FUNCTIONAL HETEROGENEITY OF CIRCULATING POLYMORPHONUCLEAR LEUKOCYTES IN ACUTE MYOCARDIAL INFARCTION

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Interaction between polymorphonuclear leukocytes (polymorphs) and macrophages with soluble and corpuscular agents converts the cells into an activated state, characterized by the appearance of a wide range of functions: orientation and oriented migration along the gradient of the stimulator, activation of oxidative metabolism (known as the respiratory burst), phagocytosis, and degranulation [14]. Similar functional manifestations arise in vivo in diseases with a course resembling that of specific or nonspecific inflammation (pneumonia, septicemia, myocardial infarction, uveitis, and so on) [2, 5]. The writers showed previously that enhanced functional activity of peripheral blood polymorphs is observed in acute myocardial infarction (MI), as shown by increased production of active forms of oxygen (AFO) by the cells and an increase in the number of Fc-receptors on their surface [2]. The increase in the activity of polymorphs observed in vivo may evidently be due to several causes: first, an increase in functional activity of the whole pool of circulating granulocytes; second, the appearance of a subpopulation of cells possessing greater functional poential in the blood stream. The first suggestion is based on existing data showing that circulating polymorphs are a morphologically homogeneous cell population [14]. However, if it is recalled that, first the life span of polymorphs in the blood stream is 5-6 h [3] and, second, that neutrophilic leukocytosis is observed during MI with a shift of the blood formula to the left [1], the observed increase in functional activity of the polymorphs found in pathology is most probably due to the appearance of a more active subpopulation of granulocytes in the blood. The available factual evidence in support of functional heterogeneity of the polymorphs in diseases with a course resembling that of specific inflammation [5] is limited and to some degree contradictory, whereas in aseptic inflammatory conditions, this phenomenon has not been demonstrated at all.

The aim of this investigation was to study functional heterogeneity of the population of mature human peripheral blood neutrophils under normal conditions and in acute myocardial infarction.

EXPERIMENTAL METHOD

Polymorphs were isolated from heparinized venous blood from healthy blood donors and from patients with acute MI [6], using a Ficoll ("Pharmacia," Sweden) – Verografin ("Spofa," Czechoslovakia; $\rho = 1.077$ g/cm^3) density gradient. Expression of surface Fc-receptors on polymorphs was evaluated by the use of immune complexes (IC), formed by human class G immunoglobulins and fluorescein isothiocyanate (FITC)-labeled monospecific rabbit antibodies against human IgG (Bacterial Preparations Production Enterprise, N. F. Gamaleya Institute of Epidemiology and Microbiology), as the fluorescent marker. Preparations for fluorescence microscopy were made as follows: to a suspension of neutrophils (10^6 cells) 1 ml of a 0.01% solution of NaN_3 in Hanks' solution (pH 7.4) was added to inhibit phagocytosis of FITC-IC. The cells were than washed with Hanks' solution by centrifugation at 200 g for 15 min. To determine binding of the fluorescent marker, 2 ml of FITC-IC (1 mg protein/ml) was added to

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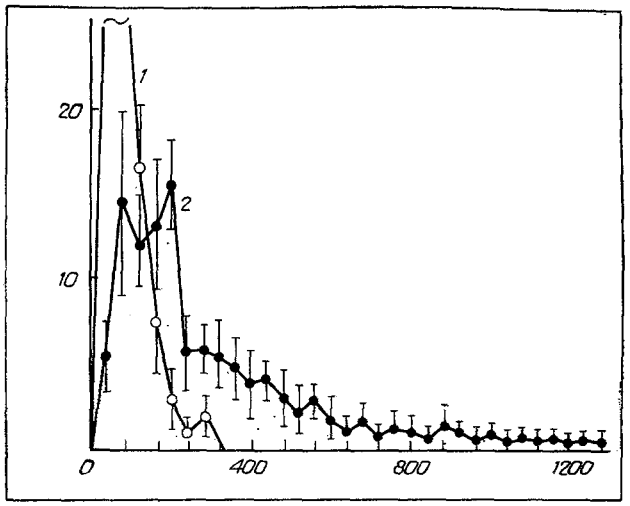

