Transcriptional and Immunologic Correlates of Response to Pandemic Influenza Vaccine in Aviremic, HIV-Infected Children

Lesley R. de Armas1, Varghese George1, Abdelali Filali-Mouhim2, Courtney Steel3, Anita Parmigiani1, Coleen K. Cunningham4, Adriana Weinberg5, Lydie Trautmann6, Rafick-Pierre Sekaly7, Mark J. Cameron8 and Savita Pahwa1*

1 Department of Microbiology and Immunology, University of Miami Miller School of Medicine, Miami, FL, United States, 2 Department of Neurosciences, CRCHUM University of Montreal, Montreal, QC, Canada, 3 Collaborative Genomics Center, Vaccine and Gene Therapy Institute, Port St. Lucie, FL, United States, 4 Department of Pediatrics, Duke University School of Medicine, Durham, NC, United States, 5 Departments of Medicine, Pathology, and Pediatric Infectious Diseases, University of Colorado School of Medicine, Aurora, CO, United States, 6 Vaccine and Gene Therapy Institute, Oregon Health and Science University, Portland, OR, United States, 7 Department of Pathology, Emory University School of Medicine, Atlanta, GA, United States, 8 Department of Population and Quantitative Health Sciences, Case Western Reserve University, Cleveland, OH, United States

People living with HIV (PWH) often exhibit poor responses to influenza vaccination despite effective combination anti-retroviral (ART) mediated viral suppression. There exists a paucity of data in identifying immune correlates of influenza vaccine response in context of HIV infection that would be useful in improving its efficacy in PWH, especially in younger individuals. Transcriptomic data were obtained by microarray from whole blood isolated from aviremic pediatric and adolescent HIV-infected individuals (4-25 yrs) given two doses of Novartis/H1N1 09 vaccine during the pandemic H1N1 influenza outbreak. Supervised clustering and gene set enrichment identified contrasts between individuals exhibiting high and low antibody responses to vaccination. High responders exhibited hemagglutination inhibition antibody titers >1:40 post-first dose and 4-fold increase over baseline. Baseline molecular profiles indicated increased gene expression in metabolic stress pathways in low responders compared to high responders. Inflammation-related and interferon-inducible gene expression pathways were higher in low responders 3 wks post-vaccination. The broad age range and developmental stage of participants in this study prompted additional analysis by age group (e.g. <13yrs and ≥13yrs). This analysis revealed differential enrichment of gene pathways before and after vaccination in the two age groups. Notably, CXCR5, a homing marker expressed on T follicular helper (Tfh) cells, was enriched in high responders (>13yrs) following vaccination which was accompanied by peripheral Tfh expansion. Our results comprise a valuable resource of immune correlates of vaccine response to pandemic influenza in HIV infected children that may be used to identify favorable targets for improved vaccine design in different age groups.

Keywords: pandemic, influenza, vaccine, pediatric, HIV, microarray, systems vaccinology
INTRODUCTION

It is well established that very young, elderly and immune compromised individuals including people living with HIV (PWH) are at higher risk of influenza infection and related complications, underscoring the need for effective vaccine intervention in these populations (1, 2). The Centers for Disease Control recommends seasonal influenza vaccination for all persons above six months of age (3), but seasonal vaccines have shown modest efficacy (4) and low antibody titers are generated in the elderly (over age 60 years) (5, 6) and in PWH (7). In particular, children and adolescents living with perinatally acquired HIV infection have impaired responses to vaccinations, including influenza vaccination, despite successful viral suppression by combination anti-retroviral therapy (ART) (8–10).

Influenza vaccination confers protection primarily via humoral immunity (11, 12). In response to natural infection, neutralizing antibodies are critical for blocking infection while cell-mediated immunity clears the virus (13, 14). Molecular and immunological factors contributing to protection induced by vaccines have been studied amply in recent years. Systems biology approaches have been used to evaluate immune responses to vaccines, e.g. yellow fever (15–17), meningococcus (18, 19), pneumococcal (18, 20) and influenza (21–23) and have been powerful tools for elucidating immunological correlates of vaccine responses. In the context of seasonal influenza vaccination, gene sets related to immunoglobulins, complement proteins, and cellular proliferation are strongly enriched in vaccine responders compared to non-responders 7 days post-vaccination (22). Ex vivo studies show that antibody-secreting B cells exhibit peak proliferation around day 7 post-vaccination (24–26), thereby validating transcriptomic analyses in vaccine biology. Based on gene signatures alone, transcriptomic analysis from pre-vaccination samples across multiple cohorts was used to predict response to influenza vaccination with accuracy above 83% (27). However, the majority of these studies focus on healthy, young adults leaving many questions still unanswered regarding PWH and other immune-compromised populations.

