Lessons Learned from Clinical Trials in 1976 and 1977 of Vaccines for the Newly Emerged Swine and Russian Influenza A/H1N1 Viruses

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Abstract An explosive local outbreak of respiratory disease in the US in 1976 with a swine influenza A/H1N1-like virus [A/New Jersey/76 (H1N1)] and the appearance of another A/H1N1 virus [A/USSR/77 (H1N1)] in the subsequent year led to extensive clinical trials of vaccines as preparations for public use. Two whole virus (WV) and two subunit (SV) vaccines of each virus were evaluated in all age groups for safety and immunogenicity. A/NJ WV vaccines were more reactogenic and more immunogenic than SV vaccines, particularly in children. Increase in the dosage led to increase in reactogenicity, which was significant for one WV vaccine that contained more antigen, but reactogenicity was best associated with the presence of WV particles of varied morphology. Although this result led to the concept that WV vaccines were not acceptable for children, WV A/USSR vaccines were not reactogenic in adults as were the A/NJ vaccines.

Patterns of antibody response by age, dosage, and number of doses were similar for both A/NJ and A/USSR vaccines. Increasing dosage increased the frequency and magnitude of responses and μg of HA related better to this finding than CCA units used initially for dosage of A/NJ vaccines. "Primed" persons (exposed to A/H1N1 viruses circulating before 1957) responded to single doses of vaccine. One dose of WV vaccine induced acceptable antibody responses among most unprimed persons but SV vaccines required two doses. For WV vaccine, one dose of high dosage vaccine (60–118 μg HA) was as immunogenic as two doses of lower dosage among adults; two doses as low as 2.5 μg HA were immunogenic in children. For "primed" persons, doses of 15–20 μg HA induced adequate responses. These principles of dosage, morphology and priming as major determinants of reactogenicity and immune responses have been replicated since 1976–1977 and

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seem likely to be replicated again in the current vaccine trials with the 2009 swine-like A/H1N1 virus.

1 Introduction

An explosive outbreak of febrile respiratory disease in military recruits occurred at Fort Dix, New Jersey, USA in January 1976. The cause of the outbreak was identified as a swine influenza A (H1N1)-like virus; it was designated as A/NJ/76 (H1N1). The outbreak was about 4 weeks in duration and primarily involved basic combat training units as very little spread to other units occurred and none to the surrounding community. Influenza authorities, government officials, and industry representatives convened and concluded that an influenza pandemic caused by A/NJ-like (A/NJ) viruses was possible; authorities proceeded to organize vaccine production and clinical trials in preparation for immunization of the public during the fall of 1976. Those clinical trials were an extensive effort coordinated by the US. Federal Drug Administration (FDA), National Institutes of Allergy and Infectious Diseases (NIAID), and the Centers for Disease Control (CDC). Results of the trials are contained in a supplement to the *Journal of Infectious Diseases* [1].

Approximately 1 year later, a new strain of influenza A/H1N1 virus was identified in the Soviet Union as a cause of influenza outbreaks, particularly among younger people. The strain was designated A/USSR (H1N1) virus and was shown to be similar to A/H1N1 viruses detected almost 30 years earlier in humans and distinct from A/NJ (H1N1) virus [2]. Vaccine clinical trials with this virus were organized by NIAID to provide guidance for vaccines for public use; results are contained in *Reviews of Infectious Diseases* [3].

These two experiences with type A/H1N1 virus vaccines preceding a potential influenza A/H1N1 pandemic are the only large, organized vaccine clinical trials with the A/H1N1 influenza virus performed prior to the current trials with the A/H1N1 virus that emerged in Mexico in the spring of 2009. Both the A/NJ/76 and the newly emerged A/H1N1/2009 virus are swine-like viruses.

The author of this report participated in both the A/NJ/76 and A/USSR/77 vaccine trials which are summarized in this chapter.

2 The Vaccines and Evaluations

The transition from quantitation of vaccine antigen using chick cell agglutinating (CCA) units to quantitation of the HA of each strain in influenza vaccines occurred with the 1976–1977 vaccine trials. The A/NJ vaccines were formulated in CCA units and later tested for HA content; the A/USSR vaccines were formulated in μg of HA and later tested for CCA units.

