PARTICIPATION OF PHAGOCYTIC FACTORS IN NATURAL AND ACQUIRED IMMUNITY AGAINST THE AGENT OF MENINGOPNEUMONIA IN MICE

A. A. Kyazimova, G. I. Il'in, and A. A. Smorodintsev

UDC 616.988-097-07:616.155.3-008.13-072.7

Phagocytic cells play an active role in protecting the body against the agent of meningopneumonia. In naturally resistant animals (rats), the principal role in this process is played by the neutrophils. In acquired immunity (mice), the infectious agent is destroyed by neutrophils and macrophages.

* * *

Some interesting relationships have been discovered between phagocytic cells and infectious agents of the psittacosis–lymphogranuloma group, occupying an intermediate position between viruses and bacteria. Using meningopneumonia on mice as experimental model, the phagocytic activity of neutrophils and their participation in the rapid destruction of the agent in immune animals, in contrast to macrophages, which are sensitive to this agent in normal mice but resistant to it in immune animals have been established [1].

In this investigation the role of leukocytes and macrophages in the protection of animals with acquired (mice) and natural (rats) immunity against the agent of meninogopneumonia was studied.

EXPERIMENTAL METHOD

An influx of neutrophils into the peritoneal cavity was produced by intraperitoneal injection of mice with 3 ml, or rats with 5 ml, of Hottinger's broth. The percentage of neutrophils in normal mice 24 h after injection of broth was 41, compared with 53 in immunized mice and 63 in rats, while the corresponding figures for macrophages were 26, 19, and 23%. Peritoneal exudate rich in macrophages was obtained by intraperitoneal injection of 2% starch solution in the same volumes. After 24 h the percentage of macrophages was 57 in normal mice, 67 in immunized mice, and 32 in rats, while the corresponding figures for neutrophils were 19, 11, and 56%. Films of the exudate were stained by the Romanovsky–Giemsa method.

To prepare a culture of macrophages, 24 h after injection of starch, the peritoneal cavity of the rats was washed out with 10 ml medium No. 199 with 20% bovine serum and 0.2 unit/ml heparin, and the washings were poured in volumes of 1 ml into test tubes. The medium was changed after 30 min for one of the same composition but without heparin, and cultivation continued at 37°.

The agent of meningopneumonia (MP), obtained in 1956 from the Serum Institute in Copenhagen, was used as the supernatant obtained after centrifugation of a 10% suspension of mouse lungs.

The material in which the content of MP agent was determined was titrated in a transplantable culture of β-cells. Intracellular MP agent was titrated after disintegration of the cells by agitation for 20 min with beads in a shaker.

Immunity against the MP agent was produced in mice by triple subcutaneous injection of 0.2 ml of MP agent containing 0.01 LD_{50} for mice at 10-day intervals. One month after the end of immunization the mice were resistant to 1700 LD_{50} of MP agent.

All-Union Influenza Research Institute, Ministry of Health of the USSR, Leningrad. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 67, No. 3, pp. 64-66, March, 1969. Original article submitted December 26, 1967.

TABLE 1. Fate of MP Agent in Peritoneal Cells of Mice and Rats after Injection of Hottinger's Broth and Starch

Time after infection (in h)	Content of MP agent (log TCD_{50}/0.2 ml) in cells of peritoneal exudate after injection of				
	broth			starch	
	in normal mice	in immunized mice	in rats	in normal mice	in immunized mice
1	3.66	3.5	3.66	3.00	3.66
3	3.00	3.33	2.66	3.5	3.33
5	2.66	2.66	< 1.00	4.66	3.00
24	4.33	0	0	5.00	< 1.00
48	4.66	0	0	5.66	0

Legend to Tables 1 and 2: < 1.0 indicates that MP agent was found only in undiluted material.

