Immune Response Following H1N1pdm09 Vaccination: Differences in Antibody Repertoire and Avidity in Young Adults and Elderly Populations Stratified by Age and Gender

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Background. The H1N1 2009 influenza (H1N1pdm09) pandemic had unexpected features, including lower morbidity and mortality in elderly populations.

Methods. We performed in-depth elucidation of antibody responses generated post–H1N1pdm09 vaccination in elderly (aged 66–83 years) and younger (aged 18–45 or 46–65 years) adults using H1N1pdm09 whole-genome-fragment phage display library and measured antibody isotype and affinity to antigenic domains within hemagglutinin (HA).

Results. H1N1pdm09 vaccination induced 10-fold higher antibody levels in elderly compared with younger adults. These antibodies primarily targeted the HA1 globular domain, including neutralizing epitopes in the receptor-binding domain. Antibody epitope repertoire, isotype, and affinity maturation after H1N1pdm09 vaccination evolved independently for HA2, HA1, and HA1 N-terminus antigenic regions. Postvaccination serum samples from elderly subjects demonstrated substantially higher avidity than from younger subjects (60% vs 30% resistance to 7 mol/L urea) and slower antibody dissociation rates using surface plasmon resonance. We also identified a gender difference in postvaccination antibody avidity (female < male subjects) in adults 65 years old.

Conclusions. This is the first study in humans that provides evidence for a qualitatively superior antibody response in elderly adults after H1N1pdm09 vaccination. These findings may help explain the age-related mortality observed during the H1N1pdm09 pandemic. The difference in gender specific avidity merits further exploration.
antibody binding and dissociation kinetics to antigenic domains of the influenza hemagglutinin (HA). To that end, we have applied whole-genome-fragment phage display libraries (FLU-GFPDLs), as well as surface plasmon resonance (SPR) technologies, and resistance to 7 mol/L urea treatment of polyclonal serum samples [17, 18, 19]. Such assays capture the majority of in vivo circulating influenza-specific antibodies, long-lived plasma cells, and newly activated naive and memory B cells, all of which contribute to the antiviral response in vivo and determine clinical outcome.

In the current study, these technologies were used to elucidate the magnitude, diversity, and affinity of polyclonal antibodies in a subset of subjects who received the inactivated split virus H1N1pdm09 HA vaccine (CSL Limited) in a clinical trial conducted in young and elderly adults [20]. Our findings provide evidence that elderly adults had antibody responses to H1N1pdm09 vaccine that were both quantitatively and qualitatively superior to those elicited in young adults. In addition, we provide evidence for a gender difference in the avidity of antibody response in younger adults.

**MATERIALS AND METHODS**

**Description of Clinical Trials and Collection of Vaccination Samples**

A phase 2, prospective, randomized, placebo-controlled, observer-blind, parallel-group clinical study of the H1N1pdm09 vaccine (monovalent, unadjuvanted, inactivated, split-virus) produced by CSL Limited [21], was conducted at 11 sites and in 1313 subjects, as described elsewhere (NCT00958126) [20]. The participants in this study included younger (aged 18–65 years) and elderly adults (aged ≥65 years). For the current study, we tested a subset of serum samples from individuals aged 24–83 years who received 2 doses of 7.5, 15, or 30 μg of H1N1pdm09 vaccine at the Johns Hopkins School of Public Health site, and these samples were used in FLU-GFPDL and antibody avidity assays described below. Table 1 contains all the relevant information for each individual in this cohort and the subgroups that were used in the assays presented in Figures 1–3. For the comparative studies of differences in antibody avidity by age group and gender we obtained serum samples from an additional 120 adults vaccinated with a 7.5- or 15-μg HA dose of the H1N1pdm09 vaccine. These individuals had preexisting HI titers of ≤1:32 and post-vaccination titers of ≥1:128. These samples were from 40 adults each in 3 age groups: <45 years (20 men aged 18–25 years and 20 women aged 18–24 years), 46–64 years (20 men aged 46–64 years and 20 women aged 48–64 years), and >65 years (20 men aged 66–82 years and 20 women aged 66–83 years).

**Immunogenicity Assessments: HI Assay**

Serum samples were obtained at baseline and 21 days after each vaccination and were tested for HI antibodies to H1N1pdm09, as described elsewhere [20].

