Inactivated influenza vaccines. 2. Laboratory indices of protection*

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Summary

The data from our 1968-69 influenza vaccine field trials are analysed and pre-challenge haemagglutinin and neuraminidase serum antibodies are evaluated as indices of protection. Prevention of flu-like disease, fever, confinement to bed, and/or seroconversion to Hong Kong was significantly related to post-vaccine A/Hong Kong/68(H3N2) haemagglutination-inhibition (HI) titres. Prevention of disease was also related, although not significantly statistically in every category, to pre-challenge A/Hong Kong/68 neuraminidase inhibition (NI) titres. The trend was the same regardless of whether the origin of the NI antibody was through A/Aichi/68 or A/Japan/62 vaccines or through pre-Hong Kong influenza infections. In summarizing the data using fever as an index of disease, the attack rate (AR) among volunteers without Hong Kong NI or HI antibody was 45%. Presence of NI antibody, in the absence of HI antibody, significantly reduced the AR to 24%. Those with both NI and HI titres experienced a still lower AR of 14%. Those with HI and NI titres both > 1 : 160 ran little risk of disease, with an AR of 7%.

It is virtually impossible for each new formulation of inactivated influenza vaccine to be evaluated in man under conditions of natural virus challenge. Aside from the formidable logistics and the element of luck inherent in most successful trials, the evaluation of a new vaccine can rarely be undertaken before the onset of the epidemic it is designed to prevent. Because of these problems, interest has continued in developing alternative laboratory procedures which may provide some index of vaccine efficacy. We

recognize, of course, that one, or even multiple, laboratory tests may be a vast oversimplification of the *in vivo* mechanism of protection. Nevertheless, any laboratory finding which can be used as an index of resistance to influenza would be highly useful.

Haemagglutination-inhibition (HI) or neutralizing titres in serum and nasal secretions were suggested very early as correlates of resistance (Francis *et al.*, 1943; Fazekas de St Groth & Donnelly, 1950). The significance of vaccine-induced nasal secretory antibody in protection against influenza is still not clear (Rossen, Kasel & Couch, 1971), but a relationship between serum HI or neutralizing antibody and protection against influenza has been consistently observed (Davenport, 1967). Very little is known about neuraminidase-inhibiting antibody in humans, although studies by Schulman, Khakpour & Kilbourne (1968) in mice and by Allan, Madeley & Kendal (1971) in chickens suggest that antineuraminidase may have some ameliorating effect. In this paper we describe the relationship of post-vaccine serum HI and neuraminidase inhibition (NI) titres to protection during the Hong Kong influenza epidemic of 1968-69.

The data presented here are from the influenza vaccine trials conducted in the Georgia State Prison in 1968-69 (Mostow *et al.*, 1969; Schoenbaum *et al.*, 1969). This trial was referred to in the preceding paper by Dr Mostow. In brief, volunteers were divided into six groups that were equivalent on the bases of age and prevaccination titre to A/Japan/170/62 (H2N2). Volunteers in each group received a single subcutaneous injection of inactivated vaccine purified by zonal ultracentrifugation. Vaccines consisted of 300 and 3000 CCA unit dosages of B/Massachusetts/3/66, A/Japan/170/62 (H2N2), and A/Aichi/2/68 (H3N2) strains. Postvaccine (pre-epidemic) serum specimens were collected from each volunteer 3 weeks after vaccination. Two weeks

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TABLE 1. Percentage attack rates (AR) among influenza vaccinees by illness category

Illness category	Vaccine received*			Efficacy†
	B/Mass/66	A/Japan/62	A/Aichi/68	A/Aichi/68
Influenza-like illness	40	41	24	40
Fever	34	40	18	47
Confinement to bed	25	24	8	68
Serodiagnosis (HI)	59	53	13	78
Illness with positive serodiagnosis (HI)	32	29	4	88
No. in group	68	78	74	

* Recipients of 300 and 3000 CCA vaccines combined.

† Percentage reduction in AR based on AR among B/Mass/66 recipients.

later a sharp outbreak of Hong Kong influenza began in the prison and lasted for 3 weeks. Postepidemic blood specimens were collected 2 weeks after the epidemic, and each volunteer was interviewed from a prepared questionnaire.

All serum specimens were examined for HI antibody (Hierholzer, Suggs & Hall, 1969) to A/Aichi/2/68, A/Japan/170/62 and B/Massachusetts/3/66. Pre- and postvaccine serum specimens were examined for NI antibody (Webster & Pereira, 1968) to the Hong Kong neuraminidase. The recombinant virus A/equine/Prague/56 (Heq1)–Hong Kong/16/68 (N2) served as the neuraminidase source.

From a previous report (Schoenbaum *et al.*, 1969), using influenza B vaccine recipients as a control group, the efficacy rate for the 3000 CCA Aichi vaccine based on reduction of influenza-like illness was calculated to be approximately 63%. The efficacy of the 300 CCA Aichi vaccine was only one-third of that. In this report we have combined the data from the groups receiving 300 or 3000 CCA units of the same vaccines in order to work with larger numbers.

