

Smallpox vaccination immunity in relation to number of insertions*

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The influence of the number of insertions of vaccine on smallpox vaccination immunity has been the subject of considerable controversy. In this study the problem was investigated in children of comparable age, sex, and smallpox vaccination history. The incidence of post-vaccinal fever and the antibody response of subjects given single and double insertions were compared. While single insertions caused fewer febrile reactions than double insertions, the difference was not significant. Successful vaccination invariably elicited a marked neutralizing antibody response. However, subjects who received two insertions developed significantly higher antibody titres than subjects with a single insertion. In the revaccinated group the difference in titre was still demonstrable 16 months after vaccination. It is concluded that the number of insertions may play a role in the duration of vaccination immunity.

The practice of multiple insertions of vaccine in smallpox vaccination has been gradually abandoned. In many countries vaccination is now performed by a single insertion, and in others two insertions are required although the vaccination is also regarded as successful if it produces one major reaction. The use of single insertions has several advantages: vaccination is more rapid, less traumatic, and leaves only one scar. In addition, it is generally believed that the smaller the area of skin involved in vaccination, the lower the incidence of postvaccinal fever. In smallpox vaccination campaigns the single insertion technique has economic benefits as well.

In all probability no objection can be raised to the efficacy of the single insertion technique provided the vaccination is performed with fully potent vaccine and perfect technique. No subjects with a recent successful single-insertion vaccination have been reported to contract smallpox. Lane et al. (1970) found a marked rise in neutralizing (NE) antibody titre in 60 % of subjects revaccinated by either single or double insertions.

However, earlier reports suggested that postvaccinal immunity increased with the number of insertions. In some cases it may be assumed that the

potency of the vaccine was inadequate and that the multiple insertion technique actually increased the likelihood of successful vaccinia infection. Some reports, however, pointed to a relationship between the number of takes or the area of reaction and the immunity produced. According to Brownlee (1905) and Rao et al. (1960) the incidence of lethal smallpox among vaccinated persons varied inversely with the number of scars. Cross (1959) has reported that resistance to revaccination develops sooner in subjects with larger primary vaccination vesicles.

In view of the importance of the question and the inconsistency of previous findings, studies were undertaken to compare the immunogenicity of the single and double insertion techniques in primary vaccinations and revaccinations and the respective incidence of adverse postvaccinal reactions.

MATERIALS AND METHODS

Vaccine

Lyophilized Lister-strain vaccine ("HUMAN", Budapest) was used. When reconstituted the vaccine had a potency of 34×10^{10} pock-forming units per litre. The same vaccine lot was used throughout the investigation.

Vaccination

Subjects were divided at random into 2 groups of similar age and sex and were given either one or two

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Table 1. Results of vaccination

Type of vaccination	Number of insertions	Number of vaccinees with			Total number of vaccinees
		no major reaction	one major reaction	two major reactions	
primary vaccination	1	1	74	0	75
	2	0	3	71	74
revaccination	1	0	107	0	107
	2	0	1	98	99

insertions of vaccine. Vaccinations were performed with the multiple puncture method using bifurcated needles. In primary vaccinations 3 strokes were applied per insertion, and in revaccinations 15 strokes.

Subjects

Primary vaccination: 149 1-year-old infants.

Revaccination: 206 12-year-old schoolchildren who had received their first vaccination at age 1 year. All had a distinctly visible primary vaccination scar.

Reading of local reactions

Reactions were read on the 7th postvaccinal day. The vaccination was regarded as successful if a pustule or an area of definite palpable induration surrounding a scab (a major reaction) could be seen.

Recording of febrile reactions

The afternoon temperature of the infants was recorded, by those caring for the children, every other day between the 7th and 14th postvaccinal days. Schoolchildren were asked, at the time when postvaccinal blood samples were taken, if they had had any fever after the vaccination.

Blood collection

Blood was taken from some infants 4 weeks after successful primary vaccination. Equal numbers of subjects from the single and double insertion groups were selected at random. The first samples from the schoolchildren were taken immediately before revaccination. Postvaccinal samples were drawn 3 weeks later from randomly selected children with successful single or double insertions. A third blood sample was collected 16 months after successful single or double revaccination, primarily from those whose first and second samples had already been examined.

Serological methods

Sera were inactivated at 56°C for 30 min and stored frozen. The haemagglutination-inhibition (HAI) and the NE test titres of the sera were determined. A 10 % preparation of normal human immunoglobulin was used as a reference preparation. It was titrated in every case simultaneously with the sera to be examined. HAI titrations were performed by the micromethod of Szathmáry & Holik (1958). Titrations of NE antibodies were made on the chorioallantoic membranes of 12-day-old chick embryos, using 60 % pock reduction as the endpoint (Nyerges et al., 1966).

RESULTS

The results of vaccination are shown in Table 1. Among the 173 subjects vaccinated at two sites there were 4 who developed only one major reaction. For this reason it was decided that in the follow-up of the antibody response to double insertions, only subjects with two major reactions would be included.

