Quantitative review of antibody response to inactivated seasonal influenza vaccines

Jessica C. Seidman, Stephanie A. Richard, Cécile Viboud, Mark A. Miller

Fogarty International Center, National Institutes of Health, Bethesda, MD, USA
Correspondence: Jessica C. Seidman, Fogarty International Center, Building16, Room 202, 16 Center Dr. Bethesda, MD 20892, USA.
E-mail: seidmanj@mail.nih.gov

Background Seasonal influenza epidemics are associated with significant morbidity and mortality each year, particularly amongst young children and the elderly. Seasonal influenza vaccines have been available for decades, yet influenza remains a major public health threat in the US, sparking interest in studies evaluating the effectiveness of vaccination.

Objectives We sought to identify determinants of serological responses to inactivated seasonal influenza vaccines including number of doses, adjuvant, and subject characteristics.

Methods We reviewed 60 articles published between 1987 and 2006. We used weighted multiple logistic regression and random-effects models to evaluate how seroconversion and seroprotection rates varied with host and vaccine factors.

Results Both children and seniors tended to have poorer immune responses compared to adults whereas use of adjuvant and a second vaccine dose tended to improve immune response. Pre-vaccination serological status had a large impact on the immune response to vaccination. We found substantial heterogeneity among studies, even with similar population settings and vaccination regimen.

Conclusions Future studies should stratify their results by pre-vaccination serological status in an effort to produce more precise summary estimates of vaccine response.

Keywords antibody response, immunogenicity, influenza, vaccine.

Background

Despite increasingly comprehensive vaccination coverage recommendations in the United States, seasonal influenza epidemics remain a major public health threat. It is estimated that seasonal influenza infection is associated with more than 36 000 deaths and nearly 294 000 hospitalizations in the United States every year.[1,2] Young children and the elderly experience the heaviest burden of severe influenza morbidity and mortality; in particular, more than 90% of influenza-related deaths occur in people >65 years.[3] Seasonal influenza vaccines are reformulated and relicensed annually because of frequent antigenic drift in circulating viruses.[4] The hemagglutination inhibition (HI) assay, a commonly used measurement of immune response to influenza vaccine, quantifies the level of serum antibody against haemagglutinin, the major surface antigen in influenza viruses. The US and European regulatory bodies accept HI titers as surrogate measures for protection against influenza illness because high titers have been observed to correlate with clinical protection.[5] In 1972, Hobson *et al.* [6] described the results of challenge studies in which the 50% protective dose of antibody (as measured by the HI assay) was found to be 18–36 for influenza A/H2 viruses. Lower titer levels may provide protection against infection with influenza A/H1 and B strains.[6,7] Hobson *et al.*'s study is routinely cited as the rationale for considering an HI antibody titer of 40 to be a marker of clinical protection; in a population of subjects with antibody titers of 40, 50% are expected to be protected.[8]

Previous reviews of inactivated seasonal influenza vaccine have identified determinants of vaccine response, including subject (age, baseline immunity) and vaccine (type, number of doses) characteristics.[9–14] Vaccine response was reduced in younger children compared with older children and in older adults compared with younger adults.[14–16] High pre-vaccination titers were correlated with high post-vaccination titers.[17] While the past research has focused on the impact of specific factors such as older age or adjuvant on vaccine response, no study has

examined the combined impact of vaccine and recipient characteristics on serological markers of immunity. We performed a quantitative review to assess the impact of number of doses, adjuvant, and subject characteristics on serological response to inactivated seasonal influenza vaccines. We also discuss sources of heterogeneity in measurements of immunological responses to influenza vaccine.

Methods

Literature review

Publications written in English and published through December 2006 were identified in PUBMED using keyword search terms 'influenza' and 'vaccine' and 'immunogenicity'. We also consulted references in papers retrieved by the PUBMED search. We excluded all studies reporting immunological responses for live-attenuated vaccines and focused on studies discussing inactivated vaccines only. Studies of inactivated influenza vaccine were included if they contained A/H1N1, A/H3N2, or B antigens, at the dosage level of currently licensed vaccines (15 μg of HA/dose), and the study population was without specific chronic conditions. Studies administering inactivated vaccines intranasally or subcutaneously were excluded. All studies meeting the inclusion criteria were published in 1987 or later. We selected studies numerically reporting the seroconversion rate (percent of vaccinees achieving a 4-fold rise in HI titer) and/or seroprotection rate (percent of vaccinees achieving an HI titer ≥40).[6,18] Studies were included if immune response was assessed within 2–8 weeks of vaccination.

