

Chemiluminescence and Morphometric Analysis of Neutrophil Granulocytes from the Blood of Patients with Purulent-Septic Complications

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It is demonstrated that the plasma membrane perimeter and the area of neutrophil granulocytes isolated from the blood of patients with a low level of nonspecific protection are significantly greater compared with these parameters in patients with a high level of nonspecific protection. These findings can be used to determine the level of nonspecific protection in patients with purulent-septic complications for correction of therapy.

Key Words: *neutrophil; chemiluminescence; morphometry; purulent-septic complications*

The need to reduce purulent-septic complications (PSC) using current methods calls for the development of a complex system for predicting postoperative complications; such a system should include tests reflecting the state of nonspecific protection of the organism. Being under the strict control of different systems of the organism and interacting with a number of cells, neutrophils (NG), the main component of nonspecific protection, participate in the maintenance of homeostasis and recognize and eliminate microorganisms and their metabolites [1,7]. The response of NG to bacterial metabolites and cytokines is accompanied by the activation of membrane structures and by the involvement of oxygen-dependent protective mechanisms against infection [2,6]. Such metabolic alterations occur not only in response to contact with foreign agents but also in response to a wide variety of biologically active substances contributing to the maintenance of homeostasis. It is important to find out how the structure of NG changes in relation to their functional activity.

Information regarding the relationship between the changes in volume and functional state of neutrophils can be gleaned from the literature. However, it is scarce, controversial, and incomplete and reflects the alterations in cell shape in response to some stimulating (most frequently chemotactic) agent [4,5,9].

We studied how the area occupied by an NG and its nucleus, the perimeters of its plasma and nuclear membranes, and the density of nucleus and cytoplasm change depending on the functional state of the cell. The purpose of the study was to establish morphofunctional characteristics for the determination of the level of nonspecific protection.

MATERIALS AND METHODS

Neutrophils were isolated from the blood of 39 patients with postoperative PSC. The formation of reactive oxygen species (an integral parameter of the functional state of NG) was measured by the method of luminol-dependent chemiluminescence (CL) in a Delta-300 spectrophotometer. The dynamics of spontaneous and stimulated CL was studied. Zymosan served as a stimulating agent. The functional state of NG was assessed from the chemilumines-

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TABLE 1. Morphometric Parameters of Neutrophils Isolated from the Blood of Patients with PSC ($M \pm m$)

Parameter	Low I_{cl} (<20)	High I_{cl} (>100)
Cell area, μ^2	195.744 \pm 6.396*	148.386 \pm 5.043*
Plasma membrane perimeter, μ	53.918 \pm 1.039*	46.584 \pm 1.065*
Cytoplasm density, arb. units	34.764 \pm 2.795	32.131 \pm 3.360
Nucleus area, μ^2	73.907 \pm 2.217*	58.525 \pm 2.626*
Nuclear membrane perimeter, μ	53.977 \pm 2.817*	45.996 \pm 3.472*
Nucleus density, arb. units	16.907 \pm 2.817	15.031 \pm 3.294

Note. *: $p < 0.01$

cence index that reflects the reserve capability of the cells [3]. The chemiluminescence index (I_{cl}) was calculated from the following formula:

$$I_{cl} = (CL_{st} - CL_{sp})/CL_{sp},$$

where CL_{sp} is the maximum spontaneous chemiluminescence and CL_{st} is the maximum stimulated chemiluminescence (cpm). This index reflects the functional state of NG. The morphometric parameters of NG were determined on fixed preparations stained by the method of Romanovskii, using a computer image analyzer and designated software.

RESULTS

The patients were divided into two groups; group 1 with a low ($I_{cl} < 20$) and group 2 with a high ($I_{cl} > 100$) reserve capability of nonspecific protection.

The data presented in Table 1 indicate that the NG of group 2 patients (low spontaneous chemiluminescence of intact NG) have a considerable potential capability to respond to an additional stimulating influence. The areas occupied by these NG and their nuclei as well as the nuclear and plasma membrane perimeters are significantly smaller than the corresponding parameters of NG of group 1 patients (low reserve capability of nonspecific protection). There were no differences in the density of the cytoplasm and nucleus. This may indicate that in PSC the influence of endogenous lipopolysaccharides is directed toward the membranes. The plasma membrane perimeter and

the area of NG increase due to the flattening out of the tiny folds on the plasma membrane which are not seen under the light microscope; this is consistent with published data [8].

Thus, the comparison of the cytometric parameters and functional state of NG confirms that there is a relationship between cell structure and function. Morphometric parameters calculated with the aid of a computer are a valuable characteristic of nonspecific protection and may be used for the prognosis and correction of therapy of patients with PSC.

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