

Homologous and heterologous antibody responses to subunit influenza virus vaccine

BY BRIAN J. FEERY AND M. G. EVERED

*WHO National Influenza Centre, Commonwealth Serum Laboratories,
Parkville 3052, Australia*

AND KATHLEEN HAYES

*Department of Pediatrics, Monash University,
Melbourne 3000, Australia*

(Received 28 November 1977)

SUMMARY

In a group of 32 adult volunteers given subunit influenza virus vaccine containing 250 international units (i.u.) of A/Victoria/3/75, 250 i.u. of A/Scotland/840/74 and 300 i.u. of B/Hong Kong/8/73, there were substantial increases in the geometric mean homologous haemagglutination-inhibiting (HI) antibody titres. There was also substantial boosting of the antibodies to the earlier variants of the Hong Kong (H3N2) series and to a later variant of the Asian (H2N2) series. There was no boosting of antibodies to the A/FM/1/47 strain, a representative member of the H1N1 series, but two individuals showed substantial rises to A/PR/8/34 (H0N1).

There were increases in the HI titre of antibodies cross reactive with two recent isolations, A/Texas/1/77, and A/Victoria/35/77, but the majority of vaccinees failed to reach antibody titres likely to be protective against such strains.

INTRODUCTION

The heterologous boosting of antibody to previously circulating influenza strains after infection with later strains was reported by Francis, Davenport & Hennessy in 1953. Francis (1960) proposed the doctrine of original antigenic sin to explain the fact that humans responded with higher titres against the strain that provided their first childhood experience of influenza than to strains causing subsequent infections. It was observed also that the response to vaccination behaved in a similar manner. Francis believed that the first infection with influenza left an indelible immunological imprint on the individual, and that subsequent infections boosted the height of the immune response to the first infection. This boosting resulted from a sharing of antigens between succeeding epidemic strains of influenza. It was shown later that there are antigenic determinants common to the A/Swine (H5W1N1), the A0 (H0N1) and the A1 (H1N1) series, the virus subtypes which had been circulating in the four decades before the development of this theory (Webster, 1966; Dowdle *et al.* 1969).

In later studies in animals (Virelizier *et al.* 1974), and in man (Morita, Suto & Ishida, 1972), heterologous antibody boosting has been demonstrated within the A/Swine, A0, and A1 series, and within the 'Asian' (H2N2) and 'Hong Kong' (H3N2) series, but not between these two groups.

The present study was undertaken to examine the antibody responses after immunization with a vaccine containing the two recent epidemic A strains, A/Victoria/3/75 and A/Scotland/840/74, and the representative B strain, B/Hong Kong/8/73. It is important to gain a greater understanding of the antibody responses to vaccines, because it is possible that the production of antibodies common to previously circulating strains may impair the development of immunity to the relevant epidemic strains. This may be one of the reasons for the varied results of previous protection studies with inactivated vaccines.

It was also decided to measure the cross-reacting antibody responses induced by the vaccine to the variant A/Texas/1/77 which has been circulating in Australia for some months, and to a more recently isolated variant, A/Victoria/35/77. Such information could be useful in determining the advisability of including the newer antigenic variants in an inactivated vaccine for the following season.

MATERIALS AND METHODS

The vaccine used in the study was an inactivated influenza virus subunit vaccine (Commonwealth Serum Laboratories) containing 250 i.u. of A/Victoria/3/75, 250 i.u. of A/Scotland/870/74 and 300 i.u. of B/Hong Kong/8/73. The A/Victoria/3/75 strain was represented by a recombinant virus obtained from Professor R. G. Webster, St. Jude Children's Research Hospital, Memphis, and the A/Scotland/840/74 was represented by a recombinant strain, MRC 12, obtained from the World Influenza Centre, London. These recombinant strains had the surface antigens of the epidemic strains, and the high growth characteristics of a laboratory strain of A/PR/8/34.

The participants in the study were 32 adult human volunteers aged between 24 and 58 years, with a median age of 31 years. Vaccine was administered by deep subcutaneous injection.

