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Generation and Protective Ability of Influenza Virus–Specific Antibody-Dependent Cellular Cytotoxicity in Humans Elicited by Vaccination, Natural Infection, and Experimental Challenge

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Background. Nonneutralizing antibodies (Abs) involved in antibody-dependent cellular cytotoxicity (ADCC) may provide some protection from influenza virus infection. The ability of influenza vaccines to induce ADCC-mediating Abs (ADCC-Abs) in adults and children is unclear.

Methods. We quantified ADCC-Abs in serum samples from adults who received a dose of inactivated subunit vaccine (ISV) targeting monovalent 2009 pandemic influenza A(H1N1) virus or live-attenuated influenza vaccine (LAIV) or who had laboratory-confirmed influenza A(H1N1) virus infection. We also measured ADCC-Abs in children who either received a dose of trivalent seasonal ISV followed by trivalent seasonal LAIV or 2 doses of LAIV. Finally, we assessed the ability of low and high ADCC-Ab titers to protect adults from experimental challenge with influenza A/Wisconsin/67/131/2005(H3N2) virus.

Results. Adults and children who received a dose of ISV had a robust increase in ADCC-Ab titers to both recombinant hemagglutinin (rHA) protein and homologous virus–infected cells. There was no detectable increase in titers of ADCC-Abs to rHA or virus-infected cells in adults and children who received LAIV. Higher titers (≥320) of preexisting ADCC-Abs were associated with lower virus replication and a significant reduction in total symptom scores in experimentally infected adults.

Conclusions. ADCC-Ab titers increased following experimental influenza virus infection in adults and after ISV administration in both children and adults.

Keywords. ADCC; influenza; vaccine; immunity.

Influenza virus infection induces a robust antibody response. The neutralizing antibody (Ab) response is mediated toward the viral hemagglutinin (HA). The protective role of neutralizing antibodies in influenza is well established, and hemagglutination inhibition (HAI) titers of ≥40 are associated with protection from influenza [1]. Additionally, influenza virus infection induces binding antibodies to HA and other viral proteins, including neuraminidase (NA), nucleoprotein (NP), and matrix proteins (M1 and M2) [2–4]. Binding antibodies can mediate nonneutralizing effector functions that include Fc-dependent functions, such as Ab-dependent phagocytosis [5], Ab-dependent complement fixation [6], and Ab-dependent cellular cytotoxicity (ADCC) [7–9]. Influenza virus–specific ADCC-mediating Abs (ADCC-Abs) were detected in the absence of neutralizing activity among healthy subjects and experimentally infected nonhuman primates [10, 11]. Additionally, ADCC function is critical to the protection provided by broadly cross-reactive HA stem antibodies in mice [12–14]. The ability of influenza vaccines to elicit ADCC-Abs in humans and their role in protection is not well defined.

To understand the role of ADCC-Abs in protection from influenza and determine whether influenza vaccines elicit ADCC-Abs, we studied sera from 5 separate clinical cohorts: (1) adults vaccinated with monovalent H1N1pdm09 inactivated subunit vaccine (ISV); (2) adults vaccinated with monovalent H1N1pdm09 live attenuated vaccine (LAIV); (3) children vaccinated with seasonal ISV followed by seasonal LAIV, or with 2 doses of seasonal LAIV containing an influenza A H1N1pdm09 component; (4) adults with community-acquired A(H1N1)pdm09 infection; and (5) adults experimentally challenged with influenza A(H3N2) virus.

METHODS

Clinical Samples

Adult and pediatric serum samples were obtained from previously performed studies; all study subjects provided informed consent/assent [15, 16]. Sera from vaccinated adults were obtained

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from the University of Rochester Medical Center. In the A(H1N1) pdm09 ISV study (clinical trials registration NCT01023711), healthy adults were enrolled in one of 3 cohorts—ages 18–32 years (n = 20), 60–69 years (n = 20), and >70 years (n = 18)—and received a single dose of 15 μg of monovalent A(H1N1) pdm09 (A/California/07/2009) ISV (Novartis). In the A(H1N1) pdm09 LAIV study (clinical trials registration NCT01023776), adults aged 18–32 years (n = 16) received 2 doses of monovalent A(H1N1)pdm09 (A/California/07/2009) LAIV (MedImmune) 28 days apart. Only baseline and post–dose 1 (day 28) sera were included in this analysis. We also analyzed sera obtained from adults presenting to the outpatient clinic at the University of Rochester Medical Center with community-acquired A(H1N1)pdm09 infection. Serum HAI titers for these subjects are presented in Supplementary Table 1.

Sera from a pediatric vaccine study (clinical trials registration NCT01246999) were obtained from the Geisel School of Medicine at Dartmouth [15]. Children were enrolled between October 2010 and February 2012. Subjects received either 2 doses of seasonal LAIV, 2 doses of trivalent influenza vaccine (TIV), LAIV followed by TIV, or TIV followed by LAIV, 28 days apart. Both TIV and LAIV contained influenza A/California/07/09(H1N1), A/Perth/16/09(H3N2), and B/Brisbane/60/08 viruses. Serum HAI titers for these subjects are presented in Supplementary Table 2.

