

# Influenza Hemagglutination-Inhibition Antibody Titer as a Correlate of Vaccine-Induced Protection

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**Background.** Antibody to influenza virus hemagglutinin has been traditionally associated with protection. Questions have been raised about its use as a surrogate for vaccine efficacy, particularly with regard to an absolute titer indicating seroprotection.

**Methods.** We examined hemagglutination-inhibition (HAI) antibody titers in subjects from a placebo-controlled trial of inactivated and live attenuated vaccines and compared titers in subjects with symptomatic influenza (cases) to those without influenza infection (noncases).

**Results.** Pre vaccination and postvaccination geometric mean titers were both significantly lower for cases compared with noncases in all intervention groups. Frequency of postvaccination seroconversion did not significantly differ for cases and noncases in either vaccine group. Among live attenuated vaccine and placebo recipients, cases were less likely than noncases to have postvaccination HAI titers  $\geq 32$  or 64. Nearly all recipients of inactivated vaccine had postvaccination titers of at least 64, and the small number of vaccine failures were scattered across titers ranging from 64 to 2048.

**Conclusions.** While HAI antibody is the major correlate of protection, postvaccination titers alone should not be used as a surrogate for vaccine efficacy. Vaccine failures from clinical trials need to be examined to determine why seemingly protective HAI titers may not protect.

**Clinical Trials Registration.** NCT00538512.

Two types of vaccines for the prevention of seasonal influenza are currently licensed, 1 containing inactivated viruses and the other containing live attenuated viruses. Both vaccines are trivalent with influenza A (H3N2), A (H1N1), and B components. Recent studies suggest that the inactivated vaccine is approximately 70% effective in preventing symptomatic influenza in young adults [1, 2]. The live attenuated vaccine appears less effective than the inactivated vaccine in adults but more effective in young children [3]. Both vaccines are updated annually as necessary based on surveillance-informed recommendations.

In Europe, the annual update is accompanied by an evaluation of vaccine immunogenicity [4, 5].

A variety of novel approaches to influenza vaccine development are currently under consideration [6]. Some are designed to produce better protection among individuals, such as the elderly, who respond poorly to existing vaccines; others are designed to use novel delivery systems or production platforms other than eggs. These developments have focused attention on laboratory markers that predict or are correlated with protection against disease [7, 8]. The traditional correlate, antibody to the influenza virus hemagglutinin (HA) surface glycoprotein, was identified in 1943 as a predictor in the first study to examine efficacy of the current type of egg-grown inactivated vaccine [9]. The HA glycoprotein binds to target cell receptors and is critical to virus infectivity; antibodies to HA inhibit binding and neutralize infectivity [10]. Studies, some conducted 40 or more years ago, indicated that few influenza infections could be identified in persons who had pre-exposure hemagglutination-inhibition (HAI) antibody

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titers >32 or 40 [11]. Of particular importance is the study by Hobson et al [12], which considered antibody derived from natural infection to determine the HAI titer associated with protection. Currently, demonstration of postvaccination HAI titers, which meet certain criteria in fixed proportions of vaccine recipients, is used in Europe as the basis for approval of the annual updated vaccine and in large part for licensing new hemagglutinin-based vaccines [4, 5, 13]. Vaccine immunogenicity data are also considered in the United States; however, criteria for licensure are more complex and may also involve demonstration of efficacy in an actual trial [4, 14].

Although preexposure HAI antibody titers are clearly important to protection, review of studies cited as the scientific basis for using a specific titer as a correlate indicates some problems with defining correlates based on these observations [9, 12, 15]. With an efficacious vaccine, the number of vaccine failures is low, and these small numbers make analysis of the distribution of postvaccination titers difficult. Therefore, studies to identify antibody levels that correlate with protection have generally focused on data from unvaccinated individuals or recipients of placebo and may not apply to vaccine-induced antibody [9, 15]. Questions about the degree of misclassification of influenza outcomes have also been raised, because some studies determined subsequent infections only by testing for rise in antibody titer. It was recently confirmed that such a method misses infections in persons who received the inactivated vaccine in the absence of virus identification [16]. In addition, the role of HAI antibody levels in protecting recipients of the live attenuated vaccine has not usually been examined in parallel with those who received the inactivated vaccine [17].