**Construction of H1N1 FLU-GFPDLs and Panning of H1N1pdm09 FLU-GFPDLs With Polyclonal Human Vaccine Serum Samples**

The H1N1pdm09 phage display libraries affinity selection was performed as described elsewhere [17, 19]. For FLU-GFPDL panning, equal volumes of serum samples from 3–10 subjects were pooled and used for each group (Table 1). These subjects were selected based on their pre- and postvaccination HI titers and were grouped by age.

**Avidity Measurements for Antibodies Resistant to 7 mol/L Urea**

Immunoglobulin (Ig) class G (IgG) avidity was determined directly for each individual pre- and postvaccination serum sample by a modified enzyme-linked immunosorbent assay (ELISA), as described elsewhere [23, 24].

**Affinity Measurements by SPR**

Steady-state equilibrium binding of post-H1N1pdm09 human vaccine serum samples was monitored at 25°C using a ProteOn SPR biosensor (Bio-Rad). Antibody off-rate constants that describe the stability of the complex (ie, the fraction of complexes that decays per second) were determined directly from the serum/plasma sample interaction with recombinant HA1 (rHA1) protein using SPR in the dissociation phase and calculated using the Bio-Rad ProteOn manager software for the heterogeneous sample model, as described elsewhere [19]. To improve the measurements, the off-rate constants were determined from 2 independent SPR runs.

**Statistical Analyses**

Differences between groups were examined for statistical significance (defined as unadjusted P < .05) using Student t test.

**RESULTS**

**Antibody Epitope Profiles in H1N1pdm09 Vaccine Recipients as Determined by FLU-GFPDL Analyses**

The large phase 2 clinical study conducted August through October 2009 demonstrated that a single 7.5-, 15-, or 30-μg dose of a monovalent, unadjuvanted H1N1pdm09 split virus vaccine (CSL Limited) induced protective HI antibody levels in adults of all ages, including very elderly adults [20]. In the current study, we evaluated serum samples from 168 adult subjects in 3 age strata. Table 2 summarizes the seroconversion rate (SCR), seroprotection rate, and geometric mean titer after each dose for subjects in 3 age groups: 18–45, 46–64, and >65 years. As expected, the SCR in the oldest age group was lower than in the 2 younger age groups (60% for >65-year age group compared with 78% and 94% in the 46–64 and 18–45-year age groups, respectively), but the geometric mean titers and seroprotection rates were similar for responders in all age groups. No significant differences in the HI responses to the vaccine dose were noted, as described elsewhere [20]. To probe the antibody repertoires elicited by the H1N1pdm09 vaccine in...
Table 1. Ages of Subjects and Hemagglutination Inhibition (HI) Titers for Samples Used for Whole-Genome-Fragment Phage Display Library (FLU-GFPDL) Analysis

<table>
<thead>
<tr>
<th>Sample ID by Group</th>
<th>Age, y</th>
<th>Gender</th>
<th>Vaccine Dose, μg</th>
<th>HI Titers</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Prevaccination (Day 0)</td>
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<tr>
<td>Group 1 (aged &lt;65 y; responders)a</td>
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<td></td>
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<tr>
<td>1</td>
<td>24.7</td>
<td>F</td>
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</tr>
<tr>
<td>3</td>
<td>34.6</td>
<td>M</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>42.9</td>
<td>F</td>
<td>30</td>
<td>&lt;4</td>
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<td>10</td>
<td>64.5</td>
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<td>&lt;4</td>
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<tr>
<td>Group 2 (aged &lt;65 y; responders)b</td>
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<td></td>
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<tr>
<td>3</td>
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<td>F</td>
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<td>Group 3 (aged &gt;65 y; nonresponders)c</td>
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<td>6</td>
<td>76.7</td>
<td>F</td>
<td>7.5</td>
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</tr>
<tr>
<td>Group 4 (aged &gt;65 y; responders)d</td>
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<tr>
<td>7</td>
<td>78.1</td>
<td>F</td>
<td>7.5</td>
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<td>8</td>
<td>82.5</td>
<td>F</td>
<td>7.5</td>
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<tr>
<td>Group 5 (aged &gt;65 y; responders)e</td>
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<tr>
<td>1</td>
<td>65.5</td>
<td>F</td>
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<td>32</td>
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<tr>
<td>5</td>
<td>83.6</td>
<td>F</td>
<td>7.5</td>
<td>64</td>
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Abbreviations: F, female; M, male; NS, no sera available.