Samples from experimental influenza virus challenge infection were obtained from a clinical study (performed at hVIVO, London, England; clinical trials registration NCT01980966) conducted by Genentech. Healthy adults 18–45 years of age were deemed susceptible based on their HAI titers were inoculated intranasally with approximately 5.0–5.5 $\log_{10}$ median tissue culture infective doses ($TCID_{50}$) of influenza A/Wisconsin/67/2005 (H3N2) virus (Baxter; Orth an der Donau, Austria) and treated with placebo. Sera were collected immediately prior to infection and 29 days later. Nasopharyngeal swab specimens were collected every 8 hours for viral quantification by molecular or virologic assays (by quantitative reverse transcription–polymerase chain reaction [qRT-PCR] or $TCID_{50}$ methods, respectively; ViroClinics, Rotterdam, Netherlands), and the area under the curve (AUC) over 8 days was calculated. Self-recorded influenza-related symptoms included runny nose, stuffy nose, sneezing, sore throat, earache, malaise, cough, shortness of breath, headache, and muscle/joint ache/stiffness. Individual symptom severity was graded on a scale of 0–3 and combined into a total symptom score, and the AUC over 8 days was calculated. Serum HAI titers for these subjects are presented in Supplementary Table 3.

High-Throughput Natural Killer (NK) Cell Activation Assay

Sera from subjects were assessed for ADCC-Abs, using a modified flow-based assay as previously described [10, 17]. Briefly, 96-well enzyme-linked immunosorbent assay plates (Maxisorp, Nunc) were coated with 400 ng/well of recombinant protein (Sino Biological or BEI Resources) overnight at 4°C. Following washing, plates were incubated with dilutions of human sera (ranging from 1:20 to 1:81920) for 2 hours at 37°C. Following washing, plates were incubated with 100–500 000 of NK-92 cells stably expressing human CD16/GFP (176 V) (NK92-CD16/GFP, kindly provided by Campbell Fox Chase Cancer Center) for 5 hours at 37°C in 10% CO_2. After incubation, cells were stained with CD107a APC-Cy7 (clone H4A3; BD) and fixed with 10% paraformaldehyde. Cells were analyzed via flow cytometry and the end point titer of ADCC-Ab was defined as the highest dilution of Ab inducing CD107a expression from NK cells at a level that was at least twice the background level in antigen-negative wells.

When virus-infected target cells were used, A549 cells were infected with influenza virus at a multiplicity of infection of 10 in OptiMEM medium supplemented with TPCK Trypsin overnight at 37°C. Following incubation, cells were trypsinized, washed, and incubated with NK92-CD16 cells at an effector cell to target cell ratio of 1:3 (typically 100 000 NK92-CD16 cells to 300 000 infected A549 cells) and serum at serial dilutions (ranging from 1:20 to 10240) for 5 hours at 37°C in 10% CO_2. Processing and analysis were performed as described above. Background activation was set as the activation of NK92-CD16 cells within virus-infected cells but without sera.

Live-Cell Imaging

ADCC-Ab–mediated effector cell interactions were imaged in real time on a Leica SP5 inverted confocal microscope equipped with HyD detectors and a heated imaging chamber. Target cells (A549) were infected as described above and labeled with eFluor670 (ThermoFisher Scientific, Waltham, Massachusetts) according to the manufacturer's instructions. Twelve hours after infection, target cells and NK92-CD16/GFP effector cells were mixed at a 1:3 ratio in a Nunc Labtek chambered coverglass (ThermoFisher Scientific) at a total volume of 200 μL and imaged at 30-second intervals with a 63× objective. To image CD107a expression and exposure, 10 μL of Pacific Blue–conjugated anti-CD107a Ab was added prior to the addition of serum. After approximately 1 hour, 10 μL of influenza virus–positive serum was added, and image acquisition continued. Images were analyzed using Imaris 8.2 (Bitplane, Concord, Massachusetts).

Statistical Analysis

Statistical analyses used Prism GraphPad, version 6 (GraphPad Software, San Diego, California). Data were analyzed by a nonparametric paired test (the Wilcoxon matched pairs signed rank test) and an unpaired test (the Mann–Whitney *U* test). Linear correlation analysis was performed with $\log_2$-transformed HAI titers and fold-change in ADCC-Ab titers.

RESULTS

ADCC-Abs Measured by High-Throughput NK Cell Activation

We modified a previously established NK cell activation assay to measure ADCC-Abs in healthy adults [10, 18] by using an NK92 cell line transfected with the human 176 V form of the CD16 receptor coupled to GFP. ADCC-Abs against influenza virus–infected cells were evaluated as a measure of total virus-

specific ADCC-Abs and those against rHA were evaluated as a measure of HA-specific ADCC-Abs. To confirm that NK cell activation/degranulation was associated with killing of influenza virus–infected cells, we performed live-cell imaging of A/California/07/09 virus–infected cells mixed with cells expressing NK92-CD16/GFP$^+$ and influenza virus–positive serum. CD107a accumulation was not observed until influenza virus–positive serum was added to cells (Supplementary Video 1). Following addition of serum, several NK92-CD16/GFP$^+$ cells formed sustained contacts with virus-infected target cells and accumulated CD107a on the cell surface (Supplementary Video 2). Over time, the virus-infected target cells associated with CD107a$^+$ NK92-CD16/GFP$^+$ cells exhibited characteristics of apoptosis, including membrane blebbing (Supplementary Video 3). As previously described [10], NK cell activation was detected in the presence of rHA or influenza virus–infected cells and influenza virus–positive test sera but not pooled naive nonhuman primate serum (Supplementary Figure 1A). Sera from 2 representative human subjects, collected on days 0 and 29 following experimental infection with influenza A/Wisconsin/67/131/2005(H3N2) virus, were serially diluted, and end point titers were determined against both rHA and A/Wisconsin/67/131/2005(H3N2) virus–infected cells (Supplementary Figure 1B and 1C, respectively).

