

Histamine release from human basophils induced by platelet activating factor: The role of extracellular calcium, interleukin-3, and granulocyte-macrophage colony-stimulating factor

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Although we have demonstrated that platelet activating factor (PAF) directly induces histamine release from human basophils, other studies have failed to report similar effects. In an attempt to understand the variability of these results, we examined the effect of some factors that could influence the basophils' response to PAF such as, extracellular Ca^{2+} and cytokines (interleukin-3 and granulocyte-macrophage colony-stimulating factor [GM-CSF]). The secretion of histamine induced by PAF was optimal when the cells were incubated in Ca^{2+} for 2 to 5 minutes, whereas it declined at longer time intervals up to 15 minutes. If cytochalasin B (5 μ g/ml) was coincubated with PAF (1 μ mol/L) to enhance the secretory response, histamine release was maximal at time 0 and decreased in parallel with the time of the basophils' exposure to Ca^{2+} , like 0.1 μ g/ml anti-IgE-induced histamine secretion but unlike 1 μ mol/L formyl-methionyl-leucyl-phenylalanine-induced histamine secretion. We found that there is synergy between interleukin-3 (1 to 3 ng/ml) and PAF (1 μ mol/L) for secretion of histamine from human basophils ($p < 0.05$) and that GM-CSF (10 ng/ml) significantly ($p < 0.02$) potentiates the secretion of histamine activated by PAF (1 μ mol/L). Our results demonstrate that: (1) the kinetics of the interaction between Ca^{2+} and the activation pathway that leads to histamine secretion are central events in the release reaction elicited by PAF in human basophils, and (2) interleukin-3 and GM-CSF can potentiate the secretory response of human basophils stimulated by PAF. (J ALLERGY CLIN IMMUNOL 1995; 95:565-73.)

Key words: Basophils, histamine, PAF, calcium, cytokines

The activation of human basophils and the consequent release of chemical mediators play central roles in the pathogenesis of allergic reactions.¹ A variety of immunologic (anti-IgE, antigen) and nonimmunologic (N-formyl-methionyl-leucyl-phenylalanine [FMLP], Ca^{2+} ionophore A23187, phorbol ester, and tetradecanoyl phorbol acetate)

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Abbreviations used

EDTA:	Ethylenediaminetetraacetic acid
FMLP:	N-formyl-methionyl-leucyl-phenylalanine
GM-CSF:	Granulocyte-macrophage colony-stimulating factor
HSA:	Human serum albumin
IL:	Interleukin
PAF:	Platelet activating factor
PAG:	1,4-Piperazinediethanesulfonic acid-albumin-glucose

stimuli can induce the release of histamine from human basophilic leukocytes.² With the exception of tetradecanoyl phorbol acetate³ and bryostatin 1,⁴ which directly activate different isoforms of

protein kinase C,⁵ these processes are dependent on the presence of extracellular Ca^{2+} .

In addition, many studies have shown that the release of inflammatory mediators from human basophils induced by different stimuli can be enhanced by pretreatment of the cells with several cytokines (interleukin [IL]-1, IL-3, IL-5, granulocyte-macrophage colony-stimulating factor [GM-CSF], interferon- γ).⁶⁻⁹ Increasing evidence suggests that the activity of human cytokines may be relevant in the pathogenesis of allergic disorders, particularly in late-phase reactions.¹⁰

Platelet activating factor (PAF) is a potent proinflammatory phospholipid synthesized by human leukocytes (neutrophils, eosinophils, monocytes, and basophils).¹¹⁻¹³ PAF can also activate these cells to release chemical mediators (lysosomal enzymes, oxygen free radicals, and arachidonic acid metabolites).¹⁴⁻¹⁸ In one series of studies, PAF was also observed to induce the secretion of histamine from human basophils, but only from cells of donors with allergic asthma and exclusively in the presence of cytochalasin B.¹⁹ In contrast, we have demonstrated that PAF can activate the release of histamine from human basophils of both allergic and normal subjects, regardless of the presence of cytochalasin B,²⁰ which does, however, significantly potentiate PAF-mediated histamine secretion.²¹ The confusion is increased by another report showing that PAF can stimulate mediator release from human basophils only when the cells are pretreated with cytokines (IL-3, GM-CSF, or IL-5).²²

