

ORIGINAL ARTICLE

Cross-Reactive Antibody Responses to the 2009 Pandemic H1N1 Influenza Virus

Kathy Hancock, Ph.D., Vic Veguilla, M.P.H., Xiuhua Lu, M.D., Weimin Zhong, Ph.D., Eboneé N. Butler, M.P.H., Hong Sun, M.D., Feng Liu, M.D., Ph.D., Libo Dong, M.D., Ph.D., Joshua R. DeVos, M.P.H., Paul M. Gargiullo, Ph.D., T. Lynnette Brammer, M.P.H., Nancy J. Cox, Ph.D., Terrence M. Tumpey, Ph.D., and Jacqueline M. Katz, Ph.D.

ABSTRACT

BACKGROUND

A new pandemic influenza A (H1N1) virus has emerged, causing illness globally, primarily in younger age groups. To assess the level of preexisting immunity in humans and to evaluate seasonal vaccine strategies, we measured the antibody response to the pandemic virus resulting from previous influenza infection or vaccination in different age groups.

METHODS

Using a microneutralization assay, we measured cross-reactive antibodies to pandemic H1N1 virus (2009 H1N1) in stored serum samples from persons who either donated blood or were vaccinated with recent seasonal or 1976 swine influenza vaccines.

RESULTS

A total of 4 of 107 persons (4%) who were born after 1980 had preexisting cross-reactive antibody titers of 40 or more against 2009 H1N1, whereas 39 of 115 persons (34%) born before 1950 had titers of 80 or more. Vaccination with seasonal trivalent inactivated influenza vaccines resulted in an increase in the level of cross-reactive antibody to 2009 H1N1 by a factor of four or more in none of 55 children between the ages of 6 months and 9 years, in 12 to 22% of 231 adults between the ages of 18 and 64 years, and in 5% or less of 113 adults 60 years of age or older. Seasonal vaccines that were formulated with adjuvant did not further enhance cross-reactive antibody responses. Vaccination with the A/New Jersey/1976 swine influenza vaccine substantially boosted cross-reactive antibodies to 2009 H1N1 in adults.

CONCLUSIONS

Vaccination with recent seasonal nonadjuvanted or adjuvanted influenza vaccines induced little or no cross-reactive antibody response to 2009 H1N1 in any age group. Persons under the age of 30 years had little evidence of cross-reactive antibodies to the pandemic virus. However, a proportion of older adults had preexisting cross-reactive antibodies.

From the Influenza Division, National Center for Immunization and Respiratory Diseases (K.H., V.V., X.L., W.Z., E.N.B., H.S., F.L., L.D., P.M.G., T.L.B., N.J.C., T.M.T., J.M.K.), and the Division of Global AIDS, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention (J.R.D.) — all at the Centers for Disease Control and Prevention (CDC), Atlanta. Address reprint requests to Dr. Katz at the Influenza Division, CDC, 1600 Clifton Rd., Atlanta, GA 30333, or at jkatz@cdc.gov.

This article (10.1056/NEJMoa0906453) was published on September 10, 2009, at NEJM.org.

N Engl J Med 2009;361.

Copyright © 2009 Massachusetts Medical Society.

ON JUNE 11, 2009, THE WORLD HEALTH Organization declared that an influenza pandemic was under way. The 2009 pandemic H1N1 virus (2009 H1N1) has a unique combination of genes from both North American and Eurasian swine lineages that has not been identified previously in either swine or human populations.^{1,2} The hemagglutinin gene of 2009 H1N1 belongs to the classical swine lineage, which was first introduced into swine populations around 1918 and shares antigenic similarity with triple reassortant swine influenza viruses that have circulated in pigs in the United States for more than a decade; these strains have been associated with sporadic human disease.²⁻⁴ The 2009 H1N1 hemagglutinin is antigenically and genetically distinct from hemagglutinins of contemporary human seasonal influenza H1N1 viruses but has greater similarity to the swine H1N1 influenza virus that caused an influenza outbreak among military recruits in Fort Dix, New Jersey, in 1976.^{2,5} This outbreak led to a national vaccination campaign in which approximately 45 million people were vaccinated.⁶