Data extraction

Studies of inactivated seasonal influenza vaccine in all age groups were included in this review. Subjects were categorized as children (<18 years), adults (18–59 years), or seniors (≥60 years). Studies of experimental vaccines were included so long as they met the licensure criteria for dosage; however, we recorded whether the vaccine was commercially licensed. We included studies of both one and two dose regimens; two dose studies were included if the second dose of the same formulation was administered within 60 days. We captured antibody response rates after first and second vaccine dose when available. We also recorded the presence and type of adjuvant; vaccines were classified as with or without adjuvant in the primary analysis.

During the period of this review (1987–2006), the WHO recommended 15 new influenza A/H3N2, five A/H1N1, and nine B strains for inclusion in seasonal vaccines.[19,20] We distinguished between new and repeated vaccine strains whenever possible. Studies including subjects living in nursing homes or other institutions were included if subjects were not suffering from specific illnesses or chronic conditions. The analysis included a variable for community vs institutional residence.

Subjects were considered to have low titers at baseline if pre-vaccination antibody levels were <40. We collected information on the proportion of subjects with pre-vaccination titers ≥40 and previous influenza vaccination rates. Some studies presented the outcomes for both the entire study population and for the baseline low titer subset; this was captured for subanalysis. Because few studies reported vaccination history, prior vaccination status was not used in multivariate analyses. Our preliminary analysis broadly categorized the pre-vaccination antibody levels of study subjects as follows: low titer (0% of subjects have pre-vaccination titers ≥40); seropositive (100% of subjects have pre-vaccination titers ≥40) and unknown (pre-vaccination titers not described). Because the bulk of studies fell into the 'unknown' category, we created a composite variable to better capture baseline antibody levels: Negative – 0% seropositive; Low – 0–49% seropositive; High – 50–<100% seropositive; Positive – 100% seropositive; Unknown – subjects' pre-vaccination titers not described.

Statistical analysis

Many studies presented outcomes for separate population subgroups based on subject age, pre-vaccination titers, or other characteristics; each of these was analyzed as a distinct study arm. In all analyses, the number of subjects was used as a weighting factor. Crude mean response rates were calculated for each antigen (A/H1N1, A/H3N2, B), outcome measure (seroprotection and seroconversion rates), and dose, without adjustment for other factors. To account for clustering by study, we generated summary measures for seroprotection and seroconversion rates using meta-analysis models with random effects.[21] Univariate and multivariate logistic regression models were fit to estimate odds ratios (OR) and 95% confidence intervals (95% CI) for the associations between seroprotection and seroconversion outcomes and vaccine and recipient characteristics. Further, we performed a similar analysis with the subset of study arms reporting results for baseline low titer subjects. We conducted sensitivity analyses to assess the impact of a more finely categorized adjuvant variable (MF59, virosomal vaccines, other, none) and to adjust for potential statistical dependence between observations from studies reporting vaccine response after one and two doses. For the latter analysis, multivariate models were refit using generalized estimating equations (GEE) with exchangeable correlation.

Results

Study characteristics

We identified 60 eligible articles published between 1987 and 2006 describing inactivated seasonal influenza vaccine

Table 1. Characteristics of 60 seasonal inactivated influenza vaccine studies included in quantitative review[*]

| | Number of study arms | | |
	H1N1	H3N2	B
Total	118	109	107
Age (years)			
<18	11	11	11
18–59	48	38	42
≥60	59	60	54
Number of doses			
1	116	107	105
2	6	7	7
Adjuvant			
No	100	89	88
Yes	18	20	19
Baseline serological status[**]			
Low titer	13	5	6
<50% Positive	42	42	33
>50% Positive	10	16	22
100% Positive	4	4	4
Unknown	49	42	42
Novel vaccine strain			
Yes	26	63	29
No	91	46	78
Missing	1	0	0
Reported % of subjects with previous vaccination	60	50	52
Reported % of subjects with previous high titers	69	67	65