Our randomized, placebo-controlled efficacy trial of inactivated and live attenuated vaccines, which was carried out during the 2007–2008 influenza season [2], provided an opportunity to reexamine the role of antibody measured by HAI in predicting protection from laboratory-confirmed influenza. This season was characterized by high influenza-related morbidity and circulation of predominately influenza type A (H3N2) viruses that were considered antigenically similar to the vaccine strain. Influenza illnesses were confirmed by virus isolation in cell culture and virus identification by real-time polymerase chain reaction (PCR). Vaccine efficacy against symptomatic laboratory-confirmed influenza was 68% (95% confidence interval [CI], 46%–81%) for the inactivated vaccine and 36% (95% CI, 0%–59%) for the live attenuated vaccine [2]. Antibody titers were measured in HAI assays, and titers in those subjects with symptomatic laboratory-confirmed influenza were compared with those without laboratory-confirmed influenza. We report here results of this analysis, with particular reference to past studies establishing the criteria currently used in evaluations of vaccine immunogenicity.

## METHODS

### Study Design

This trial enrolled healthy men and women aged 18–49. Persons with any health condition for which the inactivated vaccine was specifically recommended and for whom either vaccine was contraindicated were excluded [18]. During October–November 2007, eligible subjects were recruited from the community and randomly assigned to receive 1 intervention: the inactivated vaccine (Fluzone, Sanofi Pasteur) or matching placebo (physiologic saline) administered by intramuscular injection or the live attenuated vaccine (FluMist, MedImmune) or matching placebo (physiologic saline) administered by intranasal spray, in ratios of 5:1:5:1, respectively. Both the inactivated and live attenuated vaccines were licensed and approved for the 2007–2008 influenza season. From November 2007 through April 2008, subjects reported influenza-like illnesses meeting a symptomatic case definition (illness characterized by presence of cough or nasal congestion plus fever/feverishness, chills, or body aches), and throat-swab specimens were collected for influenza virus isolation in cell culture and virus identification by real-time PCR. Blood specimens for serologic studies were collected 3 times: immediately before receipt of assigned intervention, approximately 30 days later, and at the end of the influenza season (April–May 2008). All 30-day postvaccination blood specimens were collected at least 14 days before local surveillance–defined influenza circulation.

Sera from a subset of all enrolled subjects were selected to be tested in the HAI assay. This subset included all subjects with laboratory-confirmed influenza (isolation in cell culture and/or identification by real-time PCR), subjects who were also participating in a pilot substudy of the cell-mediated immune response to vaccination, and a randomly selected sample of the remaining participants who provided all 3 blood specimens. Sera were tested from 728 of 1952 subjects (37%) enrolled during the 2007–2008 influenza season. Results from 658 of the 728 subjects (90%) were included in this analysis including 105 subjects with symptomatic influenza A (H3N2) (cases) and 553 subjects without laboratory-confirmed influenza (noncases). Excluded subjects included those with laboratory-confirmed influenza A (H1N1) or type B, those without postseason blood specimens, and those with serologic evidence of influenza infection ( $\geq 4$ -fold increase in HAI titer between postvaccination and postseason sera) that was not confirmed by virus isolation or identification by real-time PCR.

### Laboratory Assay

The HAI assay takes advantage of the influenza viruses' ability to agglutinate red blood cells from certain birds (eg, turkeys) and mammals (eg, guinea pigs) via HA binding to sialic acid residues on red blood cells [10, 19, 20]. Antibody directed to strain-specific HA antigen is produced in response to influenza infection or

vaccination and can inhibit this hemagglutination [10, 19, 20]. The HAI assay allows quantification of these antibodies and has also been used to determine the antigenic relatedness of influenza virus strains [10, 19, 20].

Prior to HAI testing, all sera were treated overnight with receptor-destroying enzyme (Denka Seiken Co) to prevent nonspecific inhibition; sera were also adsorbed with red blood cells to remove nonspecific agglutinins [10, 19, 20]. Serial 2-fold dilutions (with an initial dilution of 1:8) were prepared for each set of 3 sera (prevaccination, postvaccination, and postseason) in 96-well plates, followed by incubation with standardized concentrations of monovalent inactivated influenza vaccine subunit materials (Sanofi Pasteur) representing the 2007–2008 A (H3N2) vaccine virus strain (A/Wisconsin/67/05) and the antigenically similar A (H3N2) virus strain that circulated (A/Uruguay/716/07) during that season. Turkey red blood cells (Lampire Biologics) were added to wells and allowed to settle. The strain-specific HAI antibody titers at each time point for each individual were calculated as the reciprocal (eg, 128) of the highest dilution of sera (eg, 1:128) that inhibited hemagglutination. HAI titers below the limits of detection (ie, <8) were denoted as half of the threshold detection value (ie, 4); titers greater than the upper test value (ie, 4096) were denoted as twice that value (ie, 8192).