The preparation of basophils usually involves suspension in Ca^{2+} -free media to prevent cell activation before the experiment begins. In common experimental procedures used for studies with human inflammatory cells (including basophils), they are subsequently incubated in a Ca^{2+} -containing medium for 5 to 10 minutes at 37° C to recreate a "physiologic" equilibrium between extracellular and intracellular Ca^{2+} levels before cell stimulation. In pilot studies we noticed that the length of this preincubation in Ca^{2+} -containing buffers affects subsequent histamine release to PAF. To our knowledge, very little is known about the relationship between reactivity of human basophils to different stimuli and time of cell preincubation in Ca^{2+} . In an effort to understand the variability in the literature on PAF-induced histamine release from human basophils, we examined some of the factors that could enhance or suppress the reactivity of basophils to this stimulus. This involved a series of experiments on the effects of Ca^{2+} and

cytokines on histamine release initiated by PAF and, as controls, anti-IgE and FMLP.

METHODS

Materials

The following were purchased: 1,4-piperazinediethanesulfonic acid, cytochalasin B, FMLP (Sigma Chemical Co., St. Louis, Mo.); crystallized human serum albumin (HSA) (Calbiochem Co., La Jolla, Calif.); PAF_{16:0} (Biomol, Philadelphia, Pa.); dextran T 70 (Pharmacia, Piscataway, N.J.); ethylenediaminetetraacetic acid (EDTA), 60% perchloric acid, and dextrose (Fisher Scientific Co., Fair Lawn, N.J.). Goat anti-human IgE was prepared as previously described.²³ PAF_{16:0} (99% pure) was resuspended in methanol and stored at -20° C in a stock solution of 1 mg/ml. Solutions of 0.1 mmol/L in buffer were prepared by drying aliquots of the stock solution under nitrogen just before the experiments.

Recombinant human IL-3 and GM-CSF were kind gifts of Dr. S. Gillis (Immunex, Seattle, Wash.).

Buffers

1,4-Piperazinediethanesulfonic acid-albumin-glucose (PAG) contains 25 mmol/L 1,4-piperazinediethanesulfonic acid, 110 mmol/L NaCl, and 5 mmol/L KCl with the addition of 0.1% dextrose and 0.003% HSA. P(10×A)GC, PAG containing 0.03% HSA, and 5 mmol/L CaCl_2 were used during histamine release experiments with cytokines. In the studies of Ca^{2+} addition after basophil stimulation, a P(10×A)G buffer was used, and the appropriate concentration of Ca^{2+} was added exogenously at the indicated time points. In studies published previously,²¹ we have shown that a concentration of extracellular Ca^{2+} of 5 mmol/L is optimal for studying PAF-induced histamine secretion.

Preparation of peripheral blood basophils

Mixed leukocytes containing basophils from human peripheral blood were prepared as previously described.²⁴ In brief, after informed consent was obtained, blood was drawn into a final concentration of 0.008 mol/L EDTA and 1.1% dextran and was allowed to sediment for 90 minutes at room temperature. The leukocyte-containing plasma layer was then removed, and the cells were pelleted (150 g for 8 minutes) and washed twice with PAG. We did not perform experiments with purified basophils because we previously observed that purification procedures abolished PAF-induced basophil histamine secretion, regardless of the cell purity achieved and the presence of cofactors (cytochalasin B, IL-3) (M. Columbo. Unpublished observations).

Histamine release

The cells were resuspended in P(10×A)GC (experiments with cytokines) or P(10×A)G and challenged in

duplicate with stimuli. The resuspended cells were prewarmed for 5 minutes at 37° C before experiments were performed. All preincubation and release reactions were performed at 37° C. The reaction was allowed to proceed for the indicated time. In the kinetic experiments, time 0 was obtained by adding cells resuspended in P(10×A)G to test tubes containing 5 mmol/L Ca^{2+} and the stimuli (PAF $\pm$ cytochalasin B, anti-IgE, or FMLP). Cell-free supernatants were obtained at the end of the incubation time and stored until they were assayed. In the experiments with cytokines, the cells were preincubated with IL-3 or GM-CSF for 10 minutes before PAF challenge. Histamine release in the absence of stimuli ("spontaneous") was normally less than 5% and was always subtracted for the calculation of the response to stimuli. Histamine was measured by an automated fluorometric technique, as previously described.²⁵

Statistical analysis

Data are presented as means $\pm$ SEM and analyzed by two-tailed Student's *t* test.

