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Platelet-activating factor-induced hydrolysis of phosphatidylinositol 4,5-bisphosphate stimulates the production of reactive oxygen intermediates in macrophages

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To investigate the relationship between inositol lipid hydrolysis and reactive oxygen-intermediate (ROI) production in macrophages we have examined the effect of platelet-activating factor (PAF) on normal bone marrow-derived macrophages. Addition of PAF to macrophages prelabelled with [^{3}H]inositol caused a marked and rapid increase in [^{3}H]inositol trisphosphate levels. Similarly when PAF was added to [^{3}H]-glycerol prelabelled macrophages there was a rapid increase in 1,2-diacyl[^{3}H]glycerol levels. These events preceded any increase in the rate of PAF-stimulated ROI production by a discernible period of several seconds. Increasing concentrations of PAF led to a markedly similar increase in both ROI production and [^{3}H]inositol lipid hydrolysis suggesting that inositol lipid hydrolysis may lead to the generation of ROI in macrophages. Further evidence that this is the case came from experiments in which pretreatment of macrophages with phorbol esters was shown to inhibit both PAF-stimulated [^{3}H]inositol phosphate production and ROI production to a markedly similar degree. Similarly pertussis toxin inhibited both PAF-stimulated ROI production and [^{3}H]inositol phosphate production. Phorbol esters were shown to activate ROI production in normal bone marrow-derived macrophages whereas the Ca^{2+} ionophore, A23187, did not. These experiments suggest that PAF stimulates a pertussis toxin-sensitive activation of inositol lipid hydrolysis leading to the formation of inositol trisphosphate and diacylglycerol. The diacylglycerol formed can then activate protein kinase C leading to the stimulation of ROI production in normal bone marrow-derived macrophages.

INTRODUCTION

Mononuclear phagocytes, or macrophages, depend on the activation of respiration and production of ROI such as the superoxide anion and hydrogen peroxide as one of the main functional responses by which they kill invading microbes or tumour cells (see Babior, 1984). This response can be induced by phagocytosis (Cooper *et al.*, 1984) or by the addition of agonists such as the *N*-formylated oligopeptides (see Snyderman, 1985).

The molecular basis for the induction of the respiratory burst in macrophages has not been well characterized when compared to the polymorphonuclear phagocyte or neutrophil. In these cells the increase in O_2 consumption observed is associated with a ligand-stimulated hydrolysis of inositol lipids (Volpi *et al.*, 1983; Yano *et al.*, 1983; Dougherty *et al.*, 1984) leading to the activation of protein kinase C (see Nishizuka, 1984) which in turn leads to the activation of NADPH oxidase and superoxide anion production (see Babior, 1984; Tauber, 1987). In macrophages some agonists (such as chemotactic peptides) do induce an increase in inositol lipid hydrolysis (Whetton *et al.*, 1986), but there is little direct evidence implicating this response in the initiation of a respiratory burst in mononuclear phagocytes. To resolve this question we have examined the response of normal bone marrow-derived macrophages to PAF, a phospholipid mediator molecular which can activate most inflammatory cells including eosinophils, neutrophils and platelets (Benveniste *et al.*, 1972; Wardlaw & Kay, 1986; Ford-Hutchinson, 1983; Gay *et al.*, 1986). There is also some evidence to suggest that PAF can stimulate a respiratory burst and chemotaxis in some preparations of macrophages (Czarnetzki, 1983; Hartung, 1983; Hayashi *et al.*, 1985); however alveolar macrophages are apparently not responsive to PAF (Hwang *et al.*, 1983). We have investigated the responses of a pure population of macrophages, freshly derived from bone marrow progenitor cells, to PAF. We show that there is a possible cause-and-effect relationship between PAF-stimulated inositol lipid hydrolysis and the production of ROI in normal bone marrow-derived macrophages similar to that previously reported in other phagocytic cells.

METHODS

Preparation of normal bone marrow-derived macrophages

Macrophages were cultured as previously described from normal (femoral) bone marrow cells (Whetton *et al.*, 1986) with the exception that for determinations of ROI production the cells were cultured in tissue culture flasks (Nunc, Roskilde, Denmark; 80 cm^2). Otherwise cells were cultured in 24-well microwell plates (Costar, Cambridge, MA, U.S.A.).