### Study Objectives and Statistical Analyses

Our objectives were to examine the serologic immune response to vaccination by determining the proportion of subjects demonstrating seroconversion (a postvaccination HAI titer of at least

32 given a prevaccination titer <8 or, alternatively, a  $\geq 4$ -fold increase in HAI titer between prevaccination and postvaccination sera if the prevaccination titer was  $\geq 8$ ) and the proportion of subjects with postvaccination HAI antibody titers at  $\geq 2$  cutpoints (32 and 64, termed “seroprotection”) for cases and noncases by intervention group [10, 21]. Both cutpoints (32 and 64) were utilized because of variation in initial dilutions used by different laboratories, and known variability in absolute HAI titer levels that occurs between laboratories [10, 21, 22]. We also calculated and compared geometric mean HAI antibody titers (GMTs) for sera collected at prevaccination, postvaccination, and postseason visits between cases and noncases by intervention group. Individual HAI antibody titers at each time point were transformed to binary logarithms, and original values were divided by 4 (undetectable titer) to set the starting point of the log scale to zero prior to transformation. Average  $\log_2$  titers at each of the 3 time points (prevaccination, postvaccination, and postseason) were calculated to obtain the GMTs by intervention across time.

Categorical data (eg, seroconversion) were analyzed with an appropriate  $\chi^2$  test or, when necessary, Fisher exact test; continuous values (eg, GMT) were analyzed using Wilcoxon rank-sum tests. Statistical analyses were conducted using SAS (release 9.2, SAS Institute) software. A *P* value <.05 was considered to indicate statistical significance. No correction for multiple testing was considered. Because the overall ratio of cases to noncases was set by the strategy used to select subjects for HAI testing, the proportion of influenza cases within each HAI titer level was higher than they would have been if the entire study population were tested and included. However, because the selection of

**Table 1. Prevaccination and Postvaccination Geometric Mean Hemagglutination-Inhibition Antibody Titers and the Number/Proportion of Subjects Demonstrating Postvaccination Seroconversion and Postvaccination Titers  $\geq 32$  and  $\geq 64$  to Vaccine and Circulating Influenza A (H3N2) Strains by Intervention Group**

Intervention and strain	Prevaccination HAI GMT, mean (SD)	Postvaccination HAI GMT, mean (SD)	Seroconversion, no. (%) <sup>a</sup>	Postvaccination HAI titer $\geq 32$ , no. (%)	Postvaccination HAI titer $\geq 64$ , no. (%)
Inactivated vaccine <sup>b</sup>					
A (H3N2) vaccine strain <sup>c</sup>	3.49 (2.78)	7.61 (2.23)	198 (76.4)	259 (100.0)	252 (97.3)
A (H3N2) circulating strain <sup>d</sup>	2.13 (2.47)	5.69 (2.47)	196 (75.7)	233 (90.0)	216 (83.4)
Live attenuated vaccine <sup>e</sup>					
A (H3N2) vaccine strain <sup>c</sup>	4.03 (2.80)	4.87 (2.38)	61 (21.1)	245 (84.8)	204 (70.6)
A (H3N2) circulating strain <sup>d</sup>	2.46 (2.34)	2.92 (2.30)	29 (10.0)	147 (50.9)	116 (40.1)
Placebo <sup>f</sup>					
A (H3N2) vaccine strain <sup>c</sup>	3.75 (2.92)	3.85 (2.98)	3 (2.7)	69 (62.7)	56 (50.9)
A (H3N2) circulating strain <sup>d</sup>	2.07 (2.29)	2.16 (2.33)	4 (3.6)	41 (37.3)	30 (27.3)

Abbreviations: GMT, geometric mean titer; HAI, hemagglutination inhibition.

<sup>a</sup> Seroconversion: either prevaccination titer of <8 and postvaccination titer of  $\geq 32$  or prevaccination titer of  $\geq 8$  and  $\geq 4$ -fold rise in strain-specific HAI antibody titer between prevaccination and postvaccination sera.

<sup>b</sup> Inactivated influenza vaccine administered as an intramuscular injection (n = 259; 22 cases and 237 noncases).

