Biochemical Pharmacology, Vol. 39, No. 4, pp. 677–683, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

THE ADHESIVENESS OF MONOCYTIC U937 CELLS IS STIMULATED BY PRO-INFLAMMATORY AGENTS AND INHIBITED BY ADENOSINE 3′:5′-CYCLIC MONOPHOSPHATE

M. A. VENTURA,* A. RIBIER, I. DELBOURG, A. M. CHAMBAUT-GUERIN and P. THOMOPOULOS

U-282 INSERM, Hôpital Henri Mondor, 94010 Creteil, France

(*Received* 17 *May* 1989; *accepted* 19 *September* 1989)

Abstract—We investigated the regulation of the adhesiveness of the human promonocytic cell line U-937, differentiated along the monocytic pathway either by 1,25-$(OH)_2$-cholecalciferol or a combination of retinoic acid and dibutyryl cAMP. Adhesion to untreated polystyrene plastic was induced by inflammatory agents like PAF, fMLP or LTB_4. The response to PAF first appeared after 48 hr of differentiation and was inhibited by PAF antagonists and protein kinase C inhibitors indicating involvement of the phosphatidyl-inositol pathway in the stimulating effect. On the other hand, all the c-AMP raising agents tested inhibited PAF-induced cell adhesion, whatever their target: membrane receptors, the G_s transducing protein, the catalytic unit of adenylate cyclase or cAMP phosphodiesterase. Direct stimulation of protein kinase A by Br^8-cAMP had a similar effect. Moreover, PAF was able to increase cAMP levels. This suggests the existence of a cAMP based negative control mechanism limiting the action of PAF.

The adhesion of monocytes to endothelial cells is the first step in their migration through the blood vessel wall. It is probably involved in the pathogenesis of inflammatory diseases and of atherosclerosis [1]. Thus, in experimental hypercholesterolemia, one of the earliest occurrences is the increased adherence of monocytes to the endothelium [2]. This increase might be related to a modification of monocytes, endothelial cells or both. In effect, several interactions have been described between these two cell types. Macrophage products like reactive oxygen intermediates or the cytokines IL-1† and TNF stimulate the secretion of PAF, PGI_2 and other mediators by endothelial cells, which in turn modulate macrophage activity [3, 4]. IL-1 and TNF also specifically induce the appearance of the Endothelial Leukocyte Adhesion Molecule 1, ELAM-1 [5].

The present work investigated the regulation of the adhesiveness of the human promonocytic cell line, U-937. The cells were induced to maturation along the monocyte pathway by use of 1,25-$(OH)_2$-cholecalciferol [6] or a combination of retinoic acid and dibutyryl-cAMP [7]. The parameter measured was cell attachment to untreated polystyrene plastic in order to avoid interference from products released by endothelial cells.

Our data demonstrate that several mediators of inflammation (platelet activating factor, chemotactic peptide and leukotriene B_4) stimulate the adhesiveness of U-937 cells. We also show for the first time that this effect can be counteracted by agents inducing an increase in cAMP levels. Finally, we report that PAF is able to increase cAMP levels, thus establishing a negative control mechanism to its action.

MATERIALS AND METHODS

Materials. RPMI 1640 medium and newborn calf serum were from Eurobio (France). 1-Oleoyl-2-acetyl glycerol, 8-bromo adenosine 3′:5′-cyclic monophosphate, cholera toxin, (−)isoproterenol bitartrate, prostaglandins E_1 and I_2, adenosine and its derivatives, theophylline, iso-butyl-methyl-xanthine, sphinganine, acridine orange, *N*-formyl-L-methionyl-L-leucyl-L-phenyl-alanine (fMLP), L-alpha-phosphatidyl choline, beta-acetyl-gamma-*o*-(octadec-9-cis-enyl) (PAF), retinoic acid, dibutyryl-cyclic-AMP, 1,25-$(OH)_2$-cholecalciferol, leukotriene B_4, dimethyl sulfoxide, were from Sigma Chimie (La Verpillière, France). Forskolin was obtained from Calbiochem (Frankfurt, F.R.G.). BN 52021 and BN 52111 were kindly provided by Institut Henri Beaufour (France). Ro 20-1724 and alprenolol were gifts from Hoffman LaRoche (Basel, Switzerland) and Ciba-Geigy (Basel, Switzerland), respectively. OAG was stored as DMSO solution (0.25 M) and the working dilutions in culture RPMI medium were briefly sonicated before use. Ro 20-1724 (5×10^{-2} M), PGE_1 (1.41×10^{-2} M), sphinganine (2×10^{-2} M), 1,25-$(OH)_2$-cholecalciferol

* To whom correspondence should be sent.

