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Clinical Reactivity and Immunogenicity of Hemagglutinin Influenza Vaccine

II. Clinical Reactions, Hemagglutination-Inhibiting and Strain and Type-Specific Complement-Fixing Antibody Responses in Infants

By

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Summary

The reactivity and immunogenicity of a polyvalent hemagglutinin influenza vaccine was studied in subjects aged 5—7 months. The vaccine was administered in three doses either subcutaneously (0.5 ml per dose) or intradermally (0.1 ml per dose) over a period of 6—7 months. Clinical reactions, both local and febrile, even after administering two or three doses, were quite mild. After two 0.5 ml doses of vaccine administered subcutaneously, most subjects possessed hemagglutination-inhibition (HI) antibodies to all the six vaccine viruses. In most subjects the antibodies dropped to undetectable levels in the course of four to five months. A booster effect was observed after the third vaccine dose. Intradermal administration of the vaccine (0.1 ml) was much less efficient. Even three doses of vaccine did not induce antibody development in most subjects. The antibody response in the complement-fixation (CF) test using strain-specific V antigens corresponded, in general, with the results of the HI test; however the CF antibodies to the A 2/Taiwan and B/Md viruses were found much less frequently than the corresponding HI antibody. Most of the infants vaccinated subcutaneously also developed antibodies reactive in the CF test with the soluble (S) antigens of influenza A and B. They were much less frequent in subjects immunized intradermally.

1. Introduction

The administration of inactivated whole-virus influenza vaccines to infants is frequently associated with severe reactions. For this reason, its use in very

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young children has been restricted, although the risk of death from influenza is higher among these than older subjects. A detailed study of the clinical reactivity and immunogenicity of a polyvalent hemagglutinin vaccine in infants has therefore been undertaken, using different routes of vaccine administration and up to three vaccine doses. The results are reported in this paper.

2. Materials and Methods

2.1. Vaccine

The same lot of polyvalent hemagglutinin vaccine as in the study reported in the previous paper was used (ZÁVADOVÁ *et al.*, 1971).

2.2. Selection of Subjects

Babies aged 5—7 months living in infants' establishments were vaccinated. A number of these establishments were monitored for three weeks prior to initiation of the study and those with an unusual incidence of febrile illnesses during this period were not included. None of the vaccinees had previously been given influenza vaccine. Before vaccination each subject passed a careful physical examination including measurement of the body temperature. Only children whose rectal temperature did not exceed 37.2°C were included.

The vaccinees were divided into four groups according to vaccination schedule:

Group I. Subjects were vaccinated subcutaneously with three 0.5 ml doses of vaccine at intervals of 6 weeks and 4—5 months between the first and second and third doses, respectively.

Group II (control to Group I). Subjects received one 0.5 ml dose of vaccine subcutaneously at the same age and time as Group I received their third injection.

Group III. Subjects were injected intradermally with three 0.1 ml doses of vaccine, at the same intervals as Group I.

Group IV (control to Group III). Subjects received one 0.1 ml dose of vaccine intradermally at the same age and time as Group III received their third injection.

In addition, non-vaccinated subjects were included. The subjects belonging to the parallel groups and the non-vaccinated subjects were distributed equally in the respective establishments.

2.3. Administration of Vaccine

The vaccine was injected in 0.5-ml doses (subcutaneously) or in 0.1-ml doses (intradermally) into the deltoid area of the left arm. The right and left arm were used alternately for subsequent injections.

2.4. Evaluation of Reactions

Local reactions were followed similarly as in the previous study (ZÁVADOVÁ *et al.*, 1971). Febrile reactions were determined by taking rectal temperature et two-hour intervals for the first eight hours and then at 24-hour intervals. All vaccinees were thereafter regularly examined by the physician of the respective establishment. Follow-up by the study team continued once a week.

2.5. Serum Sampling

Up to five serum samples were taken from each subject. They were denoted A-E. Sample A was taken immediately before the administration of the first dose; sample B six weeks later, immediately before the second dose. Sample C was obtained four weeks after B; sample D 3—4 months after C, immediately before the third dose; and sample E 4—5 weeks after D, *i.e.* 7—8 months after A. All these samples were taken in Groups I and III. In Groups II and IV only three sera (A, D and E) were collected. They were taken at the same time as the respective sera in Groups I and III. Thus, samples D were taken immediately before administering the first vaccine dose and samples E 4—5 weeks later. Because of a high turn over in the infants

establishments, the initial number of subjects decreased considerably in the course of the study. This was especially marked in Groups II and IV. For this reason, the late administration of the first dose of vaccine (simultaneously with the third dose of vaccine in Groups I and III) and the late samplings (D and E) were also performed in children originally not included in the study. Accordingly, the numbers of D and E samples collected in these two groups markedly exceeded the A samples.