<sup>c</sup> Influenza A (H3N2) vaccine strain (A/Wisconsin/67/05).

<sup>d</sup> Influenza A (H3N2) circulating strain (A/Uruguay/716/07).

<sup>e</sup> Live attenuated influenza vaccine administered as a nasal spray (n = 289; 53 cases and 236 noncases).

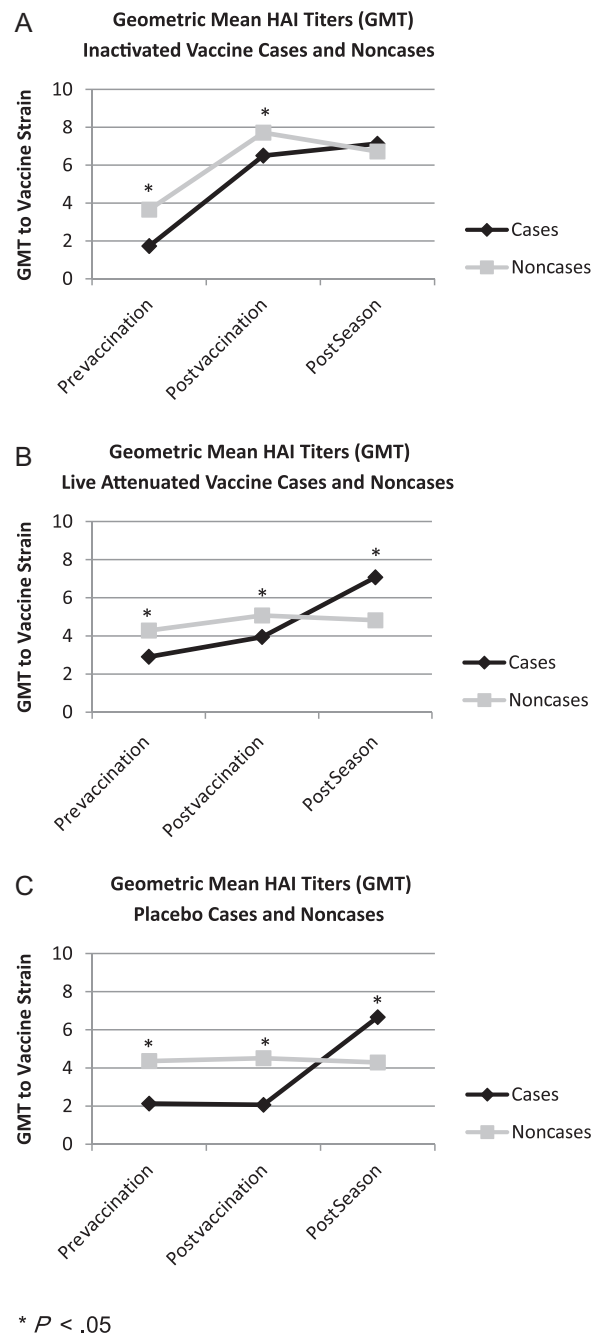
<sup>f</sup> Placebo: physiologic saline administered as a nasal spray or an intramuscular injection (n = 110; 30 cases and 80 noncases).

noncases for testing was essentially random, it was assumed that the proportion of influenza cases would have decreased evenly across HAI titer levels if the entire study population were tested.

## RESULTS

Table 1 shows prevaccination and postvaccination GMTs to both vaccine and circulating influenza A (H3N2) strains and the proportion of subjects demonstrating postvaccination seroconversion and HAI titers  $\geq 32$  and  $\geq 64$  cutpoints by intervention group. Although initial GMTs were similar across interventions, postvaccination GMTs to both vaccine and circulating strains were significantly ( $P < .001$ ) higher in sera from inactivated vaccine recipients compared with those who received the live attenuated vaccine. Approximately 76% of inactivated vaccine recipients demonstrated seroconversion to both vaccine and circulating strains, but significantly ( $P < .001$ ) fewer (approximately 21% to the vaccine strain and 10% to the circulating strain) live attenuated vaccine recipients had similar titer increases, as expected from past studies [1, 17]. All recipients of the inactivated vaccine had postvaccination HAI titers of at least 32 to the vaccine strain, as did 85% of live attenuated vaccine recipients and 63% of placebo recipients. These percentages were reduced for HAI titers of at least 64 and for titers to the circulating strain. Differences noted for vaccine compared with circulating strains could have been due to the slight antigenic variation between the 2 strains or, alternatively, as a result of differing red blood cell avidities, which is always an issue when interpreting HAI results [23]. Because of the similarity of the patterns of titers within intervention groups, additional presentations are limited to results using the vaccine strain.