ADCC-Abs Are Elicited by ISV but Not LAIV

To determine whether ISV or LAIV could induce ADCC-Abs in healthy adults, we measured ADCC-Abs in specimens collected from 3 clinical cohorts in 2009 before the second wave of the 2009 pandemic: those vaccinated with a dose of A(H1N1)pdm09 ISV, those vaccinated with a dose of a dose of A(H1N1)pdm09 LAIV, or those who had PCR-confirmed A(H1N1)pdm09 infection. At day 0, all subjects had some preexisting cross-reactive ADCC-Abs toward A(H1N1)pdm09 (A/California/07/2009) virus–infected cells and rHA (Figure 1*A*–*C*) [18]. Following ISV, there was a significant increase in titers of ADCC-Abs toward both virus-infected cells and rHA, from a median titer of 80 to 320 in each case ($P < .0001$, by the Wilcoxon matched pairs signed rank test; Figure 1*A*). The fold-change in ADCC-Ab titers to A(H1N1)pdm09–infected cells from days 0 to 28 after ISV did not correlate with preexisting (day 0) HAI titers ($R^2 = 0.0018$; $P = .83$). The kinetics of the response to A(H1N1)pdm09 ISV was rapid: an increase in ADCC-Ab titers was detected within 3 days following vaccination (Supplementary Figure 2A), and ADCC-Abs cross-reacted with rHA of A/Anhui/01/05(H5N1) and A/Brisbane/59/07 (H1N1) viruses but not with rHA from influenza A/Brisbane/10/2007(H3N2) or B/Brisbane/60/2004 viruses (Supplementary Figure 2C). In contrast, H1N1pmd09 LAIV did not elicit a significant increase in ADCC-Ab titers toward A(H1N1)pdm09-infected cells (median titer, 320 on days 0 and 28 after LAIV receipt) or rHA (median titer, 80 on days 0 and 28; $P > .05$, by the Wilcoxon matched pairs signed rank test; Figure 1*B*). Outpatients with confirmed A(H1N1)pdm09 infection had high ADCC-Ab titers against rHA (median, 320) and virus-infected cells (median, 320) on the day of presentation (indicated as day 0, although this was typically 2–3 days after symptom onset) and 28 days later, suggesting that ADCC-Ab titers may have risen prior to the clinic visit ($P > .05$, by the Wilcoxon matched pairs signed rank test; Figure 1*C*). It is likely that ADCC-Abs were generated early (within 2–3 days) following influenza virus infection, and therefore increases in ADCC-Ab titers may be difficult to measure without preinfection serum samples in community-acquired influenza.

Rise in ADCC-Ab Titers to Surface and Internal Viral Components by Seasonal TIV but Not LAIV in Children

To further compare ISV and LAIV, we investigated ADCC-Abs in a previously described pediatric cohort [15]. Children received either a dose of seasonal TIV followed by a dose of seasonal LAIV or 2 doses of seasonal LAIV, 28 days apart. The proportion of subjects with undetectable ADCC-Abs to influenza A(H1N1), influenza A(H3N2), and influenza B viruses prior to vaccination was higher in children than adults prior to monovalent vaccine (7 of 25, 15 of 25, and 6 of 25, respectively; Figure 2*A*). TIV induced a low but significant increase in ADCC-Ab titers against A(H1N1)pdm09 and influenza B virus–infected cells ($P = .0195$ and .0117, respectively, by the Wilcoxon matched pairs signed rank test; Figure 2*A*). However, 1 or 2 doses of LAIV did not induce significant increase in titers of ADCC-Abs to cells infected with any of the 3 vaccine viruses ($P > .05$, by the Wilcoxon matched pairs signed rank test; Figure 2*A*). The ADCC-Ab titers were modest in both vaccine groups, with median titers following TIV ranging from 20 to 80. The fold-change in ADCC-Ab titers to the 3 vaccine viruses from days 0 to 28 after TIV did not correlate with preexisting (day 0) HAI titers ($P > .05$; Supplementary Table 2). Interestingly, an increase in ADCC-Ab titers following ISV was detected with rHA, as well as rNA and rNP (Figure 2*B*). These data support previous findings that small amounts of other viral proteins present in the inactivated influenza vaccine formulations could elicit antibodies [19, 20].

