We have assessed the effect of platelet-activating factor (PAF), a biologically active phospholipid present in the human marrow, on the growth of human marrow and blood CD34\(^+\) progenitors. While the metabolism rate of PAF by CD34\(^+\) cells is low (weak acetylhydrolase and acylation processes) it is readily catabolized by the acetylhydrolase activity present in the growth medium (10\% fetal calf serum + 10\% 5637-conditioned medium). Treatment of marrow CD34\(^+\) cells with the non-metabolizable PAF agonist C-PAF (1 nM to 100 nM) immediately before semi-solid culture significantly \((P < 0.01)\) decreased the number of BFU-E but not of CFU-GM colonies. Treatment of marrow or blood CD34\(^+\) cells with C-PAF (10–100 nM) for 3 days in liquid medium before semi-solid culture significantly \((P < 0.01)\) decreased the number of BFU-E and CFU-GM colonies. Treatment of blood CD34\(^+\) cells with the two PAF receptor antagonists CV 3988 and BN 52021 (1 mM) had no significant effect on the number of BFU-E and CFU-GM colonies suggesting no role of endogenous PAF in these processes. These results show that exogenous PAF downregulates human erythropoiesis and myelopoiesis, a result that might be of importance during inflammatory states.

Key words: PAF, CD34\(^+\) cells, Myeloid progenitor, Erythroid progenitor

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**Effect of platelet-activating factor on the growth of human erythroid and myeloid CD34\(^+\) progenitors**

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**Introduction**

Studies highlight the role of lipidic compounds on human haematopoiesis.\(^1\)–\(^6\) They demonstrate that leukotrienes and prostaglandin E upregulate and downregulate human myelopoiesis, respectively. By contrast leukotrienes and prostaglandin E downregulate and upregulate human erythropoiesis, respectively. Platelet-activating factor (PAF) is a phospholipid molecule produced by inflammatory stimulated cells.\(^7\) PAF levels are regulated by an acetylhydrolase activity (AHA) which converts PAF into lysoPAF which is then reacylated into membrane phospholipids by an acyltransferase activity (ATA).\(^7\) Numerous studies report the immunoregulatory properties of PAF: Its inhibiting or activating effects are shown in various cell types such as monocytes/macrophages, polymorhonuclear neutrophils, eosinophils and T- and B-lymphocytes.\(^7\)–\(^9\) Studies report that complex interrelations exist between PAF and cytokines. Thus, PAF stimulates the production of several cytokines such as interleukin (IL)-1, IL6 and tumour necrosis factor-alpha (TNF-\(\alpha\)) which, in turn, may enhance PAF synthesis.\(^7\)–\(^9\)

While PAF is present in the human bone marrow,\(^10\)\(^,\)\(^11\) its effects on the growth of myeloid and erythroid progenitors is not documented. CD34, a surface antigen expressed on haemopoietic stem/ progenitor cells that disappears at later stages of differentiation can be used to select this population.\(^12\)\(^,\)\(^13\) In this study we have examined the effect of PAF on human haematopoiesis by investigating its effects on the growth of granulocyte-macrophage (CFU-GM) and erythroid progenitor cells (BFU-E) from purified human marrow and blood CD34\(^+\) cells. We also examined PAF metabolism by CD34\(^+\) cells.

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**Materials and Methods**

Collection and preparation of mononuclear cells

Sternal bone marrow cells were harvested by aspiration into heparinized tubes (Vacutainer system, Becton Dickinson, Meylan, France) from patients referred for diagnosis according to the Helsinki recommendations. Mononuclear cells were isolated by separation on a Ficoll gradient (400 \(\times\) g, 20 min) and washed twice with Hank’s balanced salts solution (Gibco, Cergy Pontoise, France). None of these patients had a haematological malignancy and their myelograms were normal. Peripheral blood stem cells were collected from lymphoma patients in complete remission undergoing apheresis for autologous trans-
planted. Several aliquots used for bacteriological and CFU-GM controls were harvested in freezing tubes from the final apheresis product just before freezing. They were then stored in liquid nitrogen with apheresis autograft bags until used.

