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Antibody-dependent phagocytosis (ADP) responses following trivalent inactivated influenza vaccination of younger and older adults

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ABSTRACT

Globally the most commonly utilised immunisation against influenza is the trivalent inactivated influenza vaccine (TIV) derived from an A/H1N1, an A/H3N2 and a B type influenza virus. Vaccine effectiveness of TIV varies year to year, depending on how well antigenically matched the strains in the vaccine are compared to circulating strains [1,2]. Moreover, vaccine effectiveness can vary within certain subpopulations such as HIV-positive, young children and the elderly. Decreased vaccine effectiveness in the elderly is associated with impaired Ab production, as measured by standard hemagglutination inhibition (HAI) assays. We investigated the level of Antibody Dependent Phagocytosis (ADP)-mediating Abs induced by the 2008-TIV in healthy Australian adults aged over and under 60 years to determine if this immune function was also reduced in the elderly. We utilised an ADP assay that measures the uptake of IgG-opsonised HA-coated fluorescent microspheres by a monocytic cell line. We also measured HA-specific Abs that are close enough to bind to dimeric FcγRIIa ectodomains in an ELISA-based assay. Furthermore, we compared the extent of cross-reactive recognition of diverse influenza strains by ADP-mediating Abs found in pre- and post-vaccination sera in both of these groups. We found that young adults and older adults mounted similar ADP activity against HAs contained in the 2008-TIV, despite older adults have diminished HI responses. The level of cross-reactive antibodies against other HAs was limited in both groups. We conclude that seasonal influenza vaccination elicits limited cross-reactive ADP to HA in both young and older adults. New influenza vaccination strategies that elicit cross-reactive and polyfunctional antibodies are needed.

1. Introduction

Influenza virus infects an estimated 5–15% of the population each year and accounts for up to 500,000 deaths worldwide [3]. Vaccination is a key method to prevent new influenza infections and/or decrease influenza disease severity. A commonly utilised immunisation against influenza is the trivalent inactivated influenza vaccine (TIV) derived from an A/H1N1, an A/H3N2 and a B type influenza strains. Vaccine effectiveness (VE) of TIV varies year to year, depending on how well antigenically matched the strains in the vaccine are compared to circulating strains [1,2]. Moreover, VE can vary within certain subpopulations such as HIV-infected subjects, young children and the elderly. The elderly are at risk for more severe complications from influenza and, although randomized controlled trials in the elderly are lacking [2], there is a general acceptance from observational trials that VE in the elderly is lower than it is in younger adults [4]. Decreased VE in the elderly is likely to be associated with impaired Ab production, as measured by standard hemagglutination inhibition (HAI) assays and with reduced isotype switching owing to immunosenescence [5,6].

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Humoral immunity elicited by TIVs predominantly targets Influenza hemagglutinin (HA) and to a lesser degree neuraminidase (NA). Neutralising Abs (nAbs) induced by TIVs primarily protect against influenza strains contained in the vaccine and are directed against highly variable epitopes of the HA protein head domain [7–9]. However, traditional methods to analyse nAb responses to TIVs do not provide information about vaccine-elicited Abs effecting functions through their Fc domains such Ab-dependent cellular cytotoxicity (ADCC) and phagocytosis (ADP). While some studies have focused on ADCC [10], anti-influenza ADP responses are not well understood.

Studies in animal models suggest that the Fc-portion of Abs plays a crucial role in influenza virus clearance *in vivo* [11–14]. ADP was implicated in protective immunity in a recent publication on H7N9-based influenza vaccination [15]. Abs forming immune complexes with viruses or virus-infected cells may engage activating FcγRs, including FcγRI (CD64), FcγRIIa (CD32a) and FcγRIII (CD16) expressed on various innate leukocytes such as on monocytes/macrophages, neutrophils and dendritic cells, triggering phagocytosis of the opsonised antigens and thereby contributing to viral clearance.

We previously developed assays to measure ADP-mediating Abs against multiple influenza subtypes following infection of macaques with influenza and in adult humans with prior exposure to influenza (10, 16). Herein, we investigated the level of ADP-mediating Abs induced by 2008-TIV in healthy Australian adults aged over and under 60 years. Furthermore, we compared the extent of cross-reactive recognition of diverse influenza strains by ADP-mediating Abs found in pre- and post-vaccination in both groups.

