Metabolism of platelet-activating factor in human haematopoietic cell lines

Differences between myeloid and lymphoid cells

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The binding and metabolism of platelet-activating factor (PAF) was studied in human cell lines resembling myeloid cells (HL60 and U937) and B and T lymphocytes (Daudi and Jurkat). All of the cell lines were found to bind and catabolize exogenous [^3H]PAF in a time- and temperature-dependent manner. PAF binding could also be demonstrated in isolated membrane fractions, which provides further evidence of the existence of true membrane receptors. Myeloid cell lines contained numbers of receptors at least 10-fold higher than in lymphoid cell lines. Biosynthesis of PAF upon challenge by ionophore A23187 could be demonstrated in HL60 and U937 cells. In contrast, lymphoid cell lines were unable to produce PAF. Incubation with [^{14}C]acetate showed incorporation of the label into three main fractions: neutral lipids, phosphatidylcholine and PAF, but the distribution of the label varied depending on the cell line. Significant incorporation into phosphatidylcholine was observed in uninduced myeloid cell lines. A phospholipase A_2 acting on 1-*O*-hexadecyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine and an acetyl-CoA:lyso-PAF acetyltransferase were expressed in the HL60 cell line and showed variations in specific activity with granulocytic differentiation. In contrast, these enzyme activities were not expressed in Daudi and Jurkat cell lines. These data indicate (1) the occurrence of PAF binding and catabolism in both myeloid and lymphoid cell lines; (2) the restriction of PAF biosynthesis to myeloid cell lines, especially HL60 cells; (3) the occurrence of differentiation-elicited changes in the specific activities of the enzymes involved in PAF biosynthesis by the remodelling pathway; and (4) the central role played by the disposal of lyso-PAF, a product of the phospholipase A_2 reaction, in PAF biosynthesis.

INTRODUCTION

The phospholipid mediator platelet-activating factor (PAF) is a potent activator of a variety of cells which take part in inflammatory reactions, e.g. endothelial cells, platelets, polymorphonuclear leucocytes (PMN) and monocytes [1–4]. These cells respond to PAF because they have specific PAF receptors [5–7], and in some cases they metabolize PAF within the time required for cell activation [8,9]. Mononuclear phagocytes can metabolize PAF at different rates depending on their functional state [10,11], and a recent report has suggested that a major role of macrophages could be the modulation of the inflammatory reaction by promoting PAF catabolism, since the intracellular concentration of PAF acetylhydrolase, the main enzyme involved in PAF catabolism, increases 260-fold after differentiation of human peripheral blood monocytes into macrophages [12]. At present, there are only two reports on the role played by lymphocytes in PAF catabolism [13,14], even though some studies indicate that PAF could play a role in the modulation of the immune response [15,16]. Thus a variety of cell types can be involved in the metabolism of PAF, and variations in the original pattern can be elicited by cell differentiation.

As an approach to these problems, some investigations have been carried out using the HL60 cell line [17–19], a human promyelocytic leukaemic cell line that behaves as a differentiative bipotent cell population [20]. Thus it can be induced to differentiate along the granulocytic pathway by incubation with dimethyl

sulphoxide (Me_2SO) or retinoic acid [21], whereas exposure to other inducers such as 1,25-dihydroxyvitamin D_3 or phorbol 12-myristate 13-acetate (PMA) results in monocytic/macrophage differentiation [22]. A report by Camussi *et al.* [17] related the capacity of HL60 cells to release PAF to the expression of membrane receptors and transformation into a phagocytic cell, whereas Billah *et al.* [18] stressed the role of defective mechanisms regulating the activity of phospholipase A_2 and acetyl-CoA:lyso-PAF acetyltransferase in uninduced cells.