2.6. Viruses

The viruses were the same as used in the previous study (ZÁVADOVÁ *et al.*, 1971).

2.7. Serological Investigations

The procedures of performing hemagglutination-inhibition (HI) and type- and strain-specific complement-fixation (CF-S and CF-V) tests and calculation of GMT have been described (ZÁVADOVÁ *et al.*, 1971). All sera were examined simultaneously in each test.

3. Results

3.1. Clinical Studies

Local reactions expressed by erythema are presented in Tables 1 and 2. The frequency of occurrence of erythema of ≥ 1 cm after subcutaneous or intradermal injection varied from 0 to 13%. The number and size of the reactions indicate that they are of minor importance only.

Table 1. *Development of Erythema ($\geq$ 1 cm) in the Course of Three Days Following Subcutaneous Vaccination of Infants Aged* **5—12** *Months*

Injection	Time after vaccination (hours)	Group I (3 × 0.5 ml s.c.)			Group II a) (1 × 0.5 ml s.c.)		
		No. of vaccinees	Occurrence of erythema	Mean size of erythema (cm)	No. of vaccinees	Occurrence of erythema	Mean size of erythema (cm)
1st injection	24 hrs.	31	4 (13)b)	1.6	21	0	0
	48 hrs.	31	2 (6)	1.3	21	1 (5)	1
	72 hrs.	31	0	0	21	0	0
2nd injection	24 hrs.	24	0	0			
	48 hrs.	24	0	0	No second and third injections given		
	72 hrs.	24	0	0			
3rd injection	24 hrs.	16	0	0			
	48 hrs.	16	1 (6)	1			
	72 hrs.	16	0	0			

a) Vaccinated at age and time when the 3rd injection was given to Group I children
b) Numbers in parentheses: mean percent.

Febrile reactions are recorded in Table 3. The percentage of reactions in the vaccinees was corrected with respect to the control groups according to the formula $p = \frac{100\,(pv - pc)}{100 - pc}$, in which p is the corrected percentage, pv is the percentage of reactions in vaccinated infants and pc is the percentage of reactions in the control non-vaccinated subjects. It can be seen that vaccination-associated body-temperature increase was infrequent both after subcutaneous and intradermal injections. After the first subcutaneous injection a reaction frequency

Table 2. *Development of Erythema (≧ 1 cm) in the Course of Three Days Following Intradermal Vaccination of Infants Aged 5—12 Months*

Injection	Time after vaccination (hours)	Group III (3×0.1 ml i.d.)			Group IV a) (1×0.1 ml i.d.)		
		No. of vaccinees	Occurrence of erythema	Mean size of erythema (cm)	No. of vaccinees	Occurrence of erythema	Mean size of erythema (cm)
1st injection	24 hrs.	31	2 (6)b)	1.5	24	2 (8)	1.3
	48 hrs.	31	1 (3)	1.0	24	1 (4)	1.0
	72 hrs.	31	0	0	24	0	0
2nd injection	24 hrs.	22	0	0			
	48 hrs.	22	0	0	No second and third injections given		
	72 hrs.	22	0	0			
3rd injection	24 hrs.	15	2 (13)	1.1			
	48 hrs.	15	1 (7)	1.4			
	72 hrs.	15	0	0			

a) Vaccinated at age and time when the 3rd injection was given to group III children
b) Numbers in parentheses: mean percent.

peak (9%) occurred in 2 hours, while after the first intradermal dose a peak (7%) appeared after 4 hours. Similar peaks were observed in vaccinees of the Groups II and IV (12 and 14% respectively). After the second subcutaneous injection reaction frequency peaks appeared in 4 hours (27%), and after the second intra-

Table 3. *Increase of Rectal Temperature (≧ 0.5° C) in Infants after Vaccination*