† Abbreviations: IL-1, interleukin-1; TNF, tumor necrosis factor; cAMP, cyclic AMP; PAF, platelet activating factor; fMLP, chemotactic peptide; LTB_4, leukotriene B_4; OAG, 1-oleyl-2-acetyl-glycerol; Br^8-cAMP, 8-bromoadenosine 3′:5′-cyclic monophosphate; iso, (−)isoproterenol bitartate; PGE_1, prostaglandin E_1; PGI_2, prostacyclin; IBMX, iso-butyl-methyl-xanthine; DMSO, dimethyl sulfoxide; NECA, adenosine 5′-ethyl-carboxamide; PIA, L-N^6-phenyl-isopropyl-adenosine; CHA, N^6-cyclohexyl-adenosine; DOA, deoxy-adenosine; EC_{50}, efficient concentration 50%; IC_{50}, inhibitory concentration 50%.

($2.5–10^{-4}$ M) were stored as ethanol solutions, fMLP (10^{-2} M) as a DMSO solution, forskolin (10^{-2} M) as a DMSO/ethanol (2:1, v/v) solution and Br^8-cAMP (10^{-1} M) as a water solution. All the above cited solutions were kept at −20°. The other drugs were freshly dissolved in water, (iso, adenosine), saline (BN 52111), ethanol (LTB_4, retinoic acid), ethanol/water (1:1, v/v, adenosine derivatives), ethanol/water (1:9, v/v, acridine orange), DMSO (BN 52021). Final concentrations of the drugs and solvents are indicated in the legends to figures.

Cell culture. U-937 cells were cultured in suspension in plastic culture flasks (Nunc, Denmark) in RPMI 1640 medium containing 10% newborn calf serum (Eurobio, France), 100 units/mL penicillin and 50 μg/mL streptomycin, at 37° in a 5% CO_2–95% air humidified atmosphere. During the induction of monocytic maturation, they were maintained in Teflon conical flasks in order to avoid attachment to the plastic; this was particularly the case with 1,25-$(OH)_2$-cholecalciferol [6]. The inducers were added and the final concentrations of 2.5×10^{-8} M for 1,25$(OH)_2$ cholecalciferol [6], 10^{-6} M for retinoic acid and 10^{-4} M for dibutyryl-cAMP [7]. The effectiveness of monocytic maturation has been established in our laboratory [8] and was in good agreement with previous studies [6, 7]. Cell density was maintained between 0.1 and 0.5×10^6 cells/mL. Cell viability was tested by the trypan blue exclusion method and was higher than 95%.

Cell adhesiveness assay. Cell adhesiveness was measured as already reported [19] on cells differentiated during 3 to 5 days. The cells were incubated overnight under their usual culture conditions in the presence of *methyl*-[^{3}H]thymidine (Amersham, Bucks, U.K.), specific activity 70–85 Ci/mmol at 0.2 μCi/mL. Before each experiment they were washed once and resuspended (3 to 5×10^5 cells/mL) in RPMI-1640 bicarbonate-free medium supplemented with 10% fetal calf serum and 25 mM Hepes at pH 7.4 (incubation medium). When indicated, they were preincubated at 37° in the presence of various drugs in Teflon flasks or in polypropylene tubes coated with 5% Surfasil (Pierce Chemical Co., Rockford, IL) to which the U-937 cells do not adhere. At the onset of the adhesion assay, 1-mL aliquots of cell suspensions were transferred to polystyrene Petri dishes not treated for tissue culture (Falcon 1008, Becton Dickinson). Drugs or solvents were added, if required, and the dishes were maintained at 0° for 10 min. This step allowed the cells to settle down and come in contact with the polystyrene substrate. No adhesion was observed at that temperature. At 0 time the dishes were carefully transferred onto a slide drying hotplate (R. A. Lamb, London, U.K.) set at 37° and incubated for the indicated time intervals in kinetics studies and for 12 min in dose–response experiments. At the end of the adhesion assay, the dishes were shaken for 3 min on a Rotatest 95220 shaker (Bioblock, France) at 100 rpm. Then the supernatants were carefully discarded, the attached cells solubilized in 2×1 mL sodium dodecyl sulfate (SDS) (0.1% in H_2O) and the radioactivity was measured after addition of 2 mL H_2O and 6 mL Ready Value (Beckman Instruments) in a Beckman liquid scintillation counter. Per cent adherent cells was calculated as [(experimental cpm-blank)/(total cpm)] × 100. Total cpm was the radioactivity of the total cell number used for each experimental point, that is, 1 mL of cell suspension, pelleted for 1 min in an Eppendorf microfuge and solubilized in SDS as above. Blank values were measured by stopping the adhesion assay at 0 time, immediately after the cells had been transferred to the dishes. The assays were carried out in triplicate.

cAMP production. cAMP content of the cells and medium was measured by a modification of a previously described method [10]. Briefly, 0.5×10^6 cells in 0.5 mL were incubated in the absence or the presence of 10^{-4} M Ro 20-1724 for 15 min, in Eppendorf tubes coated with 5% Surfasil. After the addition of the stimuli the incubation of the cells was terminated by the addition of ice-cold IBMX (5×10^{-4} M final concentration), and immersion of the tubes in liquid nitrogen. After thawing, the mixture was boiled for 5 min and centrifuged for 30 sec at 13,000 *g*. The supernatants were assayed for cAMP using the acetylation method described for use with the radioimmunoassay kit purchased from ERIA Diagnostics Pasteur (France).

