Immunology 1991 **72** 181–187 ADONIS 001928059100032B

Platelet-activating factor (PAF-acether) enhances the concomitant production of tumour necrosis factor-alpha and interleukin-1 by subsets of human monocytes

P. E. POUBELLE, D. GINGRAS,* C. DEMERS,* C. DUBOIS,* D. HARBOUR, J. GRASSI† & M. ROLA-PLESZCZYNSKI* *Inflammation and Immunology-Rheumatology Research Unit, Centre de Recherche du Centre Hospitalier de l'Université Laval Ste-Foy, QC, Canada, the †Immunopharmacology Section, Department of Biology, CEN-Saclay, Gif-sur-Yvette, France and *Immunology Division, Department of Pediatrics, Faculty of Medicine, University of Sherbrooke, Sherbrooke, QC, Canada*

Accepted for publication 22 October 1990

SUMMARY

The production of the cytokines tumour necrosis factor (TNF) and interleukin-1 (IL-1) by human monocytes was analysed following their stimulation with muramyl dipeptide (MDP; 1 μg/ml), in the absence or presence of graded concentrations of platelet-activating factor (PAF). Significantly enhanced production of both TNF and IL-1 was observed at two concentration ranges of PAF: a major enhancement was observed at 10^{-8}–10^{-6}M and this was blocked by the PAF antagonist BN 52021 (10^{-4}M). A second enhancement was observed at 10^{-15}–10^{-14}M PAF, which was not blocked by BN 52021. Monocytes isolated either by adherence or counterflow elutriation had similar responses to PAF. The biologically inactive precursor-metabolite, lyso-PAF, had no effect on cytokine production. PAF was shown to augment the production of both bioactive TNF and IL-1 and immunoreactive TNF-α and IL-1α and β. Fractionation of monocytes on a discontinuous Percoll gradient yielded a denser subpopulation, which responded preferentially to higher PAF concentrations, while the less dense subpopulation responded to both concentration ranges. These data indicate that PAF can modulate monocyte functions as related to cytokine production, and may thus contribute to amplification of inflammatory reactions and regulation of immune responses by interacting with subsets of human monocytes.

INTRODUCTION

When monocytes or macrophage are appropriately activated by inflammatory, phagocytic or toxic stimuli, they produce a variety of substances which comprise cytokines, such as interleukin-1 (IL-1)[1] and tumour necrosis factor (TNF),[2] and lipid mediators such as the arachidonic acid metabolites prostaglandins (PG)[3] and leukotrienes (LT)[4] and the phospholipid platelet-activating factor (PAF).[5–7] There is actually increasing evidence suggesting that the production of these substances may be inter-related: IL-1 and TNF induce PG synthesis in various cells[8–10] and PG, in turn, down-regulate cytokine production.[11,12] In contrast, we have shown in specific experimental conditions that LT can augment IL-1[13] and TNF[14] production and that endogenous LT production may play a role in TNF synthesis.[15]

IL-1 and TNF can also induce synthesis of PAF in several cells types, including endothelial cells, neutrophils and macrophages.[16,17] We and others have shown that PAF can, in turn, augment IL-1 production by rat and human monocytes/macrophages,[18–21] suggesting a positive feedback loop at this level, with potential for amplification of immune or inflammatory responses.

TNF and IL-1 have several synergistic or overlapping activities.[22] Moreover, both TNF and PAF are potent mediators of numerous inflammatory responses, such as endotoxic shock[23] and bowel necrosis,[24] and TNF may play a role in immune modulation[25] and anti-tumour defences.[26] PAF has also been proposed as a potent immunoregulatory mediator.[27,28]

In the present report, we present evidence that PAF enhances the production of both IL-1 and TNF by subsets of human monocytes in a bimodal manner.

Abbreviations: LT, leukotriene(s); PAF, platelet-activating factor, 1-O-hexadecyl-2-acetyl-sn-glycero-3-phosphocho-line; lyso-PAF, 1-O-hexadecyl-sn-glycero-3-phosphocholine.

Correspondence: Dr M. Rola-Pleszczynski, Immunology Division, Faculty of Medicine, University of Sherbrooke, 3001, 12th Avenue north Sherbrooke (Québec), Canada J1H 5N4.

MATERIALS AND METHODS

Chemical reagents

PAF (hexadecyl-) and lyso-PAF were obtained from Bachem (Torrance, CA). BN 52021 [9H-1, 7a-(epoxymethano)-1H, 6aH,

cyclopenta(c) furo (2-3-6-)furo-(3,2:3,4) cyclopenta (1,2-d) furan-5,9,12-(4H)-trione, 3-tert-butyl hexahydro 4,-76,-11 hydroxy-8 methyl] was a gift from Dr P. Braquet, Institut H. Beaufour (Le Plessis Robinson, France). Muramyl dipeptide (MDP: N-acetyl-muramyl-L-alanyl-D-isoglutamine) was obtained from Behring Diagnostics (La Jolla, CA).

PAF and lyso-PAF were dissolved in ethanol followed by RPMI-1640 medium containing 2·5 mg/ml human serum albumin. Final ethanol concentrations in assays were < 0·08%. MDP was dissolved in saline.