In 2009, the WHO declared the pandemic influenza A H1N1 swine-origin influenza virus a novel strain. Children were found to have no pre-existing immunity to the new strain but older adults (over age 60 years) had some degree of immunity attributed to cross reactivity to past influenza strains (28). A clinical trial (P1088) launched by the International Maternal Pediatric and Adolescent Clinical Trials (IMPAACT) Network evaluated safety and efficacy of a monovalent pandemic H1N1 (pH1N1) vaccine in perinatally HIV-1-infected children and adolescents (29). We utilized a systems biology approach to evaluate gene signatures from peripheral blood before and after pH1N1 vaccination in participants of the IMPAACT P1088 study with integration of serum antibody titer data from the same individuals. Multiple gene set enrichment databases were used to correlate gene expression patterns with antibody titers induced by vaccination and create this resource for this unique patient cohort. In light of the SARS-CoV-2 pandemic beginning in 2019, this study may have further relevance to the study of vaccine responses to novel antigens in children and adolescents living with HIV infection.

MATERIALS AND METHODS

IMPAACT P1088 Clinical Study Participants and Immunogenicity Assessments

Specimens from the P1088 clinical trial “Safety of and Immune Response to an H1N1 Influenza Virus Vaccine in HIV Infected Children and Youth”, aged 4-24 years (n=40, mean age 13.7 yrs, 17 females and 23 males), were obtained from IMPAACT sites in the United States and Puerto Rico. All participants in the current study were HIV positive and receiving stable ART for at least 90 days before entry and had HIV RNA copies/ml ≤50. Other exclusion and inclusion criteria were described in the original study (29). In the trial, 155 participants received two doses (30ug) of 2009 Novartis influenza A (H1N1) monovalent vaccine separated by 21-28 days, each delivered as two 0.5 ml (15ug) injections into the thigh muscle. This study used blood samples collected pre-vaccination (baseline, BL) and 21-28 days post-first vaccination (visit 1, V1). Blood was processed for PBMC and plasma and an aliquot (2.5ml) was collected in PAXgene tubes and shipped overnight to the Miami IMPAACT laboratory at room temperature. Immunogenicity was determined by specific hemagglutination inhibition (HAI) titers in serum. The HAI assay was adapted from previously described methods (30).

Microarray Experiments on Whole Blood

Total RNA was isolated using PreAnalytix PAXgene Blood RNA Isolation Kits (Qiagen), globin removed using GLOBINclear Kit (Ambion), and the quantity and quality of the RNA was confirmed using a NanoDrop 2000c (Thermo Fisher Scientific) and an Experion Electrophoresis System (BioRad). Samples (50 ng) were amplified using Illumina TotalPrep RNA amplification kits (Ambion). The microarray analysis was conducted using 750 ng of biotinylated complementary RNA hybridized to HumanHT-12_V4 BeadChips (Illumina) at 58°C for 20 h. The arrays were scanned using Illumina’s iSCAN. All microarray data is available under GEO reference number GSE167893.

PBMC Culture and Flow Cytometry

Cryopreserved PBMC from BL and V1 were thawed and allowed to rest overnight at 37°C in culture medium (RPMI containing 10% FBS and pen/strep). For surface staining: PBMC were labeled with fluorescently-conjugated antibodies to human CD3, CD4, CD8, CD38, CD45RO, CXCR5 and HLA-DR. For 12 hr stimulation and intracellular cytokine staining: PBMC were cultured with or without 5ug/ml H1N1 (A/California/09) for 12h at 37°C prior to the staining procedure. Data was acquired on BD Fortessa Instrument and analyzed using FlowJo software version 9.7.6 (TreeStar).
Statistics
Quantile normalization, followed by a log2 transformation using the Bioconductor package LIMMA was applied to process microarrays. The LIMMA package was used to fit a linear model to each probe and perform (moderated) t tests or F tests on the groups being compared. To control the expected proportions of false positives, the FDR for each unadjusted P value was calculated using the Benjamini and Hochberg method implemented in LIMMA. Multidimensional scaling was used as a dimensionality reduction method in R to generate plots for evaluation of similarities or dissimilarities between datasets. For data mining and functional analyses, genes that satisfied a p-value (<0.05) with ≥ 1.3 fold change (up or down) were selected. The differentially expressed genes selected based on above criteria were mapped to ingenuity pathway knowledge base with different colors (red: up-regulated; blue: down-regulated). Significance of the association between the dataset and canonical pathway was measured in two ways (1): A ratio of the number of genes from the dataset that map to the pathway divided by the total number of genes that map to the canonical pathway; (2) over-representation analysis where Fisher’s test was used to calculate a p-value determining the probability that the association between the genes in the dataset and the canonical pathway is explained by chance alone.

