Functional Activity of Human Leukocytes Exposed to a Hypotonic Medium

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This study, in which zymosan-stimulated luminol-dependent chemiluminescence of leukocytes was used to examine the osmotic resistance and functional activity of peripheral blood leukocytes from 23 patients with purulent septic lesions and 11 healthy donors before and after exposure of these cells to a hypotonic medium (0.45% NaCl), showed that this medium stimulated their spontaneous chemiluminescence while reducing their reserve capacities. The effects of the hypotonic medium on cells from the patients were more strongly marked.

Key Words: *leukocytes; chemiluminescence; hypotonic solution*

In the past few years increasing significance has been attached to evaluating the resistance of neutrophilic granulocytes in various disease states [1,2,5, 7,8]. The objective of the study reported here was to explore how hypotonic medium as a nonspecific factor that alters leukocyte activity might influence one of the earliest and most characteristic metabolic changes in phagocytes, namely the levels of their oxygen metabolism and their cytolysis.

MATERIALS AND METHODS

Peripheral blood leukocytes from 23 patients with purulent septic lesions and 11 healthy subjects (donors) were examined. As a nonspecific factor eliciting leukolysis and altering the production of intracellular reactive oxygen species, a 0.45% NaCl solution was used [3], in which the percentage of osmotically resistant leukocytes was calculated by the formula:

$$n = (B/A) \times 100\%,$$

where A is the baseline (pre-exposure) leukocyte count and B is the leukocyte count after a 30-minute exposure to the hypotonic medium. Leukocytes before and after exposure to this medium (at 37°C) were counted conductometrically using a blood analyzer.

Morphological control of the changes undergone by formed elements under the influence of the hypotonic medium was carried out in whole blood smears and in smears prepared from blood samples incubated in the hypotonic solution.

Functional activity of leukocytes was estimated from changes in spontaneous and zymosan-stimulated luminol-dependent chemiluminescence (CL) of intact cells and cells exposed to the hypotonic medium for 30 min and then transferred to isotonic conditions through the addition of more NaCl to the medium. The results of these tests were evaluated by recording both absolute changes in spontaneous and stimulated CL and changes in the chemiluminescence index I_{cl}, which reflects the reserve capacities of leukocytes [6] and is defined by the formula:

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

where CL_{sp} is the maximal spontaneous CL and CL_{st} is the maximal stimulated CL, both expressed in counts per minute (cpm).

RESULTS

Incubation of formed elements of blood in the hypotonic medium led to a strongly marked cytolysis:

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the mean leukocyte count in samples from patients with purulent septic lesions decreased from (5.48±±0.46)×10^9 cells/liter before incubation to (4.14±±0.30)×10^9 after it, i.e., it amounted to 78.2±2.8% of the preincubation value. Comparison of leukocyte functional activity in unexposed samples and those exposed to the hypotonic medium indicated that the osmotically resistant leukocytes were in an active state and retained their potential to reorganize cell metabolism, responding with a "respiratory burst" to the additional stimulation by zymosan.

When the integral functional activity of leukocytes from patients with purulent septic conditions was examined, it was found that CL_{sp} was 4524±±1265 cpm and CL_{st} was 371,590±70,252 cpm, giving an I_{cl} value of 532.1±150.5; recalculation of these CL_{sp} and CL_{st} values on a per leukocyte basis (sample volume 0.2 ml) gave 4.72±1.45 and 331.16±54.07 cpm, respectively.

After exposure to the hypotonic medium, the maximal CL_{sp} reached 12,000±3007 cpm, i.e., it exceeded the pre-exposure level 2.6-fold ($p \leq 0.01$), whereas the maximal CL_{st} was only 14,036±4235 cpm, i.e., decreased by a factor of 26.4 ($p \leq 0.01$); the I_{cl} dropped 7.8-fold to 68.53±42.84. On a per cell basis, spontaneous activity following incubation in the hypotonic medium increased 3.4-fold to 15.97±4.29 cpm, whereas stimulated activity was 166.68±41.39 cpm, which is 1.9 times lower than that shown by the intact leukocyte.

For the group of 11 healthy donors, the following results were obtained before exposure to the hypotonic medium: proportion of osmotically resistant leukocytes, 83±2%; CL_{sp}, 423±113 cpm; CL_{st}, 96,623±21,439 cpm; spontaneous and stimulated activities on a per cell basis, 0.38±0.07 and 69.91±10.46 cpm, respectively. After incubation, CL_{sp} increased to 1828±463 cpm, CL_{st} decreased to 59,428±21,265 cpm, CL_{sp} per leukocyte increased 0.2-fold to 1.83±0.72 cpm, while CL_{st} per leukocyte decreased to 59.43±12.68 cpm.

These figures indicate that incubation in the hypotonic medium altered the functional state of leukocytes, raising integral spontaneous activity and the spontaneous activity per leukocyte, while decreasing the response of these cells to zymosan stimulation.

Comparison of the data obtained for the healthy donors and patients shows that the spontaneous activity of intact leukocytes was lower than that of cells exposed to the hypotonic medium in both groups, but that the difference recorded for the patients is much larger, apparently as a result of priming of the neutrophils by endogenous lipopolysaccharides and other endotoxins present in patients' blood [4].

The present results suggest that exposure to the hypotonic medium altered structural and functional properties of the leukocytes that had not been lysed. The heightened spontaneous activity exhibited by the exposed leukocytes indicates that the activation process involves a complex set of metabolic and biochemical events.

Exposure to the hypotonic medium caused leukocytes to pass into a new functional state, in which they were producing oxygen metabolites that determine cytotoxic and bactericidal functions of the cells. However, the maximal response to zymosan stimulation shown by such leukocytes was lower. Possibly, incubation in the hypotonic medium boosted lipid peroxidation, which prevented the attainment of high CL_{st} values in response to zymosan; this led to a decrease in I_{cl}, which reflects the reserve capacities of leukocytes.

In summary, hypotonic medium, being a destabilizing factor, exerts a lytic action on a certain proportion of leukocytes, and this proportion depends on the condition of their membranes and reflects the resistance and barrier functions of the cells. Exposure to a hypotonic medium produces a stimulating effect on the surviving leukocytes, but their reserves are diminished. The characteristics of leukocyte functional activity described above should be taken into consideration in various disease states that alter the body's homeostasis as well as in the undertaking of measures aimed at its correction.

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