2. Methods

2.1. Influenza vaccine cohort

We retrospectively studied banked pre-2009 pandemic plasma samples from 91 TIV vaccinated adults. Fifty healthy younger adults (age: 18–59 years) and forty-one healthy older adults (age: 60–79 years) were recruited by the Australian WHO Collaborating Centre for Influenza to assess the immunogenicity of the 2008-TIV, approved by the WHO human ethics committee (number: CSLCT-WHO-08-51). Volunteers were vaccinated with the southern hemisphere 2008 seasonal TIV (CSL Behring, Australia), containing 15 μg of H1N1 A/Solomon Islands/3/2006, H3N2 A/Brisbane10/2007 and B/Florida/4/2006. Blood was collected at day 0 and 21 ± 4 after immunisation. HAI titres were measured for each plasma sample as previously described [17]. IgG was purified from 50 μl of plasma samples (Protein G HP Multitrap, GE Healthcare, UK).

2.2. HA-specific ADP-SHIP assay

ADP of HA-coated beads by a monocytic cell line was measured with the ADP-SHIP assay as previously described [16]. Briefly, yellow-green NeutrAvidin® FluoSpheres® (Invitrogen, Carlsband, CA) were incubated with Cy5 fluorescent internalisation probe (FIP_{Cy5};5′-Cy5-TCAGTTCAGGACCCTCGGCT-3Bio-3′; Integrated DNA Technologies) for 10 min. Biotinylated monomeric influenza HA or SIV-gp120 control (Sinobiological, China) were added to FIP-coated fluorescent beads overnight. Washed coated beads were added to 96-well plates and opsonised with IgG for 2 h. 10^5 THP-1 cells (ATCC TIB-202) were added to each reaction for 16 h at 37 °C. The Cy5 fluorescence of surface bound beads was quenched with a complementary probe (5′-AGCCGAGGGTCCTGAACTGA-BHQ2-3′). Cells were fixed and acquired using a LSR Fortessa (BD Biosciences).

2.3. FcγRIIa (CD32a) dimer-binding ELISA

HA-specific Ab engagement of FcγRIIa was measured as previously described [10,18,19]. Briefly, recombinant influenza-HA or SIV-gp140 proteins were coated on a 96-well plates (Nunc, Rochester, NY), washed, blocked and plasma samples added. Following incubation, wells were washed and biotinylated dimeric recombinant FcγRIIa added. Binding was detected by HRP-Streptavidin (Thermo Fisher, MA). Plates were developed and absorbance read at 450 nm. To normalise results between plates we used wells coated with 5 μg/ml IVIG (Intragam, bioCSL, Australia). Responses were considered positive when above cut-off levels of 0.1 normalised OD at 450 nm.

2.4. Data analysis

Statistical analyses were performed with Prism GraphPad v5 (San Diego, CA). Data were analysed by paired *t*-test, unpaired *t*-test or one-way ANOVA followed by a Bonferroni's comparison, Pearson's product-moment correlation coefficient or Chi-square as indicated in figure legends. Positive ADP values were those over mean + 2SD over SIV-gp140 control.

3. Results

3.1. Subjects and influenza virus HAI activity

Elderly individuals are at higher risk of developing severe influenza disease and can respond poorly to seasonal TIV. Since influenza-specific ADP is associated with protection from influenza in mice [15], we investigated whether TIV elicits ADP activity in young and older adults. We studied plasma samples from 50 young adults and 41 older individuals vaccinated with 2008 Southern Hemisphere TIV (Table 1). We first evaluated the HAI activity of each individual at baseline (day 0) and 3 weeks following vaccination for each vaccine strain and for two other non-circulating strains that were (Fig. 1).

HAI levels were analysed by 3 methods: seroconversion rates (a 4-fold rise in HAI titres regardless of baseline or endpoint titre), "seroprotection" rates (a titre ≥40) and by geometric mean titre (GMT) HAI levels (shown in Fig. 1 in 3 columns). Seroconversion rates post-vaccination for the 3 vaccine strains (H1N1, H3N2 and B) were 68%, 52% and 44% in young adults respectively and 54% 49% and 32% in older individuals. Interestingly, seroconversion to B/Florida/4/2006 was lower for both groups compared to the 2 Influenza A strains in the vaccines. For heterologous H1 and H3 strains, HAI responses were lower as expected. The seroconversion rate in young adults was 12% for the non-vaccine strain H1N1pdm09 A/Auckland/1/2009 and 18% for H3N2 A/Texas/50/12. In older adults the seroconversion rates were higher, at 29% for H1N1pdm09A/Auckland/1/2009 and 32% for H3N2 A/Texas/50/12, consistent with improved priming of the older adults to H1N1pdm09 strain [20,21].

Table 1
Baseline characteristics of the cohort.