RESULTS

Influence of the time of interaction between cells and Ca^{2+} on PAF-activated histamine secretion from human basophils

PAF-induced histamine release was dependent on the time that the cells were incubated in Ca^{2+} -containing medium before PAF challenge. Fig. 1, *A* shows that the histamine release induced by PAF (1 $\mu\text{mol/L}$) was minimal at the shortest preincubation times (0 to 1 minute) and optimal when the cells were incubated in Ca^{2+} for 2 to 5 minutes before cell stimulation. If the basophils were incubated in Ca^{2+} for longer times (up to 15 minutes) before the PAF challenge, a significant, time-related decrease in histamine secretion was found (e.g., $p < 0.05$ between the release obtained at the 3-minute and 15-minute time points). When these experiments were repeated with cells stimulated with PAF (1 $\mu\text{mol/L}$) and 5 $\mu\text{g/ml}$ cytochalasin B, the secretion was maximal at time 0; subsequently, there was a time-dependent decrease in histamine secretion that appeared to be more rapid when PAF was a less effective secretagogue (Fig. 1, *B*). In experiments in which cytochalasin B was already present at the time of PAF addition, there was a time-related decrease in PAF-induced histamine release, similar to the studies of co-addition of cytochalasin B and PAF ($n = 2$, data not shown). The histamine release induced by cytochalasin B alone, when significant, did not appear to correlate with the decrease of PAF-elicited histamine secretion (data not shown).

To ascertain whether the dependence of the

basophils' response on the time of cell incubation in Ca^{2+} was unique for PAF, we performed similar experiments with two common stimuli for human basophils, anti-IgE and FMLP. There was a slight effect on histamine release induced by anti-IgE (0.1 $\mu\text{g/ml}$): at 5 to 15 minutes the secretion was significantly decreased when compared with that obtained at time 0 (Fig. 2, *A*). We repeated these experiments with anti-IgE, using concentrations of extracellular Ca^{2+} of 1 mmol/L, and obtained analogous results ($n = 3$, data not shown); results appeared to be reproducible even when the basophils' response was moderate, although the magnitude of the effect was much less than that with PAF (Fig. 2, *A*). In contrast, no significant time-related change in the histamine secretion was found if FMLP (1 $\mu\text{mol/L}$) was used, regardless of its efficacy as an inducer of basophil degranulation (Fig. 2, *B*).

Fig. 3 shows the time course of the effect of Ca^{2+} on histamine release from human basophils when the cation was added after challenge of the cells with either PAF alone or with the combination of cytochalasin B (5 $\mu\text{g/ml}$) and PAF (1 $\mu\text{mol/L}$). Under both of these conditions, there was a rapid and progressive decrease in the basophils' response (half-life $\cong$ 1 minute), and the secretion was essentially abolished if Ca^{2+} was added 3 to 5 minutes after the initial stimulation. When PAF was coincubated with cytochalasin B (5 $\mu\text{g/ml}$) to enhance the histamine release, a slightly slower decrease in the Ca^{2+} -dependent secretion was detected (control release at time 0 = 67% $\pm$ 8%, $n = 3$) (data not shown). As previously demonstrated,²⁶ when Ca^{2+} was added after basophil challenge with anti-IgE (0.1 $\mu\text{g/ml}$) or FMLP (1 $\mu\text{mol/L}$), there was a time-related decrease of the secretion of histamine ($n = 3$, data not shown). Thus this phenomenon, called *desensitization*, occurs similarly with all secretagogues considered in this study.

Effect of human IL-3 and GM-CSF on PAF-induced histamine release from human basophils

The literature is also discordant with respect to the effects of IL-3. We and several other investigators have reported that IL-3 induces the release of histamine from human basophils.²⁷⁻²⁹ In contrast, other investigators have failed to observe this effect.²² In preliminary studies we confirmed that moderate concentrations of IL-3 could activate histamine secretion from human basophils. In a series of nine experiments, IL-3 (0.03 to 3 ng/ml)

