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

I. Clinical and Serologic Response to Primary Vaccination

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Extract

A comparative study of two smallpox vaccines, standard calf lymph vaccine, and an attenuated vaccine, CVI-78, was performed in 95 children. Primary vaccination with CVI-78 resulted in a more attenuated response than primary vaccination with standard vaccine. Sixty-one percent of those vaccinated with CVI-78 and 96% of those vaccinated with standard vaccine developed a major dermal reaction; 16% of those vaccinated with CVI-78 and 89% of those vaccinated with standard vaccine developed post-vaccination neutralizing antibodies. Twenty-seven percent of the children vaccinated with CVI-78 demonstrated neither a dermal nor serologic postvaccination response, whereas only 2% of those vaccinated with standard vaccination demonstrated no postvaccination response.

Speculation

Smallpox vaccine strain CVI-78 produces a modified local and serologic reaction in children. This vaccine may be overattenuated and thus is not useful as a replacement for standard vaccine. The vaccine in limited circumstances may be useful as a protective vaccine for those who might be expected to develop complications after challenge with standard vaccine. This speculation requires further study.

The last known case of smallpox in the United States was reported in 1949 (5); consequently, interest now centers on the complications of vaccination. Available data have indicated a need to reduce the number of untoward reactions following primary vaccination (1, 6, 8, 9, 11-13). Many complications may be prevented by more careful screening for known contraindications, but only two alternatives exist to reduce the number of nonpreventable complications. First is discontinuation of routine smallpox vaccination except for high risk groups such as foreign travelers and medical and hospital personnel. Second is development of modified vaccines (7, 14, 17, 18, 20).

Most complications occur as a result of primary vaccination with standard vaccinia and many occur because of accidental vaccination contact in individuals who have dermatologic conditions that potentiate the generalized spread of the vaccinia virus (13). These complications, especially those that occur in individuals with eczema, are preventable either by more careful screening or theoretically by the introduction of an attenuated vaccine which can be administered before vaccination with standard vaccinia. An attenuated vaccine used in this way might protect an individual from complications that could result from an initial inoculation with standard vaccinia were this inoculation to be either accidental

or required because of potential travel to a smallpox endemic area.

In 1930 Rivers *et al.* (18) developed a modified vaccinia strain, CVI, with the intention of using this strain in place of standard vaccinia. He subsequently discarded this modified strain because it was thought to be too attenuated to be protective (18).

In 1968 Kempe *et al.* (7) employed a further modification of this strain, now identified as CVI-78, in the primary protective vaccination of 1,009 patients with eczema and other skin disorders. The standard multiple pressure technique with a vaccine of titer $10^{8.4}$ TCID₅₀/ml was used, as well as subcutaneous administration of vaccines with titers of $10^{3.0}$ - $10^{4.5}$ TCID₅₀. Control groups were not included. In this study Kempe reported milder local and systemic reactions than would be expected with standard vaccination of healthy children, 100% seroconversion (387 patients tested), and two cases of mild erythema multiforme. Revaccination (104 patients) at 1-6 months with a standard strain vaccine resulted in modified cutaneous reaction.

It is the purpose of this study to compare primary vaccination with CVI-78 with primary vaccination with a standard strain of vaccinia in normal subjects using standard multiple pressure vaccination techniques. This inquiry may be helpful in determining whether CVI-78 is a candidate vaccine for wider use in preventing complications that would occur in individuals after challenge with standard vaccine.

METHODS

STUDY POPULATION

Forty-nine subjects received primary vaccination with reconstituted CVI-78 and 46 subjects with reconstituted standard calf lymph vaccine (CL) (24). All subjects were healthy children between 1 and 5 years of age who received their medical care at the Johns Hopkins Hospital Comprehensive Child Care Clinic. All were screened for routine contraindications and prior smallpox vaccination by history and physical examination. Parents were advised of the intent of the study and consent was obtained. Subjects were randomly vaccinated with either CL or CVI-78 vaccine. The vaccines were color coded and the vaccinators did not know which vaccine was being used until the end of the study. All vaccinations were performed between February 19, 1970, and May 15, 1970.

VACCINE

Both CVI-78 and CL vaccines were stored at 5° and reconstituted within 24 hr before use. Reconstitution was made in 50% glycerine solution containing 0.25% phenol and 0.005% brilliant

green. Prevacination and postvaccination vaccine titers were measured in association with the Division of Biologics Standards reference strain at the Center for Disease Control. These titers are exhibited in Table 1.

VACCINATION TECHNIQUE

All vaccinations were administered by two vaccinators (WCS and RBW). Each vaccination was on the left arm over the lateral aspect of the deltoid. The area was cleansed with acetone, and a drop of vaccine was placed on the skin. Five double punctures were made through the vaccine with a bifurcated needle directed perpendicular to the skin. Excess vaccine was removed with a dry cotton ball. Parents were instructed in appropriate care of the vaccination site (16).

