Original Research

Comparison of one- and two-dose regimens of influenza vaccine for elderly men

Marc Levine,* PhD
B. Lynn Beattie,† MD, FRCPC
Donald M. McLean,‡ MD, FRCPC

In November and December 1984, 102 male residents of a long-term care facility (mean age 74.6 [extremes 59 and 97] years) received 0.5 ml of trivalent inactivated whole-virion influenza vaccine, containing 15 μg of the hemagglutinin of each of A/Philippines/2/82 (H3N2), A/Chile/83 (H1N1) and B/USSR/83. A second dose of the vaccine was administered to a subgroup of 55 randomly chosen subjects 8 weeks later. Serum samples were collected from all the subjects before and 4, 8, 12 and 16 weeks after administration of the first dose and were assayed for hemagglutinin-inhibiting (HAI) antibody to each of the three antigens. At 8 weeks there were significant increases ($p < 0.05$) in the geometric mean titre of antibody and in the proportion of subjects with HAI antibody titres of 1:40 or more (except to the B/USSR antigen) in both groups. There were no differences between the groups at 8 weeks or at 16 weeks (8 weeks after administration of the second dose of vaccine) in the frequency of seroconversion, the geometric mean titre or the proportion of subjects with HAI antibody titres of 1:40 or more. Overall, 60%, 32% and 13% of the 102 subjects had titres of 1:40 or more to the A/Philippines, A/Chile and B/USSR antigens respectively at 16 weeks. The results suggest that a second dose of influenza vaccine given 8 weeks after the first does not enhance the immune response in elderly men and that a substantial proportion of this population remains unprotected against infection (having HAI antibody titres of less than 1:40) during the influenza season.

*From *the Faculty of Pharmaceutical Sciences and †the Department of Geriatrics and ‡the Division of Medical Microbiology, Faculty of Medicine, University of British Columbia, and the departments of *Pharmacy, *Medicine, and †Geriatrics and Continuing Care, Shaughnessy Hospital, Vancouver*

Reprint requests to: Dr. Marc Levine, Faculty of Pharmaceutical Sciences, University of British Columbia, 2146 East Mall, Vancouver, BC V6T 1W5

En novembre et décembre 1984 on administre à 102 pensionnaires masculins d'un établissement pour soins prolongés, âgés de 59 à 97 ans (moyenne 74,6 ans), 0,5 ml d'un vaccin anti-grippal inactivé trivalent à virion entier, soit 15 μg de chacune des hémagglutinines de A/Philippines/2/82 (H3N2), A/Chile/83 (H1N1) et B/USSR/83. Au bout de 8 semaines on répétera la dose chez 55 sujets choisis au hasard. On pratique le dosage des anticorps inhibiteurs de l'hémagglutination (AIH) pour chaque antigène dans le sérum prélevé sur tous les sujets avant la première vaccination et 4, 8, 12 et 16 semaines plus tard. Au bout de 8 semaines les deux groupes montrent une augmentation significative ($p < 0,05$) de la moyenne géométrique des concentrations de ces anticorps et du nombre des sujets où celle-ci est d'au moins 1:40 (sauf à l'égard de l'antigène B/USSR). Aucune différence n'est notée dans ces chiffres ou dans la fréquence de séroconversion à 8 et à 16 semaines (soit 8 semaines après la seconde dose). Dans l'ensemble, on observe des concentrations d'au moins 1:40 à 16 semaines à l'égard des trois antigènes chez 60%, 32% et 13% des sujets respectivement. Nos résultats font penser que chez les hommes âgés la seconde dose de vaccin anti-grippal n'augmente pas la réponse immunitaire et qu'une fraction importante d'entre eux aborde la saison de l'influenza sans protection, c'est-à-dire avec une concentration de AIH inférieure à 1:40.