Among the 105 cases of influenza A (H3N2), 53 cases were detected in live attenuated vaccine recipients and 22 cases in inactivated vaccine recipients. Thirty cases were detected in the smaller placebo group. Figure 1A–C compares the GMTs for influenza A (H3N2) cases and noncases at prevaccination, postvaccination, and postseason time points by intervention. Prevaccination GMTs to the vaccine strain were significantly lower for cases compared with noncases, and these differences were present regardless of intervention received. Postvaccination GMTs were slightly increased from prevaccination levels for cases and noncases that received the live attenuated vaccine and, more dramatically, for cases and noncases that received the inactivated vaccine. As expected, recipients of placebo did not show a similar increase. Despite the increases in GMTs from prevaccination to postvaccination in both vaccine groups, postvaccination GMTs were significantly lower for cases compared with noncases in all intervention groups. Postseason GMTs increased from postvaccination levels for all cases regardless of intervention, although the magnitude of the increase was lower for cases that received the inactivated vaccine. Postseason GMTs decreased from postvaccination levels for all



**Figure 1.** Geometric mean hemagglutination-inhibition (HAI) titers (GMT) to the A (H3N2) vaccine strain among influenza A (H3N2) cases and noncases at prevaccination, postvaccination, and postseason time points by intervention group.

noncases regardless of intervention, with the greatest decrease being among those who received the inactivated vaccine.

Table 2 presents the numbers and proportions of cases and noncases that demonstrated postvaccination seroconversion and postvaccination HAI titers  $\geq 32$  and  $\geq 64$  cutpoints by intervention group. Surprisingly, since seroconversion is used as a major indicator of the activity of a vaccine [5, 14, 21], the proportion of subjects demonstrating this outcome did not

**Table 2. Number and Proportion of Cases<sup>a</sup> and Noncases<sup>b</sup> Demonstrating Postvaccination Seroconversion<sup>c</sup> and Postvaccination Hemagglutination-Inhibition Titers  $\geq 32$  and  $\geq 64$  to the Influenza A (H3N2) Vaccine Strain by Intervention Group**

Intervention and Outcomes			P value
Inactivated vaccine	Cases (n = 22)	Noncases (n = 237)	
Seroconversion	20 (90.9)	178 (75.1)	.095
Postvaccination HAI titer $\geq 32$	22 (100)	237 (100)	...
Postvaccination HAI titer $\geq 64$	22 (100)	230 (97.0)	1.00
Live attenuated vaccine	Cases (n = 53)	Noncases (n = 236)	
Seroconversion	13 (24.5)	48 (20.3)	.499
Postvaccination HAI titer $\geq 32$	37 (69.8)	208 (88.1)	<.001
Postvaccination HAI titer $\geq 64$	31 (58.5)	173 (73.3)	.032
Placebo	Cases (n = 30)	Noncases (n = 80)	
Seroconversion	0 (0.0)	3 (3.8)	.561
Postvaccination HAI titer $\geq 32$	9 (30.0)	60 (75.0)	<.001
Postvaccination HAI titer $\geq 64$	6 (20.0)	50 (62.5)	<.001

All data are presented as no. (%).

Abbreviations: HAI, hemagglutination inhibition; PCR, polymerase chain reaction.

<sup>a</sup> Cases: subjects with symptomatic influenza A (H3N2) laboratory-confirmed by isolation in cell culture or identification in real-time PCR assay.

<sup>b</sup> Noncases: subjects without cell culture, real-time PCR, or serologic evidence of influenza infection.

<sup>c</sup> Seroconversion: either prevaccination titer of  $<8$  and postvaccination titer of  $\geq 32$  or prevaccination titer of  $\geq 8$  and  $\geq 4$ -fold rise in strain-specific HAI antibody titer between prevaccination and postvaccination sera.

significantly differ for cases and noncases in either vaccine group. In contrast, the proportion of subjects with postvaccination titers consistent with seroprotection significantly differed for recipients of the live attenuated vaccine and the placebo, with cases less likely than noncases to have HAI titers  $\geq 32$  or  $\geq 64$ . All recipients of the inactivated vaccine had postvaccination HAI titers  $\geq 32$ , and most had titers  $\geq 64$  with no differences by case status.