[*]The 60 included studies were published between 1987 and 2006 and reported results from 129 independent study arms.
[**]Pre-vaccination categorical variable coded as follows:
Low titer – 0% of subjects have pre-vaccination antibody titers ≥40.
<50% Positive – 0–50% of subjects have pre-vaccination antibody titers ≥40.
>50% Positive – 50–<100% of subjects have pre-vaccination antibody titers ≥40.
100% Positive – 100% of subjects have pre-vaccination antibody titers ≥40.
Unknown – subjects' pre-vaccination antibody titers not described.

immunogenicity studies conducted in the United States, Canada, Europe, Israel, and Russia.[10,22–80] We included 129 independent study arms of which 119 reported data on response to A/H1N1, 109 on influenza B, and 108 on A/H3N2 vaccine strains (Table 1). More than 84% of study arms assessed serological response within 3–4 weeks of vaccination; the minimum and maximum intervals were 2 and 6 weeks, respectively. The number of subjects ranged from 2 to 595 in each arm (mean 85, SD 101). The most prevalent age group was seniors (50% of study arms), followed by adults (42%) and children (9%). Only one study focused on young children (age range 6 months to

5 years)[47]; all other studies concentrated on older populations. A total of 127 study arms measured response after one dose; seven arms measured after two doses. Nearly 16% of study arms assessed response to adjuvanted vaccines; the most common adjuvants were the oil-in-water emulsion MF59 approved for use in Europe and virosomal vaccine preparations (40% of adjuvanted vaccine study arms each).

Vaccine response rates

The crude mean response rates after one dose of influenza vaccine were similar across the three antigens for both seroprotection (range 75–81%) and seroconversion (range 51–62%) (Table 2). The proportion of subjects achieving seroprotection was greater than that of seroconversion for subtype A, and response rates were higher after two doses. The summary effect size estimates from the random effects models were generally higher than the mean estimates (Table 2). The test for variance heterogeneity was highly significant for nearly all antigen-outcome combinations. To reduce the variance between studies, we repeated the random effects analysis on the subset of studies reporting responses after a single dose of commercially licensed vaccine administered to adults, published between 1990 and 2006. The summary estimates for seroprotection were remarkably consistent across the antigens (A/H1N1 86%; A/H3N2 88%; B 89%) (Figure 1), and the estimates for individual studies ranged from 49 to 100%. The summary estimates for seroconversion were also consistent across subtype (A/H1N1 72%; A/H3N2 73%; B 70%); however, there was more variation between individual studies with seroconversion rates ranging from 20 to 100% (Figure 2). The test for variance heterogeneity was highly significant for all combinations of antigens and serological outcomes.

Factors associated with vaccine response

The number of study arms included in the univariate regression models ranged from 40 to 115. A second vaccine dose significantly increased the odds of both seroprotection (OR range 1·2–2·1, $P < 0·01$) and seroconversion (OR range 1·8–2·7, $P < 0·01$) (Table S1). Seniors had significantly decreased odds of either serological outcome compared with adults (OR range: 0·2–0·6, $P < 0·01$). Children had reduced odds of seroprotection and seroconversion compared with adults, however, many of the 95% CIs overlap 1 (OR range: 0·3–1·1, P value range: <0·01–0·58). The use of adjuvant boosted serological response to A subtypes but was associated with weaker response to B strains. In contrast, serological response to B strains improved when vaccine recommendations were updated and a new B strain was included. Both institutional residence and previous influenza vaccination were associated with lower odds of protective responses. Higher baseline

Table 2. Mean serological response rates to seasonal inactivated influenza vaccines by antigen and dose

Antigen	Dose	Seroprotection				Seroconversion			
		No. of study arms	No. of subjects	Mean (95% CI)	Random Effects Summary	No. of study arms	No. of subjects	Mean (95% CI)	Random Effects Summary
H1N1	1	74	7949	78 (74, 82)	82 (78, 86)	111	9987	58 (53, 63)	61 (55, 66)
	2	6	503	88 (79, 97)	88 (81, 96)	3	252	84 (66, 100)	85 (78, 92)
H3N2	1	71	7800	81 (77, 85)	83 (79, 87)	101	9664	61 (58, 65)	64 (60, 67)
	2	7	518	86 (75, 98)	82 (70, 95)	4	267	70 (61, 79)	70 (65, 75)
B	1	72	7972	75 (69, 80)	83 (79, 88)	101	9914	51 (45, 56)	56 (51, 62)
	2	7	518	80 (59, 100)	89 (79, 99)	4	267	93 (87, 100)	93 (90, 97)