The variables evaluated in the trials were vaccine manufacturer (four manufacturers), vaccine type [whole virus (WV) or split virus (SV)], dosage, schedule (one or two doses), and age (children, adults, or elderly people). Two manufacturers supplied whole virus vaccines [Merck Sharp and Dohme (MSD) and Merrell National[1] (MN) for A/NJ that became Connaught[1] (C) for A/USSR] and two supplied subunit vaccines [Wyeth and Parke-Davis (PD)]. In addition to CCA and HA content, evaluations of the vaccines included protein quantity, endotoxin content, and viral mass of each WV A/NJ vaccine [4, 5]. Clinical evaluations were of reactogenicity and serum anti-HA antibody responses.

3 Reactogenicity

A tendency for increasing reactogenicity with increasing dosage was noted in the A/NJ trials but the most striking difference was between the WV and SV vaccine reactogenicity in children [6]. The WV vaccines clearly induced greater systemic reactions in children than did the SV vaccines. Reactogenicity of 1,567 children in the initial single-dose trial is summarized in Table 1.

Systemic reactogenicity after the SV vaccines was considered similar to those of placebo recipients (although more mild local reactions occurred with SV). However, both WV vaccines induced significantly greater systemic reactogenicity among both the 3–5 and 6–10 year age groups, including more instances of fever; the MSD vaccine was more reactogenic than the MN vaccine. A later study with smaller numbers of children evaluated dosages considered acceptable for reactions (low dosages for WV vaccines); a second dose was given one month later, and an

Table 1 Comparison of reactogenicity among children after whole and split virus A/New Jersey (H1N1) inactivated influenza vaccines

Vaccine	Dosage[a]		Reaction Index[b]	
	CCA	HA	3–5 Years (N)	6–10 Years (N)
Wyeth	43		0.13 (30)	
	87	4	0.24 (38)	0.36 (50)
PD	54		0.04 (26)	
	108	–	0.07 (43)	0.20 (50)
MN	32		0.55 (64)	
	64	6	0.52 (50)	0.68 (95)
MSD	46		0.99 (71)	
	93	14	0.47 (17)	1.28 (89)
Placebo	–		0.25 (104)	0.28 (93)

PD Parke-Davis, *MN* Merrell National; *MSD* Merck Sharp and Dohme
[a]Approximate CCA and HA/0.5 ml; PD not available as was A/Swine/31 virus
[b]Mean score for fever, headache, malaise, abdominal symptoms; >0.6 considered significant

[1]Now Sanofi Pasteur.

11–18-year-old group was added. Dosages were not identical but the higher reactogenicity after WV vaccine was confirmed and included the 11–18 years group. The reaction index was generally lower after the second vaccination; this was most notable for the WV vaccines.

Reactogenicity among adults with A/NJ vaccines was varied among dosages and groups. Only the MSD vaccine exhibited a significant increase in systemic reactogenicity (Table 2); 12.8% of those given the 800 CCA vaccine developed fever, more than double that of any other group [7]. Local reactogenicity was increased with increasing dosages but severe reactions were rare.

A number of laboratory studies of the A/NJ vaccines were conducted to better understand the differences in reactogenicity and immunogenicity between vaccines. There was no correlation between systemic reactivity and endotoxin concentrations, rabbit pyrogenicity, protein concentration, or neuraminidase content [4]. Vaccine mass (for WV vaccines) was determined by chromatographic separations; an increase in mass correlated with an increase in reactivity of the MSD vaccines. In further comparisons of the two WV vaccines, electron micrographs of each WV vaccine were obtained; the MSD vaccine contained more intact virus particles with a greater variety of shapes than did the MN vaccine. This difference was associated with a greater HA concentration, a higher viral mass and greater systemic reactivity for the MSD vaccine than for similar CCA levels of the other WV vaccine and both SV vaccines.

A comparison of reactogenicity among adults for the A/NJ and A/USSR vaccines prepared by the four manufacturers is shown in Table 2. The Reaction Index (RI) for all A/USSR vaccines was low. There is no consistent increase in reactogenicity with increasing dosage, as seen with the A/NJ vaccines. An increase in the RI with increasing dosage was noted among children but all were clinically acceptable [8]. Protein and endotoxin content of the A/USSR vaccines varied; viral mass was not reported.

