The Journal of Infectious Diseases

MAJOR ARTICLE

Differential Induction of Interferon-Stimulated Genes by Cell-Based Versus Egg-Based Quadrivalent Influenza Vaccines in Children During the 2018–2019 Season

Judith M. Martin,[1] Krissy Moehling Geffel,[2] Marianna A. Ortiz,[1] Dhivyaa Rajasundaram,[1] Mary Patricia Nowalk,[2] Richard K. Zimmerman,[2] and John F. Alcorn[1,3]

[1]Department of Pediatrics, UPMC Children's Hospital of Pittsburgh, Pittsburgh, Pennsylvania, USA; [2]Department of Family Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania, USA; and [3]Department of Immunology, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

Background. Cell-based quadrivalent-inactivated influenza vaccine has been shown to have higher vaccine effectiveness than traditional egg-based quadrivalent-inactivated influenza vaccine. This is observed despite similar levels of serum hemagglutinin antibodies induced by each vaccine.

Methods. In this study, we examine peripheral immune activation after egg-based or cell-based influenza vaccination in a clinical trial in children. Peripheral blood mononuclear cells were isolated, and ribonucleic acid was sequenced from 81 study participants (41 Fluzone, egg based and 40 Flucelvax, cell based) pre- and 7 days postvaccination. Seroconversion was assessed by hemagglutinin inhibition assay. Differential gene expression was determined and pathway analysis was conducted.

Results. Cell-based influenza vaccine induced greater interferon-stimulated and innate immune gene activation compared with egg-based influenza vaccine. Participants who seroconverted had increased interferon-signaling activation versus those who did not seroconvert.

Conclusions. These data suggest that cell-based influenza vaccine stimulates immune activation differently from egg-based influenza vaccine, shedding light on reported differences in vaccine effectiveness.

Keywords. blood; immunity; influenza; interferon; vaccine.

Influenza poses a global health challenge with millions of cases of severe illness and hundreds of thousands of deaths worldwide annually before the coronavirus disease 2019 pandemic [1]. The 2022–2023 influenza season saw a re-emergence of influenza infection worldwide. Influenza vaccines directed at the viral surface protein hemagglutinin represent the best available prevention for severe disease. Traditionally, inactivated influenza vaccines have been prepared using propagation of predicted circulating influenza strains in chicken eggs. There are several potential disadvantages to egg-based influenza vaccines that include production time and capacity and allergies to egg components. Furthermore, the process of growing influenza viral strains in chicken eggs can result in mutations in the desired hemagglutinin protein, which, in turn, may alter vaccine match with circulating strains [2, 3]. Specifically, a change to a specific glycosylation site (T160K) in the 2016–2017 H3N2 vaccine strain due to growth in chicken eggs resulted in poor antibody binding and neutralization of circulating H3N2 that season [4].

These challenges have led to the development of alternative methodologies that include the growth of vaccine viral strains in mammalian cells (cell-based, Flucelvax) and a vaccine composed of only recombinant hemagglutinin protein (recombinant, Flublok). Recombinant influenza vaccine contains increased hemagglutinin antigen content and has been shown to generate superior antihemagglutinin antibody responses compared with egg-based vaccines [5–9]. Cell-based inactivated influenza vaccine has been shown to induce similar levels of antibody as inactivated egg-based vaccines [5, 9, 10] but better vaccine effectiveness in children and adult recipients [11–24]. Furthermore, cell-based inactivated influenza vaccine has been shown to be effective against A(H1N1), A(H3N2), and B strains over the course of multiple recent influenza seasons [25]. Cell-based influenza vaccine induced superior antibody responses compared with live-attenuated influenza vaccine in children [26].

Improved vaccine effectiveness of cell-based vaccines, despite no change in antibody response, suggests that other arms of the immune system may be involved in providing protection. We performed a randomized clinical trial of

Received 30 March 2023; editorial decision 28 August 2023; accepted 31 August 2023; published online 4 September 2023

Correspondence: John F. Alcorn, PhD, Division of Pulmonary Medicine, Department of Pediatrics, UPMC Children's Hospital of Pittsburgh, 9127 Rangos, 4401 Penn Ave., Pittsburgh, PA 15224 (john.alcorn@chp.edu). Judith M. Martin, MD, Children's Hospital Office Building, Floor 3, 3414 Fifth Avenue, Pittsburgh, PA 15213-3205 (judy.martin@chp.edu).

The Journal of Infectious Diseases®

https://doi.org/10.1093/infdis/jiad380

quadrivalent egg-based inactivated influenza vaccine (IIV4) compared with quadrivalent cell-based inactivated influenza vaccine (ccIIV4) in children aged 4–20 during the 2018–2019 influenza season [10]. From this parent study, we isolated peripheral blood mononuclear cells (PBMCs) from a subset of participants and sequenced ribonucleic acid (RNA) to examine the immune response to each vaccine. Gene expression in response to vaccination was compared between vaccine types and between those with and without significant vaccine-induced antibody production (seroconversion).