Abbreviations used: PAF, platelet-activating factor; ROI, reactive oxygen intermediate(s); TPA, tetradecanoylphorbol acetate.

Loading of macrophages with [^{3}H]inositol

The culture medium was removed from adherent layers of normal bone marrow derived-macrophages in microwell plates. This was replaced by 0.5 ml of fresh Fischers medium containing 1% (v/v) horse serum, 20% (v/v) L-cell conditioned medium (as a source of colony stimulating factor 1) and 4 μCi of [^{3}H]inositol (Amersham International, Amersham, U.K.) in each well. The microwell plate was then incubated at 37 °C in a 1:19, CO_2/air atmosphere for 18 h. The medium was removed and the adherent layer of cells was washed twice with Fischers medium containing 1% (w/v) bovine serum albumin. The cells were finally incubated in 500 μl of the above medium for 2 h at 37 °C prior to the addition of agonists which was performed as previously described (Whetton *et al.*, 1986). Preincubations were performed in Fischers medium plus bovine serum albumin, followed by two washes of the cells prior to the addition of agonists.

Loading of macrophages with [^{3}H]glycerol

Macrophages were loaded with [^{3}H]glycerol using the methods described above except that 4 μCi of [^{3}H]-glycerol was added to each well instead of [^{3}H]inositol.

Measurement of [^{3}H]inositol phosphates

Perchloric acid-treated samples were collected and in general the contents of two microwells were pooled. Each microwell was washed out with 100 μl of water and this was added to the sample, which was then centrifuged and 700 μl of supernatant removed. To this was added 400 μl of a freon/tri-*n*-octylamine mixture (1:1, v/v) to neutralize excess perchloric acid (Sharpes & McCarl, 1982). The resulting solution was centrifuged and 600 μl of the upper phase removed and made up to 5.0 ml with water. EDTA was also added to a final concentration of 1 mM. This solution was taken for anion exchange chromatography on Dowex-1 formate columns as previously described (Downes *et al.*, 1986). The following fractions were eluted: water (10 ml, containing free inositol); 60 mM-ammonium formate/5 mM-disodium tetraborate (10 ml, to elute any deacylated inositol lipids); 200 mM-ammonium formate/100 mM-formic acid (10 ml, which elutes inositol monophosphates); 400 mM-ammonium formate/100 mM-formic acid (10 ml, which elutes inositol bisphosphates); 800 mM-ammonium formate/100 mM-formic acid (10 ml, which elutes inositol trisphosphates). Where elution of total inositol mono-, bis- and tris-phosphates was required only the 800 mM-ammonium formate/100 mM-formic acid (10 ml) of the last three eluants was applied to the columns. H.p.l.c. analysis was employed to confirm the identity of labelled components of the acid-soluble fractions from macrophages using the methods previously described (Hawkins *et al.*, 1986).

Measurement of diacyl[^{3}H]glycerol

After incubating [^{3}H]glycerol-labelled cells with the additives previously described (Whetton *et al.*, 1986) the incubations were terminated by the addition of trichloroacetic acid [10% (w/v), 50 μl] followed by vigorous mixing. The resulting pellet was extracted with 400 μl of chloroform/methanol (2:1, v/v) for 1 h at 4 °C. An aqueous solution containing EDTA (5 mM, 80 μl) was added to the chloroform/methanol extract and the two phases were mixed vigorously. The organic phase was then dried (Folch *et al.*, 1957). The dried lipid was resuspended in 50 μl of chloroform/methanol (9:1, v/v).

A portion of this (25 μl) was applied to silica-gel t.l.c. plates (Merck) and developed in chloroform/acetone (96:4, v/v) (Thomas *et al.*, 1965).

The location of 1,2-diacylglycerol was established (on every t.l.c. plate) by running a 1,2-diacylglycerol standard. The area containing 1,2-diacylglycerol was cut out and the radioactivity determined by liquid-scintillation counting.

Determination of ROI production

The measurement of ROI over the short time courses required was performed using the chemiluminescence technique described by Trush *et al.* (1978). The chemiluminescent compound luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) reacts with ROI generated during the respiratory burst of phagocytic cells. These emissions were recorded in a liquid-scintillation counter (Packard 3000 Tricarb) in the out-of-coincidence mode (Trush *et al.*, 1978).