In Table 1 the attack rates among vaccine recipients were calculated on the basis of (a) reported influenza-like illness, (b) fever, (c) confinement to bed with influenza-like illness, (d) serodiagnosis (four-fold or greater increase in Hong Kong HI antibody titre between pre- and postepidemic paired sera) without regard to illness, and (e) serodiagnosis (by HI) with reported clinical illness. These categories were not mutually exclusive. The only attack rates which were significantly lower in any of these categories occurred among the Aichi vaccinees.

The attack rate based on influenza-like illness among the Aichi vaccine recipients (300 and 3000 CCA doses combined) was only 40% of the attack rate of the influenza B control group. This reduction can be considered the crudest index of protection, since influenza-like illness may include illnesses caused by other respiratory viruses as well. Fever ($>37.6^{\circ}\text{C}$) is generally a more specific criterion of influenza than of other respiratory illnesses in adults,

but the percent reduction of attack rates for febrile respiratory illness was only slightly better than the percent reduction for overall influenza-like illness. The attack rate based on confinement to bed with an influenza-like illness was 68% lower in the Aichi vaccine recipients than in the control B group. The occurrence of four-fold rises in antibody titre against the epidemic strain was 78% lower in Aichi recipients than in the control group. However, serologic evidence of infection is not synonymous with illness. Nearly a third of the B/Mass vaccinees who had a four-fold rise in antibody titre against the epidemic strain reported no influenza-like symptoms during the 3 week observation period.

In theory, a reduction in the amount of illness proven by serodiagnosis to have been causally related to the Hong Kong virus should provide the best measure of efficacy. Vaccine efficacy in this category was 88%. However, this high rate may be misleading. It suggests that there were many more 'nonflu' illnesses among the Aichi vaccinees than any other group. This observation is examined more closely in Table 2, which shows the percentage of volunteers in each disease category with no serologic evidence of infection with influenza. The percentages among the control B/Mass vaccinees were small; 6% with influenza-like illness, 3% with fever, and 3% confined to bed. The percentages among the A/Japan vaccinees were higher and among the Aichi vaccinees were two to three times higher than the percentages among the B vaccine recipients.

TABLE 2. Percentage incidence of 'noninfluenza' respiratory illness among vaccinees*

Illness category	Vaccine received†		
	B/Mass/66	A/Japan/62	A/Aichi/68
Influenza-like illness	6	11	16
Fever	3	9	10
Confinement to bed	3	4	7
No. in group	64	77	70

* Defined as individuals with influenza-like illness in the absence of a $\geq$ four-fold rise in HI antibody titre to A/Aichi.

† Recipients of 300 and 3000 CCA vaccines combined.

An increased number of influenza-like illnesses which cannot be diagnosed by conventional laboratory tests is not uncommonly observed in influenza vaccine field trials. Edmondson *et al.* (1971) recently described their 1968–69 study in which the Aichi vaccine efficacy rate based on laboratory data exceeded 90%, yet the total number of clinical illnesses was not reduced. The author offered several possible explanations for this phenomenon. First, influenza was prevented by vaccine but was replaced by illnesses caused by other respiratory viruses. Second, all groups suffered equally from 'colds', but the nonprotected individuals had influenza superimposed and therefore both illnesses were recorded as a single event.

The studies of Rapmund *et al.* (1959) suggest a third explanation. They found that some recipients with high vaccine-induced serum antibody titres became ill upon subsequent exposure to influenza. These individuals shed virus but failed to demonstrate a further four-fold rise in antibody titre in either the HI or complement fixation (CF) test.

Efficacy based on serodiagnosis then, indicates only that the number of four-fold rises in antibody titre was reduced; it does not indicate how the reduction occurred or how much illness was prevented. Thus, in field studies of this type a precise figure for efficacy cannot be assigned. We concede that efficacy of the Aichi vaccine for preventing influenza-like illness was greater than 40%, but we also submit that the 88% based on serologic findings is unrealistic.

Because definition of clinical influenza is imprecise, some errors will inevitably enter into our observations on the relationship between protection from disease and the presence of neuraminidase and haemagglutinin antibodies. However, any possible bias arising from such errors would suggest no relationship and therefore would only serve to strengthen findings that the presence of antibodies indicates protection.

The relationship of A/Aichi serum HI titre to attack rates for each of the illness categories is shown in Fig. 1. Subjects with Aichi HI titres ≥ 20 were exclusively Aichi vaccine recipients. In each of the illness categories the relationship between post-vaccine Aichi HI titres ≥ 80 and reduction of attack rate was statistically significant ($P = < 0.05$). This would be expected. However, we cannot rule out any possible effects of antibody to the Aichi neuraminidase.

