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Enhanced human monocyte cytotoxicity by platelet-activating factor

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SUMMARY

The capacity of platelet-activating factor (PAF) to enhance human monocyte cytotoxicity for WEHI 164 cells was examined. Spontaneous monocyte cytotoxicity was 24±2% (mean±SEM, $n=9$). Preincubation of monocytes with 1 pM–1 nM PAF for 18 hr significantly enhanced cytotoxicity in a dose-related manner, whereas less enhancement was observed at PAF concentrations above 1 nM. Maximal PAF-induced cytotoxicity was 68±6%, which was similar to that induced by optimal concentrations of tumour necrosis factor (TNF) and interferon-gamma. The specific PAF antagonist kadsurenone inhibited PAF-induced cytotoxicity but not TNF-induced cytotoxicity. The inactive PAF analogues lysoPAF and enantioPAF did not increase monocyte cytotoxicity. Two observations suggest that TNF mediates PAF-induced cytotoxicity: specific anti-TNF antibodies inhibited PAF-induced cytotoxicity toward WEHI 164 cells, and PAF did not enhance cytotoxicity to TNF-resistent cells. PAF represents a distinct class of phospholipid monocyte activators that increase monocyte cytotoxicity by TNF-dependent mechanisms.

INTRODUCTION

Human monocyte cytotoxicity is enhanced by a variety of cytokines, including interferon (IFN)-gamma and IFN-alpha, tumour necrosis factor (TNF), interleukin-1 (IL-1) and interleukin-2 (Onoraki *et al.*, 1985; Tweardy *et al.*, 1986; Philip & Epstein, 1986; Sone, Lopez-Berestein & Fidler, 1986; Malkovsky *et al.*, 1987). These monocyte activators are proteins that must be synthesized before release during immunological reactions. Maximal release may require several hours (Matsushima *et al.*, 1986) so only low concentrations of these agents will be present at the initiation of an immunological reaction. Platelet-activating factor (PAF) is a phospholipid mediator of acute and subacute immunological reactions whose synthesis and release is maximal within the first few minutes of an inflammatory response (Grandel *et al.*, 1985). Thus, monocytes may be exposed to PAF before stimulation by protein cytokines such as TNF and IFN. Several studies have examined PAFs effects on monocytes. PAF stimulates monocyte (or macrophage), chemotaxis (Valone & Goetzl, 1983), aggregation (Yasaka, Boxer & Baehner, 1982), superoxide production (Hyaski, Kudo & Inoue, 1985), prostaglandin biosynthesis (Rola-Pleszczynshi *et al.*, 1987) and IL-1 production (Salem *et al.*, 1987; Pignol *et al.*, 1987; Barthelson *et al.*, 1988). We have recently begun to examine PAFs effects on monocytes in detail. The studies described in this communication demonstrate that PAF enhances monocyte-mediated tumour lysis *in vitro*.

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MATERIALS AND METHODS

PAF (1-0-hexadecyl-2-acetyl-sn-glycero-3-phosphocholine), lysoPAF (1-0-hexadecyl-sn-glycero-3-phosphocholine) and enantioPAF (3-0-hexadecyl-2-acetyl-sn-glycero-1-phosphocholine) (Bachem, Torrance, CA) were prepared as stock solutions in sterile phosphate-buffered saline containing 2·5 g/dl bovine albumin (Sigma Chemical Co., St Louis, MO). Kadsurenone (gift of T. Y. Shen, Merck and Co., Rahway, NJ), BN52021 (gift of P. Braquet, IHB, Paris, France) and triazolam (Upjohn, Kalamazoo, MI) were prepared as stock solutions in dimethylsulphoxide. Recombinant interferon-gamma ($1{\cdot}6\times10^7$ U/mg, Amgen, Thousand Oaks, CA), recombinant TNF alpha (5×10^7 U/mg, Genentech, S. San Francisco, CA) and polyclonal antibody to TNF (Genentech) were obtained as noted. Cell culture media were obtained through the University of California cell culture facility.

Human monocyte cytotoxicity was quantified using actinomycin D-treated WEHI 164 cells as the target cells, as described previously (Philip & Epstein, 1986). Briefly, mononuclear leucocytes were prepared from fresh citrate-anti-coagulated blood by dextran sedimentation, centrifugation on Ficoll cushions (Pharmacia, Piscataway, NJ) and adherence to plastic dishes. Monocyte purity was greater than 95% as assessed by non-specific esterase staining. There were fewer than one platelet per 10 monocytes as assessed microscopically. The monocytes were suspended at a concentration of 1×10^6/ml in RPMI-1640 (plus 10% fetal bovine serum) and incubated for 18 hr at 37° in 5% CO_2 in polypropylene tubes without or with different stimuli. After preincubation, the monocytes were washed and 10^5 monocytes were mixed in triplicate with 5000

actinomycin D-treated, ^{51}Cr-labelled WEHI 164 cells in 96-well, round-bottomed plates. The plates were centrifuged at 350 *g* for 3 min and incubated for 12 hr at 37° after which ^{51}Cr release was quantified. Spontaneous release was determined by incubating target cells with medium alone and maximum release was determined by lysing target cells with 100 μl saponin with 2% EDTA.

