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DNA priming and influenza vaccine immunogenicity: two phase 1 open label randomised clinical trials

Julie E Ledgerwood, Chih-Jen Wei*, Zonghui Hu, Ingelise J Gordon, Mary E Enama, Cynthia S Hendel, Patrick M McTamney, Melissa B Pearce, Hadi M Yassine, Jeffrey C Boyington, Robert Bailer, Terrence M Tumpey, Richard A Koup, John R Mascola, Gary J Nabel, Barney S Graham, and the VRC 306 Study Team*

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*These authors contributed equally to this work

Vaccine Research Center (J E Ledgerwood DO, C-J Wei PhD, I J Gordon RN, M E Enama PA-C, C S Hendel CRNP, P M McTamney PhD, H M Yassine PhD, J C Boyington PhD, R Bailer PhD, R A Koup MD, J R Mascola MD, G J Nabel MD, B S Graham MD), **and Biostatistics Research Branch, Division of Clinical Research** (Z Hu PhD), **National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA; and Influenza Division, National Center for Immunization and Respiratory Diseases, US Centers for Disease Control and Prevention, Atlanta, GA, USA** (M B Pearce PhD, T M Tumpey PhD)

Correspondence to: Dr Julie E Ledgerwood, Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA **ledgerwood@mail.nih.gov**

Summary

Background Because the general population is largely naive to H5N1 influenza, antibodies generated to H5 allow analysis of novel influenza vaccines independent of background immunity from previous infection. We assessed the safety and immunogenicity of DNA encoding H5 as a priming vaccine to improve antibody responses to inactivated influenza vaccination.

Methods In VRC 306 and VRC 310, two sequentially enrolled phase 1, open-label, randomised clinical trials, healthy adults (age 18–60 years) were randomly assigned to receive intramuscular H5 DNA (4 mg) at day 0 or twice, at day 0 and week 4, followed by H5N1 monovalent inactivated vaccine (MIV; 90 μg) at 4 or 24 weeks, and compared with a two-dose regimen of H5N1 MIV with either a 4 or 24 week interval. Antibody responses were assessed by haemagglutination inhibition (HAI), ELISA, neutralisation (ID_{80}), and immunoassays for stem-directed antibodies. T cell responses were assessed by intracellular cytokine staining. After enrolment, investigators and individuals were not masked to group assignment. VRC 306 and VRC 310 are registered with ClinicalTrials.gov, numbers NCT00776711 and NCT01086657, respectively.

Findings In VRC 306, 60 individuals were randomly assigned to the four groups (15 in each) and 59 received the vaccinations. In VRC 310, of the 21 individuals enrolled, 20 received the vaccinations (nine received a two-dose regimen of H5N1 MIV and 11 received H5 DNA at day 0 followed by H5N1 MIV at week 24). H5 DNA priming was safe and enhanced H5-specific antibody titres following an H5N1 MIV boost, especially when the interval between DNA prime and MIV boost was extended to 24 weeks. In the two studies, DNA priming with a 24-week MIV boost interval induced protective HAI titres in 21 (81%) of 26 of individuals, with an increase in geometric mean titre (GMT) of more than four times that of individuals given the MIV-MIV regimen at 4 or 24 weeks (GMT 103–206 *vs* GMT 27–33). Additionally, neutralising antibodies directed to the conserved stem region of H5 were induced by this prime-boost regimen in several individuals. No vaccine-related serious adverse events were recorded.

Interpretation DNA priming 24 weeks in advance of influenza vaccine boosting increased the magnitude of protective antibody responses (HAI) and in some cases induced haemagglutinin-stem-specific neutralising antibodies. A DNA-MIV vaccine regimen could enhance the efficacy of H5 or other influenza vaccines and shows that anti-stem antibodies can be elicited by vaccination in man.

Funding National Institutes of Health.

Introduction

The worldwide burden of influenza remains substantial and an estimated 250 000–500 000 people die of influenza every year.[1] The substantial public health effect of influenza infections is compounded by the potential for pandemics caused by emerging virus strains for which no immunity exists in the population. Such episodes occurred in 1918, when the influenza A subtype H1N1 was first identified in human beings, causing the Spanish flu pandemic, with mortality estimated at 40 million deaths or more worldwide[2] and again in 2009, when a related H1N1 virus caused a pandemic derived by a triple reassortment of genes from swine, avian, and human influenza viruses.[3–5] This type of adaptation of animal influenza viruses to man represents a global threat to public health.[6] Another example of zoonotic spread includes the highly pathogenic avian influenza A H5N1 viruses, which cause morbidity and mortality in bird populations and have caused sporadic human disease. WHO, as of June 16, 2011, has reported 561 confirmed human H5N1 cases and 328 deaths.[7] In addition to representing a highly pathogenic strain, the absence of background H5N1 immunity in the general population makes the H5 antigen ideal for the assessment of novel influenza vaccine approaches.