To prepare normal bone marrow-derived macrophages for chemiluminescence determinations the cell monolayers were washed twice and prepared as detailed above for incubations prior to [^{3}H]inositol phosphate determinations. The sole difference was that the cells were gently suspended by agitation with a rubber policeman after the second wash. The suspension cells were then centrifuged and resuspended to 5×10^5 cells/ml in Fischers medium containing 1% (w/v) bovine serum albumin. After preincubation (as described in Whetton *et al.*, 1986) the cells were divided into 1 ml portions. After another 10 min preincubation, luminol (10 μl, 20 mM) was added and the cells left for a further 1 min. The appropriate stimuli were then added to the cells and the vial placed immediately in the liquid-scintillation counter and the relative rate of ROI production determined from the chemiluminescence readings obtained. All readings were on cells which were maintained at 37 °C. Cellular viability (determined by Trypan Blue exclusion) was > 95% in macrophages prepared for chemiluminescence assays. All values shown are net values obtained by subtracting the chemiluminescence obtained when macrophages were incubated in the absence of luminol.

Unless otherwise shown, results are the means of duplicate determinations, and are representative of at least three such experiments.

RESULTS

PAF can stimulate inositol phosphate production

We have previously shown that the chemotactic peptide, formylmethionyl-leucylphenylalanine, stimulates the hydrolysis of [^{3}H]phosphatidylinositol 4,5-bisphosphate to form the second messenger [^{3}H]inositol 1,4,5-trisphosphate. To establish whether PAF stimulates a similar hydrolysis of [^{3}H]phosphatidylinositol 4,5-bisphosphate we measured the production of [^{3}H]inositol phosphates. In PAF (50 μM)-treated macrophages there was a rapid increase in [^{3}H]inositol trisphosphate levels which reached a maximum point within 15 s of the addition of the agonist (Fig. 1). The production of

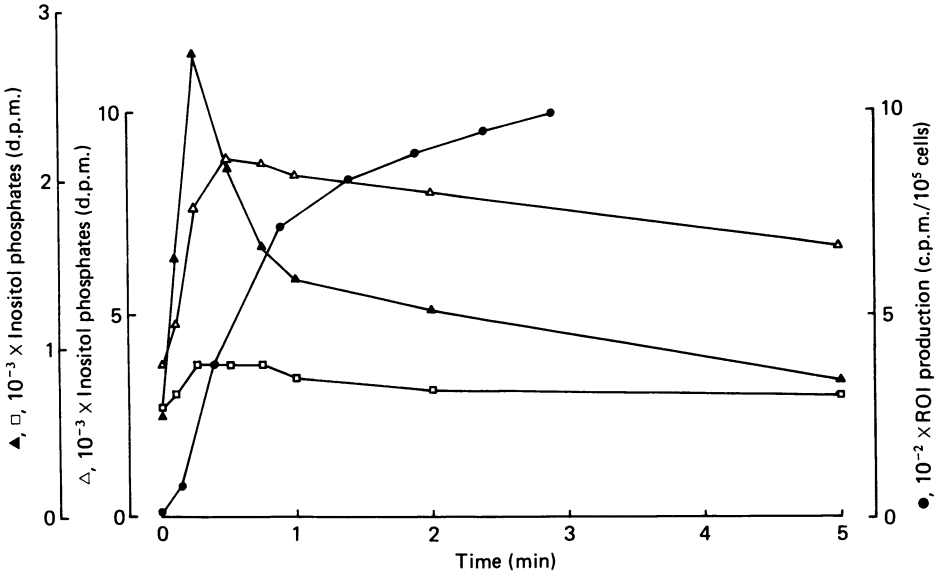

Fig. 1. Effect of PAF on [^{3}H]inositol phosphate levels and ROI production

A time course of the effect of PAF (50 μM) on [^{3}H]inositol trisphosphate (▲), [^{3}H]inositol bis- plus mono-phosphates (△), [^{3}H]inositol tetrakis- plus pentakis-phosphate levels (□), and ROI production (●), in normal bone marrow-derived macrophages. Results shown are the means of triplicate determinations from one of four similar experiments. The S.D. was < 10% of the values shown for all the experimental points displayed.

