EFFECT OF PHOSPHOLIPID PLATELET ACTIVATION FACTOR ON PROLIFERATION OF PERIPHERAL BLOOD B LYMPHOCYTES OF NORMAL INDIVIDUALS AND BRONCHIAL ASTHMATICS

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Platelet activation factor (PAF), or 1,0-alkyl-2-acetylglycero-3-phosphocholine, is a lipid mediator of immediate type. Its biological effects as a mediator of allergic reactions are realized through activation of circulating cells (basophils, neutrophils, monocytes, platelets) and of the lung macrophages [4]. It has recently been shown that PAF has a regulatory action on peripheral blood lymphocytes and has a suppressor effect on PHA-stimulated proliferation in a total lymphocyte population [2, 3, 7] or in the SD4+ subpopulation of T lymphocytes [5]. PAF can also exert an immunoregulatory action by modulating cellular secretion of lymphokines (interleukins 1 and 2) or other mediators (prostaglandins, tumor necrosis factor), affecting cells of the immune system [4]. Although PAF is a recognized mediator of allergic reactions and inflammatory processes, its effect on cells of the immune system in various allergic or other diseases connected with the immune system is virtually unknown.

In the investigation described below the effect of PAF on lymphocyte proliferation induced by pokeweed mitogen (PWM) was studied in normal blood donors and also in patients with atopic bronchial asthma (ABA) and with infectious-allergic bronchial asthma (IBA).

EXPERIMENTAL METHOD

Peripheral blood was obtained from patients with ABA (of pollen and dust types) and IBA during a period of quiescence. The group of patients consisted of 14 men and 35 women aged from 20 to 50 years. The group of healthy individuals consisted of 12 persons (six men and six women) aged from 20 to 35 years.

Mononuclear cells (MNC) were isolated by centrifugation in a one-step Ficoll-Hypaque gradient. MNC were cultured in a 96-well planchet (10^5 cells per well) for 7 days in the presence of PAF in concentrations of 10 pg to 10,000 ng per culture. Medium RPMI-1690 with additives, as described previously [2], was used for cell culture in vitro. The cells were activated by PWM ("Servan", which is a T-dependent polyclonal B-cell activator, in a concentration of 2000 ng per culture. Indomethacin ("Serva") in a concentration of 1 g/ml was used as cyclo-oxygenase inhibitor. The intensity of cell proliferation was judged from incorporation of ^{3}H-thymidine into cellular 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 glass-fiber filters by a "Harvester" apparatus. Radioactivity was measured on a Mark III liquid scintillation counter. To remove adhesive monocytes, MNC in a concentration of 10^7 cells/ml were incubated in medium RPMI-1640 containing 5% fetal calf serum, in plastic Petri dishes at 37°C for 1.5 h, and then washed off with medium. PAF was obtained by hydrogenation of natural beef heart plasmalogen-lecithin, deacylation, followed by acetylation of the alkyl-lysophosphatidylcholine with acetic anhydride, as described previously [1]. The experimental results were subjected to statistical analysis. The results of three or four experiments were used to calculate the statistical mean with confidence interval for $p = 0.05$. The significance of differences between the means was determined by Student's test.

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TABLE 1. Comparison of Results of Action of PAF on Level of Proliferation of Blood Lymphocytes from Normal Individuals and Patients with ABA and IBA

PAF to culture	Normal blood donors	Patients with ABA	Patients with IBA
	Degree of proliferation, percent of control		
0,01—10	103,3±1,3	104,4±2,5	77,5±2,7
100	115,3±2,4	124,2±5,0	77,9±2,9
1000	185,5±3,5	185,7±12,3	91,0±4,2
5000	15,5±3,7	196,8±17,6	177,2±17,0
10000	7,0±1,0	64,6±8,9	25,3±7,2

Legend. Averaged results of 3-4 experiments shown. Degree of incorporation of ^{3}H-thymidine in control groups was 4000-20,000 cpm/10^5 cells for healthy blood donors, 3000-22,000 cpm/10^5 cells for blood lymphocytes from patients with ABA, and 8000-16,000 cpm/10^5 cells for blood lymphocytes from patients with IBA.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that against a background of PAF administration within the concentration range of 0.1-10 ng per culture, the level of activated proliferation of healthy human blood MNC remained unchanged, and was the same as the corresponding level of proliferation in patients with ABA. However, in patients with IBA this level was approximately 30% lower.

The study of the effect of PAF on the proliferative response of PWM-initiated peripheral blood lymphocytes from normal blood donors revealed dose-dependent activation of the proliferative response, the maximal activity of which corresponded to a concentration of PAF of 1000 ng per culture, and which averaged 200% of the initial level (PWM+ PAF-). With a further increase in the dose of PAF activation was replaced by dose-dependent suppression of the proliferative response. A concentration of PAF of 5000-10,000 ng per culture caused virtually total suppression of proliferation. It must be stated that the effect of a change in cell proliferation was not connected with the cytotoxic action of PAF, for the survival rate of the cells, as shown by the trypan blue test, was virtually identical in the control (without PAF) and the experimental (with PAF) cultures. Our results on the effect of PAF on B-cell function of normal blood donors confirmed data obtained previously by other workers [7].