Protection against influenza is mainly antibody-mediated, and responses to influenza vaccines are typically measured by haemagglutination inhibition assays (HAI). These antibodies are directed against well defined antigenic sites in the globular head region of influenza haemagglutinin, and are largely strain-specific.[8,9] HAI titres of 1:40 or more are typically associated with at least a 50% reduction in the risk of infection with strain specific influenza viruses in human beings.[10] By contrast, neutralising antibody assays detect functional antibodies

with the capacity to inhibit viral entry into cells. In the case of influenza, neutralising antibodies can be specific for the head of haemagglutinin or directed against a conserved region in the stem of haemagglutinin and are able to neutralise multiple subtypes of influenza. Specifically, a localised region of the haemagglutinin stem has been identified as an important antigenic site capable of inducing broadly neutralising antibodies.[11–13] This region is highly conserved among group 1 influenza type A viruses (H1, H2, H5, H6, H8, H9, H11, H12, H13, and H16).[12] Although neutralising stem-specific haemagglutinin antibodies have been identified in man,[11,12,14,15] such stem antibody responses have not been elicited by vaccination. A vaccine regimen that could induce antibodies against a conserved antigenic target would represent an important step toward universal influenza vaccine development.

Gene-based vaccinations have been shown to induce cross-neutralising antibodies directed against the conserved region of the haemagglutinin stem and are protective against infection from multiple strains of influenza in animals.[16] Here, we have assessed a similar vaccination regimen in man and investigated its ability to elicit HAI and neutralising antibody responses, including those directed to the highly conserved stem region. Because of the background response due to previous infection with H1 and H3 viruses, we addressed this question with DNA vaccine encoding H5 as a priming vaccine (prime) followed by an H5N1 monovalent inactivated vaccine (MIV; boost) in phase 1 safety and immunogenicity studies. We tested the ability of this prime-boost regimen to induce improved antibody responses against autologous virus, heterologous virus, and to a conserved haemagglutinin stem epitope.

Methods

Study design and participants

VRC 306 and VRC 310 were single-site, phase 1, open label, randomised clinical trials done at the National Institutes of Health (NIH) Clinical Center by the National Institute of Allergy and Infectious Diseases (NIAID) Vaccine Research Center, NIH, Bethesda, MD, USA.

VRC 306 was initiated in November, 2008, to assess the potential of DNA to prime for an MIV boost. The study fully enrolled in November, 2009, and preliminary data indicated a need to enrol two more groups to confirm the findings. VRC 310 was initiated in March, 2010, and included two groups designed to validate the findings in VRC 306. VRC 306 and VRC 310 were both designed to examine the safety, tolerability, and immunogenicity of an investigational influenza DNA vaccine encoding H5 (H5 DNA) boosted with MIV subvirion H5N1 vaccine, in healthy adults aged 18–60 years with no history of H5 influenza vaccination, a body-mass index lower than 40, normal baseline blood counts, and normal liver and renal function laboratory measurements. The studies were reviewed and approved by the NIAID Institutional Review Board. Individuals provided written informed consent and completed a study specific assessment of understanding before enrolment. We followed human experimental guidelines for conducting clinical research from the US Department of Health and Human Services.