Little is known about the level of preexisting immunity to 2009 H1N1 in humans, one of the determining factors for susceptibility to a novel influenza virus. Our preliminary studies suggested that children under the age of 10 years may have little or no preexisting immunity but that adults over the age of 60 years may have some level of cross-reactive antibody to the pandemic strain.⁷ To inform a public health response to the pandemic, we further assessed the extent of cross-reactive antibodies against 2009 H1N1 that were present in pediatric, adult, and older adult populations as a result of previous influenza infection or recent vaccination with seasonal non-adjuvanted or adjuvanted vaccines. To better define the age distribution and possible origin for cross-reactive antibody against 2009 H1N1, we also evaluated serum samples from the general population, spanning birth decades during the past 130 years, as well as a cohort of subjects who received the 1976 swine influenza vaccine.

METHODS

STUDY DESIGN

We collected stored-serum panels from vaccine trials conducted in 1976 or between 2005 and 2009 from academic, government, and industry

partners. Serum samples were collected with approval from the institutional review board at each contributing institution, and written informed consent was provided. The testing of serum samples at the Centers for Disease Control and Prevention (CDC) was considered to be a public health, nonresearch activity that was exempt from human-subjects review. Details of the vaccine study cohorts and the vaccine products that were used are provided in the figure in the Supplementary Appendix, available with the full text of this article at NEJM.org. In addition, we also tested 417 serum samples that were collected anonymously in the United States, consisting of 59 samples that were collected in 1971⁸ and 358 samples that were collected from July 2002 through February 2009 (for details, see the Methods section in the Supplementary Appendix).

Using a microneutralization assay and a hemagglutination-inhibition assay with 0.5% turkey erythrocytes for a subgroup of serum samples,^{9,10} we tested the samples for antibody responses to 2009 H1N1, A/California/04/2009, seasonal H1N1 viruses, and A/New Jersey/8/1976 (A/NJ/76) virus (for details about the assays and the hemagglutination-inhibition assay results, see the Methods section and Table 1 in the Supplementary Appendix). Because the microneutralization assay correlated well with the hemagglutination-inhibition assay ($r=0.7$) but generally yielded higher titers and more seroconversions among vaccinated subjects, the microneutralization assay was the primary serologic test used. The seasonal influenza A H1N1 viruses and the swine influenza A/NJ/76 virus were propagated in embryonated chicken eggs. A/California/04/2009 was propagated in Madin-Darby canine kidney (MDCK) cells, as detailed in the Supplementary Appendix.

STATISTICAL ANALYSIS

To estimate the value of the microneutralization titer corresponding to a hemagglutination titer of 40 (a measure that has been associated with at least a 50% reduction in the risk of infection or disease with influenza viruses in human populations), we performed a correlation analysis using linear regression models.¹¹⁻¹³ Analyses were performed to fit linear regression and multivariable models, to perform t-tests, and to estimate geometric mean titers (GMTs) with confidence intervals and corresponding P values with the use of SAS software (version 9.1). For analysis of the

data for the 417 serum samples, we evaluated titers on the basis of the birth decade of the serum donor and computed the cumulative GMT by averaging the mean log microneutralization titers for that specific decade and preceding decades, giving each decade equal weight regardless of varying sample sizes. A P value of less than 0.05 was considered to indicate statistical significance. (For details regarding the statistical analysis, see the Supplementary Appendix.)

RESULTS

CROSS-REACTIVE ANTIBODIES BEFORE AND AFTER SEASONAL INFLUENZA VACCINATION

We detected little or no preexisting cross-reactive antibody against 2009 H1N1 in 124 samples from children ranging in age from 6 months to 9 years (Table 1, and Table 2 in the Supplementary Appendix). Among 13 samples from a subgroup of children between the ages of 5 to 9 years, in which prevaccination antibodies to the seasonal H1N1 virus were evident, the GMT of antibodies against 2009 H1N1 was 10; titers of 40 or more were observed in only 1 child (8%) in this age group. After vaccination with seasonal vaccine, the GMT of antibodies against 2009 H1N1 did not increase by a factor of four or more in any of the 55 children who received trivalent inactivated vaccine, although a robust response to seasonal vaccine strains was detected in 67 to 100% of the children. Likewise, no seroconversion to antibodies against 2009 H1N1 was detected in any of the 24 children between the ages of 6 months to 9 years who were vaccinated with live attenuated influenza vaccine (Table 2 in the Supplementary Appendix). However, only 7 of 24 recipients of the live attenuated vaccine (29%) had an increase by a factor of four or more in the antibody titer against the seasonal vaccine strain, and all the children had a lower postvaccination GMT, as compared with recipients of the inactivated vaccine, as reported previously.¹⁴

