PRIMATOLOGY

Epidemiology of SV-40 Simian Virus in Different Regions of the Russian Federation

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 148, No. 12, pp. 687-689, December, 2009
Original article submitted September 7, 2009

Multiplication of poliomyelitis virus for vaccine production in 1955-1961 was realized in kidney cell culture from *M. rhesus* naturally infected with SV-40 simian virus. Hence, some lots of the vaccine were contaminated with this virus. It was found that SV-40 is oncogenic for laboratory rodents. Since 1963, in accordance with WHO recommendation, green monkey kidneys containing no SV-40 were used instead of *M. rhesus* kidneys. Overall vaccination of the population with poliomyelitis vaccine in 1955-1961 led to infection of many humans in Russia and many foreign countries with SV-40. The possibility of horizontal transmission of the virus was demonstrated. As a result, virus (its DNA sequences) was detected in individuals who were never vaccinated. Hundreds of reports, often contradictory, discuss this problem. Our study is based on the analyses of 460 blood specimens from subjects living in different regions of Russia (Krasnodar region, Moscow, Novosibirsk region, Krasnoyarsk territory). The percent of individuals infected with SV-40 varies from 16 to 49%.

Key Words: *SV-40; vaccination; poliomyelitis; epidemiology*

Due to overall vaccination against poliomyelitis with a highly effective vaccine created in the middle of the 20th century, poliomyelitis as an epidemic disease was liquidated virtually within several years. On the other hand, contamination of the vaccine with SV-40 (simian virus) highly oncogenic for laboratory rodents was revealed.

Both vaccines, D. Salk's killed formol vaccine and A. Sabine "live" vaccine based on attenuated strains, were cultured in kidney cells from *M. rhesus*, often infected with this virus. It was found later that the method of formalin inactivation of the vaccine used by D. Salk did not ensure 100% inactivation of SV-40, and hence, the vaccines were partially contaminated with this virus. A. Sabine "live" vaccine was initially contaminated with SV-40 [3,7].

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During the very first years after creation of poliovaccine in Russia (USSR), more than 70 million people, mainly children and adolescents, were vaccinated, to say nothing about the population of the friendly nearest and distant foreign countries. During the same period, more than 100 million people were immunized in the USA [3].

Apprehensions caused by the fact of poliovaccine contamination with SV-40 gradually resolved, because statistics did not record increased incidence of tumors in vaccinated individuals. The incidence of tumors, characteristic of hamsters infected with SV-40, did not increase in humans. Despite these facts, WHO in 1963 recommended the manufacturers of poliovaccine to replace the *M. rhesus* kidneys with green monkey kidneys containing (according to the then data) no SV-40. The results of analyses of contamination of vaccines prepared at the beginning of 1960 are presented in many reports [3,7,11,12].

0007-4888/09/1486-0924

Hence, liquidation of poliomyelitis as an epidemic disease was associated with unintentional introduction of SV-40 into human population.

The attitude to the problem of human infection by SV-40 largely changed during recent 10-15 years, when the presence of SV-40 DNA sequences in some malignant tumors of humans was demonstrated by PCR. This problem is now discussed in numerous publications, often contradictory. However, we are not going to discuss here possible cause-effect relationship between SV-40 and human tumors, but will speak only about the epidemiology of this virus [3,6,10-12].

Immunological and PCR studies in the USA, UK, Italy, Netherlands, Japan, and other countries demonstrated the presence of antibodies to SV-40 and the SV-40 DNA sequences in the blood of healthy subjects immunized and not immunized with the poliovaccine. The release of SV-40 into the environment with excretions and the possibility of its horizontal transmission were demonstrated [5,12].

Thorough analysis of the Russian publications failed to detect reports about SV-40 infection of healthy subjects vaccinated and not vaccinated against poliomyelitis. It seems there is only one report (ours) in Russia about SV-40 infection of the population [1].

In order to evaluate the rate of SV-40 infection in the population of Russia, we carried out screening for SV-40 DNA sequences of blood specimens from healthy subjects of different age and sex from different regions of Russia.