RESULTS
Baseline Molecular Profiles Are Associated With pH1N1 Responsiveness
The primary goal of our study was to identify gene expression signatures that correlated with immunogenicity of pH1N1 monovalent vaccine in PWH of younger age groups. Vaccine response was determined by measuring hemagglutination inhibition (HAI) titers in serum before and after immunization (Figure 1). High responders were distinguished by exhibiting a ≥ 4-fold increase at week 3 (visit 1, V1) compared to week 0 (baseline, BL), while low/non-responders failed to increase titer at least 4-fold between these timepoints (31).

To investigate gene expression profiles predictive of antibody response to vaccination, we performed regression analysis using microarray data from BL samples against fold change pH1N1 titers (V1/BL). Two-way hierarchical cluster analysis of the top genes correlating with responder status (Supplementary Table S1) divided participants into 2 distinct clusters (Figure 2A). Downstream gene set enrichment analysis (GSEA) analyses of differentially expressed genes was performed on participants in the clusters using Ingenuity Pathway Analysis (IPA) and the immunologic signature module from Molecular Signatures Database (MSigDB), the latter of which contains published, manually curated gene sets from the Gene Expression Omnibus (GEO) that represent cell types, states, and perturbations of the human and mouse immune system. We found that the Low/non-responder (LNR) group exhibited pathway enrichment in mitochondrial dysfunction, oxidative phosphorylation, cytokine signaling modulation (LTB, IL-4), macrophage signaling (Fc-gamma receptor-mediated phagocytosis), and EIF2 signaling (stress-related signaling) at BL compared to high responder (HR) group (Figure 2B). In MSigDB analysis several gene sets identified were derived from studies with Flu-vaccinated HIV-negative adults (GSE29617 (21) and day 21 yellow fever vaccine responses in human PBMCs (GSE13485) (Figure 2C). The gene BCL2L1 encoding the cell death inhibitor protein Bcl-2 like protein was highly enriched in HR. HR displayed gene signatures resembling pre-vaccination signatures from HIV-negative individuals (GSE29617_CTRL_VS_TIV_FLU_VACCINE_PBMC_ 2008). Some genes from this pathway (ATP5J, UQCRQ, PSMA4, and NDUFB10) overlapped with IPA analysis as members of the mitochondrial dysfunction and oxidative phosphorylation pathways. Overall, the BL gene expression data suggests that enrichment of mitochondrial or oxidative stress transcriptional pathways at the time of vaccine administration may confer poor responses to vaccination.

![Figure 1](image_url)
Post-Vaccination Molecular Profiles Associated With pH1N1 Responsiveness

Given that pH1N1 was a novel antigen in the P1088 study cohort, participants received a boost at 21-28 days after the first vaccination. We investigated molecular signatures at this timepoint using differentially expressed gene profiles from PBMC at V1 prior to the boost vaccination. Regression analysis was performed and supervised based on the fold change in pH1N1 titer (V1/BL), as in Figure 2 (top genes listed in Supplementary Table S2). This analysis also generated two clusters with one containing all non-responders and some low responders and the other with high responders (Figure 3A). IPA analysis of the top correlating genes revealed lower expression of the activation marker CD69 (as a member of the ‘Crosstalk between Dendritic cells and Natural Killer cells’ pathway) and LY96, whose protein associates with TLR4 to respond to LPS in HR compared with LNR (Figure 3B). MSigDB analysis (Figure 3C) showed that expression of multiple IFN-inducible genes (IFI16, IFI27, IFI44, IFI44L1, IFIT1, ISG15, OAS1, OAS2, MX1) were higher in LNR. These genes are upregulated in PBMC during acute viral and bacterial infections (GSE6269) (32).