Preparation of monocytes

Human venous blood from healthy medication-free volunteers was collected on citrate/dextrose/adenine. Cell preparation was performed at room temperature. The platelet-rich plasma was removed after centrifugation of the blood at 250 ***g*** for 15 min and the cell pellet was diluted 1/1 with 0·9% NaCl solution before dextran sedimentation (Dextran T-500; Pharmacia Fine Chemicals, Uppsala, Sweden) of the erythrocytes. The leucocyte-rich supernatant was centrifuged (350 ***g***, 10 min), the pellet was resuspended in isotonic solution, layered over a Ficoll–Paque (Pharmacia) cushion, and centrifuged at 700 ***g*** for 20 min according to the method of Böyum.[29] The peripheral blood mononuclear leucocytes (PBML) were collected at the interface and were washed twice in Ca^{2+}/Mg^{2+}-free Dulbecco's phosphate-buffered saline (PBS). Mononuclear cells were then purified by either adherence or counterflow centrifugal elutriation depending on the experiments.

Purification by adherence

PBML were suspended in RPMI-1640 medium with 10% heat-inactivated foetal bovine serum (FBS). The cells were then allowed to adhere to the surface of plastic Petri dishes previously coated with baby hamster kidney cell microexsudate. This was effective in enriching the adherent population to > 90% monocytes as assessed by non-specific esterase staining. This population was removed with EDTA (0·01 M) in RPMI-1640 medium and resuspended in RPMI-1640 medium, 5% FBS, at a final concentration of 1×10^6 cells/ml. This concentration proved to be optimal in our assays. The cells were allowed to rest overnight to allow them to return to baseline activity following initial activation by adherence. The cells were then incubated for 24 hr in the absence or the presence of MDP (1 μg/ml), BN 52021 (10^{-4}M) or PAF (10^{-16}–10^{-6}M) alone or in combination. After incubation, the cell suspensions were centrifuged and the cell-free supernatants were used for the TNF and IL-1 assays. In selected experiments, monocytes were further fractionated on a discontinuous Percoll gradient. Five 2-ml aliquots of Percoll/PBS of densities 1·045, 1·050, 1·055, 1·060 and 1·065 were overlaid with $20–25 \times 10^6$ monocytes in 1 ml and centrifuged at 400 ***g*** for 25 min. Bands 1 + 2 correspond to cells at 1·045 and 1·050 interfaces, and bands 3 + 4 correspond to cells at 1·055 and 1·060 interfaces.

Purification by counterflow centrifugal elutriation

PBML were purified using a Beckman JE-6 elutriator rotor.[30] The elutriation buffer consisted of Ca^{2+}/Mg^{2+}-free PBS supplemented with 0·25% bovine serum albumin (BSA) and 2 mM EDTA. The cells were injected into the separation chamber at a flow rate of 8 ml/min and constant rotor speed of 2500 r.p.m. Platelets were washed out after 1/2 hr of elutriation at the injection flow rate. Fractionation of PBML was achieved by increasing the flow rate from 8 to 20 ml/min using steps of 1 ml/min (leading to lymphocytes) and from 20 to 25 ml/min with steps of 0·25 ml/min (leading to enriched monocyte fractions). Fifty millitres of medium were collected at each step, centrifuged (350 ***g***, 10 min) and the cell pellet was washed once in isotonic solution and finally resuspended in the incubation medium. Enriched monocyte fractions (lymphocytes < 20%) were pooled and adjusted to 1×10^6 monocytes/ml. Incubations were performed in 12 × 75 mm sterile polypropylene culture tubes at 37° under humidified atmosphere with 5% CO_2. Differential cell counts of leucocytes were performed by (i) flow cytometry (EPICS-C, Coulter, Hialeah, FL) using forward angle and right angle light scatter characteristics;[30] (ii) non-specific esterase and Wright's stains; and (iii) phase-contact microscopy analysis for platelet contamination, which was found to be less than one platelet per 10 monocytes in pooled fractions of enriched monocytes. Cell viability was routinely assessed by trypan blue exclusion test and was greater than 95%. Incubation medium was RPMI-1640 supplemented with L-glutamine, 5% FBS and pen-strep; Ca^{2+} and Mg^{2+} chlorides were added at final concentrations of 2 and 0·5 mM, respectively.

Purified monocytes were then cultured in the absence or presence of 1 μg/ml MDP at 37°, in a humidified atmosphere of 5% CO_2. In preliminary experiments, this MDP concentration proved to be optimal for IL-1 and TNF production.

Thymocyte proliferation assay for IL-1

The IL-1-like material in monocyte supernatants was assessed by using the mouse thymocyte proliferation assay.[31] Briefly, $1·5 \times 10^6$ C3H/HeJ mouse thymocytes were cultured in a final volume of 200 ml in RPMI-1640 medium containing 7·5% FBS with dilutions of monocyte supernatants in the absence or presence of PHA (0·05%). Appropriate controls contained medium, MDP or PAF or antagonists, alone or in combination. Cultures were incubated for 66 hr in a humidified CO_2 atmosphere at 37°, pulsed with 1 μCi of [^{3}H]thymidine (specific activity 2 Ci/mmol; New England Nuclear, Boston, MA) and were harvested at 72 hr onto glass fiber filters with a Skatron cell harvester. Total cell-associated radioactivity was measured in a Beckman scintillation spectrophotometer, and bioassay data are expressed as units per ml, derived from a standard curve with human rIL-1β (Genzyme, Boston, MA). Rabbit anti-human IL-1β antibodies (Genzyme) neutralized > 94% of experimental IL-1-like activity.