Figure 2 shows a significant ($P = < 0.05$) relationship between postvaccine Aichi NI titres of ≥ 160 and prevention of fever and confinement to bed. A significant reduction ($P = < 0.05$) in influenza-like illness occurred only with an NI titre ≥ 1000 . Anti-Aichi neuraminidase serum antibody could have originated in these volunteers from three possible

FIG. 1. All vaccinees, postvaccine, pre-epidemic serum haemagglutination inhibition (HI) titres to A/Aichi/2/68 (H3N2) and their relationship to attack rates based on (a) serodiagnosis without regard to illness (AR seroconversion); (b) fever (AR fever); (c) confinement to bed (AR bed); and (d) influenza-like disease (AR flu). Numbers in columns are the numbers of vaccinees in each titre category.

sources: first, vaccination with Aichi—and in this group we cannot eliminate the additional presence of Aichi HI serum antibody; second, vaccination with Japan/170, since the neuraminidase antigens of Aichi and Japan/170 are related; and third, prior natural infection with pre-Hong Kong influenza A strains which may also have had neuraminidase antigens related to Aichi.

Regardless of the source of neuraminidase antibody, some protective effect is suggested by its presence (Fig. 3). The numbers of volunteers in three vaccine groups are small, and therefore the differences in attack rates among those with and without NI antibody in the B/Mass and Japan/170 vaccine groups were not statistically significant ($P = < 0.05$).

FIG. 2. All vaccinees, postvaccine, pre-epidemic serum neuraminidase inhibition (NI) titres to A/Aichi/2/68 [A/equine/Prague/56 (Heq1) – Hong Kong/16/68(N2)] and their relationship to attack rates based on (a) serodiagnosis without regard to illness (AR seroconversion); (b) fever (AR fever); (c) confinement to bed (AR bed); and (d) influenza-like disease (AR flu). Numbers in columns are the numbers of vaccinees in each titre category.

However, if the B/Mass and Japan/170 vaccine groups are combined, which still eliminates any possible effect of Aichi HI antibody, protection against fever and against confinement to bed becomes significant ($P = < 0.05$).

In summary, volunteers without serum antibodies to either Aichi haemagglutinin or the neuraminidase antigens had nearly a 50% chance of becoming ill and over a 30% chance of confinement to bed during the Hong Kong epidemic (Fig. 4). Volunteers with serum antibodies to only the Aichi neuraminidase had less chance of becoming ill or being confined to bed, but the differences in attack rates were significant ($P = < 0.05$) only for fever. If antibodies to both the haemagglutinin and neuraminidase were present, the

FIG. 3. Postvaccine, pre-epidemic serum neuraminidase inhibition (NI) antibody to A/Aichi/2/68 [A/equine Prague/56 (Heq1)–Hong Kong/16/68 (N2)] according to vaccine group. The relationship of the presence of antibody to attack rates based on (a) serodiagnosis without regard to illness (AR seroconversion); (b) fever (AR fever); (c) confinement to bed (AR bed); and (d) influenza-like disease (AR flu). Numbers in columns are the numbers of vaccinees in each titre category.

chance of becoming ill was considerably less, and the differences in attack rates were significant ($P = < 0.05$) for all illness categories.

If haemagglutinin and neuraminidase serum antibodies were present at titres equal to or greater than 160, chances of an influenza-like illness in this epidemic were 13%, chances of having fever were 7%, and chances of becoming sick enough to be confined to bed were nil. In effect, then, those Aichi vaccine recipients who developed HI and NI titres of 160 or greater had a 72% reduction in influenza-like illness, an 85% reduction for fever, and a 100% reduction for confinement to bed as compared to those without detectable titres. These figures are even more impressive when we consider that they were based

FIG. 4. Postvaccine, pre-epidemic serum haemagglutination inhibition (HI) and neuraminidase inhibition (NI) antibody to A/Aichi/2/68. The relationship of the presence of antibody of any titre (+) and the relationship of both HI and NI titres ≥ 160 to attack rates based on (a) serodiagnosis without regard to illness (AR seroconversion); (b) fever (AR fever); (c) confinement to bed (AR bed); and (d) influenza-like disease (AR flu). Numbers in columns are the numbers of vaccinees in each titre category.

entirely on raw clinical data. No adjustments were made for influenza-like illnesses which may have been caused by other viruses, and no adjustments were made for possible malingerers. Whereas it is true that recipients of 300 and 3000 CCA Aichi vaccines were able to achieve HI and NI titres of 160 or greater, such titres were produced uniformly only by the recipients of 3000 CCA units.

This study has confirmed previous reports (Salk, Menke & Francis, 1945) of an apparent association

of HI titre with protection against influenza. Because of the composition of the vaccines, we were unable to analyse the relationship of HI antibody to protection in the absence of NI antibody. However, we have shown that neuraminidase antibodies may be associated with amelioration or possible protection against disease. We have also shown, without implying a causal relationship, that the ability of inactivated influenza vaccine to stimulate high-titred neuraminidase and haemagglutinin antibody was a reasonable assessment of its protective efficacy during the Hong Kong epidemic of 1968–69.

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