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## Immune Correlates of Protection Against Influenza in the Human Challenge Model

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### SUMMARY

Intranasal administration of laboratory passaged wild-type influenza viruses to adults induces a mild upper respiratory illness accompanied by nasopharyngeal shedding of virus. A second model has used inoculation of children with attenuated viruses as a surrogate for wild-type infection. Generally, results of studies of antiviral or vaccine approaches in the challenge model have been predictive of efficacy in the real world, but it is recognized that the model does not reproduce naturally acquired illness well and both viral shedding and symptoms in the challenge model are mostly restricted to the upper respiratory tract. Serum antibody plays a major role in protection in this model and it is difficult to infect adults who have serum HI titres of > 1:8. Among adults with lower levels of HI antibody, a variety of protective factors have been described, including nasal IgA and serum NI antibody, and CTL. In some challenge studies, subjects have been protected despite lack of a measurable immune response to vaccination. Because measurement of mucosal antibody has been difficult to standardize, there is not a level that can be considered a cut-off for susceptibility. Use of a consistent method for assessing mucosal IgA between studies may help to derive a target level for vaccination.

## INTRODUCTION

Experimental infection of humans with influenza virus has been used as a model system for exploring the pathophysiology of disease and the efficacy of vaccines and antiviral agents for many years. Generally, the model involves intranasal inoculation with wild-type influenza virus and assessment of subsequent symptoms and nasopharyngeal viral shedding. These types of studies possess unique advantages in evaluation of the relationship between markers of immunity and protection from infection and illness. The time of sampling, the time of exposure, and the dose of inoculum are under the control of the investigator, so that the temporal pattern of virus shedding, symptoms, and immune response can be fully appreciated. In the following review, we present an analysis of the relationship between antibody and infection in a recent study of influenza vaccines and summarize the results of previous studies evaluating the role of serum antibody, mucosal antibody, and cellular immunity in protection against experimental challenge.

## MATERIALS AND METHODS

This paper analyses the relationship between pre-challenge antibody and protection from infection in a challenge study performed to evaluate the protective efficacy of trivalent, cold-adapted influenza vaccine (CAIV) [1]. In that study, healthy young adult subjects were screened for low levels of antibody to either H1N1, H3N2, or influenza B viruses by the standard microtitre haemagglutination-inhibition (HI). Susceptible subjects were randomized to receive either: (i) intranasal placebo and intramuscular placebo; (ii) intranasal cold-adapted vaccine containing approximately  $10^7$  TCID<sub>50</sub> of each of the cold-adapted influenza A/Shangdong/9/93 (H3N2), A/Texas/36/91 (H1N1) or B/Panama/45/90 viruses with intramuscular placebo; or (iii) intranasal placebo with intramuscular trivalent inactivated vaccine containing 15 µg of each of the hemagglutinins of the above viruses. Twenty-eight days after vaccination, subjects were challenged with  $10^7$  TCID<sub>50</sub> of a wild-type virus corresponding to that to which they were initially susceptible upon screening. Nasopharyngeal secretions for assessment of virus shedding were obtained daily for eight days after challenge, and subjects who shed wild type virus, manifested a four-fold or greater serum HI antibody response to challenge, or both were considered infected.

Serum HI antibody to each of the three vaccine viruses was determined by microtitre HI before vaccination, after vaccination and before challenge, and after challenge. Nasal wash for antibody was also obtained at the same time points, and nasal wash HA-specific IgA determined by ELISA as described previously. The results of the ELISA were compared to a standard control, and reported as arbitrary ELISA units after correction for total IgA content. Initial inspection of the data showed that results of the IgA ELISA for antibody to influenza B/Panama/90 were approximately half the levels seen for the two influenza A viruses. Therefore, the IgA units against influenza B were doubled to allow data from all three viruses to be used together.

