MECHANISM OF SUPPRESSION OF HUMAN PERIPHERAL BLOOD LYMPHOCYTES BY PLATELET ACTIVATION FACTOR

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Platelet activation factor (PAF), which has the structure 1-O-alkyl-2-acetyl-Sn-glycero-3-phosphocholine, is a phospholipid mediator of allergic reactions of immediate type. PAF under these circumstances exhibits a broad spectrum of biological activity against cells present in the blood stream. For example, it has been found that PAF stimulates histamine release from human blood basophils [1] and chemotaxis of human blood leukocytes [2]. PAF has a cytotoxic action on strain HL-60 of human leukemic cells [4] and suppresses spontaneous proliferation of K-562 human erythromyeloleukemia tumor cells [3].

It has recently been shown that PAF can exert a regulatory action on cells of the immune system. It has been found, for instance, that PAF has a suppressor action on proliferation of human peripheral blood lymphocytes [3, 5]. The mechanism of PAF-induced suppression of lymphocytes is therefore an interesting problem for study.

The aim of this investigation was to study the role of certain mononuclear cell populations in PAF-induced suppression of lymphocyte proliferation and its dependence on cyclo-oxygenase metabolites of arachidonic acid.

EXPERIMENTAL METHOD

Peripheral blood was taken from patients with bronchial asthma (pollen and dust forms) during remissions of the disease. The group of patients consisted of nine men and nine women aged from 20 to 40 years. The group of healthy blood donors consisted of six subjects (three men and three women) aged from 22 to 32 years.

Mononuclear cells were isolated by centrifugation in a one-step Ficoll–Hypaque density gradient. The population of T lymphocytes was separated from other lymphocyte populations by the rosette-formation method with sheep's red blood cells, treated with neuraminidase [6]. Mononuclear cells were cultured in a 96-well panel (1×10^5 cells/200 µl) for 72 h in the presence of PAF in concentrations of 100 or 1000 ng/culture. The cells were cultured in vitro in medium RPMI-1640 with additives, as described previously [3]. The cells were activated with PHA ("Difco") in a concentration of 10 µg/ml. Indomethacin ("Sigma") in a concentration of 1 µg/ml was used as cyclo-oxygenase inhibitor.

The intensity of cell proliferation was judged by incorporation of ^{3}H-thymidine into the cell DNA. ^{3}H-thymidine (1 µCi) was added to the cultures 4 h before the end of incubation. After incubation the cells were transferred to the glass fiber filters of a "Harvester" apparatus. Radioactivity was measured on a "Mark III" liquid scintillation counter. The index of suppression (IS) of lymphocyte proliferation was determined by the following equation:

$$IS\,(\%) = \left(1 - \frac{\text{number of cps in experiment}}{\text{number of cps in control}}\right) \times 100.$$

To remove adhesive cells (monocytes) the cells were incubated in a concentration of 10^7/ml in medium RPMI-1640 containing 5% embryonic calf serum, in plastic Petri dishes at 37°C for 1.5 h and then washed off with the medium.

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TABLE 1. Effect of PAF on Proliferation on Peripheral Blood Lymphocytes from Patients with Bronchial Asthma

	PAF concentration (ng/culture)			
	100	1000	1000 + indomethacin*	
	IS (%)			
Total lymphocyte population	−14,4±4,4	−20,6±1,2	+6,6±3,2	$p<0,01$
Lymphocytes after removal of monocytes	+6,7±0,5	+8,7±1,5	—	$p<0,01$

Legend. *) Concentration of indomethacin in medium was 1 μg/ml.

PAF was obtained by hydrogenation of natural beef heart plasmalogen—lecithin, deacylation, and subsequent acetylation of the alkyllysophosphatidylcholine with acetic anhydride as described previously [1]. The PAF antagonist BL 8705, which is an analog of PAF of lipid nature, was synthesized at the Research Institute of Biomedical Technology, Ministry of Health of the USSR.

The experimental results were subjected to statistical analysis. The arithmetic mean with confidence interval for p = 0.05 was calculated from the results of three of four experiments. The significance of differences between mean values was determined by Student's test.

EXPERIMENTAL RESULTS

Taking account of the fact that PHA is a mitogen for the mature T-cell population, it might be supposed that the phenomenon of suppression of human lymphocyte proliferation described previously [3] is due to the effect of PAF on the T lymphocyte population. To test this hypothesis, blood lymphocytes from patients with bronchial asthma were divided into a T lymphocyte population and a fraction of lymphocytes not containing T cells, after which the action of PAF was studied on the two lymphocyte populations. It was found that PAF if added to a cell culture enriched with T lymphocytes, had a suppressor action only on the T lymphocyte population, similar in character to the effect of PAF on the unfractionated lymphocyte population. For example, in the unfractionated lymphocyte population the percentage of suppression under the influence of PAF in a concentration of 1000 ng/culture was 76.5-81.0, whereas in the T cell population it was 73.9-84.0% of the initial value.

