EFFECT OF AURANTIN ON PHAGOCYTOSIS AND INDUCTION OF SYNTHESIS OF HEMOLYSINS

M. I. Grutman and M. Ya. Orgel'

UDC 615.332.015.46

Aurantin inhibits phagocytosis of sheep's erythrocytes by macrophages of the peritoneal exudate in vitro and depresses the ability of macrophages to induce antibody formation in vivo.

Recent investigations suggest that macrophages play an important role in antibody formation [1-3, 6, 9].

The object of this investigation was to study the effect of inhibition of RNA synthesis in a culture of macrophages on the ingestion of antigen and on the ability of these cells, having ingested antigen in vitro, to transfer the "immunogenic information" to cells of the lymphoid series.

EXPERIMENTAL METHOD AND RESULTS

Aurantin, the active component of which is actinomycin D, was used as the inhibitor of RNA synthesis in vitro [5], for its specifically inhibits RNA synthesis on a DNA template [4]. Noninbred albino rats weighing 200-250 g were used in the tests. The immune donors of macrophages were animals which received an intraperitoneal injection of 10^9 sheep's erythrocytes 28-30 days before the main experiment. The normal and immune rats received an intraperitoneal injection of a sterile mixture of 3% starch solution and 1.5% suspension of bentonite 48 h before the experiment. On the day of the experiment, peritoneal exudate was taken from these same rats under ether anesthesia; its character is described in Table 1. The operation wounds were sutured in layers. Cell suspensions of each individual animal were then cultivated in vitro in a volume of 5 ml at 37°C for 4 h with antigen (sheep's erythrocytes) in the ratio of 1 : 5. Sterile carbogen was passed through the layer of culture fluid (medium No. 199 with 20% normal and 0.1 ml immune homologous antiserum against sheep's erythrocytes (titer 1200 units of 50% hemolysis in 1 ml) was added to the cultures of macrophages in order to produce opsonization of the antigen [14]. Aurantin was added to the experimental cultures (10 μg/ml). At the end of cultivation, cells of the peritoneal exudate were treated with 0.25% trypsin solution, freed from unphagocytosed antigen [14], and washed to remove aurantin. The cell suspensions were counted, their viability assessed [11], and injected into the animals from which they were taken. Antibodies in the blood of recipients of autologous macrophages, cultivated with antigen in vitro, were determined by the 50% hemolysis test [12].

The cell composition of the cultures was unchanged during cultivation (Table 1). The results showing the effect of aurantin on phagocytosis are given in Table 2. They show that aurantin sharply depressed the phagocytic activity of macrophages of both normal and immune animals. Aurantin also suppressed the inducing ability of the macrophages. Experimental cultures of macrophages, having ingested antigen in the presence of aurantin, produced less intensive antibody synthesis in vivo than control cultures (Table 3).

A special control experiment was carried out to determine whether the antibodies which appeared were the result of induction of antibody formation by macrophages preliminarily incubated with antigen, or whether cells of the peritoneal exudate themselves produce antibodies. In these experiments, the donors of macro-

Laboratory of Immunobiology, Kiev Institute of Epidemiology, Microbiology, and Parasitology. (Presented by Academician of the Academy of Medical Sciences of the USSR L. V. Gromashevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 69, No. 6, pp. 69-71, June, 1970. Original article submitted March 24, 1969.

TABLE 1. Characteristics of Cultures of Peritoneal Exudate Cells from Rats Injected into Autologous Recipients after Incubation with Sheep's Erythrocytes

Index	Experiment (with aurantin)	Control (without aurantin)
Number of cells ($\times 10^7$) per culture at beginning of cultivation	16.28±0.65	15.95±0.57
Viability (in percent)	98.7 ±0.84	97.5 ±1.03
Qualitative composition (in percent):		
Macrophages	76.8 ±1.25	77.5 ±1.11
Heterophils	10.9 ±0.89	10.3 ±0.59
Lymphocytes	10.0 ±0.80	11.4 ±0.72
Other types of cells	1.0 ±0.26	1.2 ±0.29
Number of cells ($\times 10^7$) per culture at beginning of cultivation	15.20±0.60	15.60±0.55
Viability (in percent)	69.0 ±1.81	78.8 ±1.16
Qualitative composition (in percent):		
Macrophages	78.2 ±1.18	77.5 ±1.14
Heterophils	9.1 ±0.70	9.7 ±1.21
Lymphocytes	11.5 ±0.64	11.0 ±0.79
Other types of cells	0.9 ±0.32	0.9 ±0.24

Note. Each value obtained in a series of 64 determinations.