Investigation of the effect of PAF on the proliferative response of initiated peripheral blood lymphocytes from patients with ABA showed that the character of the response resembled that in healthy blood donors. Initially, for instance, dose-dependent potentiation of the proliferative response was found, but this began to be manifested as a lower PAF concentration than in healthy subjects, namely 100 ng per culture. Maximal potentiation of proliferation was observed with concentrations of 1000 and 5000 ng per culture. The level of proliferation in some cases reached 300% of the initial value or more, higher than the corresponding values in healthy blood donors. Starting with a concentration of 10,000 ng per culture, dose-dependent suppression of proliferation took place. However, only in rare cases was this suppression total, even with a high PAF concentration: 10,000 ng per culture.

Investigation of the effect of PAF on the level of proliferation of peripheral blood lymphocytes from patients with IBA revealed a lower initial level of activated proliferative response compared with healthy donors. PAF activity was shown to begin to be manifested when its concentration in the extracellular medium was 1000 ng per culture, and reached a maximum with a concentration of 5000 ng per culture. A dose of 10,000 ng per culture did not give total suppression of proliferation.

Comparison of the time course of proliferation of the different groups of MNC showed that MNC from patients with ABA are more reactive than those from normal donors: they react more strongly to PAF in a dose of 100 ng and they greatly exceed the response to doses of 5000 and 10,000 ng. At the same time, the response of MNC of patients with IBA to PAF differed from that both of healthy donors and of patients with ABA. The initial sensitivity of MNC to PAF in patients with IBA was considerably depressed compared with the sensitivity of MNC of other groups: in response to the action of PAF in a con-

centration of 1000 ng the degree of the response of MNC was 50% lower. Sensitivity of MNC in response to the action of PAF in a concentration of 10,000 ng exceeded the sensitivity of MNC from healthy donors, but was less than the sensitivity of MNC of patients with ABA. To sum up the results showing the effect of PAF on the level of proliferation of blood MNC from healthy subjects and patients with ABA and IBA, it can be postulated that PAF induces dose-dependent activation and suppression of proliferation, and that there is a marked difference in the character of the changes in these three groups. Our data on the effect of PAF on the level of proliferation of B-cells from healthy blood donors confirm those obtained by other workers [7], suggesting an immunoregulatory function of PAF toward both T-cells, as was shown previously, and B-cells.

In the study of the mechanism of the effect of PAF on lymphocytes initiated by PWM, and on the basis of a previous study [3], we investigated the possible role of monocytes and prostaglandins. Adhesive cells (macrophages/monocytes) are known to be very important for realization of T-cell and B-cell functions. We studied the possibility of their involvement in suppression induced by PAF. After removal of adhesive cells the character of the response of B-lymphocytes from patients with ABA to the action of PAF was found to change. For instance, whereas PAF in a concentration of 1000 ng per culture activates the total lymphocyte population on average by 120 ± 10.1%, after removal of the monocytes activation was enhanced to 147.6 ± 9.6% ($p < 0.05$). It can accordingly be concluded that activation of blood cells of the proliferative response takes place with the active participation of monocytes. Monocytes and macrophages are known to contain biologically active substances with a powerful effect on lymphocyte proliferation. Prostaglandin E_2, for instance, has a strong immunosuppressive action on lymphocyte proliferation. Indomethacin, a cyclo-oxygenase inhibitor, in a concentration of 1 μg/ml, is known to enhance lymphocyte activation against a background of the action of PAF in a concentration of 1000 ng per culture by 200.4 ± 106.1% (compared with 165.2 ± 39.0% without the addition of indomethacin). Despite the more intensive activation of proliferation, the difference was not significant ($p > 0.05$).

Thus when the mechanism of the suppressor action of PAF is assessed, its role in the suppression of monocytes and, to a lesser degree, of prostaglandins, can be postulated.

The mechanism of suppression is evidently not simply one involving the two factors, but participation of lymphokines also is possible. It was shown previously that PAF can suppress the proliferative response of lymphocytes, initiated by PHA [2, 7]. Considering results of the present investigation, we can postulate a broadening of the immunomodulating function of PAF relative not only to T-, but also to B-lymphocytes.

The possibility cannot be ruled out that PAF, by modifying B-lymphocyte proliferation, may exert an influence also on immunoglobulin synthesis by these lymphocytes. Synthesis of IgE and IgG de novo by B-lymphocytes of patients with ABA has been demonstrated by the method described previously [8]. PAF in a concentration of 50 $\mu g/10^6$ cells has been shown to influence immunoglobulin synthesis by B-lymphocytes of patients with ABA in 7-day culture: IgE synthesis was increased to 200%, whereas IgG synthesis in the same cultures was depressed by 60%.

It is thus logical to suggest that the considerable changes caused by PAF in both B- and T-cells are further proof of its involvement in immunoregulation, and as yet another mediator of immediate allergy in addition to histamine and other immunoactive mediators in the mechanism of the immunologic changes associated with bronchial asthma.

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