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Smallpox
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Evaluation of Two Kinds of Smallpox Vaccine: CVI-78 and Calf Lymph Vaccine

II. Clinical and Serologic Observations of Response to Revaccination with Calf Lymph Vaccine

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Extract

Revaccination with standard calf lymph vaccine was performed on 26 children who had received a primary vaccination with an attenuated smallpox vaccine, CVI-78, and 22 children who had received primary vaccination with standard calf lymph. Revaccination resulted in a vesicular reaction in 96% of those who had been vaccinated previously with CVI-78 and 73% of those vaccinated previously with standard calf lymph. All children had a positive hemagglutination-inhibition (HI) antibody titer either after primary vaccination or revaccination. Only 65% of those initially vaccinated with CVI-78 vaccine had positive neutralizing antibodies after revaccination. All children who received primary vaccination with standard calf lymph had postrevaccination neutralizing antibodies. The children who had neither a dermal nor a serologic response after primary vaccination responded as primary vaccinees on challenge with standard calf lymph.

Speculation

Vaccination with CVI-78 alone offers only partial protection against variola and revaccination with standard calf lymph is

necessary to offer full protection. The incomplete neutralizing antibody response in the CVI-78 group after revaccination with standard vaccine may indicate, however, that revaccination protection in this group is incomplete. Vaccination with CVI-78 also is not completely protective against the complications that could result from primary standard calf lymph vaccination since a significant percentage of those who received primary vaccination with CVI-78 had no postvaccination serologic or dermal response and responded like primary vaccinees when challenged with standard vaccine.

In the preceding paper the clinical and serologic results of a study comparing primary vaccination with CVI-78, an "attenuated" strain of vaccinia, to the standard calf lymph vaccine (CL) of a similar titer, $10^{8.0}$, were reported (6). Effectiveness of the vaccine was measured by the presence and size of the cutaneous reaction and by the development of HI and neutralizing (Neut) antibodies. CVI-78 vaccination resulted in an attenuated dermal and serologic response and a complete absence of any dermal or serologic reaction in 27% of the subjects vaccinated as compared with only 2% of those vaccinated with standard calf lymph. This

apparent lack of immunologic response to this attenuated vaccine in over a fourth of the population vaccinated indicates that the administration of this vaccine alone might not offer sufficient protection against smallpox and that revaccination with standard vaccinia should be performed.

METHODS

POPULATION

Forty-nine children, ages 1-5, who had been vaccinated for the first time 5-9 months earlier with either CVI-78 or calf lymph vaccine returned for revaccination with high titer standard CL (7). All of these children had been vaccinated in the study reported previously (6). Twenty-six subjects who had received primary vaccination with CVI-78 vaccine returned for revaccination with standard calf lymph vaccine. These were designated the CVI-78/CL group. Twenty-two children who had received primary vaccination with standard calf lymph vaccine returned for revaccination with standard calf lymph vaccine. They were designated the CL/CL group.

VACCINE

The calf lymph vaccine used for revaccination was obtained in lyophilized form from Wyeth Laboratories and had a chorioallantoic membrane titer of $10^{8.4}$ plaque-forming units (PFU)/ml. Reconstitution was made in a 50% glycerine solution containing 0.25% phenol and 0.005% brilliant green.

VACCINATION TECHNIQUE

The vaccine was stored 5° and used within 8 hr of reconstitution. Two vaccinators (WCS and RBW) administered all revaccinations. The vaccine was administered over the lateral aspect of the right deltoid; primary vaccination had been in the left arm. The area was cleansed with acetone and a drop of vaccine was placed on the skin. Fifteen double punctures were made through the vaccine with a bifurcated needle directed perpendicularly to the skin. Excess vaccine was removed with a dry cotton ball, and the parents were instructed in the appropriate care of the revaccination site.

CLINICAL EVALUATION

The children were seen at 7 and 28 days after revaccination. The 7-day follow-up consisted of a brief history and physical, rectal temperature, and measurement of the extent of erythema, induration, and vesiculation at the vaccination site. The 28-day follow-up consisted of measurement of the revaccination scar.

