Melnick JL (ed): Prog Med Virol. Basel, Karger, 1989, vol 36, pp 191–202

Potential Use of Nonpathogenic Enteroviruses for Control of Human Disease

Marina K. Voroshilova[1]

Institute of Poliomyelitis and Viral Encephalitides, Academy of Medical Science, Moscow, USSR

Photograph taken in 1971 of Prof. Marina Konstantina Voroshilova, Professor and Member-Correspondent of the USSR AMS.

[1]Prof. Voroshilova died on November 19, 1986. This manuscript was submitted posthumously.

Contents

I. Introduction

We began our work in 1945, when strains of poliomyelitis virus were first isolated in the USSR at our laboratory. Over the years we studied the pathogenesis of and immunity to this disease in experiments using *Macaca rhesus*, *Macaca cynomolgus*, *Papio hamadryas*, and chimpanzees. We tried to simultaneously obtain nonpathogenic variants of strains of nonpoliomyelitis enteroviruses by isolation of viruses from feces of children who had been in contact with poliomyelitis patients but developed no disease as well as from feces of normal children.

In the course of the broad application of Sabin's live oral poliovirus vaccine (OPV) we found that the majority of children showed good results, but in some children the vaccine virus did not grow in the intestinal tract and no antibodies developed in the blood. In the late 1950s it was clear from virologic studies carried out in our and in many other laboratories that immunization against poliomyelitis with OPV may be unfavorably influenced by the competition of nonpoliomyelitis types of enteroviruses with one or another type that had existed in the intestinal tract of many children since birth without affecting their health. This lack of pathologic manifestations in children bearing enteroviruses in their intestinal tract was also appreciated by others and led Melnick [1, 2] to intro-

duce the term 'orphans' for these viruses. We suggested that symbiotic relationships may exist between the host and these nonpathogenic, saprophytic enteroviruses which were capable of competing with Sabin live poliomyelitis vaccine as well as with pathogenic viruses [3, 4]. In this connection, it occurred to us that the interfering effect of nonpathogenic enterovirus strains could be used for the elimination of pathogenic strains of enteroviruses, the causative agents of epidemic outbreaks of various diseases that occur predominantly in children.

It should be noted that the effective prevention of enterovirus diseases, such as aseptic meningitis, epidemic myalgia (pleurodynia, Bornholm's disease), herpangina, poliomyelitis-like paralytic diseases, hemorrhagic conjunctivitis, etc., is extremely difficult due to the existence of a large number (>72) of distinct enterovirus types that do not produce reciprocal seroimmunity. Since it is impossible to use specific vaccines against a virus family of so many different antigenic types, we proposed a novel principle for the control of enterovirus diseases by replacing pathogenic viruses with nonpathogenic enteroviruses – symbionts competing for their habitat – and on this basis the so-called live enterovirus vaccines (LEV) were developed [3, 5]. These strains were obtained at the USSR Institute of Poliomyelitis and Viral Encephalitides (Academy of Medical Sciences, AMS) as a result of examinations of feces from healthy newborn babies in maternity clinics and from older children in preschool institutions. The OPV strains can be regarded as examples of LEV, since they are similar in properties to natural nonpathogenic strains of various enterovirus types. It was demonstrated that under certain conditions LEV inhibited pathogenic viruses of different classes and stimulated many protective systems of the body – general nonspecific resistance and different types of immunity. They were also shown to be active inducers of endogenous interferon, and some of them were capable of destroying malignant cells in vitro.

To find out whether viral interference could be used as the basis for the control of enterovirus diseases, we carried out numerous investigations of biological properties of vaccine enterovirus strains, aspects of their safety, characterisitics of the vaccination process, their interfering and interferon-inducing activity, and their effects on humoral and cell-mediated immunity and other protective systems of the body.

In this paper I review the results of these studies carried out during 30 years of investigations, first at the USSR AMS Institute of Poliomyelitis and Viral Encephalitides and later in collaboration with 18 research and demonstration institutions in 10 large towns of 6 Soviet Union Republics. These results have been published in Russian in paper collections issued by the Institute [6, 7] and in monographs [3, 8–10].