Increase in ADCC-Abs in Experimentally Infected Subjects Is Dependent on High Virus Replication and Symptom Scores

We failed to detect increases in ADCC-Ab titers in subjects presenting to outpatient clinics with confirmed influenza virus infection, possibly because ADCC-Ab titers increased in the days between the onset of infection and the clinic visit (Supplementary Figure 2A). To investigate this further, we obtained sera from 31 subjects who were experimentally infected with influenza A/Wisconsin/67/131/2005(H3N2) virus and treated with a placebo. The study subjects had been screened for undetectable or low HAI Ab titers against the challenge virus (Supplementary Table 3). There was a subtle but significant increase in ADCC-Ab titers for both A/Wisconsin/67/131/2005(H3N2) virus–infected cells and rHA ($P = .0002$, by the Wilcoxon matched pairs signed rank test; Figure 3*A*). Again, the fold-change in ADCC-Ab titers to influenza A(H3N2) virus–infected

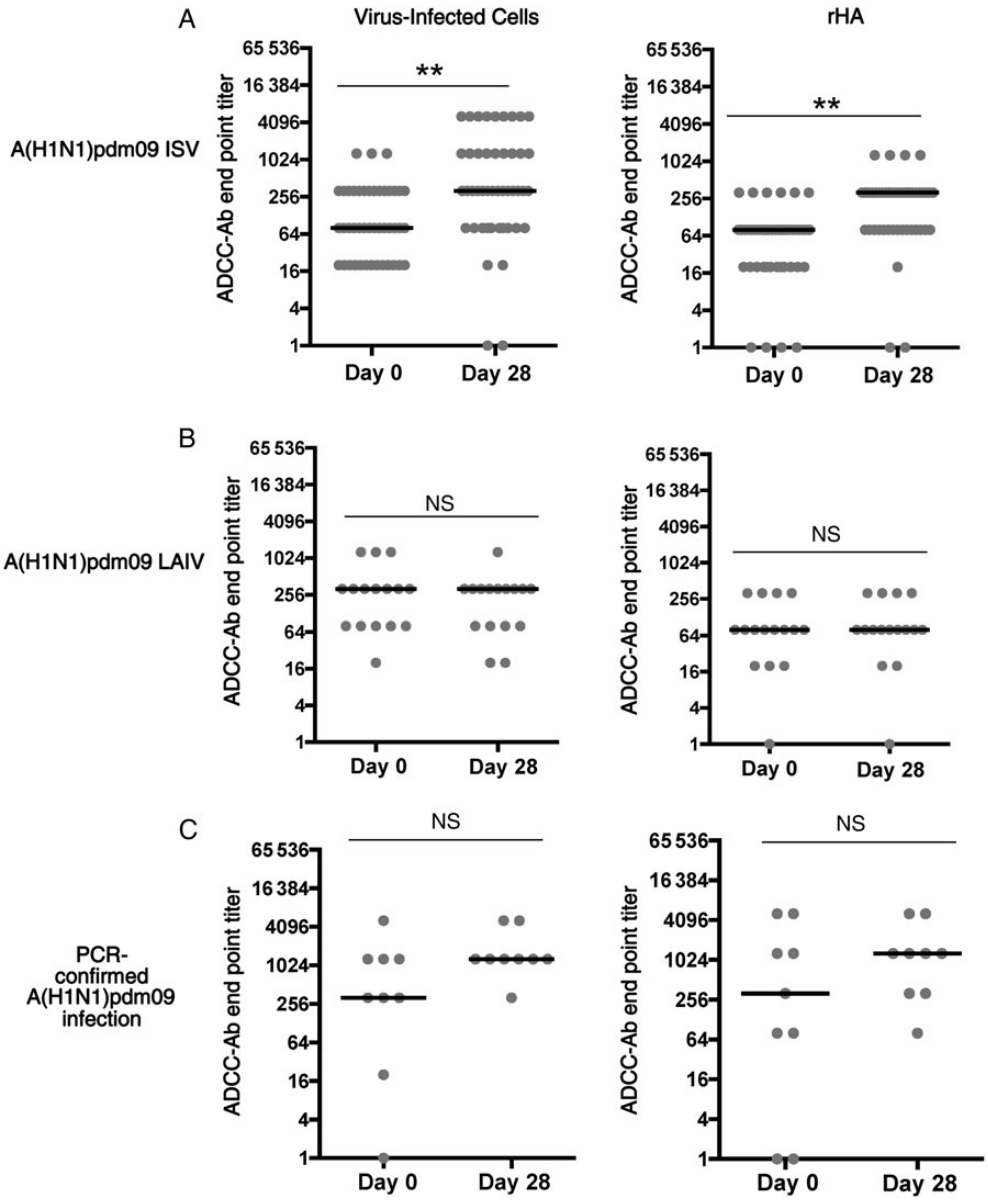

Figure 1. Antibody-dependent cellular cytotoxicity–mediating antibody (ADCC-Ab) titers following monovalent vaccination against or confirmed infection with 2009 pandemic influenza A(H1N1) virus (A[H1N1]pdm09). ADCC-Ab end point titers against A/California/07/2009 virus–infected A549 cells (virus-infected cells) or A/California/07/2009 recombinant hemagglutinin (rHA) were measured in sera obtained from subjects on days 0 and 28 after receipt of a single dose of either A(H1N1)pdm09 monovalent inactivated subunit vaccine (ISV; n = 48; *A*) or live-attenuated influenza vaccine (LAIV; n = 16; *B*) or from individuals who had polymerase chain reaction (PCR)–confirmed A(H1N1)pmd09 infection (n = 9; *C*). Bars indicate median titers. $^{**}P < .05$, by the Wilcoxon matched pairs signed rank test. Abbreviation: NS, not statistically significant.

cells did not correlate with preexisting HAI titers ($R^2 = 0.02$, $P < .45$; Supplementary Table 3). This contrasts with our findings in acute sera from patients with community-acquired influenza for whom we did not have a paired preinfection sample ($P = .019$, by Wilcoxon matched pairs signed rank test; Figure 3*A*, compared with Figure 1*C*).