Fig. 1. Histogram of distribution of polymorphs from healthy individuals (1) and patients with acute MI (2) by intensity of F1 of FITC-labeled IC bound with their surface. Abscissa, intensity of F1 (in relative units); ordinate, number of polymorphs in healthy subjects (1) and in patients with acute MI (2), %.

the cell suspension previously treated with sodium azide (10^6 cells). The samples were incubated for 30 min at 37°C. The binding reaction was stopped by diluting the samples with Hanks' solution to 10 ml followed by centrifugation at 200g for 15 min. The supernatant was removed and the sedimented cells were twice washed with Hanks' solution (pH 7.4). The cells were then added to 1 ml of a 50% solution of glycerol in Hanks' solution and carefully resuspended. One or two drops of the cell suspension in glycerol were applied to defatted slides, and coverslips wre fixed with paraffin wax. Fluorescence (F1) of FITC-IC was measured on a "Lyuman I-2" fluorescence microscope, using FS1-4, S3S24-4 and UFS filters to excite F1, and the corresponding interference filters to emit F1. To evaluate the functional activity of the polymorphs, a test of reduction of nitroblue-tetrazolium by superoxide anions to formazan (the nitro-BT test) [10] was used in the modification in [4], using the nitro-BT analog p-nitrotetrazolium violet. For this purpose 5 ml of heparinized blood was incubated in a narrow test tube inclined at 45° at 37°C for 20 min to sediment the erythrocytes. To the leukocyte-enriched plasma a suspension of latex particles was added in the proportion of 0.2 ml to 5×10^5 polymorphs (80 particles per cell) and the samples were incubated for 15 min at 37°C. Films were prepared from the residue of the incubated mixture, dried, and fixed for 1 min with saturated formalin vapor. The films were then transferred for 1 h at 37°C into medium containing 1.25 mg of p-nitrotetrazolium violet, 1.25 mg EDTA-Na, 375 mg sucrose, 425 mg NaCl, and 10 mg NADPH or NADH in 50 ml phosphate buffer (pH 7.2). The films were then washed with distilled water, the cell nuclei were stained for 15 min with 0.5% methyl green solution, after which the films were again washed with distilled water, dried, and the formazan granules in the cells were counted under the microscope. The experimental results were presented in histogram form.

EXPERIMENTAL RESULTS

Investigation of the ability of healthy human polymorphs to bind FITC-IC showed that the distribution of the cells by intensity of F1 of the marker bound to their surface can be represented by a unimodal curve (Fig. 1).

The largest number of cells (38.0 ± 3.28%) was found within the range in which the intensity of F1 was 41-80 relative units. No cells with F1 of more than 300 relative units were found. Fluorometric analysis of polymorphs from patients with MI showed (Fig. 1) that the distribution is bimodal in character, with maxima within the ranges in which the intensity of F1 was 41-80 and 161-200 relative units, and which contained 14.78 ± 5.71 and 15.95 ± 2.31% of cells respectively. A few polymorphs capable of binding 10 times more FITC-IC than normally also were found. The writers showed previously that polymorphs of patients with MI and containing a larger number of Fc-receptors possess stronger functional activity

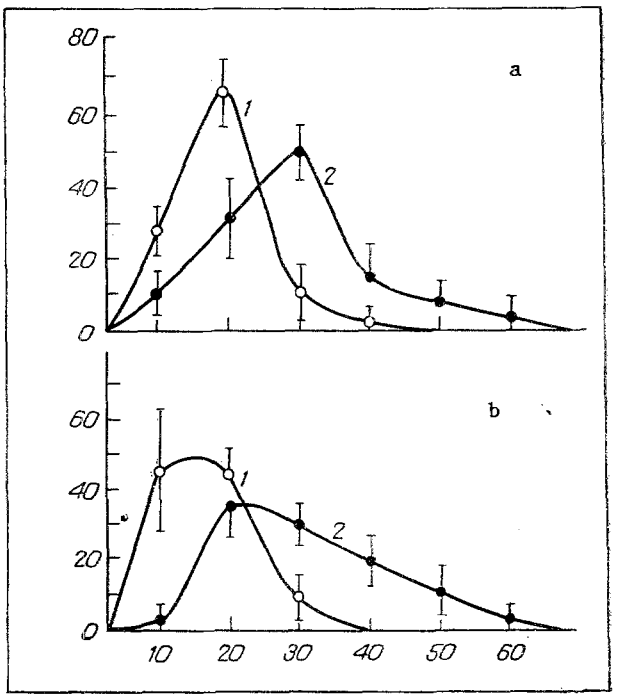

Fig. 2. Histogram of distribution of polymorphs of healthy individuals (1) and patients with acute MI (2) by number of formazan granules formed in them in the presence of NADH (a) or NADPH (b). Abscissa, number of formazan granules in cell; ordinate, number of polymorphs in normal subjects (1) and patients with acute MI (2), %.