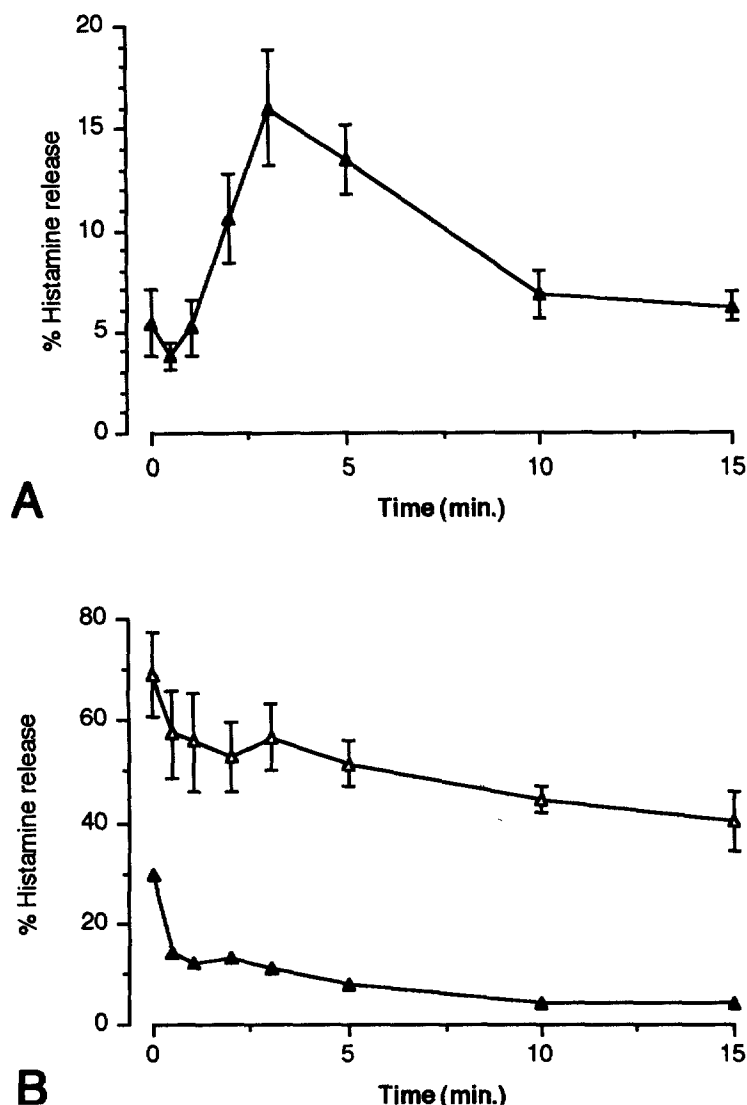


FIG. 1. A, Effect of the time course of cell incubation in a Ca^{2+} -containing medium before the addition of the stimulus on the release of histamine induced by PAF ($1 \mu\text{mol/L}$). Cells were incubated in Ca^{2+} for the indicated times, then PAF was added and the incubation was continued for an additional 30 minutes. Each point represents the mean $\pm$ SEM of five experiments. **B,** Effects of the kinetics of cell incubation in a Ca^{2+} -containing medium before the addition of the stimulus on the release of histamine induced by cytochalasin B ($5 \mu\text{g/ml}$) + PAF ($1 \mu\text{mol/L}$). *Open triangles* indicate the mean $\pm$ SEM of three experiments, and *closed triangles* show the values from a single, separate experiment. Cells were incubated in Ca^{2+} for the indicated times, then cytochalasin B and PAF were added and the incubation was continued for an additional 30 minutes.

induced significant ($\geq 10\%$) histamine release in about one half, as previously reported (data not shown). The response was quite variable among the different donors and was significantly above unstimulated controls. We also found that when IL-3 (3 ng/ml) was used in combination with cytochalasin B ($5 \mu\text{g/ml}$), histamine secretion was enhanced from $5\% \pm 1\%$ to $26\% \pm 10\%$ (control

release with cytochalasin B alone = $5\% \pm 4\%$, $n = 4$).