Statistical analysis. The statistical analysis included: (a) basic calculations (mean, SE etc); (b) *F*-max test for homogeneity of variances; (c) analysis of the variance (ANOVA), one or two way model I, according to the design of each experiment; (d) comparisons within groups using the least significant difference (LSD) between means (alpha = 0.05). All these procedures are described in Biometry [11]. The calculations were carried out using the program Statview 512+ and a Macintosh SE microcomputer.

RESULTS

Effect of PAF on cell adhesiveness

The effect of PAF on cell adhesiveness was studied during the differentiation of U937 cells by 1,25-$(OH)_2$-cholecalciferol. PAF increased the number of adherent cells after 48 hr of differentiation. This effect was maximal at 72 hr and remained constant for at least 7 days (Fig. 1). 2-Chloroadenosine, an adenosine analog able to raise cAMP levels completely inhibited PAF-induced adhesion all along the studied period (Fig. 1). PAF-induced adhesion was rapid and transient, reaching a peak between 12 and 15 min. For this reason cell adhesion was scored at the time 12 min in subsequent dose–response experiments. The EC_{50} of PAF was 3.7×10^{-8} M and the maximal effect was observed at 10^{-7} M (Fig. 2). The stimulation of adhesiveness was inhibited by specific PAF antagonists [12] BN 54021 and BN 54111 with IC_{50} of 5.3×10^{-6} M and 1.1×10^{-7} M, respectively (Fig. 3). It was also prevented by the inhibitors of protein kinase C, sphinganine and acridine orange [13, 14] (data not shown).

Effect of cAMP-increasing agents on cell adhesiveness

The inhibitory action of 2-chloroadenosine (Fig. 1) led us to investigate the effect of several other adenosine analogs (Table 1). Similar inhibitory effects were observed with NECA, PIA and CHA, while DOA, a specific agonist for the P site was

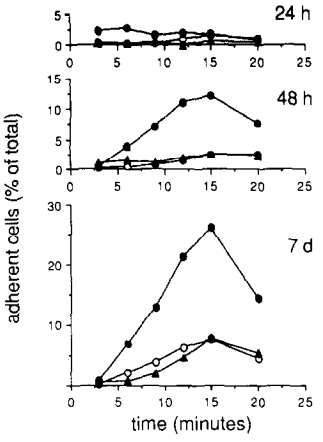

Fig. 1. Appearance of the adhesive response of U-937 cells to PAF along differentiation induced by 2.5×10^{-8} M 1,25-$(OH)_2$-cholecalciferol for 24 hr, 48 hr or 7 days. At these time points, the kinetics of cell adhesion were determined after addition of: 10^{-7} M PAF (●), 10^{-7} M PAF + 10^{-4} M 2-chloroadenosine (▲) or 0.005% DMSO, the solvent of PAF (○). Means ± SE of triplicates. Two-way ANOVA for each day (as well as for days 4, 5 and 6, not shown): treatment (PAF vs PAF + 2 chloroadenosine vs control) and time (kinetics) factors as well as interaction, $P < 0.001$ except for time factor at 24 hr, not significant.

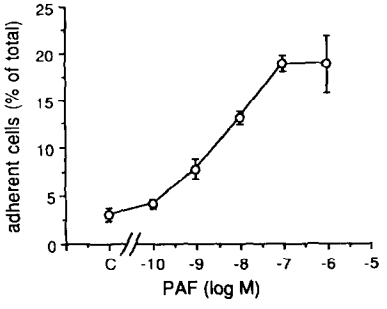

Fig. 2. Induction of adhesiveness of U-937 cells by PAF: dose–response relationship. The cells were cultured in the presence of 10^{-6} M retinoic acid and 10^{-4} M dibutyryl-cAMP for 5 days. Adhesion was scored (see Materials and Methods) in the presence of increasing concentrations of PAF or its solvent 0.005% DMSO (C). Mean ± SE of triplicates. One-way ANOVA: $P < 0.001$. LSD test: the effect of PAF became significant beyond 10^{-9} M. The experiment was repeated seven times.

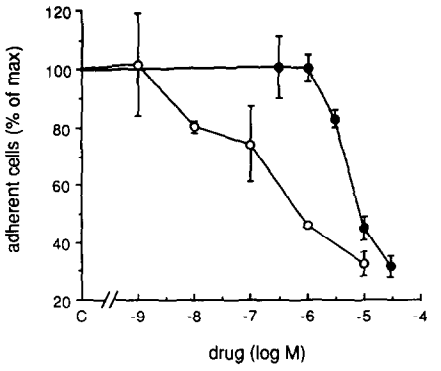

Fig. 3. Effect of PAF antagonists on PAF-induced adhesiveness of U-937 cells. The cells were cultured in the presence of 10^{-6} M retinoic acid and 10^{-4} M dibutyryl-cAMP for 5 days. Adhesion was scored (see Materials and Methods) in the presence of increasing concentrations of the PAF antagonists BN 52021 (●) and BN 52111 (○) or their solvents 0.03% DMSO or saline (C) followed by 10^{-7} M PAF. The response to PAF treatment only was set at 100%. Mean ± SE of triplicates. One-way ANOVA: $P < 0.001$ for both drugs. LSD: the effect of the antagonists became significant beyond 10^{-5} M (BN 52021) or 10^{-8} M (BN 52111). The experiment was repeated eight times.