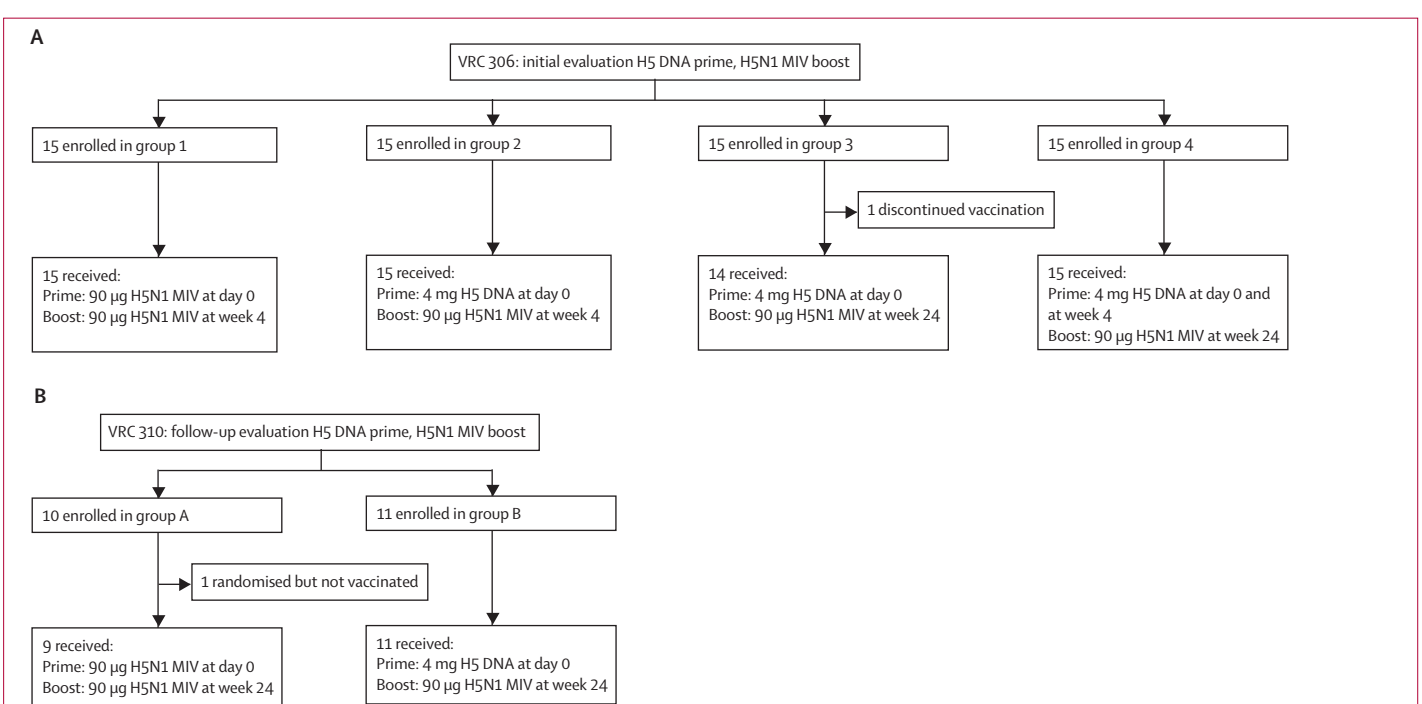

Figure 1: **Trial profile for VRC 306 (A) and VRC 310 (B)**

	VRC 306				VRC 310	
	Group 1 (n=15)	Group 2 (n=15)	Group 3 (n=15)	Group 4 (n=15)	Group A (n=9)	Group B (n=11)
Individuals with a four-times increase	6 (40%)	4 (27%)	11 (73%)	11 (73%)	4 (44%)	10 (91%)
Individuals with a post vaccination titre >1/40	6 (40%)	4 (27%)	11 (73%)	11 (73%)	4 (44%)	10 (91%)
Before vaccination (GMT)	<1/10	<1/10	<1/10	<1/10	<1/10	<1/10
After vaccination (reciprocal GMT; 95% CI)	33 (1–455)	20 (3–126)	103 (3–2827)	248 (4–13 604)	27 (12–63)	206 (77–550)

GMT=geometric mean tritre. Group 1 received H5N1 MIV at day 0 and week 4. Group 2 received H5 DNA at day 0 followed by H5N1 MIV at week 4. Group 3 received H5 DNA at day 0 followed by H5N1 MIV at week 24. Group 4 received two doses of H5 DNA (one at day 0 and the other at week 4) followed by H5N1 MIV at week 24. VRC 310 group A received H5N1 MIV at day 0 and week 24 and group B received H5 DNA at day 0 followed by an H5N1 MIV boost at 24 weeks.

Table 1: **HAI response by clinical trial and group assignment**

Randomisation and masking

During enrolment, study participants were randomly assigned as per protocol design (figure 1) with a computer-generated block randomisation. The study statistician and pharmacists developed and maintained the randomisation code.

Procedures

See Online for webappendix

60 individuals were enrolled in VRC 306, into one of four groups. The first group served as a control based on published data assessing H5N1 MIV vaccines. The other three groups served as experimental groups designed to determine the value of DNA priming before MIV boosting and to assess the potential effect of boost interval or number of doses of DNA on the response. Group 1 received H5N1 MIV at day 0 and week 4. Group 2 received H5 DNA at day 0 followed by H5N1 MIV at week 4. Group 3 received H5 DNA at day 0 followed by H5N1 MIV at week 24. Group 4 received two doses of H5 DNA (one at day 0 and the other at week 4) followed by H5N1 MIV at week 24. Partly designed as a follow-up study, two groups in VRC 310 were enrolled to specifically validate the findings in VRC 306 and data from those two groups are presented here. VRC 310 group A received H5N1 MIV at day 0 and week 24 (a control group not included in VRC 306). Group B received H5 DNA at day 0 followed by an H5N1 MIV boost at 24 weeks, identical to group 3 in VRC 306. All DNA vaccinations were 4 mg and given via needle-free Biojector device (Bioject; Tualatin, OR, USA) and all H5N1 MIV vaccinations were 90 μg, administered by needle and syringe. The dosages of DNA vaccine and MIV vaccine were based on previous trials.[17–19] All injections were given intramuscularly in the deltoid.

We assessed local and systemic reactogenicity for 5 days after every vaccination. We recorded adverse events for every individual until 28 days after their final vaccination the primary safety and immunogenicity endpoint, and coded the adverse events using the Medical Dictionary for Regulatory Activities (severity scale 0–5).

We assessed H5 neutralising antibodies by measuring the ability of serum samples to prevent the infection of 293A cells by replication-incompetent haemagglutinin-pseudotyped virus.[20] The pseudotyped virus expressed the H5 antigen and the luciferase reporter gene. Neutralisation activity was quantified by relative decrease in the luciferase activity compared with infection of 293A cells in the absence of sera based on previously described methods.[16] We calculated the 80% inhibition serum titre (ID_{80}) relative to the signal in the absence of sera using five-parameter curve fitting. We assessed vaccine-induced antibody to the haemagglutinin stem as previously described (webappendix pp 3, 7–8).