CI, Confidence Interval.
Seroprotection rate: % of vaccinees achieving a HI titer ≥40.
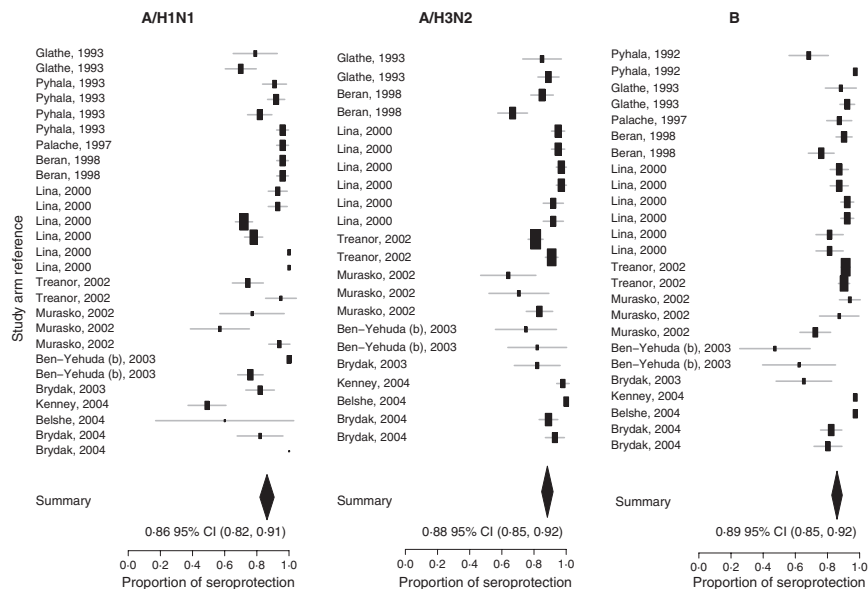Seroconversion rate: % of vaccinees achieving a 4-fold rise in HI antibody titer.

Figure 1. Meta-analysis plot of seroprotection rates from single dose, commercially licensed, inactivated vaccine studies in adults (18–59 year). Box sizes are proportional to the number of subjects in each study arm. The summary effect estimates were obtained from the random effects meta-analysis models. The test for variance heterogeneity was highly significant for all antigens ($P < 0.001$).

antibody titers were significantly positively associated with seroprotection (OR range: 1·8–6·3, $P < 0·01$), but inversely associated with seroconversion (OR range: 0·2–0·8, $P < 0·01$).

The number of study arms included in each multivariate model ranged from 76 to 114 (Table 3). The impact of a second dose was less consistent after controlling for other factors, significantly increasing the odds of protective responses for A/H1N1 and B, but not A/H3N2. Both seniors and children had significantly reduced odds of both outcomes compared with adults (OR range: 0·1–0·7,

$P < 0·01$). Institutional residence increased the odds of seroconversion by 20–80%; this factor increased the odds of seroprotection for B, but not type A strains. The inclusion of new B strains was associated with two to fivefold increased odds of protective responses. High baseline HI titers were significantly associated with increased odds of seroprotection but reduced odds of seroconversion.