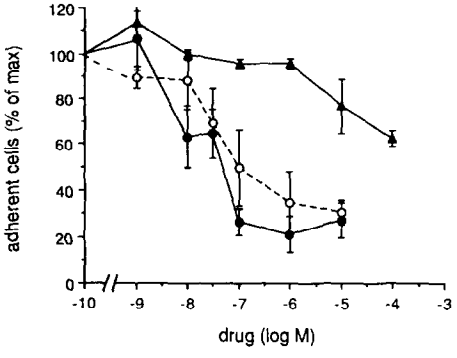

Fig. 4. Effect of activators of adenylate cyclase on PAF-induced cell adhesiveness. The cells were cultured in the presence of 2.5×10^{-8} M 1,25-$(OH)_2$-cholecalciferol for 5 days. Adhesion was scored (see Material and Methods) in the presence of increasing concentrations of adenosine (▲), PGE_1 (○) and isoproterenol (●), followed by 10^{-7} M PAF. The response to PAF treatment only (C) was set at 100%. Mean ± SE of triplicates. One-way ANOVA: $P < 0.001$ for all drugs. LSD: the inhibitory effect became significant beyond 10^{-5} M (adenosine), 10^{-7} M (PGE_1) or 10^{-8} M (isoproterenol). The experiment was repeated twice.

found inactive (Table 1). The action of these compounds was observed in the 10^{-5} M range. This suggests that they were acting through A_2 receptors, which stimulate adenylate cyclase [15]. Adenosine was also inhibitory (Fig. 4), but to a lesser degree than its stable analog 2-chloro-adenosine. Since adenosine is produced by many cell types and acts as an autocrine factor, cell attachment was measured in the presence of adenosine deaminase (1 U/mL). A 107% increase in the number of adherent cells over the controls ($P < 0.005$) was observed under these conditions (data not shown).

The notion of an inhibitory effect of cAMP on PAF-induced adhesiveness was further sustained by the results obtained with several cAMP-increasing agents, whatever their target was: membrane receptors [isoproterenol, PGE_1, adenosine (Fig. 4), PGI_2 (Table 1)], the G_s transducing protein (cholera toxin), the catalytic unit of adenylate cyclase (forskolin) (Table 1) or cAMP phosphodiesterase (theophylline, Ro 20-174) (Fig. 5). All these agents were found to be able to inhibit the induction by PAF of cell adhesiveness.

Direct stimulation of protein kinase A by Br^8-cAMP had a similar effect (Table 1). The IC_{50} for PGE_1 and isoproterenol were 7.8×10^{-8} M and 1.4×10^{-8} M, respectively (Fig. 4). The action of the

Table 1. Inhibitory effect of cAMP on PAF induced cell adhesion

Treatment	Adherent cells* (% of total) PAF 10^{-7} alone	PAF 10^{-7} M + test drug	P‡
2-Chloroadenosine 10^{-4} M	47 ± 1.3	17 ± 1.8	<0.001
NECA 10^{-5} M	42 ± 0.9	31 ± 0.5	<0.001
PIA 10^{-5} M	37 ± 0.5	27 ± 1.3	<0.005
CHA 10^{-5} M	45 ± 4.0	32 ± 3.4	<0.05
DOA 10^{-4} M	42 ± 1.2	39 ± 0.6	>0.05
PGI_2 10^{-5} M	33 ± 0.3	13.7 ± 1.8	<0.001
Cholera toxin 0.5 μg/mL	33 ± 0.3	6.1 ± 0.3	<0.001
Forskolin 10^{-5} M	14 ± 0.6	6.4 ± 0.6	<0.001
Br^8-cAMP 10^{-3} M	32 ± 12	16 ± 5	<0.05

* Values are mean ± SE (N = 3).

‡ One-tailed *t*-test.

The drugs were added just before PAF (or fMLP 2×10^{-6} M in the forskolin experiment) except for cholera toxin and forskolin, which were preincubated with the cells at 37° for 3 hr and 15 min, respectively. Adhesion was scored at time 12 min of the assay (see Materials and Methods). The data on adenosine analogs come from dose–response curves, the rest from kinetics studies. The experiments were repeated at least twice.