GSEA Using Cell-Type Specific Gene Database

A caveat of systems biology approaches using whole blood samples is the inability to evaluate the contribution of specific cell populations to the observed transcriptomic profiles. We employed a cell-type specific database for gene set enrichment (21) to clusters identified in previous analysis that associated with Responder groups at each timepoint. Gene signatures related to B cells, NK, and monocytes were enriched in HR at BL, while DC subsets and T cells were enriched in LNR (Figure 4A). At V1, DC subsets were enriched in HR along with B cells, NK, and monocytes, while T cell signatures remained enriched in LNR (Figure 4B). Monocyte-associated gene expression
accounted for most of the observed genes and showed enrichment at BL and V1 in HR (Figures 4C, D, respectively). This signature shared multiple genes (ACTG1, ALDOA, ATP6V06, CD151, CSF3R, CTSD, FKBP8, GRN, MARK2, SLC6A10P, TSPO, TYMP, and UBXN6) between the two timepoints (Figure 4B).

Pathway Analysis by Age of Study Participants

The P1088 study enrolled participants representing a broad age range from 4 to 24 years old, however age was not associated with Responder status (29). In the subset of participants analyzed by microarray, there was similarly no correlation between age and fold change of antibody titers (r=0.015). However, we reasoned that puberty may affect gene expression profiles in HIV-infected children and adolescents and therefore divided the donors into two age groups for further pathway analyses: 4-12 years (“children”; n=16) and 13-24 years (“adolescents”, n=24). For this analysis individuals were compared in each age group based on responder status: fold change of ≥ 4 were considered high responders (HR) and < 4 were considered low/non-responders (LNR). IPA analysis of gene expression at BL revealed a group of molecular pathways that were induced in both age groups (e.g. age-independent) as well as age-dependent pathways for each age group (Figure 5). Age-independent pathways enriched in HR were related to metabolic pathways (Pentose Phosphate-Oxidative branch, Aryl hydrocarbon receptor, Vitamin D/Retinoic acid receptor), and cell survival and protein synthesis pathways (PI3K/AKT, eIF4 and p70S6K, mTOR) confirming data from regression and cluster analysis in Figure 2. HR in the adolescent group demonstrated enrichment in more pathways than children including numerous pathways involved in cell

**FIGURE 3** | Correlates of Vaccine-induced Antibody Responses Post-Vaccination. (A) Heatmap representation of the top significantly correlated transcripts from regressing baseline expression from all participants and fold change difference in pH1N1 antibody titers at V1 compared to BL. The expression intensities are represented using a blue-white-red color scale. Rows correspond to probes and columns correspond to profiled samples (p ≤ 0.05). “High” responder and “low/ non” responder clusters were compared and subjected to gene set enrichment analysis using the IPA database (B) and MSigDB Immunologic signature model (C).
growth (RAR activation, Cdc42, Rho, G beta gamma signaling),
cell adhesion and mobility (remodeling of Epithelial Adherens
Junctions, Integrin and Tight Junction signaling) and hormonal
and growth factor signaling (prolactin, IGF-1, NGF, BMP and
GNRH signaling).

Consistent with BL data, HR in the younger age group
exhibited distinct molecular signatures from the adolescent
subset at V1, sharing only one gene; Forkhead box O3
(FOXO3) amongst the top 10 enriched pathways (Figure 6).
Molecular pathways related to cell cycle and protein translation
were enriched in children HR including EIF2 signaling, an
indicator of ER stress and unfolded protein response (UPR)
(Figure 6A). In the adolescent group, classic inflammatory
markers such as TNF, FASLG, and CXCL10 had higher
expression in LNR compared to HR (Figure 6B). In the
younger group, ‘classical’ inflammatory markers were not
identified, however other inflammation-related genes such as
ADAM17, GSTP1, and PPP1R15A were upregulated in LNR,
suggesting that different, age-dependent mechanisms of
inflammation may be responsible for poor influenza
vaccine responses.

Markers of T Follicular Helper Cells Are
Enriched in High Responders to pH1N1
Vaccine

Higher expression of the CXC chemokine receptor type 5 (CXCR5)
was noted at V1 in HR (Figure 6B). CXCR5 is a homing marker of
T follicular helper cells (Tfh), a CD4+ T cell subset essential for
supporting B cell function and differentiation via abundant
production of IL-21 in germinal centers (GC). A proportion of
circulating CD4+ T cells express CXCR5 and exhibit functional properties of GC Tfh (33–35). To explore the significance of CXCR5 expression in the microarray data in HR at V1, we investigated peripheral (pTfh) frequencies and function by flow cytometry. We did not observe differences in the frequency of pTfh prior to vaccination, however frequencies of pTfh (CD4+CD45RO+CXCR5+) were significantly higher at V1 compared to BL in HR only (Figure 7A). Upon in vitro stimulation of PBMC with pH1N1 antigen, pTfh from HR produced significantly more IL-21 at V1 compared to LNR and frequency of IL-2-producing pTfh positively correlated with HAI titer at V1 (Figures 7B, C, respectively).

