

A graded-dose study of inactivated, surface antigen influenza B vaccine in volunteers: reactogenicity, antibody response and protection to challenge virus infection*

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SUMMARY

One hundred and nineteen volunteers were divided into five groups, and each volunteer inoculated subcutaneously with an aqueous subunit B/Hong Kong/73 vaccine containing 40, 20, 10, or 5 μg of HA or saline alone in a 0.5 ml volume. The incidence of reactions was recorded 24 h after inoculation. One month following immunization the serum HI antibody to B/Hong Kong/73 virus was measured; each volunteer was inoculated intranasally with live, attenuated influenza B (RB77) virus; and the incidence of infection by the challenge virus was determined by HI antibody response.

The results showed that the incidence of reactions to all doses of vaccine were relatively low, the severity mild, and the duration short. However, the incidence of reactions was highest for those given 40 μg HA and least for those given 5 μg HA. The serum HI antibody responses to vaccine showed a dose-response relationship. For volunteers given 40 μg HA, 22 (96%) showed a fourfold rise in antibody titre and all volunteers had antibody titres of > 40 following immunization: for volunteers given 5 μg HA the g.m.t. increased from 16.6 to 86.1; and for those given 10 and 20 μg HA the response was intermediate. Following challenge, the lowest incidence of infection was seen in volunteers given the highest dose of vaccine. However, all doses of vaccine induced some protection against challenge virus infection, and the incidence of infection was directly related to the serum antibody titre at the time of challenge. The 50% protection titre of serum HI antibody was estimated as 15 to 20.

INTRODUCTION

Extensive studies of influenza A viruses carried out over the last 50 years have established a large body of information on the properties and epidemiology of these

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agents. In addition, several types of influenza A vaccine have been developed and tested, and shown to protect against natural disease (Stuart-Harris & Schild, 1976; Kilbourne, 1975). Studies carried out on influenza B virus vaccines have been less extensive, and it has been assumed that the results obtained in studies with influenza A vaccines also apply to influenza B virus and vaccines. This may not be fitting or advisable, since influenza A and B viruses have distinctive properties. In particular, it has been reported that the haemagglutination-inhibiting antibody response to infection or immunization with influenza B viruses is not a good index of immunity to reinfection (La Montagne, 1980). Because of this and other differences, the practice of using data obtained from experiments with influenza A to standardize influenza B virus vaccines is questionable, and it is necessary to establish the formulation of influenza B vaccines specifically and independently.

In the present study, the reactogenicity and serum haemagglutination-inhibiting (HI) antibody-response of volunteers to graded doses of aqueous influenza B vaccine was evaluated. In addition, 1 month after immunization all volunteers were challenged intranasally with live attenuated influenza B vaccine to determine the susceptibility of the volunteers to infection.

MATERIALS AND METHODS

Virus and virus vaccine

Purified aqueous subunit vaccine was kindly prepared and supplied by Sandoz Virusforschungsinstitut, Vienna. Briefly, influenza virus B/Hong Kong/73 grown in embryonated eggs was harvested, pooled and purified by rate-zonal centrifugation; inactivated virus particles were treated with cetyl-trimethyl bromide (CTAB) and the released haemagglutinin (HA) and neuraminidase (NA) subunits purified in linear sucrose gradients (Bachmayer, 1975). The subunit preparation was diluted in saline to give vaccine containing concentrations of 40, 20, 10 and 5 μg of HA per 0.5 ml dose; these concentrations of HA were determined by the single radial diffusion test (Schild, Wood & Newman, 1975; Wood *et al.* 1977). A control solution without virus antigen was also prepared. All vaccines were supplied under code.

Live attenuated influenza B virus RB77, a recombinant of cold-adapted B/Ann Arbor/66 and B/Tecumseh/77 and bearing the HA and NA antigens of the latter virus, were used for virus challenge studies; this virus strain is very similar to influenza virus B/Hong Kong/73. The virus was supplied in ampoules by Sandoz Virusforschungsinstitut, Vienna, and used without passage in this laboratory. The virus had a titre of $10^{7.5}$ 50% egg-infectious doses (EID₅₀)/per ml, and volunteers were each inoculated intranasally and dropwise with 0.5 ml of virus suspension, as described previously (Potter *et al.* 1977; Jennings *et al.* 1978).