We and others have also reported that in addition to directly causing release, pretreatment of cells with IL-3 enhances the basophil histamine release caused by other secretagogues.^{7,8} Therefore we attempted to determine whether a brief preincubation (10 minutes) with IL-3 (0.03 to 3

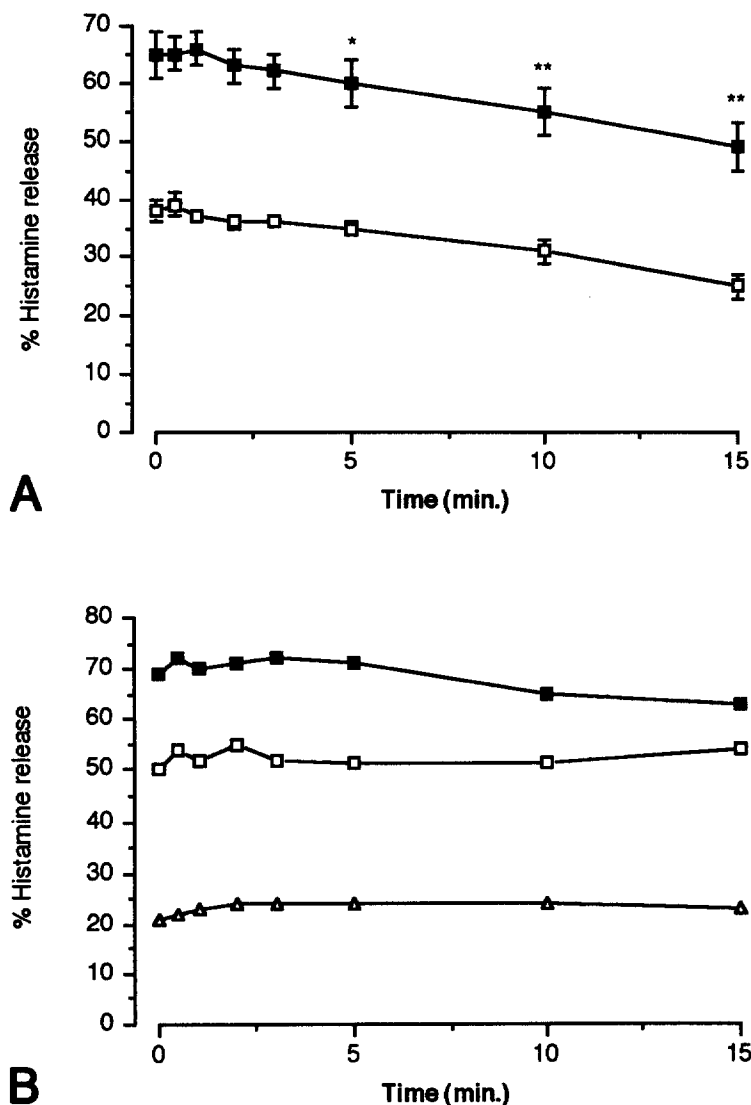


FIG. 2. A, Effect of the time of exposure of the cells to a Ca^{2+} -containing buffer before the addition of the stimulus on the secretion of histamine induced by anti-IgE ($0.1 \mu\text{g/ml}$). Cells were incubated in Ca^{2+} for the indicated times, then anti-IgE was added and the incubation was continued for an additional 30 minutes. Each point represents the mean $\pm$ SEM from 11 (■) and 3 (□) experiments, respectively. * $p < 0.03$ compared with the secretion obtained at time 0. ** $p < 0.001$ compared with the secretion obtained at time 0. **B,** Effect of the time of exposure of the cells to a Ca^{2+} -containing buffer before the addition of the stimulus on the secretion of histamine induced by FMLP ($1 \mu\text{mol/L}$). Cells were incubated in Ca^{2+} for the indicated times, then FMLP was added and the incubation was continued for an additional 30 minutes. Three typical experiments are shown from a total of six performed with similar results.

ng/ml) could also affect the release of histamine from human basophils induced by PAF ($1 \mu\text{mol/L}$). Fig. 4 shows that there was synergy between the secretory responses induced by PAF and IL-3, and this effect was statistically significant at IL-3 concentrations of 0.3 to 3 ng/ml ($p < 0.05$). However, it must be underscored that 0.3 ng/ml IL-3 did not release significantly more histamine than did un-

stimulated controls ($p < 0.08$, not significant). For this reason, the activity of 0.3 ng/ml IL-3 on PAF-induced histamine secretion should be defined as a potentiating rather than a synergistic effect.

