Effect of platelet activating factor (PAF) on the migration of human lymphocytes

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Abstract

***Background* – There is growing evidence to suggest the importance of the lymphocyte in the pathogenesis of asthma, particularly in late phase reactions and ongoing bronchial hyperreactivity. Platelet activating factor (PAF) has also been identified as a potentially important mediator in asthma.**

***Methods* – The migration of human peripheral blood lymphocytes obtained from normal volunteers in response to PAF and the effect of PAF antagonists was studied in a well standardised in vitro assay using nitrocellulose micropore filters in a microchemotaxis chamber.**

***Results* – PAF is a potent stimulus to in vitro human lymphocyte migration; at an optimal concentration of 1 nM it augmented lymphocyte chemokinesis to 310% (SE 33%) of control values. The response to PAF appears to be specific since lyso-PAF and other related membrane phospholipids had no effect. PAF-induced migration could be abrogated by specific PAF receptor antagonists such as WEB 2086 (100 nM), and was partially blocked by the cyclooxygenase inhibitor flurbiprofen at a concentration of 1 μM.**

***Conclusions* – PAF stimulates the in vitro migration of human lymphocytes through a specific PAF receptor. Part of the response may be due to the generation of cyclooxygenase products. PAF may play a part in the recruitment of lymphocytes to asthmatic airways.**

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Platelet activating factor (PAF) is a naturally occurring phospholipid which can induce bronchoconstriction in both asthmatic and normal subjects.[1–4] In contrast to other exogenous bronchoconstrictors, PAF has been reported to produce prolonged bronchial hyperresponsiveness which may persist for up to two weeks following exposure.[3 4] Not all investigators have been able to reproduce this latter effect.[5 6] The mechanism whereby PAF affects bronchial reactivity is unknown, but it is likely to involve the presence of inflammatory cells in the airways of asthmatic patients.

Mast cells and eosinophils and, to a lesser extent, neutrophils have been implicated as important participants in the asthmatic response. There is increasing evidence that lymphocytes are also involved in the pathogenesis of asthma, particularly in late phase reactions and ongoing bronchial hyperreactivity.[7 8] A decrease in circulating CD4+ T lymphocytes is seen for up to 72 hours after allergen challenge in sensitised individuals,[9] and circulating T cells in patients with acute asthma have been shown to bear markers of cell activation.[10 11] In studies employing bronchoalveolar lavage a differential recruitment of CD4+ T lymphocytes to the lungs of atopic asthmatic patients has also been reported,[12 13] particularly in those experiencing late phase reactions. Large numbers of lymphocytes have also been detected in bronchial biopsy samples from asthmatic subjects.[7 14 15]

PAF can be produced by different cells including eosinophils, basophils, neutrophils, monocytes, platelets, mast cells, and endothelial cells,[16 17] and is also released in increased quantities from the alveolar macrophages of asthmatic patients challenged with allergen.[18] PAF is a pro-inflammatory mediator as it can increase vascular permeability and activate platelets, eosinophils and neutrophils,[16 19 20] while PAF receptor antagonists can down-regulate the inflammatory response.[17 21] It is present in the bronchoalveolar lavage fluid of some patients with asthma, and especially in those subjects with raised lymphocyte counts.[22] PAF-induced bronchospasm is also associated with an increase in CD4+ lymphocytes in bronchoalveolar lavage fluid.[23]

PAF is a potent stimulus to the in vitro migration of both human neutrophils[20 24] and eosinophils,[24–26] but as no previous study has examined whether it affects the migratory response of human lymphocytes[27] we have evaluated its effect in a well standardised in vitro lymphocyte chemokinesis assay.

Methods

CELL SEPARATION

After informed consent was obtained, blood was taken from the antecubital vein of normal human volunteers and layered onto cushions of Mono-Poly resolving medium (ICN Biomedicals, Mississauga, Ontario) and centrifuged at 400 *g* for 30 minutes to separate the peripheral blood leucocytes into a polymorphonuclear cell (neutrophil) band and a mononuclear cell band. This latter band typically contains 68 (SE 5)% lymphocytes and 26 (4)% monocytes. The mononuclear cells were harvested, washed twice, and resuspended in RPMI 1640 medium with 100 U/ml penicillin, 100 μg/ml streptomycin, 2·5 μg/ml

fungizone, and 0·1% bovine serum albumin (RPMI-BSA). In some experiments the mononuclear cells were applied to a 0·3 g nylon wool column to obtain a lymphocyte-rich cell pool (98–99% lymphocytes).