Enzyme immunoassay for IL-1α and IL-1β

The measurements of IL-1α and IL-1β were performed using two highly specific enzyme immunometric assays (EIA) with acetylcholine esterase (AChE) as label.[32] Briefly, selected monoclonal anti-IL-1α and 1β antibodies were used for a two-site immunometric assay. The EIA was performed in 96-well microtitre plates coated with one of the monoclonal antibodies (mAb). Covalent conjugates of mAb coupled to the tetrameric form of AChE from electric eel *Electrophorus electricus* were used as tracers. AChE activity was measured using the colorimetric method of Ellman and the absorbance at 414 nm of each well was assessed using an automatic reader Multiscan MCC Titertek (Flow Lab. Mississauga, Ontario, Canada). One-hundred μl of IL-1 solution (as a recombinant standard or a sample) were added to wells together with 100 μl of mAb-AChE

conjugate. Reactions were then allowed to proceed overnight at 4° with agitation. Plates were then extensively washed using phosphate-Tween buffer. Solid-phase bound AChE activity was determined by addition of 200 μl of Ellman's reagent. Both EIA for IL-1α and IL-1β detected concentrations as low as 2 pg/ml without any significant cross-reactivity (<0·01%) for the other IL-1 and other cytokines.[32] Concentrations of each IL-1 were obtained by comparison to a standard curve drawn from measurements of homogeneous human recombinant IL-1α or IL-β (a gift from Dr A. Shaw, Glaxo, Geneva, Switzerland) diluted in the incubation medium RPMI-1640 with 5% FBS.

TNF assay

L929 cells (ATCC, Bethesda, MD) were used as target cells in the TNF assay. This bioassay was performed essentially as described elsewhere.[33] Briefly, L929 cells were seeded into flat-bottomed 96-well microtitre plates (Costar, Cambridge, MA) at a density of 5×10^4 cells per well. After overnight incubation, spent medium was removed and 100 μl of twofold dilutions of human monocyte supernatants were added to each well. One-hundred of microlitres of medium containing actinomycin D were then added at a final concentration of 5 μg/ml. Plates were incubated for 20 hr, medium removed and cells stained with crystal violet (0·5%) in methanol/water (:4 v:v) for 10 min. The amount of cell lysis was determined by measuring absorbance at 540 nm using a microplate EL 310 Autoreader. Percentage of cytotoxicity of a given dilution against L929 cells was calculated using the following formula:

$$\%\ \text{cytotoxicity}_{dil}: \quad \frac{A_{cont} - A_{dil}}{A_{cont}} \times 100$$

where percentage cytotoxicity$_{dil}$ is the amount of L929 cell destruction at a particular dilution, A_{cont} = absorbance in control wells and A_{dil} = absorbance at a particular dilution of human monocyte supernatants. A standard of human recombinant (r)TNF was used (Cistron, Pine Brook, NJ) and results are expressed as units of TNF, based on the rTNF dilution curve. Anti-TNF-alpha antibody (rabbit anti-human TNF-alpha, polyvalent, 10,000 neutralizing units/ml: 1/40 final dilution; Endogen, Boston, MA) was used to identify the monokine being assayed on L929 target cells. Neither PAF, PAF antagonists nor their vehicles had any direct effect on L929 cells.

Enzyme immunoassay for TNF-α

Production of TNF-α was also measured in terms of immunoreactive TNF-α using a commercial ELISA immunoassay (Endogen).

Statistical analysis

Statistical significance of differences between group of experiments was assessed by Student's *t*-test for paired data. Confidence level was at $P < 0.05$.

RESULTS

When adherence-purified human monocytes were exposed to graded concentrations of PAF, enhanced production of TNF was observed in response to 1 μg/ml MDP (Fig. 1a). Two distinct peaks of enhanced activity were actually evident at PAF concentrations of 10^{-15}–10^{-13}M and 10^{-9}–10^{-7}M. TNF production was significantly augmented by 50–90% in monocyte

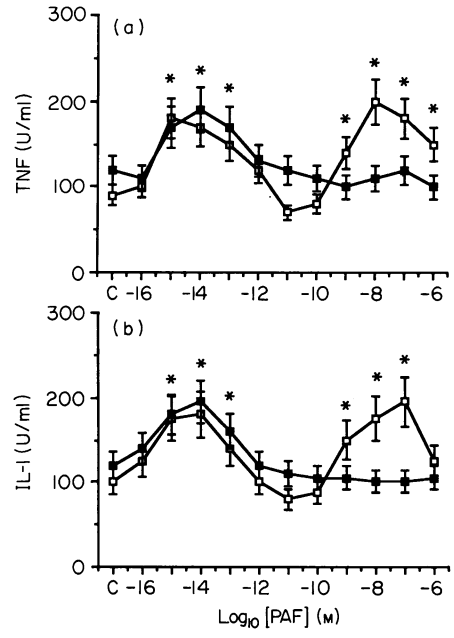

Figure 1. Production of TNF (a) and IL-1 (b) by human monocytes, isolated by adherence to microexsudate-coated plastic dishes, stimulated with MDP (1 μg/ml) and incubated for 24 hr in the absence (□) or presence (■) of 10^{-4}M BN 52021 and graded concentrations of PAF. Data represent means ± SEM of six experiments. Asterisks indicate significant differences ($P < 0.05$) between control (C) and PAF-stimulated cultures. TNF and IL-1 production in the absence of MDP was 22 ± 2 and 18 ± 2 U/ml, respectively. BN52021 had no effect on these levels.

