REACTIVITY OF CIRCULATING HUMAN PERIPHERAL BLOOD NEUTROPHILS

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Granules of neutrophils are involved not only in intracellular degradation of phagocytosed objects, but also in extracellular defensive reactions. These reactions are considered [1, 3, 7, 11, 14] to be effected through the "discharge" of their contents into the extracellular medium. This process, known as intravital secretory degranulation, is currently regarded as a component or, more accurately, the initial stage of any phagocytic act [6, 7, 13, 15]. Its functional significance is not yet clear. All that has been suggested is that it is aimed at rendering harmless any extracellular bacteria or at creating conditions for resorption of an antigen by cells of the mononuclear phagocytic system [5].

There is no doubt that phagocytic and extracellular secretory activity of the neutrophils is most marked in the loose connective tissue, into which they migrate in response to activation of chemotaxic mechanisms. It must not be considered, however, that this property is a feature only of cells outside the circulation, assuming the unlikelihood of their functional "inertia" when brought into contact with circulating factors that are disturbing homeostasis.

These problems have not been adequately reflected in the literature, although their urgent importance for both theory and practice is not in question.

The aim of this investigation was to study the dynamics of distribution of peroxidase-containing granules (PO granules) of the blood neutrophils and the character of their extracellular secretion as parameters of functional activity of the cells, during contact with antigens both outside and inside the circulation.

EXPERIMENTAL METHOD

Leukocytes isolated from the peripheral blood of 22 healthy blood donors and 37 patients with acute pneumonia, in a Ficoll–Verografin density gradient were investigated. Myeloperoxidase (MPO) activity in leukocyte films was detected by Karnovsky's method [16], using DAB as the substrate, and Klebanoff's method [17] was used to determine activity of the enzyme in solution and in a cellular homogenate [17]. The spectrum of peroxidase fractions in the packed leukocytes and in the supernatant was demonstrated by disk-electrophoresis, followed by determination of enzyme activity in gel strips by Schrauwen's method [19]. Experiments to study phagocytosis in vitro were conducted by the method developed by Zhekova [3]. The object of phagocytosis in this case was a suspension ($2 \cdot 10^9$) of a living and killed culture of *Staphylococcus aureus*.

EXPERIMENTAL RESULTS

MPO activity in the granules 5 min after addition of a living culture of *Staph. aureus* to the leukocyte suspension was detected more clearly and more quickly than in intact cells: after exposure of 15-20 min a consider-

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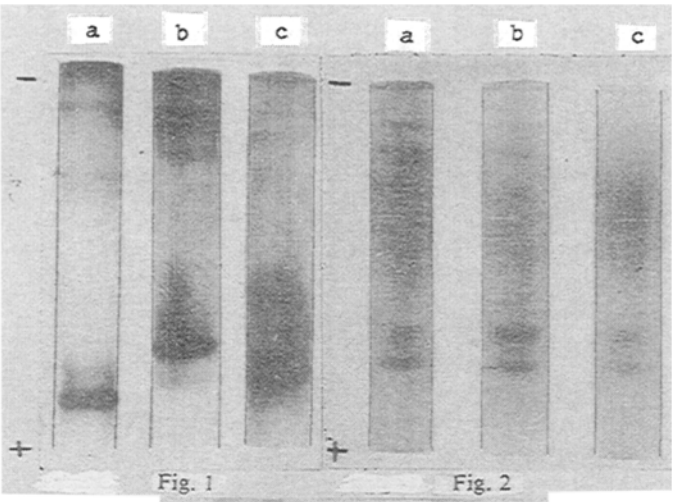

Fig. 1 Fig. 2

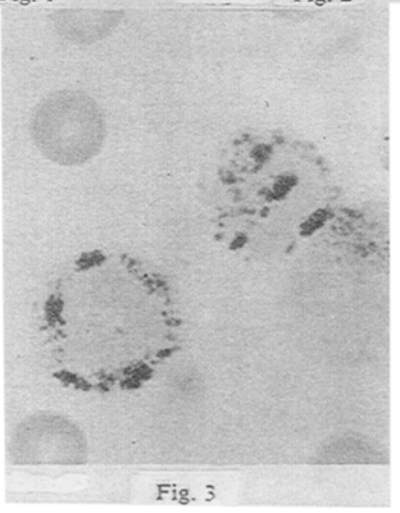

Fig. 3

Fig. 1. Disk electrophoresis of peroxidases of human peripheral blood leukocyte suspension after 3 min of contact with living (a) and killed (b) culture of *Staph. aureus*; c) control. Peroxidase fractions visible at starting (−) end of gel strips.

Fig. 2. Disk electrophoresis of peroxidases in supernatant during phagocytosis by neutrophils in vitro of living (a) and killed (b) cultures of *Staph. aureus*; c) control.

Fig. 3. Film of blood leukocyte suspension from patient with acute pneumonia at height of disease. Reaction for MPO. Signs of degranulation of neutrophils well defined. Objective 90, ocular 10. Photomicrograph.

able diffuse component was present in the cytoplasm, and not until after 30 min could a decrease in the number of granules and, correspondingly, a decrease in MPO activity, be clearly identified. Under these circumstances there was a characteristic unevenness of distribution of the granules in the cytoplasm of the neutrophils, and some zones, especially at the periphery of the cells, were free from enzyme-containing structures. These features, reflecting the character of extra- and intracellular degranulation and its intensity, were more marked after incubation for 1 h.

Contact with a killed culture of microorganisms led only after 60 min to a significant decrease in MPO activity, and granules usually of average clarity were located against a diffuse weak background. Their distribution in the cytoplasm of most cells was uniform.

