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Antibody response to immunization with influenza A/USSR/77 (H1N1) virus in young individuals primed or unprimed for A/New Jersey/76 (H1N1) virus

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SUMMARY

A group of 269 pupils of the Harbour and Transport Training Institute in Rotterdam (group A), aged 13–20 years, and of 109 patients of the Dr Mr Willem van den Bergh Foundation at Noordwijk (group B), aged 11–21 years, were immunized with a whole virus vaccine containing 10, 20, or 40 μg HA of A/USSR/92/77 (H1N1) influenza virus. A booster vaccination was administered 6 weeks later with 20 μg HA of the same virus. Many of the participants had been immunized during the two preceding years with a whole virus vaccine containing A/New Jersey/8/76 (H1N1) (A/NJ/76) virus. The side-effects, mostly of a moderate nature, increased with the dose of virus in the vaccine. In group A side effects were least frequent in the vaccinees who had never received A/NJ/76 vaccine. A single dose of A/USSR/77 vaccine did not produce satisfactory levels of homologous antibodies. After booster immunization with 20 μg HA of A/USSR/77 virus participants showed a higher homologous antibody response in all vaccine-dose groups if they had not been immunized with A/NJ/76 virus in previous years. After primary and especially after booster immunization with A/USSR/77 virus, a very high response against A/NJ/76 virus and adequate levels of A/NJ/76 antibody were found in participants who had been immunized previously with A/NJ/76 virus. Those who had not been immunized with this virus previously showed no or a very low antibody response to A/NJ/76 virus.

INTRODUCTION

Since 1977 the epidemiological characteristics of the influenza A virus have been different from those described in virological, serological, and epidemiological studies carried out from 1933 to 1977. The H1N1 virus has remained throughout

the world as a common infective agent alongside the H3N2 virus, which had the monopoly from 1968 to 1977. This is in contrast with the situation in 1957, when the H1N1 virus was succeeded by the H2N2 virus as an epidemic agent, and in 1968, when the H3N2 virus took over from H2N2. Both former viruses disappeared without trace in human populations. Currently, the H3N2 virus is still circulating widely as an epidemic agent in all age groups in the world population.

When the H1N1 virus reappeared in The Netherlands in 1977, serological studies revealed that individuals born after 1949 did not possess antibodies against this virus, although on epidemiological grounds this would have been expected solely for persons born after 1957 (Masurel & André, 1978). Subsequently, it was found that 75% of people aged 70 years or older possessed no antibodies at all against the H1N1 virus, and only 2% showed a protective level of antibodies (Masurel, 1979).

The present study was directed towards two population groups of young people born after 1957 who had been exposed in their lifetime to influenza epidemics caused by H2N2 or H3N2 virus only. Part of the study group had been immunized in 1976 and/or 1977 with the A/New Jersey/8/76 (H1N1) (A/NJ/76) virus (Masurel, de Jong & Verhoeff, 1977). In retrospective studies it has been found that the agent of the 1918–1919 pandemic, which was similar or identical to the A/Swine/Iowa/15/30(H1N1) virus, is clearly related to all influenza A viruses emerging from 1918 to 1957, including the H1N1 viruses prevailing from 1946 to 1956 (Masurel, 1962). The results presented here show the serological response to influenza A/USSR/77 vaccine in individuals born after 1957 with or without a history of previous A/NJ/76 immunization.

MATERIALS AND METHODS

Study group

Participants to the study were 269 pupils of the Harbour and Transport Training Institute in Rotterdam (group A) and 109 mentally handicapped patients of the Dr Mr Willem van den Bergh Foundation at Noordwijk (group B), aged 13–20 years and 11–21 years, respectively. In both groups the parents' consent was obtained before the trial. In group A, 145 pupils had been immunized in 1976 with a trivalent vaccine containing 20 μg HA A/New Jersey/8/76 (H1N1), 20 μg HA A/Victoria/3/75 (H3N2) and 18 μg HA B/Hong Kong/8/73 virus. This vaccine had also been administered to 97 patients of group B in 1976 and 1977 (Masurel, de Jong & Verhoeff, 1977). Age distribution among different vaccine-dose groups is similar for participants immunized or not immunized previously with A/NJ/76 virus.

Immunization

In February 1978 group A was randomly immunized with 0·5 ml monovalent whole virus vaccine (kindly supplied by Duphar, Weesp, The Netherlands) containing 10, 20 or 40 μg HA A/USSR/92/77 (H1N1) virus. A booster immunization of 20 μg HA of this virus was given to some of the volunteers six weeks later. In March 1978 group B received 0·5 ml monovalent whole virus vaccine containing 20 μg HA of the above mentioned virus and six weeks later all patients

were given a booster dose. Vaccines were administered intramuscularly in the upper arm.