The biosynthesis of PAF in PMN and peripheral blood monocytes mostly occurs through the remodelling pathway. This pathway includes a phospholipase A_2 (EC 3.1.1.4)-catalysed hydrolysis of 1-alkyl-2-acyl-*sn*-glycero-3-phosphocholine (1-alkyl-2-acyl-GPC) and acetylation of lyso-PAF by an acetyl-CoA:lyso-PAF acetyltransferase (EC 2.3.1.67). Since the acyl moiety released by phospholipase A_2 is most often arachidonate, PAF metabolism is tightly coupled to the formation of eicosanoids [23]. In this study we have selected a number of both lymphoid and myeloid cells resembling either T (Jurkat) and B (Daudi) lymphocytes or yielding granulocytes and macrophages upon differentiation, in order to characterize the features of PAF metabolism in each cell type. Our findings indicate: (1) the ability of all the cell types so far tested to metabolize exogenous PAF by a mechanism mediated by receptors, at least in part; (2) the restriction of PAF biosynthesis to myeloid cells; (3) the occurrence of variations in the expression of the enzymes involved in PAF biosynthesis at various stages in the differentiation

Abbreviations used: 1-alkyl-2-acyl-GPC, 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphocholine; lyso-PAF, lyso-platelet-activating factor; PAF, platelet-activating factor; Me_2SO , dimethyl sulphoxide; PC, phosphatidylcholine; PMA, phorbol 12-myristate 13-acetate; PMN, polymorphonuclear leucocytes.

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process; and (4) the central role played by a phospholipase A_2 activity in the modulation of PAF biosynthesis in HL60 cells.

MATERIALS AND METHODS

Cells

HL60 and U937 cells were grown at 37 °C in a humidified atmosphere containing CO₂/air (1:19) in RPMI 1640 medium supplemented with 10% (v/v) heat-inactivated fetal calf serum, 2 mM-L-glutamine, 100 units of penicillin/ml and 24 µg of gentamicin/ml. The cell suspensions were divided in order to maintain cell density between 5×10^5 and 1×10^6 cells/ml. Cells were induced to differentiate towards the monocyte/macrophage lineage by incubation with 32 nM-PMA for 24–48 h. The extent of monocyte/macrophage cell differentiation was assessed by morphology, cell cluster formation, adhesion to culture plates, and cell-surface expression of the Mo1 differentiation antigen (a glycoprotein heterodimer that is involved in cellular adhesion processes and that functions as the C3b receptor in human myeloid cells [24]). Granulocyte differentiation of HL60 cells was induced by adding 1.3% (v/v) dimethyl sulphoxide (Me₂SO) for 6 days unless mentioned otherwise. Granulocytic differentiation was assessed by morphological examination of Wright's-stained cytocentrifuge preparations, as well as by cell-surface expression of Mo1 antigen. This was determined by immunofluorescence flow cytometry as previously described [25]. Daudi and Jurkat cell lines were maintained at 37 °C in RPMI 1640 medium supplemented with 10% heat-inactivated calf serum, L-glutamine and antibiotics as described above.

Materials

Ionophore A23187 was from Calbiochem–Behring, La Jolla, CA, U.S.A. Acetyl-CoA was from Boehringer, Mannheim, Germany. [³H]Acetyl-CoA (2 Ci/mmol), [¹⁴C]acetate (55 Ci/mmol) and [³H]hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine (81 Ci/mmol) were from Amersham International, Amersham, Bucks., U.K. PMA was from Sigma Chemical Co., St. Louis, MO, U.S.A. RPMI 1640, fetal calf serum and L-glutamine were from Flow Laboratories, Irvine, Scotland, U.K. Fluorescein isothiocyanate-conjugated F(ab')₂ fragment of rabbit anti-mouse immunoglobulin was from Dakopatts, Glostrup, Denmark. The anti-(Mol α subunit) (CD11b) Bear 1 monoclonal antibody was used for flow cytometry studies. P3 × 63 myeloma culture supernatant was used as a negative control. PCA 4248 [2-(phenylthio)ethyl-5-methoxycarbonyl-2,4,6-trimethyl-1,4-dihydropyridine-3-carboxylate], a specific and competitive antagonist of PAF binding with a K_i of 15 nM for inhibition of [³H]PAF binding to rabbit platelets [26], was synthesized by Alter S. A., Madrid, Spain.

