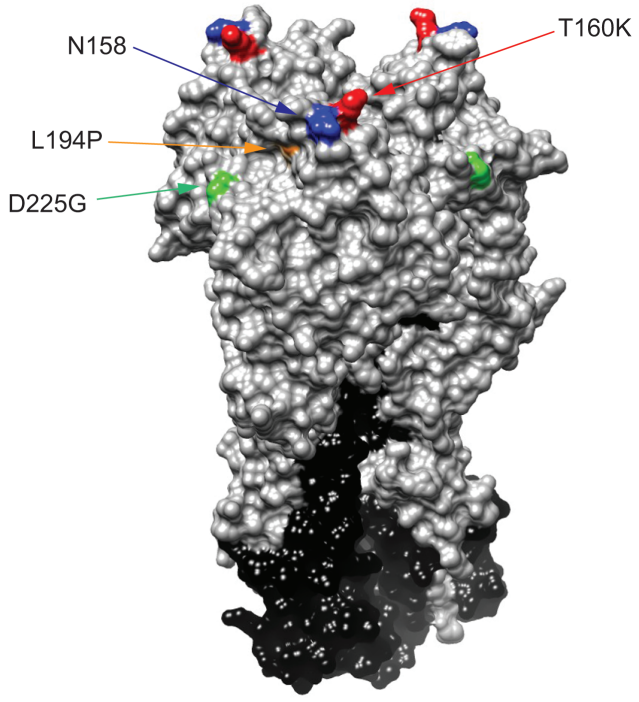

Figure 2. Substitutions and glycosylation sites highlighted on the H3 hemagglutinin trimer. The locations of T160K, L194P, D225G, and the N158 for glycosylation on H3 HA head are highlighted in color. Structure is from A/Michigan/15/2014 (H3N2), PDB: 6BKT. Abbreviation: HA, hemagglutinin.

We investigated the extent to which each of the T160K, L194P, and D225G substitutions affected neutralization titers in the context of SGP/19/16 WT HA pseudoviruses (Table 4, Figure 3 and Figure S1). In both pre- and postimmunization sera, the T160K and L194P substitutions conferred higher neutralization titers individually, and more so with T160K and L194P substitutions together, against SGP/19/16 HA pseudoviruses with these changes compared with wild-type. For example, the rHA vaccine group GMT was 668 against HA pseudoviruses with both T160K and L194P substitutions, whereas the GMTs were 293 and 458 against HA pseudoviruses with only T160K or L194P substitutions, respectively (Table 4). In contrast, the D225G substitution significantly mitigated the effect of T160K and L194P individually, though there was a trend toward enhanced titers when combined with T160K and L194P together (Supplementary Figure 2). How different combinations of these 3 amino acid substitutions influence neutralization by polyclonal sera are unclear.

To further investigate the effect of K160 and P194 residues on HA antigenicity, we analyzed neutralizing antibody titers in ferret sera from a reference panel generated by infecting ferrets with 2 different A(H3N2) viruses (Supplementary Table 3). These viruses were propagated in eggs and had T160K and L194P substitutions in HA. Using A/Hong Kong/50/2016 HA and A/Hong Kong/4801/2014 HA, with and without both T160K and L194P substitutions, we found that all ferret antisera generated higher neutralization titers against HA pseudoviruses with bothT160K and L194P substitutions compared to HA pseudoviruses without those substitutions, regardless of the infecting strain used to generate the antisera. Antisera tested against HA pseudoviruses with a single substitution also had higher and similar titers with either substitution alone compared with HA pseudoviruses without either substitutions. These findings confirm the changes in antigenicity caused by these residues.

DISCUSSION

Most influenza vaccines used worldwide are manufactured in eggs, but unfortunately some egg-adaptive mutations can

Table 4. Neutralization Responses to A/Singapore/INFIMH-16-19/2016 HA Pseudoviruses with Various Residue Substitutions