METHODS

Study Design and Cohort

Healthy participants aged 4–20 years old were enrolled during the fall of 2018 from 5 primary care health centers (1 pediatric clinic and 4 family medicine practices). Eligibility criteria included no allergies to eggs or influenza vaccine components and willingness to be randomized to receive one of the two 2018–2019 US Food and Drug Administration-approved study influenza vaccines. Exclusion criteria included the following: weight <37 lbs; known pregnancy; an immunosuppressive health condition or taking immunosuppressive medications; having already received the 2018–2019 influenza vaccine; or not able to complete all study visits [10]. After screening and consent, a blood sample was drawn. Participants were then randomized in blocks of 4 using a computer-generated 1:1 randomization assignment to receive 1 of the 2 vaccines: ccIIV4 (Flucelvax) or the IIV4 (Fluzone). Both vaccines included A/H1N1/Michigan/45/2015-pdm09-like virus, A/H3N2/Singapore/INFIMH-16–0019/2016-like virus, B/Colorado/06/2017-like virus (Victoria lineage), and B/Phuket/3073/2013-like virus (Yamagata lineage). Both vaccines contained 15 μg of hemagglutinin per strain. Participants were immediately vaccinated in the deltoid muscle onsite with study-provided vaccine, according to the randomization scheme. Additional blood samples were collected 7 (range, 6–9) and 21 (range, 21–35) days after vaccination. Baseline patient demographics including age, sex, and vaccination history the prior year were collected from the electronic medical record. The study was approved by the Institutional Review Boards at the University of Pittsburgh and the Centers for Disease Control and Prevention. The clinical trial was registered at Clinicaltrials.gov, registration number NCT03614975.

Blood Sample Processing

Blood samples were collected using BD Vacutainer serum separator (for antibody titer at day 0 and day 21 postvaccination) and CPT tubes (for PBMC collection at day 0, 7, and 21 postvaccination). Tubes were maintained at room temperature and were delivered for processing within 4 hours of blood draw. Hemagglutinin inhibition (HAI) antibody titer for A(H1N1) and B vaccine influenza strains and microneutralization (MN) titers for A(H3N2 egg- and cell-grown strains) was performed as previously published [10]. Microneutralization titers were used for H3N2 due to agglutination concerns with the egg-based strain. Seroconversion was defined as a 4-fold rise in HAI or MN titers postvaccination given a prevaccination titer ≥10 [10]. Seroprotection was defined as a HAI titer ≥110 for A(H1N1) or B strains, or microneutralization titer ≥110 for A(H3N2) [27]. The PBMCs were isolated from whole blood, washed, counted, and banked in freezing medium (heat-inactivated fetal bovine serum with 10% dimethyl sulfoxide) in liquid nitrogen until isolation of RNA was performed using a RNeasy Minikit (QIAGEN). Laboratory staff conducting the HAI and MN assays were blinded to vaccine assignment.

Ribonucleic Acid Sequencing

Peripheral blood mononuclear cell RNA was assessed for integrity and concentration using an Agilent Tape Station and Qubit Fluorometric Quantification. Ribonucleic acid sequencing was performed using the Illumina NovaSeq 6000 platform. Quality-controlled RNA sequencing reads were aligned to the human genome (hg38/GRCh38) using STAR aligner (version 2.6.1) [28], and gene-level read counts were calculated using featureCounts based on Ensembl annotation (GRCh38.96) [29]. The assembled count values were used as the input for the differential gene expression analysis between the different conditions and time points using DESeq2 [30]. The resulting differentially expressed genes between the groups were defined at cut-off criteria of $|\log_2 \text{fold-change}| \geq 1.5$ and $P < .05$. Significantly enriched pathways were examined using the gene set enrichment analysis through Clusterprofiler (v3.16.1) [31, 32]. All statistical analyses were performed using R software 4.0.1. In addition, Ingenuity Pathway Analysis (IPA) software was used to identify significantly enriched canonical pathways based on the Ingenuity Knowledge Base. Graphical network analysis was generated using the IPA web tool. Gene Set Enrichment Analysis (GSEA) was performed to identify potential enriched pathways across conditions. The gene sets collection C5 (ontology gene sets) available from the Molecular Signature Database ([MSigDB] www.gsea-msigdb.org) was used in the enrichment analysis. The functional enrichment analysis was performed and visualized using clusterProfiler (4.9.0.2) 4.0 and customized scripts. Multiple testing was corrected using the Benjamini-Hochberg method and genes and pathways with FDR <0.05 were considered significant.

RESULTS

Patient Cohort Demographics and Antibody Data Analyses

Antibody response to influenza vaccination in the full study cohort was previously reported [10]. Paired RNA samples (day 0

Table 1. Demographics, Seroconversion, and Seroprotection Status Overall and by Vaccine Received

Variable	Overall N = 81	ccIIV4 n = 40	IIV4 N = 41	*P* Value
Age (years), median (IQR)	13.9 (4.8)	13.5 (5.9)	14.2 (4.3)	.43
Age 9–20 years, ≥9, n (%)	72 (88.9)	36 (90.0)	36 (87.8)	.75
Sex, female, n (%)	45 (55.6)	19 (47.5)	26 (63.4%)	.15
2017 influenza vaccine status				
IIV receipt, n (%)	49 (60.5)	26 (65.0)	23 (56.1)	.67
Not vaccinated/no record, n (%)	32 (39.5)	14 (35.0)	18 (43.9)	
Seroconversion to 1 or more strain	44 (54.3)	17 (42.5)	27 (65.9)	.035
Seroprotection to 4 strains[a,b]	30 (37.0)	16 (40.0)	14 (34.1)	.59
Seroprotection to H3N2 cell[b]	54 (66.7)	27 (67.5)	27 (65.9)	.88

Abbreviations: ccIIV4, quadrivalent cell-based inactivated influenza vaccine; IIV4, quadrivalent egg-based inactivated influenza vaccine; IQR, interquartile range.

[a]Seroprotection is HAI (for H1N1, B/Colorado, B/Phuket) or microneutralization (MN) (for H3N2 cell) ≥110 where HAI is hemagglutinin inhibition and MN is microneutralization activity.

[b]n = 80; 1 participant missing MN titer data.