## RESULTS

Interpretation of this study was complicated by serological evidence that some of the subjects in the placebo group may have been infected with H1N1 or H3N2 viruses between the time of vaccination and challenge, when these viruses were known to be circulating in the community. Therefore, several individuals in the placebo group had levels of pre-challenge serum HI antibody of >1:8 against the relevant challenge virus, and the rates of infection with all three of the challenge viruses were lower than expected. In addition, challenge virus infection was largely asymptomatic, making it difficult to draw conclusions regarding the effect of vaccine (and of pre-challenge antibody) on protection against illness. Nevertheless, as shown in Table 1, the results of the study [1] suggested that both the inactivated and cold-adapted vaccine were protective against experimental infection.

Table 1: Protective efficacy of trivalent cold-adapted (CAIV) and inactivated (TIV) vaccines against experimental wild-type virus infection.

		No. of subjects (%) with the following response to wild-type virus challenge		
Vaccine received	No. of subjects	Virus shedding	Antibody response	Infection <sup>a</sup>
Placebo	31	10 (32)	13 (42)	17 (55)
CAIV	29	7 (24)	6 (21)	9 (31)*
TIV	32	5 (16)	0*	5 (16)*

Previously reported in [1]  
<sup>a</sup> – virus shedding, 4-fold or greater HI antibody response, or both  
 \* P >.05 compared to placebo

Analysis was carried out on 80 of the subjects from this study whose serum HI and nasal IgA results were readily available. The relationship between the relevant pre-challenge nasal and serum antibody and the response to wild-type virus challenge for each of the three vaccine groups is shown in Figure 1. As expected, pre-challenge (i.e., post-vaccination) serum HI titres were highest in those who had received parenteral inactivated vaccine, while nasal antibody levels were somewhat higher in those who had received intranasal cold-adapted vaccine. Placebo recipients had relatively low levels of both serum and nasal antibody. Infected individuals, shown in black, were concentrated in the lower left corner of the graph for all three groups, i.e., were those with relatively low levels of both nasal and serum antibody. Inspection of these graphs suggested that a pre-challenge nasal IgA level of 1,000 units, and a pre-challenge serum HI level of >1:8 provided a rough separation of those who became infected from those who remained uninfected following challenge.

Levels of pre-challenge serum and nasal antibody among infected and uninfected subjects in the three groups are shown in Table 2. Regardless of vaccine received, infected subjects tended to have had lower levels of both nasal IgA and serum HI before challenge than did those who remained uninfected. Because of the relatively small numbers of infected subjects, the difference between infected and uninfected individuals was only statistically significant for serum HI antibody among placebo recipients (P=.003); this antibody presumably is derived from previous wild-type virus infection. In addition, the difference in levels of nasal IgA between infected and uninfected subjects also approached statistical significance in both the CAIV group (P=.088) and placebo group (P=.064).

The presence of pre-challenge nasal or serum antibody above the cutoff was also somewhat more common in those who remained uninfected following challenge. In the placebo group, only 6% of those who were infected had serum HI antibody of >1:8 before challenge, compared to 64% of those who remained uninfected (P=.01), and in TIV recipients, none of the four subjects who became infected had nasal IgA titres of >1000, compared to 56% of those who remained uninfected (P=.01). Almost all TIV recipients had serum HI of >1:8, but titres of >1:32 were seen in only one of the four individuals who became infected, compared to 22 of 25 who remained uninfected (P=.006, Fisher exact test).

