PLATELET ACTIVATION FACTOR INHIBITS IRRITANT-INDUCED MIGRATION OF LEUKOCYTES INTO THE MOUSE PERITONEAL CAVITY

A. G. Melikyants, O. N. Kut'kova, and I. I. Votrin UDC 612.339.612.112.2].064:612.111.7.063].085.1

KEY WORDS: platelet activation factor; leukocytes; migration; inflammation

Platelet activation factor (PAF; 1-alkyl-2-acetyl-sn-glycero-3-phosphocholine) produced by many cells, is one of the most powerful mediators of inflammatory and allergic reactions [3]. The mechanism of its action in vivo has begun to be studied only comparatively recently. The results have been contradictory. In particular, the role of PAF in infiltration of the inflammatory focus by leukocytes is not clear. It has been shown, on the one hand, that intradermal injection of PAF induces accumulation of these cells in the rabbit [5, 6] and human [2] dermis, and on the other hand, that intraperitoneal injection of PAF does not increase the number of neutrophils in peritoneal washings [5, 9], whereas an increase in the number of these cells in the peritoneal cavity is a characteristic effect of most known irritants and chemical attractants.

To continue the study of the role of PAF in cellular reactions of inflammation we studied its effect on leukocyte accumulation in the mouse peritoneal cavity in the absence and presence of irritants.

The high-molecular-weight irritants which we used, namely carrageenan and a copolymer of acrylic acid and pentaerythritol (carbopol), induced a typical reaction after intraperitoneal injection, namely accumulation of leukocytes in the peritoneal cavity; neutrophils predominated in the washings 6 h after injection, and macrophage after 72 h.

Intraperitoneal injection of PAF 1 h before the irritants did not change their effect. Conversely, injection of PAF simultaneously with the irritants or 3 and 6 h after their injection appreciably inhibited leukocyte accumulation in the peritoneal cavity induced by these irritants, and/or increased their adhesion to the peritoneum.

The results cannot be regarded as evidence in support of a role for PAF in the inflammatory reaction, as a direct chemical attractant [2, 4]. It has been suggested that these effects are due to changes in leukocyte adhesion under the influence of PAF.

EXPERIMENTAL METHOD

Male BALB/c mice weighing 19-21 g were kept under ordinary conditions. A solution of PAF was prepared immediately before use by diluting a concentrated alcoholic solution of this substance with Hanks' solution. The final ethanol concentration in the injected solutions did not exceed 1%. Carrageenan and carbopol, of Soviet manufacture, also were dissolved in Hanks' solution to concentrations of 1 and 20 mg/ml respectively, the pH was adjusted to 7.4, and the samples were sterilized. The gels of these irritants thus obtained were injected intraperitoneally (0.5 ml), either alone or together with PAF. After an assigned time peritoneal washings were obtained with the aid of the nutrient medium lactalbumin-hydrolysate by the standard method [8] and of the total concentration of cells in the suspension (in a Goryaev's counting chamber, total count) and the number of neutrophils, macrophages, and lymphocytes (on films stained by Romanovsky's method: differential count) were counted For every experimental group of animals there was a corresponding control group. The number of animals in each group was 5-7. Statistical analysis was carried out by Student's t test and by nonparametric statistical tests.

Institute of Cell Biochemistry and Physiology, Biotechnology Center, Ministry of Health of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 5, pp. 481-483, May, 1991, Original article submitted July 25, 1990.

0007-4888/91/0005-0619$12.50

TABLE 1. Kinetics of Change in Cell Concentration in Peritoneal Washings after Intraperitoneal Injections of PAF in the Dose Range 0.1-1.0 μg/Mouse (M ± m)

Dose, μg/mouse	Cell concentration in peritoneal washings (in %) at different times after injection of PAF			
	5 min	1 h	6 h	72 h
—	100,0±7,0	100,0±10,3	100,0±10,6	—
0,1	85,0±9,8	94,0±10,6	76,0±3,0	—
0,5	62,0±4,4*	87,0±9,3	86,0±10,0	—
1,0	88,0±5,0	103,0±12,6	76,0±4,5	116,0±9,0

Legend. Number of cells in control (injection of Hanks' solution) at each time of observation taken as 100%. *$p < 0.05$ compared with corresponding control.

EXPERIMENTAL RESULTS

In the series of experiments whose results are given in Table 1, cells were counted in peritoneal washings obtained 5 min and 1 and 6 h after injection of PAF in doses of 0.1-1.0 μg per mouse. A statistically significant decrease in the number of leukocytes was observed only after 5 min and only with one dose (0.5 μg per mouse). The duration of this effect did not exceed 1 h. The opposite effect, namely accumulation of cells, was not observed in response to injection of PAF.

Injection of irritants into the peritoneal cavity as a rule led to accumulation of cells in it (Table 2). Carrageenan, a polysaccharide of natural origin, and extracted from Irish moss, is the classical agent used to induce inflammation. Carbopol is a water-soluble synthetic polymer, widely used as a carrier and stabilize of drugs, and which is also a powerful adjuvant [1]. The results obtained by the use of these dissimilar polymers were qualitatively similar.

Injection of PAF 1 h before injection of one of them (carbopol), in four series of experiments whose results are given in Table 2, did not affect cell accumulation in the peritoneal cavity induced by it.