LYMPHOCYTE MIGRATION

Lymphocyte migration was studied using a 48 well microchemotaxis chamber (Neuro Probe Inc, Cabin John, Maryland, USA). Human mixed peripheral blood mononuclear cells or nylon wool non-adherent lymphocytes were separated from test substances in the lower wells by nitrocellulose micropore filters (8 μm pore size). The loading cell concentration was 8–10 million/ml in RPMI-BSA. The chambers were incubated for three hours at 37°C in a moist 5% carbon dioxide atmosphere. Lymphocyte migration in response to RPMI-BSA with 0·1% ethanol (control) and to test substances at a known concentration in RPMI-BSA was evaluated simultaneously. For these purposes 1-0-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine (platelet activating factor, PAF) was dissolved in RPMI-BSA containing 0·1% ethanol. Most of our experiments employed a commercially available preparation of PAF (Biomol Inc, Plymouth Meeting, Pennsylvania, USA) that comprises a mixture of active compounds of various chain lengths (predominantly C16 and C18 PAF).[28] In separate experiments, however, we showed that both hexadecyl-PAF (C16) and octadecyl-PAF (C18) were equipotent in our chemokinesis assay. All subsequent experiments employed the mixed PAF as a reference agonist. We also studied the migratory effects of (a) other membrane phospholipids (10 pM–10 μM): lysophosphatidylcholine, phosphatidic acid and phosphatidylserine (Sigma Chemical Co, St Louis, Missouri, USA) and lyso-PAF (Biomol Inc, Plymouth Meeting); (b) autacoids released by PAF (1 pM–100 μM): histamine, serotonin, and substance P (Sigma Chemical Co); and (c) eicosanoid metabolites (1 pM–10 nM): hydroxyeicosatetraenoic acids (5-HETE, 12-HETE, 15-HETE), and leukotrienes B_4 and C_4 (Biomol Inc), and 12-L-hydroxy-5,8,10-heptadecatrienoic acid (HHT) (Cayman Chemicals, Ann Arbor, Michigan, USA).

Migration was quantitated by counting the total number of cells moving beyond a fixed distance into the filter in five high power fields in duplicate filters stained with haematoxylin. This distance was set at a point to which 10–15 cells per high power field had migrated in control wells. Results are expressed as the mean (SE) percentage of cell migration in control wells for comparison among experiments. A p value of <0·05 was considered statistically significant for comparison of means using a paired *t* test. For studies of neutrophil migration, 3 μm micropore filters, a one hour incubation time, and a chromotrope 2R counterstain were used.

For blocking experiments lymphocytes were preincubated with various concentrations of inhibitors for 15–30 minutes before being loaded into the upper well of the microchemotaxis chamber opposite PAF. The following were studied: (a) PAF receptor antagonists (1 nM–10 μM): CV 3988 and SRI 63072 (Bachem Inc, Torrance, California, USA) and WEB 2086 (Boehringer Ingelheim Ltd, Burlington, Ontario); (b) inhibitors of eicosanoid production (10 nM–100 μM): flurbiprofen and nordihydroguaiaretic acid (Sigma Chemical Co) and U-60 257B (Upjohn Co, Kalamazoo, Michigan, USA); and (c) an inhibitor of transmethylation reactions (1–100 μM): 3′-deazoadenosine (Southern Research Institute, Birmingham, Alabama, USA).

Results

We have confirmed the results of previous investigators[20 24] that PAF augments the in vitro migration of human neutrophils; 1 nM PAF stimulated human neutrophil migration to mean (SE) levels 339 (57)% of control values. We have extended these observations to human peripheral blood lymphocytes. Figure 1 shows the effect of various concentrations of PAF on the in vitro migration of lymphocytes purified by density centrifugation followed by nylon wool depletion; the response of mixed peripheral blood mononuclear cells before nylon wool depletion was qualitatively similar. As little as 100 pM PAF resulted in a significant increase in lymphocyte migration when compared with the response to buffer alone, and a peak response was seen with 1 nM PAF which augmented migration to levels 310 (33)% of control values.

