Acta virol. 27: 154—159, 1983

VACCINIA VIRUS IN POSTVACCINAL ENCEPHALITIS

E. B. GURVICH, I. S. VILESOVA

L. A. Tarasevich State Research Institute for Standardization and Control of Medical and Biological Preparations, 121002 Moscow, U.S.S.R.

Received January 4, 1981; revised May 26, 1982

Summary. — Results of virological examination of 239 samples taken from 84 children with neurological complications after smallpox vaccination are described. In postvaccinal encephalitis, vaccinia virus was isolated from blood, cerebrospinal fluid and pharyngeal secretions of 23 out of 40 children (57.5%) as well as from autopsy specimens sampled between 10—35 days after vaccination. During the acute period of disease, virus was detected in 17 out of 31 (54.2%) cerebrospinal fluid specimens. In 3 postvaccinal encephalitis cases the virus was present in brain and in a case of encephalomyelitis — in the spinal cord. These results confirmed the participation of vaccinia virus in the pathogenesis of postvaccinal encephalitis. The pathogenicity of vaccinia virus may be manifested only under a changed reactivity of the vaccinated host.

Key words: *live vaccine*; *adverse effect; postvaccinal encephalitis*; *vaccinia virus* (*Poxviridae*)

Introduction

Despite of the efforts of many authors in the long course of smallpox vaccination, the mechanism of the development of postvaccinal encephalitis has not been elucidated. Especially the exact role of vaccinia virus in pathogenesis of the disease remains obscure, although the presence of the virus itself seems to be important (Herzberg-Kremmer and Herzberg, 1930; Siegert, 1957; Marennikova and Matsevich, 1974; Gurvich *et al.*, 1975). As shown by a study of 450 children, vaccinia virus participated in different complications involving skin and mucous membranes, indicating the specific nature of different somatic lesions associated with vaccination (Gurvich *et al.*, 1974, 1975).

Materials and Methods

Eighty four children were followed. Of these, 79 became ill after the first vaccination (1 to 3-year-old were 63 children; 3 to 9-year-old 16 children) and 5 after revaccination (aged of 8, 10, 15 and 16 years respectively). The clinical diagnoses (in all cases confirmed by pediatrician and neuropathologist) were as follows: postvaccinal encephalitis — 31 cases; encephalomyelitis — 5; serous menigitis — 2; myelitis — 1; myelopathy — 1; encephalopathy with seizures — 44 cases.

The incubation period of the postvaccinal encephalitis lasted for 3—24 days (average of 12.2 days). The clinical course was severe in the majority of cases; 7 children showed sequelae, 6 children died. The incubation period of postvaccinal encephalopathies ranged from 4—13 days (average 7.5 days). Two children died. Blood, cerebrospinal fluid and pharyngeal secretions were examined; in addition blood, brain and visceral samples were taken at autopsy. Altogether 96 out of 145 samples examined in the course of postvaccinal encephalitis were taken from the 11th to 20th days, the rest (46, i.e. 32.8%) from 3 weeks after vaccination (71 samples were obtained in the first 5 days of illness). In the course of postvaccinal encephalopathies the materials were taken from day 5 to day 25 after vaccination; 51 samples out of 94 ones (54.2%) were examined till day 10, the rest of 42 samples (44.7%) between days 11—20 and one sample on day 25 post vaccination. The isolation attempts were made in 12-day-old chick embryos (Westwood *et al.*, 1957). The methods of handling of the samples and identification of the isolates are described elsewhere (Gurvich *et al.*, 1979). The samples were examined immediately, rarely they were stored for a few days at 4 °C.

