It can be concluded on the basis of these facts that adaptation of the lymphoid system to the hypomagnetic state is manifested as desynchronization of circadian rhythms on the basis of differences in sensitivity of the organs; reorganization of the rhythms is realized through strengthening of the ultradian components with periods of about 15 h; the data indicate indirectly the acceleration or increased power of recirculation of the lymphoid cells and also of acidophilic granulocytes.

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ROLE OF INTERCELLULAR CONTACT INTERACTIONS AND THE RESPONSE OF MACROPHAGES TO ACTIVATORS CONTAINING AND NOT CONTAINING THE Arg–Gly–Asp SEQUENCE

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The superoxide anion occupies a special place in the performance of the effector functions of phagocytes. Meanwhile the mechanisms controlling its production by activated cells under normal and pathological conditions have been inadequately studied. A definite role here may be played by signals transmitted by direct intercellular contact. It has been shown that this is one way by which functional interaction can take place between lymphocytes [6], T-lymphocytes and macrophages [15], and T-lymphocytes and granulocytes [10], but only indirect information was available on the role of direct contacts between phagocytes and the regulation of their activity [5, 11].

The initial aim of this investigation was accordingly to determine whether the level of production of the superoxide radical depends on contacts between macrophages. Since a key role in various kinds of adhesive processes is known to be played by a special group of cell receptors, recognizing the amino-acid sequence Arg–Gly–Asp found in the composition of their characteristic ligands [2, 9, 14], in order to obtain preliminary information on a possible role of receptors of this type in reception of a contact activating signal, we therefore compared the role of intercellular interactions during the response of macrophages to stimuli con-

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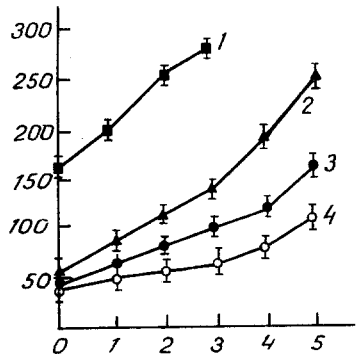

Fig. 1

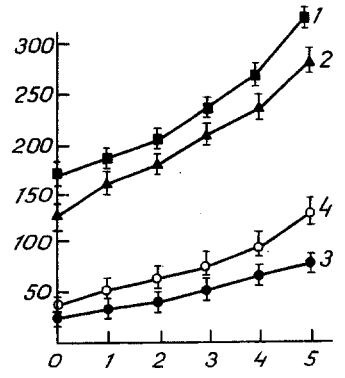

Fig. 2

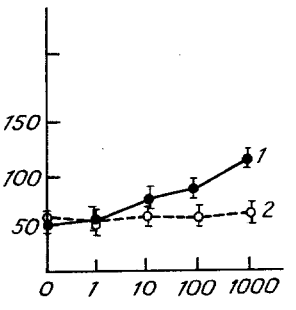

Fig. 3

Fig. 1. Dependence of reduced nitro-BT level in macrophages on intercellular contacts with different ways of activation: 1) opsonized zymosan, 2) phorbol myristate acetate, 3) peptide Form—Meth—Leu—Phe, 4) without activator. Here and in Fig. 2: ordinate, optical density at 565 nm (in relative units); abscissa, number of contacts.

Fig. 2. Dependence of reduced nitro-BT level on intercellular contacts in macrophages adherent to different substrates. 1) Gelatin + fibronectin (75 μg/ml), 2) gelatin + fibronectin (25 μg/ml), 3) gelatin, 4) without treatment.

Fig. 3. Effect of peptide Arg—Gly—Asp—Ser on reduced nitro-BT level in single macrophages, activated by phorbol myristate acetate. 1) Arg—Gly—Asp—Ser; 2) Arg—Asp—Ser—Lys. Abscissa, concentration peptides (μg/ml); ordinate, optical density at 565 nm.

taining and not containing the Arg—Gly—Asp sequence, and we also analyzed the effect of a peptide, which incorporates this grouping.