The effect of various concentrations (10 pM–100 nM) of compounds related to PAF in the lymphocyte chemokinesis assay was studied. Lyso-PAF is both the immediate precursor and a catabolite of PAF, but this compound had no effect on in vitro lymphocyte migration. Related membrane phospholipids such as lysophosphatidylcholine, phosphatidylserine, and phosphatidic acid also had no effect. PAF has been reported to stimulate the release of histamine, serotonin, and substance P, but these compounds did not alter lymphocyte migration over a wide range of concentrations.

The chemokinetic response of lymphocytes to PAF could be blocked by various PAF re-

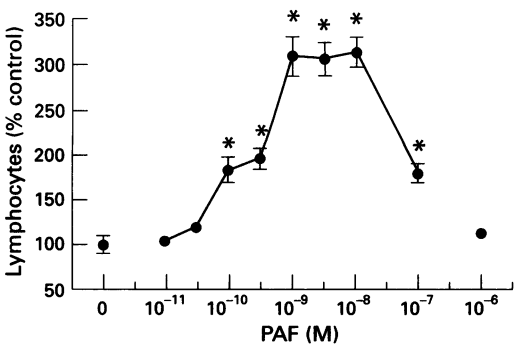

*Figure 1 In vitro migratory response of human nylon wool non-adherent mononuclear cells (lymphocytes) in response to various concentrations of platelet activating factor (PAF), expressed as a mean (SE) percentage of control migration (response to buffer alone); n = 5–18. *p<0·05 versus control.*

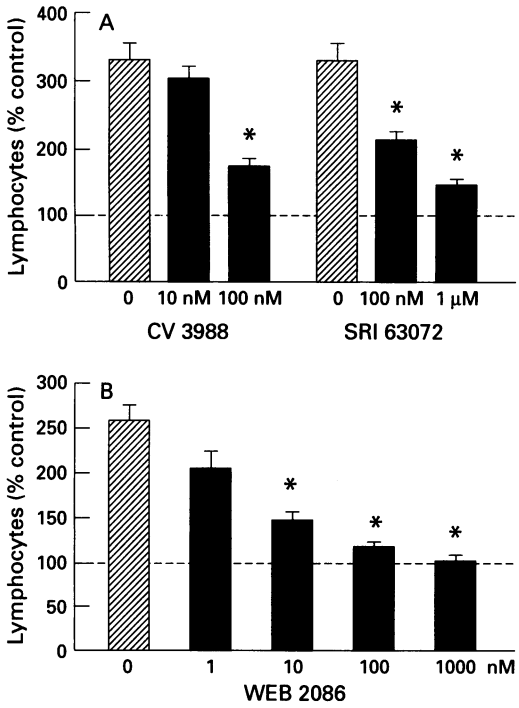

*Figure 2 Mean(SE) lymphocyte chemokinetic response to 1 nM platelet activating factor (PAF) alone (hatched bars) and in the presence of three PAF antagonists (solid bars): (A) CV 3988 and SRI 63072 and (B) WEB 2086 compared with control migration (broken lines); n=3–5. * p<0·05 versus PAF alone.*

Effect of various cyclooxygenase and lipoxygenase products on in vitro lymphocyte migration as mean (SE) of control (n=3–5)

	10^{-8} M	10^{-10} M	10^{-12} M
5-HETE	118 (9)	95 (8)	110 (5)
12-HETE	116 (7)	124(12)	102 (6)
15-HETE	81(12)	91 (6)	107 (5)
HHT	211(21)*	148(16)	138(30)
LTB_4	207(12)*	221(16)*	85 (9)
LTC_4	68 (9)*	86 (9)	106 (5)

HETE = hydroxyeicosatetraenoic acid; HHT = 12-L-hydroxy-5, 8,10-heptadecatrienoic acid; LT = leukotriene.
* p<0·05 *v* control.