Table 2. Seroconversion by Influenza Strain by Vaccine Received

Variable	Overall N = 81	ccIIV4 n = 40	IIV4 N = 41	*P* Value
H1N1, n (%)	13 (16.0)	5 (12.5)	8 (19.5)	.39
H3N2-egg, n (%)[a]	10 (12.5)	2 (5.1)	8 (19.5)	.09
H3N2-cell, n (%)[a]	18 (22.5)	7 (18.0)	11 (26.8)	.34
B/Vic (Colorado), n (%)	25 (30.9)	9 (22.5)	16 (39.0)	.11
B/Yam (Phuket), n (%)	13 (16.0)	2 (5.0)	11 (26.8)	.007

Abbreviations: ccIIV4, quadrivalent cell-based inactivated influenza vaccine; IIV4, quadrivalent egg-based inactivated influenza vaccine.

NOTE: Seroconversion is hemagglutinin inhibition (HAI) (for H1N1, B/Colorado, B/Phuket) or microneutralization (MN) (for H3N2 egg and cell) where HAI is hemagglutinin inhibition and MN is MN activity.

[a]n = 80; 1 participant missing MN titer data.

and 7 postvaccination) were available for a subset of 81 participants (Table 1). Forty participants (mean age 13.5 years) received ccIIV4 and 41 participants (mean age 14.2 years) received IIV4. There were no significant differences in participant age, sex, or 2017 influenza vaccination status between vaccine groups. Seroconversion to 1 or more strains of the quadrivalent vaccine was significantly greater in the IIV4 group (65.9% IIV4% vs 42.5% ccIIV4, $P = .035$). Seroconversion to individual vaccine strains was then analyzed (Table 2). There was no significant difference in seroconversion to A(H1N1), A(H3N2), or B/Victoria between vaccine types. The IIV4 induced greater seroconversion to B/Yamagata compared with ccIIV4. The 2017 and 2018 influenza vaccines shared the same H1N1 and B/Yam (Phuket) strains, which may impact antibody titers. The interval of timing between prior year vaccination and 2018–2019 vaccine was assessed in those with confirmed 2017 vaccination (Supplementary Table 1). The low frequency of seroconversion observed to conserved vaccine strains between the 2 seasons obviated conclusions regarding the impact of prior year vaccination on our results. Seroprotection against all 4 vaccine strains or against cell-based influenza A (H3N2) did not differ between vaccine types.

Greater Interferon-Stimulated Gene Responses Were Induced by ccIIV4 Than IIV4 at Day 7 Postvaccination

Gene set enrichment was performed from PBMC from participants 7 days after vaccination with egg-based (IIV4) versus cell-based (ccIIV4) influenza vaccine. All differentially expressed genes were used as an input ranked from top to bottom based on their $\log_2$-fold change. Many gene ontology pathways were identified as activated or suppressed in participants who received egg-based versus cell-based influenza vaccine (Supplementary Figure 1). These included a large number of cellular processes but also immunology pathways. Among the immunology pathways were type I interferon response, interferon gamma-mediated signaling, cytokine activity, and regulation of T-cell activation, which were all suppressed in recipients of egg-based versus cell-based vaccine. Furthermore, gene expression analysis identified 45 genes (false-discovery rate < 0.05) that were significantly differentially expressed by vaccine type in PBMC 7 days after vaccination (Supplementary Table 2). These included 15 canonical interferon-induced genes: *ifi44l*, *cxcl10*, *ifi27*, *oasl*, *ifi6*, *ifi44*, *mx1*, *ifit1*, *ifit3*, *stat1*, *oas3*, *oas2*, *ddx60*, *mx2*, and *ifit2*. All differentially expressed genes were lower in patients who received IIV4 versus ccIIV4. Pathway network analysis revealed a decreased interferon response induced by IIV4 compared with ccIIV4, characterized by interferon-α, -γ, and -λ and IRF1 and IRF7 nodes consistent with increased interferon signaling (Figure 1).

Greater Immune Activation Was Induced by ccIIV4 Than IIV4, Accounting for Baseline Values

We next examined the PBMC gene expression signature induced by influenza vaccination by identifying differentially expressed genes between day 0 and day 7 postvaccination for each vaccine type. This analysis revealed 428 genes with a $P < .01$ between vaccine types for use in pathway network analysis (Supplementary Table 3). Several patterns were identified including higher immune response gene expression, higher levels of proinflammatory nodes including interferon signaling, myeloid cell activation, recruitment of monocytes, hypercytokinemia, and innate immune response among ccIIV4 recipients than among IIV4 recipients (Figure 2). In addition, the role of pattern recognition receptors in recognition of bacteria and viruses was a node. This included differential expression of *c1qa*, *c1qb*, *c1qc*, *ddx58*, *eif2ak2*, *ifih1*, *il15*, *il1a*, *il1b*, *irf7*, *mapk10*, *nlrp3*, *oas2*, and *oas3*. Consistent with day 7 results, interferon signaling (including