Injection	Group of vaccinees	No. of infants	Time of evaluation after vaccination							
			2 hours		4 hours		6 hours		8 hours	
			No. %	Cor.[1] %	No. %	Corr. %	No. %	Corr. %	No. %	Corr. %
1st	I	Vaccinees 31	5 16.1	9	4 12.9	1	5 16.1	5	4 12.9	0
		Controls 52	4 7.7		6 11.5		6 11.5		8 15.4	
	III	Vaccinees 31	1 3.2	0	6 19.3	7	5 16.1	0	4 12.9	0
		Controls 39	3 7.7		5 12.8		7 17.9		8 20.5	
2nd	I	Vaccinees 24	5 20.8	6	7 29.2	27	5 20.8	18	5 20.8	18
		Controls 31	5 16.1		1 3.2		1 3.2		1 3.2	
	III	Vaccinees 22	3 13.6	7	5 22.7	17	5 22.7	20	3 13.6	3
		Controls 28	2 7.1		2 7.1		1 3.6		3 10.7	
3rd	I	Vaccinees 16	4 25.0	5	3 18.7	15	2 12.5	0	4 25.0	14
		Controls 24	5 20.8		1 4.2		4 16.7		3 12.5	
	III	Vaccinees 15	2 13.3	0	1 6.7	0	1 6.7	0	1 6.7	0
		Controls 26	4 15.4		3 11.5		5 19.2		5 19.2	
1st	II	Vaccinees 21	3 14.4	12	2 9.5	5	2 9.5	5	2 9.5	7
		Controls 43	1 2.3		2 4.6		2 4.6		1 2.3	
	IV	Vaccinees 24	3 12.5	3	4 16.7	14	5 20.8	14	3 12.5	10
		Controls 41	4 9.8		1 2.4		3 7.3		1 2.4	

[1] With respect to the controls

dermal injection at six hours (20%). The reactions after the third dose were weaker than after the second dose; this especially applies to those after the intradermal inoculation.

The reactions, when present, were mild. An increase of temperature of $\geq 1.5°C$ above the normal level was only detected in six subjects dispersed over all four groups. In no instance this increase lasted over a period of six hours.

The vaccination did not influence the weight curve of the children.

3.2. Serological Studies

The results of HI tests in Group I and II (0.5 ml doses of vaccine given subcutaneously) are summarized in Table 4. It can be seen that prevaccination antibody to A_2/Taiwan was only detected. Six weeks after the first dose administered to Group I, A_2 antibody was present in nearly all subjects. More than half of

Table 4. *Results of HIT with Sera Taken from Infants Inoculated Subcutaneously*

Group	Serum	No. subjects	Antigen					
			Swine	PR-8	AA	Taiwan	B-Lee	B-Md
	A	12	0[1] (—)	0 (—)	0 (—)	75 (16)	0 (—)	0 (—)
	B	9	0 (—)	0 (—)	11 (6)	90 (20)	33 (6)	55 (9)
I	C	11	90 (36)	70 (12)	70 (12)	100 (50)	90 (66)	100 (110)
	D	10	10 (6)	0 (—)	10 (6)	80 (13)	30 (8)	80 (16)
	E	12	75 (13)	42 (9)	50 (10)	100 (40)	83 (26)	100 (46)
	A	6	0 (—)	0 (—)	0 (—)	50 (8)	0 (—)	0 (—)
	—	—	— —	— —	— —	— —	— —	— —
	—	—	— —	— —	— —	— —	— —	— —
II	D	22	0 (—)	0 (—)	0 (—)	95 (35)	0 (—)	5 (5)
	E	23	0 (—)	0 (—)	4 (5)	100 (50)	22 (6)	65 (10)

[1] First number: percentage of subjects with antibody; number in brackets: GMT (Geometric mean titre)

them developed antibody to the B/Md virus and a few also the A_1/AA and B/Lee viruses. The administration of the second vaccine dose resulted in the formation of detectable antibody levels to all six strains in the majority of subjects. As revealed by D samples, these antibodies mostly did not persist over the period of four to five months. The third dose induced seroconversions in the majority of subjects; however, they were less frequent and the GMT's were lower than after the second injection. The results obtained in the control Group II sera indicate that one dose of vaccine at the age of one year was as effective as in subjects younger by approximately 6 months. The increased incidence of A_2 antibody in D samples as compared with A samples suggests that most of the children had experienced an infection with this virus in the interval between the first and third dose.