In the experiments of series II PAF was incorporated into the structure of gels formed by these polymers in the chosen concentrations, and the two components (PAF and one of the polymers) were injected simultaneously. The result was appreciable inhibition of induced accumulation of both neutrophils (up to 6 h) and macrophages (after 72 h, Table 2).

In the experiments of series III PAF was injected 3 h after induction of chemotaxis, and cell washings were obtained after a further 3 h, at the time of maximal accumulation of neutrophils. Cell accumulation was found to be inhibited in this case also.

In the experiments of series IV PAF was injected 6 h after the irritants, and washings were obtained after only 15 min. The number of cells in the peritoneal washings obtained from animals receiving the irritants and PAF again proved to be less than in the peritoneal washings of control animals receiving irritants alone.

PAF is known to be inactivated in vivo in the course of some tens of minutes [3]. The results of the experiments of series I and the data in Table 1 confirmed this conclusion: 1 h after injection of PAF in solution the effect of this substance either was eliminated or was not exhibited at all. However, after its incorporation into gels of both irritant polymers PAF exhibited activity for at least 3 days. This prolongation of the effect was probably due to gradual release of PAF from the gels.

The results show that PAF causes virtually no change in the number of cells in the peritoneal cavity, unless they have been subjected to preliminary activation by irritants. However, induced migration of both neutrophils and macrophages is appreciably inhibited. This effect may be due to the fact that PAF prevents the outflow of cells from the blood stream, as shown by the experiments of series III, in which PAF was injected into the peritoneal cavity during its active colonization by neutrophils. Meanwhile the decrease in the number of cells washed from the peritoneal cavity 5-15 min after injection of PAF could hardly be linked with the inhibition of their accumulation.

The process of immigration of leukocytes from the blood stream into the tissues involves other constantly interchanging stages such as the formation and destruction of adhesive connections with other cells and with the matrix. The mechanisms of monitoring of their adhesive behavior is only just beginning to be studied. Yet it is clear that dominance of one stage may prevent the process as a whole from being completed, due, for example, to increased adhesion of the

TABLE 2. Changes in Cell Concentration in Peritoneal Washings (in %) after Injection of High-Molecular-Weight Irritants and PAF in Various Combinations and under Different Conditions (M ± m)

Series of experiments	Time of injection of PAF	Time of measurement	Agent used			
			carbopol	carbopol + PAF	carrageenan	carrageenan + PAF
I	60 min before injection of irritants	6 h after injection of irritants	221,0±21,1	177,0±19,6	—	—
II	Simultaneously with irritants in composition of gel	The same	243,0±21,7	127,0±11,7*	212,0±11,2	135,0±9,1*
		72 h after injection of irritants	529,0±35,3	160,0±8,1**	166,0±11,5	133,0±7,9*
III	3 h after injection of irritants	6 h after injection of irritants	185±20	94±12*	—	—
IV	6 h after injection of irritants	6 h 15 min after injection of irritants	201±31	117,0±15,6*	162±7,0	118,0±9,0*

Legend. Mean values of cell concentration in peritoneal washings in control taken as 100% in each series of experiments. *$p < 0.05$ compared with action of irritant and combined action of irritant with PAF.

leukocytes to the vascular endothelium or to other cells It can be suggested that this cause would lead to rapid (in the course of 5-15 min) disappearance of the cells from the peritoneal cavity This hypothesis indirectly confirms the fact discovered previously in vitro that adhesion of neutrophils to endotheliocytes is increased by the action of PAF [7, 10]. Increased adhesiveness of the leukocytes due to PAF can be explained both by inhibition of their induced accumulation, as revealed in the experiments of series III, and by a decrease in their number on washings immediately after injection of this mediator, as was found in the experiments of series I and IV. Prolonged release of PAL from the gels into the peritoneal cavity probably prevented accumulation there of leukocytes, induced by these same gels in the experiments of series II.

It can naturally be suggested that the effect of PAF on the adhesive interactions of leukocytes with surrounding cells and the matrix ought to depend on this environment and it may differ, for example, in the skin and the peritoneal cavity. This hypothesis could explain the opposite effects of PAF on leukocyte accumulation in these two tissues.

LITERATURE CITED

1. M. T Alyushin, K. V. Alekseev, and V. N. Li, Farmatsiya, No. 1, 71 (1986).
2. C. B. Archer, F. M. Cunningham, and M. W. Greaves, J. Invest. Derm., **91**, No 1, 82 (1988).
3. P. Braquet, L. Touqui, T. Y. Shen, et al., Pharmacol. Rev., **39**, 97 (1987)
4. I. G. Colditz and H. Z. Movat, J. Immunol., **133**, No. 4, 2169 (1984).
5. F. Q. Cunha, N. E. Heluy-Neto, A. Klein, et al., Agents and Actions, 25, No. 1/2, 191 (1988).
6. D. M. Humphrey, D. J. Hanahan, and R. N. Pinckard, Lab Invest., **47**, No 3, 227 (1982).
7. T. M. McIntyre, G. A. Zimmerman, and S. M Prescott, Proc. Nat. Acad. Sci. USA, **83**, 2204 (1986).
8. D. O. Adams et al., Methods for Study of Mononuclear Phagocytes, New York (1981), p. 64.
9. B. A. Spicer, P. A. Hatt, and H. Smith, Int. Arch. Allergy, **85**, 364 (1988).
10. M. G. Tonnensen, D. C. Anderson, and T. A. Springer, Fed. Proc., **45**, No. 3, 379 (1986).