However, a reticulocyte reaction was absent, indicating inadequate erythron stimulation and/or a reinforcement of ineffective erythropoesis. Sodium nucleinate is known to be a stimulator of leukopoesis [6], but in the model studied no granulocytopoesis activation was registered in the bone marrow.

The injection of tactivin led to the development of lymphocytosis, an increased cell count in the thymus, and a simultaneous decrease of erythropoesis indicators. At the same time the reaction of granulocytopoesis was revived. This corresponds to data on the effect of the preparation on the bone marrow of untreated rats [2]. The latter probably reflects the phenomena of competitive relationships between immunopoesis and erythropoesis under the influence of tactivin.

The opposite data were obtained in the case of a single injection of the maximal dose of levamisole. This preparation, possessing immunomodulator properties, caused the suppression of all the hemopoesis indicators in the experimental animals, this being one of its known side effects [6].

Thus, use of the immunomodulators resulted in a certain modification of hemopoesis in the animals, vis-a-vis monotherapy with iron. Tactivin caused the activation of granulocytopoesis accompanied by the suppression of erythropoesis, while sodium nucleinate produced another effect, namely a certain erythron stimulation with a slight suppression of leukopoesis in the bone marrow. Levamisole inhibited erythropoesis. Hence, the immunomodulators have a nonuniform effect on the rehabilitative processes in the bone marrow. The mechanisms of such an influence may be diverse: immunological (cell-mediated and antibody-dependent), metabolic, etc. [1,8,9,11]. The data obtained should be taken into account in the pathogenetic substantiation of immunocorrection of hemopoetic disturbances.

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Enchancement of the Oxygen Metabolism of Human Blood Phagocytes under the Influence of Taftsin-like Peptides from a C-Reactive Protein Molecule.

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C-reactive protein (CRP) is one of the main humoral defense factors which have a regulatory effect on cells involved in nonspecific and immune reactions [3,7]. A sharp rise of the serum pentameric CRP (p-CRP) concentration during the first 24-48 h from the beginning of an inflammatory reaction, p-CRP accumulation in the focus followed by disintegration to monomers under acidic conditions in the focus, under the influence of extracellular enzymes, or due to limited proteolysis

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in neutrophils (Nph) occur simultaneously with the activation and attraction to the inflammation focus of blood phagocytes: Nph and monocytes (Mn) [8,10,11].

The purpose of the present work was to study the effect of taftsin-like peptides from the CRP molecule [11] on one of the earliest and most characteristic metabolic changes in neutrophils, namely, changes in the level of their oxygen metabolism [2].

MATERIAL AND METHODS

Taftsin-like peptides from the CRP molecule (TKPL in position 27-30, GKPR in position 113-116, TKPQ in position 200-203 [11], and taftsin TKPR were synthesized by Vector (Novosibirsk).

Nph and Mn were isolated from heparinized donor blood. The mononuclear suspension obtained in accordance to [5] served as a source of Mn. Mn were isolated from lymphocytes by means of adhesion on a plastic surface at 37°C for 1 h, followed by the removal of nonadhering cells. Mn were removed from the plastic surface with 10 mM EDTA during incubation for 30 min at 37°C.

Nph were extracted from the deposit of erythrocytes and granulocytes prepared by fractionation [9]. Erythrocyte impurities were destroyed by lysis with NH_4Cl [6].

The level of oxygen metabolism was determined using the nitro blue tetrazolium reduction method (NBT) adapted to automatic calculations on a multichannel spectrophotometer [12].

Based on the calibration curve reflecting the dependence between the NBT-diformazan concentration and the optical density, as well as the biochemical components of the reaction [2,4], O_2-anion production, expressed in 10^6 cells for 30 min reaction time, was calculated. The statistical processing of the data obtained was performed using an MK-58 microcomputer using developed software [1].

RESULTS

Results shown in Fig.1 attest to the stimulating influence of taftsin and related peptides from the CRP molecule on O_2-anion production by neutrophils. TKPR, TKPQ, and TKPL were found to have a similar effect on intact cells, giving rise to a 1.3-2.2-fold increase in oxygen metabolism in a concentration ranging from 10^{-8} to 10^{-12}M. TKPR was found to show a less stimulating effect under these conditions, resulting in a 1.4-1.7-fold increase compared with the control.

The nature of peptide influence remained under condition of costimulation with medicinal prodigiosin in a final concentration of 15 μg/ml (see Fig.1). In the range of effective concentrations of TKPR,TKPQ, and TKPL (10^{-8}-10^{-10}M) a 1.3-1.8-fold enchancement of Nph oxygen metabolism was detected; however, no essential differences of O_2 production in absolute indexes were found either with or without additional costimulation with prodigiosin. GKPR peptide exerted no reliable influence on the oxygen metabolism of prodigiosin-stimulated Nph.