Characteristics	Younger adults (n = 50)	Older adults (n = 41)
Age (yr)		
Mean	40	64
Range	18–59	60–79
No. (%) of male gender	15 (30)	14 (43)

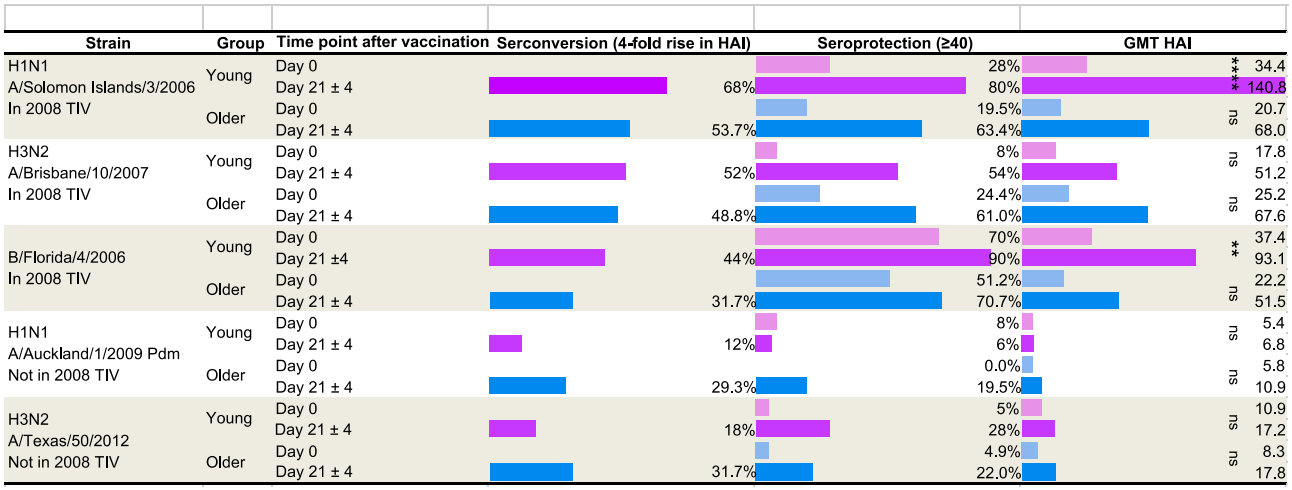

Fig. 1. Serological responses of the cohort at day 0 and 21 ± 4 post-vaccination. Seroconversion was considered positive when hemagglutination inhibition (HAI) titre increased at least 4-fold after TIV. Individuals with a HAI titre of ≥40 were defined as seroprotected. Statistical analysis performed utilising one-way ANOVA and Bonferroni's multiple comparison for geometric mean (GMT) HAI comparisons. Non-significant differences ($P > 0.05$) = ns, ** = $P \leq 0.01$, and **** = $P \leq 0.0001$.

When analysed using "seroprotection", at baseline 28% of young adults and 19.5% of older adults were seroprotected to H1N1 A/Solomon Islands/3/2006, 8% and 24% to H3N2 A/Brisbane/10/2007, 70% and 51% to B/Florida/4/2006. For heterologous strains, 8% of young adults and 0% of older adults were seroprotected to H1N1pdm09 A/Auckland/1/2009, and 5% and 5% to H3N2 A/Texas/50/2012. Following vaccination, 80% of young adults and 63% of older adults were seroprotected to the vaccine H1N1, 54% and 61% to H3N2, 90% and 71% to B/Florida/4/2006. For heterologous strains, seroprotection post-vaccination was 6% and 20% to H1N1 and 28% and 22% to H3N2.

We also analysed HAI levels by GMT. Both young and older adults, had a GMT of <40 prior to vaccination (Fig. 1, last column). After TIV, both groups reached GMT higher than 40, although there was a significant rise only in young adults to the H1N1 and B vaccine antigens. For the heterologous strains, neither the young or older adults reached a GMT higher than 40 post-vaccination.

Overall, HAI seroconversion levels were slightly higher in young adults to all vaccine strains. Seroprotection levels pre- and post-vaccination were usually higher in young adults than in older adults, except to H3N2 A/Brisbane/10/2007. Rise in GMT was non-significant for most HAs in both groups, except to the H1N1 and B vaccine antigens for young adults.

3.2. HA-specific antibodies binding FcγRIIa after TIV

To allow high-throughput assessment of Fc-functional Abs in larger cohorts, we developed an ELISA-based assay that measures HA-specific Abs binding to dimeric FcγRs. We previously demonstrated that FcγRIIIa binding strongly correlates with NK activation through FcγRIIIa [18,19] and FcγRIIa dimer binding correlates with the ADP-SHIP assay [10]. We evaluated endpoint FcγRIIa dimer binding ELISA titres in both groups (Fig. 2). At baseline, we found no significant differences in endpoint titres of HA-specific FcγRIIa-binding Abs across the 2 groups for all 3 strains in the TIV. Both groups had a significant increase in HA-specific FcγRIIa-binding Abs post-vaccination to the vaccine antigens H1 and B, but not to H3. The fold increase in FcγRIIa-binding Abs was not different among the groups (inset graphs, Fig. 2). Interestingly, after TIV immunisation, there was a significantly higher FcγRIIa-binding endpoint titre to H1 A/Solomon Islands/3/2006 HA in young adults compared to older adults (Fig. 2A, $P \leq 0.05$). Overall, these data show that 2008-TIV elicited similar titres of HA-specific Abs able to engage FcγRIIa dimers in young and older adults.

