Vaccine xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Plasma cell and serum antibody responses to influenza vaccine in preterm and full-term infants ☆

Carl T. D'Angio [a,*], Claire P. Wyman [a], Ravi S. Misra [a], Jessica L. Halliley [b], Hongyue Wang [c], Julianne E. Hunn [a], Caitlin M. Fallone [a], F. Eun-Hyung Lee [b,d]

[a] Department of Pediatrics, University of Rochester School of Medicine and Dentistry, 601 Elmwood Avenue, Rochester, NY 14642, USA
[b] Departments of Microbiology & Immunology, University of Rochester School of Medicine and Dentistry, 601 Elmwood Avenue, Rochester, NY 14642, USA
[c] Department of Biostatistics, University of Rochester School of Medicine and Dentistry, 601 Elmwood Avenue, Rochester, NY 14642, USA
[d] Department of Medicine, Emory University School of Medicine, 1648 Pierce Drive NE, Atlanta, GA 30307, USA

ARTICLE INFO

Article history:
Received 10 June 2017
Received in revised form 28 July 2017
Accepted 29 July 2017
Available online xxxx

Keywords:
Influenza vaccine
Premature infant
Antibody-secreting cell
Antibody titer
Plasma cell

ABSTRACT

Background: Preterm (PT) infants are at greater risk for severe influenza infection and experience decrements in long-term antibody responses to vaccines. This may related to defects in antibody secreting cell (ASC) generation.
Objective: To investigate the relationships among the frequencies of influenza-specific antibody secreting cells, ASC numbers and subsets, and antibody responses to influenza vaccines (IV) among PT and full-term (FT) infants.
Design/methods: We enrolled 11 former PT (≤32 weeks′ gestation, ≤1500 g′ birth weight) and 11 FT infants, 6–17 months of age, receiving their first influenza immunizations. Infants received two doses of inactivated trivalent (T)IV or quadrivalent (Q)IV during the 2012–2013 and 2013–2014 influenza seasons, respectively, at 0 and 28 days, and blood was drawn at 0, 10, 35, and 56 days and 9 months. Vaccine-specific antibody was measured by hemagglutination inhibition (HAI) at 0 and 56 days and 9 months, vaccine-specific ASC numbers by enzyme linked immunospot (ELISPOT) at 10 and 35 days, and ASC subsets by flow cytometry at 0, 10 and 35 days.
Results: PT infants had post-vaccine HAI titers to all 4 vaccine strains at least equal to FT infants at 56 days and 9 months after beginning immunization. Influenza-specific ASC ELISPOT responses at 35 days were higher among PT than FT infants (median 100 v. 30 per 10^6 PBMC, $p = 0.04$). ASC numbers at 35 days were positively correlated with serum HAI titers at 56 days ($\rho = 0.50–0.80$). There were no statistical differences between PT and FT infants in the frequency of five ASC subsets and no specific ASC subset correlated with durability of serum antibody titers.
Conclusions: Influenza-specific ASC numbers in both FT and PT infants correlated with peak antibody titers, but ASC subsets did not correlate with durability of antibody response.

1. Introduction

Infants, particularly preterm (PT) infants, are at a greater risk for severe influenza infection and related complications [1]. PT infants are born with immature immune systems, including defects in B-cell function, yet it is unclear how prematurity affects the quality of the cellular vaccine responses or when immune function matures after birth [2]. Compared to full term (FT) infants, extremely PT infants have decrements in serum titers to a number of vaccines that can persist at least to school age [3–18]. Although PT and FT infants exhibit similar acute serological responses to influenza vaccines [19], no studies have assessed long term immunogenicity or cellular immunity following influenza vaccine among PT infants.