CLINICAL EVALUATION

Each subject was seen by one of the two vaccinators 7 and 28 days after vaccination. The 7-day follow-up consisted of a brief history, rectal temperature, and examination for rash. Local erythema, induration, and vesiculation at the vaccination site were measured and photographed. The 28-day follow-up consisted of a brief history and measurement of the vaccination scar. These measurements are exhibited as the product of the major and minor axes (mm²).

SEROLOGIC EVALUATION

Venous blood was obtained from each child at 28 days postvaccination. Blood was allowed to clot; the serum was removed and stored at -70° until antibody titers were determined. Hemagglutination-inhibition (HI) antibody titers were measured using the technique described by Hierholzer *et al.* (4). Neutralizing (Neut) titers were measured with the technique of Cutchins *et al.* (2) as modified and described by Wulff *et al.* (23).

RESULTS

CLINICAL RESULTS

Forty-nine children who received CVI-78 and 46 who received CL were examined 7 days postvaccination. Vesicle formation, denoting a major reaction as defined by WHO (22), was observed in 30 (61%) CVI-78 vaccinees and 44 (96%) CL vaccinees. The sizes of the vesicles at 7 days are exhibited in Figure 1. Induration size at 7 days is exhibited similarly in Figure 2. Scar size at 28 days is shown in Figure 3. In general, vesicle, induration, and scar were smaller after CVI-78 than CL vaccine.

At 7 days fever (rectal temperature 38°) was noted in 4 of 49 (8%) subjects with vesiculation after CVI-78 vaccination and in 12 of 46 (26%) with vesiculation after CL vaccination. General malaise or anorexia or both were noted in 7 children of each group with vesiculation. No fever, malaise, or anorexia was noted in those who did not have vesiculation. Skin rashes which had cleared without therapy by 28 days were observed in 4 children at 7 days. Three were vaccinated with CL. One child had maculopapular lesions on the posterior thorax which occurred after therapy of tonsillitis with penicillin and phenobarbital (patient had prior

history of reaction to phenobarbital); another had erythematous papular lesions on the cheeks with excoriations secondary to pruritus; and the third had erythematous papular lesions on both cheeks and forehead associated with pruritus. One child vaccinated with CVI-78. After CL vaccination, 45 (98%) had positive HI right antecubital fossa.

SEROLOGIC RESULTS

Serum samples were obtained at 28 days postvaccination from 49 subjects vaccinated with CVI-78 and 46 subjects vaccinated with CL. Positive HI titers ($\geq 1:8$) were noted in 33 (67%) and positive Neut ($\geq 10^{0.9}$) titers in 8 (16%) of the subjects vaccinated with CVI-78. After CL vaccination, 45 (98%) had positive HI titers and 41 (89%) had positive Neut titers as recorded in Table 2. The postvaccination neutralization titers in those that converted were also lower with CVI-78 vaccination than with CL vaccination.

CORRELATION OF DERMAL AND POSTVACCINATION ANTIBODY RESPONSE

Table 3 compares the vaccination groups by seroconversion and vesiculation. Forty-nine children who received CVI-78 were followed clinically and serologically; 30 of these had vesiculation at 7 days. All of the 30 had positive postvaccination HI titers, but only 6 (20%) had positive Neut titers. Of the 19 subjects without vesiculation at 7 days, 3 had positive HI and negative Neut titers and 1 had a negative HI and a Neut titer of $10^{0.9}$. Two of these children had detectable scars at 28 days and in all probability represent late takes. Of the 15 remaining children who had no vesiculation at 7 days, all had negative postvaccination HI and Neut titers. Two of these 15 children did have detectable scars at 28 days. There were 13 remaining children (27%) who after vaccination with CVI-78 vaccine had no antibody response by either serologic test and no dermal response as measured by 7-day vesiculation and 28-day scar. Forty-six children who received CL were followed both clinically and serologically. Forty-four of these had vesiculation at 7 days. All of the 44 had positive postvaccination HI titers and 39 (85%) had positive Neut titers. One child without vesiculation had negative HI and negative Neut titers and another child without vesiculation at 7 days had positive HI and positive Neut titers. The latter had a detectable scar at 28 days and in all probability represents a late take while the former did not have any scar. Thus, only one child (2%) after vaccination with CL vaccine had no antibody or dermal response.

DISCUSSION

The purpose of this study was to compare vaccination with CVI-78 vaccine with vaccination with standard calf lymph vaccine using comparable techniques and vaccines of similar titer. The data have shown that CVI-78 vaccine results in a clearly modified response both clinically and serologically. Postvaccination reactions as measured by 7-day erythema, induration, and vesiculation were milder, and the 28-day scars were smaller after CVI-78 than standard calf lymph vaccination. Similarly, postvaccination neutralizing and HI antibodies were lower with CVI-78. Systemic reactions were also less, and there was only one rash illness associated with CVI-78 vaccination, compared with three associated with calf lymph vaccination. These rashes were very mild and possibly were not related etiologically to vaccination. Thus, by all measurements, vaccination with CVI-78 resulted in fewer adverse reactions than vaccination with standard calf lymph.