Vaccination of 344 adults with inactivated seasonal vaccine resulted in seroconversion against the seasonal H1N1 vaccine strain in 65 of 83 adults between the ages of 18 and 40 years (78%), in 111 of 148 of those between the ages of 18 and 64 years (75%), and in 9 of 49 (18%) and 34 of 63 (54%) of those 60 years of age or older, depending on the year (Table 1). Seroconversion to antibodies against 2009 H1N1 was observed in

10 of 83 adults (12%) between the ages of 18 and 40 years, in 33 of 148 adults (22%) between the ages of 18 and 64 years, and in 3 of 63 (5%) or none of 50 adults 60 years of age or older, depending on the year. The ratios between the GMT after vaccination to the GMT before vaccination for the response to 2009 H1N1 ranged from 1 to 2 in both the adult and older-adult age groups, as compared with the GMT ratios observed for the seasonal H1N1 vaccine component ranging from 2 to 19 (Table 3 in the Supplementary Appendix). However, 6 to 7% of 231 adults and up to one third of 113 older adults had prevaccination microneutralization antibody titers of 160 or more against 2009 H1N1. Vaccination with the 2007–2008 seasonal vaccine, but not with the 2008–2009 seasonal vaccine, resulted in a modest boost in the cross-reactive antibody response, which was probably driven by the higher prevaccination GMTs detected in 2007–2008 samples, as compared with 2008–2009 samples. Interestingly, the prevaccination antibody GMT of older adults against 2009 H1N1 was significantly higher than the GMT in the seasonal 2007–2008 H1N1 vaccine component ($P < 0.001$). A similar trend was seen with the prevaccination GMT of older adults who received the seasonal 2008–2009 vaccine. Vaccination of adults with either the 2007–2008 or 2008–2009 live attenuated vaccine resulted in only a marginal effect on the levels of the microneutralization antibody titer against the respective seasonal H1N1 vaccine strains and no effect on the levels of antibodies against 2009 H1N1 (Table 3 in the Supplementary Appendix).

We next determined whether the seasonal inactivated vaccine that was formulated with oil-in-water adjuvants would enhance the level of cross-reactive antibody response to 2009 H1N1 when administered as a single dose to adults or as two doses to children. Only a modest increase in cross-reactive antibody response against 2009 H1N1 was detected in serum samples from 45 children between the ages of 6 months and 59 months who received the 2008–2009 inactivated vaccine formulated with an oil-in-water adjuvant (Table 1). Although seroconversion was detected in 43 children (96%) and 100% had a titer of 40 or more against the seasonal H1N1 component, seroconversion to antibodies against 2009 H1N1 occurred in only 1 child (2%), and 2 children (4%) had postvaccination antibody titers of 40 or more against 2009 H1N1. In adults and older

adults, although a formulation of the seasonal inactivated vaccines with oil-in-water adjuvants provided either a dose-sparing benefit or an enhanced rate of seroconversion to the seasonal H1N1 vaccine component, the adjuvanted vaccines showed no substantial enhancement of the cross-reactive antibody response to 2009 H1N1 (Table 4 in the Supplementary Appendix).

Table 1. Cross-Reactive Microneutralization Antibody Response against Pandemic Influenza A (H1N1) Virus in Pediatric and Adult Recipients of Seasonal Trivalent Inactivated Influenza Vaccines.*