During phagocytosis of the living microorganisms in vitro activity of the enzyme in the supernatant increased significantly during the first 5-15 min of the experiment, but later its level did not change significantly. An increase in MPO activity in the supernatant also took place when a killed culture of the microorganisms was used. However, it was much smaller than in the first version of the experiments.

Parallel biochemical investigation of a homogenate of the leukocyte suspension clearly revealed conflicting changes in MPO activity at different times of the experiment. During the first 5-10 min, for instance, some increase in activity (5.3 ± 0.03) was observed compared with the control level (5.2 ± 0.02), and continued incubation led to a significant fall.

The spectrum of the peroxidase fractions in the leukocyte homogenate and also in the supernatant was studied by electrophoresis 3 min after contact with the microbial culture. Under normal conditions, four peroxidase fractions, located at the starting end of the gel strip, can usually be determined in a homogenate of the leukocyte suspension: 1) platelet, 2 and 3) monocytic and neutrophilic (myeloperoxidase), and 4) eosinophilic. After addition of the living culture of *Staph. aureus* to the leukocyte suspension, only two fractions of the peroxidase spectrum could be detected: 1 and 3. As the photographs of the gel strips show (Fig. 1a), the volume of the first fraction was much greater than the control, and the third fraction (myeloperoxidase) also was well marked. These fractions, except in a smaller volume, also were determined after contact with the killed culture of microorganisms (Fig. 1b), and in the control gel strips, instead of a microbial suspension, 0.85 NaCl solution was used, the peroxidase spectrum was similar to that in the control.

When the supernatant was used after contact between leukocytes and living bacterial culture, two fractions also could be determined after electrophoresis, corresponding in level to fractions 3 and 4. They were indistinctly outlined when a killed culture of microorganisms was used, and could not be identified at all in the control version of the experiments (Fig. 2).

At the height of the disease the degree of degranulation of the neutrophils in the leukocyte suspension from patients with acute pneumonia was high as early as on the 2nd day. Marked unevenness of distribution of the granules and their aggregation, and a decrease in their density or even their total absence in some parts of the cell cytoplasm were characteristic features (Fig. 3). The pattern of distribution of the PO granules described above continued to be found for 2 and sometimes 3 weeks. At these same times activity of the enzyme was also correspondingly reduced: 1.46 ± 0.01 compared with 2.1 ± 0.01 in the control.

The character of the biochemical changes in MPO activity in the leukocyte homogenate coincided in general with the cytochemical changes: a decrease in MPO activity in the homogenate accompanied by a simultaneous increase in the blood serum.

The results of disk electrophoresis correlate with the observations described above. In particular, a characteristic feature was an increase in activity of fraction 1 in the leukocyte homogenate: 5.7 conventional units compared with the normal level of 3.5, accompanied by a sharp decrease in volume of the remaining three fractions, to 0.2, 3.0, and 0.4 (1.2, 3.1, and 0.8 respectively under normal conditions). At the same time there was an increase in the volume of the fractions in the blood serum. It is difficult, however, to determine to what category of blood cells they belong because of superposition of many haptoglobulin fractions.

The results of these experiments thus show that changes in enzyme activity in the cells and in the extracellular medium, and also the distribution of PO granules in the cytoplasm of the neutrophils during their contact with a microbial culture of *Staph. aureus,* and also of circulating neutrophils from patients with acute pneumonia, are similar.

The first point connected with this similarity is the intensity of the signs of degranulation in both cases, with the result that there was a decrease in the number of granules in the cells and, correspondingly, a decrease in enzyme activity in them. Correlations also were found between the degree of reduction of intracellular MPO activity and an increase in it in the supernatant.

The hypothesis given above also is confirmed by the results of parallel disk electrophoretic investigations. On contact between neutrophils and microbial culture in vitro, as was pointed out above, a myeloperoxidase fraction and a fraction of eosinophilic peroxidase were found in the extracellular medium. Whereas this fact is fully in line with modern views on the course of phagocytic reactions, with the inevitable activation of the degranulation process, the

functional significance of the data showing marked activation under these circumstances of the thrombocytic peroxidase in the leukocyte homogenate is not quite clear. The only possible suggestion is that circulating antigen evidently stimulates conformational changes in peroxidase molecules located in the dense bodies of the platelets, and this may perhaps be the cause of their activation. Whatever the case, these results can be taken as weighty confirmation of the views expressed by some workers on the role of platelets in reactions to antigen.

However, the decrease in the peroxidase activity in the circulating neutrophils of patients and also in experiments in vitro, detected by cytochemical and biochemical methods, cannot be explained purely by exocytosis into the extracellular medium. Some activity also is evidently lost as a result of intracellular phagocytic reactions, although it must be pointed out that the ability of circulating neutrophils to carry out phagocytosis is disputed by some investigators [10, 12, 14]. In our opinion, phagocytosis in the circulation is perfectly possible, for peripheral blood cells, as investigations have shown [2, 4, 8, 9, 18], are morphologically and functionally mature. This has been proved by experiments to study phagocytosis in vitro. Consequently, it must be assumed that on contact between them and the object of phagocytosis, the corresponding reactions must be triggered in the circulation also. It is not clear, however, what in this case is the object of phagocytosis. Most probably it is the products of interaction between microorganisms and tissue structures of the affected organ, although the possibility of neutrophilic attack on circulating microorganisms also cannot be ruled out.

The results described above thus show that the antigen-induced extra- and intracellular degradation of human peripheral blood neutrophils, taking place both in experiments in vitro and in the circulation in patients with acute inflammatory diseases of the lungs, is an indicator of the intensity of functional activity of the peripheral blood neutrophils, and is evidently part of the general reaction of the organism to removal of a circulating antigen.

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