Table 2 Comparison of reactogenicity among adults after A/New Jersey (H1N1) and A/USSR (H1N1) inactivated influenza vaccines

Vaccine	Strain	CCA/HA[a]	RI[b]	CCA/HA[a]	RI[b]	CCA/HA[b]	RI[b]
Wyeth	NJ	174/8	0.47	335/23	0.30	661/65	0.28
	USSR	107/16	0.26	451/61	0.39		
PD	NJ	217/–	0.34	431/–	0.31	739/–	0.52
	USSR	102/10	0.37	296/43	0.00		
MN/CL	NJ	128/12	0.44	301/26	0.27	697/51	0.58
	USSR	186/19	0.34			805/59	0.31
MSD	NJ	185/28	0.49	356/60	0.90	736/118	2.15
	USSR	81/12	0.21	391/45	0.34		

PD Parke-Davis, *MN*/*CL* Merrell-National which became Connaught Laboratories and is currently Sanofi Pasteur, *MSD* Merck Sharpe and Dohme

[a]CCA/HA CCA = Actual chick cell agglutinating units/μg HA per 0.5 ml; PD for A/NJ not available as was A/Swine/31 virus

[b]Reaction Index = Mean score for fever, headache, malaise, nausea; A/USSR RI includes children

Table 3 Summary of reactogenicity of inactivated influenza A/H1N1 vaccines in the 1976–1977 clinical trials

Local reactions after all A/NJ vaccines and dosages in all age groups were clinically acceptable and were frequently within the range for placebo
A trend for increasing systemic reactions with increasing dosage of A/NJ vaccine was noted, particularly among children. The trend related better to HA than CCA content
Whole virus A/NJ vaccines induced more systemic reactions than split-product vaccines, particularly among children. These reactions occurred 6–24 h after vaccination
One whole virus A/NJ vaccine was the most reactogenic vaccine and related best to high viral mass, characterized as intact viral particles of varied morphology that included filamentous forms
Reactions after A/USSR vaccines were clinically acceptable and about the same for whole and split virus among both adults and children although children were only given SV vaccines
Systemic reaction scores were lower after a second dose than the first dose for both A/NJ and A/USSR vaccines
Reactions among adults after a dose of 200 CCA of the A/NJ vaccine and 20 μg of HA of the A/USSR vaccine were about the same and were low for all vaccines

This experience with influenza A/H1N1 inactivated vaccines provided an understanding of reactogenicity that influenced many vaccine-related decisions that are still in place today. A summary of the major findings regarding reactogenicity is in Table 3. WV vaccines are considered more reactogenic than SV vaccines, particularly among children. While this is generally true, the comparisons of reactions for the various vaccines and dosages in Table 2 indicate that this is not a uniform finding for WV vaccines, at least not among adults. Reports from trials of A/Hong Kong/68 (H3N2) vaccines indicate that high reactogenicity of WV vaccines was not seen among children [9]. Thus, rejecting WV vaccines as an option for inactivated vaccines because of a potential for increased reactogenicity seems inappropriate, particularly since available data suggest WV vaccines are generally more immunogenic than SV vaccines (see below). Using the HA content as a basis for vaccine dosage provided a better correlate for reactogenicity than did the CCA content.

4 Immunogenicity

Both one-dose and two-dose immunogenicity studies with the A/NJ inactivated vaccines were conducted in children [6]. The one-dose trial exhibited a high frequency and magnitude of responses only in the children given high dosages of WV vaccine, but the level of reactogenicity was considered unacceptable. For this reason, a second trial was done in children that used dosages of the vaccines considered acceptable for reactogenicity. Results of that two-dose trial are shown in Table 4. The lower dosages used were lower for both CCA and μg HA in each age group for the two WV vaccines than for the two SV vaccines. Despite the lower dosages, responses to the WV vaccines were similar to, and usually better than, those for higher dosages of SV vaccine. In general, responses to one dose were deficient for all vaccines at the dosages tested but were satisfactory after two doses.

Table 4 Comparison of serum antibody responses after A/New Jersey (H1N1) inactivated influenza vaccines among children

Vaccine	CCA/HA[a]	Ab 6–36 Mo[b]		CCA/HA[a]	Ab 3–5 Yr[b]		CCA/HA[a]	Ab 6–10 Yr[b]		CCA/HA[a]	Ab 11–18 Yr[b]	
		GMT	% ≥40		GMT	% ≥40		GMT	% ≥40		GMT	% ≥40
Wyeth												
1 dose[c]	97/6.5	5.4	0	194/13	8.6	7	194/13	8.7	43	388/26	14.1	25
2 doses[c]		15.9	25		65.9	83		65.6	79		90.6	82
PD												
1 dose[c]	95/10	9.3	10	190/20	17.1	18	190/20	23.3	38	380/40	27.9	41
2 doses[c]		63.1	89		65.9	78		102.3	93		86.7	88
MN												
1 dose[c]	26/2.5	18	21	51/5	13.2	15	102/10	14	13	204/20	17.8	52
2 doses[c]		86.3	89		51.3	84		41.2	77		53.7	74
MSD												
1 dose[c]	14/2.5	18.5	29	28/5	26.7	47	55/10	25.9	44	110/20	41.5	54
2 doses[c]		59.8	92		82.4	91		54.7	84		71.6	96