Type of Vaccine, Influenza Season, and Influenza Virus Used in Assay	Age Group	No. of Subjects	Increase in Antibody Titer by a Factor of ≥ 4	Geometric Mean Titer†		Microneutralization Titer of ≥ 40 for Children or ≥ 160 for Adults‡	
				Before Vaccination (95% CI)	After Vaccination (95% CI)	Before Vaccination	After Vaccination
			%				%
Children							
Trivalent inactivated influenza vaccine							
2005–2007	6 mo to 9 yr	33					
Seasonal H1N1			67	26 (16–40)	267 (171–418)	45	94
Pandemic H1N1			0	5 (5–6)	6 (5–6)	0	0
2007–2008	5 yr to 9 yr	13					
Seasonal H1N1			85	42 (22–80)	575 (303–1093)	54	100
Pandemic H1N1			0	10 (7–15)	12 (8–17)	8	15
2008–2009	6 mo to 23 mo	9					
Seasonal H1N1			100	5 (4–7)	285 (202–402)	0	100
Pandemic H1N1§			0	5	5	0	0
Trivalent inactivated influenza vaccine with adjuvant							
2008–2009	6 mo to 59 mo	45¶					
Seasonal H1N1			96	12 (8–18)	193 (134–280)	24	100
Pandemic H1N1			2	6 (5–7)	8 (7–9)	0	4
Adults							
Trivalent inactivated influenza vaccine							
2007–2008	18 yr to 64 yr	148					
Seasonal H1N1			75	48 (40–58)	598 (497–720)	29	93
Pandemic H1N1			22	25 (21–31)	54 (44–65)	7	25
2008–2009	18 yr to 40 yr	83					
Seasonal H1N1			78	29 (22–38)	546 (418–713)	20	88
Pandemic H1N1			12	11 (9–14)	21 (16–26)	6	7

Table 1. (Continued.)

Type of Vaccine, Influenza Season, and Influenza Virus Used in Assay	Age Group	No. of Subjects	Increase in Antibody Titer by a Factor of ≥4 %	Geometric Mean Titer†		Microneutralization Titer of ≥40 for Children or ≥160 for Adults‡	
				Before Vaccination (95% CI)	After Vaccination (95% CI)	Before Vaccination	After Vaccination
Older adults							
Trivalent inactivated influenza vaccine							
2007–2008	≥60 yr	63					
Seasonal H1N1			54	31 (22–42)	143 (105–194)	14	54
Pandemic H1N1			5	92 (71–121)	97 (74–127)	33	43
2008–2009	≥60 yr						
Seasonal H1N1		49**	18	22 (17–28)	51 (39–66)	6	14
Pandemic H1N1		50**	0	47 (36–61)	51 (39–65)	8	8

* All children received two doses of vaccine unless they had received influenza vaccination in a previous year; those between the ages of 6 and 35 months received two half-doses of vaccine (7.5 μ g of hemagglutinin). The pandemic H1N1 virus that was used for all assay was A/California/04/2009. Seasonal H1N1 viruses that were used were A/New Caledonia/20/99 (2005–2007), A/Solomon Islands/3/2006 (2007–2008), and A/Brisbane/59/2007 (2008–2009).

† A titer of 1280 was used for all samples with a titer of 1280 or more.

‡ A hemagglutination-inhibition antibody titer of 40 corresponds to a microneutralization titer of 40 for children and of 80 to 160 for adults.

§ A confidence interval could not be calculated because all titers were 5.

¶ Serum samples were obtained from subjects residing in Central America.

|| Fifty serum samples were obtained from subjects residing in Europe.