supernatants. Cell-associated TNF was, however, undectable (data not shown). Under identical conditions, concomitant production of IL-1 was also augmented by PAF, with two peaks of activity at identical concentrations (Fig. 1b). Lyso-PAF, the biologically inactive precursor metabolite of PAF, had no effect on either TNF or IL-1 production (data not illustrated).

When monocyte cultures were pretreated for 5 min with the PAF receptor antagonist BN 52021 (10^{-4}M) before addition of PAF and MDP, enhanced production of TNF (Fig. 1a) and IL-1 (Fig. 1b) was effectively blocked at PAF concentrations of 10^{-9}–10^{-7}M, while only partial, non-significant reduction was observed at the lower concentrations of PAF. Similar results were observed with a structurally different PAF antagonist, WEB 2086 (10^{-5}M, gracious gift of Dr H. Heuer, Boehringer, Mannheim, FRG).

Monocytes isolated by counterflow elutriation also showed two peaks of enhanced production of TNF in the presence of 10^{-15}–10^{-14}M and 10^{-8}–10^{-6}M PAF (Fig. 2). Responses of elutriated monocytes to PAF in terms of IL-1 production were also similar to those of adherence-purified monocytes. Pretreatment with BN 52021 blocked the augmented TNF and IL-1 production at higher PAF concentrations, while having no effect on enhanced cytokine production at lower PAF concentrations (data not illustrated).

Analogous results were obtained using a commercial ELISA-immunoassay for TNF-α and the newly developed EIA for IL-1 α and β (data not shown). Interestingly, PAF was found to stimulate TNF (Fig. 3) and IL-1 (Fig. 4) production in elutriate monocytes in the absence of MDP [all reagents were

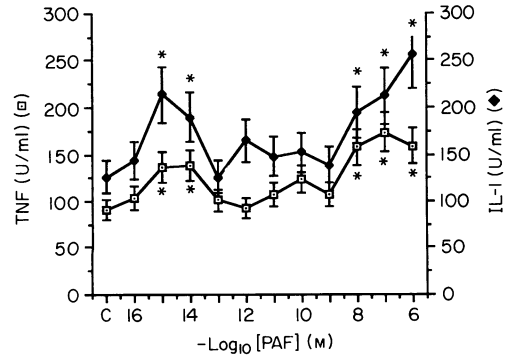

Figure 2. Comparative production of TNF (□) and IL-1 (◆) by human monocytes isolated by elutriation, stimulated with MDP (1 μg/ml) and incubated for 24 hr with increasing concentrations of PAF. Data represent means ± SEM of four experiments. Asterisks indicate significant differences ($P < 0.05$) between control (C) and PAF-stimulated cultures. TNF and IL-1 production in the absence of MDP was 21 ± 3 and 15 ± 2 U/ml, respectively.

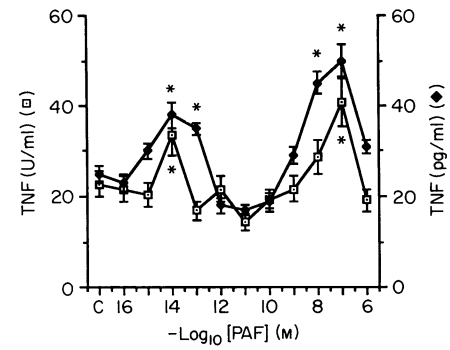

Figure 3. Comparative measurements of immunoreactive TNF-α and bioactive TNF in same supernatants of human monocytes isolated by elutriation and incubated for 24 hr with increasing concentrations of PAF in the absence of MDP. Data represent means ± SEM of four experiments. Asterisks indicate significant differences ($P < 0.05$) between control (C) and PAF-stimulated cultures.

endotoxin free (< 0·1 ng/ml) by the Limulus amoebocyte assay], as measured both by bioassays and immunoassays. Addition of rabbit anti-human TNF-α and IL- 1β antibodies abolished by greater than 90% the bioassay values for TNF and IL-1, respectively.