PD Parke-Davis, *MN* Merrell-National which became Connaught Laboratories and is now Sanofi Pasteur, *MSD* Merck Sharpe and Dohme

[a]CCA/HA Actual chick cell agglutinating units/μ HA per 0.5 ml

[b]Ab responses by age group for GMT = geometric mean hemagglutination-inhibition titer and % with titer ≥1:40

[c]1 dose no. = 10–63, 2 doses no. = 9–31

Despite an estimated dosage of only 2.5 μg of HA, the good responses to two doses of either of the two WV vaccines among 6–36-month-old children were notable. A 10 μg HA dosage of the PD vaccine was comparable for this age group but the responses to the 6.5 μg HA dosages of Wyeth vaccine were lower.

One- and two-dose trials were also performed in subjects aged 17–24 years (Table 5). A trend for increasing antibody responses with increasing dosages was seen and, as was seen in children, satisfactory antibody responses to one dose were seen only in the group given the higher dosages of the reactogenic MSD vaccine [7]. The HA dosages for the two higher CCA dosages of MSD vaccine were 60 and 118 μg. Responses to two doses of the lower dosages (20–40 μg HA) were satisfactory for the PD, MN, and MSD vaccines but not for the SV Wyeth vaccine that was of lower dosage (13 μg HA; GMT 39, 52% ≥1:40).

Older persons (≥25 years) were given a single dose only of each vaccine as these older persons exhibited good antibody responses to one dose [7]. Prior to vaccination, HAI titers ≥1:10 were seen in 15.4% of those aged 25–34 years, in 28.1% of those aged 35–51 years, and in 94.9% of those ≥52 years [7]. A comparison of responses of a group aged 22–43 years and one ≥52 years old at one clinical trial site is shown in Table 6 [10, 11]. Satisfactory responses to A/Swine/31 (H1N1) were seen among those given one and two doses of the inappropriate A/Swine/31 (H1N1) vaccine provided by Parke-Davis. Among those aged 22–43 years, vaccines of higher dosage (>20 μg HA) induced satisfactory responses and, again, were highest for the reactogenic MSD vaccines. One dose (12–60 μg HA) induced very good responses among all subjects ≥52 years of age.

Table 5 Comparison of serum antibody responses after A/New Jersey (H1N1) inactivated influenza vaccines among subjects 17–24 years of age

Vaccine	CCA/HA[a]	Antibody[b]		CCA/HA[a]	Antibody[b]		CCA/HA[a]	Antibody[b]	
		GMT	% ≥40		GMT	% ≥40		GMT	% ≥40
Wyeth									
1 dose[c]	174/8	10	21	335/23	19	31	661/65	24	34
2 doses[c]	194/13	39	52						
PD									
1 dose[c]	217/ND[d]	13	34	431/ND[d]	8	20	739/ND[d]	17	45
2 doses[c]	190/20	94	82						
MN									
1 dose[c]	128/12	22	44	301/26	34	51	697/51	31	46
2 doses[c]	204/20	72	83						
MSD									
1 dose[c]	185/28	42	56	356/60	66	84	736/118	82	91
2 doses[c]	221/40	125	94						

PD Parke-Davis, *MN* Merrell-National (which became Connaught Laboratories and is now Sanofi Pasteur), *MSD* Merck Sharpe and Dohme

[a]*CCA/HA* Actual chick cell agglutinating units/μ HA per 0.5 ml

[b]*GMT* Geometric mean hemagglutination-inhibiting antibody titer; ≥40 = % with ≥1:40 titer

[c]Separate clinical trials

[d]HA not determined as HA was A/Swine/31 not A/New Jersey/176

Table 6 Comparison of serum antibody responses after A/New Jersey (H1N1) inactivated influenza vaccines among subjects 22–43 and ≥52 years of age

Vaccine[a] Age Gp (yrs)[b]	CCA/HA[c]	Antibody[d]		CCA/HA[c]	Antibody[d]		CCA/HA[c]	Antibody[d]	
		GMT	% ≥40		GMT	% ≥40		GMT	% ≥40
Wyeth	174/8			335/23			661/65		
22–43		26	39		48	75		94	76
PD	217/ND[e]			431/ND[e]			739/ND[e]		
22–43		89	95		181	94		166	85
≥52		265	98		286	100			
MN	128/12			301/26			697/51		
22–43		40	63		26	57		84	75
≥52		164	98		232	100			
MSD	185/28			356/60			736/118		
22–43		105	78		174	100		136	88
≥52		191	98		246	98			
Placebo	0/0								
22–43		<10	0						
≥52		52	72						