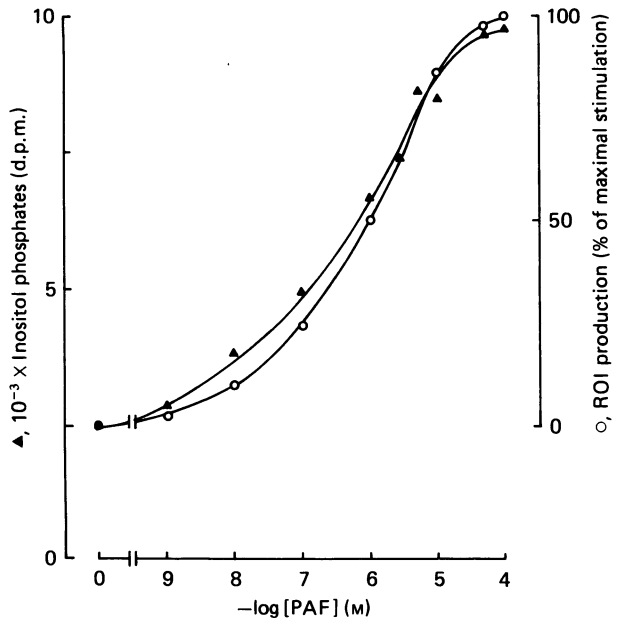

Fig. 2. Effect of increasing doses of PAF on [^{3}H]inositol phosphate production and ROI production

The effect of increasing PAF concentrations on [^{3}H]-inositol phosphates production (▲, over a 30 s time course), and ROI production (○, over a 1 min time course) are shown. Results shown are the means of duplicate determinations from one of four similar experiments.

[^{3}H]inositol bisphosphate plus [^{3}H]inositol monophosphate was somewhat slower, reaching a maximal value after 30 s. However, the rather smaller increases in the higher inositol phosphates ([^{3}H]inositol tetrakisphosphate plus [^{3}H]inositol pentakisphosphate), also reached a maximal value after 15 s and fell only very slowly over the next 4 min. The [^{3}H]inositol trisphosphate peak, on the other hand, fell precipitously after the initial 15 s to values approaching control incubations after 5 min. These results are consistent with a PAF-mediated hydrolysis of an inositol lipid (probably phosphatidylinositol 4,5-bisphosphate) to yield inositol trisphosphate plus diacylglycerol. The inositol trisphosphate formed is then rapidly phosphorylated to inositol tetrakisphosphate, or sequentially dephosphorylated to inositol bis- and mono-phosphates respectively. Furthermore it is clear that normal bone marrow-derived macrophages will respond to PAF.

This response is dependent on the dose of PAF added to the macrophages (Fig. 2). The dose required to achieve a half maximal rate of [^{3}H]inositol phosphate production was approx. 1 μM-PAF. However, it should be noted that the 1% bovine serum albumin present in the assay medium (which is essential for the maintenance of cellular viability) may bind PAF and therefore the free PAF concentration may differ from that shown in Fig. 2.

PAF can stimulate diacylglycerol formation

The agonist-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate will yield not only inositol trisphosphate but also diacylglycerol (see Downes & Michell, 1985). To confirm that diacylglycerol is produced by the action of PAF on macrophages we have labelled the cells with [^{3}H]glycerol. The 16 h incubation described was of sufficient duration to achieve a maximal incorporation of [^{3}H]glycerol (d.p.m./cell), which probably reflects full equilibration of this isotope with unlabelled glycerol. Changes in incorporated radioactivity can thus be taken to reflect changes in the cellular mass of lipid.

