ROLE OF MACROPHAGES AND NEUTROPHILS OF IMMUNE MICE IN PHAGOCYTOSIS OF VIRUS-INFECTED CELLS

A. A. Kyazimova, G. I. Il'in,
L. V. Solilova, T. I. Fomina,
and A. A. Smorodintsev

UDC 612.112.3-06:612.017.12

Macrophages of the peritoneal exudate of immune mice posses increased phagocytic activity against virus-infected cell particles. The reaction is characterized by strict specificity.

* * *

Recently during the study of virus infections much attention has been paid to the macrophages. A close connection has been found between the susceptibility of animals and that of their macrophages to certain viruses, which is transmitted as an inherited characteristic [5, 6]. The view has been expressed that the character of interaction between macrophages and viruses may even determined the outcome of an infectious disease [8].

The important role of macrophages in the absorption of incompatible tissues and tumors has also been demonstrated [4, 7].

It may be assumed that macrophages play an equally active role in the phagocytosis of rejected cells and their fragments, infected with viruses. The present investigation was carried out to examine the problem.

EXPERIMENTAL METHOD

Vesicular stomatitis virus (VSV) was used as the culture fluid of an infected primary culture of chick fibroblasts. Newcastle disease virus (NDV) was used as the allantoic fluid of infected 10-day chick embryos fed from hen tissue components. For this purpose the virus was adsorbed on formalin-treated hen erythrocytes, which were washed with cold physiological saline, and the virus was then eluted in 5% sodium chloride solution (1-10th part of the original volume of virus).

The mice were immunized by intraperitoneal injection: three times with VSV virus, 9 times with NDV virus (at intervals of 3 days). The titer of virus-neutralizing antibodies in the blood of the experimental immune animals was 1 : 1024 or more.

Particles of check fibroblasts infected with VSV and NDV viruses were used as the object of phagocytosis. Cell particles freed from culture fluid were stained for 18 h with fluorochrome (fluorescein isothiocyanate), washed carefully to remove dye, and then suspended in bovine serum in a concentration of 12 million cells/ml.

An intraperitoneal injection of 6 million cell particles, infected with virus, was given to the mice 24 h after intraperitoneal injection of 3 ml of 2% starch solution. One hour later, at the height of development of the phagocytic reaction, the animals were killed and the exudate aspirated. The percentage of peritoneal cells engaged in phagocytosis was calculated for 200 cells examined in a moist chamber under the ML-4 luminescence microscope. The mean intensity of luminescence of each cell in mV was determined for 50 luminescent cells by means of a special attachment on the ML-4 microscope. The results obtained were subjected to statistical analysis by Strelkov's method [3].

The total number of cells in the peritoneal exudate was counted in a Goryaev's chamber and their composition was determined in films fixed in methanol and stained by the Romanovsky – Giemsa method.

All-Union Research Institute of Influenza, Ministry of Health of the USSR, Leningrad. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 68, No. 9, pp. 70-72, September, 1969. Original article submitted April 1, 1968.

TABLE 1. Mean Number of Cells and Relative Percentages of Macrophages and Neutrophils in Peritoneal Exudate of Mice 1 h after Injection of Hen Fibroblasts

Group of mice	Contents of phagocytes in peritoneal exudate after injection of hen fibroblasts								
	uninfected			infected with VSV			infected with NDV		
	mean number of cells in group (in millions)	%		mean number of cells in group (in millions)	%		mean number of cells in group (in millions)	%	
		content of macrophages	content of neutrophils		content of macrophages	content of neutrophils		content of macrophages	content of neutrophils
Immunized with BBC	76	44	46	66	25	46	50	39	48
Immunized with NDV	57	31	40	58	39	43	60	37	43
Control	70	28	47	66	30	40	75	34	49

TABLE 2. Activity of Phagocytic Macrophages and Neutrophils of Peritoneal Exudate from Mice