To investigate the relationship immune activation and vaccine response, we measured co-expression of CD38 and HLA-DR on CD4+ T cells. At BL, LNR exhibited increased frequencies of CD38+HLADR+ CD4+ T cells compared to HR (2.5 +/-0.29 vs. 1.6 +/-0.26, respectively, p=0.04). CD38+HLADR+ CD4+ T cells at BL showed negative correlations with IL-21-producing pTfh and pH1N1 titer at V1 (Figures 7D, E, respectively). These data provide a link between known immunological correlates of influenza vaccine response to immunological and transcriptional signatures at pre-vaccination.

**DISCUSSION**

In this study, transcriptomic analyses of whole blood were applied to identify gene signatures related to novel pH1N1 vaccine responses in HIV-infected children and adolescents under suppressive ART. We used multiple GSEA platforms to generate a comprehensive resource of the transcriptomic changes related to antibody responses in young PWH. Our hypothesis was that chronic immune activation would influence vaccine responses (36). Previously, our group has shown that prior to vaccination markers of immune activation, including CD38+HLA-DR+ T cells and serum levels of TNF and other inflammatory markers, negatively correlate with antibody responses to seasonal influenza vaccination (including pH1N1) in multiple cohorts of HIV-infected ART-treated adults (37–40).
**FIGURE 6** | Pathways Analysis in two Age Groups at Post-Vaccination. Top 10 (or top selected) significant pathways and their gene members enriched when comparing high responder versus low/non-responder at Visit 1 in 4-12yr group (A) and 13-24yr group (B). Each row is a regulated canonical pathway (Ingenuity software); each column represents an up-(red) or down-(blue) regulated genes (p-value <= 0.05 and |FC|>1.3) induced in 1 or more pathways(s). Over representation test was performed using Fisher Exact Test; significance, displayed on the right, is achieved for p<0.05 (-log(p)1.3).

**FIGURE 7** | Increase in pTfh Frequency and Function Post-Vaccination in pH1N1 Responders. (A) Frequencies of CD4+CD45RO+CXCR5+ peripheral T follicular helper cells (pTfh) without stimulation and (B) IL-21+pTfh after 12-hour stimulation with H1N1 from responders (n=14) and non-responders (n=14) at baseline (BL) and post vaccination (visit 1, V1). Pearson correlations between (C) IL-21+pTfh at V1 following H1N1 stimulation with HAI titers at V1 (D) IL-21+pTfh at V1 following H1N1 stimulation with baseline CD4 Immune activation (E) HAI titers at V1 with baseline CD4 Immune activation. P-values were calculated with Student’s t-test or Mann-Whitney test as appropriate. Box plots include median with 25th and 75th percentile borders, and error bars represent 10th and 90th percentiles. Stars indicate the level of significance: *p < 0.05, **p < 0.01.
with vaccine responses to influenza (55). Our results did not address monocyte function since samples were analyzed without stimulus; however, we found increased IFN-inducible gene expression in LNR at V1 by multiple analysis platforms (IPA, MSigDB, and Age-specific) which could be attributed to the monocyte population. Proteins encoded by IFN-inducible genes are essential antiviral effectors with capabilities to block at various steps of the viral life cycle (56), however unregulated IFN responses can lead to immune dysfunction. The role of type I IFN in HIV infection is complex; it is important in controlling viral replication very early following infection while contributing to pathogenesis in chronic infection (57). Because the difference in IFN-inducible gene expression was not present prior to vaccination, our findings beg the question of what effect routine vaccination is having on viral replication and the existing HIV infection. Influenza vaccination has been shown to increase plasma viremia transiently following vaccination (peak 2 weeks post-vaccination) (58). Future studies monitoring vaccine responses in PWH on ART should consider a possible effect on viral recrudescence. It will be important to evaluate the role of monocytes in influencing antibody-driven vaccine responses. CSF3R encodes the receptor for granulocyte-colony stimulating factor (G-CSF) and is expressed on circulating, classical monocytes (CD14+CD16−) (59) suggesting that enrichment of this predominant monocyte subset could be an immunological correlate for antibody responses as well as a biomarker or predictor of response.