II. Biological and Morphological Properties of Vaccine Strains of Nonpathogenic Enteroviruses

Comparative studies of biological and morphological properties of strains of several enterovirus types isolated from normal human beings supported our view that the isolates possessed the characteristics of nonpathogenic strains. Some of these strains of the echovirus group [1, 2], belonging to types 1 (strain GS-1) and 12 (strain L-572), were subjected to exhaustive additional studies of their properties, and the progeny of the naturally attenuated variants were cloned in green monkey kidney cell cultures. They were considered to be vaccine strains and were used as seed for manufacture of live enterovirus vaccines (LEV-4 and LEV-7) that were prepared at the Experimental Production Unit of the USSR Institute of Poliomyelitis and Viral Encephalitides.

In addition to these strains of echovirus types 1 and 12, ten other strains of enteroviruses from the echovirus and coxsackievirus A and B groups proved to be suitable for cloning and were selected as candidate LEV strains. All had been isolated in tissue culture from the intestinal contents of normal children.

All the cloned enterovirus strains were examined by inoculation of monkeys and small laboratory animals, and all proved to be nonpathogenic for them and produced no histopathological lesions, even after intracerebral and intraspinal inoculation of monkeys. Consequently, we regarded these strains as naturally attenuated enteroviruses that were saprophytes of the human intestinal tract. Investigations of their other biological, morphological, and physicochemical properties showed that they were typical enteroviruses and, in addition, possessed the markers of stable attenuated vaccine strains.

III. Observations on Safety and Nonreactogenicity of LEV

Initial observations of LEV were carried out in institutions for mentally retarded children aged 1–5 years. In 1967–1968 careful clinical observations of 248 vaccinated children in two such institutions, where LEV-4 and LEV-7 were used, showed complete nonreactogenicity of the vaccines [3].

Subsequently, these observations were expanded to include normal children. Conclusions on vaccine safety and nonreactogenicity were based on small groups of vaccinated and thoroughly examined children. In this way 593 vaccinated children and 1,045 of their contacts were examined. In the course of large-scale epidemiologic surveys on the effec-

tiveness of LEV-4 and LEV-7 vaccinations performed in Moscow and Kharkov 6,131 children aged 1–14 years were placed under continuous medical observation [3]. These studies showed that the LEV were completely safe. Periodic examinations of the children for 3 months postvaccination and later for several years revealed no changes in their health that could be associated with the LEV vaccination.

The complete safety and nonreactogenicity of the LEV led the Vaccine and Sera Committee of the USSR Ministry of Health to permit the oral use of LEV for nonspecific prevention during seasonal outbreaks of influenza and acute respiratory diseases (ARD) for a contingent of up to 320,000 subjects [3].

IV. Virologic and Serologic Studies in Vaccinations with LEV: Interfering Activity and Stimulation of Protective Systems of the Body

One of our requirements for LEV preparations is the active multiplication of vaccine strains in the intestinal tract of the vaccinees and the elimination of 'wild' enteric viruses from it. These properties of different LEV types were studied by virologic examinations of fecal specimens collected from vaccinees and their contacts twice, one week prior to vaccination and again at 3, 7, 10, 14, 21, and 28 days postvaccination. It was found that excretion of the vaccine strains from the intestinal tract of the vaccinees began within the first few days after vaccination and peaked by the end of the month, similar to the response of children to OPV.

Detailed virologic and serologic studies during oral vaccinations with LEV showed that after administration of 100,000 plaque-forming units the vaccine virus grew in the intestinal tract of the majority of vaccinees and thier contacts. Oral administration of LEV-4 and LEV-7 induced more intensive production of specific antibodies than did intranasal administration. Viremia of short duration was observed in 16% of adults given LEV-4 orally. The initial implantation of vaccine virus did not depend on the presence of antibodies. The latter, however, had an influence on the duration of excretion of the vaccine virus both in LEV vaccinees and in their contacts. Examinations of some groups of vaccinees 3–4 months after feeding showed that by then their intestinal tracts were practically free (3.5% carrier rate) from the vaccine strains [3, 8, 9].

