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Impact of pre-existing immunity on the induction of functional cross-reactive anti-hemagglutinin stalk antibodies following vaccination with an AS03 adjuvanted pandemic H1N1 vaccine

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ABSTRACT

The 2009 pandemic H1N1 (A(H1N1)pdm09) virus had a highly divergent hemagglutinin (HA) compared to pre-2009 seasonal H1N1 strains. Most peoples were immunologically naïve to the A(H1N1)pdm09, although hospital workers were exposed early in the pandemic before pandemic vaccines became available. Here, we evaluated how pre-existing antibodies influence the induction of cross-functional HA stalk antibodies following A(H1N1)pdm09 vaccination.

Fifty-six healthcare workers vaccinated with AS03 adjuvanted A(H1N1)pdm09 vaccine were chosen by their pre-vaccination priming status (primed HI titers $\geq$ 40 or unprimed < 40). We analyzed the HA head- and stalk-specific serum IgG subclasses at pre- and 21 days post-vaccination. We also assessed the functionality of the HA stalk-specific antibodies to neutralize virus and mediate antibody dependent cellular cytotoxicity (ADCC).

Primed individuals had higher pre-existing HA head- and stalk-specific IgG1, as well as higher ADCC functionality of stalk antibodies. However, following vaccination with the adjuvanted pandemic vaccine, only the quantity of HA head specific IgG1 antibodies were significantly higher than in unprimed individuals. The priming status did not impact upon the cross-reactive HA stalk specific IgG antibodies or their ability to neutralize virus or induce ADCC post-vaccination. In conclusion, a single dose of AS03 adjuvanted pandemic vaccine elicited similar levels of functional antibodies in naïve and primed individuals. These findings are important for understanding the immunological factors that impact or modulate pandemic vaccine responses in humans.

1. Introduction

Influenza causes substantial morbidity and mortality during seasonal epidemics [1]. While seasonal influenza vaccines are effective, they need to be reformulated each year to match the influenza virus strains predicted to circulate during the next season. Furthermore, the vaccine may provide lower protection in cases of antigenic mismatch between the vaccine and the circulating viral strains. At unpredictable intervals, influenza pandemics can also arise. Therefore, it is critical to understand the mechanisms of immunity to influenza virus to optimize vaccination strategies especially if new strains emerge with pandemic potential.

Antibodies are directed towards the major surface glycoprotein, hemagglutinin (HA), consisting of a immunodominant head and stalk domain. The HA head domain is composed of the majority of the HA1 subunit while the HA stalk domain is primarily composed of HA2 subunit and the N- and C-terminal ends of HA1 [2]. Antibodies to the HA head inhibit virus attachment to the host cell receptors and these hemagglutination inhibition (HI) antibodies are measured as surrogate correlates of protection. Pandemic A (H1N1)pdm09 vaccination preferentially induced HA stalk-specific antibodies that were broadly protective, in contrast to seasonal vaccines that induce HA head-specific antibodies and little or no HA stalk-specific antibodies [3–5].

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IgG is the predominant antibody class induced in response to inactivated influenza vaccines. However, IgG subclasses differ in terms of their antigen binding capacity, complement activation, recruitment of effector cells and half-life [6]. The four human IgG subclasses have variations in amino acid sequence in the hinge region and N-terminal CH2 domain, which mediate the effector functions such as antibody dependent cellular cytotoxicity (ADCC) [6]. Therefore, different IgG subclasses have different binding strengths and functionality.

As there are frequent antigenic changes in the HA of seasonal influenza viruses and exposure to more variant influenza antigens can confer broader protection [7], it is important to understand how pre-existing immunity influences the response to changing influenza virus strains. There have been reports that pre-existing immunity negatively influences vaccination responses [8–10]. Here, we investigated the extent to which pre-existing antibodies influenced the cross-reactive antibody response to pandemic H1N1 vaccination in healthcare workers. Based on pre-existing HI antibodies, which are a surrogate correlate of protection, individuals were assigned to two groups (primed or unprimed). Since pandemic H1N1 vaccination has been shown to induce antibodies to the conserved HA stalk domain, we analyzed in detail how priming history affects the IgG subclass response towards the HA stalk and head domains. We further assessed the functionality of the HA stalk antibodies to neutralize virus, and activate natural killer (NK) cells by antibody dependent cellular cytotoxicity (ADCC).

2. Methods

2.1. Study design

Fifty-six healthcare workers at Haukeland University Hospital (Bergen, Norway) were selected for this study, which commenced in October 2009 prior to the peak of pandemic activity. All study participants were intramuscularly vaccinated with a single low dose (3.75 μg HA) of the monovalent pandemic H1N1 vaccine (Pandemrix®) adjuvanted with AS03 (GlaxoSmithKline (GSK), Wavre, Belgium). Serum samples were collected immediately prior to vaccination (D0) and 21 days (D21) post-vaccination. All participants provided written informed consent before inclusion in the study, which had ethical and regulatory approval (ClinicalTrials.gov NOT01003288).