Experimental procedure

One hundred and nineteen student volunteers aged 18–19 years were recruited from the Science and Medical Faculties of the University of Sheffield; all were in good health, and none had allergy to eggs or egg products. A specimen of blood was obtained from each volunteer and the haemagglutination-inhibition (HI) antibody titre to influenza virus B/Hong Kong/73 determined. From these results

Table 1. Incidence of reactions to immunization with aqueous subunit influenza B/Hong Kong/73 vaccine

No. of volunteers	Vaccine (μg HA/dose)	Incidence of reactions (%)		
		Local* reactions	Local† pain	Systemic‡ reactions
23	40	24	19	—
24	20	19	16	—
24	10	11	12.5	—
25	5.0	9	7	—
23	Saline	4	6	—

* Redness, swelling, itching.

† Pain on pressure, pain on contact, continuous pain.

‡ Sweating/shivering, insomnia, headache, muscle pain.

the volunteers were divided into five groups, each containing approximately equal numbers of volunteers with similar titres of serum HI antibody. Volunteers in the first group were inoculated by the deep subcutaneous route with 0.5 ml of aqueous subunit B/Hong Kong/73 vaccine containing 40 μg HA; and volunteers in the other four groups received 20 μg HA, 10 μg HA, 5 μg HA, or saline in a 0.5 ml volume by the same route. The vaccines were all administered under code, and neither the volunteers nor the inoculator knew which vaccine was given. Reactions to immunization were determined from a questionnaire completed by the volunteer 24 h after immunization (Jennings *et al.* 1978). No reactions were reported after 48 h, but further questionnaires were not completed. One month after immunization a second blood sample was obtained from each volunteer to determine the serum HI antibody response to vaccine. Subsequently, each volunteer was inoculated intranasally with live attenuated RB77 virus. This virus vaccine in a 0.5 ml volume was inoculated intranasally and dropwise to volunteers lying flat on their backs with neck extended. A final blood sample was obtained from each subject 1 month after challenge to determine the serum HI antibody response to the challenge virus infection.

Haemagglutination-inhibiting (HI) antibody titrations

Serum samples from volunteers were coded and forwarded to the National Institute of Biological Standardization and Control for haemagglutination-inhibiting (HI) antibody assays: these were carried out by standard tests following treatment with cholera filtrate (Philips-Duphar-B.V.) for 18 h at 37 °C, and subsequently heating at 56 °C for 1 h (W.H.O., 1953). Reference antisera were included in each test to standardize the test sensitivity.

RESULTS

Response to immunization

Reactions

The incidence of reactions to subcutaneous inoculation with aqueous subunit influenza B/Hong Kong/73 virus vaccine were assessed at 24 h post-inoculation

Table 2. Serum HI antibody response of volunteers to immunization with aqueous surface antigen influenza B/Hong Kong/73 vaccine

Dose of vaccine (μg HA)	No. of volunteers	Serum	Serum HI antibody titre to B/Hong Kong/73 virus					
			< 10	10-20	30-40	60-120	160-480	> 480
40	23	Pre-immun.	17	2	2	2	—	—
		Post-immun.	—	—	1	3	14	5
20	24	Pre-immun.	16	2	4	1	1	—
		Post-immun.	1	2	—	10	7	4
10	24	Pre-immun.	12	3	5	1	3	—
		Post-immun.	2	3	2	4	9	4
5	25	Pre-immun.	12	5	—	7	1	—
		Post-immun.	1	4	2	10	6	2
None	23	Pre-immun.	15	1	3	3	1	—
		Post-immun.	15	1	2	4	1	—

and were analysed under three headings: these were local reactions of redness, swelling or itching; local pain which was continuous or pain on contact or pressure; and systemic reactions of fever, headache, insomnia, or muscle pain. The results were expressed as the number of reactions reported as a percentage of the total number of possible reactions, and are shown in Table 1. For volunteers inoculated with 40 μg HA of virus vaccine, 23.6% reported local reactions, 19.4% reported local pain, and none experienced systemic reactions. For volunteers given 5.0 μg HA of virus vaccine the incidence of local reactions was 9.3%, local pain 6.7% and no systemic reactions were reported. The results for volunteers given 10 μg HA or 20 μg HA of virus vaccine were intermediate; and the incidence of reactions for the four doses showed a clear dose-response relationship for both local reactions and local pain (Table 1). In all cases reported reactions were mild in nature, of short duration, and did not persist beyond 48 h post-inoculation.