The intensive multiplication of the vaccine virus in the intestinal tract of children given LEV was accompanied by its regular excretion into the environment and extensive infection of contacts. The maximum number of virus carriers among contacts (61.9%) was found on the 14th day post-

vaccination, and 35.4% were still excreting virus on the 28th day. The average duration of excretion by the contacts was 19 days.

Field results on the interaction of LEV with 'wild' cytopathic viruses in the intestinal tracts of vaccinated children were obtained during 1967–1970. Examination of the children before vaccination showed 24% of them to be carriers of unidentified cytopathic agents (UCA). Vaccine virus multiplied in 86 of 95 children without prior UCA in the intestinal tract and in 28 of 30 UCA carriers (90.5 and 93.3%, respectively). However, the excretion of 'wild' UCA markedly decreased in the majority of children within a few days after vaccination and was detected only occasionally thereafter.

In another study of 767 vaccinated children, 27 of them proved to be UCA carriers before vaccination. Administration of LEV-4 and LEV-7 vaccines completely eliminated 'wild' strains from their alimentary tract, which we considered to be due to the interfering effect of LEV. Data on the rate of isolation of 'wild' UCA virus at various intervals after the introduction of LEV vaccine strains into a children's community are presented in table 1. Before vaccination, cytopathic agents were isolated in 29.3% of children; after vaccination cytopathic agents were isolated in only 7.7%, i.e. nearly 4 times less often.

Similar data were also obtained by others in examinations in children's institutions in different climatic zones of the USSR. Not only was there elimination of wild enteroviruses, but there was also a decrease in isolations of influenza, parainfluenza, respiratory syncytial, rhino-, adeno-, and herpesviruses. These observations suggested the possibility of using LEV for nonspecific rapid prophylaxis aimed at a significant reduction of the incidence of enterovirus and respiratory infections in emerging epidemic outbreaks.

V. Use of LEV for Control of Outbreaks of Enterovirus Diseases in Children and for Nonspecific Rapid Prophylaxis during Seasonal Outbreaks of Influenza and ARD

The prophylactic use of LEV for control of enterovirus infections was investigated in children's communities in which outbreaks of acute febrile infections had been caused by coxsackieviruses A7, B3, B4, or B5 or by other pathogenic enteroviruses. An inhibition of the pathogenic enteroviruses and rapid interruption of the outbreaks were observed [3, 6].

Specific influenza vaccines are unable to prevent noninfluenza ARD which often accompany influenza outbreaks. In this connection we considered auxiliary means of active prevention of influenza and concomitant

Table 1. Unidentified enteric cytopathic agents isolated at various intervals after introduction of the vaccine strains into a children's community

Preparation	Before vaccination			After introduction of the vaccine strain								
				in the 1st week			in the 2nd week			total		
	total number of specimens tested	among them positive		total number of specimens tested	among them positive		total number of specimens tested	among them positive		total number of specimens tested	among them positive	
		n	%		n	%		n	%		n	%
LEV-4	19	4	21.1	42	3	7.1	77	11	14.0	119	14	11.0
LEV-7	22	8	36.3	33	2	6.0	55	0	0.0	88	2	2.2
Total	41	12	29.3	75	5	6.7	132	11	8.3	207	16	7.7

Table 2. Possible prophylactic effectiveness of LEV and OPV in influenza and acute respiratory diseases

Group	Vaccinated with LEV or OPV			Internal control (no LEV)			Reduction of influenza incidence		Number protected from influenza
	total	developed disease		total	developed disease				
		n	%		n	%	ratio	%	
I	11,789	418	3.55	8,218	486	5.91	1.7	41.2	347
II	40,678	6,305	15.50	18,880	5,456	28.90	1.9	46.4	5,451
III	99,575	5,634	5.71	40,419	7,163	17.71	3.1	67.7	16,504
Total	152,042	12,407	8.16	67,517	13,105	19.41	2.4	57.4	22,302

ARD by use of the nonspecific interfering properties of LEV and OPV. If OPV is used for interference against influenza and ARD, it must be taken into account that previous multiple immunizations against poliomyelitis of all children reduce the chances of subsequent successful implantation of vaccine poliovirus strains. This may be overcome by successively using a number of other LEV strains of different immunologic types.