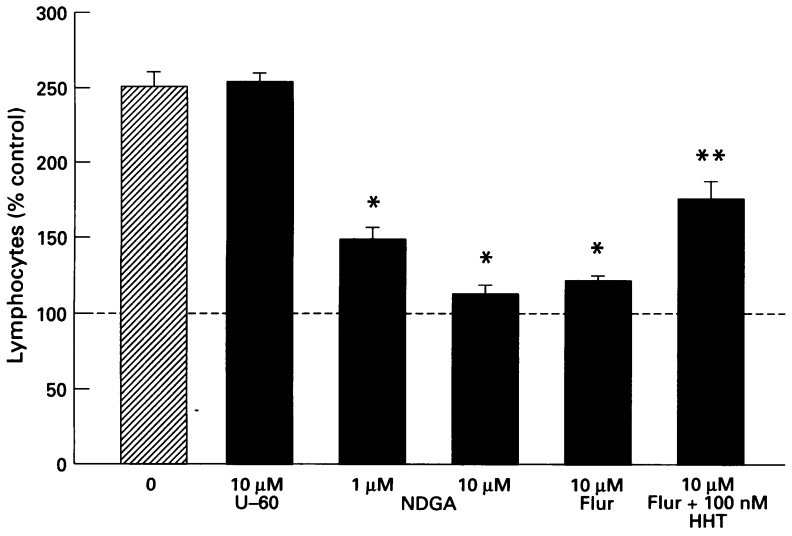

*Figure 3 Mean (SE) lymphocyte chemokinetic response to 1 nM platelet activating factor (PAF) alone (hatched bar) and to 1 nM PAF in the presence of U-60 257B, a lipoxygenase inhibitor (U-60), nordihydroguaiaretic acid, a combined lipoxygenase/cyclooxygenase inhibitor (NDGA), and flurbiprofen, a potent cyclooxygenase inhibitor (Flur) compared with control migration (broken line); n=3–4. In the presence of flurbiprofen, PAF-induced migration could be restored in part by 100 nM 12-L-hydroxy-5, 8,10-heptadecatrienoic acid (HHT). *p<0·05 versus PAF alone; **p<0·05 versus PAF alone and versus PAF+flurbiprofen.*

ceptor antagonists. The presence of both CV 3988 and SRI 63072 resulted in a significant inhibition of the response to 1 nM PAF (fig 2A). In these experiments PAF at a concentration of 1 nM stimulated lymphocyte migration to levels 330 (25)% of control values. In the presence of 100 nM CV 3988 the response to PAF was reduced significantly to a level 175 (12)% of control. Similarly, in the presence of 1 μM SRI 63072 the response to PAF was reduced significantly to a level 148 (9)% of control. It can be seen in fig 2B that the most potent PAF antagonist was WEB 2086. In this set of experiments PAF at a concentration of 1 nM augmented lymphocyte migration to levels 259 (18)% of control values. WEB 2086, 100 nM, completely blocked the chemokinetic response to 1 nM PAF (118 (6)% of control). The concentration of WEB 2086 required to inhibit migration to levels 50% of the PAF-stimulated response (IC_{50}) was calculated by interpolation to be approximately 2·5 nM. None of the PAF receptor antagonists had any effect on random lymphocyte migration in the absence of PAF.

PAF has been reported to augment the release of prostaglandins or leukotrienes from many different cells, and we have confirmed that the in vitro migration of human lymphocytes is stimulated by the lipoxygenase product leukotriene B_4 (LTB_4) and the cyclooxygenase product 12-L-hydroxy-5,8,10-heptadecatrienoic acid (HHT) (table). In addition we have examined the effect of various concentrations (100 nM–10 μM) of inhibitors of arachidonic acid metabolism on the chemokinetic response to 1 nM PAF and have found that PAF-induced migration was inhibited by flurbiprofen (a potent cyclooxygenase inhibitor) and nordihydroguaiaretic acid (a combined cyclooxygenase/lipoxygenase inhibitor), but not by U-60 257B, a specific lipoxygenase inhibitor. The effect of representative concentrations of these antagonists is depicted in fig 3. These data imply that the PAF-induced release of prostaglandins, presumably from contaminating monocytes or macrophages, may be partially responsible for the migratory response of lymphocytes to PAF. This supposition is supported by the fact that the inhibition of PAF-induced lymphocyte migration effected by flurbiprofen could be prevented, at least partially, by the addition of 100 nM HHT (fig 3).

Unlike other chemokinetic agonists, the lymphocyte migratory response to PAF could not be blocked by 10 μM 3'-deazoadenosine, a potent inhibitor of membrane transmethylation reactions.

