NUCLEIC ACID CONCENTRATION IN CULTURES OF MACROPHAGES WITH ANTIGEN

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In cultures of macrophages from the peritoneal exudate of normal and immune rats antigen (sheep's erythrocytes) produced a significant increase in the RNA concentration in vitro. Aurantin, containing actinomycin D, reduced the RNA concentration in macrophages in contact with antigen. Neither antigen nor aurantin affected the level of macrophagal DNA.

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In the light of the discussion regarding the nature of the "immunogenic information" supplied by macrophages, after ingesting antigen, to lymphoid cells [1, 5, 6], it is important to determine whether the RNA content is increased in macrophages during phagocytosis of antigen.

In the investigation described below changes in the content of nucleic acids in cultures of macrophages with antigen (sheep's erythrocytes) are described.

EXPERIMENTAL METHOD

Experiments were carried out on albino rats weighing 200-250 g. In some experiments immune animals were used. Cells of the peritoneal exudate of rats were cultivated at 37° for 4 h in an atmosphere of carbogen. To each culture ($5 \cdot 10^7$ nucleated cells, 76% macrophages) $25 \cdot 10^7$ sheep's erythrocytes were added. Aurantin was added to the experimental cultures of macrophages up to a concentration of 10 μg/ml. The total nucleic acid concentration was determined by Spirin's method [4], and the DNA concentration by the method of Orlov and Orlova [3]. The RNA concentration was calculated from the difference between the total nucleic acid concentration and the DNA concentration.

TABLE 1. Changes in DNA Concentration in Cultures of Macrophages in the Presence of Antigen and Aurantin

Animals	DNA concentration (in $\mu g/10^7$ cells)				
	macrophages before experiment (control)	macrophages + antigen	P (compared with control)	macrophages + antigen + aurantin	P (compared with control)
Normal	61.2±0.84	59.9±1.03	> 0.05	61.4±1.20	> 0.05
Immune	59.8±1.11	60.1±0.95	> 0.05	58.7±1.02	> 0.05
	> 0.05	> 0.05	–	> 0.05	–

Note. Here and in Table 2 each value is the mean of sixteen determinations.

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TABLE 2. Changes in RNA Concentration in Cultures of Macrophages in the Presence of Antigen and Aurantin

Animals	RNA concentration (in $\mu g/10^7$ cells)				
	macrophages before experiment (control)	macrophages + antigen	P (compared with control)	macrophages + antigen + aurantin	P (compared with control)
Normal	31.6±0.93	38.6±0.81	< 0.001	22.9±0.69	< 0.001
Immune	34.1±0.89	40.8±0.72	< 0.001	24.7±0.65	< 0.001
P	> 0.05	> 0.05	–	> 0.05	–

EXPERIMENTAL RESULTS

Cultivation of macrophages of normal and immune animals in the presence of antigen, whether with or without aurantin, did not lead to significant changes in the DNA content in these cells (Table 1).

Investigations by microspectrophotometry [11] and using thymidine-H^3 [11] have shown that antigen increases the rate of DNA synthesis in cultures of peritoneal exudate cells, including macrophages. The resolving power of the method now used was probably too low to detect relatively small changes in the DNA concentration which can be observed in cells in the S- and G_2-periods of interphase [8]. It was also found that antigen caused a significant increase in the RNA concentration both in normal and in immune macrophages (Table 2). If, however, aurantin was added to cultures of macrophages, the RNA concentration in the cells was significantly lowered. When much smaller doses of actinomycin D were used, no depression of RNA synthesis was found during contact between rabbit macrophages and antigen [9]. In other experiments, however, actinomycin D [7] and aurantin [2] significantly inhibited the inducing power of peritoneal exudate cells incubated with antigen.

It can be concluded from the results of these experiments that intact synthesis of macrophagal RNA is essential for the normal course of antigen "processing" and induction of antibody formation.

LITERATURE CITED

1. E. A. Kabanova and I. N. Kokorin, Abstracts of Proceedings of the International Congress of Microbiology [in Russian], Moscow (1966), p. 572.
2. M. Ya. Orgel', in:: Problems in Immunology [in Russian], Vol. 4, Kiev (1969), p. 13.
3. A. S. Orlov and E. I. Orlova, Biokhimiya, No. 5, 834 (1961).
4. A. S. Spirin, Biokhimiya, No. 5, 656 (1958).
5. F. L. Adler, M. Fishman, and S. Dray, J. Immunol., 97, 554 (1966).
6. B. A. Askonas and J. M. Rhodes, Nature, 205, 470 (1965).
7. D. C. Bishop, A. Pisciotta, and P. Abramoff, Nature, 99, 751 (1967).
8. E. de Robertis, W. Nowinski, and F. A. Saez, Cell Biology [Rusian translation], Moscow (1967), p. 353.
9. G. Harris, Nature, 211, 154 (1966).
10. J. Lokaj and J. Feit, J. Immun.-Forsch., 134, 120 (1967).
11. D. Rowley and C. Leuchtenberger, Lancet, 2, 734 (1964).