Tested HA Pseudovirus	Vaccine Type	SCR (% 4-Fold Rise)	Pre-GMT	Post-GMT	% Pre ≥80	% Post ≥80	% Pre ≥160	% Post ≥160
SGP/19/16	eIIV	4.3	30	41	13.0	23.9	4.3	4.3
(Wild-type)	cIIV	16.7	35	55	13.9	41.7	5.6	11.1
	rHA	56.9	40	181	19.6	80.4	3.9	45.1
SGP/19/16	eIIV	4.3	72	104	43.5	56.5	23.9	28.3
T160K	cIIV	8.3	72	118	47.2	69.4	22.2	41.7
	rHA	47.1	65	293	37.3	88.2	19.6	72.5
SGP/19/16	eIIV	4.3	136	231	82.6	95.7	34.8	76.1
L194P	cIIV	5.6	185	313	88.9	94.4	52.8	77.8
	rHA	25.5	164	458	84.3	98.0	51.0	94.1
SGP/19/16	eIIV	4.3	44	63	21.7	37.0	13.0	17.4
D225G	cIIV	8.3	63	99	41.7	69.4	5.6	19.4
	rHA	33.3	50	175	27.5	78.4	5.9	43.1
SGP/19/16	eIIV	6.5	202	366	87.0	97.8	60.9	80.4
T160K	cIIV	5.6	278	439	91.7	97.2	72.2	83.3
L194P	rHA	25.5	217	668	92.2	100	64.7	96.1
SGP/19/16	eIIV	4.3	56	74	39.1	50.0	15.2	26.1
T160K	cIIV	2.8	54	86	33.3	44.4	8.3	19.4
D225G	rHA	39.2	55	183	33.3	74.5	17.6	51.0
SGP/19/16	eIIV	4.3	92	146	63.0	87.0	17.4	45.7
L194P	cIIV	5.6	121	211	66.7	88.9	33.3	63.9
D225G	rHA	41.1	99	370	62.7	98.0	25.5	80.4
SGP/19/16	eIIV	8.7	274	480	89.1	100	67.4	91.3
NIB-104	cIIV	13.9	280	511	86.1	97.2	69.2	80.6
(Egg)	rHA	47.1	266	1011	86.36	98.0	70.6	98.0

SGP/19/16: A/Singapore/INFIMH-16-0019/2016. % indicates percent of persons with the indicated titer.
Abbreviations: cIIV: cell-derived inactivated influenza vaccine (Flucelvax); eIIV: egg-derived inactivated influenza vaccine (Fluarix); GMT, geometric mean titer; HA, hemagglutinin; post, postimmunization; pre, preimmunization; rHA, recombinant influenza vaccine (Flublok); SCR, seroconversion rate.

change HA antigenicity and potentially reduce vaccine effectiveness. In this study, we compared neutralizing antibody responses in 133 paired pre- and postimmunization sera from adult military beneficiaries who received either the eIIV, cIIV, or the rHA vaccine during the 2018–2019 influenza season. We found that the eIIV and cIIV elicited similar neutralization titers and SCRs against most antigens tested and that the standard dose of the rHA vaccine elicited higher neutralization titers and SCRs against each H3 HA vaccine antigen compared with the standard dose of the eIIV or cIIV.

These findings highlight several key issues. First, our data demonstrate that the responses elicited by the eIIV and cIIV are similar to each other but less than those elicited by the rHA vaccine, suggesting that the higher HA content, rather than absence of egg-adaptive substitutions, is the main reason for increased immunogenicity. Others have similarly suggested that higher HA content in the recombinant HA may contribute to higher responses [20, 21]. The overall comparable responses elicited by the eIIV and cIIV also suggest that the egg-adaptive substitutions had little effect on the immunogenicity against the egg- and cell-grown SGP/19/16-like viruses in these adults who likely had prior vaccinations.

Second, the high neutralization titers against HAs with egg-adaptive substitutions both before and after immunizations indicate that the egg-adaptive changes may have affected HA immunogenicity and antigenicity in 2 ways: (1) by boosting responses in those with preexisting immunity to antigens with egg-adaptive substitutions and (2) by introducing structural changes involving conformational dynamics and glycosylation changes [7, 8]. Such structural changes may introduce new neutralizing epitopes or increase exposure of cross-reactive neutralizing epitopes, or both. In this regard, the preimmunization sera had higher neutralization titers against egg-derived H3 HA containing T160K, L194P, and D225G substitutions, and immunization boosted these neutralization titers, regardless of the vaccine used.