Subanalysis of baseline low titer subjects

Given the apparent importance of baseline serological status, we conducted a subanalysis of subjects with low titers
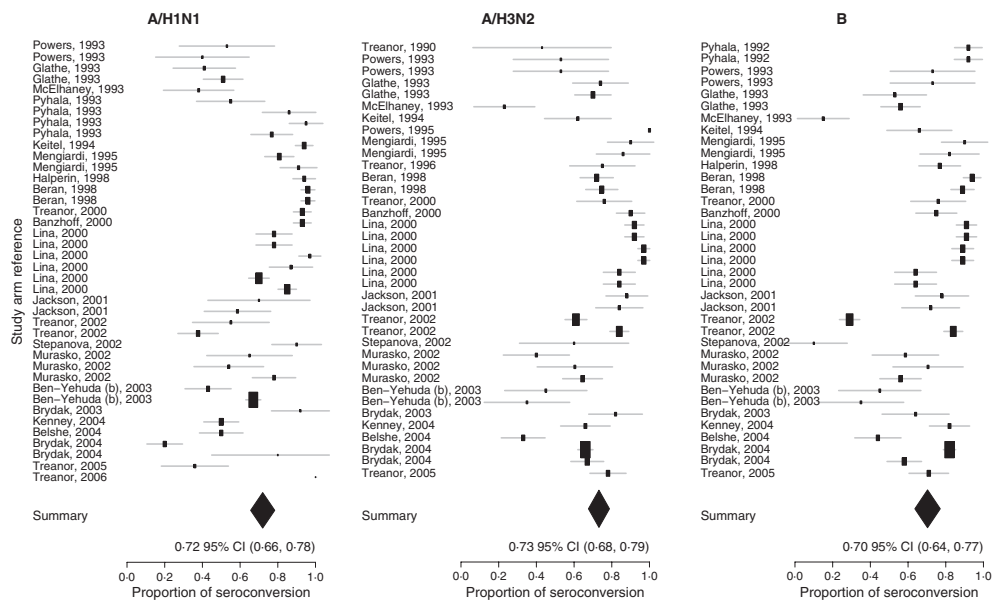
Figure 2. Meta-analysis plot of seroconversion rates from single dose, commercially licensed, inactivated vaccine studies in adults (18–59 year). Box sizes are proportional to the number of subjects in each study arm. The summary effect estimates were obtained from the random effects meta-analysis models. The test for variance heterogeneity was highly significant for all antigens ($P < 0.001$).

Table 3. Multivariate regression analysis of subject and vaccine characteristics associated with protective serological responses[*]

	Seroprotection			Seroconversion		
	H1N1	H3N2	B	H1N1	H3N2	B
	Odds ratio (95% CI)	Odds ratio (95% CI)	Odds ratio (95% CI)	Odds ratio (95% CI)	Odds ratio (95% CI)	Odds ratio (95% CI)
Dose (2 versus 1)	2·5 (1·7, 3·6)	0·6 (0·4, 0·9)	3·1 (2·3, 4·3)	2·0 (1·3, 3·0)	1·1 (0·8, 1·5)	5·5 (3·2, 9·3)
Age (ref = 18–59 years)						
≥60 years	0·4 (0·4, 0·5)	0·4 (0·3, 0·5)	0·1 (0·1, 0·2)	0·2 (0·2, 0·3)	0·3 (0·3, 0·4)	0·2 (0·1, 0·2)
<18 years	0·4 (0·3, 0·6)	0·3 (0·2, 0·5)	0·1 (0·1, 0·1)	0·7 (0·6, 0·9)	0·5 (0·4, 0·7)	0·6 (0·5, 0·7)
Adjuvant	3·3 (2·8, 3·8)	2·1 (1·7, 2·4)	1·6 (1·4, 1·8)	2·7 (2·4, 3·0)	1·7 (1·5, 1·9)	1·9 (1·7, 2·2)
Institutional residence	1·0 (0·9, 1·2)	1·0 (0·9, 1·2)	3·2 (2·7, 3·7)	1·3 (1·1, 1·4)	1·7 (1·5, 1·9)	1·8 (1·6, 2·0)
Baseline serostatus composite (ref = Low titer)[**]						
<50% Positive	2·6 (2·1, 3·1)	2·1 (1·6, 2·7)	4·6 (3·7, 5·6)	0·8 (0·7, 1·0)	0·7 (0·5, 0·9)	0·8 (0·6, 1·0)
>50% Positive	4·5 (3·5, 5·9)	6·1 (4·3, 8·8)	12·6 (9·8, 16·1)	0·3 (0·2, 0·4)	0·3 (0·2, 0·4)	0·6 (0·5, 0·8)
100% Positive	27·2 (11·7, 63·2)	>100[***]	>100[***]	0·3 (0·2, 0·6)	0·2 (0·1, 0·4)	1·4 (0·7, 2·8)
Unknown	2·3 (1·8, 2·8)	4·1 (3·2, 5·4)	2·2 (1·8, 2·8)	0·8 (0·6, 1·0)	0·6 (0·4, 0·8)	0·5 (0·4, 0·6)
New strain year	0·9 (0·8, 1·0)	0·6 (0·5, 0·7)	3·8 (3·2, 4·6)	1·3 (1·2, 1·4)	0·8 (0·8, 0·9)	2·0 (1·8, 2·2)

CI, confidence interval.

