Inhibitors of proteolytic enzymes thus effectively depress proteinase activation during the development of inflammation in the lungs. The further study of the possibility of the clinical use of acid-stable inhibitors which, because of their stability and high antielastase activity, may be effective in the treatment of bronchopulmonary diseases, would seem to be promising.

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EFFECT OF PLATELETS ON GENERATION OF ACTIVE FORMS OF OXYGEN BY LEUKOCYTES

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Active forms of oxygen (AFO), including the superoxide anion-radical $O_2^{\cdot-}$, the hydroxyl radical $OH^{\cdot}$, singlet oxygen $^1O_2$, and hydrogen peroxide, generated by leukocytes, play an important role in the development of several pathological processes [5, 10].

AFO realize their pathogenic action through the initiation of free-radical lipid peroxidation (LPO) and by their direct action on cell components and the intercellular substance (collagen, hyaluronic acid) [9].

During a study of AFO generation by leukocytes under normal and pathological conditions [3, 4, 7] we observed that addition of platelet-deprived plasma (PDP) to the leukocytes stimulates AFO generation by a greater degree than addition of platelet-enriched plasma (PEP). This observation evidently indicated that platelets can influence the stimulating action of plasma on AFO generation by leukocytes. We found a report in the literature of the ability of plasma to stimulate AFO generation by leukocytes [2], but the role of platelets was not analyzed. Only

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in one publication is it noted that "a purified fraction of platelets affects neither the magnitude nor the kinetics of AFO generation by leukocytes" (cited in [2]).

In the light of the above facts, it was decided to study the role of platelets in AFO generation by leukocytes, and the investigation described below was undertaken for this purpose.

EXPERIMENTAL METHOD

AFO generation was studied in leukocytes obtained from the blood of 34 healthy blood donors by two methods: the luminol-dependent chemiluminescence method (ChL-method) [2, 6] on an LKB "Wallac 1251" luminometer at 37°C (18 experiments) and a chemical method – based on the reaction of reduction of nitro-blue tetrazolium (nitro-BT reaction) on a spectrophotometer (16 experiments) [6]. By the ChL method it was possible to determine total AFO generation by the leukocytes. The nitro-BT reaction enabled selective generation of superoxide anion-radicals to be determined from the quantity of formazan formed during the reaction.

The buffy coat was obtained from 8-10 ml of heparinized blood taken from the cubital vein by centrifugation of the blood at 180*g* for 5-7 min or by allowing it to stand for 1.5 h at 4°C. After separation of the blood into layers of plasma, buffy coat, and erythrocytes the first two layers were drawn off and after centrifugation at 600*g* for 10 min, the supernatant consisted of PEP [1]. To obtain PDP, the PEP was centrifuged for 20 min at 2500*g* and the residue thus obtained consisted of packed platelets: platelets 96%, granulocytes 2%, lymphocytes 1%, and erythrocytes 1%. The concentration of platelets was adjusted by the addition of physiological saline, pH 7.35, to 60,000 cells/μl. The residue of leukocytes obtained after the 2nd centrifugation was diluted with physiological saline, pH 7.35, to a concentration of 2500/μl.

The ratio of leukocytes to platelets was therefore 1:25, which is close to the physiological value.

AFO generation by leukocytes was studied in standard volumes: for the ChL investigations 0.2 ml of the leukocyte suspension, 0.2 ml of packed platelets, and 0.2 ml of zymosan, incubated with PEP or PDP, were used; for the nitro-BT reaction, the difference lay in the volume of leukocyte suspension (0.1 ml). The zymosan was prepared and opsonized by the standard method [11]. For opsinization, PEP or PDP from each donor separately was used.

A basal indicator of AFO generation by leukocytes on the addition of PEP and PDP, and stimulated by addition of zymosan, opsonized with PEP or PDT, was studied. In each experiment the increase in the values of the parameters of AFO generation by the leukocytes was calculated as a percentage [6].

EXPERIMENTAL RESULTS

The addition of both PEP and PDP to the leukocytes suspended in physiological saline caused an increase in the basal ChL parameters of AFO generation by leukocytes in all the experiments (18), but PDP had this effect more strongly than PEP. PDP stimulated AFO generation by 2.2 times more than PEP. Similar results were obtained in all the experiments (16) to study the effect of PEP and PDr on generation of the superoxide anion-radical in the nitro-BT reaction. PDP also caused an increase in the parameters studied by 2.4 times more than PEP.

The results obtained by the ChL and chemical methods show that platelets inhibit the stimulating action of plasma on the basal parameters of AFO generation by leukocytes. To explain the inhibitory effect of platelets on AFO generation in plasma, we made two assumptions. According to the first, this process is due to secretion by platelets of a factor which has a direct inhibitory action on AFO generation by leukocytes. According to the second, this is due not to a direct action, but to the uptake of plasma factors stimulating AFO generation by leukocytes, and their blocking by platelets. To test the first assumption experiments were carried out on leukocytes from 10 healthy blood donors, and the effect of their packed platelets on AFO generation by leukocytes was determined.

Experiments using the ChL method showed that platelets increased the ChL parameter of AFO generation in eight of 10 experiments by 1.2 times. These changes were not significant by the parametric test, but were significant by the signs test ($p < 0.05$).

The parameters of AFO generation based on the results of the nitro-BT reaction and under the influence of packed platelets were significantly increased by both statistical tests. These results did not confirm our assumption of a direct inhibitory action of platelets on AFO generation by leukocytes. To test the second assumption, experiments were carried out to study the ability of platelets to adsorb and block plasma factors stimulating AFO generation by

leukocytes. These factors, as we know, include complement, its fractions, and immunoglobulin G [11]. To assess this we compared changes in the parameters of AFO generation by leukocytes caused by zymosan incubated with PEP and PDP, assuming competitive relations between the platelets and zymosan during adsorption of the above plasma factors in the process of opsonization of zymosan. The investigation was conducted by ChL and chemical methods.

In all experiments (18) using the ChL method and in all experiments (16) using the nitro-BT reaction, the parameters of AFO generation by leukocytes rose, but the increase caused by zymosan, incubated with PDP, was higher for the ChL method by 1.4 times and for the nitro-BT reaction by 1.8 times. It follows from these results that platelets in plasma inhibit basal and zymosan-stimulated generation of AFO by leukocytes. At the same time, the difference between the degree of increase of AFO generation in the presence of PEP and PDP can be regarded as indicators of the inhibitory effect of platelets. Our results suggest that platelets realize their inhibitory action through adsorption of stimulating plasma factors. The biological significance of this phenomenon may perhaps lie in the protection of the endothelium and of themselves against the damaging action of AFO by platelets.

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