a Subjects in group 1 were seronegative responders, with HI titer of ≤4 before vaccination and 128–1024 after the first vaccination.
b Subjects in group 2 were responders, with HI titer of 32 before vaccination and 512–2048 after the first vaccination.
c Subjects in group 3 were nonresponders, with HI titer of <2 before the first vaccination.
d Subjects in group 4 were seronegative responders with HI titer of <4 to 8 before vaccination and 128–1024 after the first vaccination.
e Subjects in group 5 were responders with HI titer of 32–64 before vaccination and 128–2048 after the first vaccination.

Different age groups, H1N1 HA FLU-GFPDL analyses were initially conducted with pooled serum samples from subjects aged 18–65 or >65 years (Table 1, group 1) or from seronegative subjects (preimmunization HI titers ≤4; Table 1, group 1) or from seronegative subjects (preimmunization titers of 32; Table 1, group 2) were used for FLU-GFPDL panning.
Prevaccination serum samples from seronegative subjects bound to low numbers of FLU-GFPDL phages whose inserts mapped to one region in HA2 and one HA1 N-terminus epitope, both of which are highly conserved between H1N1pdm09 and multiple seasonal H1N1 strains (91% and 80% homology, respectively). In contrast, prevaccination serum samples from seropositive subjects bound to additional phages with inserts mapping to the receptor binding domain (RBD) (Figure 1A). The total number of phages bound by postvaccination serum samples was substantially increased compared with prevaccination serum samples, with a 9–18-fold expansion in the total number of eluted clones displaying epitopes in both HA2 and HA1 (Figure 1A and 1C). Importantly, there was a clear increase in the number of clones bound to large RBD spanning inserts predicted to contain conformational epitopes [17, 18].

In subjects aged >65 years, we analyzed serum samples from subjects with prevaccination HI titers of ≤8 (group 4) or with preexisting titers of 32–64 (group 5). Subjects in both of these groups responded well to the first vaccination (HI titers between 128–2048). We also analyzed a group of nonresponders (group 3). In subjects aged >65 years, substantially greater numbers of phages (~5–10-fold) were bound by both pre- and postvaccination serum samples compared with the younger age group (Figure 1B and 1C). Nonresponders showed an ~4-fold increase in the number of bound phages, but the inserts still mapped only to the conserved regions in HA2 and HA1 (1-102), strongly suggesting that such antibodies do not contribute to HI activity (Figure 1B, group 3). In contrast, postvaccination serum samples from the vaccine responders aged >65 years (groups 4 and 5) bound 20–26-fold more phages than the prevaccination serum samples, with broadening of the epitopes in HA2 and HA1, including a pronounced increase in the RBD-spanning epitopes (Figure 1B and 1C).