Influenza continues to be an important contributor to illness and death in the elderly and other groups at high risk.[1,2] Despite evidence that vaccination can reduce the incidence and severity of influenza-related illness in those at risk, only a minority of these people are being vaccinated each year. It is recommended that all people over 65 years of age receive one dose of trivalent inactivated whole-virion influenza vaccine each

fall.[1] Only one dose is thought to be necessary, since most adults have previously been exposed to the main influenza subtypes in circulation. Annual vaccination is required owing to the frequency with which variant strains arise through antigenic drift and the fact that titres of hemagglutinin-inhibiting (HAI) antibody decline fairly rapidly. Titres of serum HAI antibody appear to correlate best with protection against infection, and a titre of approximately 1:40 is generally considered necessary for minimum protection.[3]

It has been observed, however, that many elderly people do not mount an adequate immune response to a single dose of influenza vaccine.[2] In some cases this appears to be due to the presence of high HAI antibody titres before vaccination, which apparently correlate with a reduced response. In such cases the patients often have protective titres of serum HAI antibody before vaccination, although these may not persist throughout the influenza season. In other cases people with low HAI antibody titres before vaccination still fail to respond adequately to the vaccine.[2] Mackenzie[4] reported that a second dose of influenza vaccine may provide additional protection to a subgroup of elderly people and recommended the use of a two-dose schedule for all patients at high risk. In 1983–84 we observed that only 31% to 73% of 62 elderly male volunteers who had been vaccinated maintained HAI antibody titres of 1:40 or greater throughout the influenza season.[5] We therefore undertook a study to compare the immune response of elderly subjects to one and two doses of influenza vaccine.

Methods

Subjects were recruited from the 600 predominantly male residents of a long-term care facility. Residents were approached if their physician felt that vaccination was indicated and that the patient or a family member was capable of giving consent. Written informed consent was thus obtained from 130 male residents, of whom 102 (mean age 74.6 [extremes 59 and 97] years) completed the study. Of the 28 who did not complete the study, 5 declined to be vaccinated after having consented, and 23 refused to have one or more blood samples drawn, were discharged or died during the study period.

In November and December 1984, prevaccination serum samples were obtained, and within 3 days the subjects received 0.5 ml of trivalent inactivated whole-virion influenza vaccine (Institut Armand Frappier, Laval, PQ, lot 3-65011, expiry date July 20, 1985), containing 15 μg of the hemagglutinin of each of A/Philippines/2/82 (H3N2), A/Chile/83 (H1N1) and B/USSR/83. Serum samples were obtained 4 and 8 weeks after vaccination. The subjects were assigned by means of a table of random numbers to two groups: those in group 1 (59 subjects with a mean age of 74.6 years) did not receive an additional dose of influenza vaccine, and those in group 2 (66 subjects with a mean age of 74.5 years) received another 0.5-ml dose of vaccine from the same lot at the end of week 8. Serum samples were taken from all the subjects at the end of weeks 12 and 16. Of the 102 subjects who completed the study, 47 received one dose and 55 received two doses of the vaccine.

In a previous study we had found that the titres of serum HAI antibody in elderly men began to decline 9 to 12 weeks after vaccination.[5] By 24 weeks only 31% of the subjects had titres of HAI antibody to the A/Philippines antigen of 1:40 or more. We therefore chose 8 weeks after vaccination as the time to administer the second dose of the vaccine to optimize our chance of observing an effect of the second dose while maintaining effective antibody titres during the influenza season.

The sample size was estimated from the proportion of subjects expected to have titres of serum HAI antibody of 1:40 or more 8 weeks after administration of the second dose of vaccine to the subjects in group 2 (week 16 of the study). With α set at 0.05 and β at 0.1 (power of 0.9), we estimated that 38 subjects would be needed in each group to detect an increase from 35% to 70% in the proportion of subjects with a titre of 1:40 or more in the group receiving two doses. The expected frequency in the group receiving one dose, 35%, was estimated from our previous observations in this population.[5]

All doses of the vaccine were administered subcutaneously in the right arm by the same nurse. The serum samples were stored at $-20°C$ until assayed for HAI antibody as previously described.[5] The samples were assayed over a range of twofold dilutions from 1:10 to 1:1280, and the HAI antibody titres were recorded as the reciprocal of the highest dilution at which hemagglutination was inhibited. Each subject's serum samples were assayed on the same day. For the calculation of geometric mean titres of antibody, titres of less than 10 were assigned a value of 5 and those of 1280 or more a value of 1280. Seroconversion was deemed to have occurred when the titre after vaccination was at least four times the titre before vaccination.