In the past 11 influenza seasons, eIIVs comprised the vast majority of influenza vaccines that were distributed in the United States [22]. The rHA vaccine, Flublok, was approved in January 2013 by the FDA and used for the first time in the United States in February 2013. The cIIV, Flucelvax, was approved in November 2012 by the FDA and used first in the United States in the 2013–2014 season. However, egg-derived A(H3N2) vaccine seed viruses used in Flucelvax vaccines were not replaced by cell-derived A(H3N2) vaccine seed viruses until the 2017–2018 influenza season. The majority of IIVs in the past decade, especially in the 2016–2019 seasons, had H3 HAs from egg-derived viruses that had T160K or L194P substitutions, or both (Table 5). Significantly, cIIV and rHA vaccine, as well as the eIIV, boosted neutralization titers against HA containing

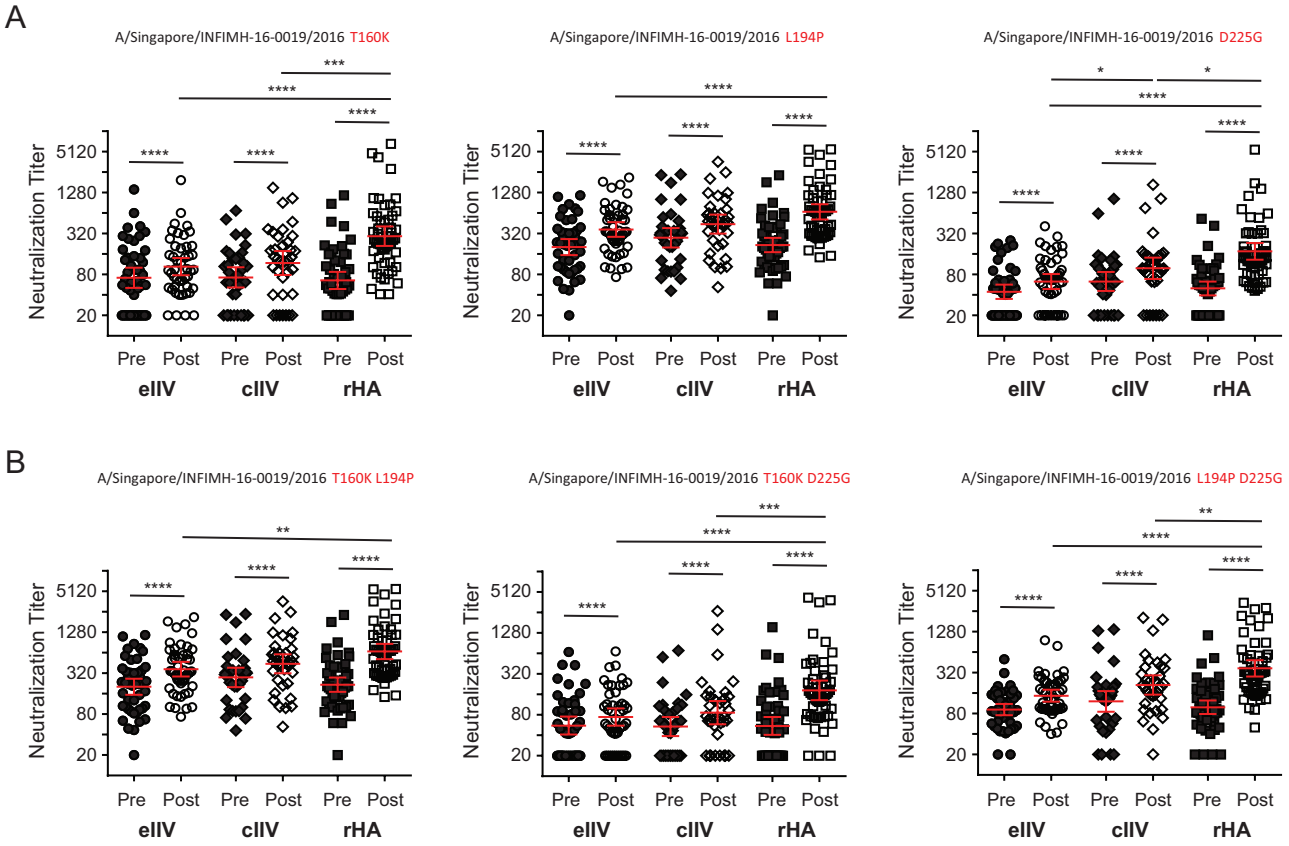

Figure 3. Neutralizing antibody titers against H3 hemagglutinin pseudoviruses with various egg-adaptive substitutions before and after immunization with egg- or cell-derived inactivated influenza vaccines or recombinant hemagglutinin vaccine. Pre- and postimmunization neutralization titers against HA pseudoviruses corresponding to A/Singapore/INFIMH-16-0019/2016 (SGP/19/16 WT) with single substitutionsT160K, or L194P, or D225G (*A*), and double substitutions T160K L194P, or T160K D225G, or L194P D225G (*B*). Bars: geometric means of titers (GMT) with 95% CI. *P* values were calculated by Mann-Whitney test for the comparison of 2 groups (unpaired) and Wilcoxon test for the comparison of 2 groups with matched pairs (pre vs post). All neutralization titers were log2 transformed before test. **P* < .05; ***P* < .01; ****P* < .001; *****P* < .0001. Abbreviations: cIIV, cell-derived inactivated influenza vaccine (Flucelvax); eIIV, egg-derived inactivated influenza vaccine (Fluarix); rHA, recombinant hemagglutinin vaccine (Flublok).

these residue changes. Prior immunizations with vaccines containing these residues may therefore have contributed to the relatively high pre- and postimmunization sera titers against HA containing these substitutions (Table 4).