Despite the intriguing findings in this cohort there were several limitations inherent in the present study. The study design catered to blood sampling coincident with important timepoints for measuring serum antibody responses. The inclusion of blood sampling at an early timepoint post-vaccination (e.g. day 2-7) would have allowed for evaluation of the innate immune response to the vaccine, however the innate response to seasonal influenza vaccine (trivalent-inactivated) has been studied extensively in HIV-uninfected populations (18, 21, 22, 60, 61). Thus, our study is focused on creating a resource of later adaptive immune biomarkers that correlate with serum antibody titers to a novel influenza antigen and, therefore, response. As one of the few transcriptomic studies in perinatal HIV infected children and adolescents, the data presented herein will undoubtedly serve as a novel resource for further immune monitoring studies in this population and may especially be important in light of the current pandemic SARS-CoV-2.

Unexpectedly, this cohort had relatively high baseline antibody responses to pH1N1 despite no documented exposure to the antigen (29), while other cohorts with participants at similar ages displayed low baseline titers and a lack of cross-reactivity (62). Despite the complexity in using systems biology to predict immunogenicity to influenza vaccines due to individual infection and vaccination histories, we believe that our transcriptomic study in young PWH fills an age gap and has yielded a valuable resource likely to provide insight into favorable and negative targets for improving vaccine design and assessment of vaccine responses in young PWH subjects.
AUTHOR’S NOTE

Parts of the work described in this manuscript have been presented at Conference of Retrovirus and Opportunistic Infections 2013 and American Association of Immunologists 2014 as poster presentations by A.P. and L.D., respectively.

DATA AVAILABILITY STATEMENT

The data presented in the study are deposited in the Gene Expression Omnibus data repository (https://www.ncbi.nlm.nih.gov/geo/) under the accession number GSE167893.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by International Maternal Pediatric Adolescent AIDS Clinical Trials Network. Written informed consent to participate in this study was provided by the participants’ legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

SP, AW, CC, R-PS, and MC were involved in conception of the work. CS, AP, VG, and AF-M were involved in data collection. LD, CS, AP, VG, AF-M, R-PS, MC, and SP, data analysis and interpretation. LD, MC, and SP drafted the article. LD, CS, VG, AW, CC, R-PS, MC, and SP provided critical revision of the article. All authors contributed to the article and approved the submitted version.

FUNDING

This work was funded by an ARRA IMPACT grant to SP. Overall support for the International Maternal Pediatric Adolescent AIDS Clinical Trials Group (IMPAACT) was provided by the National Institute of Allergy and Infectious Diseases (NIAID) [U01 AI068632], the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) and the National Institute of Mental Health (NIMH) [AI068632]. This work was supported by the Statistical and Data Analysis Center at Harvard School of Public Health, under the National Institute of Allergy and Infectious Diseases cooperative agreement #5 U01 AI41110 with the Pediatric AIDS Clinical Trials Group (PACTG) and #1 U01 AI068616 with the IMPAACT Group. Support of the sites was provided by the National Institute of Allergy and Infectious Diseases (NIAID) and the NICHD International and Domestic Pediatric and Maternal HIV Clinical Trials Network funded by NICHD (contract number N01-DK-9–001/HSN267200800001C). This publication was made possible by support for the Miami Center for AIDS Research (CFAR) at the University of Miami Miller School of Medicine funded by a grant (P30AI073961) and to SP (R01AI108472) from the National Institutes of Health (NIH) and R01, which is supported by the following NIH Co-Funding and Participating Institutes and Centers: NIAID, NCI, NICHD, NHLBI, NIDA, NIMH, NIA, NIDDK, NIGMS, FIC AND OAR. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

