EFFECT OF DOUBLE STIMULATION ON MACROPHAGE FUNCTION

A. I. Braude, L. A. Shevnyuk,
and S. L. Fainshtein

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The phagocytic activity of macrophages, their ability to produce a substance with a protective antiviral action, and the acid phosphatase content in the macrophages are all increased to a greater degree after combined administration of 8-mercaptoadenine and prodigiosan to albino mice than after administration of these substances in the same doses, but separately. Macrophage function is greatly stimulated by preliminary oral administration of three doses of 8-mercaptoadenine (2.5 mg/kg) followed by parenteral administration of prodigiosan (250 μg/kg).

The vital protective function of macrophages determines the importance of factors aimed at increasing the phagocytic activity of these cells. Progress in this direction has been made after the discovery of the connection between the digestive power of the macrophages and the intensity of their synthesis of lysosomal proteins [1, 2, 8]. It must therefore now be asked whether the response of macrophages to the agent stimulating phagocytosis can be strengthened by increasing the intensity of protein synthesis in these cells.

In the present investigation the effect of a compound with anabolic action (8-mercaptoadenine [5]) and a stimulator of phagocytosis, the bacterial polysaccharide prodigiosan [5], on macrophage function (phagocytic activity, acid phosphatase activity, and ability to produce a substance with protective antiviral action) was studied.

EXPERIMENTAL METHOD

Following pilot studies to determine optimal schemes of administration and doses of the two compounds to albino mice, 8-mercaptoadenine was given initially by mouth for 3 days in a daily dose to 2.5 mg/kg, and 24 h after the last dose, prodigiosan was injected intraperitoneally in doses of 50 and 250 μg/kg. The animals of the remaining groups received either 8-mercaptoadenine alone, prodigiosan alone, or physiological saline. The cells contained in the peritoneal cavity were removed 24 h after this injection by the usual method [7], their total number was counted, and the percentage of macrophages was determined in stained films. The acid phosphatase content in a standard number of cells from the peritoneal cavity was determined by the biochemical method of Aerts et al. [9]. Acid phosphatase was detected cytochemically by Gomori's method in the modification of Allison and Malluci [10], and the intensity of the reaction was estimated after incubation of the cells in substrate for 25 and 40 min. The phagocytic activity (ingestive and digestive separately) of the macrophages was determined by the method described previously [3], using Escherichia coli strain No. 94 as the test strain for phagocytosis.

To determine the ability of the macrophages to produce a substance with protective antiviral action, cells from the peritoneal cavity were cultivated for 24 h in a mixture of medium No. 199 (90%) and bovine serum (10%), assuming that during this period the substance synthesized intravitally in the cells would be excreted into the culture medium. The culture medium was then decanted and added in dilutions of 1:50,

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TABLE 1. Number and Functional Activity of Macrophages from Animals of Compared Groups (M ± m; n = 12)

Group of animals	Character of treatment	Number of macrophages/ml (in millions)	Phagocytic activity		Activity of substance with antiviral action	
			ingestive ability	index of digestive activity (in conventional units)	per million peritoneal cells	per million macrophages
1	8-Mercaptoadenine	1.21 ± 0.19	3.77 ± 0.29	38.1 ± 1.7	65.0 ± 2.06	89.9 ± 0.9
2	Prodigiosan (50 μg/ml)	1.46 ± 0.36	2.98 ± 0.14	37.0 ± 4.1	60.4 ± 0.8	77.4 ± 1.0
3	8-Mercaptoadenine + prodigiosan (50 μg/ml)	2.29 ± 0.24	4.26 ± 0.40	54.9 ± 3.2	140.4 ± 1.5	165.0 ± 1.7
4	Prodigiosan (250 μg/ml)	2.13 ± 0.40	4.27 ± 0.19	43.6 ± 2.9	90.3 ± 0.7	109.2 ± 0.8
5	8-Mercaptoadenine + prodigiosan (250 μg/ml)	4.31 ± 0.41	5.41 ± 0.33	72.2 ± 4.7	180.6 ± 4.7	200.3 ± 5.3
6	Control	0.60 ± 0.27	2.63 ± 0.26	28.2 ± 3.0	–	–

1:80, and 1:120 for 24 h to cultures of line L mouse fibroblasts. The fibroblasts were then transferred into fresh medium No. 199 and serum, and inoculated with vesicular stomatitis virus (Indiana strain). During the next three days the number of living and dead fibroblasts was counted after staining with 0.1% erythrosin solution. The number of living fibroblasts not subjected to the cytopathic action of the virus indicated the activity of the substance with protective antiviral action, liberated by cells from the peritoneal cavity. The unit of activity of this substance was therefore taken as the reciprocal of the highest dilution of culture medium of the peritoneal cells protecting 50% of fibroblasts against virus degeneration.