3.3. TIV vaccination induced ADP in young and older adults

We next evaluated whether TIV elicited Fc-functional anti-HA Abs in young and older adults that mediate ADP in a cell-based assay. Here we used uptake of HA-coated beads by a monocytic cell line as a measure of ADP purified IgG samples towards the 3 vaccine HA proteins (Fig. 3). A significant rise in functional ADP activity was detected post-vaccination in both young and older adults against all 3 vaccine HAs (Fig. 3A–C). There was significantly higher increase in ADP activity elicited by TIV to HA B/Florida/4/2006 in the young adults compared to the older adults ($P \leq 0.001$; right panel on Fig. 3C), but not to the other 2 vaccine proteins. Overall, these results show that 2008-TIV induced HA-specific ADP-mediating Abs in both young and older adults. There was a positive correlation between FcγRIIa binding endpoint titres and ADP activity by the ADP-SHIP assay for both young ($P < 0.0001$, $r = 0.47$; Fig. 3D) and older adults ($P = 0.0005$, $r = 0.37$; Fig. 3E) as previously found [10]. There are also positive correlations between HAI antibodies and between FcγRIIa binding endpoint titres and ADP activity for most vaccine antigens across both age groups, however the strength of the correlations is always stronger for the younger adults than the older adults (Supplementary Fig. 1).

3.4. Breadth of TIV-induced ADP responses

Since the most prevalent circulating strain of influenza is uncertain in advance, an important issue is the number of strains to which individuals respond. We subsequently categorised responses according to the percentage of individuals on each group that had at least 5% increase in ADP activity to zero, one, two or all three HA subtypes in the 2008 TIV (Fig. 4). Only 6% of young individuals and 12% of older adults lacked ADP to any of the HA proteins in TIV after vaccination (Fig. 4). 32% of young and 42% of older adults had an increase in ADP activity against only one HA subtype. Interestingly, 28% and 34% of young adults had a rise in ADP activity against two or all three HA proteins respectively, whereas 34% of older adults had in increase in ADP to two strains and only 12% to all three strains in the TIV. Overall, young adults had a broader increase in vaccine-induced ADP compared to older adults ($P = 0.002$).