Treatment of macrophages with PAF significantly increased the cellular content of 1,2-diacyl[^{3}H]glycerol

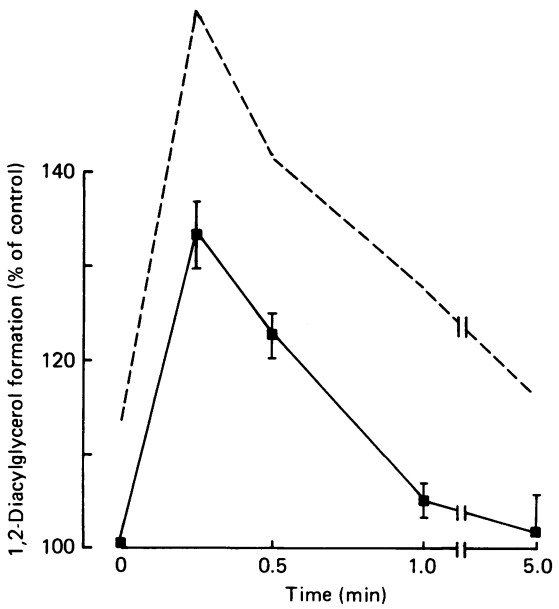

Fig. 3. Time course following the effect of PAF (50 μM) on diacyl[^{3}H]glycerol levels in normal bone marrow-derived macrophages

Total radioactivity per well was approx. 85000 d.p.m. Control wells contained 1200 d.p.m. of diacyl[^{3}H]glycerol. The broken line is the shape of the time course for PAF-stimulated [^{3}H]inositol trisphosphate production taken from Fig. 1, superimposed here for comparison. Results shown are the means ± S.D. from four observations.

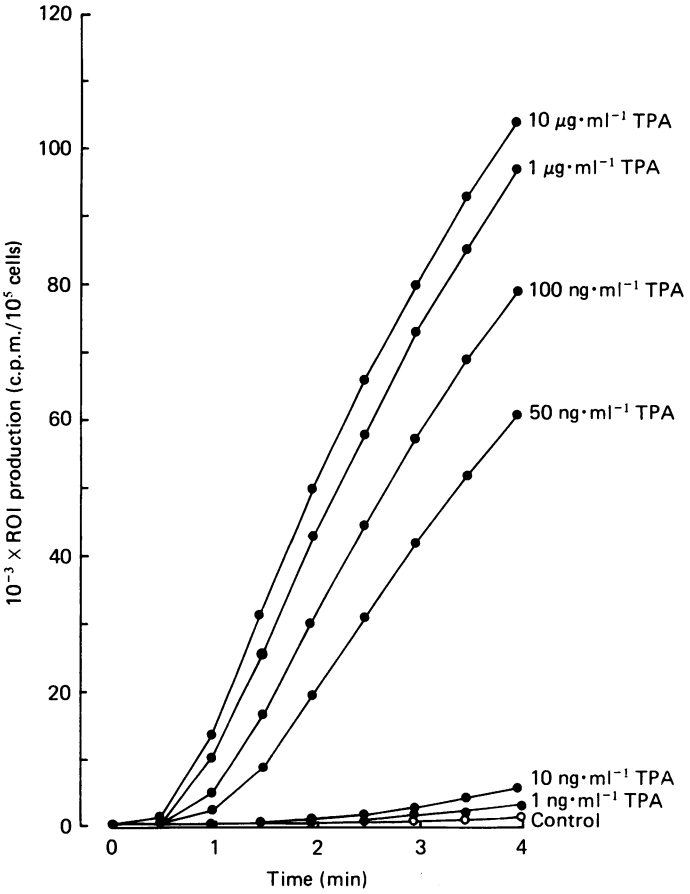

Fig. 4. Effect of the phorbol ester, TPA, on ROI production in normal bone marrow-derived macrophages

Results shown are from one of four similar experiments.

over a time course which was similar to that shown for the PAF-stimulated increase in [^{3}H]inositol trisphosphate levels (Fig. 3). Also the rapid increase in 1,2-diacyl-[^{3}H]glycerol was a transient phenomenon in that the rise elicited by PAF was significantly above control levels for less than 5 min. In no instance was there a significant increase or decrease in triacyl[^{3}H]glycerol in PAF-treated macrophages.

Effect of PAF on production of ROI

To examine the relationship between the putative second messengers produced within macrophages by the action of PAF and the biological effects of this agent on macrophages we have studied the production of ROI in these cells.

PAF stimulated a marked and rapid increase in ROI production, as assessed by luminol-dependent chemiluminescence (Trush *et al.*, 1978). There was a noticeable lag for the first 5–10 s after PAF addition (Fig. 1) followed by a maximal rate of ROI production for approx. 1 min. After this time there was a steady fall in this rate until control values were reached after about 5 min. The available evidence indicates that the PAF-stimulated hydrolysis of phosphatidylinositol 4,5-bisphosphate preceded any increase in ROI production.

