ATTEMPT TO DETECT FOWL LEUKEMIA VIRUS IN LIVING VIRUS VACCINES PREPARED IN EGGS

N. P. Leshchinskaya, N. A. Ivanova, and A. A. Smorodintsev

UDC 615.371:576.858.57.07

Investigation of specimens of liquid and lyophilized influenza vaccine and of normal allantoic fluid failed to reveal visceral lymphomatosis virus. No case of development of tumors was recorded in hamsters, albino rats, and mice injected after birth with living lyophilized vaccines against influenza and epidemic parotitis during observations over a period of 2 years.

An urgent yet little studied problem in connection with the specific prophylaxis of virus infections is that of spontaneous contamination of prepared vaccines with oncogenic viruses. This is particularly true of living vaccines obtained from developing chick embryos or chick embryonic tissue cultures. These substrates may become infected by visceral lymphomatosis virus, which is widespread in poultry farms supplying embryos for virological laboratories [1, 2, 10].

In the present investigation an attempt was made to detect fowl leukemia virus in some living vaccines.

EXPERIMENTAL METHOD

The test material consisted of freshly prepared liquid and lyophilized living vaccines against influenza of type A2 + B and against epidemic parotitis (made at the Leningrad Institute of Vaccines and Sera and in the Department of Virology, Institute of Experimental Medicine). In addition several batches of normal allantoic fluid from 30-40 developing embryos were examined.

Suspensions of chick embryonic fibroblasts (CEF) obtained by trypsinization of 9-11-day embryos were divided into several equal parts. The control (untreated) culture was obtained from one part. The remaining suspensions were treated with 5-10% (by volume) of the test vaccine or normal allantoic fluid and then seeded in flasks. Influenza vaccine was investigated without preliminary neutralization of the influenza virus to avoid incidential immunologic inactivation of the contaminant virus which was being sought. Attempts to detect influenza virus in the medium of cultures incubated for long periods, using the hemagglutination reaction((HR) with 1% hen erythrocytes gave negative results in every case. The course of four seedings of treated and control cultures was studied to compare their sensitivity to the transforming action of a standard preparation of Rous virus (Bryan and Shmidt-Rupin strains), by counting foci of transformed cells and determining the logarithm of the number of plaque-forming units (log PFU/ml) for each culture after incubation for 8 days at 40°.

Biological tests of the oncogenic activity of the egg vaccines were carried out by injecting the corresponding preparations into newborn Syrian hamsters, noninbred albino rats, and mice, after which the experimental and control animals remained under observation for long periods. Liquid undiluted vaccines and twice concentrated lyophilized preparations were injected into the animals on the first day after birth, subcutaneously in a volume of 0.1-0.2 ml. The control animals received physiological saline.*

*Pathological and sample histological investigations of the organs of dying and sacrificed animals were conducted by G. I. Il'in (Department of Pathological Anatomy, Institute of Experimental Medicine), and A. M. Dyad'kova (Leningrad Oncologic Institute).

Department of Virology, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 66, No. 8, pp. 89-92, August, 1968. Original article submitted February 13, 1967.

TABLE 1. Sensitivity of Cultures of Chick Embryonic Fibroblasts to Transforming Action of Rous Virus before and after Treatment with Living Influenza Virus or with Normal Allantoic Fluid*

Expt. No.	Ingredient added to culture of chick fibroblasts	Titers (log PFU/ml) of 2 strains of Rous virus in cultures of chick fibroblasts after			
		1 passage of culture		4 passage of culture	
		Bryan	Shmidt-Rupin strain	Bryan	Shmidt-Rupin strain
1	Control	5,4	4,2	5,2	4,1
	Normal allantoic fluid	4,5	5,5	5,4	5,0
2	No. 1				
	Control	4,6	2,1	3,9	2,1
	Normal allantoic fluid	4,4	3,4	4,6	3,1
3	No. 2				
	Control	5,1	2,6	4,9	2,7
	Normal allantoic fluid No. 3	5,2	2,1	5,4	3,1
4	Control	5,6	4,8	5,7	4,6
	Normal allantoic fluid No. 4	6,1	5,2	5,2	4,4
5	Vaccine No. 1 (liquid)	5,5	4,9	5,8	4,1
	Control	2,7	4,8	2,6	5,1
6	Vaccine No. 2				
	liquid	5,0	4,2	5,1	4,7
	dried	5,3	5,5	5,4	5,4
	Control	4,5	3,2	4,2	3,1
	Vaccine No. 3				
	liquid	3,8	4,2	3,7	3,7
	dried	4,2	4,1	3,8	4,4
	Vaccine No. 4				
	liquid	4,5	3,8	4,1	4,1
	dried	3,7	3,0	3,4	3,1

* Pathological and sample histological investigation of organs from dying and sacrificed animals were carried out by G. I. Il'in (Dept. of Pathological Anatomy, Institute of Experimental Medicine) and A. M. Dyad'kova (Leningrad Oncologic Institute).