The L194P substitution has been reported to alter loops surrounding the receptor binding site and change site B epitopes in the context of A/Brisbane/10/2007 HA [7], whereas the T160K substitution eliminates potential glycosylation of residue N158 and can change antibody binding to clade 3C.2a viruses [8]. Past circulating viruses and vaccine strains have contained K160 in H3 HA, although the circulating viruses in 2016–2019 seasons contain mostly T160. Influenza vaccines in the past decade have contained P194 or L194 in H3 HA, though most contained L194 (Table 5). The volunteers in our study likely received egg-derived vaccines in prior years. Military beneficiaries generally have high vaccine coverage, though the vaccination histories of subjects in this study were not known. Interestingly, Wu et al reported that 2015–2016 influenza season vaccine responses to A/Switzerland/9715293/2013, which contained an H3 antigen with L194, reacted better to HA antigens with L194 compared with P194, which may suggest boosting from prior exposures to H3 antigen containing L194 before 2015–2016 season (Table 5) [7].

Apart from immunogenicity, the antigenicity of HA with egg-adaptive substitutions in some influenza strains may affect titer measurements. For example, if egg-adaptive changes increase exposure of neutralizing epitopes, then neutralization titers against viruses with HA egg-adaptive substitutions might be higher than titers against wild-type viruses that lack those changes, regardless of prior immunity. Perhaps this contributed to the higher neutralization titers against HA pseudoviruses with egg-adapted substitutions in those immunized with the cIIV or rHA vaccine. Neutralization responses against circulating strains, rather than against vaccine-matched antigens that may contain adaptive substitutions, however, are clearly most relevant in interpreting potentially protective antibodies titers elicited by vaccines.

Ultimately, antibody titers need to be correlated with protection. A study among the elderly immunized in the 2017–2018