After vaccination, protective antibody titers originate from naïve B cell differentiation into antibody secreting cells (ASC) and memory B cells in infants. In adults, most circulating ASC demonstrate vaccine antigen specificity with little bystander response

Abbreviations: ASC, Antibody Secreting Cells; ELISPOT, Enzyme-Linked Immunospot; FT, Full-Term; GA, Gestational Age; HAI, Hemagglutination Inhibition; LLPC, Long-Lived Plasma Cells; PBMC, Peripheral Blood Mononuclear Cell; PT, Preterm; QIV, Quadravalent Inactivated Influenza Vaccine; TIV, Trivalent Inactivated Influenza Vaccine.

☆ This research was supported in part by NHLBI R01 HL097141-01, 1R01 AI121252, P01 AI125180-01, P01 A1078907-04.

* Corresponding author at: Box 651, Neonatology, Golisano Children's Hospital, University of Rochester Medical Center, 601 Elmwood Avenue, Rochester, NY 14642, USA.
E-mail address: carl_dangio@urmc.rochester.edu (C.T. D'Angio).

http://dx.doi.org/10.1016/j.vaccine.2017.07.115
0264-410X/

[20], and influenza-specific ASC frequency correlates with the rise in protective antibody titers [21]. Among adults, there is significant heterogeneity in the ASC ($CD19^{+}CD27^{hi}CD38^{hi}$) subsets after vaccination that include $CD138^{+}$ and $CD138^{neg}$ subsets [22,23]. We have also identified $CD19^{neg}$ ASC populations after vaccination that resemble a recently described human bone marrow long-lived plasma cell (LLPC) phenotype by cell surface markers [24].

We evaluated the relationships among the frequencies of influenza-specific antibody secreting cells, ASC numbers and subsets, and antibody responses to influenza vaccines (IV) among PT and full-term (FT) infants.

2. Materials and methods

2.1. Subjects

In this prospective study, infants were recruited during the 2012–2013 and 2013–2014 influenza vaccine seasons at the University of Rochester. We enrolled very-low-birth-weight (≤1500 g at birth), PT (≤ 32 weeks′ gestation at birth) infants and normal-birth-weight (>2500 g at birth), FT (35 0/7 to 41 6/7 weeks′ gestation at birth) infants into the study. Subjects were 6–17 months chronological age, previously un-immunized for influenza, and eligible for influenza immunization. Subjects with known immunodeficiency, concurrent systemic corticosteroid administration or physician-diagnosed influenza illness were excluded. The Institutional Review Board approved the study, and informed parental permission was obtained.

Children had six study visits over 9 months: at 0 (Visit 1), 10 (Visit 2), 28 (Visit 3), 35 (Visit 4) and 56 (Visit 5) days, and 9 months (Visit 6) from the first dose of influenza vaccine (Fig. 1). Children received two doses (prime/boost) by intramuscular thigh injection with a 1-inch needle of trivalent (TIV) (H1N1, H3N2, B-Yamagata, 2012–2013 season, multiple lots) or quadrivalent (QIV) (H1N1, H3N2, B-Yamagata, B-Victoria, 2013–2014 season, multiple lots), inactivated, split-virion influenza vaccine (Fluzone®, Sanofi Pasteur, Swiftwater, PA), about 4 weeks apart. TIV components were A/California/7/2009 (H1N1), A/Victoria/361/2011 (H3N2), and B/Wisconsin/1/2010 (B/Yamagata lineage). QIV components were A/California/7/2009 (H1N1)-like virus, A/Victoria/361/2011 (H3N2)-like virus, B/Massachusetts/2/2012-like virus (B/Yamagata lineage), and B/Brisbane/60/2008-like virus (B/Victoria lineage). Since only QIV contained the B/Victoria strain, B/Victoria-related assay results are reported only for children receiving QIV. Blood was obtained at five time points (Fig. 1). An interim medical history, focused on influenza symptoms, was obtained at each study visit.

2.2. Peripheral blood mononuclear cell (PBMC) isolation

PBMC were isolated as previously described [25]. Briefly, plasma was first separated by centrifugation at room temperature. The blood, diluted 1:2 with phosphate buffered saline, was centrifuged over a Ficoll gradient. The buffy coat layer was transferred to a 15-mL tube, and the cells were washed with HBSS. Cells were counted and viability assessed by Trypan Blue exclusion. All enzyme-linked immunospot (ELISPOT) and flow cytometry assays were done on fresh cells.