[a]*PD* Parke-Davis, *MN* Merreill-National (which became Connaught Laboratories and is now Sanofi Pasteur), *MSD* Merck Sharpe and Dohme. 22–43 years given A/NJ vaccine; ≥52 years given A/NJ/76 – A/Victoria/75 (H3N2) vaccine. Wyeth vaccine not provided

[b]22–43 years, all 224 were <1:10 HAI in prevaccination sera; 6/405 ≥52 years were <52 years with a high risk condition

[c]*CCA/HA* Actual chick cell agglutinating units/μ HA per 0.5 ml

[d]*GMT* Geometric mean hemagglutination-inhibiting antibody titer; ≥40 = % with ≥1:40 titer

[e]HA not determined as HA was A/Swine/31 not A/New Jersey/76

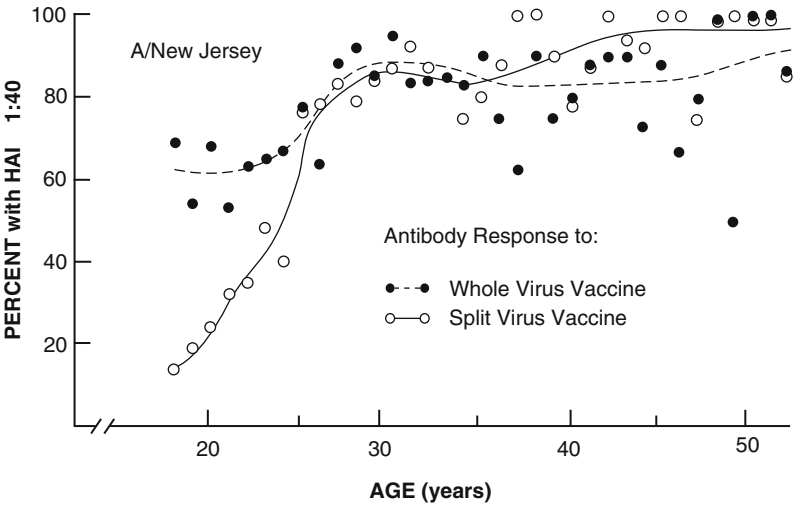

Fig. 1 Relation of age to serum hemagglutinin-inhibiting (HAI) antibody responses. Percent $\geq$ 1:40 after vaccination with one dose of vaccine. Results pooled for WV vaccines (Merrell-National and Merck Sharp and Dohme) and SV vaccines (Parke-Davis and Wyeth). Reprinted from Parkman et al. [7]

The effect of age on antibody responses is shown in Fig. 1 from Parkman et al. [7]. Responses after one dose are variable but generally good for both SV and WV vaccines among those $\geq$25 years of age but were significantly better after one dose of WV vaccine than for SV vaccines among those $\leq$25 years.

Antibody responses to A/USSR (H1N1) vaccines are summarized in Table 7. Dosages were proposed to be 2.3, 7, 20, and 60 μg of HA; results for the three lower projected dosages are shown in the table. Because of the reactogenicity seen with WV A/NJ vaccines, SV only was given to children; results for those aged 3–6 and 7–12 years are shown [8]. None of the single-dose groups developed a satisfactory response (1.3–16 μg HA), while all of the groups exhibited relatively good responses after two doses. Responses to one dose among those 20–25 years old of either SV or WV were generally good but not to the lower dosages (3–6.3 μg HA); responses to two doses were very good including to the lower dosages [12]. Responses to one dose of vaccine containing 10–19 μg HA in those aged 55–88 years old were very good and a second dose of the SV and WV vaccines added very little to the response. Patterns of responses to one and two doses of vaccine for all subjects in the A/USSR vaccine trials are shown in Fig. 2 from La Montagne et al. [5]. Increasing dosages induced increasing frequencies of antibody response for those $\leq$25 years after one dose. For those aged 13–25 years, an optimal response frequency, which was equivalent to the response after two doses of the middle-dose (10–19 μg HA) vaccine, was seen after one dose of a high-dosage (43–61 μg HA) vaccine; no benefit ensued in this age group from a second dose of a high-dosage vaccine. The greatest value for a second dose was seen in those $\leq$12 years of age.

