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Skin reactivity and antibody response following vaccination against smallpox

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SUMMARY

Revaccination between 2 and 5 years after the last vaccination induced higher complement-fixation titres than revaccination 7–63 years after the last vaccination. The highest CF titres were reached during the 3rd to the 5th week after vaccination.

Fifty-two serum samples, taken 137 or more days after a successful vaccination, all showed CF titres lower than 1/2.

In a group of successfully vaccinated persons with no post-vaccinal complications, 65 out of 66 had a successful 'take' after one or two subsequent vaccinations.

Sera of persons receiving primary vaccination showed significantly lower CF titres than sera of revaccinated persons. Cases of post-vaccinal encephalitis showed greater antibody response than uncomplicated successfully vaccinated cases in both the primary vaccination and revaccination groups. The antibody response in revaccinated persons with post-vaccinal encephalitis was greater than that in the group of successfully revaccinated cases without encephalitis.

A group of 26 naturally hyporeactive persons completely failed to 'take' even after vaccination repeated between 3 and 10 times (average 5 times per person). The frequency of seropositives in this group, and the height of their CF titres, were significantly lower than in the group of successfully vaccinated persons. These findings support the view that untreated persons who repeatedly fail to 'take' with the vaccine must not be considered immune.

In vaccinated persons treated with immunoglobulin (with or without simultaneous treatment with Marboran) antibody production was apparently diminished. Treatment of patients with Marboran significantly lowers the capacity of the vaccine to 'take'.

INTRODUCTION

At the very beginning of the smallpox outbreak in Yugoslavia conflicting opinions emerged regarding several problems.

In Voyvodina, the following two rules were adopted by most of the vaccinators during the vaccination campaign in 1972: (1) Any person who had been successfully vaccinated within the last 3 years who failed to 'take' with four successive revaccinations was considered to be protected. (2) If vaccination failed in primary contacts or persons at high risk of being contacts, such persons were vaccinated

repeatedly until the vaccine 'took' or until the end of the campaign. Generally, repeated failures to 'take' with a vaccine of proven potency, after introduction by a well-controlled technique, have been ascribed to: (1) a solid, specific, cellular or humoral immunity of the host; (2) a natural 'skin immunity factor' or an increased local production of interferon in an otherwise receptive organism, or (3) some other defence mechanisms of the skin.

Cases of post-vaccinal encephalitis were treated conservatively by some, though the majority advocated the use of antivaccinal immunoglobulin.

All contacts were vaccinated and most were also treated with immunoglobulin. Most of the contacts were also treated with Marboran, but some physicians did not use this drug.

As a result of these differences of opinion, both theoretical and practical, we studied the appearance of skin reactivity and antibody response to vaccination, as well as the correlation of these findings with the vaccination status, in different categories of people.

MATERIALS AND METHODS

Serum donors

The vaccine was introduced by two parallel scratches. A total of 193 serum samples, collected from 186 individuals, were tested by complement-fixation (CF) test. The age of these persons varied from 5 to 68 years, the mean age being 39 years. Of the 186 persons tested 166 (89 %) had been vaccinated 2–68 years (average about 30 years) before 1972, and 20 (11 %) of them were first vaccinated in 1972.

According to the case-histories, they were divided into five groups.

Group 1. Vaccinated successfully April 1972, with no complications (66 persons).

Group 2. Not vaccinated in 1972, but a history of vaccination 3–63 years earlier (47 persons).

Group 3. Vaccinated repeatedly without success. 'Naturally hyporeactive' (28 persons).

Group 4. Vaccinated successfully and given 500–700 units of immunoglobulin (36 persons). Some of these received Marboran (26) or cortisone (2) as well.

Group 5. Vaccinated successfully and developed post-vaccinal encephalitis (9 persons).

In group 3, three of the hyporeactive persons had failed with their previous vaccinations, attempted 2, 9 and 20 years ago respectively. Sixteen others in this group carried marks of successful previous vaccinations. In group 5, sixteen serum samples were collected from the nine cases, and from five of these cases six samples of cerebrospinal fluid were obtained. Results with these CSF samples are described in a previous paper (Terzin, Mašić, Vuković & Mudrić, 1974). The onset of encephalitis took place 7–16 days (average 11·6 days) after vaccination. The duration of illness varied from 5 to 33 days (average 17 days).

Sera were preserved by sodium azide (final concentration 0·08 %), stored at 4° C and inactivated at 57° C for 30 min. No serum-sample with anticomplementary activity (even in dilution 1/1) was used for these studies.

Complement-fixation (CF) technique

This was a modification of Hoyle's technique, described earlier (Terzin *et al.* 1954), with overnight fixation at 4° C. Titres are expressed as dilutions of serum before addition of other reagents (i.e. the initial dilution and not the final).

