The biological activity of this fraction is 50-100 times greater than that of the original preparation, thymarin. This substance, in a dose of 20 μg per mouse, stimulates the immune response intensively to injection of a thymus-dependent antigen, namely sheep's erythrocytes, and in a dose of 1 μg stimulates the responses of the host connected with IgM and IgG formation, but does not increase the circulating antibody level.

It will be noted that these experiments were performed on immunologically normal animals; possibly under conditions of immunological deficiency, due in particular to thymus insufficiency, this substance would have a stronger action.

LITERATURE CITED

1. G. A. Belokrylov, V. G. Morozov, V. Kh. Khavinson, et al., Byull. Éksp. Biol. Med., No. 2, 202 (1976).
2. E. V. Gyulling and I. S. Nikol'skii, Byull. Éksp. Biol. Med, No. 4, 82 (1975).
3. N. N. Kuznetsova, K. M. Genender, G. V. Samsonov, et al., Byull. Izobret., No. 36; USSR Authors' Certificate No. 322332 (1971).
4. V. G. Morozov and V. Kh. Khavinson, Eksp. Khir., No. 2, 49 (1974).
5. Y. A. Asanuma, A. L. Goldstein, and A. White, Endocrinology, 86, 600 (1970).
6. J. Comsa, Am. J. Med. Sci., 250, 79 (1965).
7. D. W. Dresser and H. H. Wortis, Nature (London), 208, 859.
8. A. L. Goldstein, F. D. Slater, and A. White, Proc. Nat. Acad. Sci. USA, 56, 1010 (1966).
9. A. L. Goldstein, A. Guna, M. L. Howe, et al., J. Immunol., 106, 773 (1971).
10. A. L. Goldstein, A. Guna, M. M. Zatz, et al., Proc. Nat. Acad. Sci. USA, 69, 1800 (1972).
11. N. K. Jerne and A. A. Nordin, Science, 140, 405 (1963).
12. S. T. Milcu, I. Potop, N. Olinici, et al., Stud. Cercet. Endocrinol., 24, 85 (1973).
13. M. Steinbuch and R. Audran, Arch. Biochem., 134, 279 (1969).
14. J. Wybran, A. S. Levin, H. H. Fudenberg, et al., Ann. N. Y. Acad. Sci., 249, 300 (1975).

LIMITS OF PHAGOCYTIC POWER OF MACROPHAGES

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UDC 612.112.3+616.155.3-008.13

The ability of peritoneal macrophages to take up different doses of antigen (sheep's erythrocytes) and of antigens differing in physicochemical properties (sheep's erythrocytes, rat erythrocytes, and typhoid vaccine) was studied. An increase in the dose of sheep's erythrocytes injected many times over had no effect on the quantity of antigen ingested by the macrophages within a definite time interval. In macrophages taken at short periods after injection of erythrocytes of the different species of animals into the mice, ability to take up these erythrocytes in vitro was sharply inhibited. Preincubation of macrophages (in vivo or in vitro) with all the antigens tested sharply increased their ability to phagocytose typhoid vaccine.

KEY WORDS: *macrophages; phagocytosis; antigen.*

It has long been established that blockade of cells of the monocyte phagocytic systems (MPS) by inorganic particles or by injection of certain agents causes depression of antibody

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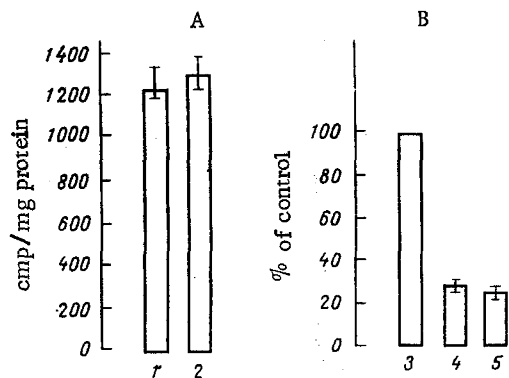

Fig. 1. Uptake of erythrocytes by intact macrophages and by macrophages previously treated with antigen. A) Uptake of different doses of ^{51}Cr-labeled SE in vivo: 1) 10^{10} ^{51}Cr-labeled SE; 2) $3.5 \cdot 10^8$ ^{51}Cr-labeled SE. B) Uptake in vitro of ^{51}Cr-labeled SE by macrophages treated with SE in vivo: 3) intact macrophages + $3.5 \cdot 10^8$ ^{51}Cr-labeled SE in vitro; 4) $3.5 \cdot 10^8$ SE in vivo + $3.5 \cdot 10^8$ ^{51}Cr-labeled SE in vitro; 5) 10^{10} SE in vivo + $3.5 \cdot 10^8$ ^{51}Cr-labeled SE in vitro.