Another human cytokine, GM-CSF, has been demonstrated to induce the secretion of histamine from human basophils of selected do-

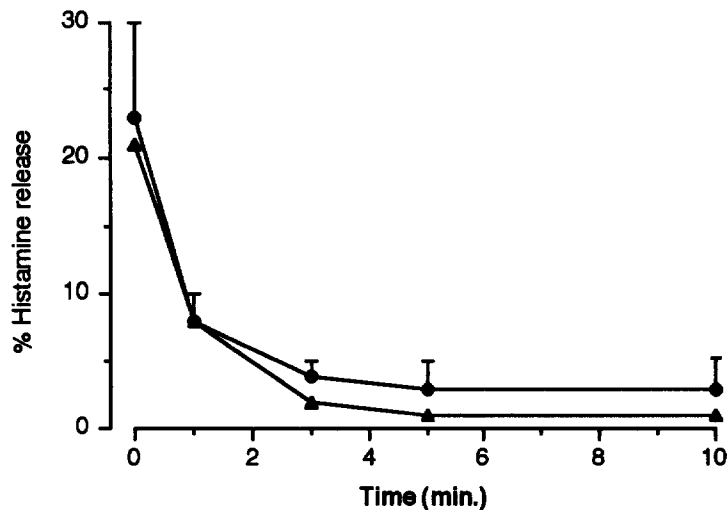


FIG. 3. Effect of the time course of cell incubation with the stimulus before Ca^{2+} addition on the release of histamine induced by PAF ($1 \mu\text{mol/L}$) in the presence (▲) or in the absence (●) of cytochalasin B ($5 \mu\text{g/ml}$). Cells were incubated with PAF $\pm$ cytochalasin B for the indicated times, then Ca^{2+} was added and the incubation was continued for an additional 30 minutes. Each point represents the mean $\pm$ SEM of three experiments for PAF alone, whereas a single experiment is shown for PAF + cytochalasin B.

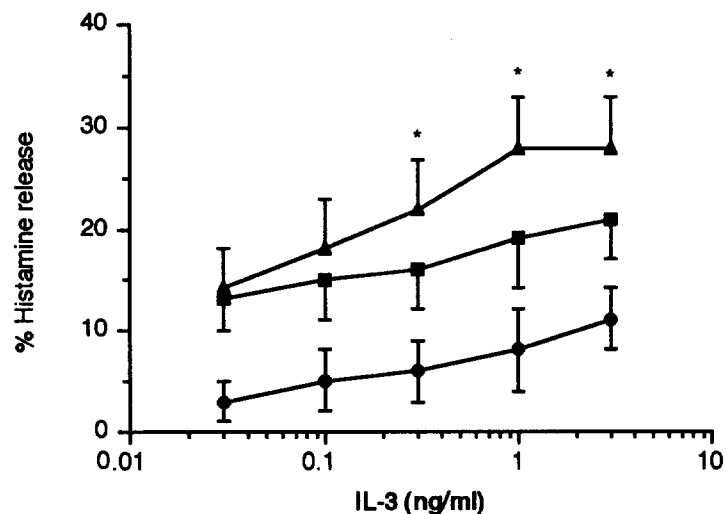


FIG. 4. Synergy between IL-3 and PAF on the release of histamine from human basophils. Triangles represent the results obtained when cells were incubated for 10 minutes with the indicated concentrations of IL-3 and then challenged with PAF ($1 \mu\text{mol/L}$) for an additional 30 minutes. The values shown as squares are a theoretic curve indicating the sum obtained by adding the histamine release induced by IL-3 to that activated by PAF. Circles represent the concentration-response curve of IL-3-induced histamine secretion. Each point represents the mean $\pm$ SEM of nine experiments. * $p < 0.05$ when compared with the sum of IL-3 and PAF-induced histamine secretion.

nors²⁷ and to enhance IgE-dependent, as well as IgE-independent, histamine release.⁸ In our experiments GM-CSF (0.1 to 10 ng/ml) was unable to activate the release of significant amounts of histamine from human basophils, but

at a concentration of 10 ng/ml, this cytokine was a histamine secretagogue when cytochalasin B ($5 \mu\text{g/ml}$) was added (from $2\% \pm 1\%$ to $26\% \pm 6\%$, cytochalasin B control release = $7\% \pm 2\%$, $n = 11$). A 10-minute preincubation of cells with

GM-CSF alone (0.1 to 10 ng/ml), however, did cause enhancement of histamine secretion induced by 1 μ mol/L PAF, which was significant ($p < 0.02$) at the highest cytokine concentration used in these studies (from $10\% \pm 3\%$ to $18\% \pm 4\%$, $n = 8$).

DISCUSSION

In this study we examined the discrepancy between our results with regard to PAF's ability to induce basophil histamine release and those of others²² by investigating the role of some factors that can influence the reactivity of the basophil: extracellular Ca^{2+} and the human cytokines IL-3 and GM-CSF.

