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Intra-seasonal antibody repertoire analysis of a subject immunized with an MF59®-adjuvanted pandemic 2009 H1N1 vaccine

Jack Ferdman [a], Giuseppe Palladino [a], Hua-Xin Liao [b,1], M. Anthony Moody [b], Thomas B. Kepler [c], Giuseppe Del Giudice [d], Philip R. Dormitzer [a,2], Stephen C. Harrison [e,f], Ethan C. Settembre [a], Pirada Suphaphiphat [a,*]

[a] Seqirus, Inc. (formerly Novartis Influenza Vaccines), Cambridge, MA 02139, USA
[b] Duke Human Vaccine Institute, Duke University Medical School, Durham, NC 27710, USA
[c] Department of Microbiology, Boston University School of Medicine, Boston, MA 02118, USA
[d] GSK Vaccines (formerly Novartis Vaccines and Diagnostics), Siena 53100, Italy
[e] Laboratory of Molecular Medicine, Boston Children's Hospital, Harvard Medical School, Boston, MA 02215, USA
[f] Howard Hughes Medical Institute, Boston, MA 02115, USA

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ABSTRACT

During the height of the 2009 H1N1 swine-derived influenza pandemic, a clinical trial was conducted in which seven subjects were immunized using a monovalent, MF59®-adjuvanted vaccine, developed from an egg-passaged candidate vaccine virus (CVV), A/California/07/2009 X-181. Whole blood was collected prior to immunization and at 8, 22, and 202 days post-vaccination, and subjects' serological responses were evaluated. Here, we reconstruct and examine the longitudinal, influenza-specific circulating B cell repertoire of one subject in that study. Genotypic analysis of 390 total subject-derived antibodies (Abs) revealed a total of 29 germline genes in use among immunoglobulin heavy chain variable regions (IgHV), with the majority of those sequences isolated representing memory recall responses and two major lineages dominating the early response. In vitro phenotyping showed a diverse set of binding epitopes on the surface glycoproteins hemagglutinin (HA) and neuraminidase (NA), many of which are considered subdominant. Strong correlations were found between IgHV germline usage among non-related lineages and both binding epitope and neutralization breadth. Results here highlight the potential for Ab responses to be misdirected to egg-adaptive artifacts on CVVs while simultaneously stressing the ability to mount potent, broadly neutralizing responses to mostly novel antigens via recall of subdominant memory responses, as well as the need for evaluating alternative endpoint assays and anti-NA responses following clinical trials.

1. Introduction

Vaccines protect from infection by establishing or modifying immune memory. In the case of influenza, these responses are directed primarily at the surface proteins hemagglutinin (HA) [1], which mediates entry into the host cell, and neuraminidase (NA), which releases newly formed virions from the host cell's membrane. Eighteen genotypically distinct subtypes of HA have been described for

Abbreviations: AA, amino acid; Ab, antibody; BCR, B cell receptor; B-mAbs, biotinylated monoclonal antibodies; BSA, bovine serum albumin; CL, clonal lineage; CVV, candidate vaccine virus; DHVI, Duke Human Vaccine Institute; DPBS, Dulbecco's Phosphate-Buffered Saline; EC_x, effective concentration at which x% of the maximum is reached; FACS, fluorescence-activated cell sorting; FLsE, full-length soluble ectodomain; HA, hemagglutinin; HCDR3, third complimentarity-determining region on the immunoglobulin heavy chain; HRP, horseradish peroxidase; Ig, immunoglobulin; IgHV, immunoglobulin heavy chain variable region; mAb, monoclonal antibody; MBC, memory B cell; NA, neuraminidase; NT, nucleotide; PBMC, peripheral blood mononuclear cell; PBS-T, DPBS + 0.05% Tween20; pdm2009, H1N1 pandemic Influenza strain; rAgs, recombinant antigens; RBS, receptor-binding site; rHA, recombinant hemagglutinin; rNA, recombinant neuraminidase; RT, room temperature; SHM, somatic hypermutation; tfx sups, transfection supernatants; TMB, 3,3′,5,5′-tetramethylbenzidine; UCA, unmutated common ancestor.

* Corresponding author at: Seqirus, Inc, 50 Hampshire St 9th Floor, Cambridge, MA, USA.
E-mail addresses: jack.ferdman@seqirus.com (J. Ferdman), giuseppe.palladino@seqirus.com (G. Palladino), tliao805@jnu.edu.cn (H.-X. Liao), tony.moody@duke.edu (M.A. Moody), tbkepler@bu.edu (T.B. Kepler), giuseppe.x.del-giudice@gsk.com (G. Del Giudice), Philip.Dormitzer@pfizer.com (P.R. Dormitzer), harrison@crystal.harvard.edu (S.C. Harrison), ethan.settembre@seqirus.com (E.C. Settembre), Pirada.Suphaphiphat@seqirus.com (P. Suphaphiphat).
[1] Currently: College of Life Science and Technology, Jinan University, Guangzhou 510632, China.
[2] Currently: Pfizer Vaccine Research and Development, Pearl River, NY 10965, USA.

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influenza A, falling into two major sub-divisions (Group 1 and Group 2), and two diverse lineages for influenza B have been described (Yamagata and Victoria). Within each subtype and lineage, influenza viruses are further subject to antigenic drift—constant, rapid viral evolution driven by mutations in the genes that encode the HA and NA. Because of this genetic and antigenic diversity, influenza-related deaths average nearly half a million people worldwide each year [2], with circulating influenza resulting in annual outbreaks; transfer from birds or swine, in new pandemics. In the case of the swine-derived H1N1 pandemic strain that emerged in 2009, estimates suggested that 20% of people worldwide were infected [3], in large part because its antigenic properties were different from those of the concurrent, seasonal circulating H1N1.