Analysis of variance was used to compare repeated measures of the geometric mean titre, and Student's *t*-test was used to compare the geometric mean titres before vaccination in the two groups. Analyses were actually performed with the logarithm of the HAI antibody titre since antibody titres vary geometrically. The frequency of seroconversion (the proportion of subjects showing seroconversion during the previous interval) and the proportion of subjects with HAI antibody titres of 1:40 or more in the two groups were compared by means of chi-squared analysis with Yates's correction for continuity. The level of significance was set at $p < 0.05$.

Results

The serologic findings before vaccination are summarized in Table I. The geometric mean titres of HAI antibody to the three antigens in the vaccine were relatively low and were similar in the two groups. Only 4% to 25% of the subjects had HAI antibody titres of 1:40 or more.

By 8 weeks after administration of the first dose of vaccine, 21% to 64% of the subjects had seroconverted (Table II). There were no significant differences between the groups in the proportion of subjects who had seroconverted with respect to each antigen. Of the subjects who had not seroconverted by 8 weeks, 8% to 53% had apparently seroconverted by week 16 — 8 weeks after administration of the second dose of vaccine to group 2 (Table III). Since there were again no significant differences between the groups in the proportion of subjects who had seroconverted, we combined the data for all the subjects and found that 22%, 17% and 26% of all instances of seroconversion with respect to the A/Philippines, A/Chile and B/USSR antigens respectively occurred later than 8 weeks after the first dose of vaccine had been administered.

The geometric mean titres of HAI antibody to all three antigens at 8 and 16 weeks were significantly higher than those before vaccination in both groups ($p \leq 0.003$) (Table IV). However, administration of the second dose of vaccine to group 2 did not result in a higher geometric mean titre of antibody to any of the antigens at 12 weeks (data not shown) or at 16 weeks.

In both groups the proportion of subjects with titres of HAI antibody of 1:40 or more to the A/Philippines and A/Chile antigens but not the B/USSR antigen was significantly greater at 8 and 16 weeks than before vaccination ($p < 0.001$ to 0.02) (Table V). However, there were no differences between the groups at 8 or 16 weeks. When the data for all the subjects were combined, 56%, 33% and 18% of the subjects had titres of HAI antibody of 1:40 or more to the A/Philippines, A/Chile and B/USSR antigens respectively at 8 weeks. The corresponding values at 16 weeks were 60%, 32% and 13%.

Discussion

Our results indicate that a second dose of influenza vaccine had no additional effect on the immune response in our population, as measured by the frequency of seroconversion, the geometric mean titre of serum HAI antibody and the proportion of subjects with HAI antibody titres of 1:40 or more. In the case of the A/Philippines antigen, 62% of the subjects who received one dose of vaccine had HAI antibody titres of 1:40 or more at 16 weeks. We therefore did not have enough subjects to observe an effect of the second dose on the titres of antibody to this antigen. However, at week 16 the proportions of subjects in group 1 with titres of HAI antibody to the A/Chile and B/USSR antigens of 1:40 or more were 26% and 6% respectively. Therefore, on the basis of our sample size estimates we could have detected an increase to 70% in the proportion of subjects with titres of serum HAI antibody to each antigen of

Table I — Serologic findings in a population of elderly men who subsequently received one (group 1) or two (group 2) doses of influenza vaccine

Antigen	Geometric mean titre of hemagglutinin-inhibiting (HAI) antibody*		No. (and %) of subjects with HAI antibody titre ≥ 1:40†	
	Group 1 (n = 47)	Group 2 (n = 55)	Group 1	Group 2
A/Philippines/2/82 (H3N2)	13.8	15.0	7 (15)	14 (25)
A/Chile/83 (H1N1)	9.4	9.2	2 (4)	4 (7)
B/USSR/83	9.0	9.3	4 (8)	5 (9)

*There were no significant differences between the groups by Student's *t*-test.
†There were no significant differences between the groups by chi-squared analysis.