The results of CF-V tests with the same sera are presented in Table 5. Serum samples from one additional subject were examined in the CF-V test. The most marked differences between these and the HIT results are the absence of Taiwan CF antibody in the pre- and post of the postvaccionation sera, and the absence of B/Md CF antibody in sera collected after one dose and before the third dose. Otherwise, however, the general pattern of antibody development and persistence resembled the findings in HIT.

The antibody responses after intradermal administration of the vaccine (0.1 ml doses) were much lower than after subcutaneous injection. The results of HIT's with sera of intradermally inoculated subjects are listed in Table 6. It can be seen that even after the administration of three doses of vaccine (Group III)

Table 5. *Results of Strain-Specific CF Test with Sera from Infants Inoculated Subcutaneously*

Group	Serum	No. subjects	Antigen					
			Swine	PR-8	AA	Taiwan	B-Lee	B-Md
	A	13	0[1] (—)	0 (—)	0 (—)	0 (—)	0 (—)	0 (—)
	B	13	8 (3)	0 (—)	0 (—)	8 (3)	0 (—)	0 (—)
I	C	12	83 (40)	66 (10)	75 (12)	50 (6)	92 (21)	100 (22)
	D	12	25 (3)	8 (3)	17 (3)	17 (3)	8 (3)	8 (3)
	E	13	62 (12)	54 (7)	62 (11)	38 (6)	83 (15)	70 (8)
	A	6	0 (—)	0 (—)	0 (—)	0 (—)	0 (—)	0 (—)
	—	—	— —	— —	— —	— —	— —	— —
II	—	—	— —	— —	— —	— —	— —	— —
	D	22	0 (—)	0 (—)	0 (—)	0 (—)	0 (—)	0 (—)
	E	23	0 (—)	10 (3)	17 (3)	13 (3)	17 (3)	10 (3)

[1] First number: percentage of subjects with antibody; number in brackets: GMT (Geometric mean titre)

no antibodies to Swine, PR-8 and AA viruses were detected. The presence of antibody to the Taiwan virus seems to be conditioned by natural infection. Antibody response to the B/Lee virus was rare and weak. Only the response to B/Md was good; after three injections all subjects possessed antibody.

Table 6. *Results of HIT with Sera from Infants Inoculated Intradermally*

Group	Serum	No. subjects	Antigen					
			Swine	PR-8	AA	Taiwan	B-Lee	B-Md
	A	9	0[1] (—)	0 (—)	0 (—)	66 (16)	0 (—)	0 (—)
	B	9	0 (—)	0 (—)	0 (—)	78 (14)	11 (6)	44 (8)
III	C	9	11 (6)	0 (—)	0 (—)	89 (37)	33 (7)	88 (18)
	D	8	0 (—)	0 (—)	0 (—)	50 (16)	0 (—)	50 (7)
	E	9	0 (—)	0 (—)	0 (—)	89 (17)	22 (7)	100 (25)
	A	5	0 (—)	0 (—)	0 (—)	60 (11)	0 (—)	0 (—)
IV	D	19	0 (—)	0 (—)	0 (—)	80 (36)	0 (—)	0 (—)
	E	19	0 (—)	0 (—)	0 (—)	74 (38)	0 (—)	0 (—)

[1] First number: percentage of subjects with antibody; number in brackets: GMT (Geometric mean titre)

In Table 7 the results of CF-V tests are presented. At variance with the HIT results, very little or none Taiwan or B/Md antibodies were detected in either pre- or postvaccination sera. On the other hand, two out of nine subjects developed CF-V antibodies to Swine virus and one to AA virus without displaying antibody detectable by HIT.

Table 7. *Results of Strain-Specific CF Test with Sera from Infants Inoculated Intradermally*

Group	Serum	No. subjects	Antigen					
			Swine	PR-8	AA	Taiwan	B-Lee	B-Md
III	A	11	0[1] (—)	0 (—)	0 (—)	0 (—)	0 (—)	0 (—)
	B	11	0 (—)	0 (—)	0 (—)	0 (—)	0 (—)	0 (—)
	C	11	18 (3)	0 (—)	9 (3)	18 (3)	18 (3)	18 (3)
	D	11	9 (3)	0 (—)	9 (3)	0 (—)	0 (—)	0 (—)
	E	11	18 (3)	0 (—)	9 (3)	18 (3)	0 (—)	0 (—)
IV	A	5	0 (—)	0 (—)	0 (—)	0 (—)	0 (—)	0 (—)
	D	19	0 (—)	0 (—)	0 (—)	0 (—)	0 (—)	0 (—)
	E	19	0 (—)	0 (—)	0 (—)	5 (3)	0 (—)	0 (—)