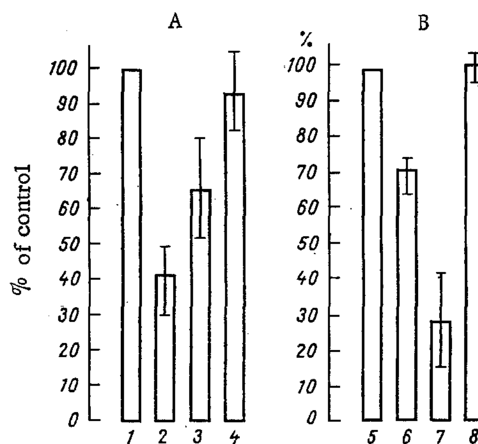

Fig. 2. Uptake in vitro of erythrocytes by macrophages of mice previously treated with various antigens in vivo. A) Uptake in vitro of ^{51}Cr-labeled SE by macrophages prepared in vivo: 1) control — intact macrophages (taken as 100%); 2) SE; 3) RE; 4) TV. B) Uptake in vitro of ^{51}Cr-labeled RE macrophages prepared in vivo: 5) control (intact macrophages); 6) RE; 7) SE; 8) TV.

formation in response to subsequent injection of antigens at both the cellular and the humoral level [9, 12].

It has been suggested that high tolerance of certain antigens or synthetic polypeptides can be explained by their weak degradation by macrophages induced by natural or artificial methods [7].

The object of this investigation was to study whether tolerance to large doses of sheep's erythrocytes is connected with blockade of cells of the MPS by them.

EXPERIMENTAL METHOD

Experiments were carried out on CBA mice. Sheep's (SE) and rats' (RE) erythrocytes, either labeled with ^{51}Cr [6] or unlabeled, in doses of $3.5 \cdot 10^8$ cells and 10^{10} cells, and also heated typhoid vaccine (TV), labeled with sodium [1-^{14}C]acetate or unlabeled, in a dose of 250 million bacterial cells, were used as the antigens. Peritoneal extract cells (PEC) were obtained on the fourth day after intraperitoneal injection of meat–peptone broth (MPB) into the mice. A monolayer of macrophages was obtained by culture of the PEC ($1 \cdot 10^7$ cells to 5 ml medium No. 199_0 at 37°C for 1 h, followed by thorough washing to remove nonadherent cells and free antigen.

Depending on the conditions of the experiments the antigens were injected in vivo or added to the culture of macrophages in the doses mentioned above. Uptake of ^{51}Cr-labeled SE was determined by a Nuclear Chicago gamma spectrometer, and uptake of ^{14}C-labeled TV by the Mark-2 instrument.

EXPERIMENTAL RESULTS

In the experiments of series I the uptake of immunogenic ($3.5 \cdot 10^8$) and tolerogenic (10^{10}) doses of SE by macrophages in vivo was determined, and for that purpose the cells were injected intraperitoneally into mice. PEC were obtained 30 min later, and after incubation for 1 h at 37°C in Hanks's solution and thorough washing, the quantity of radioactive material taken up by the macrophages was determined. The result was expressed per number of cells and also per milligram protein. As Fig. 1A shows, despite the enormous difference between the numbers of SE injected, in the course of the first 30 min the macrophages ingested practically equal numbers.

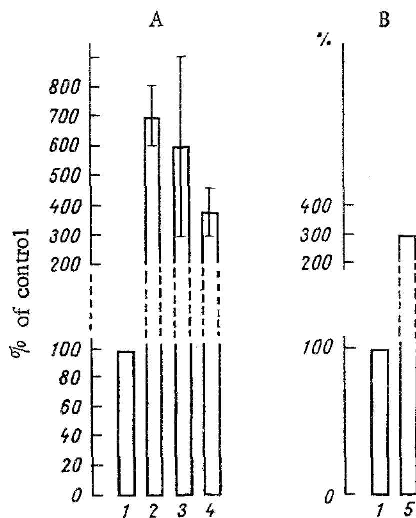

Fig. 3. Uptake in vitro of ^{14}C-labeled TV by macrophages of mice treated with different antigens: A) uptake of ^{14}C-labeled TV by macrophages prepared in vivo: 1) control (intact macrophages); 2) TV; 3) SE; 4) RE. B) Uptake of ^{14}C-labeled TV by macrophages prepared in vitro: 1) control (intact macrophages); 5) SE + ^{14}C-labeled TV.

The results of the experiments of series II, in which the animals were given intraperitoneal injections of the same doses ($3.5 \cdot 10^8$ or 10^{10}) of unlabeled SE, are given in Fig. 1B. The PEC obtained 30 min later were transferred to tubes and the monolayer of macrophages, after removal of nonadherent cells, was incubated with labeled SE in a dose of $3.5 \cdot 10^8$ per tube (about 35 erythrocytes per macrophage). After incubation for 30 min the macrophages were washed to remove uningested radioactive antigen and the level of uptake of ^{51}Cr-labeled SE was determined. The results were expressed as percentages of the uptake of the same dose of antigen by a culture of macrophages obtained from intact animals. The results show that macrophages taken from animals 30 min after injection of different doses of SE ingested only one-quarter as much antigen as intact macrophages, regardless of the dose of SE (immunogenic or tolerogenic) with which the donors of the macrophages were treated. The impression was obtained that in a certain period of time (in these experiments 30 min) the macrophage population was able to take up a limited number of SE.