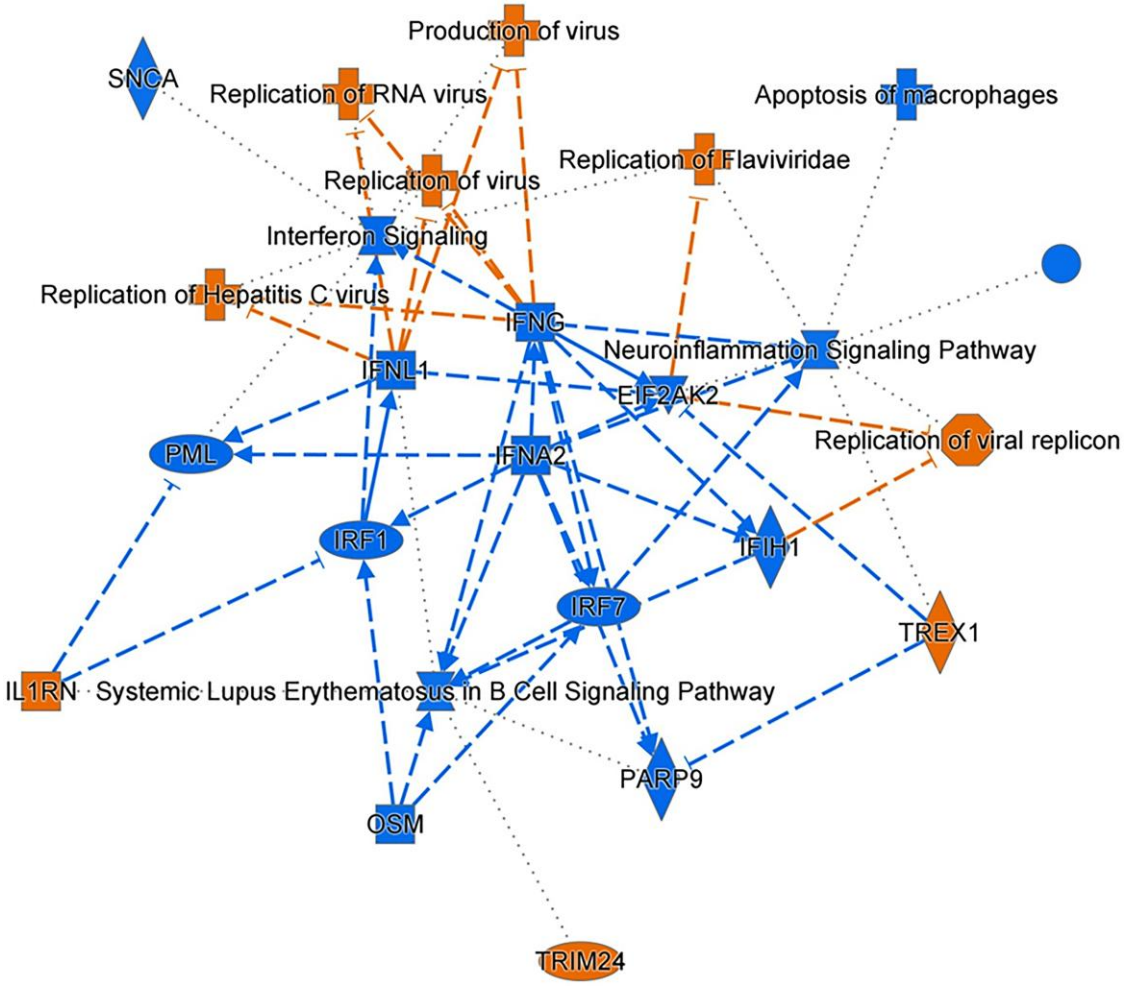

Figure 1. Quadrivalent cell-based inactivated influenza vaccine (IIV4) recipients had decreased interferon signaling compared with quadrivalent egg-based inactivated influenza vaccine (ccIIV) recipients. Peripheral blood mononuclear cell differential gene expression between vaccine types were examined at day 7 postvaccination. *P* value has been adjusted to correct for false-discovery rate ($P < .05$, 45 total genes). Ingenuity Pathway Analysis was used to examine signaling nodes and pathways that were differentially expressed. Directionality of the fold changes is indicated by Z-score (blue = decreased, orange = increased) in IIV4 (n = 41) versus ccIIV recipients (n = 40). Network interactions are depicted as follows: dotted lines indicate inferred relationships, dashed lines indicate indirect interaction, and solid lines indicate direct interaction.

bak1, *ifi35*, *ifi6*, *ifit1*, *ifit3*, *ifitm3*, *isg15*, *mx1*, *stat1*, *stat2*, and *tap1*) was significantly elevated in ccIIV4 recipients, when accounting for prevaccination levels.

Seroconversion to Vaccine Strains Was Associated With Elevated Gene Expression for Interferon Signaling

Finally, we examined gene expression between participants who seroconverted to at least 1 influenza vaccine strain (44 participants) versus those who did not (37 participants) regardless of vaccine type received. Gene expression analysis revealed 713 differentially expressed genes with a $P < .01$ (Supplementary Table 4). Gene expression analysis showed that seroconverters expressed higher levels of interferon stimulated genes (*ifi6*, *ifit1*, *ifit3*, *ifitm3*, and *isg15*) compared with participants who did not seroconvert to any influenza vaccine strain (Figure 3). Pathways for type I and III interferon were elevated in participants that seroconverted, regardless of vaccine type. Central upstream nodes were the canonical interferon signaling transcription factors IRF1 and IRF3.

DISCUSSION

Cell-based influenza vaccines have been widely available for several years. Because of their egg-free production method, they avoid the egg adaptations that have resulted in lower vaccine effectiveness of egg-based vaccines, especially against the rapidly mutating A(H3N2) strain. This strain is responsible for more severe disease and increased mortality in seasons when it is the predominant circulating strain. Thus, there has been keen interest in ccIIV4's vaccine effectiveness. With few exceptions [33, 34], published studies suggest higher vaccine effectiveness among ccIIV4 recipients than IIV4 recipients [11–24].