In the immediate aftermath of the 2009 pandemic (pdm2009), several studies analyzed how immune repertoires had been affected by vaccination against that wildtype strain, A/California/07/2009. Most of those studies focused on B-cell repertoires and the breadth of reactivity of the antibodies produced. Certain subjects exhibited an unexpected antibody (Ab) binding phenotype specific for HA epitopes other than the immunodominant globular head, and this profile correlated with expanded heterotypic neutralization breadth [4]. It was further shown that these broadly-neutralizing, heterotypic, non-head Abs preferentially use the germline gene 1–69 to encode the variable region of their immunoglobulin heavy chains (IgHV) [5–9]. Most of these studies involved large patient cohorts and therefore could examine only snapshots of each subject's repertoire. These snapshots were never intended to assess the composition of the flu-specific B cell repertoire throughout a single season.

In the current study, we took the opportunity to reconstruct and examine a longitudinal intra-seasonal antibody repertoire using samples, originally collected for another study, from a cohort of seven subjects who received an MF59-adjuvanted single-component pdm2009 subunit vaccine made from egg-passaged, A/California/07/2009-derived, reassortant CVV NYMC-X181 (hereafter, "X-181") [10]. The first report focused on T-cell and polyclonal responses. However, the structure of that study also provided an opportunity to trace the subjects' serological responses to pdm2009 in real time, to assess the presence of broadly protective heterotypic antibodies, and to determine which responses were converted into long-term memory. We provide here an analysis of the complete intra-seasonal antibody repertoire of a single individual in that study—the subject who exhibited the lowest pre-vaccination baseline titer (a 32-year-old male hereafter known as Subject 7). We examined this repertoire using paired heavy and light chain sequences from single cells and characterized each antibody's epitope, neutralization breadth, clonal relationships, and IgHV germline usage. Thus, we were able to trace the rise and fall of expanded clonal lineages as well as orphan clones from non-expanded lineages at up to six months post-vaccination. We believe that these analyses are the most complete case study to date on the rapidly developing immune response following vaccination against influenza within a season.

2. Materials and methods

2.1. Study design and sample preparation

The study design, a complete description of sample collection procedures, and statements of Ethics Committee approval and informed consent have all been previously described [10].

2.2. Cell sorting

Cell sorting was completed at the Duke Human Vaccine Institute (DHVI) using methods previously published [11–13]. PBMCs from Day 8 were sorted for all plasmablasts in an unbiased fashion via FACS. Gating for plasmablasts was as described [11]. Antigen-specific sorting of memory B cells (MBCs) from Days 0, 22, and 202 was performed using a recombinant X-181 protein produced in insect cells via a baculovirus expression system by collaborators at Harvard Medical School [15]. The X-181 protein was labeled in two fluorochrome colors by N-hydroxysuccinimide ester chemistry. Antigen-specific MBCs were sorted as viable singlet events gated as CD3/14/16/235a negative, CD19 positive, surface IgD negative that were double-positive for the X-181 protein. Sample plots and gating for antigen-specific sorting are presented in Raymond et al., Fig. S7 [16]. Single cell sorting, PCR isolation of IgHV, V_κ, and V_λ from sorted cells, and synthesis of linear expression cassettes were also completed at DHVI as previously published [14]. Cell culture supernatants from 293 T cells transfected with paired heavy and light chain linear cassettes were harvested at DHVI and sent for characterization.

2.3. Clonal lineage clustering

Paired Ig heavy and light sequences from cell transfections were grouped into clonal lineages using Clonalyst software [17,18] from single-cell, paired heavy- and light-chain sequences. Antibodies are designated by heavy-chain sequence number.

2.4. Antigen production

Monobulks: Unblended, monovalent lots of subunit vaccine antigen were produced in embryonated chicken eggs by Novartis Vaccines (now Seqirus) in either pilot or engineering batches as previously described [19].

Recombinant protein: HA gene segments were cloned into pCMV-KM2l and featured a foldon trimerization domain and a 6x-His tag. NA gene segments were cloned into pRS5α featuring a tetramerization domain and a 6x-His tag. All vectors were sequence verified. Plasmid DNA was transfected into Expi293F cells (ThermoFisher, A14527) using the Expi293 Expression System (Life Technologies, A14524) according to the recommended protocol. Culture media were harvested three days post-transfection by centrifugation. Recombinant antigens (rAgs) were purified with HisTrap Excel columns (GE Healthcare, 17-3712-05) by FPLC (GE, AKTA Pure 25) according to the recommended protocol. Purified proteins were characterized by SDS-PAGE and SEC-HPLC.

2.5. Production of human monoclonal antibodies (mAbs)

Linear DNA cassettes for Ig V_H, V_κ, and V_λ segments were synthesized as GBlocks at Integrated DNA Technologies and cloned into pRS5α expressing full-length IgG_1 heavy, kappa light, or lambda light chains where appropriate, by Gibson assembly (New England Biolabs, E5510S) according to the recommended protocol. Variable regions of Ig chains were sequence verified. Plasmid DNA for paired Ig heavy and light chains were co-transfected into Expi293F cells using the Expi293 Expression System according to the recommended protocol. Culture media were harvested three days post-transfection by centrifugation. mAbs were purified with HiTrap MabSelect Columns (GE Healthcare, 28-4082-53) by FPLC according to the recommended protocol. Purified mAbs were characterized by SDS-PAGE and SEC-HPLC.

2.6. Binding affinity by simple ELISA

Monobulk antigens were diluted to 1.0 μg/ml in Dulbecco's Phosphate Buffered Saline (DPBS; Lonza, 17-512F); rAgs to 1.5 μg/ml. Diluted antigen was coated onto 96-well (Nunc, 439454) or 384-well (Greiner Bio-One, 781097) plates and stored at 4 °C

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