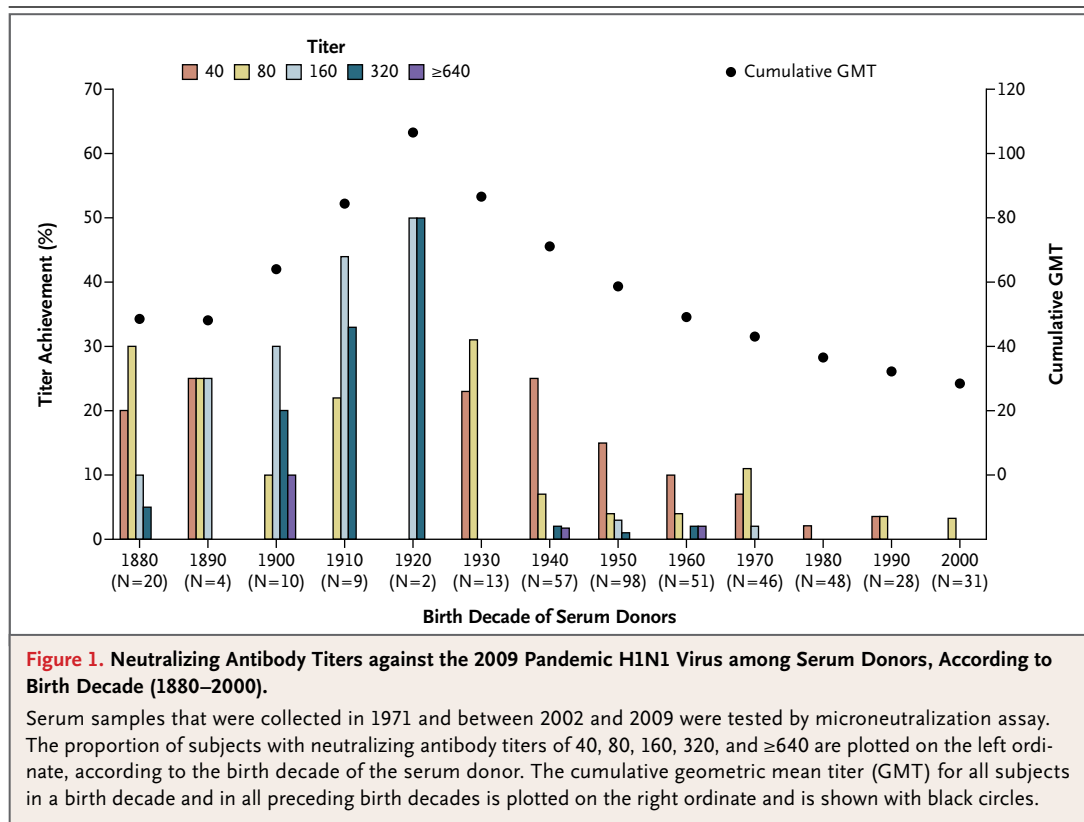
** All serum samples were obtained from subjects residing in Europe.

CROSS-REACTIVE ANTIBODY RESPONSE FROM PREVIOUS INFECTION OR VACCINATION

To further investigate the age distribution of cross-reactive neutralizing antibodies against 2009 H1N1 in U.S. populations, we tested a collection of 417 samples from anonymous donors born between 1880 and 2004 for antibodies against 2009 H1N1. The peak of antibody responses, presented as the frequency of titer achievement, occurred in 11 donors who were born between 1910 and 1929 (100% with titers of 80 or more), although the study was limited by the small number of samples from donors born between 1880 and 1920 (Fig. 1). The cumulative GMT, which includes data from subjects born in any one decade and those born in previous decades, peaked in the 1920s and gradually declined afterward. Approximately 39 of 115 subjects (34%) who were born before 1950 had antibody titers of 80 or more against 2009

H1N1. However, only 4 of 107 subjects (4%) who were born after 1980 had titers of 40 or more.

In 1976, approximately 20% of the U.S. population was immunized with the A/NJ/76 (H1N1) vaccine.¹⁵ We tested archived serum samples from 83 adults who were at least 25 years of age at the time that the sample was obtained and who had received one dose (400 chicken-cell agglutinating units) of a monovalent, split A/NJ/76 vaccine.¹⁶ Vaccination with the A/NJ/76 vaccine resulted in seroconversion to antibodies against A/NJ/76 virus in 67 subjects (81%) and a corresponding seroconversion to antibodies against 2009 H1N1 in 45 subjects (54%) (Table 5 in the Supplementary Appendix). Whereas 59 subjects (71%) achieved a postvaccination microneutralization antibody titer of 160 or more against the vaccine strain, 52 subjects (63%) achieved a postvaccination antibody titer of 160 or more against



2009 H1N1. These results showed that vaccination with the A/NJ/76 vaccine of persons who were primed by previous natural infection with influenza H1N1 virus led to the generation of serum antibodies that were broadly cross-reactive against 2009 H1N1.