A summary of the immunogenicity findings for the A/NJ and A/USSR vaccines is presented in Table 8. The patterns of serum anti-hemagglutinin antibody

Table 7 Comparison of serum antibody responses after A/USSR (H1N1) inactivated influenza vaccines

Vaccine[a] Age Gp (Yr)	CCA/ HA[b]	Antibody[c] GMT	% ≥40	CCA/ HA[b]	Antibody[c] GMT	% ≥40	CCA/ HA[b]	Antibody[c] GMT	% ≥40
Split virus[d]	W 12/2			W 37/6			W 107/16		
3–6 yr[e]	PD 12/1.3			P 36/3.4			P 102/10		
1 dose		10	10		13	22		15	22
2 doses		40	61		54	71		57	67
7–12 yrs[e]									
1 dose		13	16		12	21		23	39
2 doses		37	74		36	58		57	77
Split virus[d]				W 37/6			W 107/16		
20–25 yr[e]				P 36/3.4			P 102/10		
1 dose					14	18		29	60
2 doses					38	82		48	87
55–88[e]									
1 dose								189	100
2 doses								215	100
Whole virus[d]				C 61/6.3			C 186/19		
20–25[e]				M 20/3			M 81/12		
1 dose					21	25		32	77
2 doses					48	83		47	92
55–88[e]									
1 dose								56	83
2 doses								61	87

[a]Vaccines were *PD* Parke-Davis, W Wyeth, Connaught Laboratories formerly Merrell-National, now Sanofi Pasteur, MSD Merck Sharpe and Dohme

[b]*CCA/HA* Actual chick cell agglutinating units/estimated μ HA per 0.5 ml

[c]Antibody responses; *GMT* geometric mean titer; % ≥40 = % with titer ≥1:40

[d]Vaccines were SV = Wyeth and Parke-Davis; responses to each vaccine pooled; WV = Connaught (C) and MSD (M); responses to each vaccine pooled

[e]No. – 3–6 = 18–31, 7–12 = 28–32 (86%.<1:10 pre); 20–25 = 12–17 (all <1:10); 55–88 = 21–23 (all >1:10 pre)

responses were similar for both the A/NJ and A/USSR vaccines. Increasing the dosage of the vaccine increased the frequency and magnitude of responses and one dose of a higher dosage vaccine frequently elicited a response similar to two doses of a lower dosage vaccine. For the most unprimed age groups (younger children), two doses were almost always needed. Among the healthy "primed," as defined by age, a single moderate dosage (15–20 μg HA) of vaccines was generally adequate. While WV vaccines appeared more immunogenic (and reactogenic) for A/NJ virus, they were not for A/USSR virus vaccines. The greater immunogenicity of A/NJ WV vaccines was at least partly due to a higher HA content.

5 Comment

These clinical trials in humans with two sets of influenza A/H1N1 vaccines constituted the most extensive experience with inactivated whole virus and split-product influenza virus vaccines ever undertaken to that time. Altogether, almost 10,000