Selection of CD34+ cells

Mononuclear bone marrow cells were used immediately after Ficoll and washing procedures. Peripheral blood stem cells were rapidly defrozen in a 3°C water bath, then diluted and washed in saline with 4% human serum albumin (FBA). CD34+ cells were obtained by magnetic cell sorting (MiniMACS, Têbu, Le Perray en Yvelines, France) as previously described. The entire procedure was made according to the manufacturer’s instructions. Briefly, cells were resuspended at a concentration of 1 × 10⁸/0.3 ml. The blocking reagent and the antibody reagent (0.1 ml for 1 × 10⁸ cells) were added to the cell suspension and incubated at 4°C for 15 min. Cells were then washed with 4 ml of washing buffer (PBS/0.5% human serum albumin (HSA)/5 mM EDTA) and centrifuged. Cells were recovered in cold washing buffer (0.4 ml for 1 × 10⁸ cells) and incubated with a suspension of submicroscopic magnetic beads (0.1 ml for 1 × 10⁸ cells) for 15 min at 4°C. After washing, cells were recovered in 0.5 ml of cold washing buffer, filtered, and incubated at 37°C in the presence of various concentrations of PAF (Sigma, St Quentin-Fallavier, France) and of the non-metabolizable PAF agonist 1-O-hexadecyl-2-N-methylcarbamyl-sn-glycero-3-phosphocholine (C-PAF) (Sigma) before methylcellulose culture. Marrow or blood CD34+ cells were grown for 3 days in liquid medium with various concentrations of PAF, C-PAF, CV 3988 and BN 52021 (two potent PAF receptor antagonists) (Têbu) or the appropriate vehicle (10 μl of 2% HSA) before colony formation in methylcellulose cultures. Marrow or blood CD34+ cells (4 × 10⁵) were grown in 96-round-bottomed well microtitre plates in 100 μ1 IMDM with 10% fetal calf serum (FCS) (Gibco), 10% 5637-conditioned medium and antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin) at 37°C in 5% CO₂ in air.

Colonies assays were performed in both experiments with CD34+ cells (5 × 10⁵) cultured in 35 mm Petri dishes in sixplicates for 14 days at 37°C in 5% CO₂ in air in 100% humidity. The culture medium (1 ml/dish) was a mixture of IMDM with 0.8% methylcellulose, 30% FCS, 1% defatted bovine serum albumin, 10% ml Stem Cell Factor (Têbu), 40 ng/ml interleukin-3 (Sandoz, Rueil-Malmaison, France), 3 U/ml erythropoietin (R&D systems, Oxfordshire, UK), α-thioglycerol (0.1 mM) and 10% 5637-conditioned medium. CFU-GM and BFU-E derived colonies were counted under an inverted microscope in the same dish. The differences of colony number between controls and test dishes were analysed using Wilcoxon’s test.

Flow cytometric analysis of selected CD34+ cells

Samples were analysed on a Profile Coulter using Epics Profile Software to determine the efficiency of the selection. One-hundred μl of cell suspension was incubated at room temperature for 30 min with 5 μl phycoerythrin (PE)-labelled anti-CD34 (HPCA2) monoclonal antibody or PE-labelled monoclonal control (mouse immunoglobulin) and washed twice with PBS supplemented with 2% HSA. Fixation with paraformaldehyde (PFA 1%) was performed at the end of staining. The purified population contained 84 ± 2% of CD34+ cells (mean ± SEM of 17 experiments, range 72–98%).

Semisolid cultures

Two experimental conditions were used for CD34+ cells. Firstly, freshly isolated marrow CD34+ cells were incubated 30 min at 37°C in 1 ml IMDM with various concentrations of PAF (Sigma, St Quentin-Fallavier, France) and of the non-metabolizable PAF agonist 1-O-hexadecyl-2-N-methylcarbamyl-sn-glycero-3-phosphocholine (C-PAF) (Sigma) before methylcellulose culture. Secondly, freshly isolated marrow CD34+ cells and frozen blood mononuclear CD34+ cells were grown for 3 days in liquid medium with various concentrations of PAF, C-PAF, CV 3988 and BN 52021 (two potent PAF receptor antagonists) (Têbu) or the appropriate vehicle (10 μl of 2% HSA) before colony formation in methylcellulose cultures. Marrow or blood CD34+ cells (4 × 10⁵) were grown in 96-round-bottomed well microtitre plates in 100 μ1 IMDM with 10% fetal calf serum (FCS) (Gibco), 10% 5637-conditioned medium and antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin) at 37°C in 5% CO₂ in air.

[³H]PAF metabolism and AHA assay

CD34+ cells (1 × 10⁵) were washed twice with HBSS and were incubated at 37°C in 1 ml of IMDM for various periods of time in the presence of [³H]alkyl-PAF (final concentration 0.05 nmol/tube; 0.5 μG) (Amersham) complexed to HSA (final concentration 2 mg/ml). Experiments were performed in duplicate. The PAF metabolism was assessed after lipid extraction. Recovery of added radioactivity after lipid extraction was 85%. The labelled compounds derived from [³H]alkyl-PAF were separated using TLC plates (Silica Gel 60 (20 × 20 cm, 0.25 mm)) eluted with chloroform/methanol/acetic acid/water (50:25:8:4, v/v). Each lane was divided in areas of 0.5 cm length which were scraped into vials and radioactivity was measured on a Packard liquid scintillation counter. PAF, lyso PAF, phosphatidylcholine (PC) and neutral lipid (NL) were used as standards. AHA was assessed in culture medium according to the method of Mwa et al. Results were expressed as picomoles PAF degraded per min per ml as means of duplicate determinations. The variation between duplicates was less than 5%.
**Results**