Since we had no medical passports or other medical documents recording the history of vaccination against poliomyelitis for virtually all the examined individuals, our main checkpoint was their age: people born after 1945 were most likely vaccinated in accordance with the state program of vaccination. Therefore, detection of SV-40 DNA sequences in them was most likely a result of injection of poliovaccine contaminated by this virus. Detection of SV-40 in older subjects, whose "vaccination age" was over before 1955 (the year of poliovaccine creation and beginning of overall vaccination) was regarded as a result of horizontal transmission of the virus.

MATERIALS AND METHODS

Blood specimens from clinically healthy subjects of different age (born in 1945-1998) were received from blood transfusion station of Hematological Center of the Russian Academy of Medical Sciences (50 specimens) and from clinical laboratories engaged in hormonal studies in Novorossiisk and Sochi, Krasnodar region (100 specimens), Krasnoyarsk territory (202 specimens), and Novosibirsk (108 specimens).

Blood for PCR analysis (100 µl) was put into a tube with EDTA and stored at 4°C until DNA extraction. After extraction, DNA specimens were stored at -20°C. Specimens from the Krasnodar region and Novosibirsk were sent in the cold with express mail.

Extraction of DNA was carried out by modified guanidine thiocyanate (GuSCN) protocol: 300 µl 5 M GuSCN, 1% Triton X-100 (v/v), 20 mM EDTA, 50 mM Tris-HCl (pH 6.4), and 10 µl SiO_2 were added to the blood specimen (100 µl), the mixture was incubated for 15 min, centrifuged, and the precipitate was washed in 5 M GuSCN (5 M GuSCN, 50 mM Tris-HCl, pH 6.4) and twice washed in 10 mM Tris-HCl (pH 7.3), 50 mM NaCl, and 50% Eton. The sample was then dried in a solid body thermostat and DNA was extracted with TE buffer (10 mM Tris-HCl (pH 8.0) and 1 mM EDTA); 5 µl sample was used for PCR amplification. The remainder was stored at -20°C.

Detection of SV-40 was carried out by the real time PCR with TaqMan probe directed to the site with 9-nucleotide deletion differing SV-40 from JCV and BKV [9].

The PCR was carried out on a BioRad I Cycler according to the following protocol: 5 min of initial denaturation, 8 sec at 94°C, 23 sec at 60°C, and 30 sec at 72°C, 50 cycles. DNA extracted from *M. rhesus* blood served as the positive control. Water (5 µl) from an open tube, which was placed in a rack with DNA specimens during DNA addition to the plate, served as the negative control.

RESULTS

Results of PCR analysis for the presence of SV-40 in DNA samples from the blood of healthy subjects, residents of different regions of Russia, are presented in Table 1.

The highest incidence (49%; Table 1) was observed in the Krasnodar region. The SV-40 infection rates in Novosibirsk and Krasnoyarsk region were 29.6 and 26.7%, respectively, in Moscow it was 16%.

The differences in the population infection rates in different regions can be caused by various factors, for example, communal, and by different living conditions in regions.

It is assumed that anti-poliomyelitis vaccine, manufactured in Russia (USSR), used for immunization since 1967, was free from SV-40 [2]. On the other hand, a group of scientists from the USA and UK screened the vaccines manufactured in "Eastern European countries" for SV-40 and detected SV-40 sequences in the vaccines manufactured in Eastern Europe before 1969 (it remains unclear from the report in which, specifically, countries the vaccine was manufactured). SV-40 DNA sequences were detected

TABLE 1. Results of Screening for SV-40 by Real Time PCR with DNA Samples Extracted from the Blood of Healthy Subjects from Different Regions of Russia

Region	Number of samples	SV-40-positive	
		abs.	%
Krasnodar region	100	49	49
Moscow	50	8	16
Novosibirsk region	108	32	29.6
Krasnoyarsk region	202	54	26.7

in A. Sabine's strain used for the preparation of the "live" vaccine up to 1980 (until SV-40-free strain was obtained from WHO) [4]. Presumably, the presence of SV-40 DNA sequences in subjects born after 1955 is caused by vaccination or horizontal transmission of the virus.

The problems of SV-40 origin in human population (zoonotic or pre-existing before the start of vaccination by contaminated vaccine) and possible relationships between SV-40 and some tumors in humans will be discussed in further reports.

The authors are grateful to Academicians A. I. Vorobyov and V. A. Trufakin and to V. T. Manchuk, Corresponding Member of the Russian Academy of Medical Sciences, for their assistance in collection of material.

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