The antigen used was the supernatant obtained by centrifuging at 1500 rev./min. for 3 min. the homogenized smallpox vaccine, i.e. vaccinia, produced by the Tashkent Research Institute for Vaccines and Sera. Its anticomplementary activity was less than 1/2. In checkerboard titration with a rabbit antiserum this antigen had a maximum titre of 1/32–1/64 so that working dilutions of 1/10 or 1/20 were selected as containing about 3 antigen units/unit volume. This antigen showed no cross-reactions with antisera against *Toxoplasma gondii*, *Mycoplasma pneumoniae* and *Rickettsia burneti*.

The statistical significance of the differences observed between frequencies of positive reactors has been evaluated by the chi-square test, and that of the differences between mean titres by the *t*-test (Chambers, 1952; Diem, 1960).

RESULTS AND DISCUSSION

Skin reactivity to vaccination

Table 1 shows the multiplicity of vaccinations applied in order to obtain a successful take, as well as the statistical significance of the findings listed.

Among the 66 successfully vaccinated persons, an unequivocal reaction occurred after one vaccination in 54 (81 %) and after two vaccinations in a further 11 (17 %). The last person required a third vaccination for a positive result. This is in contrast to the group of naturally hyporeactive persons in whom repeated vaccinations failed completely, as well as the group of persons treated with Marboran, who showed significantly inferior reactivity to vaccination (in both cases, at a significance level of $P < 0{\cdot}0001$), than the group of successfully vaccinated persons.

Twenty-six of the 28 naturally hyporeactive persons failed entirely to 'take' the vaccine, even after 3–10 times (average 4·5 times) repeated vaccinations per individual.

Most (77 %) of the patients treated simultaneously with Ig and Marboran had to be vaccinated 3–6 times (average 3·4 times), and the rest (23 %) twice subsequently, in order to attain a positive reaction. However, the group treated with Ig alone proved to be as responsive to vaccination as the untreated, successfully vaccinated, group of persons. It appears that treatment with Marboran supresses an adequate propagation of vaccinia virus, thus making the vaccine behave as one of diminished potency.

Both in the group of successfully vaccinated persons, with no post-vaccinal complications, and in the group of vaccinees displaying post-vaccinal encephalitis, the multiplicity of effective vaccinations was the same, amounting on the average to about 1·1. In about 83 % of the cases belonging to these two groups, the vaccine produced unequivocal takes at its first application.

Table 1. *Types of reactivity to vaccination in* 1972

Groups of vaccinees		No. of persons with multiplicity of vaccination						Mean no. of vaccinations per person (significance of difference)
		1×	2×	3×	4×	5-10×	Total	
Successfully vaccinated (v.)	Primary	5	1	—	—	—	6	1·17 ($P > 0·2$)
	Revaccinated	49	10	1	—	—	60	1·20 (null hypothesis)
Hyporeactive to revaccination		1	1	2	16*	8	28*	4·48 ($P < 0·0001$)
Revaccinated persons treated with	Anti-vacc. Ig	7	—	—	1	—	8	1·38 ($P > 0·2$)
	Cortisone and anti-vacc. Ig	2	—	—	—	—	2	1·0 ($P > 0·2$)
	Marboran and anti-vacc. Ig	—	6	14	1	5*	26*	3·38 ($P < 0·0001$)
Post-vaccinal encephalitis	Primary	3	—	—	—	—	3	1·11 ($P > 0·2$)
	Revaccinated	5	1	—	—	—	6	—
Totals		72	19	17	18	13	139	—

* One of these persons was receiving primary vaccination.

Table 2. *Complement-fixing antibody response in groups of vaccinated persons*

Groups of vaccinated persons		Successfully vaccinated			Hyporeactive to vaccination		Post-vaccinal encephalitis			Treated with immunoglobulin	
		13–33*	34–136*	137–163*	13–33*	85–121*	13–33*	34–136*	140*	13–33*	40–121*
Revaccinated 2–5 years after last vaccination	Pos/tstd†	2/2	2/2	—	—	0/4	—	—	—	—	5/15
	(%)	—	—	—	—	—	—	—	—	—	(33)
	g.m.t.‡	22·6	11·3	—	—	1·7	—	—	—	—	3·0
	Range	16–32	8–16	—	—	< 2–4	—	—	—	—	< 2–16
Revaccinated 7–63 years after last vaccination	Pos/tstd†	12/17	10/35	0/4	0/4	0/19	5/6	3/3	—	1/2	2/19
	(%)	(71)	(29)	—	—	—	(83)	—	—	—	(11)
	g.m.t.‡	10·2	3·1	1·7	1·7	1·4	22·6	16·0	—	5·7	2·2
	Range	< 2–128	< 2–32	< 2–2	< 2–4	< 2–4	4–64	8–32	—	4–8	< 2–16
Primary vaccination	Pos/tstd†	0/2	0/4	—	0/1	—	5/5	1/1	0/1	—	—
	(%)	—	—	—	—	—	(100)	—	—	—	—
	g.m.t.‡	1·0	1·7	—	1·0	—	13·9	8·0	1·0	—	—
	Range	< 2	< 2–2	—	< 2	—	8–32	8	< 2	—	—
No. of serum samples tested		21	41	4	5	23	11	4	1	2	34

* Days after the last vaccination in 1972 when the serum was tested.
† Pos/tstd = number of sera positive at > 1/4/number of sera tested.
‡ g.m.t. = geometric mean titre.