Results

Vaccinia virus was isolated from 22 out of 36 patients (61.1%) with postvaccinal encephalitis or encephalomyelitis and in 1 out 2 cases of serous meningitis. No virus was isolated from the blood and pharynx of children suffering of myelitis or myelopathy (Table 1). The relatively frequent virus isolation from cerebrospinal fluid (17 positives out of 31 samples, i.e. 54.2%) and blood (18 positives out of 42 samples, i.e. 42.2%) were noteworthy as the positive isolations at later intervals, i.e. from blood until the 34th day, from cerebrospinal fluid until the 32nd day and from pharynx until the 35th day. In 15 children the virus was isolated simultaneously from 2 or 3 samples (blood, cerebrospinal fluid and pharynx); in another 3 patients the virus was repeatedly isolated from blood and cerebrospinal fluid within 11—18 days, while in another 4 the pharyngeal secretions were positive at subsequent intervals between 1—13 days.

In 3 out of 6 cases with lethal outcome, the vaccinia virus was isolated from the brain tissue (on days 12, 19 and 40 after vaccination; in one case the titre of the virus in the brain was 1.5×10^5 PFU/g. The examination of internal organs (lungs, liver, heart) fell out positive in two cases. In 1 case of encephalomyelitis vaccinia virus was isolated from the spinal cord (22 days after vaccination). No virus was found in the cerebellar tissue and brain stem. In 2 autopsy cases no virus was isolated from the brain and visceral organs;

Table 1. Isolation of vaccinia virus from children with neurological complications after smallpox vaccination

Clinical diagnosis	No. of patients positives	total	per cent
Postvaccinal encephalitis including:	23	40	57.5
meningoencephalitis, encephalomyelitis	22	36	61.1
serous meningitis	1	2	
myelitis, myelopathy	0	2	
Encephalopathy	7	44	15.9

despite of negative isolation attempts vaccinia virus antigen was detected by direct immunofluorescence in lungs and respiratory airways, but no antigen was found in the brain of this particular case. It is possible that in the latter cases the immune state of the vaccinees was essential; the virus-neutralizing antibody titre in the children 12 days after revaccination reached the value of 80. In one case the virus had been detected in the content of brain ventricles — which confirmed the presence of virus in the absence of blood contamination — while in another, the vaccinia virus antigen was seen in a few neurons by immunofluorescence.

The virus isolations from these materials, except of the autopsy samples, were made in some cases in serial passages. From the original samples the vaccinia virus was isolated in 16 cases (8 from the cerebrospinal fluid), while a second passage was necessary in 23 cases (5 from cerebrospinal fluid) and in 7 cases even more subpassages were performed. In some cases the virus was easier isolated from the cerebrospinal fluid than from the blood. In 3 children revealing virus in the cerebrospinal fluid no viraemia was found.

As shown in Table 2, in postvaccination encephalopathy vaccinia virus

Table 2. Isolation of vaccinia virus from specimens of children with neurological complications after smallpox vaccination

Interval after vaccination	Clinical diagnosis: postvaccinal encephalitis	serous meningitis	myelitis myelopathy	encephalopathy
Till day 11:				
blood	0/2	—	—	4/18
cerebrospinal fluid	—	—	—	0/1
pharyngeal secretions	0/1	—	—	2/32
From 11 to 20 days:				
blood	13/24	1/1	0/2	2/12
cerebrospinal fluid	12/22[a]	1/1	—	1/2
pharyngeal secretions	7/26	2/2	0/1	0/17
brain tissue	3/6	—	—	0/3
internal organs	4/11	—	—	0/8
Later than 21 days:				
blood	5/14	0/2	—	—
cerebrospinal fluid	4/6	0/2	—	—
pharyngeal secretions	3/14	0/2	—	1/1
brain tissue	2/4	—	—	—
internal organs	2/2	—	—	—
Total	55/132	4/10	0/3	10/94
%	41.7	40.0	—	10.6

Numerator: number of positive samples; denominator: total number of samples.
[a]) including 1 positive out of 2 specimens from brain ventricles
n.t. — not tested

was isolated from the blood or pharyngeal secretions of 7 out of 44 children. From 4 children (9%) the virus was isolated later than 10 days after vaccination; in 2 children it was present in the blood (days 12 and 16), in 1 in the pharynx only (day 25) and in 1 in the cerebrospinal fluid (day 15). No viraemia was found in the latter case. It cannot be excluded that in children, the blood and cerebrospinal fluid of which contained vaccinia virus on the day of their deaths, encephalitis would have developed.