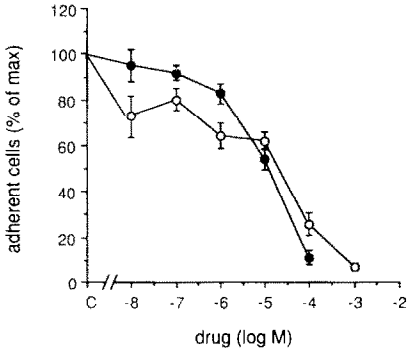

Fig. 5. Effect of cAMP phosphodiesterase inhibitors on PAF-induced adhesiveness of U-937 cells. The cells were cultured in the presence of 10^{-6} M retinoic acid and 10^{-4} M dibutyryl-cAMP for 5 days. The cells were preincubated for 15 min with the indicated concentrations of theophylline (○), Ro 20-1724 (●) or the solvent, 0.2% ethanol (C). Adhesion was scored (see Materials and Methods) in the presence of 10^{-7} M PAF. The response to PAF treatment only was set at 100%. mean ± SE of triplicates. One-way ANOVA: $P < 0.001$ for both drugs. LSD: the inhibitory effect became significant beyond 10^{-4} M (theophylline) or 10^{-5} M (Ro 20-1724).

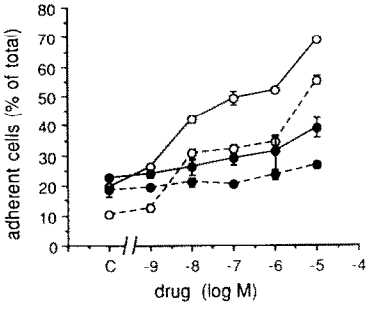

Fig. 6. Stimulatory effect of fMLP and LTB_4 on adhesiveness of U-937 cells and its inhibition by PGE_1. The cells were cultured in the presence of 10^{-6} M retinoic acid and 10^{-4} M dibutyryl-cAMP for 5 days. Adhesion was scored (see Materials and Methods) in the presence of 10^{-6} M PGE_1 (----) or the solvent, 0.007% ethanol (——), followed by the indicated concentrations of fMLP (○) or LTB_4 (●). Mean ± SE of triplicates. Two-way ANOVA: treatment (±PGE_1) and time factors as well as interaction, $P < 0.001$ for both fMLP and LTB_4. LSD: the effect of fMLP became significant at 10^{-9} M (without) or 10^{-8} M (with PGE_1). For LTB_4, at 10^{-7} M and 10^{-5} M, respectively. The experiment was repeated twice.

latter was competitively inhibited by alprenolol, with an IC_{50} of 1.2×10^{-7} M (data not shown).

The role of basal cAMP levels in unstimulated cells was also demonstrated by the action of two inhibitors of cAMP-phosphodiesterase (Fig. 5). Theophylline and Ro 20-1724 prevented the stimulation of cell adhesiveness by PAF, with an IC_{50} of 2.8×10^{-5} M and 6.7×10^{-6} M, respectively.

Effect of fMLP, LTB_4 and OAG

In addition to PAF, the adhesiveness of U-937 cells was found to be increased by two other pro-inflammatory substances, fMLP and LTB_4. Their action was also inhibited by PGE-1 (Fig. 6). Furthermore, OAG which is an analog of diacyl glycerol and acts directly on protein kinase C was found to stimulate cell adherence; at 12 min, the peak time of the kinetics assay, 47 ± 4% of the cells were attached in the presence of 10^{-5} M OAG. 2-Chloroadenosine (10^{-4} M) inhibited almost completely the effect of OAG: 8 ± 3.0% of the cells were adherent ($P < 0.001$) after the simultaneous addition of OAG and 2-chloroadenosine (data not shown).

Production of cAMP

In the absence of phosphodiesterase inhibitors, isoproterenol and PGE_1 induced a burst of cAMP

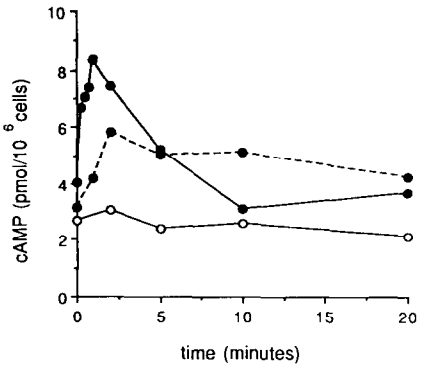

Fig. 7. Effect of 2-chloroadenosine and PAF on cAMP production. The cells were cultured in the presence of 2.5×10^{-8} M 1,25-$(OH)_2$-cholecalciferol for 4 days. Cell suspensions were preincubated for 15 min in the presence of 10^{-4} M Ro 20-1724. Total cAMP levels (incubation medium plus cells) were measured at the indicated time intervals, after addition of 10^{-4} M 2-chloroadenosine (--●--), 10^{-7} M PAF (—●—) or solvent, 0.005% DMSO (—○—). Similar results were found in another experiment.

peaking at 30 sec (data not shown). Under these conditions no effect of 2-chloroadenosine was observed. However, in the presence of 10^{-4} M Ro 20-1724 basal cAMP levels were clearly increased and 2-chloroadenosine induced a doubling of this value, which remained stable for at least 20 min (Fig. 7).

