CHANGES IN PHAGOCYTIC ACTIVITY OF PERITONEAL EXUDATE CELLS OF MICE IMMUNIZED WITH INFLUENZA VIRUS

G. I. Il'in, A. A. Kyazimova,
L. V. Solilova, and A. A. Smorodintsev

UDC 615.371:576.858.75].
015.4:612.339:612.112.3

Influenza virus inhibits phagocytosis of cell fragments by peritoneal macrophages in mice without having any effect on phagocytic activity of neutrophils under these same conditions. The development of immunity against influenza is characterized not only by an increase in the titer of specific antibodies and in the intensity of phagocytosis with respect to cell fragments infected with homologous virus, but also by the development of resistance to influenza virus among peritoneal macrophages.

* * *

In severe forms of influenza in animals and man, there is intensive desquamation of infected cells of the upper respiratory tract, which readily undergo phagocytosis.

The object of this investigation was to study the phagocytic activity of peritoneal exudate cells of normal immune mice against cell fragments infected with influenza virus.

EXPERIMENTAL METHOD

Allantoic fluid of chick embryos infected with type A (3711) influenza virus was used to immunize mice which received 5 intraperitoneal injections, each of 1 ml at intervals of 3 days. Experiments were carried out on mice with an antihemagglutinin titer of 1:320 or higher. Allantoic fluid of uninfected chick embryos was used as the control.

Cells of the chorioallantoic membrane of chick embryos, either uninfected or infected with influenza virus, served as the object of phagocytosis. Minced chorioallantoic membranes were treated with 0.25% trypsin solution on a magnetic mixer. The cells were then sedimented by centrifugation (10 min, 1000 rpm) and treated with 0.3% acetic acid solution to produce lysis of contaminant erythrocytes. After removal of the acid, the cells were shaken for 3 min with beads in order to obtain fragments more amenable to phagocytosis. Experiments in vivo and in vitro were carried out by the method described previously [2]. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The results of the experiment in vivo (Table 1) show that influenza virus present in the fragments of cells of the chorioallantoic membrane can inhibit their phagocytosis by peritoneal macrophages of mice. At the same time, cell fragments of the chorioallantaoic membrane, whether uninfected or infected with influenza virus, were phagocytosed with equal activity by the neutrophils of intact mice. Immunization of the mice completely abolished the unfavorable effect of influenza virus on the phagocytic activity of the macrophages. As a result, the phagocytic indices of these cells for animals immune to cells of the chorioallantoic membrane infected with influenza virus were very close to (percentage of cells carrying out phagotytosis)

All-Union Research Institute of Influenza, Ministry of Health of the USSR. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 68, No. 12, pp. 59-62, December, 1969. Original article submitted June 7, 1969.

TABLE 1. Phagocytic Activity of Peritoneal Exudate Cells of Mice Immunized with Influenza Virus

Experimental conditions	Object of phagocytosis (cell fragments)	Mice from which phagocytic cells were taken	Addition of specific immune serum	Percent carrying out phagocytosis				Mean intensity of luminescence (in mV)			
				of macrophages		of neutrophils		of macrophages		of neutrophils	
				$M \pm m$	P	$M \pm m$	P	$M \pm m$	P	$M \pm m$	P
In vivo	Uninfected chorioallantoic membrane	Intact		19±1		15±1,3		79±5		17±3	
		Immunized:									
		with allantoic fluid		18±1	>0,05	13±17	>0,05	85±8	>0,05	21±7	>0,05
		with influenza virus		16±1,1	>0,05	13±1	>0,05	98±2	>0,05	17±2	>0,05
	Infected with influenza virus	Intact		10±0,7	<0,05	12±0,8	>0,05	34±2	<0,05	20±2	>0,05
		Immunized:									
		with allantoic fluid		11±1,1	<0,05	9±1	>0,05	40±6	<0,05	21±3	>0,05
		with influenza virus		23±1,5	>0,05	13±0,8	>0,05	195±20	<0,05	19±1	>0,05
In vitro	Uninfected chorioallantoic membrane	Intact	—	10±2		16±2		81±16		13±2	
		Immune[a]	—	15±1	>0,05	13±1	>0,05	90±24	>0,05	14±3	>0,05
	Infected with influenza virus	Intact	—	3±0,5	<0,05	12±1	>0,05	27±7	<0,05	15±3	>0,05
		Immune	—	12±1	>0,05	14±1	>0,05	99±13	>0,05	13±2	>0,05
		Intact	+	15±2	>0,05	11±2	>0,05	62±6	>0,05	19±2	>0,05
		Immune	+	35±2	<0,05	12±3	>0,05	158±19	<0,05	22±0	>0,5