RESULTS

Enhancement of monocyte cytotoxicity by PAF

PAFs capacity to activate monocyte cytotoxicity was examined in dose–response studies (Fig. 1.). Monocyte cytotoxicity was enhanced significantly by PAF concentrations as low as 1 pM ($P<0{\cdot}01$, paired *t*-test, $n=9$). Enhancement was maximal at 1 nM PAF with lesser enhancement observed at higher concentrations. Maximal cytotoxicity induced by PAF was $68\pm6\%$ (mean $\pm$ SEM) and was comparable to that elicited by 100–1000 μ/ml of IFN-γ or TNF (Fig. 1).

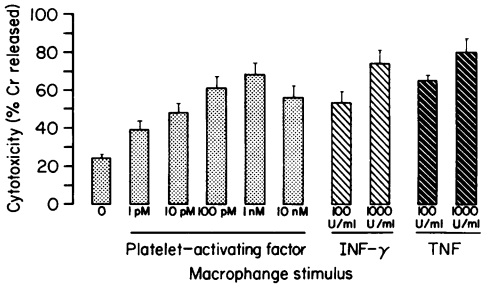

Figure 1. Enhanced human monocyte cytotoxicity by platelet-activating factor. Each bar represents the mean ($\pm$SEM) of seven to nine experiments using monocytes from different donors. Compared to buffer alone, all stimuli increased cell lysis significantly ($P<0{\cdot}01$, paired *t*-test).

PAF analogues and PAF antagonists

PAF analogues and PAF antagonists were used in studies designed to confirm that PAF itself enhanced monocyte cytotoxicity. The capacity of the inactive PAF analogues enantio-PAF and lysoPAF to enhance monocyte cytotoxicity were examined in four dose–response studies analogous to those shown in Fig. 1. Spontaneous cytotoxicity was $24\pm3\%$ and monocyte incubation with 1 pM or 1 nM PAF increased cytotoxicity to $37\pm4\%$ and $62\pm3\%$, respectively. In contrast neither analogue enhanced monocyte cytotoxicity at concentrations between 1 pM and 10 μM. The capacity of selective PAF antagonists to inhibit monocyte-activation was examined. In three studies spontaneous cytotoxicity was $20\pm6\%$ and activation with PAF increased cytotoxicity to $45\pm3\%$. When the monocytes were stimulated with 1nM PAF in the presence of 10 μM kadsurenone cytotoxicity was $18\pm2\%$. The selectivity of kadsurenone's effects was confirmed by the observation that monocyte activation by 300 U TNF/ml in the absence or presence of 10 μM kadsurenone yielded $43\pm2\%$ and $44\pm3\%$ cell lysis, respectively. In two additional studies, 10 μM BN52021 and 40 μM triazolam completely inhibited PAF-induced cytotoxicity (data not shown).

Endotoxin is a potent monocyte activator (Sone *et al.*, 1986; Matsushima *et al.*, 1986) and is a common contaminant in biological preparations. To exclude the possibility that endotoxin contamination contributed to PAFs activity different concentrations of PAF were preincubated for 30 min with 10 μg/ml polymyxin B, which binds and inactivates endotoxin (Sone *et al.*, 1986). Monocytes were then stimulated with 1 pM–1 nM PAF and cytotoxicity was assessed. In four experiments, spontaneous cytotoxicity was $26\pm3\%$ (mean $\pm$ SEM). Monocyte stimulation with 1nM PAF without or with polymyxin B-pretreatment elicited cytotoxicity of $79\pm2\%$ and $78\pm6\%$, respectively. Polymyxin also did not inhibit enhanced cytotoxicity by lower concentrations of PAF (data not shown).