After primary immunization, participants of group A recorded local reactions, redness, swelling, and pain at the injection site, and systemic reactions, headache, fever and malaise. In group B redness, swelling, and tenderness at the injection site were monitored and body temperature was measured by the medical staff.

For both groups, local reactions were considered positive if one or more local symptoms were reported. Systemic reactions were scored positive in group B if the body temperature was over 37·5 °C, and in group A if one or more systemic reactions were reported.

Serological studies

Blood samples were collected from all participants on the day of primary immunization (SI) and four weeks later (SII). From 176 participants sera were sampled three weeks after booster immunization (SIII). All sera from each participant were simultaneously examined in the haemagglutination inhibition (HI) test for antibodies against the H1N1 virus A/Hong Kong/117/77 (identical to the vaccine strain A/USSR/77) and against the A/NJ/76 (H1N1) virus present in the influenza vaccines of previous years. Before testing, the sera were treated with *Vibrio cholerae* filtrate for 18 h at 37 °C and subsequently heated at 56 °C for 1 h to remove non-specific inhibitors (Masurel, 1969). In all experiments reference antisera were included. In establishing the geometric mean titre (*GMT*) an HI titre of < 9 was recorded as 8, and of > 2150 as 2200. A titre $\geqslant 100$ found by our titration method was regarded as protective (Wesselius-de Casparis, Masurel & Kerrebijn, 1972).

RESULTS

Table 1 shows the results of the HI test using the influenza virus A/Hong Kong/117/77 (H1N1) on sera of groups A and B, obtained before, after primary, and after booster immunization with the antigenically identical virus A/USSR/77 (H1N1). Group A is divided in three subgroups according to the amount of virus in the vaccine given. Furthermore, distinction has been made in groups A and B between subjects that did or did not receive a vaccine containing A/NJ/76 virus in 1976 and/or 1977. All pre-immunization sera (SI) were negative with regard to H1N1 antibody.

In group A, after primary immunization with 10 μg, 20 μg, or 40 μg HA A/USSR/77 virus the serological responses showed little difference between subjects that had or had not received A/NJ/76 virus containing vaccine previously. After booster immunization *GMT*s were 1·5-fold to 3-fold higher, and frequency of titres above 100 1·5-fold to 2-fold higher in the participants not previously immunized with A/NJ/76 virus.

In group B, after booster immunization patients immunized previously with A/NJ/76 virus showed a response similar to that of participants after primary immunization with A/USSR/77 virus without previous A/NJ/76 immunization. After booster immunization *GMT*s were 4-fold higher and frequency of titres above 100 2·5-fold higher in patients not immunized previously with A/NJ/76 virus.

Table 1. *HI antibodies against influenza virus A/Hong Kong/117/77 (H1N1) after immunization with 10, 20, or 40 μg HA, and booster immunization with 20 μg HA of A/USSR/92/77 (HN1) virus in individuals primed or unprimed for A/NJ/76 virus*

Immunization status	No. of participant	% 4-fold titre increase I* → II*	GM titre II	% HI titres II		Booster immunization	No. of participants	% 4-fold titre increase I → III*	GM titre III	% HI titres III	
				< 18	⩾ 100					< 18	⩾ 100
Group A											
10 μg A/USSR/77						20 μg A/USSR/77					
A/NJ/76+†	42	36	23	57	10	A/NJ/76+†	13	85	77	15	46
A/NJ/76−†	45	38	20	60	7	A/NJ/76−†	15	100	266	0	93
20 μg A/USSR/77						20 μg A/USSR/77					
A/NJ/76+	60	55	29	40	13	A/NJ/76+	14	86	115	14	56
A/NJ/76−	38	58	34	37	18	A/NJ/76−	6	100	237	0	83
40 μg A/USSR/77						20 μg A/USSR/77					
A/NJ/76+	43	70	43	28	26	A/NJ/76+	13	100	133	0	54
A/NJ/76−	41	46	31	41	17	A/NJ/76−	10	100	198	0	90
Group B											
20 μg A/USSR/77						20 μg A/USSR/77					
A/NJ/76+	95	44	31	47	20	A/NJ/76+	95	73	59	17	31
A/NJ/76−	10	70	66	30	50	A/NJ/76−	10	90	217	0	80

* I, pre-immunization serum; II, post-immunization and pre-booster immunization serum; III, post-booster immunization serum.