**Binding of Pre- and Postvaccination Serum Samples to HA1 and HA2 Proteins Using SPR**

We next evaluated the capacity of polyclonal serum samples from the H1N1pdm09 vaccine cohort to bind to recombinant proteins encompassing the main targets that were identified in the FLU-GFPDL analyses. The location of these domains within the trimer structure is shown in Figure 2, with the surface exposed regions highlighted in red (HA1 1-102), orange and yellow (HA1 1-330, RBD), and blue (HA2). Pooled serum samples from groups 1 and 4 (with prevaccination titers of ≤8)
Figure 2. Main antigenic clusters recognized by post–H1N1pdm09 immune serum samples using whole-genome-fragment phage display libraries (FLU-GFPDLs) in hemagglutinin (HA) with marked amino acid residues: HA1 (1-102) (A); HA1 (1-330) encompassing RBD (B); and HA2 (331-514) (C) are shown as surface-exposed colored patches on one HA monomer within the HA trimer structure (Protein Data Bank identifier 3LZG) [22]. Binding of post–H1N1 vaccination human serum to HA1 (1-330), N-terminal HA1 (HA 1-102) fragment, and HA2 (331-514) proteins in SPR. D–F Steady-state equilibrium analysis of human vaccine serum to HA proteins [18] were measured using surface plasmon resonance. Ten-fold diluted pooled pre- and post–H1N1pdm09 vaccination serum samples from vaccine responders (groups 1 and 4 in Table 1) among subjects aged <65 years and those aged >65 years (which were used for FLU-GFPDL) were injected simultaneously onto HA 1-102 (D), HA2 (331-514) (E), and HA1 (1-330) (F). Prevaccination serum samples were used as controls in each assay. Binding of the antibodies to immobilized proteins is shown as resonance unit (RU) values. G, Isotype of serum antibodies bound to HA 1-102, HA2 (331-514), and HA1 (1-330) after vaccination with inactivated H1N1pdm09 vaccine is shown for the 2 age groups. Data shown are means ± standard deviations for each serum sample from 2 independent experiments. Ig, immunoglobulin.
that were analyzed by FLU-GFPDL bound to all 3 purified recombinant proteins in SPR (Figure 2D–F). Surprisingly, in the case of HA1 N-terminus (1-102) peptide, no increase in maximal binding was observed between pre- and postvaccination serum samples of responders in either age group (Figure 2D). In contrast, increase in maximal resonance unit (RU) values for the HA2 (Figure 2E) and HA1 (Figure 2F) proteins were demonstrated with postvaccination serum samples from both age groups. The increase of binding to the HA1 globular domain was much more pronounced for the pooled serum samples from the group aged >65 years (group 4) compared with the serum samples from the group aged ≤65 years (group 1) (Figure 2F, red and blue curves, respectively), in agreement with the FLU-GFPDL results (Figure 1C). Isotyping of postvaccination antibodies demonstrated that the majority (>90%) of HA 1-102 binding antibodies were IgM, whereas binding to the HA1 globular domain (1-330) and to HA2 was mediated by IgG antibodies, with a small contribution (≤10%) from IgA antibodies (Figure 2G).

When pooled serum samples are used for analysis, there is a possibility of variability among individuals. Therefore, additional SPR analyses were conducted with individual serum samples in groups 1–5. As can be seen in Figure 3, minimal variation was observed within groups. Again, no significant increase in the binding to the HA (1-102) N-terminus was found in post- versus prevaccination serum samples of either nonresponders (group 3) or vaccine responders (groups 1, 2, 4, and 5) (Figure 3A). In the case of HA2, significant prevaccination binding was measured in both age groups, with similar increase of binding observed for responders (groups 1, 2, 4, and 5) and nonresponders (group 3) (Figure 3B). In contrast, SPR binding to the HA1 globular domain demonstrated a very significant increase in antibody binding after vaccination of responders in groups 4 and 5 (4.5- and 7.8-fold, respectively) but not in nonresponders (group 3) in the group aged >65 years. Importantly, the mean maximal binding observed with the postvaccination serum samples of responders from the subjects aged >65 years in groups 4 and 5 (1678 and 2294 RU, respectively) was significantly higher than that observed in the subjects aged ≤65 years in groups 1 and 2 (465 and 586 RU, respectively) (P= .0007).

Antibody Avidity Measurements Using 7 mol/L Urea Treatment in Different Age Groups

To further investigate the effect of age on the quality of antibody responses, we extended our analysis to ELISA. In ELISA, as in other equilibrium-based assays (eg, HI and microneutralization; MN), it is not feasible to discriminate between the contributions of antibody affinity versus antibody concentration to the binding titer. It is possible, however, to approximate antibody avidity by measuring the effect of denaturants on antibody binding because low-avidity antibodies are more rapidly eluted [23–25].

To this end, individual serum samples obtained from vaccine responders in 3 age groups (Table 2) after first and second vaccinations were analyzed by ELISA with recombinant intact HA (HA0), HA2 (331-514), or HA1 (1-330) [26]. To evaluate relative avidity of bound serum antibodies, the antibody-antigen complexes were briefly exposed to 7 mol/L urea before addition of the
Table 2. Postvaccination Hemagglutination Inhibition Titers Against H1N1pdm09 in Different Age Groups

