

The Effect of Prior Inoculation with an Enterovirus (LEV 4) on Rhinovirus Infection of Volunteers

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With 1 Figure

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Summary

Twenty-four volunteers at the Common Cold Unit were divided into two groups of twelve. One group was vaccinated orally with an enterovirus (LEV 4) and the other with nutrient broth. Both groups were challenged three days later with intranasal rhinovirus 4 and they were observed clinically and monitored by laboratory tests to see if any modification of the rhinovirus infection occurred.

All the vaccinated volunteers were successfully infected with LEV 4 and were excreting the enterovirus in the faeces at near maximum titres at the time of the rhinovirus infection, following which 67 per cent of the volunteers were infected and 29 per cent developed symptoms. However, the vaccinated group did not differ from the unvaccinated in respect of the illness induced, the excretion of rhinovirus type 4 or the rise of RV4 antibody titre. LEV 4 was isolated from the nasopharynx of some of the volunteers, but the rhinovirus infection was not modified even in these. Interferon was present in the serum and nasal washings of nine volunteers in all, of whom only 3 had received the LEV 4 vaccination.

Two additional volunteers were shown to be insusceptible to reinfection with LEV 4. It was concluded that live enterovirus vaccination does not induce viral interference.

1. Introduction

Since many serotypes of viruses cause acute respiratory disease, vaccination against most of them is impracticable. The exploitation of viral interference for prophylaxis is especially attractive because of its wide application and the possibility of offering protection against agents for which no other means of prophylaxis is currently available.

It appears that acute respiratory disease in man can be prevented by viral interference. TYRRELL and REED (1) showed that volunteers inoculated with influenza during the incubation period of a rhinovirus were protected against

influenza infection, although this protection did not occur during the incubation period of a coronavirus. However, both viruses primarily affect the respiratory system, so that purely local factors may have operated. Interference by a heterologous virus was observed by VELLA *et al.* (2) when they showed that pre-school children vaccinated against rubella were protected against acute upper respiratory tract illness for at least 12 weeks after vaccination. This protection was greater than that afforded by a parainfluenza virus vaccine.

Recent epidemiological evidence from the U.S.S.R. has suggested that interference may be exploited by giving a live enterovirus as a vaccine to prevent viral respiratory disease, and that the protection induced is mediated by circulating interferon. VOROSHILOVA (3) reported that an enterovirus vaccine had been successfully used for the prophylaxis of influenza and other acute respiratory diseases. In many trials, a 1.9 to 5.1-fold reduction in the incidence of these diseases was observed in vaccinees. Furthermore, interferon was measurable in the blood, nasal washings and urine of vaccinees, the maximum titres being observed on the 5th to 8th days. However, no studies of the efficacy of the procedure in an isolation unit have been reported. We thought it important to study the effect of a vaccine in isolated volunteers both by measuring interferon production and by the results of challenge with an interferon-sensitive virus.

2. Materials and Methods

2.1. Vaccine

The enterovirus, live epidemiological vaccine type 4 (LEV 4), was kindly supplied by Professor M. K. Voroshilova. The virus was completely neutralised by W.H.O. reference echo type 1 antiserum in monkey kidney tissue culture. Other agents were excluded by cultivation in standard bacteriological media and tissue cultures. The pathogenicity of LEV 4 for the central nervous system of cynomolgus monkeys (*M. irus*) was tested at the National Institute for Biological Standards and Control. The vaccine was injected directly into the thalamus and lumbo-sacral region of the spinal cord of 24 animals. There were no clinical or histological responses attributable to viral activity.

2.2. Challenge Virus

The challenge virus, rhinovirus type 4 (RV 4) was prepared from nasal washings of infected volunteers and had not been passaged in tissue culture.

2.3. Procedures in Volunteers

The subjects were healthy adults aged 18—50 who were housed in isolation at the Common Cold Unit, Salisbury, and observed by standardised methods described by TYRRELL (4). All clinical assessments were made under double blind conditions and at the end of the trial a clinical symptom score was calculated for each volunteer. We excluded volunteers with serum haemagglutination inhibition (HAI) titres of 1 in 4 or more against RV 4 using the method of REED and HALL (5).

2.4. Specimens and Isolation of LEV 4, RV 4 and Interferon

Throat swabs and faeces for enterovirus titration were collected before LEV 4 inoculation and before and after RV 4 challenge. Nasal washings for RV 4 titration and interferon were collected before and on several occasions after the RV 4 challenge. Blood was taken before the LEV 4 inoculation, on alternate days for 6 days afterwards, and about 17 days after rhinovirus challenge. The first and last sera were used to measure HAI and neutralising antibodies to RV 4; interferon was measured in all specimens. All specimens were collected from both test and control groups.