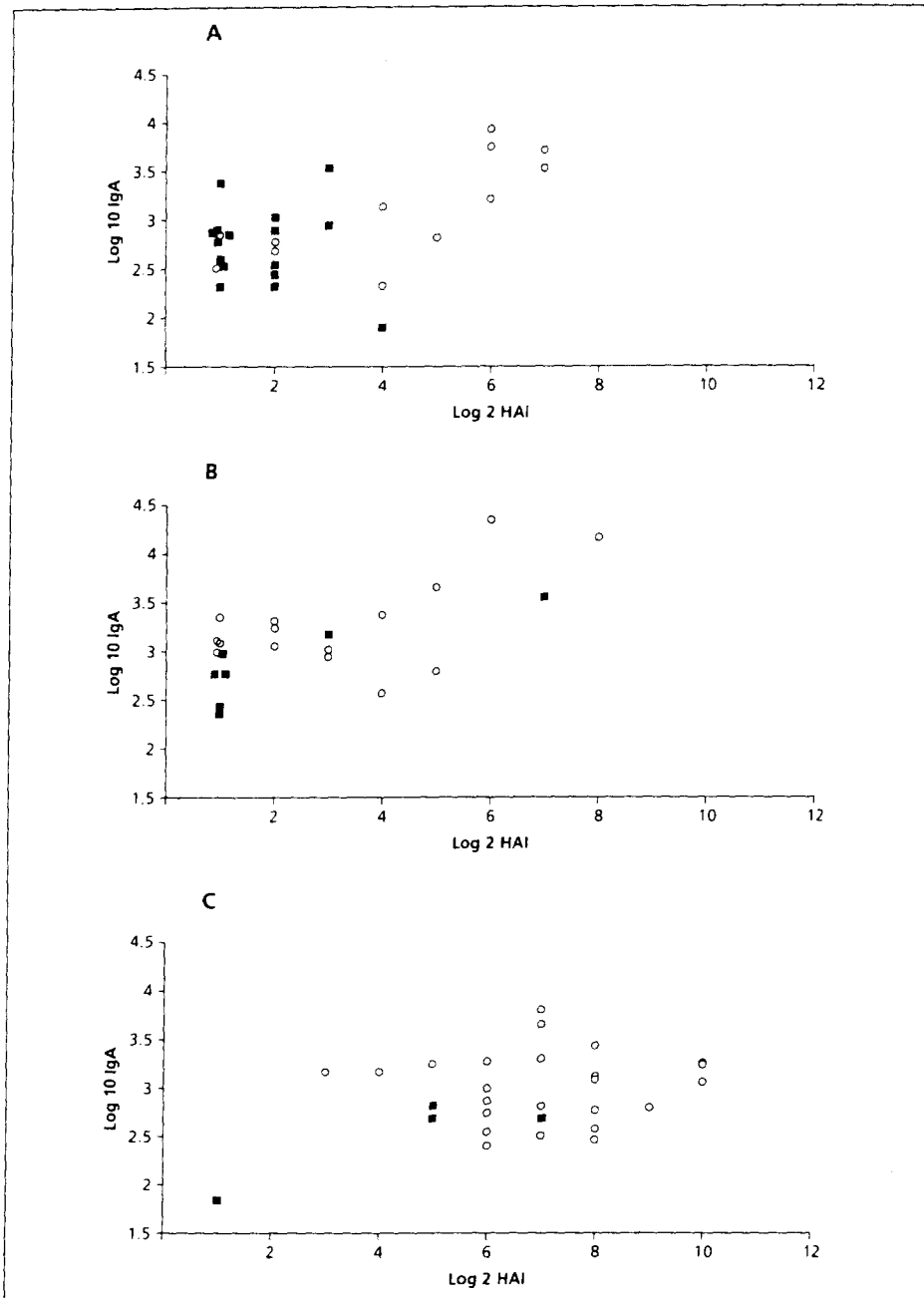


Fig. 1: The relationship between nasal IgA, serum HI and infection in: A, placebo recipients; B, CAIV recipients; C, TIV recipients. Black squares represent individuals with evidence of wild-type virus infection (virus shedding, antibody response, or both) while open circles represent those who remained uninfected after challenge.

Table 2: Relationship between infection and pre-challenge serum and mucosal antibody.