<table>
<thead>
<tr>
<th>Age, y</th>
<th>Samples, No.</th>
<th>SCR, %</th>
<th>GMT (Range)</th>
<th>SCR, %</th>
<th>GMT (Range)</th>
<th>Seroprotection Rate, %</th>
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</thead>
<tbody>
<tr>
<td>18–45</td>
<td>51</td>
<td>94</td>
<td>510 (80–5120)</td>
<td>94</td>
<td>499 (40–2560)</td>
<td>100</td>
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<tr>
<td>46–64</td>
<td>55</td>
<td>78</td>
<td>300 (&lt;10–2560)</td>
<td>84</td>
<td>273 (20–3220)</td>
<td>98</td>
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<tr>
<td>&gt;65</td>
<td>62</td>
<td>60</td>
<td>177 (&lt;10–5120)</td>
<td>74</td>
<td>214 (20–5120)</td>
<td>97</td>
</tr>
</tbody>
</table>

Abbreviations: GMT, geometric mean titer; SCR seroconversion rate.

*All samples had prevaccination hemagglutination inhibition (HI) titers of ≤32, except for 1 outlier with titer of 64.

b Collected on study day 21.

c The seroconversion rate (SCR) is the percentage of subjects demonstrating a ≥4-fold increase in HI titer compared with prevaccination titer.

d The GMT of DF HI end-point titers using postvaccination human serum samples.

*The seroprotection rate is the percentage of subjects demonstrating an HI titer ≥40.

secondary labeled antihuman IgG antibody, as described elsewhere [19]. The percentages of antibodies resistant to 7 mol/L urea were calculated from the dose-response curves (half maximal effective concentration; EC50) of samples treated with 7 mol/L urea compared with untreated samples (Figure 4). In the case of binding to rHA0, 7 mol/L urea treatment reduced antibody binding by 40%–50%, with no statistically significant differences between the 2 age groups or between the serum samples obtained after the first and after the second vaccinations (Figure 4A). Similarly, 7 mol/L urea treatment reduced antibody binding to rHA2 by only 10%–20% across all age groups (Figure 4B). Remarkably, when these same individual serum samples were tested for avidity of binding to rHA1 (1-330), significantly higher proportions of antibodies in the responders aged >65 years were resistant to 7 mol/L urea after the first vaccination (mean, 58%) compared with the postvaccination serum samples from the groups aged 46–64 years or <45 years (mean, 18% and 24%, respectively; \( P = .0008 \) and \( .0003 \)) (Figure 4C). After the second immunization, only minimal increase in antibody avidity were observed in all age groups (Figure 4C). Only 4%–5% of pre-vaccination serum antibodies (all with HI titers of ≤32) were resistant to 7 mol/L urea in the rHA1 ELISA (data not shown).

To further corroborate the 7 mol/L urea ELISA data with rHA1, these findings were extended to SPR-based measurements of antibody off-rates against rHA1 (1-330), as recently described [19]. As can be seen in Figure 5D, the average dissociation off-rates for the subjects aged <45 years or 46–64 years were significantly higher compared with those for subjects aged >65 years, reflecting lower HA1-specific antibody affinity in the younger age groups.

**Gender Differences in Avidity of Antibody Binding to HA1 (But Not HA2) in Young But Not Elderly Adults**

Recent work has highlighted the potential importance of gender differences in susceptibility to influenza and in response to influenza vaccination [27, 28, 37]. In our original study of this inactivated H1N1pdm09 vaccine, we found no gender-based differences in HI antibody response [20]. However, the HI assay does not detect differences in antibody avidity, which may have a significant impact clinically [23]. The data in Figure 5 are based on all male and female vaccine responders (4-fold increase in HI titers at day 21 versus day 0 and titer of >1:128 after vaccination) stratified according to age as indicated in Table 2. The percentage of 7 mol/L urea-resistant antibodies against HA1 and HA2 in individual serum samples of the 3 age groups after H1N1pdm09 vaccination (n = 51–58 per group) were equally high for men and women aged >65 years against either HA1 (60%) or HA2 (80%) (Figure 5C and 5F). Surprisingly, serum samples from women in the 2 younger age groups (n = 51–55 per group) had significantly lower percentages of 7 mol/L urea-resistant antibodies against rHA1 compared with samples from men (mean, 11%–14% vs 26%–33%, respectively; \( P = .009 \) and \( .011 \)) (Figure 5A and 5B). In contrast, no gender difference in avidity of binding to HA2 was identified in any age group (Figure 5D–F). All preimmunization serum samples had HI titers of ≤32 and contained ≤5% 7 mol/L urea-resistant antibodies, irrespective of age or gender (data not shown).