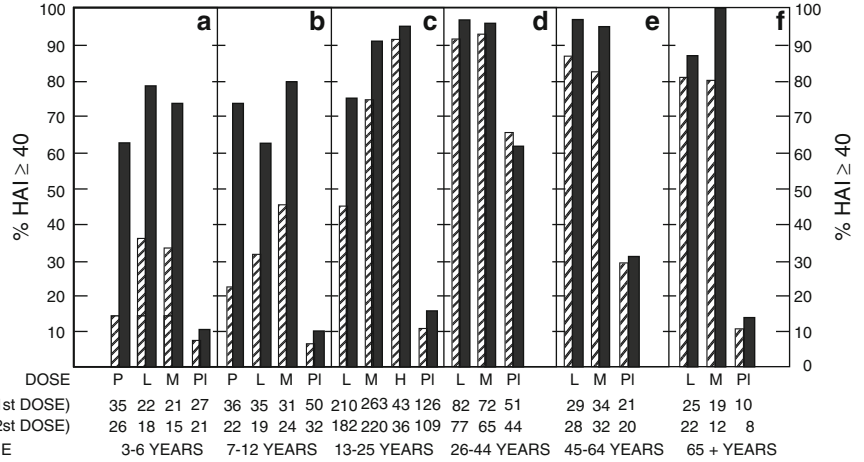

Fig. 2 Cumulative serum hemagglutinin-inhibiting (HAI) antibody responses to the A/USSR/77 (H1N1) vaccines for all subjects in the trial. Each panel illustrates the proportion of individuals who attained serum titers of HAI antibody of ≥1:40 after the first injection (hatched bar) and after the second injection (*solid bar*). The results are shown by ages of the subjects. The HA dosage of vaccine administered [*P* pediatric (SV, 1.3–2 μg), *L* low (*SV*, 3.4–6 μg; *WV*, 3–6.3 μg), *M* medium (SV, 10–16 μg; WV, 12–19 μg), or *H* high (*SV*, 43–61 μg; *WV*, 45–59 μg)], ages of the subjects, and number of individuals who received the first and second vaccinations are shown along the abscissa. Reprinted from La Montagne et al. [5]

Table 8 Summary of immunogenicity of inactivated influenza A/H1N1 vaccines

The patterns of antibody responses by age, dosage, and number of doses were similar for both sets of vaccines – A/New Jersey (H1N1) and A/USSR (H1N1) virus vaccines
Increasing the dosage of the vaccine increased the frequency and magnitude of antibody responses
Expressing vaccine dosage as μg of HA related better to responses than CCA units
A higher dosage of vaccine frequently induced antibody responses similar to two doses of lower dosage vaccine
Two doses of vaccine were almost always needed for satisfactory antibody responses among children
A single dose of vaccine of moderate dosage (15–20 μg HA) induced a satisfactory antibody response among those "primed" because of past exposure to related HA antigens. Age was adequate for this determination
Whole virus vaccines appeared more immunogenic than split-product vaccines for A/New Jersey virus but not for A/USSR viruses although A/USSR WV vaccines were not tested in children
Long-term persistence of A/H1N1 serum antibody was demonstrated (≥25 years)

volunteers of ages 0.5–100 years participated in the trials. The trials confirmed many immunological concepts noted in earlier vaccine trials and expanded our understanding of vaccine responses. Reinforced principles included that increasing dosage increases antibody responses and two doses of a low dosage (<10 μg HA) will induce a greater response than one dose. These concepts were particularly noticeable

among persons unprimed to H1 antigens. Priming was shown to be a major determinant of enhanced responses and was age-related as determined by a likely exposure to H1 antigens of influenza viruses circulating in earlier years; the years of A/H1N1 circulation were 1918–1956.

Responses to WV vaccines were not consistent in that reactogenicity and immunogenicity were both greater for A/NJ vaccines than for SV vaccines while for A/USSR they were similar. The increased reactogenicity and immunogenicity for the A/NJ WV vaccines could at least partly be caused by the increased HA content. However, for the MSD vaccine, the increased reactogenicity and apparent immunogenicity appeared to be also partly due to the structure of virus particles in the vaccine; the MSD vaccine contained more intact particles, including both spherical and filamentous particles, than did the MN whole virus vaccine. This adjuvant-like effect for WV vaccines was not seen for the A/USSR WV vaccines but virus particle structure of the two WV vaccines was not provided. Thus, WV vaccines may not be uniformly more reactogenic nor immunogenic than SV vaccines.

A number of additional variables were examined with the A/NJ vaccines. An Intradermal (ID) immunization study with 0.1 ml vaccine of WV vaccine (MN vaccine) was inferior to 0.5 ml IM among unprimed persons after one dosage and an ID–IM sequence was no better than an IM–IM sequence [13]. Responses to ID were similar to IM among those with some prior antibody. Local reactions were greater among those given ID vaccine but systemic reactions were greater and also among those given vaccine IM. A number of studies were conducted among persons of "high risk" [1]. The increased reactogenicity of WV vaccines was noted in these studies, but the vaccines were otherwise considered safe and immunogenic in subjects with multiple sclerosis, asthma, pulmonary disease, heart disease, cancer, and some other disorders.

The 1976–1977 vaccine trials did not explore some other vaccine variables of interest; these include timing for dose two. A 3-week interval between doses is commonly used by European investigators but a 4-week interval was used in the two dose studies of 1976–1977. A 2-week interval between vaccinations was explored in the past and responses were suboptimal ([14, 15] and own unpublished data). The value of adjuvants was not explored in the 1976–1977 trials but incomplete Freund's adjuvant was used in some A/H2N2 vaccines in 1957 and an adjuvant effect was demonstrated [15]. It was stated that using IFA with a 100 CCA dose induced an antibody response similar to a 400 CCA dose without IFA.

