THE JOURNAL OF INFECTIOUS DISEASES • VOL. 140, NO. 2 • AUGUST 1979

Comparison of Responses to Influenza A/New Jersey/76-A/Victoria/75 Virus Vaccine Administered Intradermally or Subcutaneously to Adults with Chronic Respiratory Disease

F. Alexander Herbert, R. P. Bryce Larke, and Edythe L. Markstad

From the Departments of Medicine and Pediatrics and the Provincial Laboratory of Public Health, The University of Alberta; and the Health Sciences Clinic, University of Alberta Hospital, Edmonton, Alberta, Canada

Serum HAI (hemagglutination inhibition) antibody responses were compared in two groups of 70 age-matched patients (age range, 17 to 82 years) who were vaccinated with bivalent influenza A/New Jersey/76-A/Victoria/75 whole-virus vaccine. The group that was vaccinated intradermally received 40 chick cell–agglutinating units of each viral antigen in 0.1 ml, and the group that was vaccinated subcutaneously received 200 chick cell–agglutinating units of each antigen in 0.5 ml. The serum HAI antibody response to A/New Jersey/76 antigen was significantly higher in the group that was vaccinated subcutaneously; this difference was particularly evident in patients ≤50 years old. The serologic response to A/Victoria/75 antigen did not differ significantly between the two groups. Levels of antibody before vaccination indicated previous widespread exposure of patients to influenza A/Victoria/75 virus, but not to influenza A/New Jersey/76 virus. Such differences in prior immunologic experience with a particular strain of influenza virus probably determine whether the intradermal route of vaccination is as effective as, or inferior to, the subcutaneous route.

The administration of influenza virus vaccine by the intradermal (id) route has been shown by some investigators to result in an antibody response superior to that elicited by sc administration, even though a smaller dose of vaccine is inoculated id [1]. The immune response to influenza vaccines is determined to an important degree by prior antigenic experience of the population to be immunized, as well as by dose and route of inoculation. Persons of different ages have different previous exposures to influenza, which may be reflected in prevaccination levels of antibody and in variations in response to immunization [1–3].

Received for publication on October 6, 1978, and in revised form February 5, 1979.

The authors thank the staff of the Health Sciences Clinic for their cooperation, particularly Dr. Brian J. Sproule for permission to investigate his patients; Ms. Donna Trenholm and Mr. Ronald Taylor for coordinating the serologic testing at the Provincial Laboratory of Public Health; and Dr. Richard L. Jones for helpful advice with statistics.

Please address requests for reprints to Dr. F. Alexander Herbert, 6-104 Clinical Sciences Building, The University of Alberta, Edmonton, Alberta, T6G 2G3, Canada.

Age was expected to be particularly important in determining the response to the bivalent influenza A/New Jersey/76-A/Victoria/75 virus vaccine that had been recommended for use in high-risk groups in 1976 [4]. Seroepidemiologic studies had shown that the majority of individuals aged ≥51 years had preexisting antibody to A/New Jersey/76–like strains of virus, whereas those <51 years generally lacked such antibodies [5, 6]. The bivalent influenza vaccine provided an opportunity to reexamine the relative merits of id and sc routes of vaccination against a wide background of previous exposure to two distinctly different influenza viruses.

Materials and Methods

Patients. The study was carried out in December 1976 on 140 patients with chronic pulmonary diseases, who were attending the Health Sciences Clinic at the University of Alberta Hospital, Edmonton. Patients ranged in age from 17 to 82 years: 64% were ≥51 years of age, 27% were 24–50 years of age, and only 9% were <24 years.

The 140 patients were divided into two groups of 70 patients; each group was carefully age-matched within two years. The patients were assigned a code number by the nursing staff of the clinic, and vaccine was administered sc to even-numbered patients and id to odd-numbered patients. Neither the attending physicians nor those supervising the serologic testing were aware of the route by which the vaccine was administered until all the data were compiled, analyzed, and interpreted.

There were no cases of naturally occurring influenza A detected in the community during the time of the clinical study.

Type and administration of vaccine. The vaccine that we used was an inactivated bivalent whole-virus type containing influenza A/New Jersey/76 and A/Victoria/75 viruses supplied by Connaught Laboratories, Willowdale, Ontario. The vaccine was inoculated by needle and syringe; the doses were either 200 chick cell–agglutinating (CCA) units of each viral antigen/0.5 ml (sc) or 40 CCA units of each antigen/0.1 ml (id).

Serologic studies. Blood samples for determination of antibody to influenza virus were obtained from each patient immediately before vaccination and again four weeks later. All sera were stored at −40 C and then assayed together for HAI antibody to influenza A/New Jersey/76 and A/Victoria/75 viruses with use of prototypic viral antigens obtained from the Laboratory Centre for Disease Control, Ottawa, and standard serologic techniques [7].