We first investigated PAF catabolism by human CD34+ cells. Cells were incubated with [3H]alkyl PAF in IMDM alone. After 24h of incubation 73%, 18%, 5% and 4% of the label migrated with PAF, lyso PAF, 1-alkyl analogue of PC and NL, respectively (mean of three experiments). When cells were incubated in IMDM + 10% FCS + 10% 5637-conditioned medium (growth medium), 0% 90% 6% and 4% of the label migrated with PAF, lyso PAF, 1-alkyl analogue of PC and NL, respectively. After 1h of incubation in growth medium, 100% of the [3H]PAF was converted into [3H]lyso PAF demonstrating a high AHA. The mean AHA levels detected in IMDM with 10% 5637-conditioned medium, 10% FCS, and 10% 5637-conditioned medium + 10% FCS were 204 ± 16, 1676 ± 190 and 2160 ± 115 pmol/min/ml, respectively (mean ± SEM of three experiments). No significant AHA was detected in IMDM alone.

We next assessed the effect of PAF and C-PAF on the growth of human CFU-GM and BFU-E. Due to its rapid metabolism, no significant effect was found with PAF. By contrast treatment of marrow CD34+ cells with the non-metabolized PAF agonist C-PAF (1–100 nM) significantly (P < 0.01, seven independent experiments) decreased the number of BFU-E but not of CFU-GM colonies (Fig. 1). Treatment of marrow CD34+ cells with C-PAF (10–100 nM) for 3 days in liquid medium before semi solid cultures, significantly (P < 0.01, three independent experiments) decreased the number of BFU-E and CFU-GM colonies (Fig. 2). In similar experimental conditions, C-PAF significantly (P < 0.01, three independent experiments) decreased the growth of BFU-E and CFU-GM colonies from blood CD34+ cells (Fig. 2B). In these experimental conditions the PAF receptor antagonist CV 3988 (1 μM) slightly (P < 0.05, four independent experiments) enhanced the growth of BFU-E colonies compared with control dishes (23 ± 2 colonies vs. 19 ± 2 colonies for CV 3988-treated cells and controls, respectively) while BN 52021 (1 μM) had no effect (20 ± 2 colonies). CV 3988 and BN 52021 had no effect on CFU-GM growth (54 ± 5 colonies and 56 ± 5 colonies vs. 52 ± 5 colonies for CV 3988-treated cells, BN 52021-treated cells and controls, respectively).

**Discussion**

Lipidic mediators (such as prostaglandins and leukotrienes) affect the growth of human CFU-GM and BFU-E progenitors.1–6 The rationale for analysing the role of PAF on the growth of human myeloid and erythroid progenitors is based on several points. First, PAF is
present in the human bone marrow. Second, PAF modulates the growth of various human cell types including adherent bone marrow cells. Third, PAF stimulates the eosinophilic and basophilic differentiation from human blood progenitors. Finally, PAF increases the erythroid colony formation from cultured CD34+ haematopoietic progenitors by enhancing the prolactin synthesis by human bone marrow stromal cell feeders.

The use of the CD34 antigen is a useful way to obtain haematopoietic stem/progenitor cells. The use of 1nM of C-PAF may be compared with a continuous stimulation with this physiologic dose of PAF. In the present study treatment of marrow CD34+ cells with C-PAF immediately before semi solid culture decreased the growth of BFU-E but not of CFU-GM colonies suggesting a direct effect of PAF on human erythroid progenitor cells. An effect of PAF on marrow or blood CFU-GM colonies was found after 3 days of treatment in liquid medium before semi solid culture suggesting an indirect effect through an elevated production of inhibitory factor(s) or a decreased production of stimulatory factor(s). In vitro studies report that PAF modulates the production of several molecules such as IL-1, IL-3, IL-4, IL-6, TNF-α, prostaglandins and leukotrienes that regulate human CFU-GM growth. No role of endogenous PAF may be documented on the growth of CD34+ cells. Thus, the two PAF receptor antagonists CV 3988 and BN 52021 have no effect on the number of CFU-GM colonies. In contrast to BN 52021, CV 3988 has a weak effect on BFU-E colonies. A similar difference between these two drugs has been found concerning their ability to inhibit superoxide anion generation in a human B cell line. In this previous study the weak effect of CV 3988 was attributed to its incorporation in cell membrane leading to a non-specific effect. Such an effect may also be suggested in our study.

Bellone et al have recently reported that PAF stimulates the erythroid colony formation from CD34+ haematopoietic progenitors by enhancing the prolactin synthesis by human bone marrow stromal cells. Our present results indicate that, in absence of a marrow stromal cell feeder, PAF decreases the growth of erythroid and myeloid CD34+ progenitors highlighting not only the putative role of PAF during early steps of human haematopoiesis but also the complexity of the regulation of the bone marrow progenitor growth.

References


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