First number: percentage of subjects with antibody; number in brackets: GMT (Geometric mean titre)

Also type-specific CF-antibodies were determined. The results in sera from Groups I and III are summarized in Table 8. The S antibody responses in general followed the HI and V antibody development patterns. In subjects vaccinated subcutaneously (Group I) S antibodies were detected rarely after the first vaccine

Table 8. *Results of Type-Specific CF-S Test with Sera from Infants Inoculated Subcutaneously or Intradermally*

Sample	S – A		S – B	
	Group I	Group III	Group I	Group III
A	0/13[1]	0/9	0/13	0/9
B	1/13	0/9	0/13	0/9
C	7/13	2/9	8/13	0/9
D	3/12	2/9	0/12	0/9
E	8/13	2/9	8/12	0/9

[1] Numerator: No. positive sera; denominator: No. sera tested.

dose, but were present in the majority of subjects after the second vaccine dose. Their incidence dropped in the course of the subsequent 4—5 months and rose again after the third dose. The antibody titres never exceeded 1:20. Antibody responses to both S antigens were markedly less frequent after intradermal vaccination (Group III).

4. Discussion

Local and febrile reactions developing in infants after repeated administration of a hemagglutinin influenza vaccine were studied in terms of the usual criteria employed for the evaluation of reactivity of the DPT vaccine (Adam *et al.*, 1970), which is the most frequently used vaccine in childhood. The local reactions observed after one to three doses given either sub- or intracutaneously were minimal both as regards frequency and intensity. Also the temperature increases were

infrequent and mild. The vaccine under study produced less unfavourable effects than the best DPT vaccines used in a comparative study of European and U.S. preparations (ADAM *et al.*, 1970). The febrile reactions in this study were less pronounced than those reported in an earlier test of hemagglutinin vaccine in infants (HENNESY and DAVENPORT, 1967).

Two 0.5-ml doses of vaccine administered subcutaneously were necessary to induce the development of HI antibodies to all six vaccine viruses in most subjects. This is in agreement with the observations of HENNESY and DAVENPORT (1967). Intradermal administration of 0.1-ml doses of vaccine represented an insufficient antigenic stimulus. A good antibody response was only recorded for B/Md virus; this might be caused by the higher content of the B/Md antigen in the vaccine (300 CCA), but also by previous natural exposure to related antigens. Thus, there was a marked difference in infants between the two routes of vaccine administration, which was not apparent in subjects aged three years and more (ZÁVADOVÁ *et al.*, 1971). It should be kept in mind, however, that only one dose of vaccine was administered to those older subjects, and that the antibody response in them was age-dependent, being limited to those virus types with which natural infection could have been experienced. On the other hand, the difference between the two immunization schedules in infants became apparent only after two doses of the vaccine had been given. The antibody response in infants, at least as regards four out of the six viruses employed, seems to be due to antibody development *de novo*, not conditioned by previous sensitization to the respective antigens.

As in the preceding study both HI and CF-V antibody responses were recorded. Little differences between the two tests was observed, with the exception of the A_2/Taiwan and B/Md viruses. Antibodies to these viruses were found less frequently in the CF-V than the HI test. This may be associated with a higher sensitivity of the HI than CF test. It is more likely, however, that the previous natural exposure of the subjects to viruses belonging to the respective virus types was involved, and that the differences encountered reflect the different strain specificities of the two tests. It may also be of interest that in two subjects Swine-V antibody was found in the absence of detectable levels of homotypic HI antibody. A similar observation was reported and discussed in the preceding paper (ZÁVADOVÁ *et al.*, 1971).

Summarizing the serologic data in infants, it seems clear that even the repeated administration of 0.5-ml of vaccine induced a low-order response. Although the antibody levels formed may be too low to prevent virus infection and development of disease they are probably high enough to modify the course of the disease and to reduce the death rate considerably. This assuption can only be confirmed in a trial in which the hemagglutinin vaccine is used on a large scale. Its very low reactivity seems to justify such a study.

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