EXPERIMENTAL RESULTS

Propagation of visceral lymphomatosis virus in naturally or artificially infected CEF cultures observed in the course of 4-6 subcultures is accompanied by the development of resistance to the transforming action of Rous virus. This is shown by a sharp decrease in titer of this indicator virus in infected cultures compared with controls [5-8]. We used this method in an attempt to determine the presence of leukemia virus in the liquid and lyophilized influenza virus and in normal allantoic fluid by induction of resistance to Rous virus by these preparations in CEF cultures highly sensitive to it. Because of absence of embryos known to be free from leukemia virus, we conventionally regarded CEF cultures permitting intensive propagation of Rous virus during 4 subcultures in the control (of the order of 4-6 log) as uninfected.

During the comparison of the transforming activity of two strains of Rous virus in treated and control CEF cultures, no appreciable difference was found between the titers of the same strain in the course of 4 subcultures (Table 1). Often the titers of Rous virus were higher in the treated cultures than in the controls, possibly because of the more favorable conditions for transformation created in such cultures by the additional feeding of the cells with components of the allantoic fluid.

Preparations of living influenza vaccine and of normal allantoic fluid thus did not contain leukemia virus in concentrations capable of inducing partial or complete resistance to the transforming action of Rous virus in highly sensitive CEF cultures. The hypothesis of the absence of antigenic identity between contaminant leukemia virus and the assistant virus (RAV) which is a constituent of indicator strains of Rous virus [3, 4, 9, 10], which is necessary for the detection of specific resistance, is unlikely to be valid, because these

TABLE 2. Results of Inoculation of Newborn Hamsters, Rats, and Mice with Living Lyophilized Vaccines Against Epidemic Parotitis and Influenza (Period of observation: December, 1962 to February, 1965)

Material tested	Animals		No. of animals dying during the 2-year period from			No. of animals surviving 2 years after inoculation (pathological and histological investigations carried out)			
	species	number	cannibalism	infectious diseases	development of tumors	total	with no pathological changes	with pathological changes but no tumors	with tumors
Parotitis vaccine	hamsters	66	21	0	0	45	39	6	0
	rats	57	17	2	1†	37	29	8	0
	mice	54	8	24	0	22	22	0	0
Leningrad Institute of Vaccines and Sera									
Influenza vaccine	hamsters	47	19	0	0	28	25	3	0
	mice	93	5	33	0	45	45	0	0
Leningrad Institute of Vaccines and Sera									
Physiological saline	hamsters	45	14	0	0	31	25	6	0
	rats	35	6	0	0	29	23	6	0
	mice	48	3	28	0	17	17	0	0
Control									

*Fatty degeneration of the liver; scarring of the myocardium; serous cysts in the liver (hamsters) and lungs (rats); helminthiasis of the liver (rats); cystic degeneration of the ovaries and uterus (rats).

†Benign encapsulated fibroma of connective-tissue origin.

strains, when used in preliminary experiments, detected naturally resistant cultures with a frequency of between 1:5 and 1:15. Meanwhile four seedings of the treated cultures must have ensured sufficiently intensive accumulation of latent virus [4, 5].

In the course of observations lasting 2 years on Syrian hamsters, which are highly sensitive to the action of various oncogenic viruses, and also on albino rats and mice, injected in the neonatal state with living parotitis and influenza vaccine, no case of tumor development was recorded among the animals of the experimental and control groups (Table 2). The only exception was the development of a benign encapsulated fibroma in one rat 18 months after inoculation with parotitis vaccine. The long periods of observation were inapplicable to albino mice, of which more than two-thirds died in the first year of life from infections and other nonspecific causes. On visual inspection and sample histological investigation of the organs of the animals sacrificed 2 years after inoculation, no signs of growth of tumors or of diseases of leukemic nature were found. The commonest pathological finding in the hamsters and rats was cystic degeneration of the liver and lungs, observed in both inoculated and control animals.

The results of the search for fowl leukemia virus in the living influenza and epidemic parotitis vaccines suggest that no such virus was present in the preparations examined. However, a final solution to

this important problem must be based on the use of more sensitive methods capable of indicating the presence of leukemia virus directly in the tested vaccines.

LITERATURE CITED

1. R. A. Mukhamedshin, In: Material on Prophylaxis and Treatment of Livestock and Birds [in Russian], Leningrad (1966), p. 268.
2. B. R. Burmester, R. F. Gentry, and W. F. Waters, Poultry Sci., 34, 609 (1955).
3. H. Hanafusa, Virology, 25, 248 (1965).
4. T. Hanafusa, H. Hanafusa, and H. Rubin, Virology, 22, 634 (1964).
5. H. Rubin, Proc. Nat. Acad. Sci. (Wash.), 46, 1105 (1960).
6. H. Rubin, Virology, 13, 200 (1961).
7. H. Rubin, A. Cornelius, and L. Fanshier, Proc. Nat. Acad. Sci. (Wash.), 47, 1058 (1961).
8. H. Rubin, L. Fanshier, A. Cornelius, et al., Virology, 17, 143 (1962).
9. H. Rubin and P. K. Vogt, Virology, 17, 184 (1962).
10. P.K. Vogt, Virology, 25, 237 (1965).