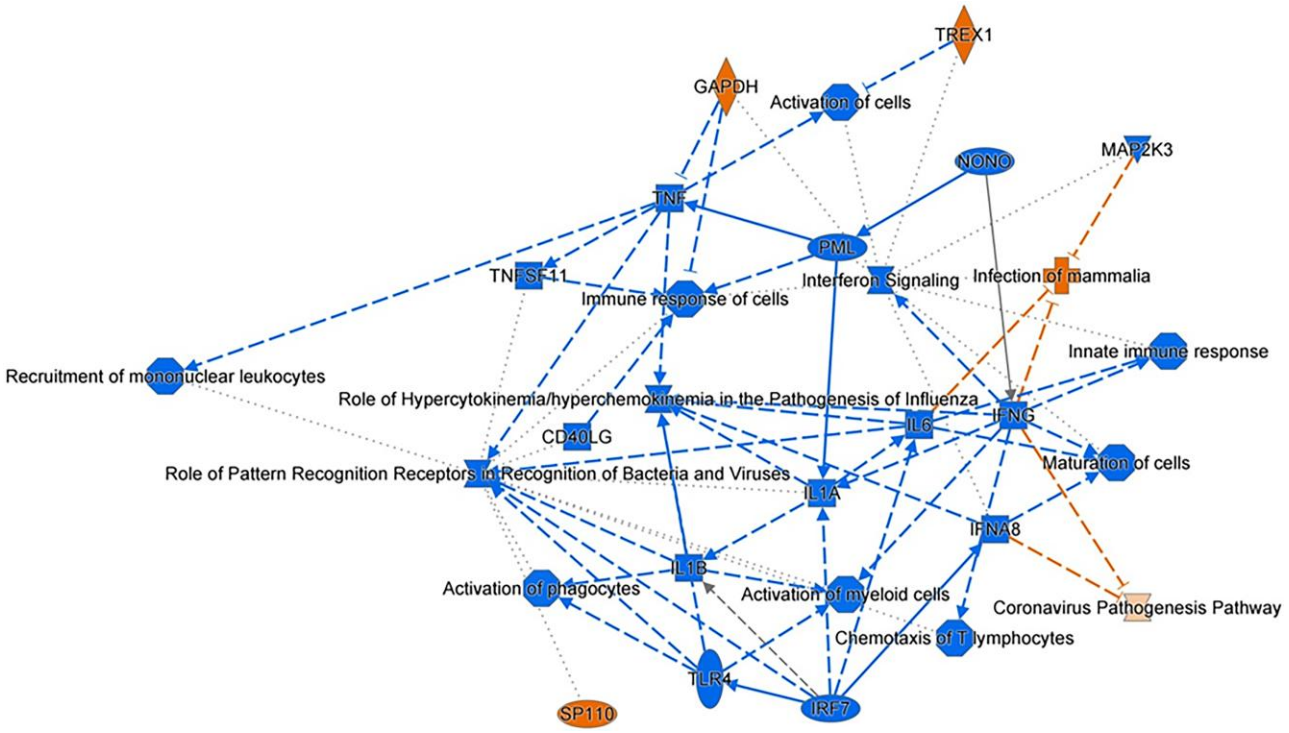

Figure 2. Quadrivalent cell-based inactivated influenza vaccine (IIV4) induced reduced interferon and innate immune activation compared with quadrivalent egg-based inactivated influenza vaccine (ccIIV). Peripheral blood mononuclear cell differential gene expression was determined by examining the change in expression between day 7 and day 0 for each vaccine type, then comparing by vaccine type: $P < .01$ (428 total genes). Directionality of the fold changes is indicated by Z-score (blue = decreased, orange = increased) in IIV4 (n = 41) versus ccIIV recipients (n = 40). Network interactions are depicted as follows: dotted lines indicate inferred relationships, dashed lines indicate indirect interaction, and solid lines indicate direct interaction.

Outcomes from these studies include influenza-related medical encounters [11–13, 17, 18], influenza-related emergency room or hospitalization [15, 16, 20, 21], influenza-like illness [14], influenza outcome [22], or polymerase chain reaction-positive influenza [19].

Several studies, all conducted in 2018–2019, have sought to explain these vaccine-effectiveness differences by comparing HAI antibody response to the vaccines. In a study of 133 adults, egg-based and cell-based influenza vaccines induced similar H3 antibody responses and neutralization activity [9]. A second study of 727 adults showed a similar result at both 1 and 6 months after vaccination [5]. In our parent study of 144 children [10], there were no differences in antibody concentration or seroconversion between vaccines, except when stratified by the source of the A (H3N2) strain tested. These studies suggest moderate to no difference in the antibody responses between vaccines.

A potential explanation for the inconsistency between antibody response and vaccine effectiveness is immune activation of additional pathways beyond antibody-producing B cells by vaccine antigens. This study is the first of its kind to compare peripheral transcriptional immune responses to cell-based and egg-based influenza vaccine in a randomized clinical trial in children. The RNA sequencing of PBMC was used to evaluate the extent of immune activation 7 days after vaccination. Analyses of differentially expressed genes revealed that ccIIV4 resulted in increased interferon signaling and innate immune activation compared with IIV4. Furthermore, participants who seroconverted to at least 1 influenza vaccine strain had higher interferon signaling than those who did not, regardless of vaccine type. These data suggest that ccIIV4 stimulates immunity differently from IIV4, despite similar HAI antibody induction.