In the absence of phosphodiesterase inhibitors PAF had no effect, neither on basal nor on isoproterenol or PGE_1 stimulated levels of cAMP (data not shown). However, in the presence of Ro 20-1724, PAF clearly induced the production of cAMP (Fig. 7).

DISCUSSION

In this study we investigated the regulation of the adhesiveness of the human promonocytic cell line, U-937. The system that we used is based on the adhesion of the cells to a polystyrene surface. The low level of cell adhesion to this substrate allows the study of its regulation. This is not the case with tissue culture substrates to which differentiated U-937 cells strongly attach (personal observation). Previous work has demonstrated that the behaviour of the cells in this system closely resembles the adhesion of myelomonocytic cells to endothelium and their recruitment to an inflammatory focus [16, 17]. Basically, the same molecules seem to be involved: the CD11a-c/CD18 family of integrins [18]. More complex systems using cocultures of monocytes, endothelial cells and smooth muscle cells [19] are certainly closer to the "*in vivo*" situation. Our model, however, avoids interactions that occur between these cells.

The human U-937 promonocytic cell line [20] differentiates along the monocytic pathway in the presence of 1,25-$(OH)_2$-cholecalciferol [5] or a combination of retinoic acid and dibutyryl cyclic AMP [6]. Both agents were equivalent as to the extent of the adhesive response to PAF or OAG, or its inhibition by cAMP. However, the response to fMLP was much weaker in 1,25-$(OH)_2$-cholecalciferol-treated cells (unpublished results).

Undifferentiated U-937 cells are able to respond to PAF, for instance, by an increase in intracellular calcium levels (Ref. 21 and personal observations). The slow appearance of PAF-induced adhesiveness during the differentiation of U-937 cells in our experiments is probably related to the pregressive expression of the integrins of the CD11a-c/CD18 family, involved in leukocyte cell–cell and cell–substrate adhesion, along the maturation process [22].

Cell adhesiveness was also stimulated by fMLP, LTB_4 and OAG. The latter is a well-known cell-permeant analog of diacyl glycerol and it directly stimulates protein kinase C. Its action mimics the already reported effect of phorbol esters on cell attachment [23, 24]. fMLP and LTB_4, like PAF, stimulate the breakdown of polyphosphoinositides in their target cells [25–27] and induce the attendant biochemical responses: mobilization of intracellular free calcium and stimulation of protein kinase C, through the production of inositol triphosphate and diacyl glycerol. An increase in permeability to calcium can also be induced [27]. Our data on the inhibition of the effect of PAF by the inhibitors of protein kinase C, sphinganine [13] and acridine orange [14] are in accordance with the above-described biochemical events.

Cyclic AMP-increasing agents usually suppress many of the functions of macrophages [28–36]. However, opposite results concerning the action of PGE_1 have been recently reported, related to the origin of the macrophages [8, 37]. We report here for the first time a clear inhibitory effect on adhesiveness of all the cAMP-raising agents used. Moreover, their effect was obtained against PAF, fMLP and LTB_4, as well as against OAG. The inhibition of monocyte adhesiveness seems to take place at or beyond the level of protein kinase C. However, our data do not rule out additional effects on more proximal steps, similar to the inhibition of inositol phospholipids metabolism and calcium flux by cAMP described in neutrophils [38] and T lymphocytes [39]. The potency of the various agents to inhibit cell adhesiveness was not proportional to their capacity to increase cAMP. Thus, adenosine agonists were much less effective in raising cAMP levels than isoproterenol or PGE_1, whereas they were equally potent in decreasing cell attachment. One possible explanation is that slight increments in cAMP are sufficient. Additional mechanisms might be operative also, since adenosine is able to modulate K^+ channels [40]. Whatever its mechanism of action might be, our results with adenosine deaminase suggest that this agent is a physiological modulator of monocyte attachment.

Our data on the increase of cAMP induced by PAF suggest the existence of a feed-back regulatory system through which the action of PAF on monocytes is limited. A similar situation has been described for the effects of PAF on polymorphonuclear leukocytes [41]. However some cell specificity exists, so that, in platelets and alveolar macrophages PAF rather inhibits the cAMP production stimulated by prostaglandins or catecholamines [42, 43]. This is reminiscent of the opposite

effects of phorbol esters on adenylate cyclase depending on the cell type [10].

In conclusion, this work investigates the regulation of the adhesiveness of monocytes. This property of the cells is stimulated by proinflammatory agents (PAF, fMLP, LTB_4) and inhibited by cAMP raising drugs. The latter are active in the range of their usual therapeutic concentration, e.g. $3–5 \times 10^{-5}$ M for theophylline. Thus, these data might lead to the design of agents acting specifically on cAMP levels of the cells of the immune system, which might allow new approaches in the treatment of diseases where the adhesion of monocytes is involved like inflammation and atherosclerosis.

Acknowledgements—We are greatly indebted to Pr. P. Braquet for the gift of PAF antagonists and to Mrs N. Scharapan for expert secretarial assistance.