Discussion

Our studies have shown that very low concentrations of PAF can result in in vitro lymphocyte migration (fig 1). PAF has been reported to increase the in vitro migration of both human neutrophils[20 24] and eosinophils,[24–26] but often employing much higher concentrations. In studies with human neutrophils the threshold concentration for a response to PAF has

been 10 nM, with optimal responses to 100 nM–1 μM PAF. Human eosinophils appear somewhat more responsive to the chemotactic effects of PAF, with threshold concentrations as low as 100 pM and optimal concentrations varying between 10 nM and 1 μM. Compared with other circulating leucocytes, human lymphocytes appear to be more responsive to PAF in an in vitro migration assay (optimal concentration 1 nM). Our study is the first to evaluate the role of PAF in in vitro lymphocyte migration. Recently, however, Renkonen and coworkers reported that 10 nM PAF augmented the adherence of rat lymphocytes to endothelial cell monolayers; they also described the migration of these lymphocytes through the endothelium and into underlying micropore filters.[29] It is not evident from their report whether this transmigration was a direct receptor-mediated effect of PAF on lymphocytes, or whether it was dependent on changes in the endothelial cell substratum or the release of other chemokinetic mediators.

We have shown that the chemokinetic response to PAF is specific in that related compounds such as lyso-PAF and other membrane phospholipids are without effect. In addition, it appears that PAF induces migration through interaction with a specific cell surface receptor, since selective PAF receptor antagonists blocked stimulated migration (fig 2). As seen in other PAF–leucocyte interactions, WEB 2086 appears to be the most potent and efficient PAF receptor antagonist.

PAF has been reported to potentiate the release of interleukin 1 from lipopolysaccharide-stimulated rat monocytes in both in vitro[30,31] and in vivo[32] experiments. In our assay system, however, neither natural nor recombinant interleukin 1 augmented human lymphocyte migration.[33] It should be pointed out that our results with interleukin 1 are in contrast to those of some authors,[34,35] but in agreement with others.[36]

Our previous studies showed that inhibitors of membrane transmethylation reactions such as 3′-deazoadenosine blocked the response of human lymphocytes to several chemokinetic agonists.[37] It is suspected that these compounds may in part alter membrane fluidity so that receptor occupation is more efficiently linked with intrinsic second messenger systems. The chemokinetic response to PAF is not affected by 3′-deazoadenosine. This may be because the prostaglandin-induced migration effected by PAF assumes a greater importance in this situation. On the other hand, the lipophilic properties of PAF may allow it to exert its effect in the absence of membrane transmethylation.

The exact mechanism whereby PAF stimulates lymphocytes to migrate is not known. PAF has been reported to stimulate the release of eicosanoid metabolites from many different types of leucocytes[38–40] and these compounds can certainly affect in vitro lymphocyte migration. Three lipoxygenase products – leukotriene B_4, 5-HETE, and 12-HETE – have been reported to augment in vitro lymphocyte migration,[41,42] as has the cyclooxygenase product HHT.[43] On the other hand, prostaglandin E_2, a cyclooxygenase product, has previously been shown to inhibit lymphocyte migration in vitro.[39] In our assay system only leukotriene B_4 and HHT stimulated in vitro lymphocyte migration (table). This raises the possibility that the migratory response to PAF may be due in part to the release of eicosanoid products from either target lymphocytes themselves or the small number of contaminating monocytes present. Our results with the cyclooxygenase inhibitor flurbiprofen (fig 3) would suggest that PAF-induced lymphocyte chemokinesis may result from the release of a cyclooxygenase product such as HHT. In fact, HHT could partially restore PAF-induced lymphocyte migration that had been blocked by flurbiprofen. PAF is inextricably linked to arachidonic acid and its metabolites in all leucocyte membranes[44] which makes direct and "indirect" effects of PAF difficult to separate. Finally, lymphocytes do not exist on their own in vivo, and the fact that nanomolar concentrations of PAF in vitro augment migration is still relevant to the inflammatory cascade seen in human disease states.

We have shown that human lymphocytes can be stimulated by PAF to migrate in an in vitro system. This suggests that the enhancement of leucocyte motility may be one of the most constant proinflammatory effects of PAF. Evidence is accumulating that PAF may play a part in many pathological conditions with a prominent inflammatory component. Although the recruitment and activation of lymphocytes is clearly dependent on multiple factors, we would suggest that PAF may be a contributor to this process. The role of PAF in leucocyte recruitment in asthma and other subacute pulmonary diseases characterised by lymphocyte and/or eosinophil infiltration should be evaluated.

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