† A/NJ/76+, immunized with A/New Jersey/8/76 (H1N1) virus in 1976 and/or 1977; A/NJ/76−, not immunized with this virus.

Table 2. *HI antibodies against influenza virus A/New Jersey/8/76 (H1N1) after immunization with* 10, 20, *or* 40 *μg HA, and booster immunization with* 20 *μg HA of A/USSR/92/77 (H1N1) virus in individuals primed or unprimed for A/NJ/76 virus*

Immunization status	No. of participants	% 4-fold titre increase I* → II*	GM titre I	GM titre II	% HI titres I < 18	% HI titres I ⩾ 100	% HI titres II < 18	% HI titres II ⩾ 100	Booster immunization	No. of participants	% 4-fold titre increase I → III*	GM titre III	% HI titres III < 18	% HI titres III ⩾ 100
						Group A								
10 μg A/USSR/77 A/NJ/76+†	42	60	54	258	29	36	2	74	20 μg A/USSR/77 A/NJ/76+†	13	46	243	8	85
A/NJ/76−†	45	2	8	9	98	0	93	2	A/NJ/76−†	15	0	9	100	0
20 μg A/USSR/77 A/NJ/76+	60	79	49	449	26	28	2	90	20 μg A/USSR/77 A/NJ/76+	14	93	621	0	100
A/NJ/76−	38	0	8	9	100	0	100	0	A/NJ/76−	6	0	8	100	0
40 μg A/USSR/77 A/NJ/76+	43	91	40	508	26	14	2	95	20 μg A/USSR/77 A/NJ/76+	13	100	570	0	100
A/NJ/76−	41	0	8	9	100	0	100	0	A/NJ/76−	10	0	8	100	0
						Group B								
20 μg A/USSR/77 A/NJ/76+	95	36	145	352	10	60	1	92	20 μg A/USSR/77 A/NJ/76+	95	34	403	1	94
A/NJ/76−	10	30	8	19	100	0	70	10	A/NJ/76−	10	20	13	70	0

* † See Table 1.

Table 3. *Local and systemic side effects after primary immunization with* 10, 20, *or* 40 *μg HA of influenza virus A/USSR/92/77 (H1N1) in individuals primed or unprimed for A/NJ/76 virus*

Immunization status	No. of participants	Percentage local (L) and systemic (S) reactions			
		L	S	L and S	No L and/or S
		Group A			
10 μg A/USSR/77					
A/NJ/76+†	40	48	25	18	43
A/NJ/76−†	43	35	19	12	58
20 μg A/USSR/77					
A/NJ/76+	57	49	33	21	39
A/NJ/76−	34	44	24	18	50
40 μg A/USSR/77					
A/NJ/76+	42	69	29	26	29
A/NJ/76−	40	55	35	30	40
		Group B			
20 μg A/USSR/77					
A/NJ/76+	97	14	21	2	67
A/NJ/76−	12	25	17	8	66

† See Table 1.

The percentage of HI titres above 100 is shown in the last column of Table 1. The Fisher test was applied to establish whether the differences between participants immunized and not immunized with A/NJ/76 were statistically significant. This resulted in P2 = 0·01 (group A, 10 μg), P2 = 0·35 (group A, 20 μg), P2 = 0·09 (group A, 40 μg), and P2 = 0·003 (group B, 20 μg). Moreover, when the Mantel–Haenszel test with continuity correction was used to combine the results of these four 2 × 2 tables: P2 < 0·0001.

Table 2 presents the serological results of the HI test using A/NJ/76 virus. In group A all participants who had been immunized in 1976 with the A/NJ/76 virus showed, after primary immunization with A/USSR/77 virus, a high response in the different vaccine-dose groups. Sixty to 91 % had fourfold increases in titre, the *GMT*s were 258–508, and 74–95 % had titres above 100. No response against A/NJ/76 virus was induced in participants not immunized previously with this virus. This was also observed after booster immunization with 20 μg HA A/USSR/77 virus, especially in those groups given 20 ~ 20 μg or 40 ~ 20 μg HA, where subjects previously receiving A/NJ/76 vaccine showed an optimum response with regard to percentage of fourfold titre increases and of titres above 100.

Participants in group B vaccinated with the A/NJ/76 virus both in 1976 and in the autumn of 1977 (71 of 95: 75 %) showed higher pre-immunization titres against this virus than participants of group A, who had been immunized in 1976 only. Consequently, the percentage of fourfold titre increases was higher in group A. Values and percentages obtained after primary immunization with 20 μg HA A/USSR/77 virus were almost identical in groups A and B. In group B booster immunization had little effect on titre values obtained after primary immunization.