Widespread controlled trials of the interfering activity and prophylactic effectiveness of OPV types 1–3 and LEV types 4 and 7 during influenza A and ARD epidemics in 1969–1971 gave good results. More than 320,000 subjects were vaccinated. The published reports [3, 11, 12] analyzed the results of LEV trials in 152,000 vaccinees. Over 67,000 subjects comprised the control groups in the communities for purposes of comparison of the incidence levels. No significant side effects or untoward vaccination reactions were detected, confirming the safety and lack of reactogenicity of LEV. Marked reduction of the total incidence of influenza and ARD was achieved by the use of OPV and LEV beginning in the first 2–3 weeks of the epidemic. Only 2 or 3 feedings of these preparations at an interval of 1–2 weeks were usually required.

Table 2 summarizes the apparent epidemiologic effectiveness of prevention of influenza and ARD by means of enterovirus vaccines. Group I includes the data for 1971 (nonepidemic season) with a relatively low incidence of influenza (5.91%) among the nonvaccinated controls. Group II presents the data on the use of OPV types 1 and 3 in 1970 at the Gorky automobile plant. Among 40,678 employees of this plant who received LEV 2 or 3 times, the overall incidence of influenza and ARD was reduced by half when compared to 18,880 persons who had not received the vaccine. As a result, 5,450 people were protected from influenza and ARD at this plant by the use of LEV. Group III summarizes the data for the 1969–1970 epidemic involving other regions (Khabarovsk, Tallinn, Kiev, Balashikha, and the Vnukovo housing estate). Among 99,575 persons vaccinated with LEV or OPV, influenza and ARD incidence was 5.71% as contrasted to 17.71% among 40,419 controls, i.e. 3.1 times lower. The use of LEV and OPV protected 16,504 persons in this group and 22,302 persons in all three groups.

In another study in Kiev [11] LEV preparations given twice at an interval of 10 days to 45,945 subjects produced no febrile reactions and protected 7,856 persons from influenza, an average 3-fold reduction of incidence when compared with that in the unvaccinated group.

According to data from the Clinic of Experimental Pathology and Therapy of the All-Union Influenza Research Institute, LEV-4 tested in 168 adult volunteers gave no complications or side effects as monitored by daily examinations. LEV-4 protected the volunteers against experimental

influenza infection, reducing the number of reactions to influenza virus by 2.6-fold.

These and many other data have demonstrated higher prophylactic effectiveness of LEV during outbreaks of ARD and influenza than that of the specific influenza virus vaccines used in the USSR and led us to propose administration of LEV preparations at the beginning of seasonal outbreaks of influenza and ARD [3, 11, 12].

In the course of widespread controlled trials of LEV the following important advantages of these preparations were established: (i) a broad protective effect involving antigenic variants of influenza virus serotypes A and B and numerous other respiratory viruses; (ii) rapidly developing and sufficiently long protection (from 1 to 2 weeks), permitting use of different types of LEV no more often than at weekly intervals; in contrast, prophylaxis with leukocyte interferon and chemoprophylaxis with amantadine require daily multiple use; (iii) standardization of LEV preparations manufactured in the USSR with a well-developed technology of production and a reliable system of biological control supported by 20 years of large-scale manufacture of live poliovirus vaccine; LEV preparations in inventory may be preserved for at least 10 years and thus represent a strategic reserve as frozen concentrates for mass use; (iv) the possibility of carrying out mass prophylaxis of influenza on any scale in the face of approaching influenza epidemics to achieve a significant (average 2.4-fold) reduction of the total incidence of influenza and ARD; (v) nonreactogenicity and safety of standard LEV preparations for children and adults, and (vi) simplicity and convenience in handling of LEV preparations, especially in the candy form, which can be produced at a low cost per dose.

VI. Therapeutic Use of LEV

In the course of prophylactic use of LEV in a contingent of 320,000 subjects, anecdotal reports of their therapeutic effect in enterovirus and respiratory infections were also reported. The reports included a shortening of the duration of illnesses in some neurologic, gynecologic, dermatologic, and stomatologic diseases with chronic or progressive courses and in gastritis, ulcerative colitis, cystitis, and pancreatitis. Faster healing of wounds, which prior to LEV administration had not healed for a long period, was also reported.