			Pre-challenge titre and percent with titres above cutoff for:			
Vaccine group	Infection	No. of subjects	Nasal IgA		Serum HI	
			Mean log <sub>10</sub> titre (± SD)	% above cutoff	Mean log <sub>2</sub> titre (± SD)	% above cutoff
Placebo	No	12	3.01 ± 0.52	50	4.25 ± 2.26	67
	Yes	16	2.74 ± 0.40*	19	1.75 ± 0.93**	6**
CAIV	No	16	3.23 ± 0.48	69	3.06 ± 2.11	38
	Yes	7	2.87 ± 0.42*	29	2.14 ± 2.27	14
TIV	No	25	3.02 ± 0.37	56	7.24 ± 1.90	96
	Yes	4	2.51 ± 0.45	0*	4.50 ± 2.52	75

\* P < .10, \*\* P < .05, two sample T test, compared to uninfected subjects

# P < .10, ## P < .05, Fisher exact test, compared to uninfected subjects

Note: 6 CAIV recipients, 3 TIV recipients, and 3 placebo recipients from Table 1 did not have both nasal IgA and serum HI results readily available and are not included in this analysis.

The relationship between the presence or absence of serum antibody and subsequent infection is shown in Table 3. In this analysis, subjects were divided into those having both serum and nasal antibody at or below the arbitrary cutoff discussed above (1000 units for IgA and 1:8 for HI), those with only nasal antibody above the cutoff, those with only serum antibody above the cutoff, and those who had both serum and nasal antibody. This analysis showed that either the presence of serum or nasal antibody was protective against infection although the best protection was seen when both elements were present. Similar results were obtained when subjects were divided on the basis of nasal antibody responses to vaccination (> twofold increase in units) and/or serum HI response (=fourfold increase in titre). Among 16 vaccine recipients (CAIV or TIV) who manifested neither response, seven (44%) were infected, compared to three/21 (14%) with a serum antibody response only, none of three of those with only a nasal antibody response, and one/12 (8%) of those with both a serum and nasal response (P = .076, Chi Square).

Table 3: Pre-challenge antibody status and protection.

Pre-challenge antibody status		No. infected/Total no. in those challenged with:			
Serum HI	Nasal IgA	H1	H3	B	ANY
Low	Low	8/10	4/5	6/10	18/25 (72%)
Low	High	1/6	2/4	1/2	4/12 (33%)*
High	Low	1/3	3/7	0/9	4/19 (21%)**
High	High	0/10	1/8	0/6	1/24 (4%)*

Low or high refers to below or above cutoff of >1:8 for serum HI and >1000 units for Nasal IgA, respectively.

\* P = .036; \*\* P = .002; # P < .001 compared to individuals with low HI and low Nasal IgA, Fisher exact test

## DISCUSSION

The results of this study suggest that the presence of either serum or nasal antibody contributes to protection from infection, although protection is not complete. Interpretation of these results is obviously complicated by the low infection rates overall, suggesting that the challenge viruses were not particularly infectious, and the pooling of data from all three challenges needed because of these low infection rates. However, the same trends towards protection with either nasal or serum antibody were seen for each of the three individual challenges, suggesting that pooling of the data was appropriate.

The results are very consistent with observations in other challenge studies made over several years. In fact, the role of serum antibody in protection against experimental infection was quickly recognized in the earliest such studies [2], in which illness was seen almost exclusively in those who lacked pre-challenge neutralizing antibody against the challenge virus. It was subsequently recognized that a critical feature of challenge experiments was pre-screening volunteers for low levels of pre-challenge antibody, to assure uniform susceptibility among groups [3]. Because serum antibody plays such an important role in protection against infection in the challenge model, these studies are almost always conducted in subjects with pre-challenge serum HI antibody levels of 1:8 or less [4,5]. Attempts to infect subjects experimentally with higher levels of serum antibody have not been very successful [6]. Rates of infection with either the wild-type A/California/10/78 (H1N1) or wild-type A/Bethesda/85 (H3N2) viruses were significantly lower in subjects with serum HI antibody of >1:8, and although it was possible to infect some subjects with higher levels of antibody, the level of virus shedding following challenge was lower than in more susceptible individuals [6].