The HAI assays were done in V-bottom 96-well plates using four haemagglutinating units of virus and 1% horse red blood cells as previously described.[21] The virus strain used for the HAI assay is a low-pathogenic, H5N1-PR8 reassortant, obtained from Ruben Donis at the Centers for Disease Control and Prevention, Influenza Branch (Atlanta, GA, USA): clade 2.1, A/Indo/5/2005(H5N1)/PR8-IBCDC-RG2.[21]

We assessed ELISA binding antibodies directed against H5 antigen (Immune Technologies Corporation, New York, USA) using 96-well Immulon2 (Dynex Technologies, Chantilly VA, USA) plates coated with a preparation of purified recombinant proteins according to methods adapted from those previously described.[18] The endpoint titre was calculated as the most dilute serum concentration that gave an optical density reading of more than 0·2 above background.

CD4 and CD8 T cell responses to H5 were assessed 4 weeks after the MIV boost for every individual, by intracellular cytokine staining for interleukin 2 or interferon γ as previously described.[22,23]

Vaccines

The H5 DNA vaccine (VRC-AVIDNA036-00-VP) was manufactured at the VRC/NIAID/Vaccine Pilot Plant operated by SAIC (Frederick, MD) and consists of a single closed-circular plasmid DNA macromolecule (VRC-9123), expressing influenza A/Indonesia/5/05 haemagglutinin sequence, derived from a human isolate (influenza sequence database number 125873, Los Alamos National Laboratory database). The plasmid contained a CMV/R promoter as previously described.[18] The plasmid DNA was prepared under Good Manufacturing Practices at 4 mg/mL in phosphate buffered saline (PBS).

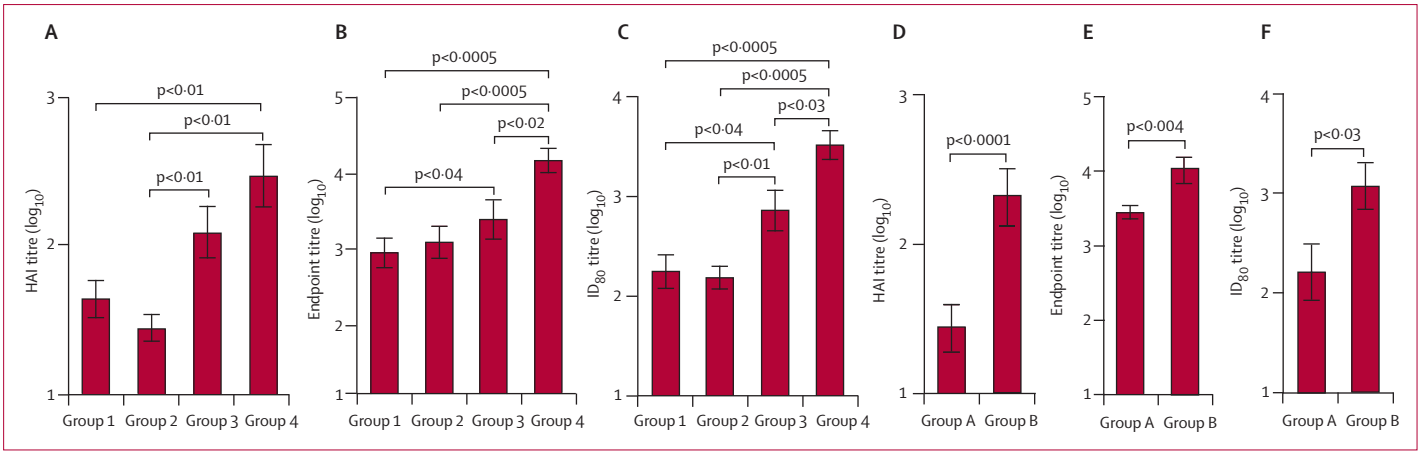

Figure 2: **Effect of prime boost immunisation on induction of homologous H5-specific antibodies**
HAI=haemagglutination inhibition assays. Mean titres with standard error of the mean are shown at 2–4 weeks after boost for groups 1–4 and A and B. VRC 306 group 1 received H5N1 MIV at day 0 and week 4. Group 2 received H5 DNA at day 0 followed by H5N1 MIV at week 4. Group 3 received H5 DNA at day 0 followed by H5N1 MIV at week 24. Group 4 received H5 DNA at day 0 and week 4 followed by H5N1 MIV at week 24 (A, B, C). VRC 310 group A received H5N1 MIV at day 0 and week 24 and group B received H5 DNA at day 0 followed by H5N1 MIV at week 24 (D, E, F): (A, D) HAI titres, (B, E) ELISA, and (C, F) neutralisation (ID_{80}).