Fractionation of monocytes on a discontinuous Percoll gradient yielded a denser subpopulation which responded preferentially to the higher degree range (10^{-8}–10^{-6}M) of PAF concentrations (bands 3+4, Fig. 5): TNF production was significantly enhanced at 10^{-8}–10^{-6}M PAF, while no effect was observed at lower PAF concentrations, in contrast to bands 1+2 or unfractionated monocytes. Table 1 presents data on phenotypic characterization of the subpopulations. Most markers are equally represented in both denser (bands 3+4) and less dense (bands 1+2) subsets, except for a newly developed

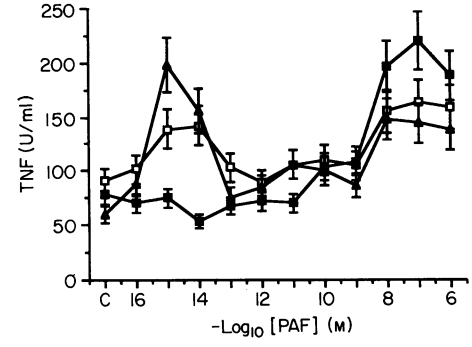

Figure 5. Production of TNF by monocytes, unfractionated (□) or fractionated on a discontinuous Percoll gradient and stimulated with MDP (1 μg/ml) and PAF. Bands 1+2 (▲) correspond to cells recovered from 1·045 and 1·050 g/ml and bands 3+4 (■) correspond to cells recovered from 1·055 and 1·060 g/ml interfaces.

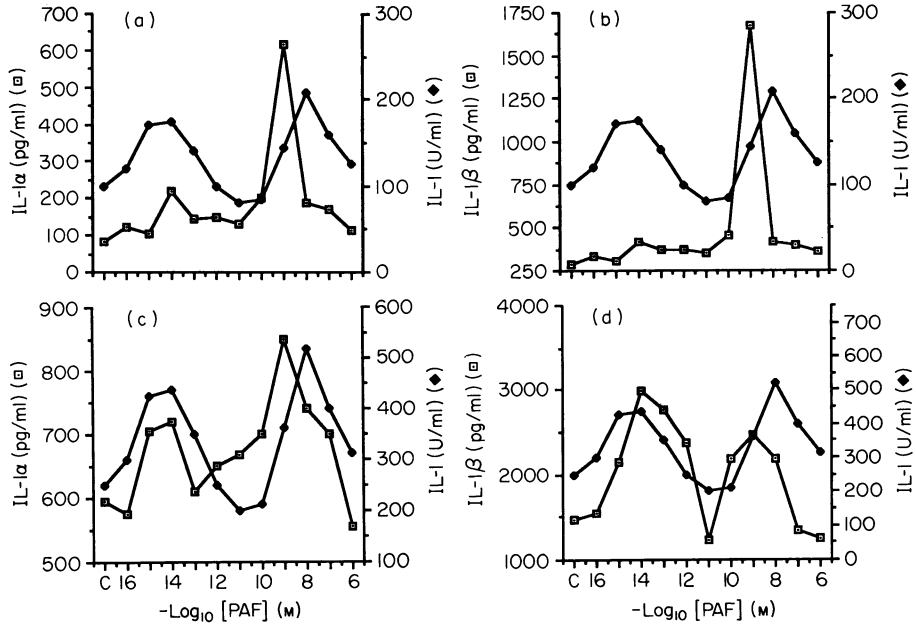

Figure 4. Comparative measurements of immunoreactive IL-1α and IL-1β and bioactive IL-1 in same supernatants of elutriated monocytes stimulated with graded concentrations of PAF in the absence (a and b) or presence (c and d) of 1 μg/ml MDP. Means of duplicate measurements of a representative experiment.

Table 1. Phenotypic profile of human monocyte subpopulations as fractionated on Percoll density gradient

Markers	Fraction 1+2	Fraction 3+4
CD11b (M0.1)	99±2	95±4
CD14 (Leu-M3)	98±2	93±2
HLA-DR	98±3	86±6
CL-7*	89±5	34±6
Naphtyl esterase	90±3	97±2
CD19 (Leu-12)	<1%	2±2
CD3 (Leu-4)	<2%	<2%

* Mouse monoclonal antibody reactive with activated macrophages.[50]

marker, identified by our mAb CL7, which labels preferentially the less dense monocytes.

This absence of response to lower PAF concentrations was not due to presence of inhibitory factors, since addition of such supernatants to the TNF bioassay using human rTNF had no effect. Furthermore, immunoassay measurements by ELISA of TNF production closely parallel the presented bioassay data.

DISCUSSION

Our present findings indicate that PAF can modulate the concomitant production, by human monocytes, of the inflammatory and immunoregulatory cytokines TNF and IL-1. Of particular interest was the finding of a second peak of PAF activity at femtomolar concentrations. In contrast to the nanomolar peak which could be blocked by the PAF receptor antagonists BN 52021 and WEB 2086, the femtomolar peak was mostly resistant to the antagonists. Its presence was consistently observed in cultures of monocytes, purified either by adherence or elutriation, from different donors at different times and was not seen with lyso-PAF, suggesting a specific effect of PAF.

The finding that a denser subpopulation of monocytes responded only to one of the two ranges of PAF concentrations suggested that there could be a differential distribution of PAF 'receptors' on distinct monocyte subsets. Human[34-39] and rat[40,41] monocyte–macrophage heterogeneity has been described in terms of prostaglandin or IL-1 production and cytotoxic activity and may represent different functional states or maturational levels.