ACKNOWLEDGMENTS

We acknowledge members of the P1088 Protocol Team which include: Patricia M. Flynn, MD, MS, St. Jude Children’s Research Hospital, Memphis TN; Sharon Nachman, MD, SUNY Health Science Center at Stony Brook, Stony Brook, NY; Petronella Muresan, MS, Statistical and Data Analysis Center, Harvard School Public Health, Boston, MA; Terence Fenton, Ed D, Statistical and Data Analysis Center, Harvard School Public Health, Boston, MA; Stephen A. Spector, MD, University of California, San Diego, La Jolla, CA and Rady Children’s Hospital; Coleen K. Cunningham, MD, Duke University Medical Center, Durham, NC; Robert Pass, MD, University of Alabama at Birmingham, Birmingham, AL; Ram Yogev, MD, Children’s Memorial Hospital and Feinberg School of Medicine Northwestern University Medical School, Chicago, IL; Sandra Burchett, MD, MS, Children’s Hospital Boston, Boston, MA; Barbara Heckman, BS, Frontier Science and Technology Research Foundation, Buffalo, NY; Anthony Bloom, BA, Frontier Science and Technology Research Foundation, Buffalo, NY; L. Jill Utech, RN, MSN, CCRC, St. Jude Children’s Research Hospital, Memphis, TN; Patricia Anthony, BS, CLS, University of Southern California, Keck School of Medicine, Los Angeles, CA; Elizabeth Petzold, PhD, Social and Scientific Systems, Silver Spring, MD; Wende Levy, RN, MS, Social and Scientific Systems, Silver Spring, MD; Jennifer Bryant, MPA, Westat, Rockville, MD; George K. Siberry, MD, MPH, Pediatric Adolescent and Maternal AIDS Branch, Eunice Kennedy Shriver National Institute of Child Health and Development, Bethesda, MD; Ruth Ebiyash, Pharm D, MS, R Ph., Division of AIDS, National Institute of Allergy and Infectious Diseases, Bethesda, MD; Judi Miller, RN, BSN, Division of AIDS, National Institute of Allergy and Infectious Diseases, Bethesda, MD; Ed Handelsman, MD, Division of AIDS, National Institute of Allergy and Infectious Diseases, Bethesda, MD; Adriana Weinberg, MD, University of Colorado, Denver, Aurora, CO.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2021.639358/full#supplementary-material
38. de Armas LR, Pallikkuth S, George V, Rinaldi S, Pahwa R, Arheart KL, et al. Reevaluation of immune activation in the era of CAR T and an aging HIV-infected population. JCI Insight (2017) 2(20). doi: 10.1172/jcinsight.95726.

39. George VK, Pallikkuth S, Parmigiani A, Alcaide M, Fischl M, Arheart KL, et al. HIV infection Worsens Age-AssOCIated Defects in Antibody Responses to Influenza Vaccine. J Infect Dis (2015) 211(12):1959–68. doi: 10.1093/infdis/jiu840

40. Pallikkuth S, Parmigiani A, Silva SY, George VK, Fischl M, Pahwa R, et al. Impaired peripheral blood T-follicular helper cell function in HIV-infected nonresponders to the 2009 H1N1/09 vaccine. Blood (2012) 120(5):985–93. doi: 10.1182/blood-2011-12-396648

41. Gonzalez-Amaro R, Cortes JR, Sanchez-Madrid F, Martin P. Is CD69 an innate immunity marker? Trends Mol Med (2013) 19 (10):625–32. doi: 10.1016/j.molmed.2013.07.006.

42. Domblices C, Cartlidge L, Faustin B. Metabolic Stress in the Immune Function of T Cells, Macrophages and Dendritic Cells. Cells (2018) 7(7):68. doi: 10.3390/cells7070068

43. Marijt KA, Sluiter M, Blijleven L, Tolmeijer SH, Scheeren FA, van der Burg SH, et al. Metabolic stress in cancer cells induces immune escape through a P13K-dependent blockade of IFN gamma receptor signaling. J Immunother Cancer (2019) 7(1):152. doi: 10.1186/s40428-019-0627-8

44. Schoeman JC, Moutloatse GP, Harms AC, Vreeken RJ, Scherpbier HJ, Van Leeuwen L, et al. Fetal Metabolic Stress Disrupts Immune Homeostasis and Induces Proinflammatory Responses in Human Immunodeficiency Virus Type 1- and Combination Antiretroviral Therapy-Exposed Infants. J Infect Dis (2017) 216(4):436–46. doi: 10.1093/infdis/jix291

45. West AP, Khoury-Hanold W, Staron M, Tal MC, Pineda CM, Lang SM, et al. Pallikkuth S, de Armas LR, Rinaldi S, George VK, Pan L, Arheart KL, et al. Dysfunctional peripheral T follicular helper cells dominate in people with impaired influenza vaccine responses: Results from the FLORAH study. PLoS Biol (2019) 17(5):e3000257. doi: 10.1371/journal.pbio.3000257

46. Schultz BT, Teiger JC, Pisani F, Oster AF, Kranias G, Alter G, et al. Circulating HIV-Specific Interleukin-21(+)CD4(+T Cells Represent Peripheral Tfh Cells with Antigen-Dependent Helper Functions. Immunity (2016) 44(1):167–78. doi: 10.1016/j.immuni.2015.12.011