REFERENCES

1. Johnston RB, Monocytes and macrophages. *N Engl J Med* **318**: 747–752, 1988.
2. Ross R, The pathogenesis of atherosclerosis—an update. *N Engl J Med* **314**: 488–500, 1986.
3. Harlan JM, Leukocyte-endothelial interactions. *Blood* **65**: 513–525, 1985.
4. Nathan CF, Secretory products of macrophages. *J Clin Invest* **79**: 319–326, 1987.
5. Bevilacqua MP, Stengelin S, Gimbrone Jr MA and Seed B, Endothelial leukocyte adhesion molecule 1: an inducible receptor for neutrophils related to complement regulatory proteins and lectins. *Science* **243**: 1160–1165, 1989.
6. Olsson I, Gullberg U, Ivhed I and Nilsson K, Induction of differentiation of the human histiocytic lymphoma cell line U-937 by 1 alpha, 25-Dihydroxycholecalciferol. *Cancer Res* **43**: 5862–5867, 1983.
7. Olsson IL and Breitman TR, Induction of differentiation of the human histiocytic lymphoma cell line U-937 by retinoic acid and cyclic adenosine 3′:5′-monophosphate-inducing agents. *Cancer Res* **42**: 3924–3927, 1982.
8. Rouis M, Thomopoulos P, Louache F, Testa U, Hervy C and Titeux M, Differentiation of U-937 human monocyte-like cell line by 1 alpha, 25-dihydroxyvitamin D_3, or by retinoic acid. Opposite effects on insulin receptors. *Exp Cell Res* **157**: 539–541, 1985.
9. Issaad C, Ventura MA and Thomopoulos P, Biphasic regulation of macrophage attachment by activators of cyclic AMP-dependent kinase and protein kinase C. *J Cell Physiol* **140**: 317–322, 1989.
10. Chambaut-Guerin AM and Thomopoulos P, Protein kinase C potentiates isoproterenol-mediated cyclic AMP production without modifying the homologous desensitization process in J774 cells. *Eur J Biochem* **170**: 381–387, 1987.
11. Sokal RR and Rohlf FJ, *Biometry* 2nd Edn. WH Freeman and Co, San Francisco, 1981.
12. Braquet P, Touqui L, Shen TS and Vargaftig BB, Perspectives in platelet activating factor research. *Pharmacol Rev* **39**: 97–145, 1987.
13. Hannun YA, Loomis CR, Merrill Jr AH and Bell RM, Sphingosine inhibition of protein kinase C activity and of phorbol dibutyrate binding *in vitro* and in human platelets. *J Biol Chem* **261**: 12604–12609, 1986.
14. Hannun YA and Bell RM, Aminoacridines, potent inhibitors of protein kinase C. *J Biol Chem* **263**: 5124–5131, 1988.
15. Daly JW, Adenosine receptors. *Adv Cycl Nucleot Prot Phosphor Res* **19**: 29–46, 1985.
16. Mentzer SJ, Crimmins MAV, Burakoff SJ, and Faller DV, Alpha and beta subunits of the LFA-1 membrane molecule are involved in human monocyte-endothelial cell adhesion. *J Cell Physiol* **130**: 410–415, 1987.
17. Rosen H and Gordon S, Monoclonal antibody to the murine type 3 complement receptor inhibits adhesion of myelomonocytic cells *in vitro* and inflammatory cell recruitment *in vivo*. *J Exp Med* **166**: 1685–1701, 1987.
18. Skoglund G, Cotgreave I, Rincon J, Patarrayo M and Ingelman-Sundberg M, H_2O_2 activates CD 11b/CD18-dependent cell adhesion. *Biochem Biophys Res Commun* **157**: 443–449, 1988.
19. Navab M, Hough GP, Stevenson LW, Drinkwater DC, Laks H and Fogelman AM, Monocyte migration into the subendothelial space of a coculture of adult human aortic endothelial and smooth muscle cells. *J Clin Invest* **82**: 1853–1863, 1988.
20. Sundström C and Nilsson K, Establishment and characterization of a human histiocytic lymphoma cell line (U-937). *Int J Cancer* **17**: 565–577, 1976.
21. Maudsley DJ and Morris AG, Rapid intracellular calcium changes in U937 monocyte cell line: transient increases in response to platelet-activating factor and chemotactic peptide but not interferon-gama or lipopolysaccharide. *Immunology* **61**: 189–194, 1987.
22. Miller LJ, Schwarting R and Springer TA, Regulated expression of the Mac-1, LFA-1, p150,95 glycoprotein family during leukocyte differentiation. *J Immunol* **137**: 2891–2900, 1986.
23. Mercurio AM and Shaw LM, Macrophage interactions with laminin: PMA selectively induces the adherence and spreading of mouse macrophages on a laminin substratum. *J Cell Biol* **107**: 1873–1880, 1988.
24. Skoglund G, Patarroyo M, Forsbeck K, Nilsson K and Ingelman-Sundberg M, Evidence for separate control by phorbol esters of CD18-dependent adhesion and translocation of protein kinase C in U-937 cells. *Cancer Res* **48**: 3168–3172, 1988.
25. Dougherty RW, Godfrey PP, Hoyle PC, Putney JW Jr and Freer RJ, Secretagogue-induced phosphoinositide metabolism in human leucocytes. *Biochem J* **222**: 307–314, 1984.
26. Holian A, Leukotriene B_4 stimulation of phosphatidylinositol turnover in macrophages and inhibition by pertussis toxin. *FEBS Lett* **201**: 15–19, 1986.
27. Naccache PH, Molski MM, Volpi M, Shefcyk J, Molski TFP, Loew L, Becker EL and Sha'afi RI, Biochemical events associated with the stimulation of rabbit neutrophils by platelet-activating factor *J Leucocyte Biol* **40**: 533–548, 1986.
28. Gallin JI, Sandler JA, Clyman RI, Manganiello VC and Vaughan M, Agents that increase cyclic AMP inhibit accumulation of cGMP and depress human monocyte locomotion. *J Immunol* **120**: 492–496, 1978.
29. Schenkelaars EJ and Bonta IL, Beta 2-adrenoceptor agonists reverse the leukotriene-C4-induced release response of macrophages. *Eur J Pharmacol* **107**: 65–70, 1985.
30. Knudsen PJ, Dinarello CA and Strom TB, Prostaglandins posttranscriptionally inhibit monocyte expression of interleukin 1 activity by increasing intracellular cyclic adenosine monophosphate. *J Immunol* **137**: 3189–3194, 1986.
31. Channon JV, Leslie CC and Johnston RB Jr, Zymosan-stimulated production of phosphatidic acid by macrophages: relationship to release of superoxide anion and inhibition by agents that increase intracellular cyclic AMP. *J Leucocyte Biol* **41**: 450–453, 1987.
32. Pearson RD, Symes P, Conboy M, Weiss AA and Hewlett EL, Inhibition of monocyte oxidative responses by bordetella pertussis adenylate cyclase toxin. *J Immunol* **139**: 2749–2754, 1987.
33. Renz H, Gong JH, Schmidt A, Nain M and Gemsa D,