	VRC 306				VRC 310	
	Group 1 (n=15)	Group 2 (n=15)	Group 3 (n=15)	Group 4 (n=15)	Group A (n=9)	Group B (n=11)
ELISA GMT	874 (32–23 137)	1156 (25–51 479)	2404 (27–208 992)	14 095 (873–227 413)	1685 (724–3920)	10 870 (5094–23 195)
Neutralising antibody (ID_{80}) GMT	176 (8–3369)	153 (20–1114)	670 (12–34 332)	3263 (264–40 237)	140 (27–732)	1179 (348–3998)

Data are GMT (95% CI). GMT=geometric mean tritre. Group 1 received H5N1 MIV at day 0 and week 4. Group 2 received H5 DNA at day 0 followed by H5N1 MIV at week 4. Group 3 received H5 DNA at day 0 followed by H5N1 MIV at week 24. Group 4 received two doses of H5 DNA (one at day 0 and the other at week 4) followed by H5N1 MIV at week 24. VRC 310 Group A received H5N1 MIV at day 0 and week 24 and group B received H5 DNA at day 0 followed by an H5N1 MIV boost at 24 weeks.

Table 2: **ELISA and neutralising antibody response by clinical trial and group assignment**

Subvirion H5N1 MIV (A/Indonesia/05/2005) 90 µg/0·5 mL was produced by Sanofi Pasteur Inc (Swiftwater, PA, USA) following the procedures and methods used to manufacture licensed influenza virus vaccine, Fluzone (Sanofi Pasteur). The investigational MIV contained no preservative or adjuvant.

Statistical analysis

The primary outcomes of the study related to safety, the secondary outcomes related to antibody responses, and the exploratory outcomes related to T-cell responses. We reported the positive response rate and the exact 95% CI for every antibody and T-cell response. We reported the response magnitude from the positive responders with the geometric mean and the 95% CIs. We assessed antibody responses to haemagglutinin by ELISA, HAI, and neutralisation assays and compared them between study groups by Fisher's exact test for response rate and Wilcoxon test for response magnitude. Computation of the exact 95% CIs was based on the exact binomial distribution by Pearson-Clopper method in statistical software R version 2.12.0.

VRC 306 and VRC 310 are registered with ClinicalTrials.gov, numbers NCT00776711 and NCT01086657, respectively.

Role of funding source

The study was funded by intramural National Institute of Allergy and Infectious Diseases (NIAID). The NIAID sponsored the trial, and the trial was conducted by the Vaccine Research Center, NIAID. The corresponding author served as the principal investigator and was responsible for the conduct of the study and the accumulation and analysis of data. She had final responsibility to submit for publication.

Results

60 healthy individuals were enrolled into clinical trial VRC 306 between Nov 17, 2008, and Nov 16, 2009 (figure 1). To directly compare vaccine regimens with a 24-week boost interval, a follow-up clinical trial (VRC 310) was initiated, and 21 individuals were enrolled between March 8, 2010, and May 13, 2010. Groups A and B were enrolled in VRC 310 to expand on the findings in VRC 306 (figure 1). The demographic characteristics were similar between groups (webappendix p 4). The vaccines were well tolerated, and no vaccine-related serious adverse events were recorded. When present, reactogenicity was mild to moderate in severity (webappendix pp 5–6).