Quantification of [³H]PAF binding to intact cells

Cell suspensions were washed three times with a Hepes-buffered medium and resuspended at a concentration of 5×10^6 cells/ml in the same medium supplemented with 1 mM-CaCl₂ and 0.1% BSA. Viability of the cells was always > 98% as assessed by Trypan Blue exclusion. Binding studies were carried out in a volume of 0.5 ml in Eppendorf microcentrifuge tubes at either 37 °C or 4 °C. The concentration of [³H]PAF was 0.12 nM and different concentrations of unlabelled PAF were added. The reaction was stopped by addition of cold incubation medium and centrifugation at 8000 rev./min for 30 s. The supernatants were removed and the cell pellets were disrupted and mixed with a scintillation solution for aqueous samples. The binding data were subjected to Scatchard analysis [27] using the program EDDBA (Elsevier–Biosoft, Cambridge, U.K.) in a PC computer.

Assay of [³H]PAF binding to membrane fractions

For preparation of a membrane fraction, the cells were washed and resuspended in the Hepes-buffered medium used for lavage and disrupted with a probe-type sonicator (three 10 s pulses). Nuclei and unbroken cells were removed by centrifugation at 27000 *g* for 15 min and the supernatant was centrifuged at 100000 *g* for 60 min. The pellet was resuspended in washing buffer in the presence of 1 mM-CaCl₂, 1 mM-MgCl₂ and 0.1% BSA, and binding studies were carried out in membrane fractions containing 100 µg of protein, measured by the method of Bradford [28]. [³H]PAF was used at a concentration of 0.25 pM, and unlabelled PAF was used at concentrations up to 800 nM. The reaction was incubated for 1 h at room temperature in a volume of 0.5 ml, and stopped by addition of 1 ml of cold medium followed by filtration in a Millipore 1225 sampling manifold, fitted with 0.22 µm pore size glass fibre filters (Millipore Iberica, Madrid, Spain). Extraction of the filter with methanol was carried out to elute bound [³H]PAF. Membrane fractions from rabbit platelets and human PMN were also studied as positive controls.

Metabolism of cell-bound [³H]PAF

A lipid extract from cells was obtained by using the Bligh & Dyer [29] procedure and subjected to t.l.c. on silica-gel plates using as developer the system chloroform/methanol/acetic acid/water (50:25:8:6, by vol.), since this allows a good separation of PAF, lyso-PAF and 1-alkyl-2-acyl-GPC, i.e. the major metabolites of PAF in the remodelling pathway. Further characterization of these products was carried out by straight-phase h.p.l.c. under the conditions described for the PAF biosynthesis assay.

Assay of [¹⁴C]PAF biosynthesis

This was carried out by incubating 10⁷ cells in 1 ml of a Hepes-buffered medium containing 140 mM-NaCl, 3 mM-KCl, 1 mM-CaCl₂, 1 mM-MgCl₂, 5.6 mM-glucose, 0.25% BSA and 20 µCi of [¹⁴C]acetate, pH 7.4. Stimulation of the cells was carried out with 10 µM-ionophore A23187 for 20 min. The lipid extract was evaporated to dryness and resuspended in the mobile phase used for straight-phase h.p.l.c. This was carried out in a Spheri-5 silica column using as a mobile phase 96% B (propan-2-ol/hexane, 1:1, v/v) and 4% A (water); the proportion of A was linearly increased to 8% during a 15 min period [30]. The column was eluted for 60 min at 2 ml/min, and the fractions collected were used for scintillation spectrometry after addition of a scintillation cocktail for non-aqueous samples. Further characterization of the [¹⁴C]acetate-containing phospholipid as authentic PAF was carried out by demonstrating loss of the label after treatment with phospholipase A_2 , which indicates that the [¹⁴C]acetate has not been elongated. The labelled phospholipid was also remarkably resistant to phospholipase A_1 treatment, which allows us to rule out the incorporation of [¹⁴C]acetate into a moiety linked by an ester bond at the *sn*-1 position. Furthermore, in two experiments carried out with ionophore A23187-stimulated HL60 cells in the absence of [¹⁴C]acetate, platelet-activating activity similar to that displayed by PAF was found to elute at the same time of retention.