Table 5. Past H3N2 Vaccine Strains Used in US and HA Residue Differences

Flu Season	H3N2 Vaccine Strain	GISAID or GenBank	Vaccine Type[a]	HA Residue[b] 160	194	225
2018–2019	A/Singapore/INFIMH-16-0019/2016 IVR-186[c]	EPI1104214	eIIV	K	P	G
	A/Singapore/INFIMH-16-0019/2016 NIB-104[c]	EPI1151864	eIIV	K	P	G
	A/North Carolina/04/2016	EPI1390880	cIIV	T	L	D
	A/Singapore/INFIMH-16-0019/2016	EPI780183	rHA	T	L	D
2017–2018	A/Hong Kong/4801/2014 NYMC X-263B	EPI765207	eIIV	K	P	N
	A/Singapore/GP2050/2015	EPI956368	cIIV	T	L	D
	A/Hong Kong/4801/2014	EPI539576	rHA	T	L	D
2016–2017	A/Hong Kong/4801/2014 NYMC X-263B	EPI765207	eIIV	K	P	N
	A/Hong Kong/4801/2014 (E5/E2)[d]	EPI614437	cIIV	K	P	D
	A/Hong Kong/4801/2014	EPI539576	rHA	T	L	D
2015–2016	A/Switzerland/9715293/2013 NIB-88	EPI653209	eIIV	K	L	D
	A/South Australia/55/2014 IVR-175	EPI674597	eIIV	K	L	G
	A/South Australia/55/2014 (E5/E2)[d]	EPI544084	cIIV	K	L	G
	A/Switzerland/9715293/2013	EPI541659	rHA	K	L	D
2014–2015	A/Texas/50/2012 NYMC X-223A	EPI731465	eIIV	K	L	N
	A/Texas/50/2012 NYMC X-223	KJ942744	cIIV	K	L	N
	A/Texas/50/2012	EPI377499	rHA	K	L	N
2013–2014	A/Texas/50/2012 NYMC X-223A	EPI731465	eIIV	K	L	N
	A/Texas/50/2012 NYMC X-223	KJ942744	cIIV	K	L	N
	A/Texas/50/2012	EPI377499	rHA	K	L	N
2012–2013	A/Victoria/361/2011 IVR-165	EPI358038	eIIV	K	L	N
	A/Victoria/361/2011	EPI349103	rHA	K	L	N
2011–2012	A/Victoria/210/2009 NYMC X187	HQ378751	eIIV	K	L	N
2010–2011	A/Victoria/210/2009 NYMC X187	HQ378751	eIIV	K	L	N
2009–2010	A/Uruguay/716/2007, NYMC X-175C	EPI162118	eIIV	K	P	N
2008–2009	A/Uruguay/716/2007, NYMC X-175C	EPI162118	eIIV	K	P	N

[a]The IIV and rHA vaccine in the United States market each year during last 11 influenza seasons using individual H3N2 vaccine strain were indicated.

[b]H3 HA number without signal peptide.

[c]The protein sequences of IVR-186 and NIB-104 HA are identical.

[d]The passage number of master seed viruses of vaccine strains on egg was E5/E2.

Abbreviations: cIIV: cell-derived inactivated influenza vaccine (Flucelvax); eIIV, egg-derived inactivated influenza vaccines; HA, hemagglutinin; rHA, recombinant influenza vaccine (Flublok).

influenza season suggested that cIIV had superior vaccine effectiveness compared with eIIVs, though the difference in relative effectiveness was small [17]. For the 2018–2019 influenza season, we show that the eIIV and cIIV induced similar neutralizing responses, which is consistent with a recent study reporting no significant difference in relative effectiveness between eIIV and cIIV among individuals 65 years and older [23]. Compared with the eIIV and cIIV, however, the rHA vaccine induced the highest neutralization titers to the strains tested. Our data support prior reports suggesting that rHA vaccines induce broader responses and better protection [21, 24].

Limitations of this study include small sample size and unknown vaccination histories among the participants. Nonetheless, our findings underscore the need to better understand how HA substitutions affect not only the immunogenicity and effectiveness of influenza vaccines, but also how HA antigenicity can affect antibody titers used for inferring vaccine responses and protective levels. Variations in vaccine effectiveness across seasons are due to a complex interplay between viral and host factors that includes prior immunity and strain changes. Additional studies comparing vaccine effectiveness to antibody responses elicited by vaccines with and without HA egg-adaptive substitutions are needed across many influenza seasons to inform vaccination policy.

Supplementary Data

Supplementary materials are available at Clinical Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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References