Next, we used HA1-based SPR to calculate the antibody dissociation off-rates in male and female subjects (n = 4–8 per group with similar HI titers). As shown in Figure 5I, in the group aged >65 years, antibody off-rates after H1N1pdm09 vaccination were not significantly different between male and female subjects (mean off-rate constant, 0.00021 vs 0.00045; \( P = .012 \)). In contrast, statistically significant differences in mean off-rate constants between men and women were found in the younger groups (Figure 5G and 5H). In subjects aged <45 years, the mean off-rate constant was 0.00071 for men and 0.00194 for women (\( P = .011 \)). In the group aged 46–64 years, the mean off-rate constant was 0.000505 for men and 0.00103 for women (\( P = .012 \)). These findings are in agreement with the results obtained in the 7 mol/L urea ELISA-based avidity assay (Figure 5G and 5H vs Figure 5A and 5B).

**DISCUSSION**

Multiple novel influenza vaccines and adjuvants are being evaluated in clinical trials in an attempt to improve immunogenicity.
against seasonal influenza as well as swine and avian strains with pandemic potential. The conventional immunological end point, HI titers, is not suitable for all types of vaccines and/or does not provide complete information about the quality and quantity of the immune responses. Thus, multiple laboratories are developing new assays that could be evaluated during efficacy trials to establish correlation with clinical end points. We demonstrated elsewhere the broad epitope repertoires of HA and neuraminidase (NA) and measurements of antibody binding avidity to the HA1 globular domain provided better understanding of the impact of oil-in-water adjuvant or alternate vaccine platform (virus-like particles) on the antibody profiles and affinity maturation after immunization with H5N1 or H1N1pdm09 vaccines.

In the current study, we used these assays to elucidate and compare the humoral immune responses of elderly (aged >65 years) and younger (aged <65 years) adults after immunization with an unadjuvanted H1N1pdm09 vaccine. Serum IgG binding to mammalian H1N1-rHA0 (A), rHA2 (331-514) (B), and rHA1 (1-330) (C) after 1 or 2 immunizations was analyzed by comparing the dose-response curves (half maximal effective concentration; EC50) of 3-fold serial serum dilutions in the absence or presence of 7 mol/L urea for responders among elderly subjects (aged >65 years; n = 18), older adults (aged 46–64 years; n = 16), and young adults (aged <45 years; n = 18; B and C only). Correlation statistics of the avidity measurement of the human serum samples between the responders from these age groups after the first or second vaccination showed significant differences only for rHA1 (C) (P < .05; t test). Data shown are means ± standard deviations for each serum sample from 3 independent experiments. D, Antibody affinity as measured by antibody dissociation rates for rHA1 (1-330) in different age groups after first vaccination. Surface plasmon resonance analysis of post–H1N1 vaccination human serum samples from 3 age groups (as in B and C) was performed with functional H1N1-rHA1 (1-330) [26]. Serum antibody off-rate constants were determined as described elsewhere and in Materials and Methods. Correlation statistics of the off-rate constants for the postvaccination human serum samples between different vaccine groups showed significant differences between the group aged >65 years and the group aged <65 years (P < .05; t test).
percentage of high-avidity antibodies to the rHA1 globular domain, as determined by increased fraction of 7 mol/L urea-resistant IgG antibodies and lower antibody-antigen dissociation rates in real-time kinetics assay using SPR. Importantly, these differences in antibody response were seen in the elderly vaccinated subjects irrespective of their preexisting HI titers against the H1N1pdm09 virus, including seronegative subjects (HI #1:8). We did not further stratify the group of adults aged >65 years into smaller age subsets owing to insufficient numbers. However, the SPR and 7 mol/L urea assays conducted on individual serum samples did not reveal significant variability among the elderly vaccinees.