Faeces were homogenised with sterile applicator sticks to make a 10 per cent suspension in nutrient broth with antibiotics. Throat swabs were collected into a similar medium. Isolations and titrations were performed in continuous monkey kidney tissue culture, either VERO or V3, in which the enterovirus produced typical cytopathic effect. Both these cell lines were insensitive to rhinovirus type 4.

Nasal washings were tested in parallel in rhinovirus-sensitive HeLa cells as described by STOTT and TYRRELL (6), and in monkey kidney cells. Specimens showing an enterovirus cytopathic effect in monkey kidney cells were re-tested in HeLa cells in the presence of antiserum to echovirus 1, used at 30 times its neutralising titre. This procedure effectively suppressed growth of at least 1000 TCD₅₀ of LEV 4 while leaving the growth of RV 4 unaffected.

Sera were screened for interferon by a plaque reduction method of MERIGAN (7) using bovine vesicular stomatitis virus in the semi-micro method of ZISMAN and MERTGAN (8). Titration of the reference human interferon preparation B 69/19 showed inhibition by 0.1 to 1 i.u. of human interferon per ml of sample tested. Samples were tested at a dilution of 1:10 to exclude non-specific interference.

3. Results

3.1. Pattern of Enterovirus Excretion of Volunteers

All volunteers in the test group were successfully infected with LEV 4 (Table 1). Their pre-inoculation specimens, and all the control group specimens, showed no enterovirus. Table 1 shows that the LEV 4 was isolated from the nasopharynx of nearly half of those inoculated. Daily titrations of faeces of 5 volunteers showed that excretion of LEV 4 was maximal on days 4 or 5; overall, excretion of LEV 4 was near maximum on days 1 or 2 so that infection was well established when the volunteers were challenged with rhinovirus.

Table 1. *Isolation and Titration of Enterovirus (LEV 4) from Volunteers: LEV 4 Vaccination on Day 0*

	Proportion of volunteers from whom LEV 4 was isolated		Mean and range of titres in faeces Geometric mean titres: log ₁₀ TCD ₅₀ per ml 10 per cent suspension	
	Faeces	Nose or throat	Day 1 or 2	Day 4 or 5
Nutrient broth (control group)	0/12	0/12	0	0
LEV 4 (test group)	12/12	5/12	3.2 (1.5—5.2)	3.9 (3.2—5.2)

3.2. Overall Effects of Rhinovirus 4 Challenge

Effects of challenge were monitored by both clinical response and virological evidence of infection, namely the isolation of RV 4 from one or more nasal washings or a four-fold or greater rise in serum HAI or neutralising antibody titre to RV 4. Although only 25 per cent developed symptoms and 60 per cent were infected of the control group, no overall reduction in illness or infection was induced by prior LEV 4 inoculation (Table 2).

Table 2. *Effects of Prior LEV 4 Inoculation on Rhinovirus 4 Infection of Volunteers*

Inoculum for volunteers	Site of excretion of LEV 4	Number of volunteers	Symp-toms present	Mean clinical score	RV 4 isolated once or more	Mean titre of RV 4 in nasal washings (log ₁₀ TCD ₅₀ per ml)	Fourfold rise in titre of antibody to RV 4	Virus isolation, antibody rise or both
Nutrient broth (controls)	None	12	3	9.7	7	1.05	4	7
LEV 4 (test group)	Faeces, or faeces and nasopharynx	12	4	10.3	8	1.10	2	9
LEV 4 (part of test group)	Nasopharynx and faeces	5	1	7.6	3	1.02	0	3
LEV 4 (part of test group)	Faeces only	7	3	12.3	5	1.15	2	6

EFFECT OF LEV 4 VACCINATION 'TAKE' IN NASOPHARYNX ON MEAN DAILY RV 4 EXCRETION.

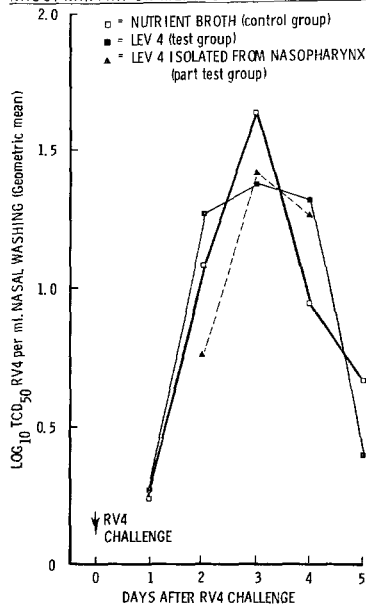


Fig. 1. Rhinovirus 4 was given by intranasal inoculation on day 0 and the daily excretion of RV 4 measured in nasal washings was followed in those given nutrient broth (control group) and LEV 4 (test group). No significant difference in excretion of the common cold virus is seen in the vaccinated group. The mean titres are from 12 volunteers on days 2, 3 and 4 and from 5 volunteers on days 1 and 5 in the control and test groups. Mean titres on days 2, 3 and 4 are also shown for the five volunteers who grew LEV 4 from the nasopharynx: they are not significantly delayed or lower than the controls