A detailed assessment of the relationship between antibody and protection against infection in the challenge model in adults was performed several years ago [7]. This study was a retrospective analysis of the relationship of pre-challenge serum and mucosal antibodies and resistance to infection with either the influenza A/California/10/78 (H1N1) or A/Washington897/80 (H3N2) viruses in studies designed to evaluate the protective efficacy of cold-adapted influenza vaccine. Subjects were divided into those whose immunity was derived by natural infection (i.e. placebo recipients), those who had received the homologous cold-adapted influenza vaccine, and those who had received inactivated vaccine. A stepwise interactive multiple regression analysis of data from 163 subjects pooled from several trials revealed that serum neuraminidase-inhibiting (NI) antibody was independently associated with protection in all three groups, and nasal HA-specific IgA antibody was associated with protection in those whose immunity was derived from infection (natural or live vaccine). Serum HI antibody was also associated with protection against infection in inactivated vaccine recipients, but not in live vaccine or placebo recipients. This was because placebo recipients were selected for low levels of pre-challenge HI antibody, and live vaccine recipients did not manifest serum HI responses to vaccination. Overall, these results were consistent with the interpretation that either serum or nasal antibody can be protective against nasal challenge.

The role of cytotoxic T lymphocytes (CTL) has also been explored in the challenge model. Shortly after the re-emergence of H1N1 viruses in 1977, McMichael and colleagues evaluated the relationship between influenza-specific CTL memory and protection from infection following experimental challenge of

healthy adults with the influenza A/Munich/1/79 (H1N1) virus [8]. In addition to demonstrating a significant protective effect of pre-challenge serum HI antibody, they found that the presence of CTL at a level of 10% lysis in a standard chromium release assay was associated with a significant reduction in the level of nasal virus shedding following challenge. This effect was particularly pronounced in the subset of subjects with low pre-challenge serum HI and NI antibodies.

The challenge model has not been used to explore immunity in children because it is obviously not possible to inoculate children intentionally with wild-type influenza viruses. However, inoculation of children with attenuated viruses has been used as a surrogate type of challenge experiment. This strategy was used recently to demonstrate the protective efficacy of trivalent cold-adapted influenza vaccine against H1N1 viruses in children [9]. In that study, children who had received either trivalent, cold-adapted influenza vaccine or placebo were challenged between five and nine months later with  $10^7$  TCID<sub>50</sub> of monovalent cold-adapted influenza A/Shenzhen/227/95-like (H1N1) virus. Previous vaccination provided 82% protection against shedding of the challenge virus. Both the presence of any detectable serum HI antibody (i.e., a titre of > 1:4) or any detectable nasal IgA antibody was associated with prevention of nasopharyngeal shedding of the challenge virus. However, previous receipt of cold-adapted vaccine appeared to be somewhat protective even in children who did not have detectable serum HI or nasal IgA antibody. Shedding of the challenge virus was detected in 16 of 35 (46%) placebo recipients who had no HI or IgA antibody prior to challenge and in only 4 of 16 (25%) of antibody negative vaccine recipients ( $P = .22$ ). This observation probably represents a low level of vaccine-induced immunity that was not detected by the relatively insensitive tests used. In fact, serum neutralizing antibody was subsequently detected in 12 of 16 of the HI and IgA negative vaccine recipients but only one of the 35 negative placebo recipients. A similar result has been reported in children challenged with a monovalent cold-adapted A/Washington/897/80 (H3N2) virus, in which infection-induced nasal HA-specific IgA was also shown to be protective against shedding of the challenge virus [10]. In that study, the challenged children were divided into four groups: those with prior natural infection, those naive to influenza, those who had received inactivated vaccine one year before and those that had live attenuated vaccine one year before. The prior recipients of live vaccine and those with prior natural infection were almost completely protected against the vaccine challenge based on an IgA response. The recipients of inactivated vaccine and those naive to influenza had prolonged shedding of vaccine virus in a higher titre on challenge indicating in this setting a superior long term protection of live versus inactivated vaccine as judged by the experimental model [10].