[*]The number of study arms included in each multivariate regression model ranged from 76 to 114.

[**]Pre-vaccination categorical variable coded as follows:

Low titer – 0% of subjects have pre-vaccination antibody titers ≥40.

<50% Positive – 0–50% of subjects have pre-vaccination antibody titers ≥40.

>50% Positive – 50–<100% of subjects have pre-vaccination antibody titers ≥40.

100% Positive – 100% of subjects have pre-vaccination antibody titers ≥40.

Unknown – subjects' pre-vaccination antibody titers not described.

[***]Odds ratios very large because of the small number of studies in this category.

Table 4. Mean serological response rates to seasonal inactivated influenza vaccines by antigen and dose, sensitivity analysis of subjects with low titers at baseline[*]

Antigen	Dose	Seroprotection			Seroconversion		
		No. of studies	No. of subjects	Mean (95% CI)	No. of studies	No. of subjects	Mean (95% CI)
H1N1	1	14	661	58 (39, 76)	17	575	70 (56, 85)
	2	1	103	85[*]	0	–	–
H3N2	1	9	434	65 (45, 85)	7	320	76 (63, 89)
	2	1	19	68[*]	0	–	–
B	1	10	647	57 (36, 78)	11	579	62 (48, 75)
	2	1	155	48[*]	0	–	–

CI, Confidence Interval.
Seroprotection rate: % of vaccinees achieving a HI titer ≥40.
Seroconversion rate: % of vaccinees achieving a 4-fold rise in HI antibody titer.
[*]No CI given because only one study arm was included.

Table 5. Multivariate regression analysis of subject and vaccine characteristics associated with protective serological responses, subanalysis of subjects with low titers at baseline[*]

	Seroprotection			Seroconversion		
	H1N1	H3N2	B	H1N1	H3N2	B
	Odds ratio (95% CI)	Odds ratio (95% CI)	Odds ratio (95% CI)	Odds ratio (95% CI)	Odds ratio (95% CI)	Odds ratio (95% CI)
Dose	10·9 (5·5, 21·6)	18·4 (3·2, 106·6)	4·5 (2·7, 7·7)			
Age (ref = 18–59 years)						
≥60 years	0·5 (0·3, 1·0)		0·3 (0·2, 0·5)	1·2 (0·7, 2·0)	0·4 (0·1, 0·9)	0·3 (0·1, 0·7)
<18 years	0·3 (0·1, 0·6)		0·0 (0·0, 0·0)	0·4 (0·1, 1·0)	1·9 (0·3, 10·7)	0·0 (0·0, 0·1)
Adjuvant	8·5 (4·8, 15·2)	6·2 (3·6, 10·6)	1·9 (1·2, 3·1)	2·6 (1·4, 4·9)	3·3 (1·8, 5·9)	2·3 (1·5, 3·4)
Institutional residence	0·7 (0·4, 1·4)		1·0 (0·5, 1·9)	0·1 (0·0, 0·1)	1·7 (0·8, 3·7)	0·1 (0·0, 0·3)
New strain year	0·6 (0·3, 1·2)	0·4 (0·2, 0·6)	4·8 (2·9, 7·9)			0·2 (0·1, 0·6)

CI, Confidence Interval.
[*]The number of study arms included in each multivariate regression model ranged from 7 to 18.

at baseline. Of the 24 study arms included, subjects had baseline titers ≤10 in 42% of study arms, and the remainder had titers between 10 and <40. Mean seroprotection rates after one dose ranged from 57 to 65% (only one study reported results after two doses); mean seroconversion rates ranged from 62 to 76% after one dose (no studies included reported results after two doses) (Table 4). We found greater heterogeneity between antigens and outcomes as compared to the main analysis, possibly due to decreased sample size.

In univariate analysis, a second vaccine dose, use of adjuvant, and previous vaccination were generally associated with significantly increased odds of protective responses (Table S2). The magnitude and direction of the effect sizes for age, residence, and new vaccine strains varied between antigens and outcomes. The number of study arms included in each multivariate model ranged from 7 to 18 (Table 5). Adjustment for the other factors strengthened the association between a second dose and immunological responses. Both seniors and children had lower odds of seroprotection compared with adults. The use of adjuvant was associated with increased odds of protective responses, and new vaccine strains increased the odds of seroprotection but decreased the odds of seroconversion for subtype B strains. Overall, this suggests that while pre-vaccination serostatus significantly affects serologic outcomes, this is not the only factor responsible for the observed heterogeneity of vaccine responses.