Blood samples were taken from all volunteers on the day of vaccination, and four weeks later. Serum samples were separated and stored at -20°C .

Antibody against the vaccine strains was measured by the haemagglutination-inhibition (HI) test using standard microtitre techniques (Palmer *et al.* 1975). Tests were also undertaken with the previously circulating epidemic H3N2 strains, A/Pt Chalmers/1/73 and A/Nth Territory/60/68, the epidemic H2N2 strains, A/Sth Australia/64, and A/Sth Australia/57; the earlier H1N1 strain A/FM/1/47, and the H0N1 strain A/PR/8/34. Subsequently, tests were undertaken with A/Texas/1/77 (H3N2), a strain widely circulating in 1977, and with a more recent isolate from Victoria, A/Victoria/35/77 (H3N2).

Sera without antibody detectable at the lowest tested dilution (1/10) were assumed to be positive at a dilution of 1/5 for the purposes of calculating geometric mean titres.

Table 1. *Homologous HI antibody responses*

Strain	Geometric mean titre*			Individual increases in titre		
	Pre	Post	Increase	< 2-fold	2- to 4-fold	≥ 4-fold
A/Victoria/3/75	9.1	84	9.2:1	0	5	27
A/Scotland/840/74	14	183	13.1:1	1	1	30
B/Hong Kong/8/73	23	67	2.9:1	12	6	14

* Reciprocal of antibody titre.

Table 2. *Homologous and heterologous HI antibody responses*

Strain	Type	Geometric mean titres*			
		Pre	Post	Increase in titres	Number with ≥ 4-fold rise
A/Victoria/3/75	H3N2	9.1	84	9.2:1	27/32
A/Scotland/840/74	H3N2	14	183	13.1:1	30/32
A/Pt Chalmers/1/73	H3N2	24	323	13.5:1	31/32
A/Nthn Territory/60/68	H3N2	18	222	12.3:1	30/32
A/South Australia/64	H2N2	6.5	54	8.3:1	22/32
A/South Australia/57	H2N2	17	26	1.5:1	4/32
A/FM/1/47	H1N1	12	13	1.1:1	0/32
A/PR/8/34	H0N1	7.4	11.1	1.5:1	2/32

* Reciprocal of antibody titre.

The viruses were obtained from the collection of the WHO National Influenza Centre at the Commonwealth Serum Laboratories.

RESULTS

The homologous HI antibody responses to the vaccine are shown in Table 1. It can be seen that there were substantial increases in the geometric mean (GM) HI titre to the A/Victoria/3/75 and A/Scotland/840/74 components, and a 2.9-fold increase in the geometric mean titre to B/Hong Kong/8/73. Twenty-seven of 32 volunteers showed 4-fold increments in antibody to A/Victoria/3/75 and 30 of 32 to A/Scotland/840/74.

The increase in the titre to the influenza B component was lower than to either of the A components, but 14 of 32 showed 4-fold or greater increases in titre, and six showed 2- to 4-fold increases. Twenty-three had final HI titres ≥ 40.

A comparison of the geometric mean homologous and heterologous HI antibody responses to the influenza A components of the vaccine, and the number of volunteers achieving a 4-fold increase in HI titre are shown in Table 2. It can be seen that there is substantial homologous and heterologous antibody boosting to the tested variants of the 'Hong Kong' H3N2 series, both in the geometric mean titre and in a high proportion of individuals who showed a 4-fold increase in titre. Twenty-two volunteers showed substantial boosting to A/South Australia/64, a later variant of the H2N2 series, but only four showed 4-fold increases in titre to the earlier H2N2 strain A/South Australia/57.

Table 3. *Heterologous HI antibody responses to recent isolations*

Strain	Geometric mean titre			Individual increases in titre		
	Pre	Post	Increase	< 2-fold	2- to 4-fold	> 4-fold
A/Texas/1/77 (H3N2)	5.6	18	3.2:1	4	11	17
A/Victoria/35/77 (H3N2)	6	15	2.5:1	6	14	12

There was no boosting in the antibody titre to the H1N1 strain A/FM/1/47, but two individuals showed substantial rises to A/PR/8/34.

