Neuraminidase-Inhibiting Antibody Response to H5N1 Virus Vaccination in Chronically Ill and Immunocompromised Patients

Richard Fritz,[1] Nicole Hetzelt,[1] Reinhard Ilk,[2] Christine Hohenadl,[1] Maikel V. W. van der Velden,[3] Gerald Aichinger,[3] Daniel Portsmouth,[1] Otfried Kistner,[1] M. Keith Howard,[1] P. Noel Barrett,[1] and Thomas R. Kreil[1]

[1]Vaccine R&D, Orth/Donau, [2]Global Quality and [3]Vaccine R&D, Baxter BioScience, Vienna, Austria

Neuraminidase-inhibiting (NAi) antibodies have been reported to be an independent correlate of protection from influenza disease, but the NAi antibody response to influenza vaccination has never been assessed in chronically ill or immunocompromised participants. Using an enzyme-linked lectin assay, we demonstrated that 2 immunizations with a Vero cell culture-derived, whole-virus H5N1 A/Vietnam vaccine induces NAi antibodies in 94.3% of chronically ill and 83.8% of immunocompromised participants. A booster with a heterologous A/Indonesia H5N1 vaccine induced comparable NAi antibody titers in both groups and resulted in 100% seropositivity. These data support prepandemic H5N1 vaccination strategies for these highly vulnerable risk groups.

Keywords. avian influenza; vero cell culture; influenza vaccine; neuraminidase; risk populations.

Highly pathogenic avian A/H5N1 influenza viruses are enzootic in Asia and the Middle East and cause sporadic human cases with a high fatality rate [1]. Although human infections typically originate from contact with poultry, there is concern that pandemic outbreaks with interhuman virus transmission could occur. This finding is substantiated by reports describing mammalian-transmissible H5N1 influenza viruses [2]. As a cornerstone of pandemic preparedness, safety and immunogenicity of vaccines against H5N1 influenza viruses have been demonstrated in healthy pediatric and adult study populations. However, in the event of a pandemic, the highest rates of severe disease complications can be expected in individuals with immunodeficiencies or chronic illness, who qualify for prioritized vaccination. The effectiveness of a cell culture-derived whole-virus H5N1 vaccine to induce hemagglutinin (HA)-specific antibodies, which provide protective immunity by preventing virus attachment to the host cell or endosomal membrane fusion, has recently been demonstrated in these special populations [3]. Because immune dysfunction might compromise vaccine responses, it is especially important to investigate the full breadth of antibodies induced by a pandemic influenza vaccine in these risk groups. Immunity to the second most abundant influenza surface protein, the neuraminidase (NA), constitutes an additional line of defense: NA-inhibiting (NAi) antibodies have been described as an independent correlate of protection in humans [4] and animal models [5], and they contribute to the amelioration of influenza disease by limiting viral spread in the host [6,7]. This function has been linked to the inhibition of HA-receptor cleavage on the host cell surface by NA, a crucial step in the influenza life cycle allowing progeny viruses to exit the infected cell [8]. Neuraminidase-inhibiting antibodies further affect viral fitness by interfering with NA-mediated viral disaggregation [9] and facilitating access to the respiratory epithelium [10].

Priming to H5 HA by exposure to H5N1 viruses is a rare event, and only low levels of cross-reactive antibodies to H5 HA are induced by seasonal influenza viruses [11]. In contrast, antibodies to N1 NA—derived from contact with H1N1 viruses—are frequently found in healthy children and adults and have been shown to be efficiently increased after H5N1 vaccination [12, 13] and to cross-protect against H5N1 challenge in mice [14] and ferrets [5]. However, the extent of immunological priming to the NA antigen has never been evaluated in persons with underlying medical conditions or immunosuppression who may not only lack antibodies towards HA—as would the general population in a pandemic situation—but also NA. In the current study, we investigated the NAi antibody response to vaccination with a nonadjuvanted cell culture-derived whole-virus H5N1 vaccine in chronically ill and immunocompromised study participants using an enzyme-linked lectin assay (ELLA).

MATERIALS AND METHODS

Clinical Study and Vaccines

Sera were derived from a phase III clinical trial assessing the safety and immunogenicity of Vero cell-derived whole-virus H5N1 vaccines, containing 7.5-µg HA doses of either clade 1 A/Vietnam/1203/2004 or clade 2.1 A/Indonesia/05/2005 strains, in chronically ill and immunocompromised individuals in Austria and Germany between August 2008 and October 2010 (EU-DRACT 2008-000558-11, ClinicalTrials.gov NCT00711295). Study participants with chronic cardiovascular, respiratory, renal or metabolic illness were included in the chronically ill study population, and participants with human immunodeficiency virus infection and CD4+ cell count $\geq 200 \times 10^6$/L or patients who were at least 6 months after solid organ or peripheral blood stem cell transplantation were included in the immunocompromised study group. All participants were immunized twice, 3 weeks apart, with the A/Vietnam vaccine, and a subset received a booster vaccination with the A/Indonesia vaccine 12–24 months after the first immunization. Blood was drawn immediately before and 21 days after each vaccination. Neuraminidase-inhibiting antibody titers were determined for all serum samples that were available after the original immunogenicity evaluation [3] from participants with at least 1 pre- and postvaccination study site visit. Written informed consent was obtained for all participants before enrollment.