Assay of phospholipase A_2 and acetyl-CoA:lyso-PAF acetyltransferase

The assay of phospholipase A_2 activity was performed as described by Billah *et al.* [18], except that the substrate was added in methanol, evaporated to dryness and dispersed into micelles by using a probe-type sonicator. The reaction mixture medium consisted of 0.1 mM-Tris/HCl, 2 mM-CaCl₂ and 2 µM-1-*O*-

hexadecyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine (81 Ci/mmol), pH 7.4. The reaction was initiated by adding the cell homogenate and was allowed to proceed for up to 30 min. The labelled substrate was from two sources, either prepared from liver microsomal fractions incubated with [3 H]arachidonic acid [31], or purchased from New England Nuclear. Both substrates were equally radiochemically pure, as assessed by h.p.l.c., and gave similar results. The reaction was stopped by addition of methanol/chloroform (2:1, v/v) and the lipids were extracted and separated by t.l.c. The silica gel areas corresponding to phosphatidylcholine (PC) and to arachidonate were scraped and counted for radioactivity by liquid scintillation counting. The assay of acetyl-CoA:lyso-PAF acetyltransferase activity was carried out as described by Wykle *et al.* [32].

RESULTS

Binding of [3 H]PAF to intact cells

All of the cell lines incubated in the presence of [3 H]PAF showed a time-dependent incorporation of 3 H radioactivity into cell lipids. This is shown for Me₂SO-differentiated HL60 cells and PMA-differentiated U937 cells in Fig. 1; similar profiles obtained for Daudi and Jurkat cell lines are not shown. The accumulation of cell-associated radioactivity reached a maximum at about 60 min, was decreased when the incubation was carried out in the presence of an excess of unlabelled PAF, and was

Fig. 1. Binding of [3 H]PAF to Me₂SO-treated HL60 cells (a) and to PMA-treated U937 cells (b)

Cells at a concentration of 5×10^6 /ml were incubated at 37 °C in the presence of 0.12 nM-[3 H]PAF, and at the times indicated cells were collected, subjected to lipid extraction and the radioactivity incorporated in the fractions migrating as 1-[3 H]alkyl-2-acyl-GPC (●) and [3 H]PAF (■) in t.l.c. was quantified. Data represent mean values of an experiment with duplicate samples.

Table 1. Metabolism of exogenous [3 H]PAF by different cell lines: effect of temperature and of a PAF receptor antagonist

Approx. 5×10^6 cells were incubated with 0.12 nM-[3 H]PAF (about 12000 d.p.m.) at 37 °C or at 4 °C for 60 min. At the end of this period, lipids were extracted and the radioactivity associated with the 1-alkyl-2-acyl-GPC fraction was assessed. * Indicates experiments carried out in the absence of 10 μ M-PCA 4248; ** indicates experiments carried out in the presence of 10 μ M-PCA 4248. Cells were incubated for 6 days in the presence of Me₂SO to promote granulocytic differentiation. Data represent mean values of two experiments with duplicate samples. ND, not determined.

Cell line	Temperature...	1-Alkyl-2-acyl-GPC (d.p.m.)		
		37 °C*	37 °C**	4 °C
Uninduced HL60		5187	2658	616
Me ₂ SO-differentiated HL60		5432	ND	ND
PMA-differentiated HL60		4569	3247	984
Uninduced U937		5985	2214	ND
PMA-differentiated U937		6002	4214	515
Daudi		3294	ND	276
Jurkat		3328	2234	143