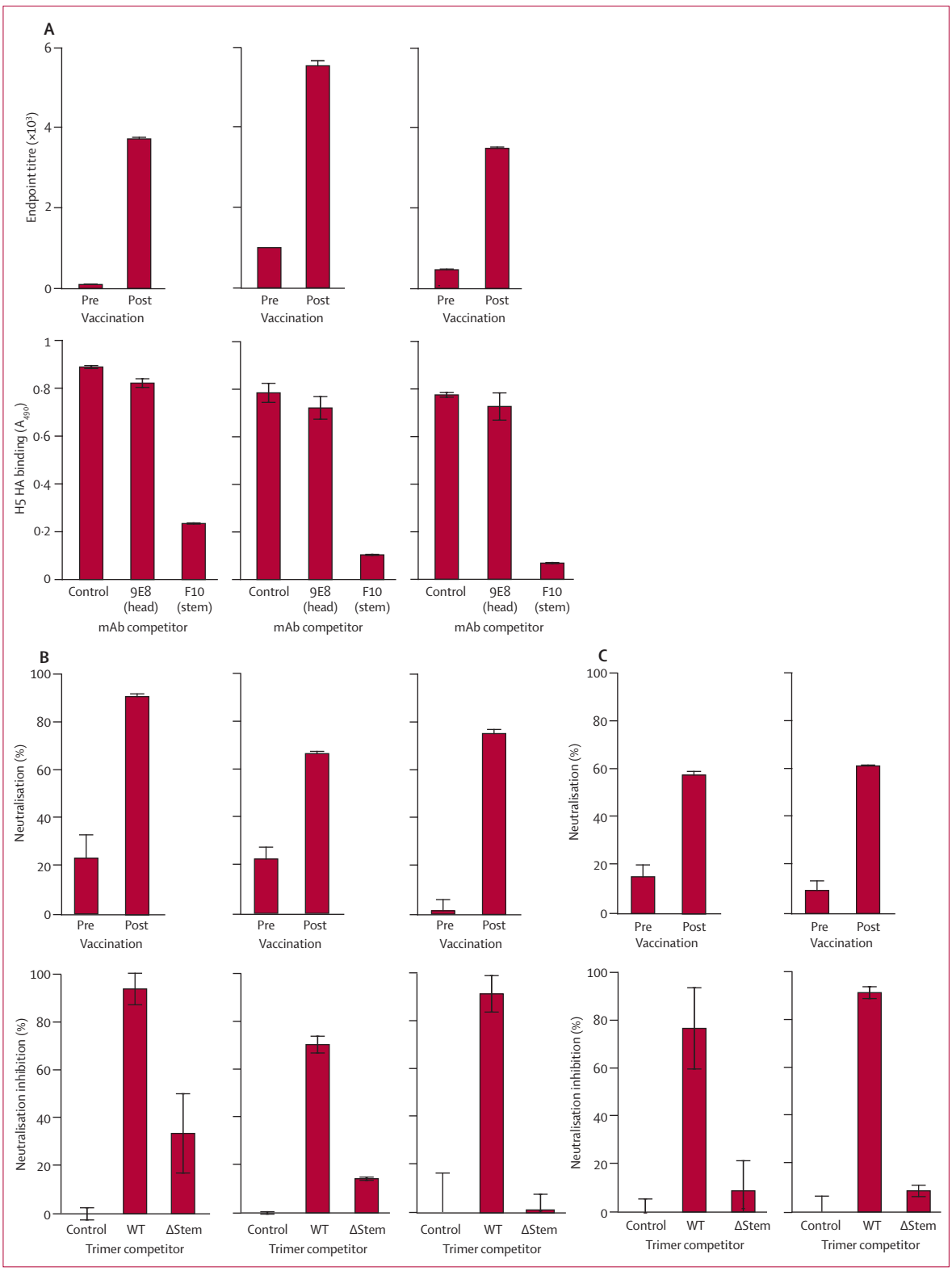

Figure 3: **Induction of stem-specific cross-neutralising antibodies after vaccination**
mAb=monoclonal antibody. WT=wild-type. (A) Three representative individuals are shown for neutralisation of A/Indonesia/05/2005 (H5N1). (B) Neutralisation of heterlogous virus, A/mallard/Pennsylvania/12180/1984 (1984 Penn H5N2), is shown for three representative individuals. (C) Neutralisation of heterologous virus, A/Hong Kong/1074/1999 (1999 HK H9N2), is shown for two representative individuals.

Antigen-specific T-cell responses were assessed by intracellular cytokine staining at 4 weeks after the MIV boost in each group. CD4 (interleukin 2 or interferon γ) T-cell responses to H5 were detected by intracellular cytokine staining more frequently in individuals who received two doses of DNA than in those who did not receive DNA priming ($p<0·05$) and CD4 responses were more frequently observed than CD8 T cell responses, which were only detected in two individuals in the study (webappendix p 2).

Antibody responses were assessed at 2–4 weeks after the MIV boost. All individuals were negative for H5 antibodies by HAI at baseline (table 1). After MIV boost, the frequency of positive H5 (Indo) HAI responses was greatest when the boost interval was longer (groups 3, 4, and B, table 1). The HAI titre (geometric mean titre [GMT]) was highest in individuals who received DNA priming and were boosted at the longest interval (groups 3, 4, and B; figure 2A and 2D). H5 binding antibodies assessed by ELISA were present in most individuals after the boost, and the magnitude of the GMT of ELISA responses (table 2) was highest in DNA primed individuals boosted after the longer 24-week interval (groups 3, 4, and B; figure 2B and E, and table 2). Neutralising antibodies to the H5 were detected in most individuals, and the greatest magnitude of response was also seen in the same groups, (groups 3, 4, and B; figure 2C and F and table 2).

To assess whether stem-directed neutralising antibodies were elicited, we analysed individuals for the presence of anti-stem antibodies before or after immunisation as previously described.[16] Sera were first absorbed with 293 cells expressing the stem mutant of the H5 (Indo) haemagglutinin to remove non-stem directed antibodies, and then tested their binding affinity to wild-type H5 (Indo) haemagglutinin.[16] Figure 3A shows vaccine-induced anti-stem antibodies for three representative individuals (one individual each from groups 2, 3, and 4) to illustrate these findings of a five-times increase in binding after immunisation (figure 3A). We confirmed the specificity of these anti-sera using a competition ELISA assay in which haemagglutinin binding was done in the presence of 9E8 (anti-head) or F10 (anti-stem) antibodies. In this assay, binding to haemagglutinin was inhibited by the anti-stem antibody F10 but not the control anti-head antibody 9E8 (figure 3A). We assessed the ability of the sera from these individuals to neutralise heterologous strains of virus on an unmatched distant low pathogenicitiy H5N2 (A/mallard/Pennsylvania/12180/1984, 1984 Penn) and on a different subtype, H9N2 (A/Hong Kong/1074/1999, 1999 HK) in three representative individuals (figure 3B). Post-immune but not pre-immune sera neutralised the unrelated 1984 Penn virus and two of these individuals also neutralised the alternative subtype 1999 HK virus (figure 3C), documenting the increased breadth of these immune