In evaluating this vaccine, however, very little can be determined about the degree of immunity which CVI-78 vaccine confers against smallpox. Standard calf lymph vaccine results in a high degree of protection against smallpox. The immune mechanisms probably involve humoral antibodies, cell-mediated immunity, and possibly interferon (3, 10, 15, 19, 21). Serum neutralizing and HI

Table 1. Vaccine titers

	Chorioalantoic membrane titers, plaque-forming unit (PFU)/ml	
	Prevaccination	Postvaccination
CL	$10^{7.9}$	$10^{8.1}$
CVI-78	$10^{7.7}$	$10^{7.6}$
DBS ¹	$10^{7.9}$	$10^{8.1}$

¹ Division of Biologic Standards reference strain.

Fig. 2. Induration 7 days after primary vaccination.

antibodies provide the best available index of humoral immunity. The presence of a cutaneous reaction may suggest that cell-mediated immunity is developing (15). By these measurements CVI-78 vaccination is not as protective as standard calf lymph vaccination. Only 61% of the children vaccinated with CVI-78 vaccine, compared with 96% of the children vaccinated with standard calf lymph, developed major dermal reactions as defined by WHO criteria. The development of an HI titer corresponded closely to the development of a major dermal reaction. Only 16% of those vaccinated with CVI-78 vaccine, however, developed neutralizing antibodies, compared with 89% vaccinated with standard calf lymph. This low neutralizing response may in part be a result of insensitivities in the test rather than the absolute lack of neutralizing antibodies in the population vaccinated. The neutralization antibody results, however, are distinctly different in the group vaccinated with CVI-78 as compared with the controls who were vaccinated with standard vaccinia. Finally, 27% of those receiving CVI-78 vaccine demonstrated neither a dermal nor

serologic postvaccination response as measured either by HI or neutralizing antibodies, compared with only 2% of those who received standard calf lymph vaccine. Although it is uncertain to what degree modified serologic or dermal reactions confirm immunity against smallpox, it must be assumed that those who show no reaction at all are not protected against smallpox or from complications that would result from standard smallpox vaccination.

These results confirm Rivers' original observation (17) that this vaccine strain, CVI-78, is too modified to be used as a replacement for standard vaccine for protection against smallpox. It still, however, may be useful as a primary and protective vaccination for those individuals who might be expected to develop complications after challenge with standard vaccinia, as suggested by Kempe *et al.* (7). It should be noted, however, that only 73% of those receiving CVI-78 vaccine in the above fashion showed an immunologic response and could be expected to have a modified vaccination response when challenged with standard vaccinia. The other

Fig. 3. Scar size 28 days after primary vaccination.

Table 2. 28-day postvaccination antibody

A. Postvaccination, neutralizing titer range, log ₁₀ TCID ₅₀ /ml						
Group	<0.6	0.6-0.9	1.0-1.9	2.0-2.9	3.0-3.9	4.0-4.9
CVI-78	41	2	6	0	0	0
CL	5	2	19	11	7	2

B. Postvaccination, HI titer range, 1:serum dilution					
Group	<1:8	1:8	1:16	1:32	1:64
CVI-78	16	6	14	11	2
CL	1	6	16	21	2

Table 3. Vesicle formation and antibody response by vaccination type¹

Antibody titer	Vesicle formation			
	CVI-78		Calf lymph vaccine	
	Present	Absent	Present	Absent
HI				
Negative	0	16	0	1
Positive	30	3	44	1
Neut				
Negative	24	18	5	1
Positive	6	1	39	1

¹ HI: hemagglutination-inhibition; Neut: neutralizing.

27% would be expected to respond on challenge as if they had never received any initial vaccination. The complication rate in this latter group would be the same as those receiving standard vaccinia for the first time.

We conclude that although CVI-78 vaccine produces a modified local reaction in healthy children, it may be overattenuated. Humoral antibody production and, quite possibly, cell-mediated immunity induction are poor after a single dose of this vaccine by the multiple pressure technique. The usefulness of this vaccine will depend on the protection it affords against challenge with standard vaccine. This question is answered in a companion paper.

SUMMARY

Primary smallpox vaccinations were performed on healthy children comparing an attenuated vaccine, CVI-78, with standard calf lymph vaccine. Both vaccines were obtained in lyophilized form and after reconstitution had similar chorioallantoic membrane titers of between $10^{7.6}$ and $10^{8.1}$ plaque-forming units/ml. Using standard vaccination techniques, 49 children received primary vaccination with reconstituted CVI-78 vaccine and 46 children with reconstituted standard calf lymph vaccine. The children were evaluated clinically on the 7th day and serologically on the 28th day postvaccination. Vaccination with CVI-78 resulted in a more attenuated response clinically and serologically and a lower percentage of dermal and serologic conversions than vaccination with standard calf lymph. Only 61% of those vaccinated with CVI-78 as compared with 96% with standard vaccine developed a major dermal reaction. Postvaccination HI antibodies correlated closely with the dermal reactions. Only 16% of the CVI-78 subjects as compared with 89% of the calf lymph subjects, however, developed postvaccination neutralizing antibodies.

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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