Object of phagocytosis (cell particles)	Group of mice	Percentage of cells engaged in phagocytosis				Mean intensity of luminescence of phagocyte (in mV)			
		macrophages		neutrophils		macrophages		neutrophils	
		$M \pm m$	P	$M \pm m$	P	$M \pm m$	P	$M \pm m$	P
Hen fibroblasts	Control	10±2		21±1		40±5		20±4	
	Immunized with VSV	17±0,8	>0,05	18±2	>0,05	33±4	>,005	20±4	>0,05
	Immunized with NDV	15±2	>0,05	21±1	>0,05	29±3	>0,05	10±8	>0,05
Hen fibroblasts infected with VSV	Control	9±2,5		21±5		29±6		15±4	
	Immunized with VSV	21±0,3	<0,05	36±4	>0,05	83±8	<0,05	27±8	>0,05
	Immunized with NDV	17±2	>0,05	18±0,8	>0,05	45±4	>0,05	11±3	>0,05
Hen fibroblasts infected with NDV	Control	17±2		15±2		33±5		15±2	
	Immunized with VSV	15±6	>0,05	16±3	>0,05	38±2	>0,05	12±1	>0,05
	Immunized with NDV	46±6	<0,05	16±5	>0,05	171±27	<0,05	19±4	>0,05

EXPERIMENTAL RESULTS

A series of preliminary experiments gave the total number of phagocytic cells present in the peritoneal exudate and the percentage content of macrophages and neutrophils in the exudate from mice receiving starch. One hour after intraperitoneal injection of uninfected particles of fibroblasts and fibroblasts and fibroblasts infected with VSV and NDV, the cell reaction of the control mice and the mice immunized with the two viruses was indistinguishable both quantitatively and qualitatively (Table 1).

A comparative study of the phagocytic exudate of the mice showed that particles of hen fibroblasts, whether uninfected or infected with VSV and NDV, can be ingested by macrophages and neutrophils. However, increased phagocytosis was visible only by macrophages of the immunized mice in respect to particles of hen fibroblasts infected with homologous virus (Table 2).

In connection with the inertia of the phagocytes as regards the ingestion of free virus particles in the presence of specific antibodies, the question of possible changes in the intensity of phagocytosis relative to cells and cell particles infected by different viruses must be considered [1, 2, 9]. To investigate this problem, interaction between phagocytic factors and cells was studied in immunized animals using as a model the phagocytic exudate formed in the peritoneal cavity of mice after injection of starch. Particles of hen fibroblasts infected with virus, homologous or heterologous relative to the immunity produced, were injected into mice preliminarily immunized against VSV and NDV.

The investigations described above demonstrated a marked increase in the intensity of ingestion of the injected cells by macrophages of the peritoneal exudate, if a specific relationship existed between the immunity of the mice and the virus present in the hen fibroblast particles. The results obtained provide evidence of an increase in the phagocytic activity of macrophages relative to cell particles infected with viruses if the antiviral immunity is specific for the virus infecting the cell. This indicates that phagocytic factors play a role in antiviral immunity, when the object of phagocytosis is represented by large fragments of infected cells or the cells themselves.

These facts may help to explain the mechanism of interaction between immunity to an oncogenic virus and suppression of activity of the cancer cells containing specific antigen.

LITERATURE CITED

1. L. A. Zil'ber, A. D. Sheboldaeva, and A. K. Shubladze, Zh. Mikrobiol., 18, No. 4, 516 (1937).
2. A. A. Smorodintsev and O. I. Shishkina, Arkh. Biol. Nauk, 59, No. 1-2, 20 (1940).
3. R. B. Strelkov, A Method of Calculation of the Standard Error and Confidence Limits of Arithmetic Means of a Table [in Russian], Sukhumi (1966).
4. R. S. Baker, J. J. Weiser, C. Evans, et al., Ann. N.Y. Acad. Sci., 101, 46 (1962).
5. F. B. Band and A. Warwick, Proc. Nat. Acad. Sci. (Washington), 46, 1065 (1960).
6. G. T. Goodman and H. Koprowski, Proc. Nat. Acad. Sic. (Washington), 48, 160 (1962).
7. P. A. Gorer, Advances Cancer Res., 4, 149 (1956).
8. C. A. Mims, Bact. Rev., 28, 30 (1964).
9. A. B. Sabin, Brit. J. Exp. Path., 16, 158 (1935).