Serum haemagglutination-inhibiting (HI) antibody response to immunization

The titres of haemagglutination-inhibiting (HI) antibody present in the serum of the volunteers before and after immunization with various doses of aqueous subunit influenza B/Hong Kong/73 vaccine are shown in Table 2. Of the volunteers given 40 μg HA of vaccine, 21 (91%) had serum HI antibody titres of ≤ 40 prior to immunization, and 22 (96%) had antibody titres of ≥ 60 following immunization: for volunteers given 5 μg of vaccine, 17 (68%) had antibody titres of $\leq 1:40$ prior to immunization, and 18 (72%) had antibody titres of ≥ 60 following immunization. The results obtained for volunteers given 10 μg HA or 20 μg HA show intermediary responses. Thus, significant increases in serum HI antibody titre were observed following immunization with all doses of vaccine (Table 2). None of the 23 volunteers given saline experienced a significant rise or fall in serum HI antibody titre to influenza B/Hong Kong/73 virus during the period of observation. This indicated that no natural infections by influenza B virus took place in the study population during the period of observation, and that rises in serum HI antibody titre were the response to vaccine.

The changes in serum HI antibody titre to influenza virus B/Hong Kong/73

Table 3. Responses of volunteers to immunization with aqueous, subunit influenza B/Hong Kong/73 vaccine

Dose of vaccine (μg HA)	No. of volunteers	No. (%) rises of ≥ 4 -fold	No. (%) titres of ≥ 40	g.m.t.		
				Pre-immun.	Post-immun.	Fold-increase
40	23	22(95.6)	23(100)	8.5	321.8	37.8
20	24	21(87.5)	21(87.5)	10.0	152.6	15.3
10	24	16(66.7)	18(75.0)	16.5	137.6	8.3
5	25	13(52.0)	19(76.0)	16.6	86.8	5.2
None	23	0(—)	6(26.1)	11.1	12.5	1.1

following immunization with varying doses of aqueous subunit vaccine are summarized in Table 3. Twenty-two volunteers (96%) given 40 μg HA of vaccine showed a \geq four-fold rise in antibody titre, and all volunteers had antibody titres of ≥ 40 following immunization. Following immunization the geometric mean titre (g.m.t.) increased from 8.5 to 321.8. For volunteers given 5 μg HA of vaccine, 13 (52%) showed a \geq four-fold increase in antibody titre, and 19 (76.0%) had titres of ≥ 40 following immunization. The g.m.t. increased from 16.6 to 86.8 after immunization. Again, the results obtained for immunization with 10 μg or 20 μg HA of vaccine showed intermediary results (Table 3). The changes of serum HI antibody related to the virus vaccine administered showed a clear dose-response relationship: for volunteers given 40, 20, 10 or 5 μg HA the g.m.t. or HI antibody at 1 month after immunization was 321.8, 152.6, 137.6 and 86.1, respectively (Table 3). In addition, the extent of the increase in serum HI antibody was related to the dose of vaccine administered: volunteers given 40 μg HA of vaccine produced a 37.8-fold increase in serum HI antibody, and volunteers given 20, 10 and 5 μg HA showed 15.3-, 8.3- and 5.2-fold increase, respectively (Table 3).

Incidence of infection following challenge virus inoculation

Vaccine-induced immunity was measured 1 month following immunization with aqueous subunit B/Hong Kong/73 vaccine by intranasal inoculation of all volunteers with $10^{7.0}$ EID₅₀ of attenuated influenza virus RB77: this challenge infection did not induce clinical symptoms in the volunteers, and a four-fold rise in HI antibody titre to the challenge virus was taken as proof of infection. The incidence of infection by the challenge virus in volunteers given different doses of inactivated vaccine is shown in Table 4. Of the 23 volunteers immunized with 40 μg HA of B/Hong Kong/73 vaccine, no infections by the challenge virus were detected: for the 24 volunteers given 20 μg HA of vaccine one infection was recorded, and this occurred in a volunteer who had no detectable serum HI antibody at the time of challenge infection. For volunteers given 10 and 5 μg HA of vaccine, three (12.5%) and five (20%) infections were found; five of these infections were in volunteers with undetectable levels of serum HI antibody, and in two volunteers with low levels of HI antibody to RB77 virus. In contrast, of the 23 volunteers given saline, 15 (65.2%) were infected by the challenge virus; 13 (68.4%) of these infections were recorded in those with no detectable antibody to the challenge virus (Table 4). In total, of 35 volunteers with no detectable serum

Table 4. *Immunity to challenge virus infection following immunization with aqueous subunit influenza B/Hong Kong/73 vaccine.*