We and others have recently reported on modulation of IL-1 production by PAF in cultures of rat and human monocytes–macrophages.[18,19,21,42] Ward *et al.*[19] and Salem *et al.*[21] showed enhanced IL-1 production by LPS- or MDP-stimulated monocytes, at PAF concentrations of 10^{-12}–10^{-8}M or > 10^{-9}M respectively. Pignol *et al.*[18] showed enhanced production of IL-1 by rat splenic macrophages at 10^{-12}M PAF. Similar responses were obtained with *ex vivo* cells from rats chronically infused with PAF.[43] Femtomolar concentrations of PAF were either not tested or not reported in these studies.

As for TNF, rat alveolar macrophages respond to PAF with a bell-shaped augmentation of TNF production in a single peak at 10^{-10}M and no effect below 10^{-13}M PAF.[44] Human large granular lymphocytes also produce augmented amounts of TNF following stimulation with certain target cells in the presence of 10^{-14}&10^{-10}M PAF.[45] Thus human monocytes may have this particularity of responding to two concentration ranges of PAF in a positive way. Recently, Barthelson *et al.*[20] showed that the THP-1 human monocytic leukaemia cell line also responded to PAF in a multiphasic dose–response curve.

Valone *et al.*[46] have shown that PAF can augment monocyte-mediated killing of a TNF-sensitive, but not a TNF-resistant, target cell. Our present findings may suggest one underlying mechanism for a such a cytotoxic activity, although only a single peak of PAF activity was reported by these investigators in their study.

Because isolation of monocytes by their adherence to plastic or fibronectin often allows for ample (5–15 platelets/monocyte) platelet contamination, certain effects of PAF on monocytes could be indirectly due, under these circumstances, to interactions with activated platelets. We therefore used elutriation to isolate monocytes in suspension, avoiding both adherence, which activates monocytes, and platelet contamination (less than 0·1 platelet per monocyte). The effect of PAF on cytokine production by monocytes isolated by either technique was similar, suggesting that neither activation by adherence nor contamination with platelets was responsible for the observed effects. It also indicated that similar monocyte populations were probably being studied with either isolation technique.[47]

Binding studies indicate a single class of PAF receptors on unfractionated human PBML (containing 10–15% monocytes), with a K_d of 6 nM and antagonism by triazolam, alprazolam and L-659,989.[48] Binding studies also indicate a single class of PAF receptors on the P388D$_1$ murine macrophage line with a dissociation constant of approximately 10^{-10} M and effective antagonism by BN 52021.[49] In contrast, human U937 or HL-60 cells also bind PAF, but binding is not antagonized by BN 52021, suggesting 'atypical' receptors for PAF may be present on certain cell types. Our findings that the effects on cytokine production mediated by femtomolar PAF were not blocked by BN 52021 suggest that normal human monocytes may also have 'atypical' but functional PAF receptors. Further studies are underway to characterize these putative receptors, but it must be stated that, at present, there is no direct proof that the biological responses observed with subpicomolar concentrations of PAF are mediated by receptors on the cell membrane.

Our findings reported herein thus indicate that PAF can directly modulate TNF and IL-1 production by human monocytes and suggest that its action may involve two types of 'receptors' of different affinity and structure with differential distribution within monocyte subpopulations.

ACKNOWLEDGMENTS

The authors are grateful to Ms Sylvie Turcotte for assistance and Mrs Carole Jacques for preparation of the manuscript.

This work was supported by grants from the National Cancer Institute, the Medical Research Council and the Arthritis Society of Canada. P.E. Poubelle and M. Rola-Pleszczynski are Research Scholars of the Fonds de Recherche en Santé du Québec. C. Dubois is a recipient of a Research Studentship from the F.R.S.Q.

REFERENCES

1. DINARELLO C.A. (1984) Interleukin 1. *Rev. Inf. Dis.* **6**, 51.
2. OLD L.J. (1985) Tumour necrosis factor (TNF). *Science*, **230**, 630.