Fifty-eight percent of subjects (18 of 31) who received an inoculum of influenza virus did not manifest an increase in ADCC-Ab titer to virus-infected cells; we hypothesized that this could be because the magnitude of virus replication and illness was insufficient to boost Ab titers. We stratified subjects into 2 groups on the basis of whether they had an increase in ADCC-Ab titer between days 0 and 29 and evaluated the magnitude of virus replication as measured by molecular (PCR; Figure 3*B*) and virologic ($TCID_{50}$; Figure 3*C*) methods and clinical illness measured by symptom score (Figure 3*D*). Subjects who showed an increase in ADCC-Ab titer showed a trend toward a higher viral load (as revealed by AUCs from PCR and $TCID_{50}$ analyses; $P = .081$ and $P = .056$, respectively, by the unpaired Mann–Whitney test; Figure 3*B* and 3*C*) and reported significantly greater clinical symptoms ($P = .0005$, by the

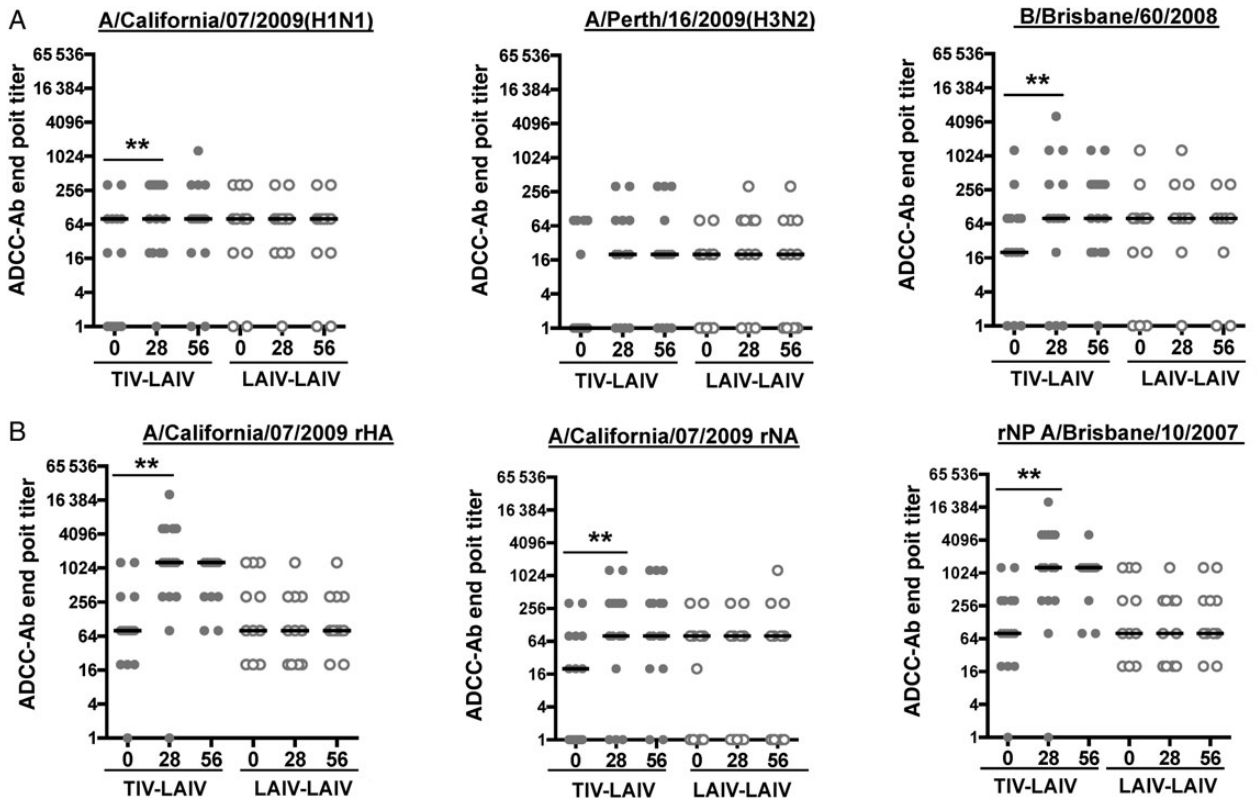

Figure 2. Antibody-dependent cellular cytotoxicity–mediating antibody (ADCC-Ab) titers following receipt of seasonal influenza vaccine in children. ADCC-Ab end point titers against A/California/07/2009(H1N1) virus–infected A549 cells (left; *A*), A/Perth/16/2009(H3N2) virus–infected A549 cells (middle; *A*), and B/Brisbane/60/2008 virus–infected A549 cells (right; *A*) and those against A/California/07/2009 recombinant hemagglutinin (rHA; left; *B*), A/California/07/2009 recombinant neuraminidase (rNA; middle; *B*), and A/Brisbane/10/2007 recombinant nucleoprotein (rNP; right; *B*) were measured in sera obtained from subjects on days 0, 28, and 56 after receipt of inactivated subunit vaccine (received on day 0) followed by live-attenuated influenza vaccine (LAIV) (received on day 28; trivalent influenza vaccine [TIV]-LAIV; n = 14; closed circles) or of LAIV (received on day 0) followed by LAIV (received on day 28; LAIV-LAIV; n = 11). Bars indicate median titers. $^{**}P < .05$, by the Wilcoxon matched pairs signed rank test.

unpaired Mann–Whitney test; Figure 3*D*). These data suggest that infections resulting in more symptoms generate larger increases in ADCC-Ab titers.