SEROLOGIC EVALUATION

Venous blood was obtained from each child at the 28-day postvaccination follow-up. The serum was separated from the clot and stored at -70° until the antibody titers were determined. Serologic tests performed were the hemagglutination antibody titer according to the method of Hierholzer *et al.* (2) and Neut according to the method of Cutchins *et al.* (1) as modified by Millar (3). The serologic tests for the revaccination study were performed simultaneously with those from the previously reported primary vaccination study (6).

RESULTS

CLINICAL RESULTS

Twenty-six children in group CVI-78/CL and 22 children in group CL/CL were seen at the 7-day postvaccination follow-up. Vesicular reactions were observed in 25 (96%) of the CVI-78/CL group and in 16 (73%) of the CL/CL group. The one child in the CVI-78/CL group and the six children in the CL/CL group who

did not demonstrate a vesicular reaction on revaccination had had a major reaction after primary vaccination (6). Figure 1 demonstrates the distribution of vesicle size from primary vaccination and revaccination comparing the CVI-78/CL group with the CL/CL group. The scattergrams depicted in Figures 2 and 3 present the individual primary and revaccination response for each of the two vaccine groups.

In general, those in the CVI-78/CL group had a smaller dermal reaction after primary vaccination and on revaccination demonstrated larger dermal reactions than those in the CL/CL group.

Seven of the 26 children in the CVI-78/CL group (27%) and 1 of the 22 children in the CL/CL group (5%) had not had a major reaction after primary vaccination and did not have scar evidence of a primary take. In the CVI-78/CL group, two of these children had positive postvaccinal HI titers. Both of these children on revaccination demonstrated a modified dermal reaction. The five children in the CVI-78/CL group (19%) and the one child in the CL/CL group (5%) who had no dermal or serologic evidence of a primary take after the initial vaccination developed typical primary like Jennerian vesicles on revaccination with CL. Four of these five CVI-78/CL subjects and the single CL/CL subject also were the only children who had a history of mild systemic symptoms after revaccination.

SEROLOGIC RESULTS

A serum sample was obtained from 20 children in the CVI-78/CL group and 19 children in the CL/CL group at the 28-day postvaccination follow-up (8). Twenty-eight-day postprimary vaccination serum samples had been obtained on all of these children as part of the study reported previously (6). Table 1 shows the frequency distribution of the postvaccination Neut and HI antibody range, and Figure 4 compares the 28-day primary postvaccination titer with the 28-day revaccination titer. After primary vaccination, only 2 of the 20 subjects in the CVI-78/CL group as compared with 17 of the 19 subjects in the CL/CL group had a positive Neut antibody response. After revaccination with standard calf lymph all of the CL/CL group developed positive neutralizing antibodies whereas 7 (35%) of the CVI-78/CL group still demonstrated no neutralizing antibody titer, and 2 (10%) had only a weakly positive titer. As in the first study, a higher percentage of the subjects demonstrated an HI conversion as compared with the neutralizing antibody conversion. Sixteen (80%) of the CVI-78/CL and all of the CL/CL group had HI antibodies after primary vaccination. After revaccination with standard calf lymph, only one child in each group had a postvaccination titer less than 1:8. Both of these children had positive HI titers after primary vaccination.

DISCUSSION

In the current study the revaccination response of children immunized previously with CVI-78, an attenuated smallpox vaccine, was determined and compared with the revaccination response of children immunized previously with standard vaccine. Seventy-three percent of the children had a major dermal reaction after primary vaccination with CVI-78 (19 of 26) and 95% had a major dermal reaction after primary vaccination with standard calf lymph vaccine (21 of 22). On revaccination with high titer standard calf lymph vaccine these rates were reversed. Ninety-six percent of those vaccinated previously with CVI-78 responded with a vesicular dermal reaction to revaccination in contrast to 73% of those vaccinated previously with calf lymph. This latter response corresponds closely with the expected rate in recent revaccinees (4) and the revaccination response of the CVI-78 approximates that which would be expected from primary or remote revaccinees (3, 5). This is not a surprising observation considering that the prevaccinal serologic profile of the CVI-78 group resembles a population of remote vaccinees. In addition, one-fifth of the CVI-78 group had no serologic or dermal evidence of a previous

Fig. 1. Vesicle size after primary vaccination. CL: calf lymph.