Table 3. *Statistical evaluation of some serological results*

Vaccination status of serum donors in 1972	Sera drawn at day following vaccination in 1972	Frequency of positive sera* (percentage)	Significance of differences between frequencies observed		
Not vaccinated; last vaccination 3–63 yr. ago	—	0/47 (0)	n.h.	< 0·0001	0·005
Successfully revaccinated (7–63 yr. after last vaccination)	13–33	12/17 (71)	< 0·0001	n.h.	0·02
	34–136	10/35 (29)	0·005	0·02	n.h.
Hyporeactive to revaccination (7–63 yr. after last vaccination)	13–33	0/4 (0)	> 0·2	0·05	—
	85–121	0/19 (0)	> 0·2	—	0·05
Encephalitis following revaccination in 1972; last vaccination 7–63 yr. ago	13–33	5/6 (83)	0·0001	> 0·2	—
	34–136	3/3 (100)	0·005	—	0·05
Revaccinated persons treated with Ig; last vaccination 7–63 yr. ago	40–121	2/19 (11)	> 0·2	—	0·2

* CF titre > 1/4.
n.h. = null hypothesis

Results of CF tests

Some data related to the antibody response of vaccinated persons and the statistical evaluation of some findings are shown in Tables 2 and 3. The frequencies of positive serum samples and/or the heights of the mean titres, observed in the various groups of vaccinees, seem to allow for the following conclusions:

CF titres reach their highest levels during the 3rd to 5th week after primary or revaccinations, subsequently falling.

After the 136th day after vaccination in 1972, no serum was found with titre higher than 1/2 (see Table 2). This finding was supported also by the fact that 47 serum specimens, drawn from patients unvaccinated in 1972, all showed CF titres lower than 1/2.

These findings agree with those of McCarthy, Downie & Bradley (1958) which were also confirmed by others.

Sera drawn from groups of persons revaccinated 2–5 years (average 3·7 years) after the last vaccination, showed suggestively higher CF titres than sera drawn from persons revaccinated 7–63 years after the last vaccination, provided that the samples were drawn at comparable intervals after revaccination in 1972 (see Table 2).

Sera drawn after primary vaccination show significantly lower CF titres than sera drawn after revaccination, with the exception of serum samples drawn from cases of post-vaccinal encephalitis.

The geometric mean of the CF titres (1/16), in sera of three patients with p.v.

encephalitis, proved to be significantly higher than the mean titre (1/3·1) found in sera of successfully vaccinated uncomplicated cases (see Table 2).

In patients with p.v. encephalitis even primary vaccination produced an antibody response comparable with, if not greater than, that shown by uncomplicated revaccinees (see Table 2).

In primovaccinees who developed p.v. encephalitis the antibody response was significantly greater than in successfully primovaccinated persons without post-vaccinal encephalitis.

In the group of naturally hyporeactive persons the frequency of positives was significantly lower than in the group of successfully revaccinated persons. Only four of the 19 hyporeactive cases (21 %) showed a titre of 1/4, and none showed titres higher than 1/4. In the group of successfully vaccinated persons (bled at comparable intervals after vaccination), 20 of the 35 cases (57 %) showed titres of 1/4 or higher. These findings are in accordance with the well-known rule, that failure to take the vaccine must not be regarded as evidence of immunity, usually supported by examples of smallpox cases, some fatal, appearing shortly after failure to take repeated vaccinations (van Rooyen & Rhodes, 1948; Dixon, 1962; Downie, 1965, and others).

The group of 36 patients treated with immunoglobulin alone, or simultaneously with Marboran, showed a suggestively weaker antibody response than the group of successfully vaccinated and otherwise not treated persons. No difference in antibody response could be detected between those treated with Marboran plus immunoglobulin, and those treated with immunoglobulin alone or with immunoglobulin and cortisone combined. The small number of vaccinees given combinations of immunoglobulin with Marboran or cortisone did not permit statistical comparisons.

The frequencies of positives shown in Table 2 were calculated by adoption of antibody titres > 1/4 (i.e. 1/8 or higher) as positive. When adopting as positive antibody titres > 1/2 (i.e. 1/4 or higher), instead of > 1/4, we find an increase in the frequency of positives, but no significant alteration of the differences revealed between the various groups of persons. For example, the group of persons revaccinated successfully in 1972, 6–63 years after the last vaccination, and bled at 13–33 days, 34–136 days and 137–163 days after revaccination in 1972, showed 70·6, 28·6 and 0 % of positives at a > 1/4 titre respectively. However, the frequency of positives at a titre > 1/2, in the same three groups of vaccinees, amounted to 88·3, 57·2 and 0 % respectively.

In order to minimize the risk of getting non-specific cross-reactions from any non-vaccinal antibodies that may have been present we took as 'positive' a titre of 1/8 or greater

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