EXPERIMENTAL METHOD

Peritoneal macrophages were flushed out of the abdominal cavity of C57BL/6 male mice weighing 16-18 g by means of phosphate-buffered 0.15 M NaCl solution. The cells were washed, resuspended in Hanks' solution not containing phenol red but containing 5 mM glucose, and transferred into polystyrene Petri dishes. After incubation for 1 h at 37°C in a humid chamber with 5% CO_2 in air the dishes were rinsed with warm Hanks' solution to remove weakly adherent cells. Production of the superoxide anion was estimated in the nitroblue-tetrazolium (nitro-BT) reduction test, for which subsequent incubation of the macrophages was carried out in the presence of nitro-BT ('Chemapol') in a final concentration of 0.5 mg/ml, with the addition of one of the following activators: zymosan (from Olaine Chemical Reagents Factory), opsonized fresh mouse serum in a concentration of 1 mg/ml, and phorbol myristate acetate ('Sigma') in a concentration of 50 mg/ml or the chemotaxic peptide Form—Meth—Leu—Phe in a concentration of 10^{-6} M. In some series the cells adhered to dishes covered with gelatin and fibronectin (25 or 75 μg/ml for 2 h at room temperature). The reaction was stopped 1 h after the beginning of incubation by rinsing the dishes and fixing the cells with 0.25% glutaraldehyde solution for 5-7 min. The peptides Form—Meth—Leu—Phe, Arg—Gly—Asp—Ser, and Arg—Asp—Ser-Lys were synthesized by the classical method using a pyridoxyl protective group [1].

The intensity of reduction of nitro-BT in individual cells was estimated by measuring absorbance at 565 nm by means of a probe, corresponding in area to the test cell, with simultaneous cytologic identification of the cells in UV light on the basis of the structure of their nuclei after staining with acridine orange. An 'MIF-K' cytophotometer, constructed in the Department of Cytology, M. V. Lomonosov Moscow University, together with a LYUMAM I-3 microscope was used for this purpose. Readings of the cytophotometer were recorded simultaneously with allowance for the number of contacts formed by the given cell with neighboring leukocytes, as was fully described by the writers previously [3]. The results were analyzed for at least 100 cells at each point of the experiment. The experiments were repeated at least 3 times.

EXPERIMENTAL RESULTS

The results given in Figs. 1 and 2 show the rise in the level of nitro-BT reduction in macrophages depending on the number of intercellular contacts. This rule was manifested both in the course of the spontaneous reaction and when stimulators with different points of application of their effect were used: formyl-methionyl-leucyl-phenylalanine [13], serum-opsonized zymosan [8], fibronectin [7], and phorbol myristate acetate [12]. It can accordingly be concluded that during intercellular contact interaction a certain signal leading to increased functional activity of the macrophages, is transmitted, at least so far as production of the superoxide radical is concerned.

The nature of the phenomenon thus discovered has not yet been explained. However, it draws attention to a definite group of surface receptors involved in interaction between cells and also with certain components of the surrounding matrix. A definite level of expression of at least some of these molecules (LFA-1, Mac-1, p 150.95) is essential for normal function of phagocytes [2]. These last data are evidence that receptors for fibronectin, laminin, vitronectin, the C3bi-component of complement and, evidently, other representatives of this family of molecules, recognize structural sites with a high degree of homology in the composition of their typical ligands, which contain as an irreplaceable component, the Arg—Gly—Asp sequence in particular [9, 14].

The reaction of macrophages to stimuli containing (fibronectin, opsonized zymosan) and not containing (chemotaxic peptide, phorbol myristate acetate) this particular sequence is characterized by definite differences with respect to dependence on intercellular contacts (Figs. 1 and 2). This is clearly shown by the example of single cells which, in the absence of intercellular contacts, can give a quite considerable reaction with activators of the first group, although reaction also is increased in the presence of contacts. The impression is obtained that certain ligands can 'replace' or 'imitate' the stimulating signal transmitted through contacts between macrophages, to a certain degree. In our view this can be explained by recognition of a certain group that is common to them all in the composition of fibronectin, the C3bi-fragment on the surface of opsonized zymosan, and an unknown ligand on the membrane of resident macrophages (which do not contain fibronectin [4]). Judging by data in the literature, this common component may be the amino-acid sequence Arg—Gly—Asp, although the effect of binding of this sequence on activation of cells has not been studied.

For the initial testing of this hypothesis we studied the effect of the peptide Arg—Gly—Asp—Ser on reduction of nitro-BT in single macrophages, activated by the phorbol ester. This peptide, but not the control (Arg—Asp—Ser—Lys) was found to induce dose-dependent stimulation of the cells (Fig. 3). Consequently, it may be that, independently of the context of recognition and, correspondingly, the concrete type of receptors involved (of the group recognizing Arg—Gly—Asp) binding of this amino-acid sequence leads to an identical or similar functional response of the cell, which, in turn, may lie at the basis of the stimulating effect of various ligands, and also of contact interactions between macrophages.

The investigation thus showed that contacts between macrophages stimulate the level of production of the superoxide radical in them, and that a high level of response in single cells is observed on their activation with opsonized zymosan or with fibronectin, but not by phorbol myristate acetate or by the chemotaxic peptide Form—Meth—Leu—Phe; a substantial rise of the level of response in single macrophages stimulated by the phorbol ester is observed in the presence of the peptide Arg—Gly—Asp—Ser.

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