TABLE 2. Phagocytosis of Sheep's Erythrocytes by Macrophages of Peritoneal Exudate of Rats*

Animals	Index	Exptl. conditions	Time of cultivation	
			0 h	4 h
Normal	Activity of macrophages (in percent)	Treatment with aurantin	0	7.91±0.39
		Control	0	24.59±0.77
	No. of erythrocytes ingested per active macrophage	Treatment with aurantin	0	1.16±0.04
		Control	0	2.64±0.14
Immune	Activity of macrophages (in percent)	Treatment with aurantin	0	10.35±0.66
		Control	0	38.22±1.23
	No. of erythrocytes ingested per active macrophage	Treatment with aurantin	0	1.32±0.07
		Control	0	3.19±0.15

*Each value obtained in a series of 32 determinations.

TABLE 3. Titer of Hemolysins (Number of units of 50% hemolysis in 1 ml) in Blood of Rats Receiving Autologous Macrophages Intraperitoneally after Incubation with Antigen In Vitro*

Animals	Exptl. conditions	Days after injection of macrophages				Titers of hemolysins in intact rats
		3	6	9	12	
Normal	Treatment with aurantin	6.6	19.6	32.8	24.4	
	Control	54.8	121.8	93.0	39.4	4.3
	P			0.05		
Immune	Treatment with aurantin	15.3	55.6	85.5	77.6	7.7
	P			0.05		

*Each value obtained in a group of 8 animals.

phages after removal of exudate were irradiated with x-rays (550 R). Intact autologous macrophages, after incubation with antigen in vitro, were injected into the irradiated recipients. Low antibody titers in the blood of the irradiated donor-recipients (not exceeding 17 units) indicated that, under the experimental conditions used, antibody formation was the result of interaction between macrophages preliminarily incubated with antigen and immunologically competent cells of the donor-recipient.

It has been stated that actinomycin D inhibits pinocytosis [8]. In the experiments with aurantin, which contains actinomycin D, phagocytosis of corpuscular antigen was adversely affected. The inhibitory action of actinomycin on these processes can be explained by its possible interference with the energy metabolism of the cell and with m-RNA synthesis [13]. In fact, injection of actinomycin D into rats 18 h before injection of antigen (sheep's erythrocytes) completely suppressed RNA synthesis in peritoneal exudate cells in contact with antigen in vivo [7]. Moreover, whereas actinomycin D had no effect on the ability of macrophages, having ingested antigen, to stimulate the rate of DNA synthesis in spleen cells of immune rabbits [10], it definitely depressed their ability to induce the formation of hemolysins in vitro [7]. In the light of these data and on the basis of the experimental results described above, it can be postulated that active synthesis of macrophagal RNA is essential for successful ingestion of antigen and transmission of "immunogenic information."

It has previously been shown that actinomycin D inhibits antibody formation in vivo only if it is injected shorly before immunization or immediately after it [15]. The results of experiments to study induction of antibody formation in vitro by means of macrophages which have ingested antigen in vivo [7], and also the results of the present experiments to study induction of antibody synthesis in vivo with the aid of autologous macrophages, stimulated by antigen in vitro, suggest that the inhibitory action of actinomycin D on antibody formation is due, at least partially, to inhibition of RNA synthesis in cells of marcophagal type after they have ingested antigen.

LITERATURE CITED

1. M. G. Avetikyan and L. G. Velikosel'tseva, in: Stimulation and Mechanisms of Biosynthesis of Antibodies [in Russian], Leningrad (1966), p. 67.
2. E. A. Kabanova and I. N. Kokorin, Abstracts of Proceedings of the 9th International Congress of Microbiology [in Russian], Moscow (1966), p. 572.
3. E. A. Kabanova, I. N. Kokorin, and N. N. Rybkina, in: Problems in Infectious Pathology and Immunology [in Russian], No. 4, 76 (1968).
4. L. A. Osterman, in: Advances in Biological Chemistry [in Russian], Vol. 7, Moscow (1965), p. 116.
5. A. B. Silaev, T. I. Orlova, I. B. Mironova, et al., in: Aurantin, an Antitumor Antibiotic Preparation of the Actinomycin Group [in Russian], Moscow (1962), p. 26.
6. B. A. Askonas and J. M. Rhodes, Nature, 205, 709 (1965).
7. D. C. Bishop, A. Pisciotta, and P. Abramoff, J. Immunol., 99, 751 (1967).
8. Z. A. Cohn, J. Exp. Med., 124, 557 (1966).
9. M. Fishman, Nature, 183, 1200 (1969).
10. E. Harris, Nature, 211, 154 (1966).
11. S. Harris, T. N. Harris, and M. Farber, J. Immunol., 72, 148 (1954).
12. E. A. Kabat and M. M. Mayer, Experimental Immunochemistry, Springfield (1964), p. 133.
13. J. Laszlo, D. S. Miller, K. S. McCarty, et al., Science, 151, 1007 (1966).
14. T. Morita and E. H. Perkins, J. Reticuloend. Soc., 2, 406 (1965).
15. C. J. Wust and M. G. Hanna, Proc. Soc. Exp. Biol. (New York), 118, 1027 (1965).