EXPERIMENTAL RESULTS

The averaged experimental results are shown in Table 1. Statistical analysis by the Student–Fisher method revealed significant differences between the digestive activity and activity of the substance with protective antiviral action in group 3, compared with groups 1 and 2, and in group 5 compared with groups 1 and 4 ($P < 0.05$).

The biochemical test showed that the content of acid phosphatase in the macrophages after combined administrations of 8-mercaptoadenine and prodigiosan in a dose of 250 μg/kg was 32 times higher than in the control, but after separate administration of the two compounds it was 13 and 19 times higher, respectively. This is in good agreement with data published previously, indicating a sharp increase in the acid phosphatase content in macrophages [11].

The highest acid phosphatase activity was also discovered cytochemically in group 5: only in the animals of this group was a definite positive reaction to the enzyme observed after incubation of the macrophages in the substrate for 25 min. The reaction in groups 3 and 4 was weakly positive, but in the rest it was negative. This correlation between the results obtained by the two methods suggests that the cytochemically detected increase in acid phosphatase activity after the procedures depends on an increase in the enzyme content. It cannot be explained entirely by an increase in permeability of the lysosomal membranes for the substrate of the cytochemical reaction, although under the conditions used this possibility is not ruled out [1]. In turn, the marked increase in acid phosphatase content in group 5 corresponds to the greatest increase in digestive power of the macrophages in this group.

With reference to all the indices used, the greatest increase in functional activity of the macrophages was thus observed after combined administration of prodigiosan and 8-mercaptoadenine. This result deserves special attention, and two circumstances must be taken into consideration. First, functional activity of the macrophages cannot be increased simply by increasing the dose of the agent acting on the cells above certain limits. In fact, the effectiveness of prodigiosan was increased by an increase in its dose from 50 to 250 μg/kg (Table 1), but as previous experiments showed, a further increase in the dose of the compound does not improve the results. Second, the phagocytic activity of the macrophages

does not increase if the same substance or a substance with a similar mechanism of action is administered repeatedly, above the level obtained by the first injection: in particular, if two injections of prodigiosan were given to animals at an interval of 24 h, the second injection not only did not increase the effectiveness of the first but, on the contrary, reduced it.

The combined administration of prodigiosan with 8-mercaptoadenine gave different results. Probably, on the basis of the facts described above, the higher effectiveness of the combination is dependent on differences between the mechanism of action of the two compounds: the absence of a direct action of prodigiosan in the doses used on the cells, by contrast with the action of 8-mercaptoadenine [4, 5]. Stimulation of the functional activity of phagocytes by bacterial polysaccharides is known to promote repair processes in an inflammatory focus [4].

The results suggest that stronger effect on regeneration can be obtained by the use of a combination of preparations of the type used in this investigation.

LITERATURE CITED

1. A. I. Braude, Uspekhi Sovr. Biol., 62, No. 1 (4), 61 (1966).
2. A. I. Braude, Zh. Mikrobiol., No. 10, 81 (1967).
3. A. I. Braude and O. V. Bukharin, Zh. Mikrobiol., No. 3, 36 (1968).
4. A. I. Braude and G. E. Vaisberg, in: Carbohydrates and Carbohydrate Metabolism [in Russian], Moscow (1962), p. 279.
5. A. I. Braude, É. G. Shcherbakova, I. I. Smertenko, et al., Antibiotiki, No. 10, 915 (1969).
6. G. E. Vaisberg, Preparation of Polysaccharide Complexes of Acetoxanes and Prodigiosan and the Study of Their Biological Activity, Author's Abstract of Doctoral Dissertation, Moscow (1964).
7. E. A. Zavenyagina and A. I. Braude, Antibiotiki, No. 1, 53 (1965).
8. V. V. Rogovin, Experimental Cytological Study of Macrophages with Different Functional Activity, Candidate's Dissertation, Moscow (1968).
9. C. Aerts, J. Guillaume, and A. Petitprez, C. R. Soc. Biol., 161, 2199 (1967).
10. A. Allison and L. Malluci, J. Exp. Med., 121, 463 (1965).
11. Z. Cohn and B. Benson, J. Exp. Med., 121, 835 (1965).