dependent on the temperature, since significant binding at 4 °C was only observed in Me₂SO-differentiated HL60 cells (results not shown). Binding of [3 H]PAF was accompanied by a rapid conversion into 1-alkyl-2-acyl-GPC in all of the cell lines studied (Fig. 1, Table 1). When the incubation was carried out at 4 °C, the incorporation of radioactivity into 1-alkyl-2-acyl-GPC was decreased by 78–95 % (Table 1). No increase of the amount of lyso-PAF associated with the cells could be detected, which is in keeping with the existence of an efficient mechanism of either reacylation or transacylation. The PAF-receptor antagonist PCA 4248 (10 μ M) lowered PAF conversion into 1-alkyl-2-acyl-GPC by 40 % in all of the cell lines (Table 1). Scatchard analysis of [3 H]PAF binding by cell lines showed a pattern consistent at first glance with the presence of receptors, but values were unacceptably high, i.e. $> 10^5$ PAF receptors/HL60 or U937 cell, which suggested, as already pointed by Valone [8], that uptake and metabolism could also occur by non-receptor-mediated mechanisms. Although these findings could allow the distinction between two kinds of cell lines as regards PAF binding (i.e. myeloid cell lines, which have a larger number of high-affinity binding sites and lymphoid cells, which have a lower number of binding sites), further experiments were planned using membrane fractions in order to overcome the possible influence on the quantification of [3 H]PAF binding of endocytosis and hydrolysis by cytosolic PAF acetylhydrolase.

Binding of [3 H]PAF to cell membrane fractions

Scatchard analysis of the binding kinetics was always consistent with the existence of a single class of high-affinity binding sites, irrespective of the variations detected in the number of binding sites. Binding of [3 H]PAF to membrane fractions from HL60 and U937 cells was consistent with a number of receptors similar to that assessed in human PMN [33], and a significant variation in the number of receptors elicited by differentiation could only be demonstrated in U937 cells after exposure to PMA. In contrast, Daudi cells had a receptor density decreased by about two orders of magnitude as compared with HL60 cells and U937 cells (Table 2). Extraction of lipids from membrane fractions used for binding showed no conversion of [3 H]PAF into 1-[3 H]alkyl-2-acyl-GPC, which is in keeping with the cytosolic location of PAF acetylhydrolase [34] and its absence from the membrane fraction used to carry out binding studies.

Table 2. Binding of [³H]PAF to membrane fractions

Data obtained from the Scatchard representation are expressed as means ($\pm$ S.D.) * Indicates cells differentiated for 2 days in the presence of 32 nM-PMA.

Cell line	$10^{-12} \times$ Receptor density (receptors/ mg of protein)	K_d (nM)	n
Uninduced HL60	1.1 ± 0.0	2.1 ± 1.9	5
PMA-differentiated HL60*	0.8	0.5	1
Uninduced U937	0.61 ± 0.27	1.5 ± 1.3	3
PMA-differentiated U937*	3.4	1.2	2
Daudi	0.04	2.1	2

Incorporation of [¹⁴C]acetate into phospholipids

Uninduced HL60 cells incorporated the [¹⁴C]acetate label into three different lipid fractions with the retention times of neutral lipids, PC and PAF, i.e. 3 min, 16 min and 21 min respectively (Fig. 2). The incorporation of the label into the PAF fraction in control resting cells was 732 ± 215 d.p.m., and this increased to 4794 ± 915 d.p.m. after stimulation with $10 \mu\text{M}$ -ionophore A23187 for 15 min. Incorporation was enhanced to 7162 ± 1109 d.p.m. when lyso-PAF and ionophore A23187 were used in combination (Table 3). The actual values for the incorporation of the label in Me_2SO -differentiated cells were lower than those detected in uninduced HL60 cells, but showed the same tendency to increase on the addition of ionophore A23187 and $10 \mu\text{M}$ -lyso-PAF (Table 3). PMA-differentiated HL60 cells showed a lower incorporation of [¹⁴C]acetate as compared with

Fig. 2. Incorporation of [¹⁴C]acetate into lipids from uninduced HL60 cells: straight-phase h.p.l.c. chromatogram showing the effects of ionophore A23187 and exogenous lyso-PAF

Cells were incubated in the absence (broken line) or presence (solid line) of both $10 \mu\text{M}$ -A23187 and $10 \mu\text{M}$ -lyso-PAF for 15 min. At the end of this period, lipids were extracted, separated by straight-phase h.p.l.c., and the radioactivity associated with each fraction was quantified by scintillation spectrometry. The arrows indicate the retention times of the standards.

undifferentiated cells. U937 cells showed the highest rate of incorporation of [¹⁴C]acetate into neutral lipids ($102498\text{--}152986$ d.p.m.) and an incorporation into PC ($7237\text{--}9891$ d.p.m.) comparable with that observed in uninduced HL60 cells. However, the incorporation of [¹⁴C]acetate into PAF was less than that observed in HL60 cell lines (e.g. $1169\text{--}1517$ d.p.m. in uninduced cells versus $698\text{--}1661$ d.p.m. in PMA-differentiated cells).

