STIMULATION OF ANTIBODY FORMATION AGAINST INFLUENZA VIRUS BY IMMUNIZATION WITH THE USE OF ASCITES TUMOR

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To obtain immune ascites fluids, rats were immunized three times with living influenza virus and vaccinated with an ascites strain of ovarian tumor (OYa). Considerable stimulation of formation of antiviral antibodies was observed compared with animals not vaccinated with the tumor strain. Immunofluorescence tests revealed an increase in titer of antibodies against both structural antigens of the virus and antigens induced by the virus in the membranes of the infected tumor cells. The phenomenon observed is evidently based on the local accumulation of virus antigens on a considerable scale in cells of the strain OYa.

KEY WORDS: influenza immunization; ascites tumor; stimulation of antibody formation.

One approach to obtaining highly active preparations of antibodies against several antigens is by the production of immune ascites fluids [6, 8, 11]. The use of transplantable strains of ascites tumors for this purpose is the most widely adopted method [9]. Most published data indicate that the spectrum of immunological activity of the ascites fluids corresponds completely with the characteristics of immune sera from animals of the same species. These views have been confirmed by experiments with paramyxoviruses, enteroviruses, and so on [1, 4, 8].

Antibody formation after immunization of rats with various strains of influenza virus with the use of ascites tumor was investigated.

EXPERIMENTAL METHOD

Virus-containing allantoic fluids with vaccine strains of A2 (Istra) 10/69 and A2 (Moscow) 16/65 influenza virus were used to immunize rats. The titers of the viruses were 10^9 EID_{50}/ml and 512-1024 A.U./ml.* Rats weighing 180-200 g were immunized by injection of virus-containing allantoic fluid in a dose of 2.5 ml (1 ml intramuscularly and 1.5 ml intraperitoneally, or the total dose of virus intramuscularly). After the first immunization, virus was injected on the 14th and 21st days in the same dose. Blood for obtaining the sera was taken on the 7th day after the last immunization. To obtain immune ascites fluids, rats were injected with 1.5 ml of a suspension of tumor cells of the ascites strain OYa of rat ovarian tumor [3], containing $10^{8.5}$ cells/ml. The ascites fluid was collected on the 7th day after vaccination with the tumor. Blood was taken simultaneously from the same rat to obtain sera. Titer of antivirus antibodies was determined after heating the pooled sera and ascites fluids at 56°C for 30 min in the inhibition of hemagglutination test (IHT) with 4 A.U. of the corresponding strain in a microtitrator of the Takacsi system. In some experiments the neutralization test (NT) in chick embryos and the inhibition of neuroaminidase activity tests were used for the same purpose. The titers of the sera were also determined in the immunofluorescent tests with acetone-fixed preparation of cells containing antigen of A2 influenza virus. For this purpose, either cultures of chick embryonic kidney cells infected with the virus, or cells of

*EID – effective infectious dose; A.U. – antigenic units.

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TABLE 1. Stimulation of Antibody Formation on Immunization of Rats with Influenza Virus and Vaccination with Ascites Tumor (results of IHT and NT)

Strain of influenza virus	Scheme of injection of virus*	Titers of antivirus antibodies					
		without injec. of virus		with injection of ascites tumor strain OYa			
		serum		serum		ascites fluid	
		IHT	NT	IHT	NT	IHT	NT
A2 (Istra) 10/69	I	32	32	N.t.†	N.t.	4 096	1024
	II (expt. 1)	256	128	16 384	8 192	32 768	8192
	II (expt. 2)	512	512	8 192	5 026	8 192	4074
A2 (Moscow) 16/65	I	64	128	N.t.	N.t.	4 096	1024
	II (expt. 1)	512	512	N.t.	N.t.	16 384	8192
	II (expt. 2)	2048	1024	7 960	N.t.	11 408	8192

*I) Intramuscular injection of virus; II) intramuscular and intraperitoneal injection of virus.
† N.t.) Not tested.

TABLE 2. Results of Immunofluorescence Test with Intracellular Antigens of Influenza Virus

Method of immunization	Preparation of antibodies	Cells with antigen used in reaction*	Antibody titer in immunofluorescence test†	
			indirect method	direct method (extinction test)
Without injection of tumor	Serum	CEK	8	N.t.
		OYa	16	8
With injection of ascites tumor strain OYa	Serum	CEK	256	64
		OYa	512	128
	Ascites fluid	CEK	256	64
		OYa	1024	512

*CEK – cultures of chick embryonic kidney cells infected with virus 48 h before fixation; OYa – squash films of OYa tumor cells infected with virus in vivo 48 h before fixation.
† Highest twofold dilutions giving appearance (extinction) of antigens in 50% of cells.

ascites strain OYa infected with virus in vivo (see below), were used as the cell substrate. To detect fluorescence by Coons' indirect method, the 7S fraction of rabbit antirat globulin, labeled with fluorescein isothiocyanate (NBCo., USA) was used. Gamma-globulin fractions of the resulting ascites fluid were labeled with the same dye for use in the direct immunofluorescence tests. To verify the specificity of the last method, the method of "extinction" of fluorescence on treatment of the cells with the corresponding unlabeled serum was used. Surface antigens were detected in the same target cells by the method described by Lezhneva [2].