The relationship between PAF concentration and ROI production was extremely similar to that between PAF concentration and [^{3}H]inositol phosphate production (Fig. 2). These results are inferential evidence of a second messenger role for inositol phosphates and diacylglycerol in PAF-stimulated ROI production.

Effect of phorbol esters and Ca^{2+} ionophore on ROI production

Further evidence of a second messenger role for inositol phosphates and/or diacylglycerol on ROI production in mononuclear phagocytes can be obtained by artificially mimicking their effects on these cells. Two major roles have been ascribed to these second messengers; inositol trisphosphate is thought to increase cytosolic Ca^{2+} levels in a wide variety of cells, including macrophages (Hirata *et al.*, 1984). This effect can also be achieved with the Ca^{2+} ionophore, A23187. Similarly phorbol esters such as tetradecanoylphorbol 13-acetate (TPA) can activate protein kinase C, as can synthetic diacylglycerols (see Nishizuka, 1984).

It has previously been demonstrated (Berton & Gordon, 1985) that TPA can stimulate ROI production in normal bone marrow-derived macrophages over a 4 h incubation period. We have shown (using the highly sensitive luminol-dependent chemiluminescence assay) that TPA initiates a rapid (< 1 min) dose-dependent increase in ROI production in normal bone marrow-derived macrophages (Fig. 4). After an initial lag phase, which is dependent on the concentration of TPA, significant increases in ROI were observed at doses of 1.0 ng/ml and above. The maximal initial rate obtained (allowing for the reduction in lag phase achieved at higher doses) was at TPA concentrations of 1 μg/ml and

Table 1. Effect of preincubation of macrophages with TPA on PAF-stimulated [^{3}H]inositol phosphate production and PAF-stimulated ROI production

Macrophages were pretreated with or without TPA (100 ng/ml) for the times indicated below, after which PAF (50 μM) was added to the cells and either [^{3}H]inositol phosphate production over a 30 s time course or ROI production over a 1 min time course was determined. PAF stimulated an increase of 7600 d.p.m. in [^{3}H]inositol phosphates over this period in control cultures. PAF also stimulated an increase in luminol-dependent chemiluminescence of 7500 c.p.m./min per 10^5 cells in control cultures. Results shown are from three experimental determinations (mean ± S.D.).

Preincubation time with TPA (h)	PAF-stimulated [^{3}H]inositol phosphate formation (% of control)	PAF-stimulated ROI production (% of control)
0	98 ± 5	100 ± 12
0.5	26 ± 17	18 ± 10
1.0	16 ± 8	10 ± 8

above. Ca^{2+} ionophore had no effect on ROI production at concentrations up to 100 ng/ml. Similarly at TPA concentrations of 10 ng/ml and higher, Ca^{2+} ionophore (0–100 ng/ml) did not increase the rate of TPA-stimulated ROI production. However at a TPA concentration of 1 ng/ml there was a small but significant synergistic interaction between TPA and Ca^{2+} ionophore (1 ng/ml) leading to enhanced ROI production.

Effect of chronic incubation with phorbol esters on PAF-stimulated [^{3}H]inositol phosphate production and ROI production

Incubation with phorbol esters for extended periods of time has been shown to inhibit agonist-stimulated inositol lipid hydrolysis in a variety of cell types (Zavoico *et al.*, 1985; Watson & Lapetina, 1985). To assess the relationship between inositol lipid hydrolysis and production of ROI, cells were pretreated with TPA (100 ng/ml) for varying periods of time (30 s and 1 h) prior to determination of PAF-stimulated [^{3}H]inositol phosphate production and PAF-stimulated ROI production respectively. This TPA preincubation led to a time-dependent inhibition of both [^{3}H]inositol phosphate production and ROI production (Table 1) which were inhibited to approx. the same degree at each time point.