The responses to the more recent variants, A/Texas/1/77 and A/Victoria/35/77, are shown in Table 3. It can be seen that there was little pre-existing antibody to these strains in 1976. Post-vaccination GM titres showed a 3.2-fold increase to A/Texas/1/77 and 2.5-fold to A/Victoria/35/75, but only 10 reached a titre of ≥ 40 to A/Texas/1/77 and five to A/Victoria/35/77.

DISCUSSION

The results of this study confirm previous reports that substantial homologous antibody responses follow one dose of subunit vaccine in adults (Feery *et al.* 1976, 1977). The study also demonstrates the fact that substantial increases in titre occur to each of the vaccine components at the dosage used in this trial. The response to the influenza B component of the vaccine was lower than the response to the influenza A components as in previous studies (Feery *et al.* 1976, 1977), but 23 of 32 achieved a titre ≥ 40 . In a protection study in Australia in 1970, Gill *et al.* (1971) found little or no vaccine-induced protection against influenza B infection, but the results of this study suggest that protective antibody titres have been induced in this group (Hobson *et al.* 1972).

The substantial heterologous HI antibody responses induced by the vaccine involved all the tested previous variants of the Hong Kong H3N2 series and a later variant of the H2N2 series A/South Australia/64. Whereas 22 of 32 vaccines showed 4-fold increases in antibody to the A/South Australia/64 strain, only four responded with similar increases to the early H2N2 strain, A/South Australia/57. This difference could not be related to the age of the vaccinees in this particular study. In a previous study in third-year medical students in which the A component of a similar vaccine was A/Victoria/3/75, a similar boosting to the earlier variants of the Hong Kong H3N2 series was demonstrated, but there was minimal boosting to the H2N2 strains (Feery *et al.* 1977). It is possible that the difference between these studies resulted from the difference in the age and experience of the participants, but it is also possible that it is due to a closer antigenic relationship between the A/Scotland/840/74 component and the earlier Asian strains. Dowdle *et al.* (1972) demonstrated an antigenic relation between the earlier H3N2 strains and the H2N2 strains which was independent of the neuraminidase; and previous studies (Morita *et al.* 1972; Marine, Workman & Webster, 1969), have shown heterologous boosting between H3N2 and the H2N2 series.

The failure to boost antibody titres to the H1N1 strains in an age group expected

to have had experience with such strains has also been demonstrated previously. The unexpected boosting of antibody to the A/PR/8/34 in two of the group is difficult to explain. A similar unexpected antibody boosting also occurred in recent vaccine studies in the United States when a small percentage of vaccinees developed antibodies to A/Jersey/8/76 (HSwN1) after immunization with vaccines containing H3N2 strains (Noble *et al.* 1977).

The heterologous boosting of antibodies to the H3N2 Hong Kong variants and to the Asian H2N2 variants, but not to the earlier H1N1 strain, confirms the findings in later studies (Webster, 1966; Morita *et al.* 1972; Virelizier *et al.* 1974), that the boosting of antibody to previous strains depends as much on antigenic relationships between the viruses as on the first infection experienced by the individual (Francis, 1960).

One of the important aspects of this study is the demonstration of low cross-reacting antibody responses to the newer variants A/Texas/1/77 and A/Victoria/35/77 in comparison with the responses to the vaccine strains and to previous variants. The geometric mean titres before and after vaccination were low, but 10 of 32 volunteers developed post-vaccination HI titres of 1/40 to A/Texas/1/77, and five developed such titres to A/Victoria/35/77. Although some studies have suggested that inactivated vaccines provide good heterologous protection when a new antigenic variant appears (Hoskins *et al.* 1973), other authors have demonstrated little or no protection (Gill, 1973; Ruben, Johnston & Streiff, 1974). Hobson *et al.* (1972) have shown that there is a good correlation between the titre of HI antibody and the degree of protection. The data in this study suggest that it is necessary to review the formulation of inactivated vaccines in order to provide optimal protection when new variants appear.