Patients who had no detectable HAI antibody in prevaccination sera that had been diluted 1:10 were considered to show seroconversion if their titer was >1:10 following vaccination; for statistical analysis, such patients were included among those having a fourfold or greater rise in antibody titer.

Evaluation of reactions to the vaccine. All patients were provided with a questionnaire that they were instructed to complete 24 hr after vaccination (135 questionnaires were returned). Questions regarding systemic reactions included the presence of headache or muscle ache, excessive feeling of warmth, chills, and a feeling of being unwell. A sixth question inquired about local pain and swelling at the site of inoculation. These items were weighted equally for development of a reaction score of zero to six, which was plotted against subsequent rises in antibody titer in an effort to determine whether individuals demonstrating the greatest immunogenic response were more likely to experience adverse effects.

Statistical analysis. Results from the two groups were analyzed statistically by the test of proportions for unpaired data. Groups of patients were also compared by Student's *t*-test for unpaired data, when appropriate.

Results

The two groups of patients were closely matched with respect to levels of HAI antibody to influenza A/New Jersey/76 and A/Victoria/75 viruses in the prevaccination serum specimens. Approximately equal numbers of patients in the two groups had been immunized previously on one or more occasions with vaccines containing influenza A/Aichi/68, A/England/72, or A/Port Chalmers/73-A/Scotland/74 virus, but their prevaccination levels of antibody to A/New Jersey/76 and A/Victoria/75 viruses were not significantly different from those of patients who had not been vaccinated previously.

Antibody response to influenza A/New Jersey/76 virus vaccine. A fourfold or greater rise in HAI antibody titer was noted in 59% of patients who were given vaccine sc and in 43% of those who were inoculated id. The difference between these two groups was statistically significant ($P < 0.05$) (table 1).

Serologic responses were also analyzed by the calculation of the geometric means of the reciprocals of HAI antibody titers and by expression of these responses as the ratio of postvaccination to prevaccination titers [1]; this ratio was referred to as the antibody response ratio. The ratio for the group given vaccine sc was more than twice that for the id group (table 1).

A level of HAI antibody of ≥1:40 is generally accepted to correlate well with resistance to infection with influenza viruses of similar antigenic compositions [8]. Such levels of antibody to influenza A/New Jersey/76 virus were achieved following vaccination in 76% of patients in the id group and 89% of those in the sc group. Ex-

Table 1. Responses of serum HAI antibody to influenza A/New Jersey/76 and A/Victoria/75 viruses among 140 patients after intradermal (id) or sc vaccination with bivalent whole-virus vaccine.

Virus, route of vaccination	No. vaccinated	HAI antibody response					
		No. (%) with ≥ fourfold rise	Geometric mean titer			No. (%) with titer ≥1:40	
			Pre	Post	Post/pre*	Pre	Post
A/New Jersey/76							
id	70	30 (43)†	39	127	3.26	45 (64)	53 (76)
sc	70	41 (59)†	28	196	7.00	40 (57)	62 (89)
A/Victoria/75							
id	70	30 (43)‡	37	106	2.86	36 (51)	58 (83)
sc	70	24 (34)‡	36	98	2.72	40 (57)	56 (80)

NOTE. Pre and post refer to vaccination.

*Ratio of postvaccination to prevaccination geometric mean antibody titers (antibody response ratio).

†Difference is statistically significant ($P < 0.05$).

‡Difference is not statistically significant ($P = 0.4$).

pressed in other terms, the vaccine induced a "protective" serologic response only in an additional eight patients who were inoculated id in contrast to 22 patients who received the vaccine sc ($P < 0.005$) (table 1).

When we analyzed the data with respect to age, we found that patients ≥51 years old had higher levels of antibody before vaccination than did younger patients, as reflected in geometric mean titers and in the percentage of subjects with titers of ≥1:40 (table 2). In the id group, eight of the 24 patients ≤50 years of age with initial antibody titers of <1:40 attained "protective" serologic levels (≥1:40) following vaccination; only one of 44 patients ≥51 years old had a prevaccination titer of <1:40, and his antibody level remained unchanged. In the sc group, of 22 patients ≤50 years of age with prevaccination antibody titers of <1:40, 14 subsequently attained titers of ≥1:40; all of the eight patients ≥51 years of age whose initial antibody titers were <1:40 responded with a rise in level to ≥1:40.