Dose of vaccine (μ g HA)	No. volunteers	Number of infections/volunteers (%) in groups determined by pre-challenge serum HI antibody titres to recombinant virus RB77						Total infections (%)
		< 10	10-20	30-40	60-120	160-480	> 480	
40	23	—	0/1	0/1	0/13	0/6	0/2	0(—)
20	24	1/3	0/1	0/5	0/8	0/7	0/1	1(4.2)
10	24	1/5	1/3	0/2	0/5	1/6	0/3	3(12.5)
5	25	4/8	1/3	0/3	0/8	0/3	—	5(20.0)
None	23	13/19	1/1	1/2	0/1	—	—	15(65.2)
Total	119	19/35(54.2)	3/9(33.3)	1/13(7.6)	0/35(—)	1/23(4.3)	0/6(—)	24(20.2)

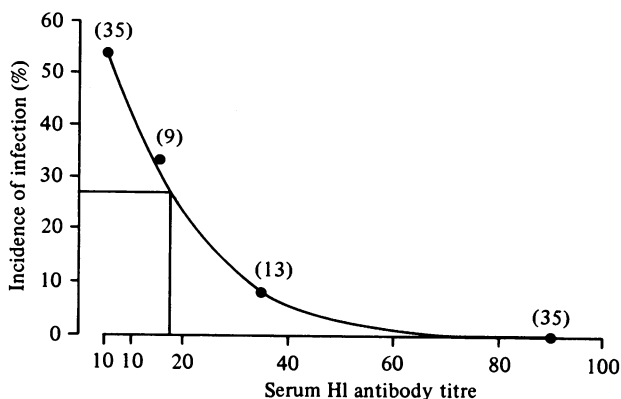


Fig. 1. Incidence of infection by attenuated influenza virus B (RB77) in volunteers related to pre-challenge serum HI antibody titre. Figures in parentheses indicate number of subjects.

HI antibody prior to challenge infection, 54.2% were infected, and the incidence of infection declined as the titres of serum HI antibody in the volunteers increased (Table 4). The relationship between the serum HI antibody titre and susceptibility to challenge virus infection is shown in Fig. 1. The results indicate a clear relationship; extrapolation from the graph indicates that the 50% protection titre of serum HI antibody to the challenge virus was approximately 15–20.

DISCUSSION

Although a large number of studies have been carried out using inactivated and live influenza A vaccines, fewer studies have been performed with influenza B vaccines. Moreover, the practice of translating data obtained in experiments with influenza A vaccines to the formation of influenza B vaccines, may not be justified, since the latter virus has distinctive characteristics (La Montagne, 1980). Thus, the dosage of HA to be included in the influenza B vaccines to induce a satisfactory serum antibody response, and the level of serum HI antibody necessary to give immunity to challenge-virus infection should be established separately for influenza A and B viruses. In the present study, volunteers were immunized with graded doses of an aqueous subunit influenza B vaccine. The vaccine was well tolerated and even doses of 40 μg HA produced only mild and transient local reactions, and no systemic reactions were reported; questionnaires relating to reactions were completed only at 24 h after inoculation but no reactions were reported after 48 h in the volunteers, all of whom were questioned. The serum HI antibody response to the influenza B/Hong Kong/73 showed a clear dose-response: thus, over 85% of volunteers given 20 or 40 μg HA of vaccine showed a four-fold increase in antibody, the g.m.t. increased to ≥ 150 and the increase in antibody was 15-fold. In contrast, only 16 of 24 (66.7%) of volunteers given 10 μg HA of vaccine showed a fourfold increase in antibody titre and the increase was 8.3-fold. The results obtained for 20 μg HA of vaccine were satisfactory, but this was not so for lower doses. In addition, the serum response to the influenza B vaccine were lower than those obtained for comparable doses of influenza A vaccine (Nicholson *et al.* 1979).

Challenge infections with attenuated RB77 virus resulted in no infections in volunteers given 40 μg HA and only one (4.2%) in the group given 20 μg HA. This was considered a satisfactory immunization. In contrast, for volunteers given 10 and 5 μg HA three (12.5%) and five (20.0%) were infected respectively, and this indicates a relatively poor response to vaccine. Thus, immunization with 20 μg HA gave satisfactory protection against challenge-virus infection, and this result is similar to that seen for immunization with influenza A vaccines.