Daudi and Jurkat cell lines were unable to incorporate the label into [¹⁴C]PAF, and significant radioactivity only appeared associated with the neutral lipid fraction in both cell lines and also with the PC fraction in Daudi cells (Table 4). Taken together, these data indicate the existence of diverse metabolic pathways for [¹⁴C]acetate. The predominant one seems to be either synthesis *de novo* or elongation of fatty acids, and this could explain both the incorporation of the label into the neutral

Table 3. Incorporation of [¹⁴C]acetate into lipid fractions from HL60 cells

HL60 cells were incubated in the presence of $20 \mu\text{Ci}$ of [¹⁴C]-acetate/ml and different additions. The incubation was stopped by addition of methanol/chloroform (2:1, v/v) and the lipid extract was evaporated to dryness and subjected to straight-phase h.p.l.c. To obtain granulocytic differentiation, HL60 cells were cultured for 6 days in the presence of 1.3% (v/v) Me_2SO . To obtain monocytic differentiation, HL60 cells were grown for 2 days in the presence of 32 nM-PMA. Data are from two to four experiments with duplicate samples, and are expressed as either mean values or means $\pm$ S.D. when $n \geq 3$. Background counts have been subtracted.

Addition	¹⁴ C Incorporation (d.p.m.)		
	Neutral lipids	PC	PAF
Uninduced cells			
No addition	67483 ± 21423	14643 ± 3921	732 ± 215
Lyso-PAF	63422 ± 19486	8428 ± 4012	711 ± 315
A23187	69367 ± 13675	9429 ± 5102	4794 ± 915
A23187 + lyso-PAF	48632 ± 12559	5124 ± 3006	7762 ± 1109
Me₂SO-differentiated cells			
No addition	20692 ± 2019	515 ± 84	61 ± 10
Lyso-PAF	18221	423	57
A23187	15083	602	1672
A23187 + lyso-PAF	14527 ± 4087	963 ± 112	3543 ± 349
PMA-differentiated cells			
No addition	14650 ± 980	483 ± 16	233 ± 26
Lyso-PAF	10884	439	261
A23187	9548	451	824
A23187 + lyso-PAF	9985 ± 1507	490 ± 23	1587 ± 263

Table 4. Incorporation of [¹⁴C]acetate into lipid fractions from Daudi and Jurkat cells

See Table 3 for experimental details. Data represent mean values of two experiments with duplicate samples.

Addition	¹⁴ C incorporation (d.p.m.)		
	Neutral lipids	PC	PAF
Daudi			
No addition	4828	202	0
Lyso-PAF	4653	164	0
A23187	5822	198	0
A23187 + lyso-PAF	5123	123	0
Jurkat			
No addition	4543	23	0
Lyso-PAF	4876	31	0
A23187	5321	43	0
A23187 + lyso-PAF	5432	47	0

lipid fraction and a subsequent transfer into PC, which is the most abundant phospholipid class. Since we have not observed incorporation of [¹⁴C]acetate into the PC fraction of human PMN under similar conditions of incubation and stimulation [35], this incorporation is probably due to a high biosynthetic rate of PC in cells rapidly dividing in culture. Alternatively, our findings could reflect an enhanced turnover of PC linked to a PC cycle such as that described in a number of cells involved in immune reactions, including Jurkat and HL60 cells [36–38].