Responses with regard to A/NJ/76 virus in participants not immunized previously with this virus were persistently low after primary or booster immunization with A/USSR/77 virus.

In Table 3 local and systemic side effects in groups A and B after primary immunization with A/USSR/77 virus are presented. In group A local reactions were principally manifested by pain at the injection site and systemic reactions by headache. The frequency of combined local and systemic side effects increased with the dose of virus in the vaccine, both in individuals primed or unprimed for A/NJ/76 virus. The percentage of participants in which side effects were absent was higher in the vaccine-dose groups that had not been immunized with A/NJ/76 virus.

In group B local and systemic side effects were less frequent than in group A. All body temperatures measured remained below 38·5 °C, most being below 38 °C. Both systemic and local reactions were reported in 2 and 8%, respectively, of patients immunized or not immunized previously with A/NJ/76 virus. Percentages of patients in which side effects were absent were identical in both contingents.

DISCUSSION

Pre-immunization antibody titres against the A/NJ/76 virus were detected in 107 sera (77%) of participants of group A and in 86 sera (90%) of patients of group B. These titres had been achieved by immunization with this virus in preceding years. The A/NJ/76 antibodies were boosted to a protective level (≥ 100) (Wesselius-de Casparis, Masurel & Kerrebijn, 1972) by A/USSR/77 immunization in 85–100% of participants in group A and in 94% of participants in group B. Volunteers who had not been primed for A/NJ/76 virus showed no or minor responses against this virus after immunization with A/USSR/77 virus. This phenomenon of immune response after immunization with an antigen (A/USSR/77) directed against a related antigen (A/NJ/76) to which the individual has already been exposed during preceding years, was described for the first time by Francis and co-workers, who called it 'original antigenic sin' (Davenport, Hennessy & Francis, 1953; Francis, 1955; Davenport & Hennessy, 1956).

The boosting described above explains the frequent presence of high antibody levels against the A/Swine/15/30 virus and the related A/NJ/76 virus in people now aged 53 years or more (Masurel, 1976). In the period 1918–1957 antibodies against the swine virus, which had been formed from around 1918 to around 1926, were boosted by antigenically related epidemic viruses, such as A/PR/8/34-like viruses from 1934 to 1946, and A/FM/1/47-like viruses from 1946 to 1957.

Volunteers in group A, immunized with the A/NJ/76 virus in 1976, showed a distinctly lower response to A/USSR/77 virus than vaccinees who did not receive A/NJ/76 vaccine previously. This was especially so after booster immunization with A/USSR/77 virus. It was even more evident for the 95 previously immunized patients of group B, 71 (75%) of whom had been immunized both in 1976 and in 1977. Presumably, the homologous antibody production after immunization with A/USSR/77 virus was inhibited by a blocking of the immune system during previous years by the related A/NJ/76 virus (Virelizier, Allison & Schild, 1974; Laver, Downie & Webster, 1976). A parallel observation was reported by Hoskins

et al. (1979) in a study in which reimmunization with a later strain did not afford protection against infection with the epidemic influenza A virus.

The reactivity of the whole virus vaccine established in the present study comprised side-effects mostly consisting of slight symptoms only. However, 30–70 % of participants in the various groups did experience some reaction. Gross *et al.* (1977) observed that prior influenza immunization reduces reactions following subsequent immunization with related antigens. In our group A it is remarkable that pupils receiving an influenza vaccine for the first time indicate less reactions than those immunized previously in 1976. In group B this difference was not found. This may be explained by the occurrence of a 'memory-effect' caused by the previous A/NJ/76 immunization in the respective participants of group A, which could not have happened in group B, since an objective registration was carried out.

The conclusions from this study are that individuals unprimed for A/USSR/77 virus should be immunized twice to afford a protective level of antibody. This is in agreement with findings in other studies (Feery *et al.* 1979; Nicholson *et al.* 1979; Potter *et al.* 1980). After two doses of A/USSR/77 vaccine, subjects primed for the related A/NJ/76 virus possess a lower degree of protection against A/USSR/77 virus than those unprimed for A/NJ/76 virus. Individuals exposed through immunization to the A/NJ/76 virus need only a single heterologous booster dose of A/USSR/77 virus to be protected against infection with A/NJ/76 virus, should the latter become epidemic (Masurel, 1976).

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