Determination of Neuraminidase-Inhibiting Antibody Titers

Neuraminidase-inhibiting antibodies were measured using an ELLA essentially as described previously [12]. Full-length A/Vietnam/1203/2004 NA (GenBank accession number EF541467.1) from Protein Sciences Corp. (Lot 1099-098) was serially diluted and incubated at 37°C for 16–18 h on fetuin-coated (Sigma) 96-well plates (Nunc). After washing the plates, peroxidase-labeled peanut agglutinin (Sigma) was added for a 2-hour incubation period at room temperature in the dark. Plates were washed and o-phenylenediamine dihydrochloride (Sigma) was added as a substrate. After 10-minute incubation in the dark, 0.5 M H_2SO_4 was added and the optical density (OD) was measured at 550 nm. The activity measured at OD 1.0 was set as the NA standard dose. Serum samples were serially diluted, and 50 µL of each dilution were applied to fetuin-coated 96-well plates (Sigma) followed by addition of the NA standard dose to each well. After incubation for 16–18 hours at 37°C, the remaining steps were performed as described above. The NAi titer of a sample was defined as 50% inhibiting titer as calculated by nonlinear regression analysis. Control sera from rabbits, either influenza naive or vaccinated with the A/Vietnam vaccine, were included on every plate with the expected results being a critical parameter for assay validity. The limit of detection (LOD) was a titer of 10, and samples below the LOD were assigned a titer of 5. Samples having a titer ≥ 10 were considered to be seropositive.
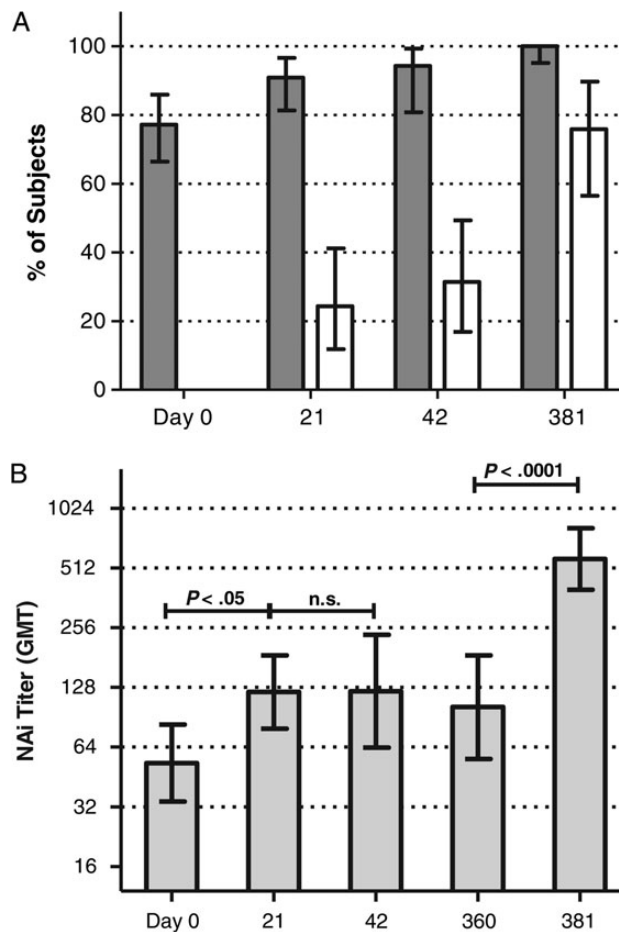

Figure 1. Neuraminidase-inhibiting antibody responses in chronically ill participants at baseline (Day 0), after the first (Day 21) and second (Day 42) primary immunizations with a whole-virus A/Vietnam vaccine, and before (Day 360) and after (day 381) a boosting immunization with a heterologous A/Indonesia vaccine. (A) Percentages of participants seropositive for N1 NA-specific NAi antibodies (grey bars), and percentages of participants with titer increases by a factor of 4 or more with respect to baseline (white bars). (B) Geometric mean NAi antibody titer (GMT). Error bars represent 95% confidence intervals.