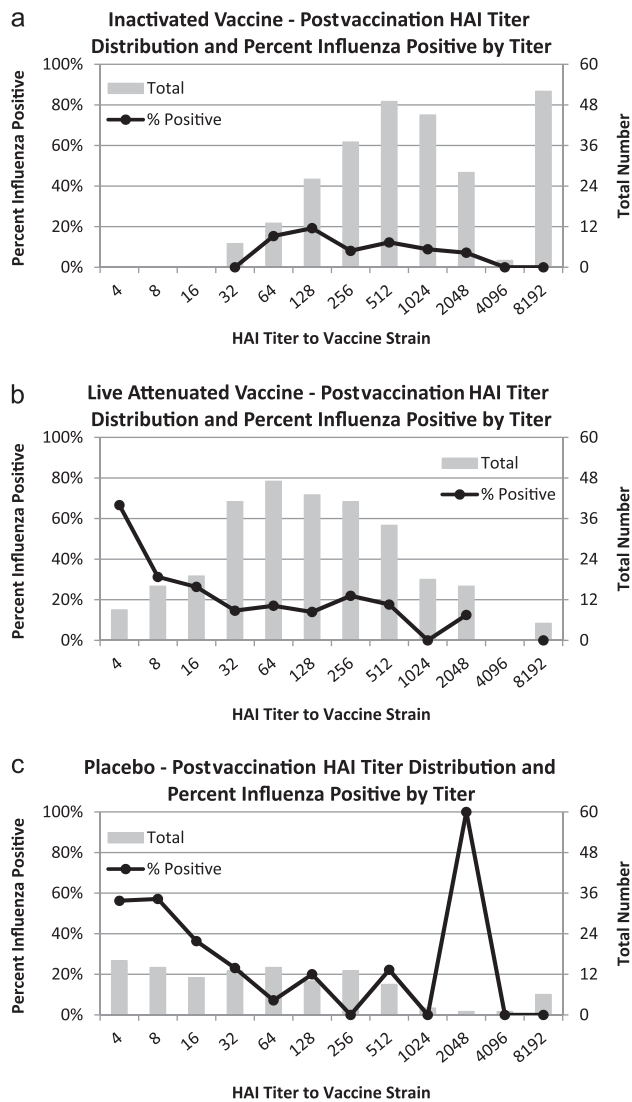
Figure 2a–c presents the distributions of postvaccination HAI titers and the proportion of subjects within each titer who were influenza-positive cases. The distributions of postvaccination HAI titers varied across interventions. Among placebo recipients, the distribution was low and relatively flat with few titers  $>512$ . Among live attenuated vaccine recipients, titers appeared normally distributed with approximately 72% of postvaccination titers in the 32–512 midrange and approximately equal numbers in the lower and upper tails. In contrast, among inactivated vaccine recipients, titers were relatively normally distributed but shifted up with no titers  $<32$ . In addition, approximately 20% of titers were greater than the upper test value of 4096 (denoted as a titer of 8192). A pattern of reductions in the proportion of cases as HAI titers increased was apparent for recipients of the live attenuated vaccine and the placebo with the largest proportions of cases among those with titers  $<64$ ; however, influenza cases were identified even among those with high HAI titers. This was particularly true among recipients of the inactivated vaccine, where no individuals had low HAI titers, and the small number of vaccine failures were scattered across titers ranging from 64 to 2048. The percentage of influenza-positive cases at titers  $>32$  was relatively similar across interventions.

## DISCUSSION

In 1943, the first efficacy trial of an inactivated influenza vaccine demonstrated that higher HAI titers were associated with protection in both vaccinated and unvaccinated subjects [9]. In the latter group, 50% of the estimated infections occurred in subjects with titers  $<64$ ; few subjects in the vaccinated group had titers  $<128$ , and the small number of infections occurred in subjects with titers  $\leq 1024$ . Another study conducted in the US military shortly thereafter also demonstrated that HAI antibody was a major determinant of protection; however, the titers found to be associated with protection were lower than those described in the previous study [15]. No infections were observed in persons who had received placebo or an irrelevant vaccine if their pre-exposure HAI titer was at least 32. Among the 8 individuals vaccinated with the relevant vaccine who were subsequently infected, only 1 had a preexposure titer of 64; all others had titers  $\leq 32$ . Similarly low titers were found to afford protection in the large influential study of Hobson et al [12], in which volunteers were challenged with a laboratory-passaged virus. As a result of these and smaller, more recent studies, a postvaccination HAI titer  $\geq 40$  (seroprotection) has been established as 1 of 3 related values used by regulatory authorities to evaluate influenza vaccine immunogenicity. The other 2 values used are postvaccination seroconversion and increase in GMT [4, 5, 14].