These reports inspired us to organize clinical investigations of the use of LEV for treatment of diseases of viral or obscure etiology that could involve immune deficiency; again, improvements in a variety of diseases were reported by a number of clinicians: A. G. Panov, G. A. Alimov,

A. A. Mikhailenko, V. I. Golovkin, M. M. Shepelev, K. S. Ivanov, S. S. Magazanik, and their colleagues.

For many years we have carried out studies on the oral use of nonpathogenic enteroviruses in cancer patients in Moscow and Leningrad. This work was based on the observations of many virologists who had demonstrated the capacity of enteroviruses to actively multiply in malignant tumor cells and induce their degeneration. This fact naturally suggested that malignant tumor cells could possibly be destroyed in the patients' bodies, i.e. oncolysis might occur in vivo. In collaboration with a number of oncologic institutions in Moscow and elsewhere, our laboratory carried out a number of investigations on the use of LEV in cancer patients. A series of favorable results has been reported in detail [13–16].

Virologic examinations of 504 blood specimens from 295 adult cancer patients showed that the vaccine virus could be isolated within 1–15 days after administration of the vaccine. In examinations of tissues and organs the virus was isolated only from tumors 3–10 days after administration of the vaccine. No virus was isolated from tissues surronding the tumor.

In a series of studies with inoculation of organ cultures of tumors, several nonpathogenic strains of enteroviruses were shown to be capable of multiplication in the cells and of destroying them [3, 17].

Thus, it appears that enteroviruses possess a number of properties that should be investigated further for the control of cancer. They have marked oncolytic properties, stimulate cell-mediated immunity, affect leukopoiesis favorably, have radioprotective properties, and may be used in conjunction with surgical, chemotherapeutic, and radiologic methods of therapy.

VII. Discussion

The results of our observations and investigations proved the existence of a novel phenomenon, namely, nonspecific inhibition of pathogenic viruses by nonpathogenic enteroviruses. The mechanism of the interfering effect of LEV against pathogenic enteroviruses and respiratory viruses in man is not yet clear. In addition to competition for habitat and interfering effect, there appears to be a nonspecific stimulation of several protective systems of the body. LEV also induced oncolysis (destruction of some types of malignant cells), and in some cases protected the leukocyte system from the harmful effect of radioactive irradiation. In most of our studies, interferon was demonstrated in the blood, urine, and nasopharyngeal washings of LEV vaccinees using several conventional methods. High titers of interferon, however, were not always observed. Our work seems to have a general biological as well as medical

importance, opening new prospects for prevention and treatment of a number of diseases.

Because of the existence of a large number of distinct immunologic types of pathogenic enteric and respiratory viruses, successful control of the diseases produced by all of them has proven to be difficult by the development and use of specific vaccines against each causative agent. LEV seem to be a potential means of overcoming the diversity of pathogenic viruses. This is all the more important, because in recent years new diseases caused by enteroviruses have emerged. For instance, in Asia and Africa recent epidemics of acute hemorrhagic conjunctivitis have occurred involving millions of people.

Examination of adult vaccinees established that after administration of LEV the activity of T lymphocytes was increased. Consequently, administration of LEV may stimulate cell-mediated immunity. Our observations support further attempts to use the immunostimulating effect of LEV preparations for correction of the leukocyte system in patients with radiation disease [3] or in other cases of immune deficiency.

The existence of numerous naturally occurring nonpathogenic enteroviruses leads us to suggest that they comprise an ecologically stable community that inhabits the human intestinal tract [4]. The intestinal tract is the organ of the body where interaction between the organism and the environment is probably most intimate; the symbiosis between the macroorganism and the microorganism is especially well developed in enteric bacteria, which posses many properties that are beneficial for the host. We propose that a similar principle governs the relationship between the macroorganism and nonpathogenic enteroviruses and suggest that further understanding of their properties will be of benefit to mankind.