Sensitivity analyses

Although only a small number of study arms used MF59-adjuvanted or virosomal vaccines, in multivariate analysis, both improved the odds of seroconversion and seroprotection compared with unadjuvanted vaccines (OR range: 1·3–3·8, Table S3). Inclusion of the more finely categorized adjuvant variable did not have an impact on the OR estimates for the other variables. Such comparisons warrant further study, especially in light of the current debate about the use of adjuvanted vaccines for pandemic influenza.

The GEE model fitting procedure generally produced similar OR estimates to the main analysis, although confidence intervals were wider than for the standard logistic regression. In this sensitivity analysis, the strength of the associations between a second dose and protective serological responses was increased although the statistical significance of the relationship between baseline serological status and vaccine response was somewhat weakened. The procedure failed to converge for three of the six antigen-outcome models (A/H3N2 for both outcomes and B models for seroprotection). Because only a small proportion of studies reported results after the first and second dose (4%), we felt that correlation because of repeated observations was limited, and that confidence interval estimates from the standard logistic regression were sufficiently robust.

Discussion

We reviewed the seasonal inactivated influenza vaccine literature to quantify the associations between antibody responses to immunization and vaccine and recipient characteristics. Despite considerable heterogeneity in study results, several patterns emerged. A second vaccine dose and the use of adjuvant generally increased the proportion of subjects achieving serological markers of protection. This concurs with previous reviews of MF59-adjuvanted vaccines, reporting increased immunogenicity in elderly subjects compared with non-adjuvanted vaccines [81,82] and suggesting similar benefits in younger age groups.[83] Virosomal vaccines have also been shown to boost immunogenicity, especially among subjects with low titers prior to vaccination.[84,85] In our study, the effects of a second dose and adjuvant were magnified in populations with low baseline titers.

Seniors were less likely to respond to vaccine compared with adults, consistent with estimates reported elsewhere.[15] Although seasonal influenza vaccines are widely used in elderly populations, there is only one randomized, controlled trial assessing vaccine efficacy in this age group that suggests decreased benefit with increasing age.[86] The youngest subjects were also less likely to achieve protective levels compared with adults. Our review included only one publication of very young children (6 month–5 year); in this study, seroconversion rates in previously vaccinated children were lower than in their unvaccinated age peers.[47] Vaccine-naïve children are recommended to receive two doses to boost immunogenicity, and older children have been shown to have significantly increased antibody responses compared with younger children.[87–89] In the baseline low titer analysis, the effect of age on responses to influenza vaccination was less consistent, likely due to small sample size.

A striking finding was the magnitude of the impact of pre-vaccination antibody titers on post-vaccination serological outcomes. Studies with large proportions of subjects with pre-vaccination titers ≥40 reported higher seroprotection rates following vaccination. In contrast, the proportion of baseline seropositive subjects was inversely correlated with seroconversion. A previous meta-analysis of annual vaccination studies found no difference in seroprotection rates between unvaccinated and previously vaccinated individuals.[90] However, individuals with high baseline titers may easily achieve the seroprotection threshold, but may not be able to generate a fourfold increase. Few studies stratify by the baseline serostatus of the study population, making it difficult to assess true vaccine immunogenicity. Beyer *et al.* [11,91] suggest linear regression or other statistical procedures to adjust post-vaccination serological measures for pre-vaccination antibody titers to facilitate meaningful evaluation of influenza vaccines.

Vaccination with new strains was associated with improved serological responses for influenza B, weaker responses for influenza A/H3N2, and had no impact on responses to influenza A/H1N1. It is surprising that vaccination with influenza B strains included in prior year formulation tends to elicit a weak immune response. A study of immune response to repeated annual vaccination with unaltered antigen composition found evidence of gradual impairment of antibody response with influenza B in elderly populations.[92] Further, modeling work suggests that vaccine efficacy may increase as the antigenic distance between the vaccine strain and strains previously encountered increases.[93] The variation in serological response to new vaccine strains of influenza A and B types warrants further study.