A common thread in our gene expression analysis for ccIIV4 versus IIV4 response was the increased expression of interferon signaling pathway genes. All 3 major families of interferon (interferon α/β [type I], γ [type II]), and λ [type III]) were identified as signaling nodes that downstream genes were induced to a greater degree by ccIIV4 than IIV4. Genes regulated by the canonical interferon-inducing transcription factors IRF1, IRF3, and IRF7 were also elevated more by ccIIV4 than IIV4. These data show that interferon signaling induction was associated with seroconversion, regardless of influenza vaccine type. Interferon induction has previously been positively correlated with antibody titers after vaccination [35–37]. Moreover, we have previously shown that

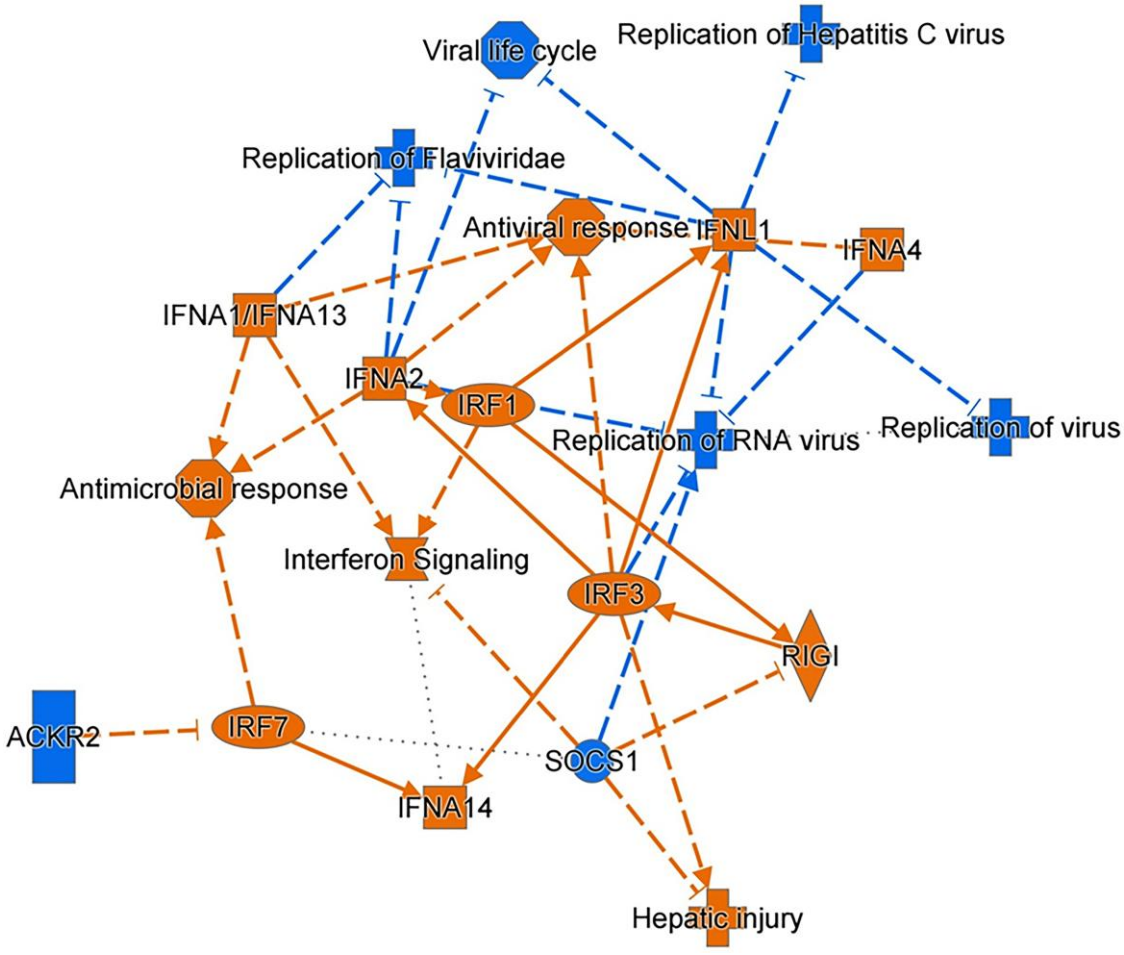

Figure 3. Participants who seroconverted to at least 1 influenza vaccine strain induced greater interferon signaling than those who did not. Peripheral blood mononuclear cell differential gene expression was determined by examining the change in expression between day 7 and day 0 for each vaccine type, then comparing by seroconversion status: $P < .01$ (713 total genes). Directionality of the fold changes is indicated by Z-score (blue = decreased, orange = increased) in nonseroconverters (N = 37) versus seroconverters (N = 44). Network interactions are depicted as follows: dotted lines indicate inferred relationships, dashed lines indicate indirect interaction, and solid lines indicate direct interaction.

expression of the interferon-inducible genes *prkra* and *ifi6* by PBMC differs between seroconverters and nonconverters in response to egg-based influenza vaccine [38]. Inactivated influenza vaccine effectiveness has also been previously associated with interferon induction [36, 37, 39, 40]. We also observed greater innate immune pathway activation in ccIIV4 recipients compared with IIV4 recipients. This potential activation of myeloid cells could precede activation of cell-mediated immunity including T-cell memory, which would be predicted to occur at a later time point than this study.

Our study has several potential limitations. Although this study is the first of its kind, it is limited to comparison of 2 influenza vaccines for the 2018–2019 influenza season. Immune response to a single vaccination may be impacted by preexisting immunity, prior immunizations, and vaccine formulation. It is also limited by the modest cohort size of 81 participants. Second, this study was performed solely in children aged 4–20 years. Adults may respond differently, and it is expected that the elderly would have altered immune activation after influenza vaccination. The aging process results in 2 major immune alterations: (1) inflammaging, low-grade persistent innate immune inflammation and (2) senescence, the loss of adaptive immune precursor cells [41]. Several functions of innate immune cells (macrophages, neutrophils, and dendritic cells) have been described to be altered in the aged in mouse and human models [42]. Differences in antigen-presenting cell migration and cytokine production could impact the vaccine response examined herein. Studies in at-risk elderly and adult populations are needed to more fully understand non-HAI titer responses to influenza vaccination. Ribonucleic acid sequencing has tremendous exploratory power, but gene transcription does not always equate

to protein expression or activity. This lack of experimental validation is a limitation, but the value in pathway identification should lead to follow-up investigations. Bulk PBMC sequencing is largely limited to immune cells; monocytes, T cells, and B cells; however, attribution of gene expression changes to specific cell types is inferred, not demonstrated. In addition, our time point examined was 6 to 9 days after vaccination. This timing is likely too early to assess adaptive immune activation differences, which may impact vaccine efficacy. Studies that incorporate influenza vaccine antigen-specific restimulation of memory T cells are needed to fully address the effect of vaccine production on this critical additional arm of immunity. Despite these limitations, this study is the first clinical trial to attempt to compare broader immune response, beyond serum antibody, to cell-based and egg-based quadrivalent-inactivated influenza vaccines.