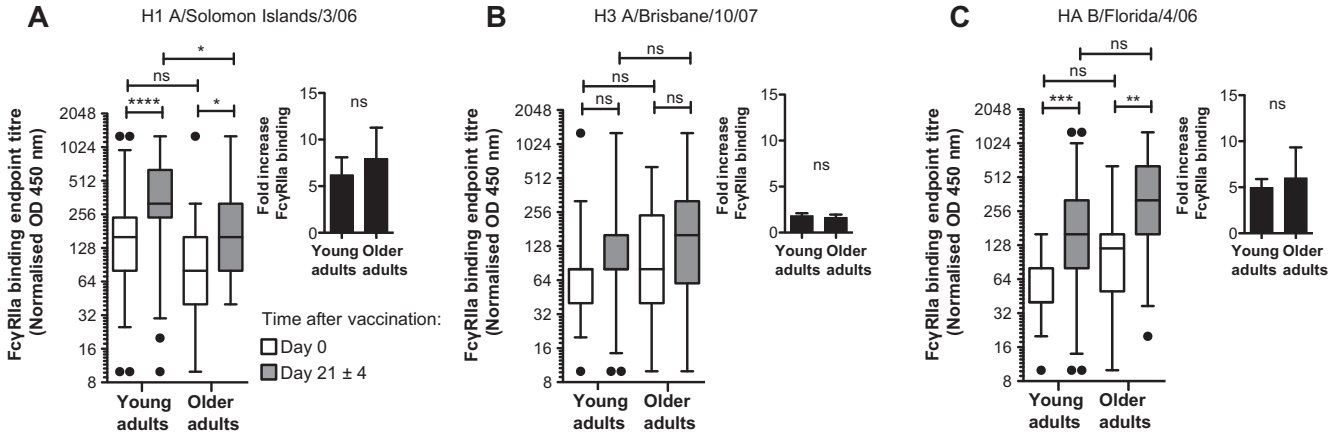

Fig. 2. HA-specific Abs with FcγRIIa binding pre and post 2008-TIV to vaccine strains. The ability of anti-HA Abs to bind to FcγRIIa dimer was assessed in plasma samples of 50 young, and 41 older individuals before and after receiving the 2008-TIV. Endpoint titres to (A) H1 A/Solomon Islands/3/2006, (B) H3 A/Brisbane/10/2007 and (C) type B/Florida/4/2006 HAs (left panels). Endpoint titres were calculated by 2-fold dilution of plasma samples and expressed as the reciprocal dilution of the lowest dilution that had a positive binding to FcγRIIa above background levels (mean of binding to SIV-gp120 + 3SD). Comparisons were performed with one-way ANOVA and Bonferroni's multiple comparisons test. Whiskers represent 5–95 percentile. Fold increase in FcγRIIa binding was calculated dividing FcγRIIa binding titre on Day 21 ± 4/Day 0 for each HA in the vaccine (right panels). Comparison were done with unpaired *t*-test. ns = $P > 0.05$, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$, and **** = $P \leq 0.0001$.

