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RESPONSE OF RAT LUNG MACROPHAGES TO ZYMOSAN STIMULATION

L. N. Shishkin and D. N. Mayanskii

UDC 616.24-008.953.3-02:615.276.4]-092.0

KEY WORDS: lung, lung macrophages, zymosan, colloidal carbon.

The course of an inflammatory process in the lung and realization of the mechanisms of defense of the lung tissue against injury are largely determined by reactivity of the lung macrophages. Not only their ability to ingest, kill, and digest microorganisms, but also the formation of a series of bactericidal principles in activated forms of macrophages, namely H_2O_2, the superoxide anion O_2^-, and singlet oxygen $^1O_2$ [4], is important under these circumstances. Secretory products of lung macrophages, on the one hand, modify the resistance of the host to infection (lysozyme, components C_2 and C_4 of complement, interferon, etc.), and on the other hand, they degrade components of the connective-tissue matrix of the lung: elastase, collagenase, enzymes catabolizing proteo- and glycosaminoglycans [1, 8]. In an inflammatory process lung macrophages cooperate with lymphocytes and fibroblasts [5]. Secretion not only of phlogogenic agents, but also of mediators of intercellular interactions, depends on reactivity of the macrophages. Accordingly it is important to study the response of the pulmonary component of the mononuclear phagocyte system (MPS) to the entry of agents activating macrophages and inducing inflammatory processes into the body. The yeast of polysaccharide zymosan is considered to be a stimulator of macrophages. If injected *in vivo* it causes a series of characteristic macrophage-dependent changes in the liver and lungs [3, 7].

The aim of the present investigation was to make a differential count of cells from broncho-alveolar washings and of loaded activated macrophages from the interstices of the lung, and to assess the general ingestive function of MPS after a single stimulation by zymosan.

EXPERIMENTAL METHOD

Experiments were carried out on 68 male Wistar rats weighing 200-250 g. The experimental animals were given a single intravenous injection of zymosan suspension in a dose of 0.1 mg/g body weight in 1 ml of 0.85% NaCl solution, and the control animals received an injection of 1 ml of 0.85% NaCl solution only. The animals were decapitated 2, 5, 7, 9 and 14 days after injection of zymosan, with simultaneous compression of the trachea. The lungs were weighed and immersed in physiological saline. The air remaining in the lungs was pumped out through a cannula introduced into the trachea, after which medium 199 with heparin (5 U/ml) was injected in a dose of 5 ml/g weight of the lungs at the rate of 0.2 ml/sec. After 30 sec

Laboratory of Pathophysiology, Department of General Pathology, Institute of Clinical and Experimental Medicine, Siberian Branch, Academy of Medical Sciences of the USSR, Novosibirsk. (Presented by Academician of the Academy of Medical Sciences of the USSR V. P. Kaznacheev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 10, pp. 454-457, October, 1985. Original article submitted November 16, 1984.

 0007-4888/85/0010-1386$09.50

TABLE 1. Number of Cells of Cell Pool Washed out of Lungs 2, 5, and 14 Days after Stimulation of MPS by Zymosan (M ± m)

Parameter	Time of investigation, days	Experimental conditions	Free alveolar cells	Monocyte-macrophages	Lymphocytes	Neutrophilis	Eosinophils
Number of cells ($\times 10^6$) per gram of lung tissue	2	Experiment (n = 4)	0,50±0,16	3,27±0,40	0,22±0,007*	0,20±0,12*	0,42±0,14*
		Control (n=4)	0,58±0,09	2,76±0,09	0,09±0,02	0,01±0,003	0,07±0,03
	5	Experiment (n = 5)	0,21±0,07	6,94±1,20*	0,14±0,12	0,10±0,06*	0,94±0,38*
		Control (n=6)	0,42±0,10	3,41±0,45	0,36±0,09	0,01±0,003	0,05±0,03
	14	Experiment (n = 5)	0,24±0,05	2,11±0,34	0,25±0,13	0,01±0,003	0,10±0,05
		Control (n=5)	0,20±0,02	1,94±0,15	0,18±0,03	0,02±0,01	0 07±0,01
	2	Experiment (n = 4)	10±2,6	71±2,3	6±2,9	5±2,9*	8±3,2*
		Control (n=4)	17±2,03	79±1,2	2±0,87	0,4±0,1	2±1,7
	5	Experiment (n = 5)	3±1,1*	83±2,8	2±0,5	1±0,2	10±3,5*
		Control (n=6)	9±2,5	83±3,6	7±2,2	0,5±0,1	1±0,6
	14	Experiment (n = 5)	6±2,2	79±4,1	7±4,3	0,5±0,4	4±1,3
		Control (n=5)	7±0,8	82±1,4	6±1,9	1±0,6	3±0,4

Legend. Asterisk indicates significant differences compared with control.