Table II — Proportions of subjects who had seroconverted by week 8

Antigen	No. (and %) of subjects*	
	Group 1	Group 2
A/Philippines/2/82 (H3N2)	30 (64)	34 (62)
A/Chile/83 (H1N1)	18 (38)	30 (55)
B/USSR/83	10 (21)	13 (24)

*There were no significant differences between the groups by chi-squared analysis.

Table III — Frequency of seroconversion by week 16 in subjects who had not seroconverted by week 8

Antigen	No. (and %) of subjects*	
	Group 1	Group 2
A/Philippines/2/82 (H3N2)	9/17 (53)	9/21 (43)
A/Chile/83 (H1N1)	3/29 (10)	7/25 (28)
B/USSR/83	3/37 (8)	5/42 (12)

*There were no significant differences between the groups by chi-squared analysis.

1:40 or more (with a power of greater than 0.9), a result that would have been clinically important.

It has been observed that in adults primed to the viral subtypes in the influenza vaccine the magnitude of the increase in titre of serum HAI antibody after vaccination is inversely related to the titre before vaccination.[3,6,7] The fact that before vaccination older adults often have high titres of antibody to the main influenza subtypes currently used in the vaccine would therefore partly explain why they tend to have a weaker response to the vaccine than do younger adults.[2] In our study the geometric mean titres and the proportion of subjects with titres of serum HAI antibody of 1:40 or more were relatively low in both groups, particularly with respect to the A/Chile and B/USSR antigens, 8 weeks after administration of the first dose of the vaccine. It is therefore unlikely that high HAI antibody titres were responsible for the lack of response in group 2 to the second dose of vaccine. As well, other studies in adults whose HAI antibody titres before vaccination were similar to the titres of our subjects at week 8 demonstrated measurable increases in titre after vaccination.[6-9] In our study the fact that the frequency of seroconversion, the geometric mean titres and the proportion of subjects with titres of serum HAI antibody of 1:40 or more (except to the B/USSR antigen) increased significantly after administration of the first dose of vaccine indicates that the lot used was effective in eliciting an immune response. Even if the strength of the vaccine was less than claimed, this would not explain the lack of effect of the second dose.

Although the first dose of vaccine produced a measurable response, the magnitude of the increase in titre of serum HAI antibody was generally less than might have been expected on the basis of recent studies in the elderly.[8,10] However, considerable variation has been observed in the immune response to influenza vaccine in various studies in elderly people.[2] Assuming that the vaccine we used was effective, the poorer response to the first dose may have been partly due to the fact that the A/Chile and B/USSR antigens were new components of the 1984–85 vaccine, although this has not been reported to be a problem in the past.

In a study of young (15 to 59 years) and older (60 to 79 years) adults Mackenzie[4] administered two doses of subunit influenza vaccine to all subjects at an interval of 4 weeks. He reported that 12% to 13% of the subjects seroconverted only after receiving the second dose and that approximately 10% seroconverted after both the first and second dose. He argued that if a second dose could improve the immune response of some people in high-risk groups, then all those in such groups should receive a two-dose regimen annually, especially when new strains occur due to antigenic drift. In our study 8% to 18% of the subjects apparently seroconverted later than 8 weeks after receiving the first dose of vaccine; however, there was no difference in the rate of late seroconversion between those who received one dose and those who received two doses. We have previously observed late seroconversion after one dose of vaccine in this population.[5] This may be due to imprecision in the HAI antibody assay, concurrent infection or, in some cases, an anomalous late response to the vaccine. In any case, late seroconversion appears to be independent of a second dose of vaccine, which may have been overlooked by Mackenzie,[4] since all his subjects received two

Table IV — Geometric mean titres of HAI antibody at 8 and 16 weeks

	Geometric mean titre*			
	Week 8		Week 16	
Antigen	Group 1	Group 2	Group 1	Group 2
A/Philippines/2/82 (H3N2)	46.4	40.5	45.7	50.2
A/Chile/83 (H1N1)	23.9	24.2	18.3	20.0
B/USSR/83	14.0	13.7	11.9	14.4

*For all three antigens the titre was significantly higher after vaccination ($p \leq 0.003$, analysis of variance); there was no significant effect of the number of doses on the titre.