Pertussis toxin sensitivity of PAF-stimulated [^{3}H]inositol phosphate and ROI production

To determine if there is a guanyl nucleotide regulatory protein associated with activation of [^{3}H]inositol phosphate and ROI production by PAF we have employed pertussis toxin. This can catalyse the ADP ribosylation of several guanyl nucleotide regulatory proteins (Ui, 1984; Van Dop *et al.*, 1984; Sternweis & Robishaw, 1984) and there is evidence to suggest that one such 'G' protein couples the chemotactic peptide receptor to stimulation of phosphatidylinositol 4,5-bisphosphate hydrolysis (Volpi *et al.*, 1983; Smith *et al.*, 1985; Paris & Pouyssegur, 1986).

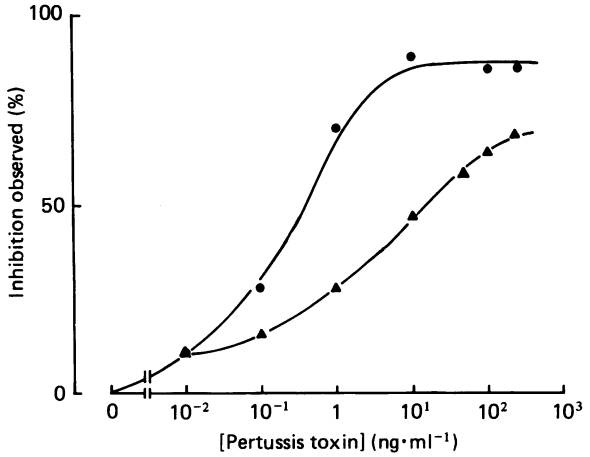

Fig. 5. Effect of pertussis toxin on [^{3}H]inositol phosphate and ROI production

The effect of increasing doses of pertussis toxin on PAF (50 μM)-stimulated [^{3}H]inositol phosphate production (▲) and ROI production (●) were measured over a 30 s and a 1 min time course respectively. Results shown are the mean values from four experiments. S.D. for all points shown were < 8%.

Incubation of normal bone marrow-derived macrophages with increasing concentrations of pertussis toxin for 4 h, followed by assay of PAF-stimulated [^{3}H]inositol phosphate and ROI production, showed a dose-dependent inhibition of both these parameters (Fig. 5). After this incubation period the maximum inhibition of [^{3}H]-inositol phosphate production recorded was 70%, whilst that for ROI production was 85%. The $I.D._{50}$ for [^{3}H]inositol phosphate production was approx. 3 ng/ml whilst that for ROI production was approx. 0.3 ng/ml. Incubation of macrophages overnight with pertussis toxin (100 ng/ml) completely inhibited both PAF-stimulated [^{3}H]inositol phosphate production and also ROI production (> 95% in both cases). TPA-stimulated ROI production was not modified by overnight treatment with pertussis toxin (100 ng/ml).

DISCUSSION

The mononuclear phagocyte is capable of a wide variety of functions including host defence against microbes and tumour cells, phagocytosis of dead cells, and a role in wound healing and atherogenesis (see Lasser, 1982). These functions are controlled by extracellular stimuli which can mediate the proliferative, secretory, locomotive, and cytotoxic properties of macrophages (see Hamilton & Adams, 1987).

Given the enormously varied role of mononuclear phagocytes it is of some interest to establish the intracellular signals generated by the wide variety of extracellular stimuli which can influence the functions of these cells and how these intracellular signals may interact with one another.

For several reasons we have chosen normal bone marrow-derived macrophages as the most suitable cell preparation for these studies. Firstly, a pure population of macrophages of approximately the same age is obtained. Secondly, tissue macrophages can develop

along several mutually-exclusive routes (Adams & Hamilton, 1984) which means that responses to extracellular stimuli may be different within the same preparation of tissue-derived macrophages. Thirdly, the normal bone marrow-derived macrophage population is still extremely responsive to proliferative stimuli such as haemopoietic growth factors (e.g. colony-stimulating factor-1) whereas tissue based macrophages are markedly less responsive (Stanley *et al.*, 1983). Thus, for comparison of the intracellular signals generated from the wide variety of extracellular stimuli to which macrophages can respond (see Hamilton & Adams, 1987), normal bone marrow-derived macrophages represent an ideal model system.