High Levels of Preexisting ADCC-Abs Provide Some Protection From Experimental Infection With Influenza Virus

The protective ability of ADCC-Abs has been assessed in mouse models of influenza but not in humans [12, 14]. In our small cohort of subjects who were experimentally infected with influenza A (H3N2) virus, we investigated whether preexisting ADCC-Abs to homologous virus–infected cells provided clinical or virologic protection or amelioration from influenza. A clear linear correlation between day 0 ADCC-Ab titers against virus-infected cells and clinical symptom score or AUCs from PCR or virus titration was not seen (data not shown). Because subjects had a median increase in homologous ADCC-Ab titers to 320 following monovalent ISV receipt (Figure 1*A*), we examined the titers in the experimentally infected study subjects with respect to this benchmark. Notably, 3 subjects with an ADCC-Ab titer of ≥320 to homologous influenza A(H3N2) virus–infected cells on day 0 had lower virus replication (as revealed by both PCR- and $TCID_{50}$-associated AUCs; Figure 4*A* and 4*B*) and a significantly lower clinical symptom score ($P < .021$, by the Mann–Whitney test; Figure 4*C*). The specificity of this finding was assessed by examining this relationship to a heterologous (A[H1N1]pdm09) virus; a similar trend was not observed between high (≥320) ADCC-Ab titers to the heterologous A(H1N1)pmd09–infected cells and influenza A (H3N2) virus load and symptom scores in the same subjects ($P > .05$, by the Mann–Whitney test; Figure 4*A*–*C*). Subjects with higher preinfection homologous influenza A(H3N2) virus ADCC-Ab titers (≥320; n = 3) were more likely to be protected from infection following experimental viral challenge, with 67% negative for virus by RT-PCR and $TCID_{50}$ methods in the 8 days following infection, compared with 34% of subjects (37) with titers <320. To extend this observation, we evaluated the relationship between preexisting ADCC-Ab titers and detectable virus within the first 24 hours following influenza challenge in an expanded cohort of 93 subjects at a time point prior to administration of a therapeutic anti–influenza virus monoclonal Ab or placebo. Subjects with higher preinfection homologous ADCC-