Analysis of the results of the incidence of challenge-virus infection with reference to the level of serum HI antibody showed that a serum HI antibody titre of 15–20 was consistent with 50% protection by challenge virus infection. In similar studies with influenza A virus, the 50% protective level of serum HI antibody was 30–40 (Hobson, Beare & Ward-Gardner, 1972; Meikeljohn *et al.* 1952; Potter & Oxford, 1979). This finding may not indicate that less serum HI antibody is necessary for protection against influenza B virus infection, but rather that the HI test is less sensitive in detecting HI antibody to influenza B viruses than to influenza A. This explanation is supported by the lower serum HI antibody responses seen following immunization, and the relative insensitivity of the HI test compared to the single radial haemolysis test in detecting antibody to influenza B virus, which is not seen for antibody to influenza A viruses (Oxford, Yetts & Schild, 1982). In addition, this would explain the finding of some studies that serum HI antibody titres are a poor indicator of immunity to influenza B infection. These present findings apply only to influenza B/Hong Kong/73 virus vaccines, and the studies should be repeated using other influenza B virus vaccines to determine if the results are typical.

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REFERENCES

- BACHMAYER, H. (1975). Selective solubilization of haemagglutinin and neuraminidase from influenza viruses. *Intervirology* **5**, 260–272.
- HOBSON, D., BEARE, A. S. & WARD-GARDNER, A. (1972). Haemagglutination-inhibiting serum antibody titres as an index of response of volunteers to intranasal infection with live, attenuated strains of influenza virus. *Proceedings of the Symposium on Live Influenza Vaccines*, Zagreb 1971, pp. 73–84. Yugoslav Academy of Sciences and Arts.
- JENNINGS, R., CLARK, A., OXFORD, J. S., HOCKLEY, D. S. & POTTER, C. W. (1978). Reactogenicity and immunogenicity of whole and ether-tween split influenza A vaccines in volunteers. *The Journal of Infectious Diseases* **138**, 577–586.
- KILBOURNE, E. D. (1975). *The Influenza Viruses and Influenza*. Academic Press: London.
- LA MONTAGNE, J. R. (1980). Summary of a workshop on Influenza B viruses and Reye's Syndrome. *The Journal of Infectious Diseases* **142**, 452–465.
- MEIKLEJOHN, G., KEMPE, C. H., TAHALMAN, W. G. & LENNETTE, E. H. (1952). Evaluation of monovalent influenza vaccines. Observations during an influenza A-prime epidemic. *American Journal of Hygiene* **55**, 12–21.
- NICHOLSON, K. G., TYRRELL, D. A. J., HARRISON, P., POTTER, C. W., JENNINGS, R., CLARK, A., SCHILD, G. C., WOOD, J. M., SEAGROATT, V., HIGGINS, A. & ANDERSON, S. G. (1979). Clinical studies of monovalent inactivated whole virus and subunit A/USSR/77 (H1N1) vaccine: serological responses and clinical reactions. *Journal of Biological Standardization* **7**, 123–136.
- OXFORD, J. S., YETTS, R. & SCHILD, G. C. (1982). Quantitation and analysis of the specificity

- of post-immunization antibodies to influenza B viruses using single radial haemolysis. *Journal of Hygiene* **88**, 325–333.
- POTTER, C. W., JENNINGS, R., NICHOLSON, K., TYRRELL, D. A. J. & DICKINSON, K. G. (1977). Immunity to attenuated influenza virus WRL105 infection induced by heterologous inactivated influenza A virus vaccines. *Journal of Hygiene* **79**, 321–332.
- POTTER, C. W. & OXFORD, J. S. (1979). Determinants of immunity to influenza infection in man. *British Medical Bulletin* **35**, 69–75.
- SCHILD, G. C., WOOD, J. M. & NEWMAN, R. W. (1975). A single-radial-diffusion technique for the assay of influenza haemagglutinin antigen. *Bulletin of the World Health Organization* **52**, 223–231.
- STUART-HARRIS, C. H. & SCHILD, G. C. (1976). *Influenza: The Virus and the Disease*. London: Edward Arnold.
- W.H.O. (1953). *Expert Committee on Respiratory Virus Disease*. World Health Organization Technical Report. no. 64. Geneva: World Health Organization.
- WOOD, J. M., SCHILD, G. C., NEWMAN, R. W. & SEAGROATT, V. (1977). An improved single radial immunodiffusion technique for the assay of influenza haemagglutinin antigen. Application for potency determination of inactivated whole virus and subunit vaccines. *Journal of Biological Standardization* **5**, 237–247.