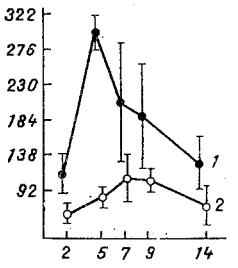

Fig. 1. Rate of clearance of blood from colloidal ink particles. Abscissa, time of investigation (in days); ordinate, velocity constant of clearance of blood from colloid (in min^{-1}). 1) After injection of zymosan, 2) after injection of 0.85% NaCl solution.

the lungs were upturned and the liquid drained into a polyethylene test tube placed in ice. The lungs were washed three times. On average 25-30 ml of fluid was obtained from each animal and centrifuged for 15 min in the cold at 3000 rpm in a TsLR-1 centrifuge. The supernatant was decanted and the cells resuspended and their number per gram weight of the lungs determined.

Into each of a series of weighing bottles with a coverslip on the bottom 4 ml of suspension (10^6 to 2×10^6 cells) in medium 199 with 20% serum for nutrient media was poured, and the bottles were then incubated at 37°C for 2 h. The coverslips with adherent cells were rinsed in medium 199. The preparations were dried, fixed with methyl alcohol, and stained with azure II-eosin. The number of free alveolar cells, monocyte-macrophages, lymphocytes, neutrophils, and eosinophils was counted under an Orthoplan (West Germany) light microscope with magnification of 1000.

Unlike macrophages, free alveolar cells contained virtually no ink particles, and with respect to shape of the cells and nucleus and the nucleocytoplasmic ratio, they corresponded more closely to criteria adopted for derivatives of type II alveolocytes [2].

Before decapitation the ingestive power of the MPS was assessed in all animals on the basis of the rate of clearance of the blood from colloidal carbon. Colloidal carbon (Gunther-Wagner, c11/1431a) was injected intravenously in a dose of 20 mg/100 g body weight. After 15 sec and 3, 6, 9, and 15 min, 0.1 ml of blood was withdrawn from the retro-orbital sinus and added to 2.9 ml of 0.1% Na_2CO_3 solution. The samples were subjected to photometry on the SF-26 spectrophotometer at a wavelength of 650 nm and the velocity constant of clearance

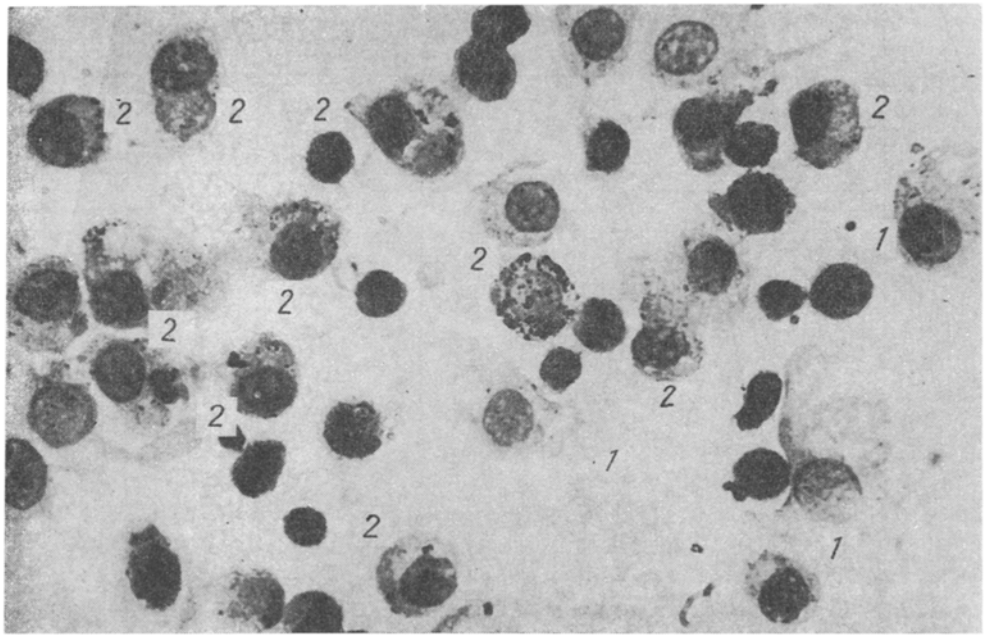

Fig. 2. Focus of mononuclear infiltration in lung 5 days after injection of zymosan. Hematoxylin and eosin. 400 ×.

of the blood from colloid was determined by the equation:

$$K = \frac{0.301}{t_{1/2}}, \text{ min},$$

where $t_{1/2}$ is the half-elimination time of the colloid from the blood [9].