Since adhesive cells (population of macrophages/monocytes) are extremely essential for realization of the functions of T and B cells, it was interesting to assess their contribution to the suppressor effect of PAF. As Table 1 shows, the character of action of PAF on blood lymphocytes from patients with bronchial asthma after removal of the cell population adherent to plastic, was abruptly changed. In this case PAF in concentrations of 100 and 1000 ng/culture could no longer induce suppression of lymphocyte of proliferation but exhibited the opposite effect, i.e., it induced stimulation of lymphocyte proliferation. In blood lymphocytes obtained from healthy donors, the same picture was observed. Removal of adhesive cells (monocytes) led to reversal of the response to PAF (1000 ng/culture) from −16.9 ± 2.2% to +8.6 ± 0.7 (IS). In the absence of PAF removal of monocytes from the blood lymphocyte population both from healthy donors and from patients with bronchial asthma did not affect the level of proliferative activity initiated by PHA. It can thus be concluded that peripheral blood monocytes both from healthy blood donors and from patients with bronchial asthma are the cells through which the suppressor action of PAF on lymphocyte proliferation is realized.

Some human and animal blood cells are known to have receptors for PAF on their surface; activation of the cells by PAF is realized, moreover, after binding of PAF with its receptors. Consequently, by blocking these receptors with PAF antagonists, it ought evidently to be possible to abolish or to considerably weaken the phenomenon of suppression of lymphocyte proliferation induced by PAF. To test this hypothesis, blood lymphocytes from patients with bronchial asthma and preincubation with the PAF antagonist BL 8705 in a concentration of 1000 ng/culture for 30 min. The cells were then washed 3 times with medium and again incubated with PAF. The results showed that BL 8705 blocked suppression of lymphocyte proliferation induced by PAF virtually completely (by 94-100%). Similar results also were obtained with blood lymphocytes from healthy donors. In this case BL 8705 also blocked inhibition of lymphocyte proliferation induced by PAF virtually completely (by 97.7-100%).

The inhibitory action of some immunosuppressors, such as histamine, is mediated through increased biosynthesis of prostaglandins. As Table 1 shows, the cyclo-oxygenase inhibitor

indomethacin, in a concentration of 1 μg/ml, abolished suppression of proliferation of lymphocytes from bronchial asthma patients, induced by PAF. Thus the effect of suppression by PAF may be connected with the fact that it induces stimulation of prostaglandin biosynthesis in monocytes; prostaglandins, especially those of type E, moreover, exhibit a marked suppressive effect on lymphocyte proliferation. However, the mechanisms of suppression of lymphocyte proliferation by PAF are evidently more varied and complex, for it has recently been shown that PAF suppresses the formation of interleukin-2, which is essential for PHA-stimulated lymphocyte proliferation, by lymphocytes [7]. The results of the present investigation and data in the literature indicate that PAF can be regarded as a new lipid bioregulator of cells of the immune system.

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SELECTIVE EFFECT OF CHORIONIC GONADOTROPHIN ON LYMPHOCYTE SUBPOPULATIONS

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Research workers are currently concentrating their efforts on the search for substances capable of regulating at will the immune response by selective action on individual stages of immunogenesis or on individual subpopulations of immunocompetent cells. The study of the immunomodulating ability of chorionic gonadotrophin (CG) is interesting from this point of view. The present writers [1, 3] and others [6-11] showed previously that CG has a dose-dependent inhibitory action in vitro and in vivo on cell-mediated immune responses. Data on the effect of CG on individual subpopulations of immunocompetent cells are not to be found in the literature.

The aim of the investigation described below was accordingly to study the effect of CG on different lymphocyte subpopulations: natural killer (NK) cells, suppressor T cells, effector T_{dth} cells.

EXPERIMENTAL METHOD

Pharacopoeial CG of Soviet origin and purified CG with biological activity of 30,000 IU/mg, obtained from the Moscow Endocrine Factory, were used. The doses used were comparable with physiological concentrations of CG determined in the blood in the early stages of pregnancy. There were four series of experiments in which 300 CBA and C57BL/6 mice were used. The effect of CG on induction and formation of specific suppressor T cells was studied by the method in

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