Our findings suggest that elderly individuals have long-term memory B cells with B-cell receptors that cross-react with the 2009 H1N1 A/California/07/2009 and were rapidly recruited and underwent selection and affinity maturation after immunization with the H1N1pdm09 vaccine [8, 32–35]. In a recent study by Sasaki et al, measurements of antibody secreting cells (ASC) and plasmablast-derived antibodies 7 days after vaccination with seasonal inactivated trivalent influenza vaccine (TIV) (2009) found that the frequency of vaccine-specific plasmablasts were lower in elderly than in young adults. However, no significant differences in the amount of vaccine-specific antibodies produced per cell or the avidity of plasmablast-derived polyclonal or

Figure 5. A–F, Antibody avidity for rHA1 and rHA2 in men and women after immunization with H1N1pdm09 vaccine. Percentage of high-avidity immunoglobulin class G (IgG) antibodies (7 mol/L urea resistant) binding to H1N1-rHA1 (A–C) and H1N1-rHA2 (D–F). Data are shown for male and female responders from young (aged <45 years) (n = 51) (A, D), older (aged 46–64 years) (n = 55) (B, E), and elderly (aged >65 years) (n = 58) (C, F) adults subjects. Reactivity to rHA1 (A–C) and rHA2 (D–F) after 1 or 2 immunizations is shown and presented as means ± standard deviations for each serum sample from 3 independent experiments. Differences between groups were examined for statistical significance using Student t test; differences were considered significant at P < .05. G–I, Serum antibody dissociation rates for rHA1 in men and women after immunization with H1N1pdm09 vaccine. Antibody off-rate constants that describe the fraction of antibody-antigen complexes decaying per second were determined directly from the plasma sample interaction with rHA1 (1-330) protein using surface plasmon resonance (SPR) in the dissociation phase. The SPR analysis of prevaccination (black dots) and postvaccination (red dots) human serum samples with unadjuvanted H1N1pdm09 vaccine from the 3 age groups included in the vaccine trial was performed with H1N1pdm09-rHA1 (A/California/07/2009). Serum antibody off-rate constants (each symbol represents one individual) were determined as described in Materials and Methods. Mean off-rate constants for the postvaccination human serum samples differed significantly between men and women only in the groups of young adults (aged <45 years) (G) and older adults (aged 46–64 years) (H) (P < .05; t test). I, Elderly adults (aged >65 years). Off-rate constants were determined from 2 independent SPR runs.
monoclonal antibodies were found between younger and elderly adults [16]. That study was not conducted in individuals who received the monovalent H1N1pdm09 vaccine, and its findings do not conflict with our results.

Our findings identified significant differences in binding avidity between elderly and younger adults to the HA1 globular domain but not to the HA0, HA2 stalk domain, or a shorter segment of HA1 (1-102). These findings are of particular importance, because in previous studies the entire HA vaccine was used to measure total ASC and binding avidity of plasmablast-derived antibodies [14, 16]. Owing to the highly conserved HA2, the level of cross-reactive binding to the stalk domain could have masked significant differences in the avidity of binding to the HA1 domain among different age groups.

Adaptation of SPR technology to follow real-time kinetics of polyclonal antibody binding to properly folded HA domains and the calculation of antibody-antigen dissociation rates (ie, direct measurement of antibody affinity), as described elsewhere [19], has several advantages over ELISA-based assays, in which only a single time point is measured. The affinity of antibodies is likely to play a key role in vivo, especially very early in infection (ie unfavorable antibody/viral load ratios). During the H1N1 pandemic, low-affinity antibodies in some infected individuals were associated with more severe disease [23]. Therefore, our finding of higher-avidity antibodies in elderly vaccine responders may help explain the unusually low rate of infection and severe respiratory disease during the H1N1 2009 pandemic in this age group, which usually represents the group most susceptible to seasonal influenza.

In addition, our study identified a gender difference in the avidity of antibodies against rHA1 measured in postvaccination serum samples: women aged <65 years developed antibodies of lower affinity and lower avidity to rHA1 compared with men in the same age group, a phenomenon that has not been described before and was not predicted by the HI titers measured in trial participants [20] and reports on HI responses to seasonal influenza vaccine [36]. On the other hand, this finding may reflect lower antibody avidity against H1N1pdm09 in young women than in men, which could partially explain a report of higher morbidity rates in younger women during the H1N1pdm09 pandemic [28].

In summary, we have shown that the epitope repertoire and affinity and avidity of HA1-bound antibodies after H1N1pdm09 vaccination were superior in elderly compared with younger adults. Use of these novel FLU-GFPDL and SPR technologies and other newly developed assays are likely to improve our understanding of the clinical outcomes of influenza vaccination in different populations, especially as new vaccine modalities and novel adjuvants are developed and evaluated.

Notes

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