3. Stenson W.F. & Parker C.W. (1980) Prostaglandins, macrophages and immunity. *J. Immunol.* **125,** 1.
4. Goldyne M.E., Burrish G.F., Poubelle P.E. & Borgeat P. (1984) Arachidonic acid metabolism among human mononuclear leukocytes. Lipoxygenase-related pathways. *J. biol. Chem.* **259,** 8815.
5. Jouvier-Marche E., Ninio E., Beaurain G., Tencé M., Niaudet P. & Benveniste J. (1984) Biosynthesis of PAF-acether (platelet-activating factor). VII. Precursors of PAF-acether and acetyl transferase activity in human leukocytes. *J. Immunol.* **133,** 892.
6. Leslie C.C. & Detty D.M. (1986) Arachidonic acid turnover in response to lipopolysaccharide and opsonized zymosan in human monocyte-derived macrophages. *Biochem. J.* **236,** 251.
7. Elstad M.R., Prescott S.M., McIntyre T.M. & Zimmerman G.A. (1988) Synthesis and release of platelet-activating factor by stimulated human mononuclear phagocytes. *J. Immunol.* **140,** 1618.
8. Mizel S.B., Dayer J.-M., Krane S.M. & Mergenhagen S.E. (1981) Stimulation of rheumatoid synovial cell collagenase and prostaglandin production by partially purified lymphocyte-activating factor (interleukin 1). *Proc. natl. Acad. Sci U.S.A.* **78,** 2474.
9. Albrightson C.R., Baenziger N.L. & Needleman P. (1985) Exaggerated human vascular cell prostaglandin biosynthesis mediate by monocytes: role of monokines and interleukin 1. *J. Immunol.* **135,** 1872.
10. Bachwich P.R., Chensue S.W., Larrick J.W. & Kunkel S.L. (1986) Tumor necrosis factor stimulates interleukin 1 and prostaglandin E_2 production in resting macrophages. *Biochem. Biophys. Res. Comm.* **136,** 94.
11. Kunkel S.L., Spengler M., May M.A., Spengler R., Larrick J. & Remick D. (1988) Prostaglandin E_2 regulates macrophage-derived tumour necrosis factor gene expression. *J. biol. Chem.* **263,** 5380.
12. Kunkel S.L. & Chensue S.W. (1985) Arachidonic acid metabolites regulate interleukin 1 production. *Biochem. Biophys. Res. Comm.* **128,** 892.
13. Rola-Pleszczynski M. & Lemaire I. (1985) Leukotrienes augment interleukin 1 production by human monocytes. *J. Immunol.* **135,** 3958.
14. Gagnon L., Filion L. & Rola-Pleszczynski M. (1987) Leukotriene B_4 augments tumor necrosis factor production by human monocytes. *Fed. Proc.* **46,** 6253.
15. Dubois C., Bissonnette E. & Rola-Pleszczynski M. (1989) Platelet-activating factor (PAF) enhances tumor necrosis factor production by alveolar macrophages: prevention by PAF receptor antagonist and lipoxygenase inhibitors. *J. Immunol.* **143,** 964.
16. Camussi G., Bussolino F., Salvidio G. & Baglioni C. (1987) Tumor necrosis factor/cachectin stimulates peritoneal macrophages, polymorphonuclear neutrophils and vascular endothelial cells to synthesize and release platelet-activating factor. *J. exp. Med.* **166,** 1390.
17. Bussolino F., Tetta C., Breviario F., Aglietta M., Mantovani A. & Dejana E. (1986) Interleukin 1 stimulates platelet-activating factor production in cultured human endothelial cells. *J. clin. Invest.* **77,** 2027.
18. Pignol B., Hénane S., Mencia-Huerta J.-M., Rola-Pleszczynski M. & Braquet P. (1987) Effect of platelet-activating factor (PAF-acether) and its specific receptor antagonist, BN 52021, on interleukin 1 release and synthesis by rat spleen adherent monocytes. *Prostagl.* **33,** 931.
19. Ward S.G., Lewis G.P. & Westwick J. (1988) Platelet-activating factor stimulates interleukin-1 production by human adherent monocytes-macrophages. In: *New Trends in Lipid Mediator Research* (ed. P. Braquet), p. 41 Karger, Basel.
20. Barthelson R., Valone F.H., Debs R.J. & Philip R. (1988) Synergy in interleukin 1 (IL 1)release by human monocytes stimulated with platelet-activating factor (PAF) plus gamma interferon or tumour necrosis factor. *FASEB J.* **2,** A1228.
21. Salem P., Derickx S., Dulioust A., Vivier E., Denizot Y., Damais C., Dinarello C. & Thomas Y. (1990) Immunoregulatory functions of pafacether. Enhancement of IL 1 production by muramyl dipeptide-stimulated monocytes. *J. Immunol.* **144,** 1338.
22. Last-Barney K. Homon C.A., Foanes R.B. & Merluzzi V.J. (1988) Synergistic and overlapping activities of tumor necrosis factor-α and IL 1. *J. Immunol.* **141,** 527.
23. Chang S.W., Fedderson C.O. & Henson P.M. (1987) Platelet-activating factor mediates hemodynamic changes and lung injury in endotoxin-treated rats. *J. clin. Invest.* **79,** 1496.
24. Sun X. & Hsueh W. (1988) Bowel necrosis induced by tumor necrosis factor in rats is mediated by platelet activating factor. *J. clin. Invest.* **81,** 1328.
25. Philip R. & Epstein L.B. (1986) Tumor necrosis factor as immunomodulator and mediator of monocyte cytotoxicity induced by itself, gamma-interferon and interleukin 1. *Nature*, **328,** 86.
26. Carswell E.A., Old L.J., Kassel R.L., Green S., Fiore N. & Williamson B. (1975) An endotoxin induced serum factor that causes the necrosis of tumors. *Proc. natl. Acad. Sci. U.S.A.* **72,** 3666.
27. Braquet P. & Rola-Pleszczynski M. (1987) Platelet-activating factor and cellular immune responses. *Immunol. Today*, **8,** 345.
28. Rola-Pleszczynski M. (1990) Modulation of immunoregulatory and cytotoxic cell functions by PAF and PAF receptor antagonists. In: *Platelet-Activating Factor in Endotoxin and Immune Diseases* (eds D. A. Handley, R. N. Saunders, W. J. Houlihan and J. C. Tomesch), p. 275. Marcel Dekker Inc., New York.
29. Boyum A. (1968) Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. Clin. Lab. Invest.* (suppl. **21**), 77.
30. Poubelle P.E., Borgeat P. & Rola-Pleszczynski M. (1987) Assessment of leukotriene B4 synthesis in human lymphocytes by using high performance liquid chromatography and radioimmunoassay methods. *J. Immunol.* **139,** 1273.
31. Mizel S.B., Oppenheim J.J. & Rosenstreich D.L. (1978) Characterization of lymphocyte-activating factor (LAF) produced by the macrophage cell line P 388D. Enhancement of LAF production by activated T lymphocytes. *J. Immunol.* **120,** 1497.
32. Grassi J., Frobert Y., Pradelles P., Chercuitte F., Gruaz D., Dayer J.-M. & Poubelle P.E. (1989) Production of monoclonal antibodies against interleukin-1α and -1β. Development of two enzyme immunometric assays (EIA) using acetylcholinesterase and their application to biological media. *J. immunol. Meth.* **123,** 193.
33. Flick D.A. & Gifford G.E. (1984) Comparison of *in vitro* cell cytotoxic assays for tumour necrosis factor. *J. Immunol. Meth.* **68,** 167.
34. Elias J.A., Schreiber A.D., Gustilo K., Chien P., Rossman M.D., Lammie P.J. & Daniele R.P. (1985) Differential interleukin 1 elaboration by unfractionated and density fractionated human alveolar macrophages and blood monocytes: relationship to cell maturity. *J. Immunol.* **135,** 3198.
35. McCarley D.L., Shah V.O. & Weiner R.S. (1983) Purified human monocyte subsets as effector cells in antibody dependent cellular cytotoxicity (ADCC). *J. Immunol.* **131,** 1780.
36. Normann S.J. & Weiner R. (1983) Cytotoxicity of human peripheral blood monocyte subsets. *Cell. Immunol.* **81,** 413.
37. Inamura N., Sone S., Okubo A., Singh S.M. & Ogura T. (1990) Heterogeneity in responses of human blood monocytes to granulocyte-macrophage colony-stimulating factor. *J. Leuk. Biol.* **47,** 528.
38. Khansari N., Chow Y.K. & Fudenberg H.H. (1985) Human monocyte heterogeneity: interleukin 1 and prostaglandin E_2 production by separate subsets. *Eur. J. Immunol.* **15,** 48.
39. Goldyne M.E. & Stobo J.D. (1979) Synthesis of prostaglandins by subpopulations of human peripheral blood monocytes. *Prostagl.* **18,** 687.
40. Chandler D.B. & Fulmer J.D. (1987) Prostaglandin synthesis and release by subpopulations of rat alveolar macrophages. *J. Immunol.* **139,** 893.