Legend: + serum present; - serum absent.

or even higher (than mean intensity of fluorescence) the phagocytic indices of phagocytosis of uninfected chrorioallantoic cells by macrophages of intact mice. Immunization with allantoic fluid alone did not change the behavior of the macrophages relative to cells of the chorioallantoic membrane infected with influenza virus.

Further investigations in vitro showed that the phagocytic activity of peritoneal exudate cells of mice immunized with influenza virus against cells of the chorioallantoic membrane infected with homologous virus coincided with the intensity of phagocytosis of uninfected chorioallantoic membrane cells by these same cells obtained from intact and immune animals. However, ingestion of cells of the chorioallantoic membrane infected with influenza virus by phagocytes of intact mice was much less marked. Addition of specific immune serum to the test system completely abolished the inhibition of phagocytosis of infected chorioallantoic membrane cells by peritoneal cells of intact mice and stimulated it by cells of immune animals.

Previous investigations with the viruses of vesicular stomatitis and Newcastle disease showed that immunization increases the intensity of phagocytosis of cell fragments infected with homologous virus, the process being strictly specific in character [2]. The present investigation revealed yet another peculiarity of the interaction between cell fragments containing influenza virus and peritoneal phagocytes of mice. It was found that influenza virus can inhibit phagocytosis not only of bacteria [1, 3], but also of cells and their fragments infected with viruses. The inhibitory action of influenza virus extended only to macrophages. The phagocytic activity of the neutrophils was unchanged in the presence of influenza virus.

Macrophages of mice immune to influenza exhibited high resistance to the unfavorable action of homologous virus. The percentage of macrophages of immune mice carrying out phagocytosis of infected cell fragments of the chorioallantoic membrane coincided with that of macrophages which carried out phagocytosis of uninfected chorioallantoic membrane cells of intact and immune mice. The mean intensity of luminescence of each phagocyte during specific phagocytosis of chorioallantoic membrane cells infected with influenza virus was much higher in macrophages of immune mice. Changes in the phagocytic activity of macrophages after immunization with influenza virus were specific in character, because injection of allantoic fluid alone had no effect on reactivity.

This feature of phagocytosis revealed by these experiments could be due either to the development of specific resistance in peritoneal cells to influenza virus under the influence of immunization or to the protective action of specific antibodies.

In experiments in vitro the peritoneal phagocytes of immune mice remained resistant to the inhibitory effect of influenza virus even after repeated washing to remove antibodies. This can be explained either by the strength of binding of antibodies by the phagocytes or by acquired resistance of the macrophages unconnected with antibodies. This latter mechanism may possibly arise during immunization of animals with active virus as the result of the preferential selection of a particular group of macrophages more resistant to the harmful toxic action of the injected virus. The mechanism of the resistance of macrophages thus brought to light is a matter for special study.

Preliminary treatment of cell fragments of the chorioallantoic membrane infected with influenza virus with specific immune serum completely abolished the inhibitory action of influenza virus on phagocytosis by macrophages of intact mice and increased its intensity in immune animals.

It may be considered that the specific increase in phagocytic activity in the immune organism can play an important role in recovery from influenza, by promoting the more rapid removal of epithelial cells of the upper and lower respiratory tracts when damaged or killed by virus. Isolation of the infected cells and their rendering harmless by phagocytosis may prevent their excretion into the external environment and thus reduce the possibility of the spread of infection.

LITERATURE CITED

1. A. D. Ado, The Pathophysiology of Phagocytes [in Russian], Moscow (1961).
2. A. A. Kyazimova, G. I. Il'in, L. V. Solilova, et al., Vopr. Virusol., No. 2, 189 (1969).
3. M. Kantoch, Zh. Gig. Épidemiol. (Prague), 6, 196 (1962).