DISCUSSION

The data from our study indicate that vaccination with contemporary seasonal influenza vaccines, even when formulated with oil-in-water adjuvants, provide little or no benefit to any age group with respect to an increase in cross-reactive neutralizing antibodies against 2009 H1N1. These findings are consistent with the substantial degree of genetic divergence of the pandemic H1N1 viruses of swine origin, as compared with recent seasonal human H1N1 viruses.² Although adjuvants enhanced serologic cross-clade reactivity of H5N1 two-dose vaccines, the degree of genetic identity between H5N1 clades is considerably higher (96 to 97%) than that for the pandemic H1N1 and seasonal H1N1 viruses.¹⁷⁻¹⁹

Children had little evidence of cross-reactive

antibodies to 2009 H1N1, but some degree of preexisting immunity to 2009 H1N1 existed, especially in older adults. Although the relatively small number of serum samples from pediatric trials was a limitation of our study, none of the seasonal vaccine formulations that we tested elicited a cross-reactive antibody response against 2009 H1N1. Subjects who were born before 1930, who were probably exposed to a 1918-like H1N1 virus, had the highest titers against 2009 H1N1. The presence of antibody titers of 80 or more against 2009 H1N1 in 34% of subjects who were born before 1950 is consistent with the higher frequency of prevaccination antibody titers of 80 or more against 2009 H1N1 that were detected in older adults in studies of seasonal influenza vaccines in the United States. These data suggest that exposure to a 1918-like H1N1 virus contributed to the induction of the cross-reactive antibody response to 2009 H1N1.

Furthermore, the data confirm the presence of some level of cross-reactive antibody in persons 60 years or more of age and the lack of such antibody in children and adults. This finding is consistent with those of previous studies show-

ing a similar effect for birth-year cohort on serum antibody responses to classical swine H1N1 viruses. These studies also showed that survivors of the 1918 pandemic had antibodies that neutralized the closely related A/swine/Iowa/30 virus.^{6,20-24} Furthermore, present-day subjects who were exposed to the 1918 virus had high-affinity neutralizing antibodies against epitopes on the hemagglutinin globular head that are conserved in both 1918 and A/swine/Iowa/30 viruses but not in human H1N1 viruses that circulated in the 1940s.²⁵ Recently, Itoh et al.²⁶ reported similar findings in that serum donors from Japan who were probably exposed to the 1918 virus or a closely related H1N1 virus had high levels of neutralizing antibodies against 2009 H1N1. However, in contrast to our findings, no appreciable cross-reactive antibody was detected in subjects born after 1920. These differences between the two studies may reflect differences in methodology or vaccination coverage rates in older adults, including receipt of the 1976 swine influenza vaccine in older adults in the United States. Likewise, the overall increased prevaccination titers for antibodies against 2009 H1N1 that we observed in older adults in the United States, as compared with their European counterparts, may reflect similar disparities in influenza vaccination history (Table 1).

Priming by previous natural infection with human H1N1 viruses in adults who were immunized with swine-origin A/NJ/76 (H1N1) virus vaccine probably contributed to the observed cross-reactive antibody response against 2009 H1N1. Serum samples from a small number of children between the ages of 6 months and 4 years who had had no previous exposure to the H1N1 virus and who received two doses (40 to 200 chicken-cell agglutinating units) of the A/NJ/76 vaccine had only modest cross-reactive antibodies against 2009 H1N1. (Only 2 of 10 subjects had seroconversion with a microneutralization antibody titer of 40 or more against 2009 H1N1, although all 10 children had seroconversion to antibodies against the A/NJ/76 virus.) It is possible that residual antibody that was induced by A/NJ/76 vaccination of adults may contribute to the observed cross-reactive antibody response in some older adults.

In a representative proportion of U.S. subjects with laboratory-confirmed 2009 H1N1 infection for whom age was known, approximately 79% of

cases occurred in subjects who were under the age of 30 years, whereas only 2% of confirmed cases were identified in those who were over 60 years of age. Therefore, the age distribution of laboratory-confirmed 2009 H1N1 infections is consistent with the observed lack of preexisting antibodies in children and adults and suggests one reason that older adults represent a minor proportion of reported cases: that cross-reactive antibody responses may provide protection against disease in this age group. Although we assessed only neutralizing antibody against 2009 H1N1 hemagglutinin, it is possible that heterotypic immunity to influenza from antibody against the neuraminidase or cellular responses to highly conserved viral epitopes may also contribute to the apparent protective effect in older adults.²⁷ Another possibility is that 2009 H1N1 has not yet spread to this older age group from populations that lack cross-reactive antibodies.

