Vaccine 34 (2016) 495–502

Contents lists available at ScienceDirect

Vaccine

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

Prior infection with influenza virus but not vaccination leaves a long-term immunological imprint that intensifies the protective efficacy of antigenically drifted vaccine strains☆

Jin Hyang Kim[a], Justine Liepkalns[a,1], Adrian J. Reber[a], Xiuhua Lu[a], Nedzad Music[a], Joshy Jacob[b,**], Suryaprakash Sambhara[a,*]

[a] Influenza Division, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, 1600 Clifton Road, Atlanta, GA 30329, USA

[b] Department of Microbiology and Immunology, Emory Vaccine Center, Emory University, 954 Gatewood Rd, Atlanta, GA 30329, USA

ARTICLE INFO

Article history:
Received 22 September 2015
Received in revised form
17 November 2015
Accepted 30 November 2015
Available online 17 December 2015

Keywords:
Influenza virus
Infection
Antigenic drift
Vaccine efficacy
Pandemic H1N1

ABSTRACT

The role of pre-existing immunity for influenza vaccine responses is of great importance for public health, and thus has been studied in various contexts, yet the impact of differential priming on vaccine responses in the midst of antigenic drift remains to be elucidated. To address this with antigenically related viruses, mice were first primed by either infection or immunization with A/Puerto Rico/8/34 (PR8) virus, then immunized with whole-inactivated A/Fort Monmouth/1/47 (FM1) virus. The ensuing vaccine responses and the protective efficacy of FM1 were superior in PR8 infection-primed mice compared to PR8 immunization-primed or unprimed mice. Increased FM1-specific Ab responses of PR8 infection-primed mice also broadened cross-reactivity against contemporary as well as antigenically more drifted strains. Further, prior infection heightened the protective efficacy of antigenically distant strains, such as A/Brisbane/59/2006 infection followed by immunization with split pandemic H1N1 vaccine (A/California/07/2009). Therefore, influenza infection is a significant priming event that intensifies future vaccine responses against drift strains.

1. Introduction

Recommendations by the US Advisory Committee on Immunization Practices (ACIP) has helped improve overall influenza vaccine coverage and reduce disease burden and mortality [1,2], yet influenza remains a significant threat to public health [3]. Influenza vaccine effectiveness (VE), currently estimated at 50–60% by the World Health Organization (WHO), is influenced by multiple confounding factors including vaccine recipients' age and health status, virulence of the circulating strain as well as the VE study design itself [4]. The antigenic relatedness between vaccine and circulating strain also impacts the VE, such that emergence of antigenically drifted strains have caused vaccine mismatches, resulting in increased infections and reduced VE [5–7]. The intermittent infections by drifted strains may seem discouraging for the vaccination effort, but it remains unclear how and to what extent these infections can influence subsequent vaccine responses. A better understanding of this issue can provide an important rationale for continual seasonal influenza vaccinations.

While the role of a primary infection on heterosubtypic immunity has been established in animal studies [8–12], its role on subsequent vaccine responses is not well-known. Human studies on this subject have been challenging, as it requires multi-year longitudinal studies in a defined cohort. However, recent studies provide valuable insights on what extent a single infection can induce hemagglutination inhibition (HI) titers against historic as well as contemporary strains in unvaccinated individuals, termed 'back-boost' [13]. Back-boost was also detectable following

Abbreviations: HA, hemagglutinin; NA, neuraminidase; HI, hemagglutination inhibition; HAU, hemagglutination unit; MN, microneutralization; LD_{50}, 50% lethal dose; PFU, plaque forming unit.

☆ This work was supported by contract HHSN266 200700006C from NIH/NIAID. Joshy Jacob is a research scholar of the American Cancer Society. The authors have no competing financial interests.

* Corresponding author at: Centers for Disease Control and Prevention, 1600 Clifton Road, NE, Atlanta, GA 30333, USA. Tel.: +1 404 639 3800; fax: +1 404 639 2350.

** Corresponding author. Tel.: +1 404 727 7919; fax: +1 404 727 8199.

E-mail addresses: joshy.jacob@emory.edu (J. Jacob), zao1@cdc.gov (S. Sambhara).

[1] Current address: Department of Microbiology and Immunology, School of Medicine, Emory University, 1518 Clifton Rd, Atlanta, GA 30322, USA.

http://dx.doi.org/10.1016/j.vaccine.2015.11.077

vaccination, but as often is the case in clinical studies, the individuals' infection history and proper controls were not readily feasible, making it difficult to delineate infection history as a compounding factor for their Ab responses. In this study, we found that prior infection, but not immunization with PR8, intensified immunogenicity and efficacy of killed FM1 vaccine and broadened Ab cross-reactivity against antigenically further drifted strains in mice. Interestingly, prior infection enhanced vaccine efficacy of even antigenically distant strains. The impact of prior infection was also long-lasting, as immunization as late as 1 year post PR8-infection enhanced FM1-specific Ab responses. Since primary infections hardly occur in naïve hosts, we also addressed whether primary infection could be modulated in immune host using vaccine mismatch scenarios. Collectively, our findings suggest that an influenza infection strengthens the subsequent vaccine responses against variants in quantity and quality, while the impact of infection can be attenuated by the host's pre-existing immunity.