CONCLUSIONS

Improved influenza vaccine effectiveness is a primary goal of the influenza research community worldwide. Given the knowledge of egg-adapted mutations in vaccine strain hemagglutinin, cell-based influenza vaccines were developed with the hope of better matching circulating influenza strains [3]. Numerous studies suggest that cell-based influenza vaccine has increased vaccine effectiveness versus egg-based influenza vaccine in the same season, despite similar levels of hemagglutinin antibody induction. Our study demonstrates that cell-based influenza vaccine induces differential gene expression of interferon and innate immune pathway genes compared with egg-based influenza vaccine. These results suggest that cell-based influenza vaccine may activate additional arms of the immune system beyond antibody production and perhaps explain the increased vaccine effectiveness of these vaccines.

Supplementary Data

Supplementary materials are available at *The Journal of 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

Acknowledgments. We thank the UPMC Children's Hospital of Pittsburgh Genetic Sequencing and Bioinformatics Cores for assistance in data collection and analyses.

Author contributions. JMM, KMG, MPN, RKZ, and JFA conceived the study and wrote the manuscript. JMM, KMG, and MPN conducted the clinical portion of the study. MAO processed clinical samples and performed assays. DR and JFA analyzed the data.

Disclaimer. This work represents the views of the authors and not the Centers for Disease Control and Prevention (CDC) or the National Institutes of Health (NIH). Fluzone was provided by Sanofi Pasteur, which had no other role in the study. No other funding was received.

Financial support. This research was supported in part by the University of Pittsburgh Center for Research Computing through the resources provided. Specifically, this work used the HTC cluster, which is supported by NIH award number S10OD028483. This work was supported by the CDC 5U01IP001035-02 and by the NIH UL1TR001857.

Potential conflicts of interest. All authors: The authors have ongoing relationships with the NIH and CDC as stated in the financial support section. The authors have a previous funding relationship with Sanofi Pasteur Inc.

References

1. Arunachalam AB, Post P, Rudin D. Unique features of a recombinant haemagglutinin influenza vaccine that influence vaccine performance. NPJ Vaccines **2021**; 6:144.
2. Robertson JS, Bootman JS, Newman R, et al. Structural changes in the haemagglutinin which accompany egg adaptation of an influenza A(H1N1) virus. Virology **1987**; 160:31–7.
3. Rockman S, Laurie K, Ong C, et al. Cell-Based manufacturing technology increases antigenic match of influenza vaccine and results in improved effectiveness. Vaccines (Basel) **2022**; 11:52.
4. 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.
5. 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.
6. Gaglani M, Kim SS, Naleway AL, et al. Effect of repeat vaccination on immunogenicity of quadrivalent cell-culture and recombinant influenza vaccines among healthcare personnel aged 18–64 years: a randomized, open-label trial. Clin Infect Dis **2023**; 76:e1168–76.
7. 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.
8. 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.