The lung tissue was fixed in 10% formalin. The number of foci of infiltration and of macrophages loaded with ink was counted in 10 fields of vision in a total area of $14 \cdot 10^4$ μ^2, in lung sections 5 μ thick, stained with hematoxylin and eosin. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

By preliminary loading with colloidal carbon, and by analyzing the characteristic morphometric parameters [2], we were able to differentiate between macrophages and free alveolocytes in the broncho-alveolar washings. Cells with an oval or, less frequenctly, a round shape, a round, loosely structured and palely stained nucleus, with pale cytoplasm and a few diffuse inclusions of phagocytised ink, with mean diameter of the nucleus of 8 ± 0.27 μ, mean cell diameter of 18 ± 0.89 μ, and nucleo-cytoplasmic ratio of 0.21 ± 0.008, were classed as free alveolar cells. Conversely, alveolar monocyte-macrophages were characterized more frequently by a round shape of the cell and nucleus, intense staining of the nucleus and cytoplasm, which was loaded with ink particles, mean diameter of the nucleus of 6 ± 0.26 μ, mean cell diameter of 11.8 ± 0.6 μ, and nucleocytoplasmic ratio of 0.31 ± 0.011.

Foci of accumulation of mononuclear cells began to be formed in the lungs 2 days after injection of zymosan. This was combined with a more than threefold increase in the number of interstitial mononuclear phagocytes, loaded with colloidal carbon (50 ± 4.4 in the experiment, 14 ± 1.4 cells/14 ± 10^4 μ^2 in the control). Meanwhile the number of cells washed out of the lungs was almost doubled (5.3 ± 0.8 million in the experiment, 3.1 ± 0.4 million/gram of lung tissue in the control). The cell composition of the washings also was changed (Table 1): the most characteristic feature was an almost 20-fold increase in the absolute number of lymphocytes and neutrophils and a fivefold increase in the number of eosinophils compared with the control. The absolute number of monocyte-macrophages remained virtually unchanged. Clearance of inert colloid by cells of MPS was more than doubled (Fig. 1).

The most marked changes in the MPS were observed on the 5th day after zymosan stimulation. Accumulations of cells in the lung stroma acquired the characteristic features of granulomas (Fig. 2). Because of the distinctness of their outlines, they could be counted: 9 ± 2 in 10 fields of vision. As before, there was a greater number of interstitial macrophages, loaded with ink, and localized mainly in the foci of inflammation (49 ± 5.0 in the experiment, 15 ± 1.4 cells/$14 \cdot 10^4$ μ^2 in the control). The number of cells escaping into the alveolar space was increased threefold, up to 11 ± 1.7 million cells per gram of tissue (4 ± 0.6 million/g in the control). There were changes also in the cell composition of the broncho-alveolar washings on account of an increase in the absolute number of macrophages and eosinophils (Table 1). The number of neutrophils remained greater than in the control. Parameters of free alveolar cells and lymphocytes were lower in value than in the control. The velocity constant of elimination of colloidal particles from the blood was increased threefold (Fig. 1).

The original values of the parameters were gradually restored 7 and 9 days after stimulation with zymosan.

On the 14th day of the experiment the lung structure was almost back to normal. Here and there islands of collagen formation were present at the site of previous foci of infiltration. The number of macrophages, loaded with colloidal carbon, in the interstices of the lung was almost back to the control level (experiment 17 ± 1.0, control 15 ± 1.3 cell/$14 \cdot 10^4$ μ^2). The number of cells obtained by washing out the lungs, both in the experiment (2.7 ± 0.4 million/g tissue) and in the control (2.4 ± 0.2 million/g tissue) was virtually identical. The absolute number of all types of cells and their relative percentages were almost indistinguishable from the control (Table 1). However, the general clearing function of MPS remained increased (Fig. 1).