Biosynthesis of PAF by different cell lines

Since PAF formation appeared to be restricted to HL60 and U937 cells, was dependent on appropriate stimulation by a secretagogue such as ionophore A23187, and was enhanced by the addition of exogenous lyso-PAF, this prompted us to look at the enzymes involved in the final steps of PAF biosynthesis. Myeloid cell lines were found to express significant amounts of acetyl-CoA:lyso-PAF acetyltransferase activity (Tables 5 and 6), whereas Daudi cells contained less than 4 pmol/min per 10⁷ cells and Jurkat cells had undetectable levels. A decrease in acetyl-CoA:lyso-PAF acetyltransferase activity was observed coincidentally with differentiation of HL60 cells into granulocytes and the increase of Mo1 antigen expression on the cell surface (Table 5). The enzyme was apparently activated upon challenge with ionophore A23187, although less markedly than has been described in human PMN [39]. PMA-induced differentiation was

Table 5. Acetyl-CoA:lyso-PAF acetyltransferase activity and Mo1 antigen cell surface expression in HL60 cells

HL60 cells were cultured in the presence of either 1.3% (v/v) Me₂SO for 6 days or 32 nM-PMA for 2 days, and then collected to assess Mo1 antigen cell-surface expression or incubated in the presence of ionophore A23187 and processed for enzyme assay. Data represent means ± s.d. of three independent experiments with duplicate samples.

Induction	Acetyltransferase (pmol/min per 10 ⁷ cells)		Mo1 expression (% positive)
	Resting	+ Ionophore A23187	
Uninduced	78 ± 18	116 ± 13	4 ± 0
Me ₂ SO	14 ± 6	34 ± 10	90 ± 4
PMA	53 ± 7	—	78 ± 6

Table 6. Acetyl-CoA:lyso-PAF acetyltransferase activity in PMA-induced U937 cells

U937 cells were kept in culture in the presence of 32 nM-PMA for 24 or 48 h. At the end of these periods the cells were collected to assess Mo1 antigen cell-surface expression or incubated with either vehicle or ionophore A23187 before sonication and acetyltransferase assay. Data represent means ± s.d. of three independent experiments with duplicate samples.

Time (h)	Acetyltransferase (pmol/min per 10 ⁷ cells)		Mo1 expression (% positive)
	Resting	+ Ionophore A23187	
0	59 ± 9	74 ± 11	2 ± 0
24	38 ± 8	36 ± 13	48 ± 9
48	34 ± 8	31 ± 10	51 ± 10

Table 7. Phospholipase A₂ activity in Me₂SO-differentiated HL60 cells

Homogenates of HL60 cells grown in the presence of Me₂SO were incubated with 1-*O*-hexadecyl-2-[³H]arachidonoyl-*sn*-GPC under the conditions described in the Materials and methods section for the phospholipase A₂ activity assay. Results are expressed as d.p.m. of [³H]arachidonate released. Data represent means ± s.d. of three experiments, or mean values for two experiments in duplicate (144 h). Mo1 cell surface expression was assessed by flow cytometry in cell samples cultured and processed in parallel. ND, not determined.

Time in culture (h)	Mo1 cell-surface expression (% positive)	Phospholipase A ₂ activity (d.p.m.)
Uninduced	2	9842 ± 3623
3	ND	13987 ± 5278
6	ND	15420 ± 6502
24	ND	20453 ± 7549
72	48	22897 ± 8326
144	88	27272

also associated with diminution of acetyltransferase activity in HL60 and U937 cells (Tables 5 and 6), but this enzyme activity was not found to be activatable upon ionophore challenge in PMA-differentiated U937 cells (Table 6). The expression of phospholipase A₂ activity markedly increased following granulocytic differentiation to reach a nearly 3-fold increase by day 6, when most of the HL60 cells had acquired the properties of mature granulocytes (Table 7). In contrast, phospholipase A₂ activity in Jurkat and Daudi cells was undetectable.