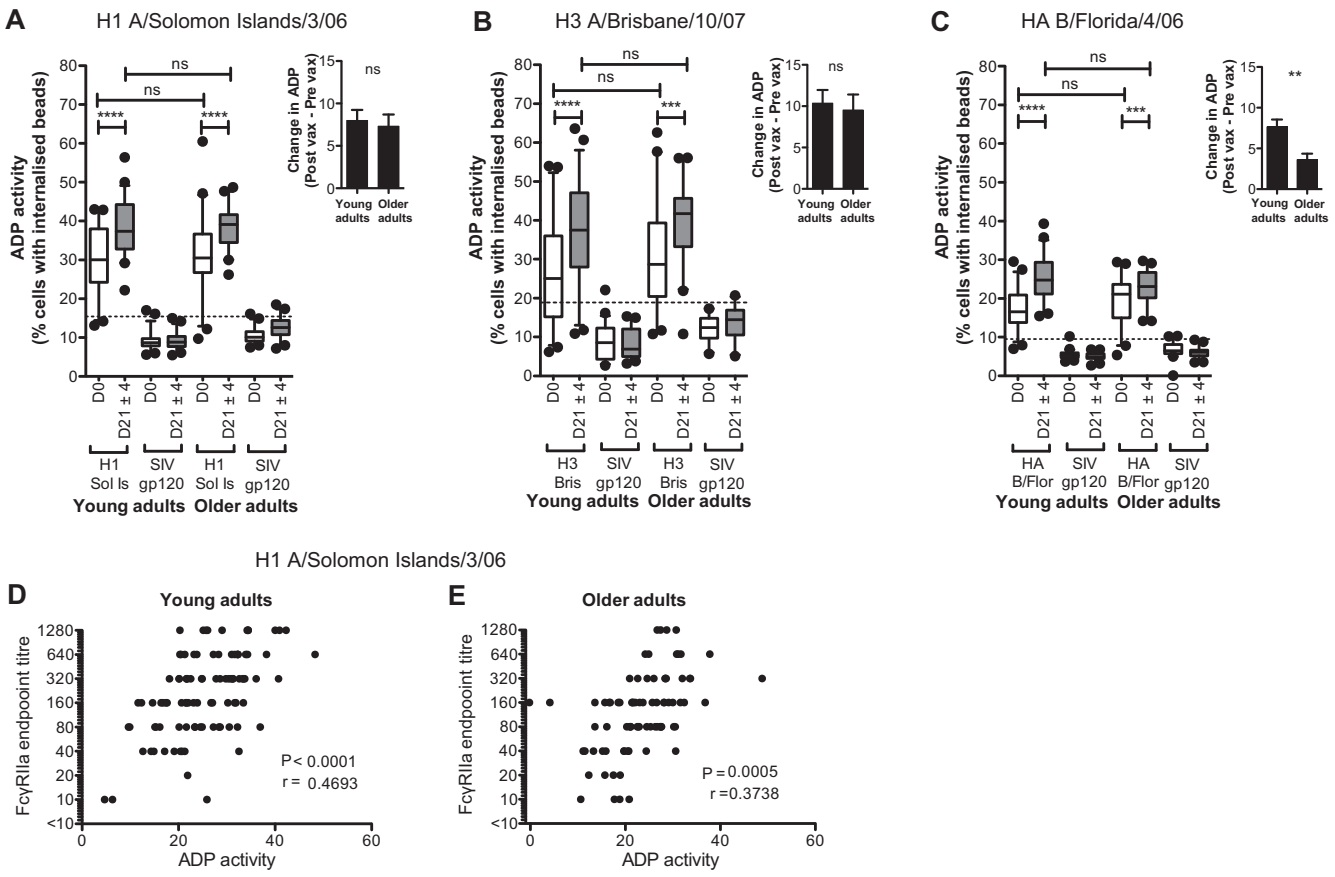

Fig. 3. Assessment of ADP activity to HAs from strains within TIV. ADP activity in plasma samples pre (Day 0) and post-vaccination (Day 21 ± 4) to (A) H1 A/Solomon Islands/3/2006, (B) H3 A/Brisbane/10/2007 and (C) type B/Florida/4/2006 HAs (left panels) using the ADP-SHIP assay. Dashed line represents mean ADP activity to SIV-gp120 + 2SD per experiment. Comparisons were performed with one-way ANOVA and Bonferroni's multiple comparisons test. Whiskers represent 5–95 percentile. Change in ADP comparisons (right panels) were performed with unpaired *t*-test. ns = $P > 0.05$, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$, and **** = $P \leq 0.0001$. Data are representative of two or three experimental repeats. (D) and (E) Correlation of HA-specific Abs binding dimeric FcγRIIa and mediating ADP at same plasma dilution, using Pearson's correlation coefficient.

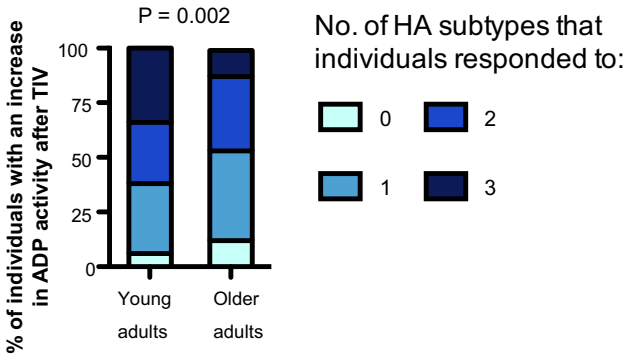

Fig. 4. Breadth of ADP activity. Percentage of individuals with an increase in ADP activity to 0, 1, 2 or 3 HAs contained in the vaccine. Comparison was done using Chi-square.

3.5. Cross-reactive ADP-mediating antibodies induced by TIV vaccination

The ability of TIV to induce cross-reactive ADP-mediating Abs has not been previously investigated. We studied the extent of cross-reactive Ab responses within our 2008 cohort by measuring FcγRIIa dimer binding ELISA Abs to H1pdm09 (a pandemic strain appearing in 2009) and H3 A/Texas/50/2012 (a drifted H3 appearing in 2012) at both single 1:40 plasma dilution (Fig. 5A and B), and by endpoint titre (Fig. 5E and F).

For the H1pdm09 pandemic strain (Fig. 5A and E), basal levels of Abs binding to FcγRIIa dimers were low in both older adults and young adults and not significantly boosted by TIV in both groups. For the seasonal H3 A/Texas/50/2012 strain (Fig. 5B), FcγRIIa-binding Ab responses were higher at baseline compared to H1pdm09 and were boosted after TIV in both young and older adults when studied at a 1:40 plasma dilution. The increase in FcγRIIa binding Abs to H3 A/Texas/50/2012 following vaccination was comparable in young and older adults. However, by endpoint titre (Fig. 5F) there was no significant increase in the H3 A/Texas/50/2012-specifc FcγRIIa dimer binding titres post vaccination.

We next determined whether the level of HA cross-reactive FcγRIIa binding is associated with the magnitude of vaccine HA FcγRIIa binding. We found a significant correlation between the FcγRIIa binding titres to vaccine H1 and H1pdm09 ($P < 0.0001$, $r = 0.40$; Fig. 5C), with a proportion of subjects responding strongly to the vaccine H1 also responding to the divergent H1pdm. For H3, we detected a stronger correlation of vaccine H3 and the heterologous H3 A/Texas/50/2012 ($P < 0.0001$, $r = 0.78$; Fig. 5D), consistent with these two H3 strains being more closely related than the H1 strains studied.

4. Discussion

Protection against influenza can be assisted through antibody Fc-mediated mechanisms such as ADP. TIV vaccination boosted HA-specific Abs that bind to FcγRIIa and mediate ADP in both young and older adults. FcγRIIa binding and ADP activity was augmented to the 3 HAs included in the 2008-TIV.

Older adults reached significantly lower titres of HAI Abs than young adults for both the H1 and B strains contained in the 2008-TIV, consistent with previous reports [22,23]. Despite this, HA-Abs mediating ADP were generally similar in both older and younger groups to all 3 HA antigens. We suggest that lifetime exposures to multiple influenza viruses prime cross-reactive Abs that mediate ADP in the absence of HI-mediating Abs [16]. We recognise that age related functional differences of Abs that we reported may be an affected by other factors that such Ab avidity [18].