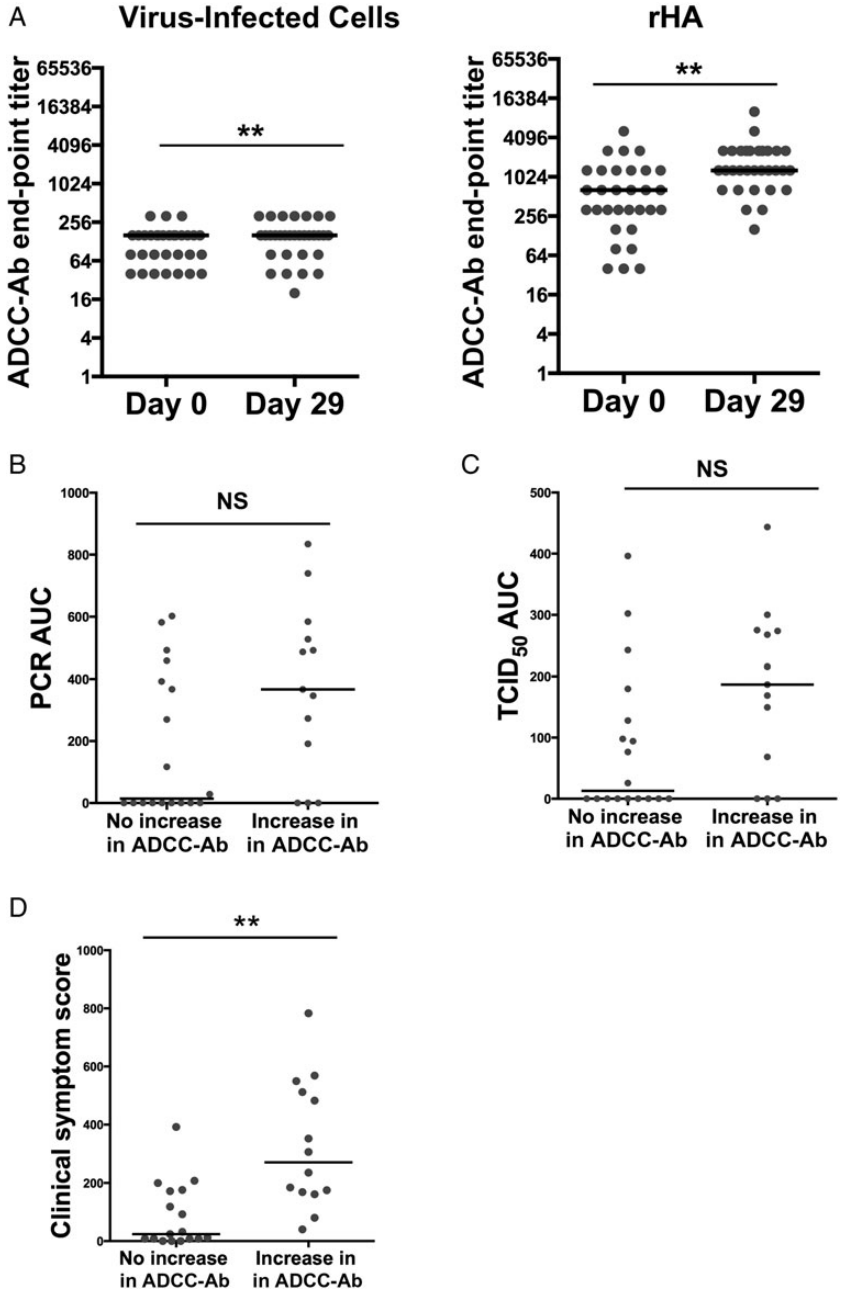

Figure 3. Increase in antibody-dependent cellular cytotoxicity–mediating antibody (ADCC-Ab) titer is associated with higher virus replication and clinical symptoms in subjects experimentally infected with influenza virus. ADCC-Ab end point titers against either A/Wisconsin/67/131/2005(H3N2) virus–infected A549 cells (left; *A*) or recombinant hemagglutinin (rHA; right; *A*) were measured in sera obtained from subjects on days 0 and 29 after experimental infection with A/Wisconsin/67/131/2005(H3N2) virus (n = 31). Subjects administered A/Wisconsin/67/131/2005(H3N2) virus were separated into 2 groups on the basis of a detectable increase in ADCC-Ab end point titer against A(H3N2)-infected A549 cells (17 had no increase and 14 had an increase) and compared for differences in polymerase chain reaction (PCR)–associated areas under the curve (AUCs; *B*), median tissue culture infective dose ($TCID_{50}$)–associated AUCs (*C*), and total clinical symptom scores (*D*). Bars indicate median titers. $^{**}P < .05$, by the Wilcoxon matched pairs signed rank test (*A* and *B*) or the unpaired Mann–Whitney test (*C, D*). Abbreviation: NS, not statistically significant.

Ab titers were less likely to have virus detected in nasopharyngeal swab specimens within the first 24 hours than subjects with lower ADCC-Ab titers (among 32 with ADCC-Ab titers of ≥320, 16% were positive for virus and 84% were negative; among 61 with ADCC-Ab titers of <320, 31% were positive for virus and 69% were negative). These data suggest that high preexisting titers of ADCC-Abs may reduce viral replication and the severity of symptoms.

DISCUSSION

We have investigated the generation of ADCC-Abs following influenza virus infection and vaccination. Our results show that

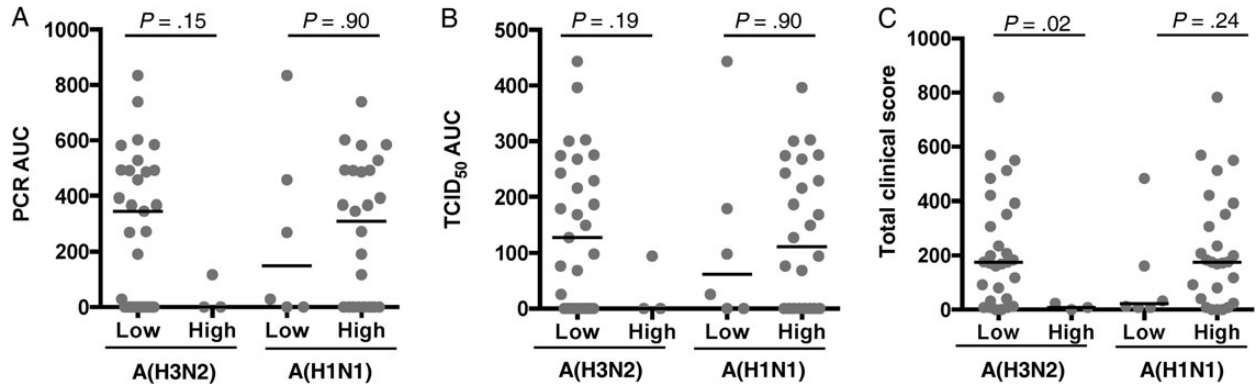

Figure 4. High levels of pre-existing antibody-dependent cellular cytotoxicity (ADCC) titers and association with disease burden in subjects experimentally infected with influenza virus. Subjects who were experimentally infected with A/Wisconsin/67/131/2005(H3N2) virus (n = 32) were separated into 2 groups: those with high ADCC (titers 320) or low ADCC (<320) against A/Wisconsin/67/131/2005(H3N2) or A/California/07/2009(H1N1) virus-infected A549 cells. The 2 groups were compared for PCR AUC (*A*), $TCID_{50}$ AUC (*B*) and total clinical symptom score (*C*). Bars indicate median titers for (*A, B, C*) H3N2 ADCC low n = 29, high n = 3, H1N1 ADCC low n = 6, high n = 26. *P* values indicated above each comparison and groups were compared by Mann-Whitney *U* test. Abbreviations: AUC, area under the curve; PCR, polymerase chain reaction; $TCID_{50}$, median tissue culture infective dose.