Fig. 2. Dermal reaction. Individual vesicle size compared in primary vs revaccination in CVI-78 groups. CL: calf lymph.

Fig. 3. Dermal reaction. Individual vesicle size compared in primary vs revaccination in CL/CL groups. CL: calf lymph.

vaccination take and on revaccination responded as primary vaccinees.

An unexplained finding in this study is the striking disparity between the HI and Neut antibody response. After primary vaccination with CVI-78 only 10% developed neutralizing anti-

bodies, and after revaccination with standard calf lymph only 65% had neutralizing antibodies. In contrast, primary vaccination with CVI-78 resulted in positive HI titers in 80% and revaccination with calf lymph resulted in positive HI titers in 95% of the subjects. In the group which had received both primary and revaccination with

Fig. 4. Serologic titers after primary and revaccination (Revacc) by type of vaccine (CVI-78 and calf lymph (CL)).

Table 1. 28-day postvaccination antibody in CVI-78 and calf lymph primary vaccinees revaccinated with standard calf lymph¹

Group	A. Postvaccination, neut titer range, log ₁₀ TCID ₅₀ /ml							Total
	<0.6	0.6-0.9	1.0-1.9	2.0-2.9	3.0-3.9	4.0-4.9	>5.0	
CVI-78/CL	7	2	5	2	2	1	1	20
CL/CL	0	0	3	5	5	3	3	19

Group	B. Postvaccination, HI titer range, 1:serum dilution						Total
	<1:8	1:8	1:16	1:32	1:64		
CVI-78/CL	1	7	5	5	2		20
CL/CL	1	12	6	0	0		19

¹ Neut: neutralizing antibody; HI: hemagglutination-inhibition.

standard calf lymph, 89% had a positive neutralizing antibody titer after primary vaccination and 100% after revaccination. This same group had 100% HI conversion after primary vaccination. From these observations it would seem that the HI test described is a more sensitive measure of circulating antibodies than the neutralization test. It is difficult to explain, however, the difference in the neutralization antibody response in the CVI-78 group as compared with the standard calf lymph group. Not only is the rate of neutralization seroconversion less in the former, but also the resulting antibody levels are lower. A tempting explanation for this finding would be that primary vaccination with an attenuated vaccine is capable of stimulating cell-mediated immunity but only a weak neutralizing antibody response. On subsequent challenge with a high titer standard strain, viral replication is limited by the cell-mediated immunity, preventing a significant revaccination neutralizing antibody response. In support of this would be the observation that the neutralization fourfold antibody rise or seroconversion rate was similar after revaccination for both the CVI-78/CL and CL/CL groups. For those initially vaccinated with CVI-78, 12 of 20, or 60%, had either a fourfold neutralization antibody response or seroconversion on revaccination as compared with 12 of 19, or 63%, for those initially vaccinated with calf lymph. Thus, it is possible that cell-mediated immunity limited viral replication and neutralization response equally in both groups regardless of the initial vaccine and that the low neutralizing antibody titer was more of a reflection of the first rather than the second vaccination.

It is also interesting to note that two children who had received a primary vaccination with CVI-78 had no dermal evidence of a take, 7-day vesicle, or 28-day scar, yet they had a positive 28-day HI titer. Both of these children had a modified dermal response when revaccinated with standard calf lymph, indicating that scar evidence of a take is not an absolute measurement of vaccine-induced immunity.