Statistical analysis

Statistical analyses were done on log-transformed data using Minitab software. Differences between vaccination-induced titers were determined by one-way analysis of variance and Tukey test. The confidence intervals (CIs) of the seropositivity and seroconversion rates were calculated according to Clopper and Pearson, based on F distributions.

RESULTS

Induction of Neuraminidase-Inhibiting Antibodies in Chronically Ill Individuals

Neuraminidase-inhibiting antibody responses in chronically ill participants ($n = 79$, mean age 47.3 years, range 19–80 years)

after 1 and 2 immunizations with the whole-virus H5N1 A/Vietnam vaccine are shown in Figure 1. At baseline, 77.2% of the participants were seropositive for N1 NA-specific NAi antibodies (Figure 1A), and the geometric mean titer (GMT) was 53.2 (95% CI, 33.9–83.2) (Figure 1B). Three weeks after the first immunization, the GMT increased significantly ($P < .05$) to 121.3 (95% CI, 79.3–185.5), corresponding to a 2.3-fold increase in GMT compared with baseline; however, no further NAi antibody titer increase was observed after the second immunization (GMT 122.5 [95% CI, 63.5–236.1]) (Figure 1B). After 1 and 2 immunizations, 90.9% and 94.3% of participants, respectively, achieved seropositivity for N1 NA-specific NAi antibodies (Figure 1A). Seroconversion rates, defined as ≥4-fold increases in NAi titers compared with baseline, were calculated as 24.3% and 31.4% after the first and second immunization, respectively (Figure 1A).

In sera from 60 chronically ill participants who received a booster immunization with the A/Indonesia H5N1 vaccine 12–24 months after the first immunization, the prebooster NAi GMT was 101.8 (95% CI, 55.8–185.8), ie, slightly decreased compared with 3 weeks after the second immunization (Figure 1B). Note, however, that 17.2% of the individuals tested showed increased prebooster NAi titers compared with that after the second vaccination. The booster immunization effectively increased the GMT to 568.3 (95% CI, 397.4–812.7) (Figure 1B), representing a 10.7-fold increase in GMT compared with baseline. All vaccinees who received the booster immunization achieved seropositivity for NAi antibodies, resulting in 75.9% seroconversion compared with baseline (Figure 1A).

Induction of Neuraminidase-Inhibiting Antibodies in Immunocompromised Patients

In the immunocompromised study population ($n = 97$, mean age 47.7 years, range 23–76 years), prevaccination NAi antibodies were detected in 63.9% of participants (Figure 2A), and the GMT at baseline was 41.4 (95% CI, 27.0–63.3) (Figure 2B). The first immunization with the whole-virus H5N1 A/Vietnam vaccine resulted in a statistically nonsignificant GMT increase to 58.6 (95% CI, 36.2–94.8), followed by a further statistically nonsignificant increase to 73.5 (95% CI, 45.5–118.6) after the second immunization (Figure 2B), representing a 1.8-fold increase in GMT compared with baseline. After the first and second immunizations, 72.9% and 83.8% of participants, respectively, achieved seropositivity for N1 NA-specific NAi antibodies, corresponding to seroconversion rates of 9.1% and 13.6%, respectively (Figure 2A).

In sera from 56 immunocompromised participants who received a heterologous booster immunization with the A/Indonesia H5N1 vaccine 12–24 months after the first immunization, the prebooster GMT was 71.4 (95% CI, 39.9–128) (Figure 2B), ie, almost unchanged from the GMT 3 weeks after the second immunization. However, 14.3% of participants had higher NAi titers before the booster than 3 weeks after the second vac-
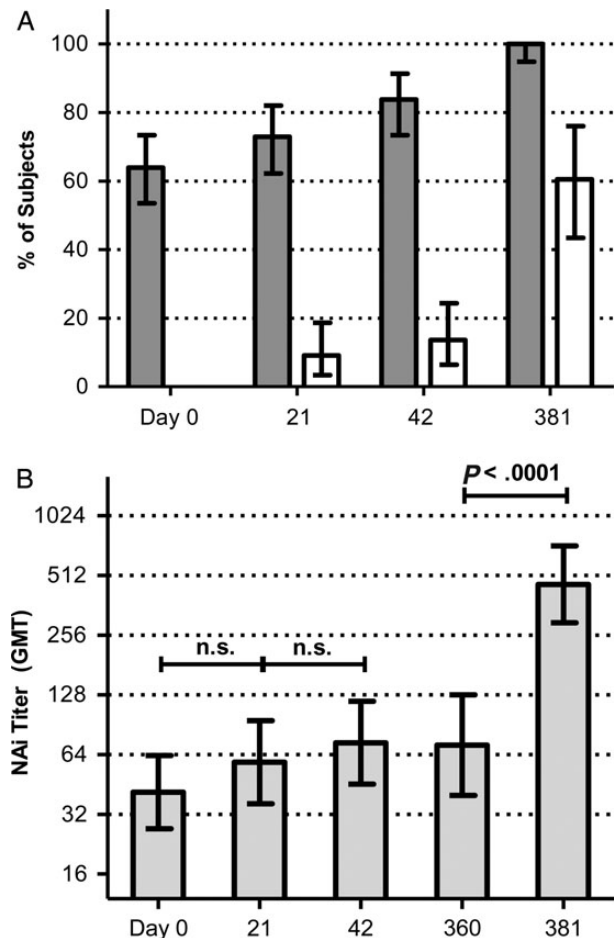