ISV rapidly induces ADCC-Abs against both homologous and heterologous influenza viruses in children and adults. In contrast, we observed no increase in ADCC-Abs following LAIV in either age group. The absence of an increase in Ab titers among adult recipients of LAIV are consistent with our findings that the A (H1N1)pmd09 LAIV was highly restricted in replication in adults and did not elicit an increase in neutralizing Ab titers (unpublished data). However, it is surprising that the seasonal LAIV did not induce an increase in ADCC-Ab titers in children, despite clear evidence of vaccine virus replication [15]. This may indicate that the antigen load was insufficient to generate class-switched antibodies that mediate ADCC. The importance of antigen load is supported by the data from the experimental challenge cohort, where subjects with higher virus titers were more likely to demonstrate an increase in ADCC-Ab titers. Further, the kinetics of ADCC-Ab generation following inactivated vaccine in adults is rapid (occurring within 3 days), suggesting that this response is derived from preexisting memory cross-reactive memory B cells recalled following inactivated influenza vaccine receipt. There was no correlation between preexisting HAI titers and the ability to generate ADCC-Abs.

It is likely that ADCC-Abs are more broadly cross-reactive than neutralizing Abs. It is still unclear whether ADCC-Abs mediate their function through binding to specific regions of influenza virus HA. Recent data suggest that cross-reactive HA antibodies mediate their function through Fc-dependent mechanisms [14]. We have shown that some ADCC-Abs can be HA subtype–specific, likely binding to the globular head, while others are more broadly cross-reactive, suggesting that they target the conserved stem of the HA. Indeed, our data show that monovalent inactivated influenza A(H1N1) virus vaccine elicits ADCC activity against heterosubtypic influenza A viruses, although their capacity to confer cross-protection is unclear. Our recent data suggest that cross-reactive ADCC-Abs to influenza A(H7N9) virus HA are less prevalent in the population than those to influenza A(H5N1) HA [17]. However, data from others have shown high levels of cross-reactivity with both influenza A(H5N1) and A(H7N9) viruses, suggesting that ADCC-Ab to internal viral proteins, as well as to NA, may be involved [7]. Our observation that vaccination of children with ISV can induce ADCC-Ab to both NA and NP is likely due to small amounts of NP and NA present in ISV preparations [19, 20]. We speculate that these antibodies are generated early in life and are expanded by infection and vaccination. We have not considered ADCC-Abs to other viral components, including M proteins, although antibodies to M2 protein have been shown to mediate their activity through ADCC [21, 22]. However, the contribution of such cross-reactive ADCC-Abs to protection in humans is still unclear and requires further investigation.

Studies in mice and nonhuman primates suggest that ADCC is critical to the protective efficacy of HA stem–binding antibodies [12–14]. The limited availability of clinical samples from subjects prior to influenza virus infection makes it difficult to assess the protective efficacy of ADCC-Abs. Access to sera from an influenza challenge study provided a unique opportunity to quantify ADCC-Abs prior to infection. The data suggest that high levels of homologous ADCC-Abs provide some reduction in viral replication and clinical symptoms. The ability of ADCC-Abs to protect is likely influenced by several factors, including concentration of immunoglobulin G in the upper respiratory tract [23], the availability of cells with CD16 receptor [24, 25], and licensing of NK cells [26, 27]. Further, ADCC-Abs may play a more significant role in lower respiratory tract infection, as seen in severe clinical disease and mouse models, rather than in the upper respiratory tract infection that occurs in influenza experimental challenge studies. Further insights will require studies that are specifically designed to address the role of ADCC-mediated protection in humans.

Although we have characterized ADCC-Abs following influenza vaccination and infection from several cohorts, there are some

limitations to our approach. First, we measured ADCC-Abs by using a CD16-transfected NK cell line. The use of the CD16-GFP NK cell line may not reflect responses from primary donor NK cells and was performed in this fashion for 2 reasons: to reduce the donor-to-donor variability of primary NK cells and to circumvent the need for a large volume of donor blood. Second, the limited volume of sera forced us to focus assessment of ADCC-Abs on homologous virus–infected cells and rHA, rather than on a broader range of HA proteins [10]. We observed ADCC-Ab responses to type B influenza virus–infected cells in children but not type B rHA–specific ADCC-Ab responses in adults. This discrepancy may be because the target of ADCC-Abs is a protein other than HA or because the quality of recombinant type B HA was not optimal. Third, the A(H1N1)pdm09 adult vaccine studies and pediatric seasonal influenza vaccine studies were performed during either the second wave of the influenza pandemic or over 2 influenza seasons (2010–2012), respectively. Subjects were not screened for influenza virus infection during this time, and therefore we cannot exclude the possibility that subjects were naturally infected with influenza viruses during this time. Fourth, we were only able to show a trend in ADCC-Abs correlating with reduced symptoms and viral load in a small cohort. This must be validated in a larger cohort with a range of ADCC-Ab titers. Future studies in larger cohorts that are specifically designed to assess ADCC-Abs could rectify the shortcomings of our study.

In summary, we have shown that high levels of preexisting ADCC-Abs provide some protection from influenza. Additionally, we have shown that ISV can induce ADCC-Abs but LAIV does not. These results extend our understanding of immunity to influenza viruses.

Supplementary Data

Supplementary materials are available at http://jid.oxfordjournals.org. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

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