There were however subtle differences detectable in ADP activity and FcγRIIa binding activity between younger and older adults. Of most interest was the weaker capacity of older adults to recognise multiple HAs in the vaccine. In addition, older adults generated poorer FcγRIIa binding Ab responses to the H1 vaccine antigen post-vaccination and poorer increases in ADP responses to the B antigen post-vaccination. The overall differences in ADP responses to vaccination were modest and further studies will be required to determine if differences in ADP responses are in part responsible for increased influenza severity in older adults. A caveat to our study was that our "older" adults were all 60–79 years old, with the average age only 64. There is a strong relationship between advanced age and influenza severity [24,25] and it will be of interest to determine if the differences we observed would be magnified in an older cohort. The modest differences in ADP activity detected after vaccination in both age groups suggests that the current inactivated influenza vaccine induced generally weak ADP responses. This weak response to vaccination may be contributed to by high pre-existing levels of ADP-mediating Abs at baseline given by past exposure to influenza virus or vaccination. Future passive transfer studies of ADP-mediating Abs (in the absence of neutralising activity) in humanised mouse or ferret models will be necessary to elucidate if the modest differences we observe after vaccination of humans are sufficient to induce protection against influenza infection or disease severity.

All three influenza strains contained in the 2008 TIV were novel HA antigens [26] which may have been a factor in the weaker capacity of older adults to mount ADP responses to multiple HA antigens. Despite this, many individuals (both young and older) had cross-reactive Abs that mediated ADP against these HAs prior to vaccination. HA-stalk and/or cross-reactive head Abs with the ability to mediate Fc-functions may explain this finding [13–15,27]. Future analyses of the fine specificity of ADP responses in older adults to TIV antigens conserved over multiple years will be of interest.

We did not assess how quickly ADP-mediating Abs decay after vaccination. Ab responses to vaccines and viral infections typically decline faster in older individuals compared to younger individuals [22,28]. This may be due to reduced germinal centre functions, including affinity maturation of B cell receptors, antibodies and memory B cells [29–32], a decline in B cell diversity [33,34], and a failure to generate long-lived effector B cells in the bone marrow [35] caused by a decrease in the size of plasma cell survival niches [36,37]. This overall immunosenescence likely contributes to increased susceptibility to (and severity of) influenza infection, as well as poorer protection by vaccination. The functionality of innate immune cells with phagocytic capacity such monocytes/-macrophages and neutrophils is also decreased in the elderly [38,39]. Diminished uptake of antigens by ADP may lead to decreased antigen presentation and stimulation of adaptive immunity. It will be of interest in future studies to evaluate ADP responses in old individuals utilising primary phagocytic cells.

A strength of our study is that we evaluated ADP response using two different assays: the ADP-SHIP assay and the FcγRIIa dimer ELISA. The ADP-SHIP assay measures the internalisation of IgG-opsonised antigens by cells expressing all FcγRs; whereas the FcγRIIa dimer ELISA detects HA-specific Abs that engage with a given FcγR. These two assays correlated reasonably well with each other in both younger and older adults (Fig. 3D and E). FcγRIIa is the main receptor that triggers ADP and blocking this receptor abrogates most ADP activity [40]. However, some of the subtle differences detected between the two assays could be explained by the presence of Abs that bind to other FcγRs such FcγRI and would not be measured by the FcγRIIa dimer ELISA. It will be of interest in