Contribution of TNF to PAF-induced cytotoxicity

TNF mediates in part enhanced cytotoxicity induced by IFN-γ, IL-1 and TNF itself (Philip & Epstein, 1986). To determine if TNF mediates enhanced cytotoxicity by PAF, monocytes were activated with 1pM–10 nM PAF, washed and mixed with target cells. Sufficient anti-TNF antibody to neutralize 1000 U TNF/ml was added to the monocyte–target cell mixture. In four studies, spontaneous cytotoxicity was $26\pm3\%$ (mean $\pm$ SEM) and monocyte stimulation with 1 nM PAF increased cytotoxicity to $79\pm2\%$. In contrast, addition of anti-TNF antibody during the cytotoxicity assay decreased cytotoxicity to less than 10% for both control (spontaneous) and stimulated cells. WEHI 164 cells are a TNF-sensitive line. It thus was of interest to examine PAFs capacity to stimulate monocyte cytotoxicity toward a TNF-resistent cell line. Raji cells were used in cytotoxicity assays in a manner analogous to the WEHI 164 cells. In two experiments, mean spontaneous monocyte cytotoxicity to Raji cells was 9% and monocyte stimulation with 1 pM–10 nM PAF did not increase cytotoxicity. In the same experiments mean spontaneous monocyte cytotoxicity to WEHI 164 cells was 22% and monocyte stimulation with 1nM PAF increased cytotoxicity to 64%. In contrast, monocyte pretreatment with 100 μ IFN-γ/ml elicited a three to four-fold increase in cytotoxicity toward both WEHI 164 and Raji cells.

DISCUSSION

These studies demonstrate that PAF stimulates human monocyte cytotoxicity. PAF concentrations as low as 1 pM significantly enhanced monocyte cytotoxicity and 1 nM PAF elicited maximal enhancement. Maximal cytotoxicity elicited by PAF is comparable to that elicited by optimal concentrations (100–1000 μ/ml) of TNF and IFN-γ. The PAF concentrations that enhance cytotoxicity are within the range of concentrations detected *in vivo* during acute immunological reactions (Grandel *et al.*, 1985; Valone *et al.*, 1987). In addition, we have observed recently that TNF, IFN-γ and IL-1 stimulate monocyte synthesis of PAF and the PAF concentrations are above those which half-maximally stimulate monocyte cytotoxicity (Valone & Epstein, 1988). So this current study may be directly relevant to *in vivo* and *in vitro* models of host immunity. PAF appears to enhance monocyte cytotoxicity only through TNF-dependent mechanisms. PAFs capacity to stimulate TNF release by monocytes has been reported recently (Braquet & Rola-Pleszczynski, 1987). In contrast to PAF, IFN-γ and IL-1

stimulate monocyte cytotoxicity through TNF-dependent and TNF-independent pathways (Philip & Epstein, 1986). PAFs effects on TNF release during cytotoxicity reactions and PAFs effects on expression of other monocyte effector functions will need to be studied in greater detail.

Recent studies demonstrate complex interactions between PAF and cytokines including TNF, IL-1 and IFN-γ. PAF stimulates IL-1 and TNF synthesis by monocytes and macrophages (Salem *et al.*, 1987; Pignol *et al.*, 1987; Braquet & Rola-Pleszczynski, 1987; Barthelson *et al.*, 1988). In turn, TNF, IL-1 and IFN-γ stimulate PAF synthesis by monocytes, macrophages and other target cells (Bussolino *et al.*, 1986; Camussi *et al.*, 1987; Valone & Epstein, 1988). This establishes a positive feed-back loop for synthesis of agents which stimulate monocyte cytotoxicity. The role of this feed-back loop in monocyte activation and the contribution of cytokine release to monocyte cytotoxicity induced by PAF and *vice versa* remain to be elucidated.

The capacity of certain PAF analogues to activate murine and human macrophage-mediated cytotoxicity *in vitro* has been reported previously (Munder *et al.*, 1983; Andreesen *et al.*, 1984; Berdel & Munder, 1987). Those studies could not definitively demonstrate macrophage-mediated cytotoxicity by the analogues because the concentrations of the analogues were so high ($\geq$1 mM) that they had substantial, direct cytotoxicity in the same assays. Similarly, macrophage function *in vivo* is enhanced by administration of high concentrations of some PAF analogues (Munder *et al.*, 1983; Berdel & Munder, 1987; Yamamoto *et al.*, 1987). This effect appears to result from the analogues direct effects on membrane lipids as the macrophage effects are shared with lysophospholipids which lack PAF's other biological actions. In contrast to these studies which used high concentrations of membrane-active PAF analogues, our studies demonstrated significantly enhanced monocyte cytotoxicity by one picomolar PAF. That PAF activates monocytes by interaction with specific receptors is demonstrated by the observation that PAF analogues which do not bind to PAF receptors (Valone, 1987) do not increase cytotoxicity, and selective PAF-binding antagonists inhibit activation. Increased monocyte cytotoxicity is probably not due to platelet-dependent monocyte activation because very few platelets are in the monocyte preparations. In addition, dose–response studies demonstrate that half-maximal monocyte activation occurs at 10–100 pM PAF, whereas half-maximal human platelet activation requires 50–100-fold higher PAF concentrations (Valone *et al.*, 1982).

In conclusion, PAF represents a distinct class of phospholipid mediators that are potent stimuli of human monocyte cytotoxicity. PAF appears to stimulate monocyte cytotoxicity primarily through TNF-dependent mechanisms.

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