Release of tumor necrosis factor-alpha from macrophages. Enhancement and suppression are dose-dependently regulated by prostaglandin E_2 and cyclic nucleotides. *J Immunol* **141**: 2388–2393, 1988.

34. Kunkel SL, Spengler M, May MA, Spengler R, Larrick J and Remick D, Prostaglandin E_2 regulates macrophage-derived tumor necrosis factor gene expression. *J Biol Chem* **263**: 5380–5384, 1988.
35. Lehmann V, Benninghoff B and Dröge W, Tumor necrosis factor-induced activation of peritoneal macrophages is regulated by prostaglandin E_2 and cAMP. *J Immunol* **141**: 587–591, 1988.
36. Tannenbaum CS and Hamilton TA, Lipopolysaccharide-induced gene expression in murine peritoneal macrophages is selectively suppressed by agents that elevate intracellular cAMP. *J Immunol* **142**: 1274–1280, 1989.
37. Richman CM and Johnson GD, Granulocyte/macrophage progenitor cells from peripheral blood and bone marrow differ in their response to prostaglandin E_1. *Blood* **70**: 1792–1796, 1987.
38. Kato H, Ishitoya J and Takenawa T, Inhibition of inositol phospholipids metabolism and calcium mobilization by cyclic AMP-increasing agents and phorbol ester in neutrophils. *Biochem Biophys Res Commun* **139**: 1272–1278, 1986.
39. Lerner A, Jacobson B and Miller RA, Cyclic AMP concentrations modulate both calcium flux and hydrolysis of phosphatidylinositol phosphates in mouse T lymphocytes. *J Immunol* **140**: 936–940, 1988.
40. Kurachi Y, Nakajima T and Sugimoto T, On the mechanism of activation of muscarinic K^+ channels by adenosine in isolated atrial cells: involvement of GTP-binding proteins. *Pflügers Arch* **407**: 264–274, 1986.
41. Hopkins NK, Lin AH and Gorman RR, Evidence for mediation of acetyl glyceryl ether phosphorylcholine stimulation of adenosine 3',5'-(cyclic) monophosphate levels in human polymorphonuclear leukocytes by leukotriene B_4. *Biochim Biophys Acta* **763**: 276–283, 1983.
42. Miller OV, Ayer DE and Gorman RR, Acetyl glycerylphosphorylcholine inhibition of prostaglandin I_2-stimulated adenosine 3',5'-cyclic monophosphate levels in human platelets. Evidence for thromboxane A_2 dependence. *Biochim Biophys Acta* **711**: 445–451, 1982.
43. Bachelet M, Adolfs MJP, Masliah J, Bereziat G, Vargaftig BB and Bonta IL, Interaction between PAF-acether and drugs that stimulate cyclic AMP in guinea-pig alveolar macrophages. *Eur J Pharmacol* **149**: 73–78, 1988.