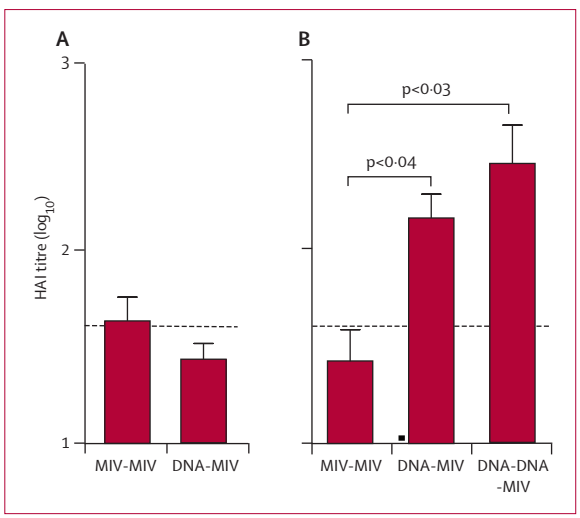

Figure 4: **Analysis of the H5 HAI mean reciprocal antibody titre in relation to the boost interval (VRC 306 and VRC 310)**
Antibody titres are shown with standard error of the mean. Response at 2–4 weeks after boost is shown for every regimen across both trials. Individuals were dosed at 4-week (A) or 24-week (B) boost intervals. Reference line represents protective HAI titre (1:40).

sera. Furthermore, wild-type H5 (Indo) haemagglutinin but not ΔStem trimer protein blocked neutralisation against each strain (figure 3B, C; lower panels, wild-type *vs* ΔStem), again showing that anti-stem neutralising antibodies were induced by vaccination in these individuals (webappendix pp 7–8).

When comparing all regimens by combining data from groups in VRC 306 and VRC 310 by boost interval, HAI titres did not differ in those who received two doses of MIV regardless of interval (figure 4). DNA priming with a 24-week boost interval induced a four-to-nine-fold increase in HAI titres (VRC 306+VRC 310 data) above DNA priming with a 4-week interval (figure 4 and table 1). A second DNA prime did not significantly increase the magnitude of HAI titre after MIV boosting at week 24 (figure 4).

Discussion

In this report, we analyse the ability of a H5 DNA vaccine to prime the neutralising antibody response elicited by an MIV H5N1 (Indo) boost. These regimens were safe and well tolerated, adding to the growing body of data that DNA vaccines carry a favourable safety profile. The safety data from the assessments of this H5 DNA vaccine are consistent with previous data generated by the Vaccine Research Centre using the same platform to assess vaccines for HIV, Ebola, Marburg, West Nile virus, severe acute respiratory syndrome virus, and seasonal influenza, in which 2100 individuals have been immunised with a total of 5700 doses of DNA vaccines.[17,18,23–30]

We have shown that the length of the interval between priming and boosting significantly affects the magnitude and breadth of the response and that DNA priming

Panel: **Research in context**

Systematic Review

We searched PubMed for "H5N1 infections", "DNA influenza vaccine", "H5N1 vaccine", "anti-stem antibody", and "universal influenza vaccine". Relevant articles are referenced in context throughout this article. Numerous DNA vaccines have been reported as safe and immunogenic. H5N1 vaccines are generally less immunogenic than seasonal influenza vaccines. DNA priming has not been previously shown to induce significant improvements in HAI responses to influenza vaccines in man.

Interpretation

We determined the data presented here by assessing H5 DNA vaccine priming with H5N1 MIV boosting in healthy volunteers in two clinical trials. The first clinical trial revealed surprising HAI responses after MIV with just one DNA prime 24-weeks earlier. The second trial allowed for enrolment of an important control group, an MIV-MIV regimen with a 24-week interval. This confirmed that the DNA primed group had higher HAI titres after MIV at 24 weeks compared directly to MIV-MIV with a 24 week interval.

HAI=haemagglutination inhibition assay. MIV=monovalent inactivated vaccine.

increases the potency of the responses. In some individuals, DNA priming can also increase breadth by eliciting antibodies directed to a highly conserved structure in the haemagglutinin stem.[11,31] DNA priming followed by MIV with a 24-week boost interval induced HAI titres more than four times higher than did two doses of MIV. Similar to other H5 antigens, this antigen generally induces low-titre and low-frequency HAI responses compared with seasonal influenza strain vaccine antigens. The DNA vaccine assessed in this study has previously been shown to induce an HAI titre of 1:40 or more in just 20% of individuals when given three times at a 4 mg dose without an inactivated vaccine boost.[32] Similarly, when given twice, the MIV boost product assessed in this study was recently shown by another group[33] to induce an HAI GMT of 27·6, and, although a 6-month boost interval improved those responses slightly (GMT 58·7), little or no cross-reactive antibody was induced.[33]