The incidence of febrile reactions is indicated in Table 2. Both after primary vaccination and after revaccination temperatures of over 38°C occurred more frequently in the double insertion group than

Table 2. Incidence of febrile reactions in successfully vaccinated children

Type of vaccination	Number of insertions	Number of subjects	Proportion of children with 38°C or higher (%)
primary vaccination	1	75	22.7
	2	74	29.7
revaccination	1	105	3.8
	2	95	8.4

Table 3. Distribution of HAI titres of successfully vaccinated children

Type of vaccination	Number of insertions	Time of blood collection	Number of subjects tested	Distribution of titres						Geom. mean of titres
				2	4	8	16	32	64	
primary vaccination	1	4 weeks after vaccination	33	0	0	4	8	13	8	27.0
	2		28	0	0	2	4	12	10	33.6
revaccination	1	before revaccination	38	19	11	5	3	0	0	3.5
		3 weeks after revaccination	38	2	4	13	10	9	0	11.5
	2	before revaccination	39	14	12	8	4	1	0	4.4
		3 weeks after revaccination	39	0	2	12	14	10	1	14.9

in those given a single insertion, although the observed differences were not statistically significant at the 5 % level. In any event, even if there was a true difference it was not a serious one.

The results of the HAI and NE tests are shown in Tables 3 and 4 respectively. No difference in HAI titre could be detected in relation to the number of insertions. The NE antibody response, however, was not identical in the single and double insertion groups. Although successful vaccination elicited considerable NE antibody production in every subject, the mean postvaccinal NE antibody titre after two insertions was significantly higher than after a single insertion for both primary vaccination and revac-

ination. This statistically significant difference was still demonstrable in the blood samples taken 16 months after revaccination.

DISCUSSION

Our finding concerning the frequency of serological reactors in the single insertion and double insertion groups is in agreement with the report of Lane et al. (1970). However, when comparing the postvaccinal NE antibody titre values, one finds that two insertions were more effective than a single insertion. Both in primary vaccination and in revaccination, subjects in the double insertion group exhibited

Table 4. Distribution of NE antibody titres of successfully vaccinated children

Type of vaccination	Number of insertions	Time of blood collection	Number of subjects tested	Distribution of titres							Geom. mean of titres
				< 4	4	16	64	256	1024	4096	
primary vaccination	1	4 weeks after vaccination	26	0	1	8	17	0	0	0	37.5
	2		26	0	0	4	17	4	1	0	70.8
revaccination	1	before revaccination	30	14	13	3	0	0	0	0	3.3
		3 weeks after revaccination	30	0	0	8	13	9	0	0	66.7
	2	16 months after revaccination	30	1	6	16	7	0	0	0	15.7
		before revaccination	29	13	13	2	1	0	0	0	3.6
	2	3 weeks after revaccination	31	0	0	0	10	14	6	1	233.8
		16 months after revaccination	29	1	6	5	12	5	0	0	32.0

significantly higher neutralization titres than persons given a single insertion of vaccine. The question arises whether this difference has any practical implications. Do the lower titres obtained after a single insertion entail any risk of imperfect protection? The answer of course is a definite no. Epidemiological data have shown that a successful vaccination confers safe protection against smallpox for 4-5 years. There has been no indication that within this period of time the immunity provided by one insertion is insufficient. Nevertheless, as the difference in titres can be demonstrated not only some weeks but even 16 months after

vaccination, it might be assumed that two takes provide more durable immunity. This would indicate that the advantage and practical value of double insertions will continue to be evident many years after vaccination. Clinical observations relating the number of lethal cases of smallpox in a vaccinated population to the number of scars are in accordance with this assumption. Though fever occurred somewhat more frequently among subjects given double insertions of vaccine, the difference was not significant and hence would not be a contraindication to the use of this technique.

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RÉSUMÉ

IMMUNITÉ CONFÉRÉE PAR LA VACCINATION ANTIVARIOLIQUE EN FONCTION DU NOMBRE D'INOCULATIONS

On a pratiqué la primovaccination antivariolique chez 149 nourrissons âgés de 1 an et revacciné 206 écoliers âgés de 12 ans en utilisant le même lot de vaccin. Dans les deux groupes, la moitié des enfants n'a reçu qu'une seule inoculation de vaccin tandis que l'autre moitié subissait deux inoculations simultanées. La réponse sérologique a été recherchée par titrage des anticorps inhibant l'hémagglutination (IH) et neutralisants (N) dans des échantillons de sang recueillis 4 semaines après la vaccination chez les primovaccinés, et, en cas de revaccination, immédiatement avant, après 3 semaines et après 16 mois.

On a relevé une incidence légèrement plus élevée des réactions fébriles postvaccinales parmi les sujets ayant

reçu deux inoculations. Chez les primovaccinés et chez les revaccinés, aucune différence des titres d'anticorps IH postvaccinaux ne s'est manifestée en fonction du nombre d'inoculations. En cas de prise, la réponse en anticorps N a été chaque fois notable. Toutefois, tant parmi les primovaccinés que parmi les revaccinés, les sujets ayant reçu deux inoculations ont présenté après 3 ou 4 semaines des titres d'anticorps N nettement plus élevés que les sujets immunisés par une seule inoculation. La différence, statistiquement significative, a été retrouvée lors des examens sérologiques pratiqués au 16^e mois.

D'après les auteurs, ces observations tendent à prouver que le nombre des inoculations joue un rôle dans la durée de l'immunité succédant à la vaccination antivariolique.

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