Intravenous injection of zymosan thus caused an incresae in the number of cells washed out of the broncho-alveolar tract and, in particular, an increase in the number of alveolar monocyte-macrophages at the peak of infiltration. Meanwhile, after zymosan stimulation the number of macrophages loaded with foreign material in the interstices of the lung was increased. After introduction of colloidal particles into the blood stream many macrophages, which had escaped into the alveolar space, contained ink particles, confirming that interstitial macrophages can migrate into the lumen of the alveoli [6]. The increase in the pool of alveolar monocyte-macropages after stimulation of MPS with zymosan may take place on account of increased production of factors inducing monocytopoiesis by activated macrophages [10, 11]. No significant accumulation of monocytes in the blood took place, according to the experimental results, after injection of zymosan. This can perhaps be explained on the grounds that newly formed monocytes, not being held up in the blood, quickly pass into the tissue as a result of the high gradient of chemical attractants in the zones where activated macrophages are located. In response to injection of a powerful stimulus for mononuclear phagocytes, foci of mononuclear and polynuclear cells, which at the times acquire the characteristic features of granulomas, are formed in the lungs. The most intensive infiltration of the lung by mononuclears was observed on the 5th day after injection of zymosan; the peak of infiltration, moreover, coincided with maximal accumulation of macrophages in the lumen of the alveoli and bronchi. Similar changes have been found not only in the lungs, but also in the liver [3, 7], evidence that the process is systemic in character.

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INTERFERON-INDUCING AND ANTIVIRAL EFFECTS OF INOSIPLEX COMBINED WITH MACROMOLECULAR INTERFERON INDUCERS

É. B. Tazulakhova, N. N. Amitina, and F. I. Ershov

UDC 615.276.4.015.2:615.339:578.245].015.4.07

KEY WORDS: inosiplex; interferon induction; antiviral action

Inosiplex* (isoprinosine, IP) is a well known immunomodulator [2, 3]. It has been extensively studied in laboratories and clinics. It has been shown that in patients with rhinovirus, herpesvirus, and influenzal infections IP alleviates the clinical manifestations of the disease [4]. IP potentiates the antitumorigenic effect of interferon [5]. A combination of IP with an interferon inducer potentiates the antiviral effect on a model of tick-borne encephalitis in mice [1].

The aim of this investigation was to study the effect of IP on interferon synthesis in animals.

EXPERIMENTAL METHOD

Noninbred male albino mice weighing 10-12 g were used. The inducers were injected intraperitoneally in a volume of 0.1 ml per mouse. Influenza virus (Aichi strain) was injected intranasally, under ether anesthesia, in a dose of 20 µl (17 LD_{50}) per mouse.

IP — the acetamidebenzoic acid salt of dimethylamine-2-propalinosine complex — was synthesized at Riga Institute of Organic Synthesis and generously supplied by Corresponding Member of the Latvian Academy of Sciences M. Yu. Lidak. Tilorone — 2,7-di-(2-diethylaminoethyoxy)-fluorenone — was the Soviet preparation in tablet (0.2 g) form. Poly(G)-poly(C), a complex of polyguanylic and polycytedylic acids, was synthesized at the Leningrad Institute of Nuclear Physics, Academy of Sciences of the USSR. RFf_2 (double-stranded RNA), the replicative form of bacteriophage, was obtained from the A. Kirchenstein Institute of Microbiology, Latvian SSR.

Interferon was titrated on a culture of L-929 cells. Marine encephalomyocarditis (EMC) virus was used as the test virus. The method of reading the cytopathic effect on plastic panels [3] was used for titration.

EXPERIMENTAL RESULTS

IP (25 mg/mouse, or 2.5 g/kg body weight) was dissolved in physiological saline and injected intraperitioneally into mice. The serum interferon titer was determined 4, 12, 18, 24, and 48 h after a single injection. The serum interferon levels were unstable and varied in different experiments, although it can be concluded from the results of independent experiments that IP possessed interferon-inducing ability. For instance, 4 h after injection of IP the interferon titer was 42 IU/ml, after 12 h it was 320 IU/ml, after 18 h 46 IU/ml, after 24 h 27 IU/ml, and after 48 h it was 18 IU/ml (mean statistical values).

A relationship is known to exist between the dose of the inducer and the level of interferon production. It was interesting to study whether the same relationship exists for IP.

*Methisoprinol.

D. I. Ivanovskii Institute of Virology, Academy of Medical Sciences of the USSR, Moscow.
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 10, pp. 457-459, October, 1985. Original article submitted June 18, 1984.

 0007-4888/85/0010-1390$09.50