Table V — Proportions of subjects with HAI antibody titres of 1:40 or more at 8 and 16 weeks

	No. (and %) of subjects			
	Week 8		Week 16	
Antigen	Group 1	Group 2	Group 1	Group 2
A/Philippines/2/82 (H3N2)*†	30 (64)	27 (49)	29 (62)	32 (58)
A/Chile/83 (H1N1)*†	16 (34)	18 (33)	12 (26)	21 (38)
B/USSR/83†	8 (17)	10 (18)	3 (6)	10 (18)

*The proportion was significantly greater at weeks 8 and 16 than before vaccination in both groups by chi-squared analysis.
†There was no difference in proportion between the groups at week 8 or 16 by chi-squared analysis.

doses. There is evidence from clinical trials reported in 1977 to 1978 that in some elderly people, particularly those with low HAI antibody titres before vaccination, titres of 1:40 or more may develop after a second dose of vaccine.[8,9] However, the number of subjects was small, and there was no comparison with a group receiving only one dose of the same vaccine.

It is generally considered that a titre of serum HAI antibody of 1:40 is necessary to provide approximately 50% protection against the particular strain of influenza virus.[3] There is also experimental and clinical evidence that the degree of protection against infection conferred by the vaccine increases with the HAI antibody titre before infection.[3,11,12] A two-dose regimen of influenza vaccine would therefore be justifiable if the population geometric mean titre or the proportion of patients with HAI antibody titres of 1:40 or more was significantly higher after the second dose. Our results suggest that this is not so when the two doses are given 8 weeks apart. Nevertheless, if a titre of serum HAI antibody of 1:40 or more is required for protection against infection, 40% to 87% of our subjects were unprotected against the three antigens in the vaccine during the study period. Recently, Ershler and colleagues[13] demonstrated that the elderly do not respond as well as younger adults to influenza vaccine but that their antibody production can be enhanced in vitro by the immune stimulant thymosin. Future studies of influenza vaccination in the elderly should perhaps include evaluation of immune stimulants or adjuvants, as recommended by Ruben,[2] to maximize the protection conferred by the vaccine. Since titres of serum HAI antibody decline relatively rapidly, and since annual vaccination does maintain higher titres, a two-dose regimen, with the doses more widely spaced than 8 weeks, may be found to increase protection against influenza in the elderly.

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Meetings

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January

Jan. 21-22, 1988

Obstetrics, Newborn and Pediatric Care
Health Sciences Centre, University of Calgary
Mrs. Jocelyn Lockyer, Continuing Medical Education, Faculty of Medicine, University of Calgary, 3330 Hospital Dr. NW, Calgary, Alta. T2N 4N1; (403) 220-7240

Jan. 21-22, 1988

Short Course on Paediatrics
Chateau Halifax
Ms. Christine Smith, program coordinator, Division of Continuing Medical Education, Sir Charles Tupper Medical Building, Dalhousie University, Halifax, NS B3H 4H7; (902) 424-2061

Jan. 24-29, 1988

Diagnostic Imaging and Monitoring Devices
Sheraton Waikiki Hotel, Honolulu
Dr. Ken Wiancko, secretary, Canadian Chapter, Pan-Pacific Surgical Association, Ste. 205, 9509-156 St., Edmonton, Alta. T5P 4J5; (403) 483-2703

February

Feb. 1-5, 1988

1st Annual Update in Emergency Medicine
Banff Springs Hotel, Banff, Alta.
Continuing Education, Faculty of Medicine, Medical Sciences Building, University of Toronto, Toronto, Ont. M5S 1A8; (416) 978-2718