[2]. With these observations and the present results in mind, it can be asserted that a subpopulation of polymorphs possessing high functional activity appears in the circulating blood stream of patients with MI. Results similar in character were obtained by the use of the modified nitro-BT test. Figure 2 gives histograms of the distribution of polymorphs from healthy persons and patients with MI depending on the number of formazan granules formed in them in the presence of NADH (Fig. 2a) and NADPH (Fig. 2b). It will be clear from Fig. 2a that the largest number of cells (65.19 $\pm$ 11.2%) from healthy persons in the presence of NADH contained 20 formazan granules per cell, and no cells containing more than 40 granules were found. Meanwhile in patients with MI the largest number of cells (45.33 $\pm$ 8.71%) contained 30 granules of dye per cell and there were a few cells containing more than 40 formazan granules per cell. Similar results were obtained by the use of the modified nitro-BT test in the presence of NADPH (Fig. 2b).

Thus in the process of the onset and development of aseptic inflammation associated with acute myocardial infarction, against the background of neutrophilic leukocytosis a subpopulation of polymorphs appears which possess increased functional activity, reflected in increased production of AFO, expression of surface Fc-receptors, and activity of intracellular oxidases, confirming the concept of functional heterogeneity of the polymorphs.

There is some experimental evidence in the literature of the existence of heterogeneity of polymorphs from the functional point of view. For example, in vitro the presence of at least two subpopulations of cells was discovered on the basis of the following parameters: buoyant density [11]; by change in membrane potential during stimulation of the cells by chemical attractants [13]; by the level of both unstimulated and stimulated (by phorbol myristate acetate, endotoxin, etc.) oxidative metabolism [5]; by expression of Fc-receptors and also of receptors to IgA and formylpeptide [8, 9, 14]; by ability to bind monoclonal antibodies [7]. In investigations in vivo, following intravenous injection of endotoxin, hemodialysis [9], and in acute bacteriemia (septicemia) [5] it has been shown that the degree of functional heterogeneity of circulating polymorphs increases due to the appearance of a subpopulation of cells with enhanced oxidative metabolism and expression of Fc-receptors.

The increase in functional activity of polymorphs in the acute period of MI may be due to several causes, of which one is the phenomenon of "priming," which is to say that cells coming into contact with a small quantity of stimulator increase their functional potential, and this is manifested during repeated stimulation [12].

The appearance of a more active subpopulation of polymorphs in vivo can evidently be attributed to more complex mechanisms, including priming of the circulating cells, and changes which may arise in granulocytopoiesis under pathological conditions.

On the basis of the data obtained we were able to formulate the possible mechanism of the increased functional activity of peripheral blood polymorphs occurring in both clinical and experimental MI, and comprising the following two stages.

1. As a result of the first cycle of ischemia and myocardial reperfusion, a number of substances capable of priming polymorphs may appear in the circulating blood: products of lipid peroxidation, fragments of complement, products of enzymic degradation of arachidonic acid, and so on. Under these circumstances primed cells with a large number of receptors on their surface, and so on, i.e., with higher functional activity, appear in the circulation.

2. An increase in power of the respiratory burst leads to increased production of AGO, interleukins, leukotrienes, and other biologically active substances, capable of acting as initiators of the release of cells from the bone-marrow depots, and as stimulators of granulocytopoiesis, by the cells, as a result of which a neutrophilic leukocytosis develops and more active cells appear from the bone-marrow depots in the circulating blood.

The increase in functional activity of polymorphs during MI is thus heterogeneous in character, and the subsequent development of this pathological process depends on the degree of this heterogeneity or, in other words, on the relative percentage of the active cell population contained in the circulating blood.

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