TABLE 2. Comparative Study in Vitro of Disappearance of MP Agent in Suspension of Peritoneal Cells of Mice and Rats and Also in a Culture of Rat Macrophages

Time after addition of MP agent (in h)	Content of MP agent (log TCD_{50}/0.2 ml)				
	in suspension of peritoneal cells after injection of broth			in culture of rat macrophages	in medium No. 199 at 37°
	in normal mice	in immunized mice	in rats		
1	4.5	4.66	4.66	4.66	4.5
3	4.39	4.5	3.00	4.00	4.33
5	4.00	4.00	2.66	3.66	4.00
24	2.66	2.00	0	2.33	2.5
48	< 1.00	< 1.00	0	< 1.00	< 1.0
72	0	0	0	0	0

EXPERIMENTAL RESULTS

The fate of MP agent was studied in mice and rats with predominance of neutrophils in their peritoneal exudate. The animals were injected intraperitoneally with MP agent 24 h after injection of broth. Mice were infected with 1 ml and rats with 5 ml of MP agent containing 5 log TCD_{50}/0.2 ml. The agent was then determined in the cells of the peritoneal washings. At each period of the experiment 5 mice and 3 rats were taken in order to obtain a pooled exudate.

The experimental results showed that relationships between the MP agent and the cells appearing in the peritoneal cavity after injection of broth depend on the level of the animal's immunity. Whereas in normal mice a slight accumulation of MP agent took place up to a limit of 2 log units, in the immunized mice the agent disappeared after 24 h. It died particularly rapidly in the peritoneal cells of the rats.

The fate of MP agent in cells of the peritoneal exudate after injection of starch was studied only in mice, because rats gave an identical cell response to both stimuli. The periotoneal cells of mice were found to acquire the ability, after immunization, to inhibit proliferation of the MP agent which takes place in normal mice (Table 1).

The inhibitory action of peritoneal cells of mice and rats to MP agent was next studied in vitro. To tubes containing 10 million peritoneal cells obtained from mice and rats 24 h after injection of broth in 1 ml medium No. 199, 5 log TCD_{50}/ml of MP agent was added in a volume of 0.2 ml, and placed in a revolving drum at 37°. Tubes containing 1 ml medium No. 199, infected with the same doses of MP agent as the experimental tubes, were used as controls.

In the experiments carried out in vitro, only the peritoneal cells of the rats had an inhibitory action on MP agent. The dynamics of its disappearance when mixed with mouse peritoneal cells coincided with death of the agent in the control tubes.

To study the dynamics of disappearance of the MP agent in a culture of rat macrophages, the growth medium in the culture was replaced by medium No. 199 and infection with MP agent was then carried out in a dose of 5 log TCD_{50}/0.2 ml. After incubation for 1 h the medium was removed, the cell layer was washed 3 times, and 1 ml fresh medium was added. After incubation for different times the content of MP agent in the cells was determined. MP agent kept at 37° in the same medium acted as control.

These experiments showed that disappearance of MP agent in a culture of rat macrophages was identical with its disappearance in medium No. 199 under similar conditions (Table 2).

The investigation showed that phagocytic cells participate in protecting animals against MP agent. Peritoneal cells of rats were particularly active in experiments both in vitro and in vivo. Since neutrophils accounted for more than half of all the cells in the peritoneal exudate of rats after injection of both stimuli (broth and starch), and macrophages had no inhibitory action on MP agent, it can be concluded that the neutrophils of rats are one of the factors of cellular protection of these animals against MP agent.

More complex relationships in mice with the MP agent were found after intraperitoneal infection. In the case of predominance of macrophages in the peritoneal exudate, MP agent multiplied in normal mice, which was not observed in the immunized animals. Experiments on mice in which neutrophils predominated revealed the more rapid death of MP agent in immunized animals than was observed in immunized mice with mobilized macrophages. In the control group of mice, with a neutrophil content in their peritoneal exudate of 41%, some decrease in the titer of MP agent was observed in the first few hours after infection, but this was followed by proliferation of the agent, evidently on account of macrophages present in the exudate and sensitive to this agent.

LITERATURE CITED

1. A. I. Drobyshevskaya, V. E. Pigarevskii, and A. A. Smorodintsev, Acta Virol., 6, 458 (1962).