Platelet-Activating Factor Modulates Interleukin-6 Production by Mouse Fibroblasts

P.	Pierre	Braquet
B.	Bernadette	Pignol
T.	Thierry	Maisonnet
J.M.	Jean Michel	Mencia-Huerta

Institut Henri Beaufour, Les Ulis, France

Abstract

We compared the effects of platelet-activating factor (PAF), interleukin-1 β (IL-1 β) and polyriboinositic-polyribocytidylic acid (poly-I:C) on IL-6 production by confluent L929 mouse fibroblasts. At concentrations above 1 μ M, PAF dose-dependently enhanced IL-6 production; at 5 μ M PAF this increase (72.7 ± 19.9 U/ml) was higher than that evoked by 100 U/ml IL-1 β (5.7 ± 0.4 U/ml) or 50 μ g/ml poly-I:C (39.3 ± 6.7 U/ml). The IL-6 production induced by 5 μ M PAF was not inhibited by addition of the specific PAF antagonist BN 52021 (10 μ M) to the incubation medium. These results demonstrate that, as this is the case for IL-1, PAF also modulates IL-6 production.

Correspondence to: Dr. Pierre Braquet, Institut Henri Beaufour, 17, Avenue Descartes, F-92350 Le Plessis Robinson (France)

\

Introduction

Although the primary source of interleukin-6 (IL-6) appears to be the monocyte/macrophage [1,2], the production of this cytokine is also induced in fibroblasts on stimulation with polyriboinositic-poly-ribocytidylic acid (poly-I:C), IL-1 and tumor necrosis factor [3, 4]. We have previously demonstrated that the phospholipid mediator platelet-activating factor (PAF) can modulate the production of IL-1 [5]. Indeed, PAF either increases or decreases IL-1 activity in cell supernatants from lipopolysaccharide-stimulated rat macrophages according to its dose and period of addition. As accessory cells produce both IL-1 [6] and IL-6 [1], we investigated whether PAF could also modulate the IL-6 activity generated by mouse fibroblasts.

Methods

The mouse embryonic fibroblast cell line L929 was cultured in DMEM containing 10% FBS. Cell cultures (2.5×10^6 ml) were incubated at 37 °C in multiwell dishes for 1 week, when the cells reached confluence. The medium was removed and 5 ml of fresh medium was added. Various agonists at defined concentrations were added, namely IL-1 β , poly-I:C, lyso-PAF (the inactive metabolite of PAF), PAF, DMSO, BN 52021 (a specific PAF antagonist) or the vehicle of PAF, i.e. DMEM containing 0.25% BSA. After incubation at 37 °C for 4, 22, 28 or 48 h as specified, the culture media were centrifuged at 400 g for 10 min, and the supernatants were collected and stored at -20°C prior to determination of IL-6 activity. No effect of the solvent of PAF or DMSO on the IL-6 release was noted. IL-6 activity was measured using the 7TD1 cell line bioassay (Dr. J. Van Snick, Brussels, Belgium), as previously described [7].

Results

After incubation for 48 h at 37 °C in the presence or in the absence of the various agonists, IL-6-like activity in the supernatants was measured using the IL-6-dependent cell line 7TD1. At concentrations above 1 µM, PAF enhanced in a dose-dependent fashion the production of IL-6 by mouse fibroblasts; at 5 µM PAF this increase (72.7 ± 19.9 U/ml) was higher than the one evoked by 100 U/ml IL-1β (5.7 ± 0.4 U/ml) or 50 µg/ml poly-I:C (39.3 ± 6.7 U/ml). The increase in IL-6 activity induced by 5 µM PAF was observed from 22 h after incubation with L929 cells. Addition of the anti-IL-6 antibody 6B4 in the bioassay on 7TD1 cells abrogated the cell proliferation induced by the various supernatants, demonstrating that IL-6 was indeed involved. Surprisingly, lyso-PAF (5 µM) increased IL-6 activity to the same extent as the one obtained with 5 µM PAF. The IL-6 activity induced by 5 µM PAF was not inhibited when the specific PAF antagonist BN 52021 (10 µM) was added to the incubation medium of L929 cells.

166

Braquet/Pignol/Maisonnet/Mencia-Huerta

Discussion

In the present study, we confirm that the fibroblast cell line L929 is capable of producing IL-6-like activity and that this production is enhanced by IL-1β, poly-I:C and PAF. The increase in IL-6 activity observed with lyso-PAF is puzzling although it could be due to the presence of acetyl-CoA:1-0-alkyl-sn-glyceryl-3-phosphorylcholine acetyltransferase in fibroblasts. The fact that 10 µM BN 52021 did not block the increase in IL-6 production induced by 5 µM PAF was unexpected; however, this might be due to an unfavorable antagonist/agonist ratio (2:1). A higher dose of BN 52021 could not be used due to the toxic effect of the vehicle (DMSO) on L929 cells.

The increase in IL-6 activity induced by PAF is in keeping with the fact that this cytokine modulates various cell functions [8]. The release of IL-6 by vascular endothelium and fibroblasts in response to mediators elaborated in areas of tissue damage may contribute to the local inflammatory response. For instance, high levels of circulating IL-6 have been observed in various inflammatory states such as severe burns [9]. Thus, the capacity of PAF to induce IL-6 production may represent a mechanism by which fibroblasts may participate in inflammatory and immune reactions.

Van Damme J, Opdenakker G, Simpson J, Rubira R, Cayphas S, Vink A, Billiau A, Van Snick J: Identification of the human 26KD protein, interferon-beta-2, as a B cell hybridoma/plasmacytoma growth factor induced by interleukin 1 and tumor necrosis factor. *J Exp Med* 1987; 165:914.

Van Damme J, Cayphas S, Opdenakker G, Billiau A, Van Snick J: Interleukin 1 and Poly (ri) Poly (re) induce production of a hybridoma growth factor by human fibroblasts. *Eur J Immunol* 1987;17:1.

Pignol B, Henane S, Mencia-Huerta J-M: Effect of platelet-activating factor and its specific receptor antagonist, BN 52021 (ginkgolide B), on interleukin-1 (IL-1) release and synthesis by rat spleen adherent monocytes; in Braquet P (ed): *The Ginkgo-lides*. Barcelona, Prous, 1988, vol 1, pp 703–712.

Gery I, Waksman B: Potentiation of thymus derived lymphocyte response to mitogen. Part 2. The cellular sources of potentiating mediators. *J Exp Med* 1972;136:143.

Van Snick J, Cayphas S, Vink A, Uyttenhove C, Coulie P, Simpson R: Purification and NH₂-terminal amino acid sequence of a T cell derived lymphokine with growth activity for B cell hybridomas. *Proc Natl Acad Sci USA* 1986;83:9679.

Wong G, Clark S: Multiple actions of interleukin-6 within a cytokine network. *Immunol Today* 1988;9:137.

Nijsten M, De Groot E, Ten Dius H, Klasen H, Hack C, Aarden L: Serum levels of interleukin-6 and acute phase responses. *Lancet* 1987;ii:921.

References

Aarden L, De Groot E, Schaap O, Lansdorp P: Production of hybridoma growth factor by human monocytes. *Eur J Immunol* 1987;17:1411.

Van Damme J, Van Beeumen J, Decock B, Van Snick J, De Ley M, Billiau A: Separation and comparison of two monokines with LAF activity (interleukin-1 beta and hybridoma growth factor): Identification of leucocyte-derived HGF as interleukin-6. *J Immunol* 1988;140:1534.