47. Pallikkuth S, Chaudhury S, Lu P, Pan L, Jongert E, Wille-Reece U, et al. A delayed fractionated dose RTS,S AS01 vaccine regimen mediates protection via improved T follicular helper and B cell responses. Elife (2020) 9:e51889. doi: 10.7554/eLife.51889

48. van Duin D, Allore HG, Mohanty S, Ginter S, Newman FK, Belshe RB, et al. Precancer determination of the expression of costimulatory B7 molecules in activated monocytes predicts influenza vaccine responses in young and older adults. J Infect Dis (2007) 195(11):1590–7. doi: 10.1086/516788

49. Sadler AJ, Williams BR. Interferon-inducible antiviral effectors. Nat Rev Immunol (2008) 8(7):559–68. doi: 10.1038/nri2314

50. Sandler NG, Bosinger SE, Estes JD, Zhu RT, Tharp GK, Boritz E, et al. Type I interferon responses in rhesus macaques prevent SIV infection and slow disease progression. Nature (2014) 511(7511):601–5. doi: 10.1038/nature13554

51. Stappers SF, Hamilton BL, Follansbee SE, Elbeik T, Barbosa P, Grant RM, et al. Activation of virus replication after vaccination of HIV-1-infected individuals. J Exp Med (1995) 182(6):1727–37. doi: 10.1084/jem.182.6.1727

52. Pallikkuth S, de Armas LR, Rinaldi S, George VK, Pan L, Arheart KL, et al. Dysfunctional peripheral T follicular helper cells dominate in people with impaired influenza vaccine responses: Results from the FLORAH study. PLoS Biol (2019) 17(5):e3000257. doi: 10.1371/journal.pbio.3000257

53. Schultz BT, Teiger JC, Pisani F, Oster AF, Kranias G, Alter G, et al. Circulating HIV-Specific Interleukin-21(+)CD4(+T Cells Represent Peripheral Tfh Cells with Antigen-Dependent Helper Functions. Immunity (2016) 44(1):167–78. doi: 10.1016/j.immuni.2015.12.011

54. Pallikkuth S, Chaudhury S, Lu P, Pan L, Jongert E, Wille-Reece U, et al. A delayed fractionated dose RTS,S AS01 vaccine regimen mediates protection via improved T follicular helper and B cell responses. Elife (2020) 9:e51889. doi: 10.7554/eLife.51889

55. van Duin D, Allore HG, Mohanty S, Ginter S, Newman FK, Belshe RB, et al. Precancer determination of the expression of costimulatory B7 molecules in activated monocytes predicts influenza vaccine responses in young and older adults. J Infect Dis (2007) 195(11):1590–7. doi: 10.1086/516788

56. Sadler AJ, Williams BR. Interferon-inducible antiviral effectors. Nat Rev Immunol (2008) 8(7):559–68. doi: 10.1038/nri2314

57. Sandler NG, Hamilton BL, Follansbee SE, Elbeik T, Barbosa P, Grant RM, et al. Activation of virus replication after vaccination of HIV-1-infected individuals. J Exp Med (1995) 182(6):1727–37. doi: 10.1084/jem.182.6.1727

58. Mobley JL, Leininger M, Madore S, Baginski TJ, Renkiewicz R. Genetic evidence of a functional monocye dichotomy. Inflammation (2007) 30(6):189–97. doi: 10.1016/j.inflamed.2007.09360-0

59. Nakaya HI, Clutterbuck E, Kazmin D, Wang L, Cortese M, Bosinger SE, et al. Systems biology of immunity to MF59-adjuvanted versus nonadjuvanted trivalent seasonal influenza vaccines in early childhood. Proc Natl Acad Sci USA (2016) 113(7):1853–8. doi: 10.1073/pnas.1519690113

60. Nakaya HI, Hagan T, Duraisingham SS, Lee WK, Kwissa M, Rouphael N, et al. Systems Analysis of Immunity to Influenza Vaccination across Multiple Years and in Diverse Populations Reveals Shared Molecular Signatures. Immunity (2015) 43(6):1186–98. doi: 10.1016/j.immuni.2015.11.012

61. Tsang JS, Schwartzberg PL, Kotliarov Y, Biancotto A, Xie Z, Germain RN, et al. Global analyses of human immune variation reveal baseline predictors of postvaccination responses. Cell (2014) 157(2):499–513. doi: 10.1016/j.cell.2014.03.031

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.