There have as yet been few studies to characterize the response of macrophages to extracellular stimuli. We have chosen to begin by characterizing the intracellular signals generated by a well-defined chemotactic stimulus and leucocyte activator, PAF. We have shown that PAF can stimulate the production of [^{3}H]inositol phosphates within normal bone marrow-derived macrophages. Furthermore the time course and dose dependency of PAF-stimulated [^{3}H]inositol phosphate and [^{3}H]-diacylglycerol production strongly suggest that this leads to the stimulation of ROI production (see Figs. 1 and 2).

The observation that TPA, but not Ca^{2+} ionophore can markedly stimulate ROI production leads us to believe that the formation of diacylglycerol (Fig. 3) by PAF, leading to the activation of protein kinase C (see Nishizuka, 1984), is the key signal for ROI production. The observation that TPA is a far more potent activator of ROI production than PAF (and also formylmethionyl-leucylphenylalanine, A. D. Whetton & S. J. Huang, unpublished work) may be due to the transient nature of the agonist-stimulated diacylglycerol formation (Fig. 2) or alternatively the diacylglycerol format may for some reason be a less potent activator of protein kinase C than phorbol esters.

The strong correlation between TPA-mediated inhibition of PAF-stimulated [^{3}H]inositol phosphate accumulation and ROI production supports our contention that inositol lipid hydrolysis has a causal link with ROI production in macrophages. This is confirmed by our investigations of pertussis toxin-mediated inhibition of [^{3}H]inositol phosphate generation and ROI production. These results suggest that PAF can activate phospholipase C via a pertussis toxin-sensitive step. Although the degree of inhibition of PAF-stimulated ROI production is somewhat different to that for [^{3}H]inositol phosphate production, this may be because adherent cells were employed for the 4 h preincubation prior to determination of PAF-stimulated [^{3}H]inositol phosphate production whereas suspension cells were preincubated prior to ROI production. The effects on these two parameters are, nevertheless, close enough for us to suggest that a relationship exists between pertussis toxin-sensitive inositol lipid hydrolysis and ROI production. The major pertussis toxin-catalysed ADP ribosylation in macrophage plasma-membrane preparations is of a 41000-M_r protein (C. M. Heyworth, unpublished work). This is the same molecular mass as the major substrate for pertussis toxin-catalysed ADP ribosylation in neutrophils. The ADP ribosylation of this protein (believed to be the α-subunit of the Gi guanyl nucleotide regulatory protein) has been correlated with the loss of *N*-formyl chemotactic peptide-stimulated neutrophil functions (Bokoch & Gilman, 1984; Okajima *et al.*, 1985). We conclude that a similar protein exists in the macrophage which can couple PAF to phospholipase C thereby eliciting a variety of responses within the cell including ROI production. In summary we have shown that PAF can stimulate ROI production in macrophages via the activation of a pertussis toxin-sensitive guanyl nucleotide regulatory protein which can activate a phosphatidylinositol 4,5-bisphosphate-specific phospholipase C leading to inositol trisphosphate and diacylglycerol production. We have previously shown that formylmethionyl-leucylphenylalanine stimulates inositol lipid hydrolysis in normal bone marrow-derived macrophages (Whetton *et al.*, 1986). TPA preincubation or pertussis toxin treatment (as described in the Methods section) inhibited both formylmethionyl-leucylphenylalanine-stimulated inositol lipid hydrolysis and formylmethionyl-leucylphenylalanine-stimulated ROI production (results not shown). This leads us to believe that activation of inositol lipid hydrolysis may be a common mechanism employed by many agonists to stimulate ROI production in normal bone marrow-derived macrophages.

The normal bone marrow-derived macrophage can be primed and activated by extracellular stimuli to enhance the response of these cells to agonists such as PAF leading to, amongst other events, a greatly increased rate of ROI production (see Hamilton & Adams, 1987). The normal bone marrow-derived macrophage has been shown to respond to stimuli like PAF, and also chemotactic peptides (Whetton *et al.*, 1986) prior to priming or activation. These cells can now be employed to determine the mechanisms whereby agents such as interferon-γ, which can prime normal bone marrow-derived macrophages (Johnson & Torres, 1985), and lipopolysaccharides, which can activate interferon-γ primed macrophages (Johnson & Torres, 1985; Cooper *et al.*, 1984), or other lymphokines can influence inositol lipid metabolism leading to enhanced production of ROI on presentation of an agonist such as PAF.

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