41. CHANDLER D.B., BAYLES G. & FULLER W.C. (1988) Prostaglandin synthesis and release by subpopulations of rat interstitial macrophages. *Am. Rev. Resp. Dis.* **138,** 901.
42. BARTHELSON R., POTTER T. & VALONE F.H. (1990) Synergistic increases in IL 1 synthesis by the human monocytic cell line THP-1 treated with PAF and endotoxin. *Cell Immunol.* **125,** 142.
43. PIGNOL B., HÉNANE S., SORLIN B., ROLA-PLESZCZYNSKI M., MENCIA-HUERTA J.-M. & BRAQUET P. (1990) Effect of long-term treatment with platelet-activating factor on IL 1 and IL 2 production by rat spleen cells. *J. Immunol.* **145,** 980.
44. DUBOIS C., BISSONNETTE E. & ROLA-PLESZCZYNSKI M. (1989) Asbestos fibers and silica particles stimulate rat alveolar macrophages to release tumor necrosis factor: autoregulatory role of leukotriene B_4. *Am. Rev. Resp. Dis.* **139,** 1257.
45. BOSSÉ J., TURCOTTE S. & ROLA-PLESZCZYNSKI M. (1988) Platelet-activating factor (PAF) enhances the production of cytotoxic cytokines during natural-cell mediated cytotoxicity. *Fed. Proc.* **47,** A415.
46. VALONE F.H., PHILIP R. & DEBS R.J. (1988) Enhanced human monocyte cytotoxicity by platelet-activating factor. *Immunology* **64,** 715.
47. DRANSFIELD I., CORCORAN D., PARTRIDGE L.J., HOGG N. & BURTON D.R. (1988) Comparison of human monocytes isolated by elutriation and adherence suggests that heterogeneity may reflect a continuum of maturation/activation states. *Immunology*, **63,** 491.
48. NG D.S. & WONG K. (1988) Specific binding of platelet-activating factor (PAF) by human peripheral blood mononuclear leukocytes. *Biochem. Biophys. Res. Commun.* **155,** 311.
49. VALONE F.H. (1988) identification of platelet-activating factor receptors in $P388D_1$ murine macrophages. *J. Immunol.* **140,** 2389.
50. TURNCOTTE S., STANKOVA J. & ROLA-PLESZCZYNSKI M. (1991) A novel myeloid activation/differentiation antigen which induces $[Ca^{++}]_i$ mobilization and synergizes with PMA for TNF production. *FASEB J.* **5** (in press).