1. Belongia EA, Simpson MD, King JP, et al. Variable influenza vaccine effectiveness by subtype: a systematic review and meta-analysis of test-negative design studies. Lancet Infect Dis **2016**; 16:942–51.
2. Paules CI, Sullivan SG, Subbarao K, Fauci AS. Chasing seasonal influenza–the need for a universal influenza vaccine. N Engl J Med **2018**; 378:7–9.
3. Katz JM, Webster RG. Efficacy of inactivated influenza A virus (H3N2) vaccines grown in mammalian cells or embryonated eggs. J Infect Dis **1989**; 160:191–8.
4. Koel BF, Burke DF, Bestebroer TM, et al. Substitutions near the receptor binding site determine major antigenic change during influenza virus evolution. Science **2013**; 342:976–9.
5. Raymond DD, Stewart SM, Lee J, et al. Influenza immunization elicits antibodies specific for an egg-adapted vaccine strain. Nat Med **2016**; 22:1465–9.
6. Skowronski DM, Janjua NZ, De Serres G, et al. Low 2012-13 influenza vaccine effectiveness associated with mutation in the egg-adapted H3N2 vaccine strain not antigenic drift in circulating viruses. PLoS One **2014**; 9:e92153.
7. Wu NC, Zost SJ, Thompson AJ, et al. A structural explanation for the low effectiveness of the seasonal influenza H3N2 vaccine. PLoS Pathog **2017**; 13:e1006682.
8. Zost SJ, Parkhouse K, Gumina ME, et al. Contemporary H3N2 influenza viruses have a glycosylation site that alters binding of antibodies elicited by egg-adapted vaccine strains. Proc Natl Acad Sci U S A **2017**; 114:12578–83.
9. Flannery B, Kondor RJG, Chung JR, et al. Spread of antigenically drifted influenza A(H3N2) viruses and vaccine effectiveness in the United States during the 2018–2019 season. J Infect Dis **2020**; 221:8–15.
10. Schild GC, Oxford JS, de Jong JC, Webster RG. Evidence for host-cell selection of influenza virus antigenic variants. Nature **1983**; 303:706–9.
11. Barr IG, Donis RO, Katz JM, et al. Cell culture-derived influenza vaccines in the severe 2017-2018 epidemic season: a step towards improved influenza vaccine effectiveness. NPJ Vaccines **2018**; 3:44.
12. Buckland B, Boulanger R, Fino M, et al. Technology transfer and scale-up of the Flublok recombinant hemagglutinin (HA) influenza vaccine manufacturing process. Vaccine **2014**; 32:5496–502.
13. Wang W, Anderson CM, De Feo CJ, et al. Cross-neutralizing antibodies to pandemic 2009 H1N1 and recent seasonal H1N1 influenza A strains influenced by a mutation in hemagglutinin subunit 2. PLoS Pathog **2011**; 7: e1002081.
14. Wang W, Butler EN, Veguilla V, et al. Establishment of retroviral pseudotypes with influenza hemagglutinins from H1, H3, and H5 subtypes for sensitive and specific detection of neutralizing antibodies. J Virol Methods **2008**; 153:111–9.
15. Wang W, Xie H, Ye Z, Vassell R, Weiss CD. Characterization of lentiviral pseudotypes with influenza H5N1 hemagglutinin and their performance in neutralization assays. J Virol Methods **2010**; 165:305–10.
16. Weiss CD, Wang W, Lu Y, et al. Neutralizing and neuraminidase antibodies correlate with protection against influenza during a late season A/H3N2 outbreak among unvaccinated military recruits. Clin Infect Dis **2019**.
17. Izurieta HS, Chillarige Y, Kelman J, et al. Relative effectiveness of cell-cultured and egg-based influenza vaccines among elderly persons in the United States, 2017–2018. J Infect Dis **2019**; 220:1255–64.
18. Levine MZ, Martin ET, Petrie JG, et al. Antibodies against egg- and cell-grown influenza A(H3N2) viruses in adults hospitalized during the 2017–2018 influenza season. J Infect Dis **2019**; 219:1904–12.
19. Wu NC, Lv H, Thompson AJ, et al. Preventing an antigenically disruptive mutation in egg-based H3N2 seasonal influenza vaccines by mutational incompatibility. Cell Host Microbe **2019**; 25:836–44 e5.
20. 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 **2019**.
21. Dunkle LM, Izikson R, Patriarca P, et al; PSC12 Study Team. Efficacy of recombinant influenza vaccine in adults 50 years of age or older. N Engl J Med **2017**; 376:2427–36.
22. US Food and Drug Administration. Vaccines licensed for use in the United States. 2020. Available at: https://www.fda.gov/vaccines-blood-biologics/vaccines/vaccines-licensed-use-united-states.
23. Izurieta HS, Chillarige Y, Kelman J, et al. Relative effectiveness of influenza vaccines among the U.S. elderly, 2018–19. In press.
24. Dunkle LM, Izikson R. Recombinant hemagglutinin influenza vaccine provides broader spectrum protection. Expert Rev Vaccines **2016**; 15: 957–66.