Taken together, these studies support the concept that either nasal or serum antibody can be protective against infection with influenza viruses, and that the levels of antibody required for protection appear to be fairly low. The unique features of the challenge model should be considered in extending these results to natural infection. The viruses used for challenge are somewhat attenuated compared to naturally occurring wild-type viruses, so that the levels of immunity required for protection could be lower than in the real world. In addition, the model uses a nasal route of administration of virus, produces relatively few symptoms, and evaluates nasal shedding of virus only, so that it is possible that these experiments could overestimate the importance of nasal antibody. However, the prediction from the challenge model that live vaccine would be protective against naturally

acquired influenza in the absence of a vigorous serum antibody response to vaccination has been validated in studies in both children [11] and adults [12].

Establishing that nasal antibody is sufficient for protection against influenza may be helpful in considering the licensure of novel vaccines that depend on induction of mucosal immunity for their efficacy. The studies certainly suggest that relying on serum antibody responses as a measure of vaccine acceptability is not uniformly appropriate. Unfortunately, it is not possible to use the existing data to determine a specific level of nasal antibody that should be set as a goal of vaccination. This is because there is no standardized method of collecting nasal secretions or measuring nasal antibodies for influenza, so that it is impossible to compare levels of antibody directly between studies. Further development of such a standard method and application to existing and future studies of experimental infection will be very important if a nasal antibody standard is to be applied for approval of vaccines in the future.

## REFERENCES

- 1 Treanor JJ, Kotloff K, Betts RF, Belshe R, Newman F, Iacuzio D, et al: Evaluation of trivalent, live, cold-adapted (CAIV-T) and inactivated (TIV) influenza vaccines in prevention of virus infection and illness following challenge of adults with wild-type influenza A (H1N1), A (H3N2), and B viruses. *Vaccine* 1999;18:899-906.
- 2 Smorodintseff AA, Tushinsky kMD, Drobyshevskaya AI, Korovin AA, Osetroff AI: Investigation of volunteers infected with the influenza virus. *Am J Med Sci* 1937;194:159-170.
- 3 Sabin AB: Amantadine hydrochloride: analysis of data related to its proposed use for prevention of A2 influenza virus disease in human beings. *JAMA* 1967;200(11):135-142.
- 4 Knight V: The use of volunteers in medical virology. *Prog Med Virol* 1964;6:1-26.
- 5 Knight V, Kasel JA, Alford RH, Loda F, Morris JA, Davenport FM, et al: New research on influenza: studies with normal volunteers. *Ann Intern Med* 1965;62(6):1307-1325.
- 6 Treanor JJ, Roth FK, Betts RF: Use of live cold-adapted influenza A H1N1 and H3N2 virus vaccines in seropositive adults. *J Clin Microbiol* 1990;28:596-599.
- 7 Clements ML, Betts RF, Tierney EL, Murphy BR: Serum and nasal wash antibodies associated with resistance to experimental challenge with influenza A wild-type virus. *J Clin Microbiol* 1986;24(1):157-160.
- 8 McMichael AJ, Gotch FM, Noble GR, Beare PAS: Cytotoxic T-cell immunity to influenza. *N Engl J Med* 1983;309:13-17.
- 9 Belshe RB, Gruber WC, Mendelman PM, Mehta HB, Mahmood K, Reisinger K, et al: Correlates of immune protection induced by live attenuated, cold-adapted, trivalent, intranasal influenza virus vaccine. *J Infect Dis* 2000;181:1133-1137.
- 10 Johnson PR, Feldman S, Thompson JM, Mahoney JD, Wright PF: Immunity to influenza A virus infection in young children: a comparison of natural infection, live cold-adapted vaccine, and inactivated vaccine. *J Infect Dis* 1986;154(1):121-127.
- 11 Belshe RB, Mendelman PM, Treanor J, King J, Gruber WC, Piedra P, et al: The efficacy of live attenuated cold-adapted trivalent, intranasal influenzavirus vaccine in children. *N Engl J Med* 1998;358:1405-1412.
- 12 Nichol KL, Mendelman PM, Mallon KP, Jackson La, Gorse GJ, Belshe RB, et al: Effectiveness of live, attenuated intranasal influenza virus vaccine in healthy, working adults: a randomized controlled trial. *JAMA* 1999;282(2):137-144.

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