DISCUSSION

The data presented in this paper are intended to provide a comprehensive view of the ability of some cell types resembling either lymphocytes or phagocytic cells to produce, bind and metabolize PAF. All of the cell lines, especially myeloid cells, bound [³H]PAF in a time-dependent manner. Binding of [³H]PAF was followed by a rapid conversion of the ligand into 1-[³H]alkyl-2-acyl-GPC. This raises the question as to whether this is due to binding to a membrane receptor followed by internalization and hydrolysis in the cytosol by PAF acetylhydrolase or whether, in turn, it is linked to another mechanism of uptake, as has been shown for leukotriene C₄ in rat hepatocytes, which binds to a protein located in the cytosol rather than to a membrane receptor [40]. The demonstration that we are dealing with a true membrane receptor in these cell lines stems from two main observations: (1) blunting of both binding and catabolism by a specific antagonist of the PAF receptor at a concentration which has been shown to block binding to PAF receptors in human platelets and PMN [26], and (2) the occurrence of binding, but not catabolism, on a membrane fraction. Furthermore, the functional significance of the putative membrane receptors has been substantiated by showing that PAF, at concentrations as low as 1 pM, increases free intracellular Ca²⁺ in a dose-dependent manner in P388D₁, U937, HL60 and Raji cells [13,41–43].

Another finding from this study is the ability of HL60 and U937 cell lines to synthesize PAF, in contrast with the absence of this property in the two lymphoid cell lines studied. The capacity of HL60 cells to generate PAF already exists in uninduced cells and persists in induced cells, at least after periods in culture of not longer than 6 days. These findings can be interpreted on the basis of our previous knowledge of the different factors involved in the modulation of PAF biosynthesis in cells taking part in

inflammatory reactions. The first requirement seems to be the expression of the enzymes involved in the final steps of biosynthesis, i.e. a phospholipase A_2 and an acetyl-CoA:lyso-PAF acetyltransferase. This can easily explain the lack of PAF biosynthesis by Jurkat and Daudi cell lines, but it does not indicate why U937 cells form PAF less efficiently than HL60 cells by the phospholipase A_2 /acetyltransferase-catalysed pathway. Our data differ in part from those reported by Billah *et al.* [18], who found uninduced HL60 cells unable to generate PAF upon challenge with ionophore A23187, whereas Me_2SO -induced HL60 cells produced PAF upon stimulation. A possible explanation for this difference could be the different procedures used to study PAF biosynthesis, since Billah *et al.* [18] carried out most studies using a bioassay with human platelets, whereas our studies have been performed by measuring the incorporation of [^{14}C]acetate into [^{14}C]PAF. Another difference with Billah's findings [18] is the variation of the expression of phospholipase A_2 and acetyltransferase activities in HL60 cells following induction by Me_2SO . In fact, a phospholipase A_2 activity acting on the alkyl-ether subclass of PC increases after induction, whereas acetyl-CoA:lyso-PAF acetyltransferase activity decreases. Since the addition of lyso-PAF at the time of ionophore A23187 challenge is an efficient mechanism with which to enhance PAF formation in uninduced cells, the most likely explanation is that a phospholipase A_2 activity, by providing lyso-PAF, plays a central role in PAF formation. This is in keeping with our findings in human PMN, in which lyso-PAF disposal is a critical event for PAF formation [35].

Our findings also show incorporation of [^{14}C]acetate into lipid fractions other than PAF, and this depends on the cell line. A possible reason could be competition of acetyl-CoA:lyso-PAF acetyltransferase with a very efficient mechanism of biosynthesis/elongation of fatty acids from acetyl-CoA, since the incorporation of [^{14}C]acetate into the neutral lipid fraction of U937 cells is 10-fold higher than that in PMA-differentiated HL60 cells, and furthermore this increases with induction. A report by Wakyl [44] has stressed the absence of acetyl-CoA carboxylase in human leucocytes, where acetate incorporation into fatty acids may only occur by chain elongation, but this enzyme activity is present in immature leukaemia blast cells, which are capable of carrying out fatty acid synthesis *de novo* [45].

In summary, this study stresses the differences between different cell lines with regard to PAF metabolism and suggests that cell lines with the ability to differentiate into granulocytes are the most efficient producers of PAF through a finely regulated phospholipase A_2 /acetyltransferase-catalysed pathway. Lymphoid cells could play a role in the modulation of inflammatory reactions by metabolizing PAF produced by other cell types.

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