REFERENCES

- DOWDLE, W. R., COLEMAN, M. T., HALL, E. C. & KNEZ, V. (1969). Properties of the Hong Kong influenza virus. Antigenic relationship of the Hong Kong virus haemagglutinin to that of other human influenza viruses. *Bulletin of the World Health Organization* **41**, 419-24.
- DOWDLE, W. R., MARINE, W. M., COLEMAN, M. & KNEZ, V. (1972). Haemagglutinin relationships of Hong Kong (H3) and Asian (H2) influenza strains delineated by antigen-specific recombinants. *Journal of General Virology* **16**, 127-34.
- FEERY, B. J., CHEYNE, I. M., HAMPSON, A. W. & ATKINSON, M. I. E. (1976). Antibody response to one and two doses of influenza virus subunit vaccine. *Medical Journal of Australia* **1**, 186-9.
- FEERY, B. J., HAMPSON, A. W., FORSYTH, J. R. L. & EVERED, M. G. (1977). Effect of dose on antibody response to subunit influenza vaccine. *Medical Journal of Australia* **2**, 324-7.
- FRANCIS, T. JR., DAVENPORT, F. M. & HENNESSY, A. V. (1953). Epidemiological recapitulation of human infection with different strains of influenza virus. *Transactions of the Association of American Physicians* **66**, 231-8.
- FRANCIS, T. (1960). On the doctrine of original antigenic sin. *Proceedings of the American Philosophical Society* **104**, 572-8.
- GILL, P. W., BABBAGE, N. F., GUNTON, P. E., FLOWER, W. & GARRETT, D. A. (1971). Efficacy of influenza virus subunit vaccine in 1970. *Medical Journal of Australia* **2**, 1005-8.
- GILL, P. (1973). Influenza in general practice. *Medical Journal of Australia Supplement* **1**, 24-8.
- HOBSON, D., CURRY, R. L., BEARE, A. S. & WARD-GARDNER, A. (1972). The role of serum haemagglutination-inhibiting antibody in protection against challenge infection with influenza A₂ and B viruses. *Journal of Hygiene* **70**, 767-77.

- HOSKINS, T. W., DAVIES, J. R., ALLCHEN, A., MILLER, C. L. & POLLOCK, T. M. (1973). Controlled trial of inactivated influenza A vaccine containing the A/Hong Kong strain during an outbreak of influenza due to the A/England/42/72 strain. *Lancet* **ii**, 116–20.
- MARINE, W. M., WORKMAN, W. M. & WEBSTER, R. G. (1969). Immunological interrelationships of Hong Kong, Asian and Equi 2 influenza viruses in man. *Bulletin of the World Health Organization* **41**, 475–82.
- MORITA, M., SUTO, T. & ISHIDA, N. (1972). Antigenic memory in man in response to sequential infections with influenza A virus. *Journal of Infectious Diseases* **126**, 61–8.
- NOBLE, G. R., KAYE, H. S., KENDAL, A. P. & DOWDLE, W. R. (1977). Age-related heterologous antibody responses to influenza virus vaccination. *Journal of Infectious Diseases* **136**, Supplement, 686–92.
- PALMER, D. F., COLEMAN, M. T., DOWDLE, W. R. & SCHILD, G. C. (1975). Advanced laboratory techniques for influenza diagnosis. *Immunology Series No. 6, US Department of Health, Education and Welfare*, 26–45.
- RUBEN, F. L., JOHNSTON, F. & STREIFF, E. J. (1974). Influenza in a partially immunized aged population. *Journal of the American Medical Association* **230**, 863–6.
- VIRELIZIER, J. L., POSTLETHWAITE, R., SCHILD, G. C. & ALLISON, A. C. (1974). Antibody responses to antigenic determinants of influenza virus haemagglutinin. II. *Journal of Experimental Medicine* **140**, 1571–8.
- WEBSTER, R. G. (1966). Original antigenic sin in ferrets. The response to sequential infections with influenza viruses. *Journal of Immunology* **97**, 177–83.