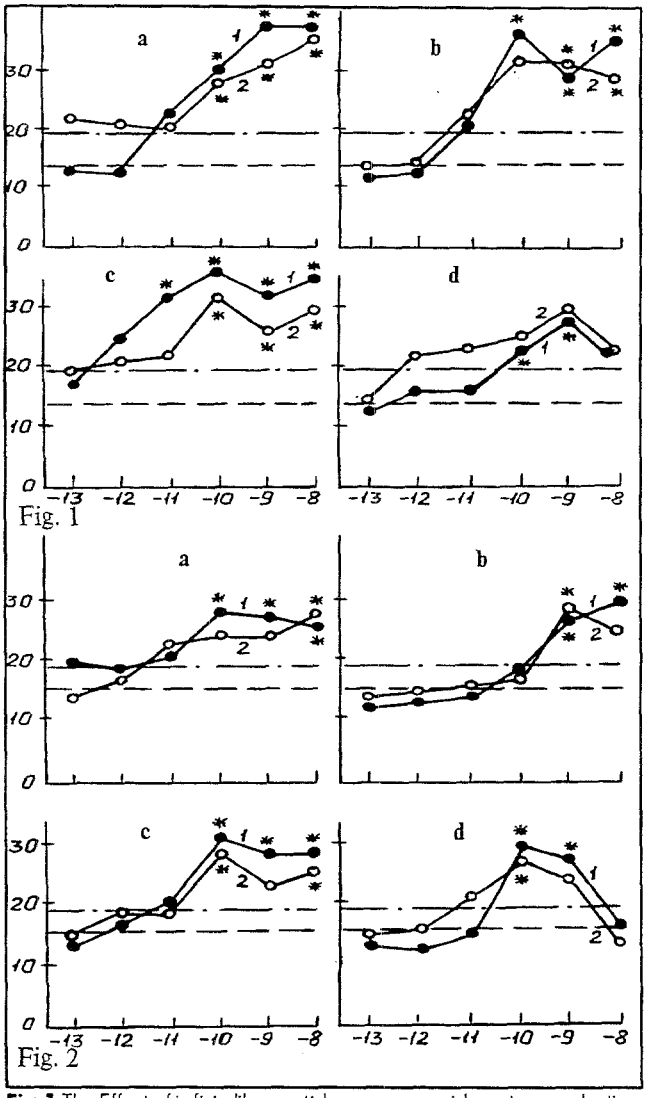

Fig. 1

Fig. 2

Fig.1 The Effect of taftsin like peptides on superoxide-anion production in neutrophils. Abscissa: log of peptide concentration (M); ordinate: O_2-anion production (nM per 10^6cells during 30-min incubation. Horizontal dashed line shows level of O_2 production by control cells (treated with buffered physiological solution instead of peptides); horizontal broken line shows O_2 production by prodigiosin-stimulated cells. Curve 1) non-stimulated cells; curve 2) Nph stimulated by 15 μg/ml prodigiosin; Asterisk: differences from control are reliable at $p<0.05$ in accordance with Fisher's z test. Number of control observations 22; in each experiments, 8; a) TKPR, b)TKPQ, c) TKPL, d) GKPR.

Fig.2 Effect of taftsin ke peptides on superoxide-anion production in monocytes. Notation as for Fig.1.

In Fig.2 the effect of the assayed peptides on superoxide-anion production by Mn is reflected. TKPR, TKPL, and GKPR were found to exhibit the most pronounced influence on the intact and prodigiozin-stimulated Mn, resulting in a 1.4-1.7-fold increase in oxygen metabolism in the concentration range of 10^{-8}-10^{-10}M. The TKPQ effect was less expressed (+45% at a concentration of 10^{-8}M). However, on the whole, the effect of the peptides under concentration on the oxygen metabolism of Mn was found to be less expressed as compared to their influence on Nph. This is confirmed by data on the Mn-peptide interaction under conditions of

prodigiosin costimulation (see Fig.2), allowing us to conclude merely that there is a tendency toward a stimulation of O_2 formation by Mn.

The study confirmed the ability of taftsin like peptides from the CRP molecule to the formation of active forms of oxygen in Mn and Nph of human peripheral blood regardless of the presence of a second stimulating signal. This conclusion is confirmed by the reliable correlation coefficients between the peptide effects on intact and stimulated Nph ($r = 0.86+0.11$) and Mn ($r = 0.85+0.11$); $p<0.001$ in both cases. In addition, the influence of the peptides from the CRP molecule was found to be not inferior to that of taftsin, a well-known activator of blood phagocytes. Therefore, the limited CRP proteolys occurring in the inflammation focus under the influence of intra- and extracellular enzymes of neutrophilic origin may be assumed to regulate phagocyte activity by means of the removal of peptides of different composition.

Peptides split off from the CRP molecule differ in their tropism with respect to phagocytes. While TKPQ and TKPL showthe greatest stimulating effect with respect to Nph, GKPR and TKRL are the most effective with respect to monocytes. Based on differences in effective concentrations on can evaluate the range of peptide influence. The fact that TKPQ show maximum activity in high concentrations (10^{-8} M) is assumed to be evidence for its activity within the bounds of the inflammation focus, whereas GKPR and TKPL are most effective in concentrations ranging from 10^{-10} to 10^{-11}M and probably have a more systemic effect. The alternative explanation of this phenomenon may consist in the differences in the affinity of the sites of different peptides on phagocytes.

Consequently, the immunoregulatory properties of CRP are not only associated with its native molecule or its subunits but are also influenced by the nature of limited proteolysis occurring in the inflammation focus. The removal of taftsin like peptides from the CRP molecule may therefore be assumed to cause the involvement of new cells in protective reactions and act on the distribution of functions between them.

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ONCOLOGY

Contribution of Tumor Necrosis Factor to the Lethality of Mice with Endotoxin Shock Presensitized by Serum from Tumor-Bearing Mice

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A combination of lipopolysaccharide (LPS) and muramyl dipeptide (MDP) administered to tumor-bearing mice has been shown to cause delayed growth and rejection of the tumor [2]. To achieve this effect, however, LPS and MDP have to be given in near-toxic doses, which interferes with their use as

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