It remains clear that optimal protection against 2009 H1N1 in persons of all ages will be achieved with the development of a strain-specific pandemic vaccine. Whether a one-dose or a two-dose vaccine regimen is needed to adequately immunize various age groups and whether the use of adjuvants will broaden the immune response against 2009 H1N1 if drifted strains emerge or provide dose-sparing benefits will ultimately be determined by the results of clinical studies that are now under way.

Dr. Hancock reports receiving research support from GlaxoSmithKline and Juvaris BioTherapeutics; Dr. Lu, research support from Juvaris BioTherapeutics; and Dr. Katz, research support from GlaxoSmithKline. No other conflict of interest relevant to this article was reported.

The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the CDC.

We thank our colleagues who shared serum samples from vaccine trials conducted under the guidance of their institutions: Robert B. Couch, Baylor College of Medicine; Zhiping Ye, Center for Biologics Evaluation and Research, Food and Drug Administration; Ventzi Vassilev, GlaxoSmithKline; Thomas P. Monath, Juvaris BioTherapeutics; Kathleen Coelingh, MedImmune; Brian R. Murphy and Linda C. Lambert, National Institute of Allergy and Infectious Diseases, National Institutes of Health; Theodore F. Tsai, Novartis Vaccines and Diagnostics; Robert B. Belshe, Saint Louis University; Cornelia L. Dekker, Harry B. Greenberg, and David B. Lewis, Stanford University School of Medicine; Arnold S. Monto, University of Michigan School of Public Health; and Peter F. Wright, John V. Williams, and Kathryn M. Edwards, Vanderbilt University School of Medicine; Patrick Blair, Naval Health Research Center, San Diego, for facilitating access to the 2009 H1N1 virus; William M. Marine, formerly at Emory University School of Medicine, for locating the archived samples from anonymous donors collected in 1971; our colleagues at the CDC who contributed to virus isolation, sequence information, and the collection of donor samples and

who provided critical assistance in handling incoming serum collections: Amanda Balish, Suzette L. Bartley, J. Brad Bowzard, Susan P. Danner, William G. Davis, Rebecca Garten, Deborah Lee, Robyn A. Stoddard, and Mayim E. Wiens; and Christopher Freedman, Lindsay F. Killmaster, and Ashley L. Sabol of other branches of the CDC for their volunteer help.