9. Wang W, Alvarado-Facundo E, Vassell R, et al. Comparison of A(H3N2) neutralizing antibody responses elicited by 2018–2019 season quadrivalent influenza vaccines derived from eggs, cells, and recombinant hemagglutinin. Clin Infect Dis **2021**; 73:e4312–20.
10. Moehling KK, Zimmerman RK, Nowalk MP, et al. A randomized controlled trial of antibody response to 2018–19 cell-based vs. egg-based quadrivalent inactivated influenza vaccine in children. Vaccine **2020**; 38:5171–7.
11. Boikos C, Fischer L, O'Brien D, Vasey J, Sylvester GC, Mansi JA. Relative effectiveness of the cell-derived inactivated quadrivalent influenza vaccine versus egg-derived inactivated quadrivalent influenza vaccines in preventing influenza-related medical encounters during the 2018–2019 influenza season in the United States. Clin Infect Dis **2021**; 73:e692–8.
12. Boikos C, Imran M, Nguyen VH, Ducruet T, Sylvester GC, Mansi JA. Effectiveness of the cell-derived inactivated quadrivalent influenza vaccine in individuals at high risk of influenza complications in the 2018–2019 United States influenza season. Open Forum Infect Dis **2021**; 8: ofab167.
13. Boikos C, McGovern I, Molrine D, Ortiz JR, Puig-Barbera J, Haag M. Review of analyses estimating relative vaccine effectiveness of cell-based quadrivalent influenza vaccine in three consecutive US influenza seasons. Vaccines (Basel) **2022**; 10:896.
14. Boikos C, Sylvester GC, Sampalis JS, Mansi JA. Relative effectiveness of the cell-cultured quadrivalent influenza vaccine compared to standard, egg-derived quadrivalent influenza vaccines in preventing influenza-like illness in 2017–2018. Clin Infect Dis **2020**; 71:e665–71.
15. Divino V, Krishnarajah G, Pelton SI, et al. A real-world study evaluating the relative vaccine effectiveness of a cell-based quadrivalent influenza vaccine compared to egg-based quadrivalent influenza vaccine in the US during the 2017–18 influenza season. Vaccine **2020**; 38:6334–43.
16. Divino V, Ruthwik Anupindi V, DeKoven M, et al. A real-world clinical and economic analysis of cell-derived quadrivalent influenza vaccine compared to standard egg-derived quadrivalent influenza vaccines during the 2019–2020 influenza season in the United States. Open Forum Infect Dis **2022**; 9:ofab604.
17. Imran M, Ortiz JR, McLean HQ, et al. Relative effectiveness of cell-based versus egg-based quadrivalent influenza vaccines in adults during the 2019–2020 influenza season in the United States. Open Forum Infect Dis **2022**; 9:ofac532.
18. Imran M, Ortiz JR, McLean HQ, et al. Relative effectiveness of cell-based versus egg-based quadrivalent influenza vaccines in children and adolescents in the United States during the 2019–2020 influenza season. Pediatr Infect Dis J **2022**; 41:769–74.
19. Klein NP, Fireman B, Goddard K, et al. Vaccine effectiveness of cell-culture relative to egg-based inactivated influenza vaccine during the 2017–18 influenza season. PLoS One **2020**; 15:e0229279.
20. Krishnarajah G, Divino V, Postma MJ, et al. Clinical and economic outcomes associated with cell-based quadrivalent influenza vaccine vs. standard-dose egg-based quadrivalent influenza vaccines during the 2018–19 influenza season in the United States. Vaccines (Basel) **2021**; 9:80.
21. Martin ET, Cheng C, Petrie JG, et al. Low influenza vaccine effectiveness against A(H3N2)-associated hospitalizations in 2016–2017 and 2017–2018 of the Hospitalized Adult Influenza Vaccine Effectiveness Network (HAIVEN). J Infect Dis **2021**; 223:2062–71.
22. Puig-Barbera J, Tamames-Gomez S, Plans-Rubio P, Eiros-Bouza JM. Relative effectiveness of cell-cultured versus egg-based seasonal influenza vaccines in preventing influenza-related outcomes in subjects 18 years old or older: a systematic review and meta-analysis. Int J Environ Res Public Health **2022**; 19:818.
23. 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.
24. Zimmerman RK, Patricia Nowalk M, Dauer K, Clarke L, Raviotta JM, Balasubramani GK. Vaccine effectiveness of recombinant and standard dose influenza vaccines against influenza related hospitalization using a retrospective test-negative design. Vaccine **2023**; 41:5134–40.
25. Nolan T, Fortanier AC, Leav B, et al. Efficacy of a cell-culture-derived quadrivalent influenza vaccine in children. N Engl J Med **2021**; 385:1485–95.
26. Williams KV, Zhai B, Alcorn JF, et al. A randomized controlled trial of antibody response to 2019–20 cell-based inactivated and egg-based live attenuated influenza vaccines in children and young adults. Vaccine **2022**; 40:780–8.
27. Black S, Nicolay U, Vesikari T, et al. Hemagglutination inhibition antibody titers as a correlate of protection for inactivated influenza vaccines in children. Pediatr Infect Dis J **2011**; 30:1081–5.
28. Dobin A, Davis CA, Schlesinger F, et al. STAR: ultrafast universal RNA-seq aligner. Bioinformatics **2013**; 29:15–21.
29. Liao Y, Smyth GK, Shi W. Featurecounts: an efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics **2014**; 30:923–30.
30. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol **2014**; 15:550.
31. Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A **2005**; 102:15545–50.

32. Yu G, Wang LG, Han Y, He QY. Clusterprofiler: an R package for comparing biological themes among gene clusters. OMICS **2012**; 16:284–7.
33. Bruxvoort KJ, Luo Y, Ackerson B, et al. Comparison of vaccine effectiveness against influenza hospitalization of cell-based and egg-based influenza vaccines, 2017–2018. Vaccine **2019**; 37:5807–11.
34. Izurieta HS, Chillarige Y, Kelman J, et al. Relative effectiveness of influenza vaccines among the United States elderly, 2018–2019. J Infect Dis **2020**; 222:278–87.
35. Bucasas KL, Franco LM, Shaw CA, et al. Early patterns of gene expression correlate with the humoral immune response to influenza vaccination in humans. J Infect Dis **2011**; 203:921–9.
36. Cao RG, Suarez NM, Obermoser G, et al. Differences in antibody responses between trivalent inactivated influenza vaccine and live attenuated influenza vaccine correlate with the kinetics and magnitude of interferon signaling in children. J Infect Dis **2014**; 210:224–33.
37. Zhu W, Higgs BW, Morehouse C, et al. A whole genome transcriptional analysis of the early immune response induced by live attenuated and inactivated influenza vaccines in young children. Vaccine **2010**; 28:2865–76.
38. Martin JM, Avula R, Nowalk MP, et al. Inflammatory mediator expression associated with antibody response induced by live attenuated vs inactivated influenza virus vaccine in children. Open Forum Infect Dis **2018**; 5:ofy277.
39. Athale S, Banchereau R, Thompson-Snipes L, et al. Influenza vaccines differentially regulate the interferon response in human dendritic cell subsets. Sci Transl Med **2017**; 9:eaaf9194.
40. Cole KS, Martin JM, Horne WT, et al. Differential gene expression elicited by children in response to the 2015–16 live attenuated versus inactivated influenza vaccine. Vaccine **2017**; 35:6893–7.
41. Haynes L. Aging of the immune system: research challenges to enhance the health span of older adults. Front Aging **2020**; 1:602108.
42. Shaw AC, Goldstein DR, Montgomery RR. Age-dependent dysregulation of innate immunity. Nat Rev Immunol **2013**; 13:875–87.