Safety and immunogenicity trials are being performed currently with the newly emerged influenza A/H1N1 virus. All vaccines in the USA 2009 trials are split-product or subunit vaccines; it seems likely that these trials will largely confirm the conclusions from the earlier A/H1N1 vaccine trials. On the basis of the 1976–1977 experience, it is predicted that persons $\geq$85 years of age have a high likelihood of possessing antibody and resistance to the 2009 A/H1N1 virus and that most $\geq$55 years old will possess antibody and a high level of "priming"; a single vaccination of moderate dosage should induce substantial responses in both of these populations. Because other A/H1N1 viruses have been causing human infections since

1977 and have been included in seasonal vaccines, a considerable portion of the population should be primed and exhibit satisfactory antibody responses to a single moderate dosage (10–20 μg of HA) of vaccine although a higher dosage would probably induce a greater response. A precise age for priming cannot be set as in the 1976–1977 trials but lower frequencies of priming will be encountered with reducing age so that two doses of vaccine will be required for an adequate response in younger persons. If these predictions are correct, the concepts and principles developed in the 1976–1977 trials with inactivated influenza virus vaccines will be reinforced.

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References

1. (1977) Clinical studies of influenza vaccines – 1976. J Infect Dis 136(Supplement)
2. Kendal AP, Nobel GR, Skehel JJ, Dowdle WR (1978) Antigenic similarity of influenza A (H1N1) viruses from epidemics in 1977–1978 to "Scandinavian" strains isolated in epidemics of 1950–1951. Virology 89:632–636
3. (1983) Clinical studies of influenza vaccines – 1978. Rev Infect Dis 5: 721–764
4. Ennis FA, Mayner RE, Barry DW, Manischewitz JE, Dunlap RC, Verbonitz MW, Bozeman FM, Schild GC (1977) Correlation of laboratory studies with clinical responses to A/New Jersey influenza vaccines. J Infect Dis 136(Suppl):S397–S406
5. La Montagne JR, Noble GR, Quinnan GV, Curlin GT, Blackwelder WC, Smith JI, Ennis FA, Bozeman FM (1983) Summary of clinical trials of inactivated influenza vaccine – 1978. Rev Infect Dis 5:723–736
6. Wright PF, Thompson J, Vaughn WK, Folland DS, Sell SHW, Karzon DT (1977) Trials of influenza A/New Jersey/76 virus vaccine in normal children: an overview of age-related antigenicity and reactogenicity. J Infect Dis 136:S731–S741
7. Parkman PD, Hopps HE, Rastogi SC, Meyer HM Jr (1977) Session V. Summary of clinical studies. Summary of clinical trials of influenza virus vaccines in adults. J Infect Dis 136: S722–S730
8. Wright PF, Cherry JD, Foy HM, Glezen WP, Hall CB, McIntosh K, Monto AS, Parrott RH, Portnoy B, Taber LH (1983) Antigenicity and reactogenicity of influenza A/USSR/77 virus vaccine in children – a multicentered evaluation of dosage and safety. Rev Infect Dis 5:758–764
9. Glezen WP, Loda FA, Denny FW (1969) A field evaluation of inactivated, zonal-centrifuged influenza vaccines in children in Chapel Hill, North Carolina, 1968–69. Bull WHO 41: 566–569
10. Cate TR, Couch RB, Kasel JA, Six HR (1977) Clinical trials of monovalent influenza A/New Jersey/76 virus vaccine in adults: reactogenicity, antibody response, and antibody persistence. J Infect Dis 136:S450–S455
11. Cate TR, Kasel JA, Couch RB, Six HR, Knight V (1977) Clinical trials of bivalent influenza A/New Jersey/76-A/Victoria/75 vaccines in the elderly. J Infect Dis 136:S518–S525

12. Cate TR, Couch RB, Parker D, Baxter B (1983) Reactogenicity, immunogenicity, and antibody persistence in adults given inactivated influenza virus vaccines – 1978. Rev Infect Dis 5:737–747
13. Brown H, Kasel JA, Freeman DM, Moise LD, Grose NP, Couch RB (1977) The immunizing effect of influenza A/New Jersey/76 (Hsw1N1) virus vaccine administered intradermally and intramuscularly to adults. J Infect Dis 136:S466–S471
14. Bayne GM, Liu OC, Boger WP (1958) Asian influenza vaccine: effect of age and schedule of vaccination upon antigenic response. Am J Med Sci 236:290–299
15. Meiklejohn GN (1961) Asian influenza vaccination: dosage, routes, schedules of inoculation, and reactions. The Amer Rev of Resp Dis 83:175–177