2.3. Influenza hemagglutination inhibition assay (HAI)

Sera were separated and frozen at −80 °C until analysis. Influenza antibody levels were measured by microtiter hemagglutination inhibition (HAI) test, as previously described [19]. Briefly, reagents for the HAI test were obtained from the WHO Collaborating Centers for Influenza, Centers for Disease Control and Prevention. Hemagglutination titer for each influenza antigen was determined and diluted to contain 4 HA units in 0.025-mL of virus suspension. H1N1, H3N2, B/Victoria, and B/Yamagata antigens were used. Serum titers were determined by the highest dilution of serum capable of inhibiting hemagglutination. A serum control was run on each plate, and all assays within a vaccine season were run using the same lot of antigen.

2.4. Antigen-specific ASC ELISPOT

The frequency of influenza antigen-specific ASCs was measured by ELISPOT, as previously described [21]. Briefly, 96-well ELISPOT plates were coated for 2 h at room temperature with the following: TIV for the 2012–2013 cohort (6 μg/mL, Sanofi Pasteur), QIV for the 2013–2014 cohort (8 μg/mL, Sanofi Pasteur), goat anti-human IgG (5 μg/mL, Invitrogen), tetanus toxoid (2 μg/ml, Calbiochem) or Bovine Serum Albumin (2% in PBS, Fisher), diluted in PBS. Plates were then washed and blocked with RPMI + fetal bovine serum + antibiotic/antimycotic for 2 h at 37 °C with 5% CO_2. Plates were incubated at 37 °C with 8% CO_2 for 18–20 h with 300,000, 100,000 or 10,000 PBMC in duplicate. Cells were then aspirated and plates washed with PBS with 0.1% Tween. Bound antibodies were detected with alkaline phosphatase-conjugated goat-anti-human IgG (1 μg/mL, Jackson Immunoresearch) for 2 h and

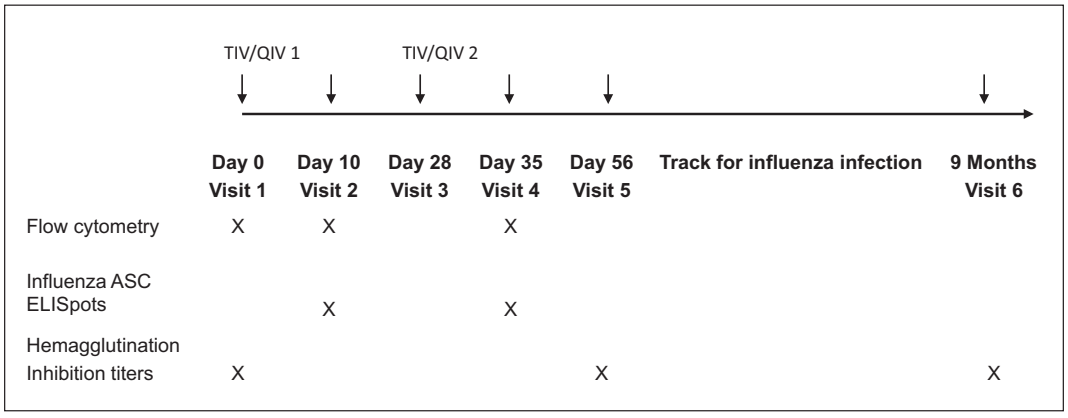

Fig. 1. Schematic of study design. TIV = Trivalent influenza vaccine, QIV = quadrivalent influenza vaccine. Arrows denote visits. X = blood draw performed for indicated analysis.

Please cite this article in press as: D'Angio CT et al. Plasma cell and serum antibody responses to influenza vaccine in preterm and full-term infants. Vaccine (2017), http://dx.doi.org/10.1016/j.vaccine.2017.07.115