2.2. Hemagglutination inhibition assay

Serum samples were treated with receptor destroying enzyme (Seiken, Japan) and run in duplicate in the HI assay against the homologous pandemic H1N1 virus strain, A/California/07/09 (H1N1), using 0.7% turkey red blood cells as previously described [11]. The HI titer was the reciprocal of the highest dilution that inhibited 50% hemagglutination. Seroprotection was defined as an HI titer $\geq$40. Titers <10 were assigned a value of 5 for calculation purposes.

2.3. Anti-hemagglutinin IgG subclass ELISA

Sera were evaluated in duplicate for IgG1, IgG2, IgG3 and IgG4 antibodies [12]. The plates were coated with capture IgG antibody (0.3 μg/ml), a proxy influenza HA1 head (A/California/06/2009 (H1N1)) hexahistidine-tagged (1 μg/ml) (eEnzyme, IA-01SW-005P) or 1 μg/ml influenza cH6/1, a chimeric HA (cHA) that combines the H1 stalk domain with the globular head domain derived from H6 influenza A virus [13]. Antibody concentrations were calculated using IgG1, IgG2, IgG3 and IgG4 standards and linear regression of the log-transformed readings.

2.4. Virus neutralization assay

Serum samples were heat-inactivated at 56 °C for 30 min and run in duplicate. The serum samples were then diluted 2-fold in virus growth medium containing Dulbecco's Modified Eagle's Medium (DMEM) with tosyl phenylalanyl chloromethyl ketone (TPCK)-trypsin, 0.14% bovine serum albumin, 100 units/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL Amphotericin B (Sigma). Egg grown cH9/1 N3 virus [3] (expressing the H1 stalk, with H9 head HA and an N3 subtype neuraminidase) was diluted to 2000 $TCID_{50}$/ml (50% tissue culture infectious dose). Sixty microliters of diluted sera were incubated with 60 μl of virus for 1 h at 37 °C. Confluent Madin Darby Canine Kidney (MDCK) cells were washed once with phosphate buffered saline (PBS) and 100 μl of serum-virus mixture was added to the cells. Cells were incubated at 37 °C for 1 h then washed once with PBS before 50 μl of appropriately diluted serum and 50 μl of virus growth medium were added to each well and incubated for 72 h at 37 °C. Fifty microliters of the supernatant was transferred to a 96-well V bottom plate and 50 μl of 0.7% Turkey red blood cells added. The highest dilution of each serum causing 100% hemagglutination was read as the neutralizing antibody titer.

2.5. Antibody dependent cellular cytoxicity (ADCC) assay

The ADCC assay measuring intracellular NK cell IFNγ and CD107a expression was conducted as previously described with minor modifications [14,15]. Briefly, 96-well plates were coated overnight at 4 °C with 1 μg/ml chimeric cH6/1 HA. Plates were washed with PBS and incubated with heat-inactivated sera at 1:40 dilution for 2 h at 37 °C. After washing, 100,000 CD16-176 V.NK-92.05 cells (human NK cell line expressing high affinity 176 V variant CD16 receptor) (kindly provided by Kerry S. Campbell, Fox Chase Cancer Centre, USA) were added to each well. NK-92.05 cells lacking the expression of CD16 were added for each sample, as negative controls. The cells were incubated in the presence of anti-CD107a-AF488 antibody (Biolegend, 328610), Brefeldin A (5 μg/ml, BD) and monensin (5 μg/ml, BD) for 16 h at 37 °C. Cells were then stained with LIVE/DEAD Fixable Aqua dead cell staining kit (Invitrogen), anti-CD3-PE CF594 (BD, 562280) and anti-CD56-APC (BD, 555518) before acquisition on a BD LSR-Fortessa. Analysis was conducted using FlowJo version-10 (treeStar).

2.6. Statistical analysis

Data analysis was performed using GraphPad Prism version-6 (GraphPad Software Inc., San Diego, USA). The unpaired nonparametric Mann-Whitney test was used to assess differences between the two groups. The paired Wilcoxon test was used to compare pre- and post-vaccination data within each group. Correlations between the assays were performed using Spearman rank test. A p-value < 0.05 was considered statistically significant.

3. Results

A total of 56 health care workers at Haukeland University Hospital (HUH) (Bergen, Norway) were selected for the current study. Of the 56 subjects, 43 (77%) were female and 13 (23%) were male, with an age range of 21–67 years old. In total, 23 subjects (41%) had been previously vaccinated at least once with seasonal trivalent influenza vaccine (Table 1).

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