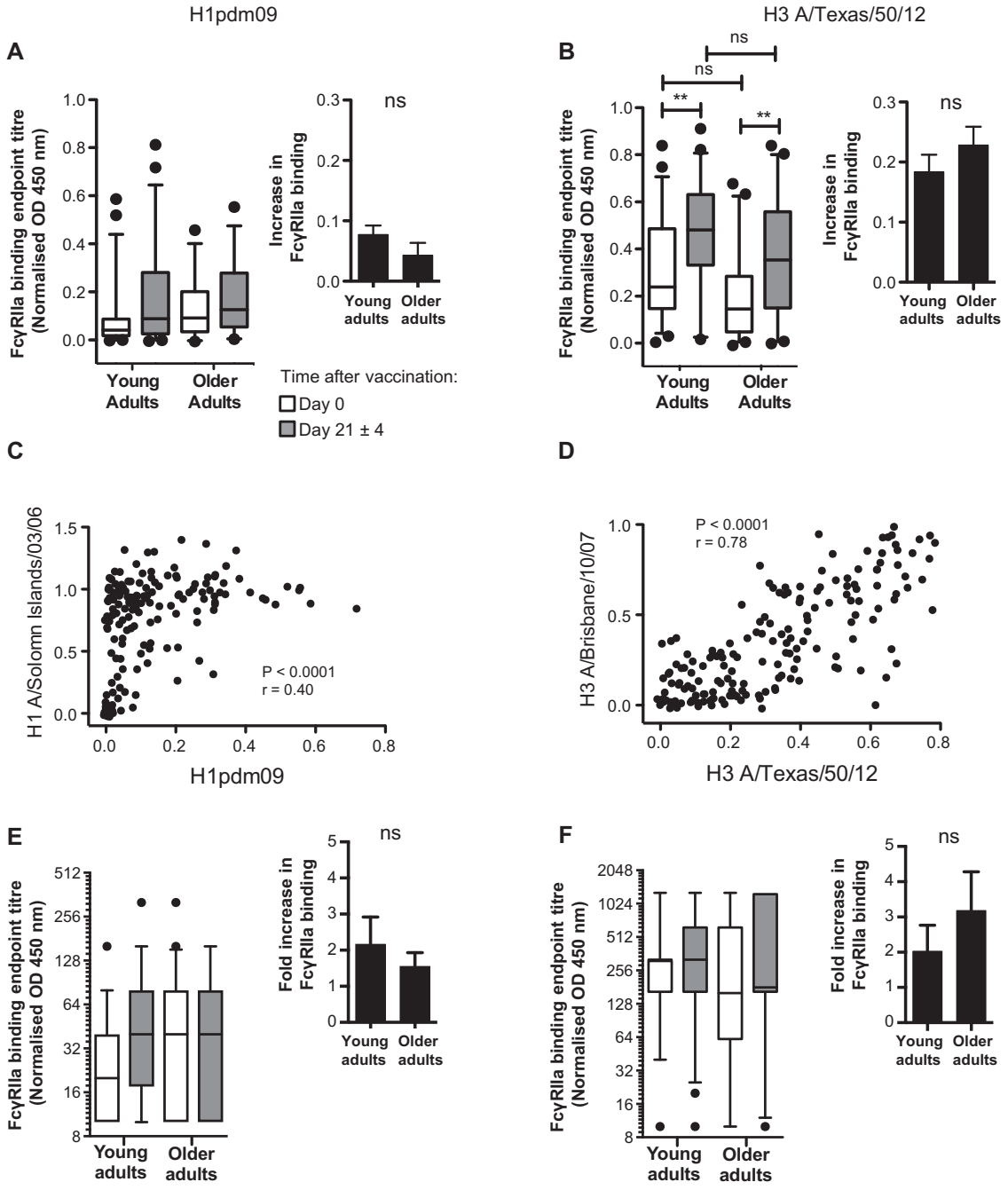

Fig. 5. Cross-reactivity of HA-specific Abs binding to FcγRIIa. The presence of cross-reactive Abs to HAs proteins not contained in the 2008-TIV – H1pdm09 (A) and H3 A/Texas/50/2012 (B) – and was assessed by the FcγRIIa dimer ELISA. Whiskers represent 5–95 percentile. Comparisons were performed with one-way ANOVA and Bonferroni's multiple comparisons test. Increase in FcγRIIa binding comparisons (right panels) were performed with unpaired *t*-test. ns = $P > 0.05$, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$, and **** = $P \leq 0.0001$. Data are representative results of two experiments. Correlation of the FcγRIIa binding titre to the homologous (C) H1 A/Solomon Islands/3/2006 and the heterologous H1pdm09 HAs, as well as (D) H3 A/Brisbane/10/2007 and H3 A/Texas/50/2012 HAs using Pearson's correlation coefficient. Endpoint FcR binding titres are shown to H1pdm09 (E) and H3 A/Texas/50/12 (F).

future studies to analyse Abs capable of binding a larger panel of FcRs. The monomeric nature of the HA proteins used in this work represents a limitation given the HA is largely presented as a trimer on the surface of the virion and infected cells. Antigenic sites of the HA trimer that are usually inaccessible to Abs could be exposed and detected by antibodies in plasma IgG by these assays when using monomeric HA. Future experiments studying native HA-specific Abs that bind to FcγRIIa and/or mediate ADP Abs could be addressed utilising viral vectors such as vaccinia virus expressing native HA timers.

More than 90% of deaths by seasonal influenza epidemics occur in the elderly, but interestingly the elderly were relatively protected from infection and subsequent disease during the 2009–10 H1N1pdm [41–45]. The elderly may possess long-lived memory B cells and plasmablasts that cross-react with HA from the H1N1pdm09 virus [20,46,47] from previous exposures to 1918-like H1N1 viruses [48] or other swine origin H1N1 viruses [49–50]. We found higher HAI responses to H1pdm09 in the older subjects in our cohort, although ADP responses to H1N1pdm09 were modest and similar across young and older individuals.

In conclusion, we found that younger and older adults mounted similar HA-specific Ab responses that bind FcγRIIa and mediate ADP ex vivo using THP-1 cells against HA contained in the 2008-TIV, despite older adults having diminished HAI responses. Further investigation into the importance of ADP-mediating Abs elicited by seasonal vaccination, as well as new vaccination strategies to induce higher levels of these Abs are warranted.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.vaccine.2017.09.062.

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