Despite these two studies, two unanswered questions still remain. What is the protective value of the attenuated vaccine

towards variola? To what degree does the attenuated vaccine reduce the incidence of complications that might be expected from standard smallpox vaccination? In the absence of a field trial one can only surmise that vaccination with CVI-78 by the multiple pressure route alone would offer an individual only partial protection against variola and that revaccination with standard calf lymph is necessary to offer full protection. The neutralizing antibody profile of the CVI-78 group after revaccination with standard vaccinia, however, still leaves some doubts about this. The second question, which relates to the incidence of complications after CVI-78 vaccination, is also unanswered. If viral replication is limited after vaccination with an attenuated vaccine, there may be a lower incidence of the type of complication characterized by the generalized spread of vaccinia, such as eczema vaccinatum or vaccinia necrosum. The severity of these complications, however, may be determined more by the immune status of the host rather than the characteristic of the vaccine. Whether the incidence of complications of unknown etiology, such as postvaccinial encephalitis or erythema multiforme, will be reduced by CVI-78 vaccine can be answered only by a very large controlled study. Finally, one must recognize that 19% of the subjects vaccinated with CVI-78 by multiple pressure did not develop a serologic or dermal reaction after primary vaccination and on revaccination behaved like primary vaccinees. This group on revaccination with standard vaccinia might have the same complication rate as standard primary vaccinees.

SUMMARY

Revaccination with standard high titer calf lymph vaccine was performed on 26 children who had received vaccination 5-9 months earlier with an attenuated smallpox vaccine, CVI-78, and on 22 who had received primary vaccination with standard calf lymph. The children were evaluated clinically at the 7th postrevaccination day and serologically at the 28th postrevaccination date. Revaccination resulted in a vesicular reaction in 96% of those who

had been vaccinated previously with CVI-78 and 73% of those vaccinated previously with standard calf lymph. All children in both groups had a vesicular reaction either after the first or the second vaccination. Likewise, all children had a positive HI titer. On the other hand, only 65% of those children initially vaccinated with CVI-78 vaccine had positive neutralizing antibodies after revaccination, whereas all children who received standard calf lymph both times had positive neutralizing antibodies. Nineteen percent of the CVI-78 group had no HI or neutralizing antibodies after primary vaccination. All of these children responded as primary vaccinees on challenge with standard calf lymph. The number of vaccinations in this trial is insufficient to determine to what degree vaccination with CVI-78 will reduce the incidence of complications associated with smallpox vaccine.

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Hyperphenylalaninemia
liver
phenylalanine hydroxylase

Phenylalanine Hydroxylase Activity in Liver Biopsies from Hyperphenylalaninemia Heterozygotes: Deviation from Proportionality with Gene Dosage

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Extract

Liver biopsy samples from the patients with hyperphenylalaninemia have an average of 5% of the normal hydroxylase activity. The parents of the patients have between 7.3% (excluding the value for one parent) and 10% of the normal hepatic hydroxylase activity. An explanation for these findings involves negative interallelic complementation, which involves protein-protein interaction between subunits in a multimeric enzyme. In support of this model is the evidence that rat liver phenylalanine hydroxylase is a multimeric protein composed of two electrophoretically distinguishable subunits.

Speculation

The finding that parents of patients with hyperphenylalaninemia have an average of 10% of the normal level of hepatic phenylalanine

hydroxylase, a multimeric enzyme, can be explained on the assumption that the liver tissue of heterozygotes has an excess of enzyme molecules that contain at least one mutant subunit.

Widespread screening of neonates for phenylketonuria (PKU) has in recent years brought to light a class of patients with an apparently milder defect in phenylalanine metabolism than is seen in classic PKU. These patients are characterized by serum phenylalanine concentrations which are well below the concentrations in PKU serum, but still severalfold higher than normal (8). The condition has therefore been called hyperphenylalaninemia. The patients can be distinguished from PKU heterozygotes, and their pedigrees suggest autosomal recessive genetics (15).

In this laboratory, a series of liver biopsies from several hyperphenylalaninemic patients was shown by direct enzyme assay