VIII. Summary

Until recently, it has been generally assumed that all human viruses are causative agents of diseases and should be regarded as harmful pathogenic agents that require control measures. In the early 1950s we began to doubt this view. In the course of experiments on virus isolations from feces of normal children, as well as in studies of isolates from animals and from tissue cultures, data accrued which suggested that some conditionally pathogenic and some completely nonpathogenic strains of enteroviruses may provide some benefit to their host by inhibition of pathogenic viruses and by activating nonspecific protective functions of the organism. The novel concept of beneficial viruses was proposed which suggested that the process of co-evolution of the host organism and its associated viral flora led to a specific interaction between them that was beneficial for both. This concept provides a potential approach to the nonspecific prevention of viral diseases by means of the interference between beneficial enteroviruses and pathogenic viruses belonging to different classes.

References

1 Melnick, J. L.: ECHO viruses. Spec. Publ. N.Y. Acad. Sci. *5:* 365–381 (1957).
2 Melnick, J. L.: Enteroviruses: polioviruses, coxsackieviruses, echoviruses, and newer enteroviruses; in Fields, Knipe, Chanock, Melnick, Roizman, Shope (eds): Virology. New York, Raven Press, pp. 739–794 (1985).
3 Voroshilova, M. K.: Enterovirus Infections of Man. Moscow, Medicina, 1979.
4 Voroshilova, M. K.: Evolution of enteroviruses and of diseases they cause. Microbiologica *2:* 179–189 (1979).
5 Chumakov, M. P.; Voroshilova, M. K., et al.: Results of large-scale controlled trials of effectivity of live enterovirus vaccines for urgent prophylaxis of influenza and acute respiratory diseases; in Chumakov (ed): Medical Virology, vol. 21. Moscow, Institute of Poliomyelitis and Virus Encephalitides, pp. 19–28 (1973).
6 Chumakov, M. P., (ed): Medical Virology. Materials of the Institute of Poliomyelitis and Virus Encephalitides. Moscow, 1971.
7 Chumakov, M. P., (ed): Enterovirus Infections. Materials of the Institute of Poliomyelitis and Virus Encephalitides, vol. XIV. Moscow, 1970.
8 Voroshilova, M. K.: Enterovirus infections; in Manual of Microbiology: Clinical Picture and Epidemiology of Infectious Diseases, vol. 8. Moscow, Medicina, pp. 168–186 (1966).
9 Voroshilova, M. K.: Immunology, epidemiology and prophylaxis of poliomyeltis and related diseases. Moscow, Medicina, 1966.
10 Voroshilova, M. K.: In Chumakov, M. P., (ed): Nonpathogenic Strains of Enteroviruses Beneficent for the Organism: Their Prophylactic and Therapeutic Employment. Moscow Institute of Medical Information, 1988.
11 Terekhov, S. N.; Stepanchuk, V. A.: Acute respiratory diseases. Materials of the Institute of Microbiology and Epidemiology, vol. 5. Kiev, pp. 62–66 (1971).
12 Prijmyagi, L. S.; Grinshpoon, L. E.: Use of live enterovirus vaccines for urgent prophylaxis of influenza; in Chumakov (ed): Medical Virology, vol. 21. Moscow, pp. 29–37 (1973).
13 Mutseniyetse, A. Y., (ed): Virotherapy and Artificial Heterogenization of Tumors: Collected Articles. Riga Zinatne, 1977.
14 Mutseniyetse, A. Y., (ed): Viruses in Oncology: Collected Articles. Riga, Zinatne, 1966.
15 Mutseniyetse, A. Y., (ed): Viruses in Therapy of Tumors: Collected Articles. Riga, Zinatne, 1978.
16 Mutseniyetse, A. Y., (ed): Immunological Aspects of Viral Oncotropism: Collected Articles. Riga, Zinatne, 1979.
17 Tsypkin, L. B.; Voroshilova, M. K.; Goryunova, A. G., et al.: The morphology of tumors of the human gastrointestinal tract in short-term organ explant cultures and the reactions of these tumors to infection with poliovirus. Cancer *388:* 179–185 (1976).

A more complete bibliography may be obtained by writing to the Institute for Poliomyelitis and Viral Encephalitides, Academy of Medical Sciences, Moscow 142782 (USSR).

Correspondence to: Institute for Poliomyelitis and Viral Encephalitides, Academy of Medical Science, Moscow 142782 (USSR)