Strain-specific analysis was limited by the small number of studies of specific strains. Evidence from the past pandemics suggests that exposure to influenza virus in childhood could provide life-long immunity. In particular, recent studies have shown that seniors enjoyed partial clinical protection during the 2009 pandemic through pre-existing cross-reactive antibodies to the 2009 A/H1N1pdm virus.[94] Here, we controlled for prior influenza exposure by including terms for age and baseline serostatus in the regression models. Current vaccine evaluation standards are independent of strain antigenic characteristics; thus, we felt

pooling studies of different strains within a subtype was valid. We only considered influenza subtypes A/H1, A/H3, and B; these results may not be generalizable to other emergent subtypes such as A/H5N1 or the recent swine origin A/H1N1 virus. In addition, it would be useful to conduct challenge studies to systematically compare seroprotective threshold titers across different influenza subtypes. Alternatively, field studies following well-characterized, serologically mixed populations through vaccination and natural exposure to influenza would further our understanding of the protection afforded by current vaccines.

There are several limitations to this study. Owing to lack of standardization in reporting, our categorical variables were broadly defined. Studies providing separate results for baseline low titer individuals used various threshold levels to define seronegativity. While previous studies have found differences in vaccine response or efficacy when comparing within age categories (younger versus older elderly; younger versus older children), we used wide age categories to allow inclusion of most studies.[14,15] Very few studies stratified by narrow age ranges, precluding more refined analysis of the impact of age on antibody responses to influenza vaccine.

We included a variable for type of residence (institutional or community dwelling) in our models. Although we excluded publications of study populations with specific comorbid conditions, it is likely that institutionalized subjects are more frail than the general population and have weakened immune response to vaccination.[15] The majority of the institutionalized populations studied were also seniors, so we were concerned about potential colinearity between these two variables. When the multivariate models were run both with and without the residence variable, the OR estimates for the association between age and vaccine response were virtually unchanged; thus, we felt that the colinearity did not affect our analysis.

We note that variation in study results was high, even in studies with similar population and vaccine characteristics, as evidenced by the meta-analysis statistics. Although our study suggests that heterogeneity in baseline antibody levels could explain some of the variability in vaccine immunological response, variability could also result from the serological assay itself. The HI assay, developed in 1941, remains the standard method for serological evaluation of influenza vaccine for licensure in both the United States and Europe.[95–97] The HI assay is highly variable and sensitive to factors such as reagents, erythrocyte source, and virus passage history, but is not standardized across laboratories.[98–100] Few studies provided enough detail on the laboratory methods used to be able to include these factors in our analyses. Additionally, studies may have used different starting dilutions to calculate a fourfold rise and report seroconversion rates, but did not systematically report this information.

In an international collaborative study of HI assay reproducibility, Wood *et al.*[99] found that relative HI titers were consistent between laboratories, but absolute levels were not. They found microneutralization assays to be even more variable.[101] Further, although high HI titers have been shown to correlate with clinical protection, HI assays are an indirect measurement of antibody levels.[6,98] This calls into question the utility of using absolute criteria, such as the presence of HI titers above 40, for the evaluation of vaccine immunogenicity, especially without the use of reference sera as advocated by Wood *et al.*[99] Improved and standardized assays are necessary for better characterization of influenza vaccine immunogenicity, as well as a better understanding of the relationship between HI titers and clinical protection against influenza virus infection.

Conclusions

We recommend that reporting guidelines for seasonal inactivated influenza vaccine licensure and relicensure studies include characterization of the baseline serostatus of the study population and stratification of post-vaccination responses by the baseline status. In addition, future studies should provide greater detail on the protocols used for the HI assay and standards for how to report on the results of influenza vaccine immunogenicity studies should be established by the research community. Our data confirm that vaccine response in seniors and children may be weaker than in adult populations. More immunogenic vaccines are warranted for these population groups at high risk of severe disease outcomes. Finally, our study also strongly emphasizes the need for more basic research into standardizing HI tests and identifying the most appropriate markers of protective humoral and cell-mediated immunity in different age groups.

Acknowledgements

Grants and financial support

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Univariate regression analysis of subject and vaccine characteristics associated with protective serological responses[*].

Table S2. Univariate regression, sub-analysis of subjects with low titers at baseline.

Table S3. Multivariate regression analysis of subject and vaccine characteristics associated with protective serological responses, including adjuvant categories[*].

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