Figure 2. Neuraminidase-inhibiting antibody responses in immunocompromised participants at baseline (Day 0), after the first (Day 21) and second (Day 42) primary immunizations with a whole-virus A/Vietnam vaccine, and before (Day 360) and after (day 381) a boosting immunization with a heterologous A/Indonesia vaccine. (A) Percentages of participants seropositive for N1 NA-specific NAi antibodies (grey bars), and percentages of participants with titer increases by a factor of 4 or more with respect to baseline (white bars). (B) Geometric mean NAi antibody titer (GMT). Error bars represent 95% confidence intervals.

cination. The booster vaccination resulted in a highly significant ($P < .001$) GMT increase to 461.7 (95% CI, 295.4–721.8) at 3 weeks postbooster (Figure 2B), with an 11.6-fold increase in GMT compared with baseline. All immunocompromised participants achieved seropositivity for N1 NA-specific NAi antibodies after the booster vaccination, with a corresponding seroconversion rate compared with baseline of 60.5% (Figure 2A).

DISCUSSION

Hundreds of millions of individuals, globally, are currently living with chronic diseases (such as cardiovascular, respiratory, renal, or metabolic disorders) or with congenital or acquired immune dysfunctions, and they must be considered when

planning for future influenza pandemics, where vaccines may be in short supply and prioritization will be necessary.

The Vero cell-derived, whole-virus H5N1 vaccine investigated here has previously been shown to be well tolerated and immunogenic in chronically ill and immunosuppressed patients [3]. The vaccine was similarly immunogenic in chronically ill patients as previously reported in healthy individuals [15], but it was lower in immunocompromised patients. In the present study, we report that the whole-virus H5N1 vaccine also induces substantial N1 NA-specific NAi antibodies in these risk populations. Similarly to a previous investigation in healthy individuals [12], the first vaccination with an A/Vietnam H5N1 vaccine significantly increased NAi antibody titers in the chronically ill by boosting preexisting immunity, and the second vaccination resulted in no further titer increases (Figure 1). In contrast, in the immunocompromised participants, 2 immunizations with the A/Vietnam H5N1 vaccine induced only insignificant NAi antibody titer increases, and, as expected, the titers achieved were lower compared with those induced in the chronically ill population (Figure 2). In both groups, before the booster immunization, the NAi antibody titers remained stable or decreased only slightly compared with 3 weeks after the second immunization, and were even increased in a substantial proportion of individuals from both populations. Because all participants who showed increased NAi antibody titers before the booster immunization compared with after the second immunization had their prebooster study site visit between July and September 2010, when H1N1pdm09 viruses were widely circulating, a possible explanation for the stability or increase in NAi antibody titer is that many subjects may have experienced an H1N1pdm09 influenza infection, which induced cross-reactive anti-N1 NAi antibodies. More importantly, the booster immunization resulted in NAi antibody titers that were comparable between the chronically ill and immunocompromised study groups, and at 3 weeks postbooster there were no nonresponders with respect to the induction of NAi antibodies, irrespective of the underlying medical condition. This finding makes the booster a critical part of the vaccination schedule and suggests that prepandemic vaccination would be the best strategy to achieve maximum contribution to protection by NAi antibodies in chronically ill and immunosuppressed populations. In this context, immunizations against seasonal influenza strains might also contribute to mitigation of pandemics by inducing cross-reactive NA-specific antibodies. Moreover, with the increasingly well recognized contributions of NAi antibodies to immune responses after influenza vaccination, determination of the NA content of vaccines, as well as their stability, seems to be an important aspect for future studies.

Taken together, the current data demonstrating the ability of the whole-virus H5N1 vaccine to induce NAi antibodies in chronically ill and immunocompromised patients, in addition to previous data showing that HA-specific antibodies are effectively induced in these populations, suggest that whole-virus H5N1 vaccines would be suitable for prepandemic priming as well as pandemic vaccination strategies in these highly vulnerable risk groups.

Acknowledgments

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