For either the id or sc route of inoculation, the antibody response ratios were greater in those ≤50 years of age than in older patients. However, among the younger patients the antibody response

Table 2. Influence of age of patient and route of vaccination on serum HAI antibody responses to influenza A/New Jersey/76 and A/Victoria/75 viruses among 140 patients after intradermal (id) or sc vaccination with bivalent whole-virus vaccine.

Virus, route of vaccination	Age (years)	No. vaccinated	HAI antibody response				
			Geometric mean titer			No. (%) with titer ≥1:40	
			Pre	Post	Post/pre*	Pre	Post
A/New Jersey/76							
id	≤50	26	4	31	7.75	2 (8)	10 (38)
	≥51	44	146	291	1.99	43 (98)	43 (98)
sc	≤50	24	3	78	26.00	2 (8)	16 (67)
	≥51	46	80	289	3.61	38 (83)	46 (100)
A/Victoria/75							
id	≤50	26	25	89	3.56	13 (50)	22 (85)
	≥51	44	50	120	2.40	23 (52)	36 (82)
sc	≤50	24	32	137	4.28	13 (54)	22 (92)
	≥51	46	39	79	2.03	27 (59)	34 (74)

NOTE. Pre and post refer to vaccination.

*Ratio of postvaccination to prevaccination geometric mean antibody titers (antibody response ratio).

ratio for those given vaccine sc was 3.35 times greater than for those who received the vaccine id (table 2).

Antibody response to influenza A/Victoria/75 virus vaccine. A fourfold or greater rise in HAI antibody titer was noted in 34% of patients who were given vaccine by the sc route and in 43% who received the vaccine id. The difference between these two groups was not statistically significant. The antibody response ratios were approximately the same for the two groups. Levels of postvaccination HAI antibody of ≥1:40 were attained in 80% and 83% of patients in the sc and id groups, respectively (table 1).

Prevaccination antibody titers to influenza A/Victoria/75 virus were similar for patients in the younger and older age groups. After vaccination, neither the antibody response ratios nor the percentages of patients who attained antibody levels of ≥1:40 were particularly different in the two age groups, whether the vaccine was given id or sc (table 2).

Vaccine reactions. Systemic reactions of ≥3+ severity were reported by 14% of the patients who were vaccinated id and 12% who were vaccinated sc. When the reaction score for each patient was plotted against the rise in titer of antibody to either influenza A/New Jersey/76 or A/Victoria/75 virus, no significant relationship was demonstrated with either vaccine component. Local reactions consisting of pain or swelling at the injection site were reported by 26% of the patients vaccinated id and 12% vaccinated sc.

Discussion

In the extensive clinical studies of influenza vaccines conducted in the United States in 1976, results were often recorded in terms of the percentage of subjects with HAI antibody titers of ≥1:40 four weeks after the subjects had received 200 CCA units of vaccine. Taking this as an index of vaccine "efficacy," we found that the immune response to either antigenic component of the bivalent influenza A vaccine used in our study (table 1) compared very favorably with the data from the United States [3, 9, 10].

We found our vaccine to be more immunogenic if given sc rather than id if there had been only limited previous exposure to the virus, as seen particularly among our patients ≤50 years of age, the majority of whom lacked detectable antibody to influenza A/New Jersey/76 virus before vaccination. In contrast, we found the id and sc routes of administration of influenza vaccine to be equally efficacious among those who had had previous experience with a strain of virus antigenically similar to that in the vaccine, as demonstrated by the serologic response to influenza A/Victoria/75 virus among both the older and the younger groups of patients. The immune response to influenza vaccine appears to be markedly influenced by levels of prevaccination antibody.

Whether the superiority of the sc route as compared to the id route of vaccine administration, as seen under some circumstances, is related directly to the antigenic mass of vaccine given, or whether it is inherent in the inoculation route itself has not been determined.

Other studies that had compared the id with an alternative route of inoculation have been reviewed by Brown et al. [11] in relation to their own results of the immunizing effect of influenza A/New Jersey/76 virus vaccine administered im or id to adults who ranged in age from 18 to 52 years. They found that, among persons 18 to 24 years old who were initially free of detectable antibody, id vaccination induced lower titers of HAI antibodies than did im vaccination. In contrast, those 24 to 52 years of age who initially lacked detectable antibody had as good a serologic response to id as to im vaccination [11]. Although Brown et al. were comparing the id to the im route rather than to the sc route of vaccination, their results would appear to be at some variance with our findings for persons ≤50 years of age, where the superiority of the sc over the id route in inducing an antibody response to influenza A/New Jersey/76 virus was most evident (table 2).