In the data from two clinical studies reported here, when a single DNA prime was boosted by MIV at 24 weeks, protective (≥1/40) HAI titres were induced in 81% of individuals with a GMT of 152 (groups 3 and B), which is a frequency and magnitude much greater than those previously induced by either vaccine alone. MIV given twice induced a protective HAI titre in six of 15 individuals in group 1, and in four of nine in group A. This finding is remarkable because of the relatively weak immunogenicity of the H5 antigen and because only one dose of DNA priming was needed to greatly improve the antibody response. Moreover, the longer boost interval significantly improved the responses seen in the DNA primed groups, but did not significantly improve the responses in individuals who received two doses of MIV. This result indicates that the biology of the priming immunisation differs between DNA and a traditional inactivated vaccine. Another surprising finding was that two doses of DNA as a prime did not greatly improve the overall responses beyond those seen with a single-dose of DNA. The two-dose DNA prime did affect the ELISA and neutralising antibody response but had no significant effect on the HAI response, which is known to correlate with protection. Gene-based antigen delivery accesses different pathways of antigen presentation and might thereby facilitate T-cell help, increase the number and diversity of CD4 clones,[34] and induce greater expansion of the relevant B-cell populations. DNA prime-inactivated vaccine boost has been shown to elicit higher haemagglutinin-specific T-cell responses compared with inactivated vaccine alone in animals, supporting the findings in this study.

DNA priming has been shown to induce improved haemagglutinin immune responses in animal studies.[16,35,36] Research in animals also suggested that gene-based influenza vaccination induces broad protection across strains; although influenza-specific broadly neutralising antibodies have been occasionally detected in man, measurement of specific influenza vaccine-elicited responses has been difficult since there is a high prevalence and variability of pre-existing influenza immunity in the general population. The benefit of assessing an H5 antigen is the low seroprevalence despite the sharing of some cross-reactive epitopes with other group 1 influenza type A viruses.

Assessment of the antibodies induced by the gene-based regimen described here indicates that antibodies against the conserved haemagglutinin stem epitope can be induced by DNA prime-MIV boost immunisation, and these antibodies are able to neutralise diverse strains, including H5N1, H5N2, and H9N2. The induction of stem-specific neutralising antibodies correlates with an overall higher magnitude of antibody response elicited by gene-based priming. Our data and previous studies[16] suggest that gene-based priming stimulates increased helper T-cell responses, perhaps by presentation in the absence of other viral components. This immune stimulation could augment the potency and breadth of the haemagglutinin-directed antibody response.

We acknowledge the following limitations of these studies: data were derived in two sequential clinical trials rather than in one trial, the variability of stem-antibody response in man remains poorly understood, and the mechanism of DNA priming and boost interval responsible for improving MIV vaccine immunogenicity remains unknown. However, an increase in the magnitude of the antibody response, either HAI titre or

activity of neutralising antibodies, induced by an influenza vaccine should translate to improved efficacy. The present findings suggest that DNA priming could substantially augment the responses otherwise seen with traditional H5 MIV vaccine approaches, and that gene-based regimens may also provide an opportunity to induce broader and potentially more potent neutralising antibodies, especially if a response can be induced against conserved influenza epitopes such as to the stem of haemagglutinin. A longer boost interval might provide an optimum immune response and allow for more flexibility in an immunisation schedule. Moreover, DNA priming might also prove to be a successful strategy in the effort to improve otherwise marginal immune responses to influenza vaccines, such as those seen in the very young or elderly.

Contributors

JEL, ZH, IJG, MEE, CSH, BSG, GJN, and JRM designed the clinical trial. JEL, IJG, MEE, CSH, and BSG conducted the clinical trial. JEL, C-JW, PMT, MBP, HMY, JCB, RB, TMT, and RAK developed assays and did immune analyses. JEL, C-JW, ZH, GJN, and BSG analysed data and conclusions. JEL, C-JW, ZH, IJG, MEE, CSH, PMT, MBP, HMY, JCB, RB, TMT, RAK, JRM, GJN, and BSG contributed to figures or tables and text.

The VRC 306 Study Team includes Sarah Hubka, LaSonji Holman, Laura Novik, Pamela Costner, Kathy Zephir, Floreliz Mendoza, Jamie Saunders, Brenda Larkin, Diane Johnson, Nina Berkowitz, Brandon Wilson, Sandra Sitar, Olga Vasilenko, Yesenia Merino, Joseph Casazza, Trishna Goswami, Raymond Cruz, Niraj Desai, Sheryl Young, Uzma Sarwar, Charla Andrews, Phillip Gomez, Becky Sheets, Judy Stein, Galina Yamshchikov, Hope Decederfelt, Judith Starling, LaChonne Stanford, Rhonda Washington-Lewis, Kathy Rhone, Hanne Andersen, and Phyllis Zaia.

Conflicts of interest

We declare that we have no conflicts of interest

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