3.3. Effect on Mean Daily RV4 Excretion

The mean titres of RV4 in nasal washings were calculated for each day (Fig. 1). Although the mean in the control group was slightly higher than in the test group on the 3rd day after rhinovirus challenge, this difference was not significant. Rhinovirus excretion was not delayed in the vaccinated group.

3.4. Effect of Presence of LEV4 in Nasopharynx

The five volunteers from whom LEV4 was isolated in the nasopharynx were considered separately to see if local interference occurred. However, none of the indices of RV4 infection was significantly lower in this group (Table 2), and calculation of the daily mean titres of RV4 in nasal washings (Fig. 1) confirmed that no interference occurred locally on any specific day.

3.5. Presence of Interferon in Serum and Nasal Washings

Interferon was found in the serum on the 5th day only (5 days after LEV4 or control inoculation and 3 days after RV4 challenge) in three volunteers, two of whom had not received LEV4. Presence of interferon in serum therefore seemed to be related to rhinovirus challenge (Table 3). Interferon in nasal washings (Table 3) also showed no relationship to LEV4 vaccination. Four of the seventeen volunteers who were symptom-free throughout the trial showed interferon in nasal washings compared with three of 7 who were symptomatic. Thus interferon in nasal washings was associated with symptoms of rhinovirus infection but the difference did not achieve statistical significance at the 0.05 level ($\chi^2 = 3.744$, $p < 0.1$).

Table 3. *Presence of Interferon (IF) in Serum and Nasal Washings: Nutrient Broth or LEV4 Vaccination on Day 0; Rhinovirus 4 Challenge on Day 3*

Inoculum for volunteers	No. of volunteers	Proportion of volunteers with IF in serum				Proportion of volunteers with IF in nasal washings			
		Before LEV4 vaccination	Days 1 and 3	Day 5	Days 7 and 21	Before RV4 challenge (Day 2 or 3)	Day 5	Day 6	Day 7
Nutrient broth (control group)	12	0	0	2	0	0	0	5	1
LEV4 (test group)	12	0	0	1	0	0	2	1	1

3.6. Effect of Revaccination with LEV4

Two additional volunteers who were not studied in isolation were successfully infected with LEV4, but they were not challenged with RV4 and are therefore not included in the previous results. LEV4 was obtained from throat swabs and faeces of both volunteers. The course of enterovirus excretion was followed at first daily, then weekly; excretion definitely ceased after 12 weeks. Neutralising antibodies to echo 1 virus in the serum rose after three weeks from titres of $<1/5$ to titres of $1/7$ and $1/14$. No interferon was demonstrated in the serum at any stage of the enterovirus infection.

These two volunteers were given 10^6 TCD₅₀ LEV 4 three months after the first infection. Virus could not be grown from any specimens of throat swabs or faeces, and it was concluded that the second dose had failed to infect.

4. Discussion

Our results do not agree with the epidemiological and laboratory data previously reported for LEV 4 by VOROSHILOVA (3). In another Russian study by OBROSOVA-SEROVA *et al.* (9) a single dose of LEV 4 was noted to be ineffective in a controlled study of the prevention of an influenza epidemic. SEIBIL *et al.* (10), in an epidemiological study involving many thousands of subjects, claimed that multiple doses of vaccine, especially live poliovirus vaccine, were more effective than a single dose in reducing respiratory illness. However, since we could not achieve reinfection with LEV 4, it is difficult to see how multiple doses could be more effective.

If viral interference is to be a useful method of prophylaxis, our study should have shown some effect, however small, on the results of rhinovirus challenge in the test group. All of the test group were infected and high titres of LEV 4 were being excreted at the time of rhinovirus challenge. Nevertheless no effect could be demonstrated on the symptoms produced, the mean total rhinovirus excretion, the mean daily rhinovirus excretion, or the number of four-fold or greater rises in RV 4 antibody titre. Furthermore the presence of the interfering virus locally in the nasopharynx conferred no apparent advantage in protection.

Interferon was present in nasal washings and serum, but its presence was not related to LEV 4 vaccination; it was probably a result of rhinovirus challenge. Interferon in the nasal washings was to some degree related to the expression of symptoms. It does not appear that vaccination with live enteroviruses will provide a satisfactory method for the prevention of acute respiratory disease due to viruses.

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