Questions have been raised concerning the use of an absolute titer and calling that value seroprotection, which implies close correlation with efficacy. This is particularly a problem in view of recognized methodological concerns with the test itself; whereas there is good within-laboratory consistency in





**Figure 2.** The distributions of postvaccination hemagglutination-inhibition (HAI) antibody titers to the influenza A (H3N2) vaccine strain and the percent of subjects with each titer who were influenza A (H3N2) positive (cases) by intervention group.

measured titers, there is great variability between laboratories [22]. Hobson et al were concerned about the low antibody titers they found to be consistent with protection and speculated that results from challenge studies with potentially attenuated viruses may not be generalizable to the protective effect of HAI antibodies against natural infection [12]. A limitation of other studies was reliance on serologically identified influenza outcomes [15]. Using increase in antibody titer between postvaccination and postseason to confirm influenza infection is a particular problem for evaluating inactivated vaccines, because postvaccination titers are already high, making additional increases associated with infection difficult to detect [16].

This does not mean that the level of HAI antibody should not be considered a correlate of protection, but rather that an absolute titer may not correlate directly with protection and

should only be viewed as a guide. In the current study, subjects who eventually became influenza cases had significantly lower prevaccination and postvaccination GMTs compared with non-cases in all 3 intervention groups. When examined by postvaccination titer cutpoints at or above levels thought to be protective, only subjects in the placebo group and, to a lesser extent, the live attenuated vaccine group were significantly less likely to become cases. In the inactivated vaccine group, all recipients had postvaccination titers  $\geq 32$ , and the small numbers of failures that did occur were in subjects with high titers. This observation is similar to that reported in the first trial of inactivated egg-based vaccine in 1943 [9]. These cases that fail at high HAI titer should be considered for study of other determinants of protection, which could be related to antineuraminidase antibody or cell-mediated immunity [24–26].

Increase in antibody titer between 2 time points has been used both to identify infection and to evaluate response to vaccination. An advantage of this measure, which examines relative change, is that variation in absolute titer should not be a concern, because the test on the 2 sera is run at the same time in the same laboratory. It was a surprise to find that seroconversion did not predict protection for either the inactivated or the live attenuated vaccine. In fact, nearly all subjects with inactivated vaccine failure had seroconverted, suggesting that vaccination might not have corrected preexisting susceptibility. This appears to be confirmed by the fact that cases had significantly lower GMTs in prevaccination sera in all intervention groups. This is another reason for vaccine failures to be further studied: to determine why seemingly protective levels of HAI antibodies do not protect.

The situation with the live attenuated vaccine was somewhat different in that seroconversion was less common and may not have occurred because infection with the vaccine virus had not taken place [2, 27]. However, that cannot be the whole explanation, because live attenuated vaccine failures had higher postvaccination titers than cases in the placebo group. Although seroconversion did not predict protection, correlation of absolute postvaccination antibody level with protection was demonstrated in both the live attenuated vaccine and placebo groups against the antigenically similar vaccine and circulating strains. Data from our study of vaccine efficacy conducted in 2004–2005 are also of interest in this regard [1]. In that year, there was moderate drift between the circulating A (H3N2) strain and that in the vaccine. Among influenza cases that received the live attenuated vaccine or the placebo, most had postvaccination titers  $\geq 32$  to the vaccine strain but  $< 32$  to the circulating strain. It is unfortunate that there have been only limited studies of correlation of protection with the live attenuated vaccine in young children, where infection with the vaccine virus should be common and the vaccine appears more efficacious [27].

Although the 2007–2008 season had the highest influenza attack rates in our 4-year study, there were still a limited number

of vaccine failures for this evaluation. Study of vaccine failures is critical to understanding how to improve influenza vaccines going forward [26]. Larger trials of vaccines are being conducted for licensure and other purposes by public and private groups, and it is important to evaluate influenza cases identified in these trials and learn why the vaccine did not protect these recipients. While HAI antibody is the major correlate of protection, it will only be through more intensive study of these cases that explanations for failure may be found. This in turn may help design more effective vaccines.

## Notes

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