There are two major previous studies that compared the id and sc routes of vaccination: both used monovalent vaccine containing influenza A/Aichi/2/68 (H3N2) virus (Hong Kong variant) in populations with low or undetectable levels of antibody to the homologous virus in prevaccination sera [1, 12]. Tauraso et al. [1] considered the antibody response to vaccine in rela-

tion to both preimmunization antibody titer and age. After one inoculation of vaccine, there was a better antibody response to 65 CCA units given id than to 320 CCA units given sc. In their study, two-thirds of those vaccinated were in an older age group that had evidence of previous exposure to antigens related to the Hong Kong variant, whereas antibody to this antigen was rare in individuals aged ≤64 years. There appeared to be an inverse relationship between preimmunization levels of antibody and antibody response determined by fourfold increases in HAI antibody titer. However, the postvaccination geometric mean antibody titer was greater in subjects ≥65 years of age, a finding presumably reflecting the higher antibody titers in their preinoculation sera [1]. Phillips et al. [12] reported that the geometric mean titer of HAI antibody was significantly higher in student nurses who were inoculated sc with 400 CCA units of influenza vaccine than in those who were inoculated id with 80 CCA units. Thus, our finding that there is an advantage in the sc over the id route for immunization of individuals who have little or no previous exposure to the strain of influenza virus contained in the vaccine supports the report by Phillips et al. [12].

Minor local reactions of swelling and erythema were more common at the site of id inoculation, but the incidence of systemic reactions to influenza vaccine was similar in the sc and id groups in our study. Thus, the smaller antigenic mass of the id inoculum did not appear to offer any advantage in reducing the few undesirable side effects from the vaccine.

References

1. Tauraso, N. M., Gleckman, R., Pedreira, F. A., Sabbaj, J., Yahwak, R., Madoff, M. A. Effect of dosage and route of inoculation upon antigenicity of inactivated influenza virus vaccine (Hong Kong strain) in man. Bull. W.H.O. 41:507–516, 1969.
2. Marine, W. M., Thomas, J. E. Age-related response to 1000 CCA unit zonally purified, inactivated influenza vaccines in volunteers in the U.S.A. Postgrad. Med. J. 49:164–168, 1973.
3. Parkman, P. D., Hopps, H. E., Rastogi, S. C., Meyer, H. M., Jr. Summary of clinical trials of influenza virus vaccines in adults. J. Infect. Dis. 136 (Suppl.): S722–S730, 1977.
4. National Advisory Committee on Immunizing Agents. Statement on 1976–1977 influenza vaccination program. Canada Diseases Weekly Report 2:117–119, 1976.
5. Laboratory Centre for Disease Control. Influenza A/swine antibody (Hsw1N1) in the Canadian population. Canada Diseases Weekly Report 2:53–56, 1976.
6. Noble, G. R., Kaye, H. S., Kendal, A. P., Dowdle, W. R. Age-related heterologous antibody responses to influenza virus vaccination. J. Infect. Dis. 136 (Suppl.): S686–S692, 1977.
7. Dowdle, W. R., Coleman, M. T. Influenza virus. *In* E. H. Lennette, E. H. Spaulding, and J. P. Truant [ed.]. Manual of clinical microbiology. 2nd ed. American Society for Microbiology, Washington, D.C., 1974, p. 678–685.
8. Delem, A., Jovanovic, D. Correlation between rate of infection and preexisting titer of serum antibody as determined by single radial hemolysis during an epidemic of influenza A/Victoria/3/75. J. Infect. Dis. 137:194–196, 1978.
9. Cate, T. R., Kasel, J. A., Couch, R. B., Six, H. R., Knight, V. Clinical trials of bivalent influenza A/New Jersey/76-A/Victoria/75 vaccines in the elderly. J. Infect. Dis. 136 (Suppl.):S518–S525, 1977.
10. Douglas, R. G., Jr., Bentley, D. W., Brandriss, M. W. Responses of elderly and chronically ill subjects to bivalent influenza A/New Jersey/8/76 (Hsw1N1)-A/Victoria/3/75 (H3N2) vaccines. J. Infect. Dis. 136 (Suppl.):S526–S532, 1977.
11. Brown, H., Kasel, J. A., Freeman, D. M., Moise, L. D., Grose, N. P., Couch, R. B. The immunizing effect of influenza A/New Jersey/76 (Hsw1N1) virus vaccine administered intradermally and intramuscularly to adults. J. Infect. Dis. 136 (Suppl.):S466–S471, 1977.
12. Phillips, C. A., Forsyth, B. R., Christmas, W. A., Gump, D. W., Whorton, E. B., Rogers, I., Rudin, A. Purified influenza vaccine: clinical and serologic responses to varying doses and different routes of immunization. J. Infect. Dis. 122:26–32, 1970.