REFERENCES

1. Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. *N Engl J Med* 2009;360:2605-15. [Erratum, *N Engl J Med* 2009;361:102.]
2. Garten RJ, Davis CT, Russell CA, et al. Antigenic and genetic characteristics of swine-origin 2009 A(H1N1) influenza viruses circulating in humans. *Science* 2009; 325:197-201.
3. Newman AP, Reisdorf E, Beinemann J, et al. Human case of swine influenza A (H1N1) triple reassortant virus infection, Wisconsin. *Emerg Infect Dis* 2008;14: 1470-2.
4. Shinde V, Bridges CB, Uyeki TM, et al. Triple-reassortant swine influenza A (H1) in humans in the United States, 2005–2009. *N Engl J Med* 2009;360:2616-25. [Erratum, *N Engl J Med* 2009;361:102.]
5. Nelson MI, Viboud C, Simonsen L, et al. Multiple reassortment events in the evolutionary history of H1N1 influenza A virus since 1918. *PLoS Pathog* 2008;4(2): e1000012.
6. Dowdle WR. Pandemic influenza: confronting a re-emergent threat: the 1976 experience. *J Infect Dis* 1997;176:Suppl 1: S69-S72.
7. Serum cross-reactive antibody response to a novel influenza A (H1N1) virus after vaccination with seasonal influenza vaccine. *MMWR Morb Mortal Wkly Rep* 2009; 58:521-4.
8. Marine WM, Thomas JE. Age-related response to 1000 CCA unit zonally purified, inactivated influenza vaccines in volunteers in the U.S.A. *Postgrad Med J* 1973; 49:164-8.
9. Kendal AP, Pereira MS, Skehel JJ. Concepts and procedures for laboratory-based influenza surveillance. Atlanta: Centers for Disease Control, 1982.
10. Rowe T, Abernathy RA, Hu-Primmer J, et al. Detection of antibody to avian influenza A (H5N1) virus in human serum by using a combination of serologic assays. *J Clin Microbiol* 1999;37:937-43.
11. de Jong JC, Palache AM, Beyer WEP, Rimmelzwaan GF, Boon ACM, Osterhaus ADME. Haemagglutination-inhibiting antibodies to influenza virus. *Dev Biol (Basel)* 2003;115:63-73.
12. Potter CW, Oxford JS. Determinants of immunity to influenza infection in man. *Br Med Bull* 1979;35:69-75.
13. Fox JP, Hall CE, Cooney MK, Foy HM. Influenza virus infections in Seattle families, 1975-1979. I. Study design, methods and the occurrence of infections by time and age. *Am J Epidemiol* 1982;116:212-27.
14. Sasaki S, Jaimes MC, Holmes TH, et al. Comparison of the influenza virus-specific effector and memory B-cell responses to immunization of children and adults with live attenuated or inactivated influenza virus vaccines. *J Virol* 2007;81:215-28.
15. Sencer DJ, Millar JD. Reflections on the 1976 swine flu vaccination program. *Emerg Infect Dis* 2006;12:29-33.
16. Massicot J, Murphy BR. Comparison of the hemagglutination-inhibiting and neutralizing antibody responses of volunteers given 400 chick cell-agglutinating units of influenza A/New Jersey/76 split-virus vaccine. *J Infect Dis* 1977;136:Suppl: S472-S474.
17. Leroux-Roels I, Bernhard R, Gérard P, Drame M, Hanon E, Leroux-Roels G. Broad Clade 2 cross-reactive immunity induced by an adjuvanted clade 1 rH5N1 pandemic influenza vaccine. *PLoS One* 2008;3(2):e1665.
18. Stephenson I, Bugarini R, Nicholson KG, et al. Cross-reactivity to highly pathogenic avian influenza H5N1 viruses after vaccination with nonadjuvanted and MF59-adjuvanted influenza A/Duck/Singapore/97 (H5N3) vaccine: a potential priming strategy. *J Infect Dis* 2005;191:1210-5.
19. Stephenson I, Nicholson KG, Hoschler K, et al. Antigenically distinct MF59-adjuvanted vaccine to boost immunity to H5N1. *N Engl J Med* 2008;359:1631-3.
20. Davenport FM, Hennessy AV, Francis T Jr. Epidemiologic and immunologic significance of age distribution of antibody to antigenic variants of influenza virus. *J Exp Med* 1953;98:641-56.
21. Shope RE. The incidence of neutralizing antibodies for swine influenza virus in the sera of human beings of different ages. *J Exp Med* 1936;63:669-84.
22. Taubenberger JK, Reid AH, Janczewski TA, Fanning TG. Integrating historical, clinical and molecular genetic data in order to explain the origin and virulence of the 1918 Spanish influenza virus. *Philos Trans R Soc Lond B Biol Sci* 2001;356:1829-39.
23. Tumpey TM, Garcia-Sastre A, Taubenberger JK, Palese P, Swayne DE, Basler CE. Pathogenicity and immunogenicity of influenza viruses with genes from the 1918 pandemic virus. *Proc Natl Acad Sci U S A* 2004;101:3166-71.
24. Cuneo-Crovati P, Gasparini R, Pellegrino C. Sero-epidemiological survey on human and swine influenza A: situation at the onset of summer 1976. *Boll Ist Sieroter Milan* 1976;55:463-70.
25. Yu X, Tsibane T, McGraw PA, et al. Neutralizing antibodies derived from the B cells of 1918 influenza pandemic survivors. *Nature* 2008;455:532-6.
26. Itoh Y, Shinya K, Kiso M, et al. In vitro and in vivo characterization of new swine-origin H1N1 influenza viruses. *Nature* 2009;460:1021-5.
27. Rimmelzwaan GF, McElhaney JE. Correlates of protection: novel generations of influenza vaccines. *Vaccine* 2008;26: Suppl 4:D41-D44.

Copyright © 2009 Massachusetts Medical Society.