Discussion

Long-term methodical and systematic virological examination of samples coming from sick children with postvaccinal encephalomyelitis relatively frequently revealed (57.5%) the presence of vaccinia virus either in acute stage of the disease or during its prolonged course. The positive isolation results can be enhanced when combined with immunofluorescence or electron microscopy, when the isolation is performed from the cell fraction of blood or from cerebrospinal fluid cells, when the immune complexes are dissociated or when tested samples are concentrated before inoculation (Kurata *et al.*, 1977).

Comparison of the results of virological studies in uncomplicated vaccination (Weisse *et al.*, 1953; Kempe, 1960; Gurvich *et al.*, 1979) to those of other different clinical complications after smallpox vaccination (Gurvich *et al.*, 1979) showed that the frequency and duration of virus isolations in postvaccinal encephalitis is essentially different as compared to uncomplicatedvaccination and resembled rather to generalized vaccinia, the viral genesis of which is doubtless. On the other hand, the same virological criteria can hardly discriminate the postvaccinal encephalopathy from uncomplicated vaccination process. This confirms the different pathogenesis of both clinical entities representing 2 different forms of postvaccinal neurological complications. In the cases of rare virus isolations from late stages of the postvaccine encephalopathy syndrome with seizures after day no clear borderline to real encephalitis could be drawn.

The most important criterion for participation of vaccinia virus in the development of pathological changes of CNS is the presence of the virus in cerebrospinal fluid during the acute period of postvaccinal encephalitis; this had already been regarded important in the thirties (Eckstein *et al.*, 1932). The virus presence in cerebrospinal fluid can be transient when occurring due to the impaired blood-brain barrier as seen in vaccinees with tuberculous or purulent meningitis (Siegert *et al.*, 1957). We isolated the vaccinia virus from 2 out of 3 children who had developed purulent meningitis in the postvaccination period (in 1 case from the blood and cerebrospinal fluid, in another case from the blood of the heart ventricle by 17 and 28 days after vaccination, respectively). In addition, from a child which developed influenza in the postvaccination period and died 22 days after vaccination, vaccinia virus was isolated from blood, cerebrospinal fluid and visceral organs. According to our data only 4 out of 40 children developing postvaccinal encephalitis had an intercurrent disease which could have altered

their susceptibility to the virus, while no concomittant disease was noted in the rest of them.

The absence of a direct relationship between viraemia and virus presence in the cerebrospinal fluid, the high efficiency of virus isolations from the cerebrospinal fluid during the acute stage of the disease points to the higher virus contents in the cerebrospinal fluid as compared to blood (as already shown by Siegert, 1957). This finding also favours the direct replication of the virus in CNS as described in monkeys (Morita *et al.*, 1957). The present data allow to claim that vaccinia virus is one of the essential links in the complicated mechanism of development of the postvaccinal encephalitis suggenting the general features of an infectious process.

It is not clear, whether the presence of vaccinia virus in brain is inevitable for the development of postvaccinal encephalitis, whether it has a triggering effect (Simon, 1979) or only an indirect influence on the brain tissue. Possibly, the changed biological properties of the virus in the sick organism might be of importance (Ehrengut and Sarateanu, 1975; Gurvich *et al.*, 1978), although no such changes had been proved till now.

No doubt that viral findings in sick vaccinees do not explain the pathogenesis of postvaccinal encephalitis. The pathogenicity of vaccinia virus can manifest itself only under favourable conditions of the changed host response, especially in immunodeficiency (Gurvich *et al.*, 1980; unpublished observations). We suggest that observations on virological examination of children showing neurological complications after smallpox vaccination can be useful in association with other live viral vaccines.

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