2. Methods

2.1. Cells, viruses and vaccines

Madin-Darby canine kidney (MDCK) cells were maintained in Dulbecco's modified Eagle's medium containing antibiotics, glutamine and 10% fetal bovine serum (FBS). Influenza viruses were propagated in 11-day-old embryonic chicken eggs and clarified allantoic fluid was used for virus infection of mice. For preparation of whole-inactivated viruses (WIV), clarified allantoic fluid was purified on discontinuous sucrose-gradient composed of 15%, 30% and 60% sucrose and inactivated with 4% w/v (=10%, v/v) formalin until no infectivity was detected in MDCK cells. After titration of the HA unit (HAU) by hemagglutination (HA) assay, WIVs (700–1400 HAU/100 μl/mouse) were used as immunogens as previously described [14]. For the experiments assessing the impact of prior infection on the immunization with antigenically distant strain, commercially available pandemic H1N1 (pH1N1) monovalent split vaccine (A/California/07/2009; Cal07, 15 μg HA/500 μl) was used to immunize mice.

2.2. Mice, immunizations, infection and tissue collection, bronchoalveolar lavage (BAL), nasal wash

Balb/c mice, infection and immunization were previously described [14]. Spleen, lung and lymph nodes were collected after euthanizing mice with a lethal dose of Avertin (Sigma-Aldrich). BAL was collected by injecting 1 mL PBS + 0.5% bovine serum albumin (BSA) through the trachea with an 18G catheter. Nasal washes were collected by passing 0.5 mL PBS + 0.5% BSA through the nasal passage. All animal studies were performed with the approval and guidance of the Institutional Animal Care and Use Committees in an Association for Assessment and Accreditation of Laboratory Animal Care International-accredited animal facility of Emory University and the CDC.

2.3. Ab responses

Serum microneutralization (MN) and HI titers were previously described [14]. Mucosal IgG and IgA responses of BAL and nasal washes were analyzed by ELISA. Nunc 96 well plates (Maxi-sorb) were coated with 100HAU whole inactivated virus (WIV), then blocked with 4% BSA in PBS-Tween for 1 h. Ten-fold dilutions of samples are added to the plates for 2 h. Plates were then developed by biotin-α-mouse IgG/IgA followed by streptavidin (SA)-HRP (Southern Biotech). The signals were developed using 1 × TMB (ebioscience) and measured at 450 nm using a plate reader (Biotek).

2.4. Ab-secreting cells (ASCs) by ELISpot assay

ELISpot plates (Millipore) were coated with 100 HAU WIV overnight and blocked with cRPMI-1640 media. Dilutions of cells were added to plates and incubated overnight at 37 °C. Plate-bound Abs were probed by biotin-α-mouse IgG, SA-alkaline phosphatase (Southern Biotech), then Vector Blue Substrate Kit (Vector Lab). Spots were counted using an ImmunoSpot® ELISPOT reader (Cellular Technology Ltd.).

2.5. Lung lysates and plaque assay

Lung lysates were prepared by homogenizing lung tissues through a 40 μm cell strainer with syringe plungers. Homogenates were spun at 450 × *g* for 20 min at 4 °C and the supernatants were assessed for virus titers via plaque assay as previously described [14].

2.6. Monoclonal Ab staining and flow cytometry

Cells were stained with CD11b, CD95, CXCR5, CD3, CD8a (BD Bioscience); CD103, I-Ad, GL7, CD138, CD69, CD86, CD19 (Biolegend); CD49b, F4/80, CD11b, CD11c, CD45, PD-1 (eBioscience). Virus-specific $CD8^+$ T cells were identified using H-$2K^d$/IYSTVASSL (HA) and H-$2K^d$/TYQRTRALV (NP) pentamers (Proimmune). For in vitro stimulation, cells were infected with PR8 or FM1 virus at a multiplicity of infection (MOI) of 1 for 1 h. After incubation overnight and addition of Golgi-Plug (BD) for the last 6 h, cells were stained with CD4 and CD8, permeabilized and stained with IFNγ, IL-2, TNFα. Cells were analyzed with a FORTESSA flow cytometer (BD) and FlowJo software (Tree Star, Inc.).

2.7. Statistics

Student's *t* test was used to compare the Ab titers between PR8 vs. FM1-specific responses following $\log_2$ transformation. For multiple groups, one way analysis of variances with Bonferroni post-test was used. For statistical designations, * denotes $p < 0.05$; ** denotes $p < 0.02$; *** denotes $p < 0.001$.

3. Results

3.1. Prior infection, but not immunization with PR8 enhanced the local and systemic Ab responses and virus-specific T cell response following FM1-WIV immunization

To compare the vaccine responses in differential priming contexts, mice were either infected or immunized with PR8. Infection dose ($0.01 \times LD_{50}$) was chosen to achieve subclinical infection (≤5% body weight (BW) loss; data not shown). At memory phase (>d28), mice were immunized with FM1-WIV and the acute local Ab responses in inguinal lymph nodes were assessed on d5 post-immunization. The %plasma cells were significantly higher in $PR8_{inf}/FM1_{imm}$ than in $PBS/FM1_{imm}$ or $PR8_{imm}/FM1_{imm}$ mice (Fig. 1A). The differential local Ab response was also reflected systemically in spleen by significant Ag-specific ASC responses (Fig. 1B). While FM1-WIV in naïve mice ($PBS/FM1_{imm}$) induced minimal ASC responses, it intensified PR8- and FM1-specific ASC responses in $PR8_{inf}/FM1_{imm}$, but not in $PR8_{imm}/FM1_{imm}$ mice. The virus-specific (NP+) CD8 T cell response in local lymph nodes was also significantly higher in $PR8_{inf}/FM1_{imm}$ compared to control groups (Fig. 1C). However, virus-specific CD4 and CD8 T cells in spleen were readily recalled upon in vitro stimulation as long as the mice were previously infected with PR8 (Fig. 1D, Supplemental Fig. 1A and B). Both PR8 and FM1 stimulated T cells at comparable levels, indicating significant cross-reactivity of T