EXPERIMENTAL RESULTS

The results of the comparative study of antibody titers in the different experimental groups are given in Table 1. In these experiments by both schemes of injection of the virus mentioned above, vaccination with the ascites tumor strain was carried out 24 h after the last injection of virus. It will be clear from Table 1 that antibody titers in rats receiving ascites tumor cells were much higher than antibody titers obtained by the same scheme of immunization but without injection of the tumor. Under these circumstances the antibody titers in the blood serum and ascites fluid from rats vaccinated with strain OYa were

TABLE 3. Detection of Surface Antigens by Immunofluorescence Method in Cells Injected with Influenza Virus

Preparation of antibodies	Target cells*	Index of fluorescence† with dilutions of antibody preparation shown below						
		1:4	1:8	1:16	1:32	1:64	1:128	1:256
Serum of rats immunized without injection of tumor	OYa	0,18	0,13	0,07	0,01	0	0	0
	OYa + virus	0,30	0,23	0,09	0,07	0	0	0
Serum of rats immunized with injection of tumor OYa	OYa	0,20	0,15	0,10	0,05	0,03	0	0
	OYa + virus	0,90	0,83	0,77	0,70	0,56	0,23	0,07
Ascites fluid of immunized rats	OYa	0,23	0,18	0,12	0,07	0	0	0
	OYa + virus	0,95	0,95	0,89	0,85	0,69	0,35	0,14

* The same as in Table 2.

† An index above 0.20 is taken as positive.

virtually identical. This phenomenon was characteristic of both strains of A2 influenza virus studied and was repeated regularly in many experiments. Increased titers of antibodies were found not only in the IHT, but also in the NT and the inhibition of neuroaminidase activity test.

To study the ability of antibodies contained in ascites fluids to localize intracellular virus antigens, optimal cell systems were first chosen. Antibodies contained in the sera and ascites fluids revealed both intranuclear and cytoplasmic virus antigens in infected cells of chick embryonic kidney cultures. The results of titration of the antibodies in the immunofluorescent test are given in Table 2. Virus antigens were also detected in the cytoplasm of strain OYa cells after infection of the tumor with the virus in vivo. In these experiments 2 ml of virus-containing allantoic fluid was injected intraperitoneally into rats with tumors 24-48 h before removal of the tumor cells. The results showed that large quantities of virus antigens accumulated in the cells of strain OYa; by the 48th hour of the experiment they were present in 80% of the tumor cells. This model proved to be the most stable source of standard preparations of the antigen for the immunofluorescence test, in agreement with observations by other workers [5]. The results of parallel titration of antibodies in the two cell systems specified are given in Table 2. Just as in the previous experiment, higher antibody titers were demonstrated after immunization with influenza virus with ascites tumor. The immunofluorescent test undoubtedly pointed to stimulation of the formation of antibodies against structural antigens of influenza viruses.

When the immunofluorescence test was set up with suspensions of living infected and control cells of strain OYa, reliable indices of fluorescence were obtained only by the use of ascites fluids and sera from rats receiving an injection of strain OYa cells in the last stage of immunization (Table 3). Antibodies against surface antigens, appearing in cells infected with influenza virus, were thus formed only when this scheme of immunization was used. Positive membrane fluorescence could be caused by interaction of the antibodies both with structural virus antigens and with virus-induced surface antigen [7]. This problem requires further study.

To obtain antivirus immune ascites fluids, the possibility of inhibiting tumor development under the influence of the virus if administered to animals in an infectious form must be taken into account [10]. Both the strains of influenza virus used in this investigation possessed this type of oncolytic action. Accordingly, to obtain the normal quantity of immune ascites fluid, the cells of strain OYa must be injected not earlier than 24 h after the last injection of virus.

Hence, during the preparation of immune ascites fluids against the vaccine strains of A2 influenza virus used in this investigation, the formation of antivirus antibodies was stimulated. This result differs from those obtained by the use of similar immunization schemes in experiments with other viruses. The phenomenon observed is evidently based on interaction between influenza virus and tumor cells in rats leading to the accumulation of large quantities of virus antigen in cells of strain OYa and to the subsequent more intensive antibody formation.

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