The next two series of experiments were devoted to the study of the phagocytic power of macrophages against one antigen after preliminary treatment with other antigens, differing from them both in size and in physicochemical nature. Different groups of mice which acted as donors of macrophages were given intraperitoneal injections of SE or RE in a dose of $3.5 \cdot 10^8$ or TV in a dose of 250 million bacterial cells. To a monolayer of macrophages obtained from these mice $3.5 \cdot 10^8$ ^{51}Cr-labeled SE were added in vitro and the level of their uptake was determined 30 min later. The results are given in Fig. 2A. The phagocytic power of the macrophages was found to be reduced most sharply after preliminary treatment of the animals with SE (42% of the level of uptake by intact macrophages). Uptake also was sharply reduced (by 40%) by macrophages taken from donors receiving RE. Only macrophages from donors receiving TV took up the same amount of ^{51}Cr-labeled SE in vitro as intact macrophages.

Testing the ability of macrophages to take up RE in vitro revealed virtually the same pattern (Fib. 2B): The ability of macrophages taken from animals treated with TV to take up RE was not reduced, whereas injection of SE into the donors sharply inhibited their ability to phagocytose RE.

Since injection of TV into the animals did not affect the ability of their macrophages to take up other antigens in vitro, an attempt was made to discover whether the ability of macrophages to take up microorganisms in vitro was modified by previous ingestion of erythrocytes or TV in vivo. For this purpose, ^{14}C-labeled TV were added in vitro to a monolayer of macrophages (intact and taken from donors after injection of SE, RE, and TV). As the results given in Fig. 3A show, preliminary injection of any of the test antigens in vivo sharply (by 4 to 8 times) increased the ability of the macrophages to take up ^{14}C-labeled TV in vitro.

After preliminary incubation of macrophages from intact animals with SE in vitro sharp stimulation of the phagocytic power of the macrophages relative to ^{14}C-labeled TV also was observed (Fig. 3B).

The results of these experiments show definite differences in the ingestive function of the macrophages depending on the type of antigen. Despite the large macrophagal population in the peritoneal cavity of the mice stimulated beforehand by injection of MPB, after intraperitoneal injection of different doses of erythrocytes the number phagocytosed within a definite time interval was limited. The existence of mechanisms limiting phagocytosis of erythrocytes can perhaps be justified physiologically, for destruction of erythrocytes is a function of phagocytes which they are constantly performing. This is confirmed also by the fact that ability to take up these erythrocytes in vitro is sharply depressed in the case of macrophages taken at short times after injection of erythrocytes from different species of animals into the mice, whereas ability to take up bacterial cells is not affected by such preliminary treatment. Conversely, any antigen preincubated with macrophages in vivo or in vitro stimulates the ability of the macrophages to take up bacterial antigen in vitro.

It is stated in the literature that macrophages secrete factors stimulating the immune response, which depend on the type of antigen previously in contact with them [5]; two different antigens can be found under these circumstances in the same phagolysosomes [10].

The results showing an increase in uptake of TV by macrophages after antigenic preliminary treatment of the animals in vivo suggest that these phenomena may be related to the mechanism lying at the basis of the phenomenon of rapidly developing resistance. The rapid increase of resistance is manifested usually by a similar type of response: an extremely rapid readjustment of the protective mechanisms of the body under the influence of the agent injected previously. Such agents may be bacteria [1, 8], vaccines [4], and viruses [3]. The nonspecific character of this phenomenon indicates that it is based on the mobilization of the physiological factors of natural immunity [2, 3, 11]. One such factor may be stimulation of the phagocytic function of the MPS. The data now obtained showing an increase in TV uptake after preliminary preparation of the animals with antigens may perhaps explain the mechanism of the phenomenon of the rapid increase in resistance, namely by stimulation of the phagocytic function of the macrophages

LITERATURE CITED

1. V. N. Krylov, Zh. Mikrobiol., No. 1, 60 (1950).
2. I. M. Lyampert, Zh. Mikrobiol., No. 6, 53 (1958).
3. G. Dalldorf, S. M. Cohen, and J. M. Coffey, J. Immunol., 56, 295 (1974).
4. D. G. Evans and F. T. Perkins, Br. J. Exp. Path., 35, 603 (1954).
5. K. Gerson and M. Feldman, Immunology, 15, 827 (1968).
6. S. Gray and H. Sterling, J. Clin. Invest., 29, 1604 (1950).
7. C. Janeway and M. Sela, Immunology, 13, 29 (1967).
8. J. Morgearoht, H. Biberstein, and R. Schnitzer, Dtsch. Med. Wschr., 46, 337 (1920).
9. P. Murray, F. Jennings, M. Murray, et al., Immunology, 27, 815 (1974).
10. J. Rhodes, Q. Lind, A. Birch-Anderson, et al., Immunology, 17, 445 (1969).
11. D. Rowley, Lancet, 1, 232 (1955).
12. T. Sabet and H. Friedman, Immunology, 19, 843 (1970).