Our previous results demonstrated that PAF-induced histamine release was a Ca^{2+} -dependent process: the secretion was optimal at 5 mmol/L Ca^{2+} ($\approx 35\%$ greater histamine release than that obtained at 1 mmol/L Ca^{2+}) and was abolished in the absence of extracellular Ca^{2+} .²¹ No release was detected in the absence of extracellular Ca^{2+} .²¹ In this study we found that PAF-induced histamine secretion was optimal when the cells were preincubated in Ca^{2+} for 2 to 5 minutes and significantly decreased at longer time intervals. From a practical point of view, these data may explain some of the discrepancies in the literature concerning the ability of PAF to stimulate human basophils in the absence of enhancing agents. From a more theoretic point of view, the data suggest that the redistribution of Ca^{2+} as the cell re-equilibrates to new conditions of extracellular Ca^{2+} affects the signal transduction cascade in a complex manner and that it is a variable that must be defined or described in future studies. Coincubation of PAF with cytochalasin B, a common prosecretory compound, which binds to actin filaments and alters their polymerization,³⁰ changed the Ca^{2+} dependence of histamine secretion induced by PAF: the basophil response was optimal when these cells were simultaneously exposed to Ca^{2+} , cytochalasin B, and PAF. Because the mechanism by which cytochalasin B exerts its effects in this and other cell systems is not clearly defined, it is difficult to explain the difference in the Ca^{2+} dependence of PAF when it is used alone or in combination with cytochalasin B. However, our results reinforce the hypothesis that cell- Ca^{2+} interactions represent a key point in the activation of human basophils by PAF. It is worth noting that a relationship between PAF-elicited biologic activities and timing of cell exposure to Ca^{2+} has been described in human neutrophils.³¹

As we previously observed (Kagey-Sobotka et al.

Unpublished observations), the IgE-mediated histamine secretion also significantly decreased in parallel with the time of exposure of the cells to Ca^{2+} , although this decrease was much less dramatic than that observed for PAF. In contrast, FMLP-induced histamine release was unaffected by the duration of the cells' preincubation in Ca^{2+} . Taken together, these results suggest that the response of human basophils to different stimuli can be significantly influenced by the duration of cellular exposure to Ca^{2+} . Therefore the changes induced by resuspending human basophils in the presence of Ca^{2+} are probably much more complex than a simple equilibration between intra- and extracytosolic Ca^{2+} levels.

The finding that the basophils' secretory response rapidly decreased with the time of Ca^{2+} addition after PAF challenge was less surprising. In fact, when anti-IgE or FMLP was used in place of PAF to stimulate histamine release, a time-dependent decrease in secretion was also observed. This decrease of reactivity to PAF, as well as to other stimuli, is due to a rapid desensitization of the leukocytes when they are challenged by Ca^{2+} -dependent stimuli in the absence of extracellular Ca^{2+} .²⁶

The results of our studies with IL-3 and GM-CSF are in agreement with previous reports that have demonstrated the ability of these cytokines to enhance human basophil mediator release.^{7,8} In fact, we found that there is synergy between basophil histamine release induced by IL-3 and PAF in human basophils. Thus it is possible that these molecules, which are synthesized by human inflammatory cells,^{11-13, 32-34} can cooperate in amplifying each other's activity on human basophil histamine release. Unlike the results obtained with IL-3, GM-CSF (at the concentrations used) did not induce significant release of histamine from human basophils in the absence of cytochalasin B. However, pretreatment with GM-CSF also enhanced PAF-elicited histamine release, confirming that GM-CSF, as well as IL-3, belongs to a group of cytokines (including IL-5), which are general potentiators of basophil histamine release.^{7,8} Although our results with cytokines are in agreement with most of the literature in the field, they do not confirm the lack of effect of PAF on human basophils reported by some authors.²² We reiterate that PAF is a secretagogue for human basophils independent of the presence of cytokines, which, in turn, potentiate PAF's releasing activity.

In conclusion, our results suggest that temporally related interactions between Ca^{2+} and the PAF-induced activation pathway, a synergistic in-

teraction with IL-3, and an enhancing effect by GM-CSF can play major roles in human basophil degranulation activated by PAF. These studies provide further information to better characterize the ability of PAF to elicit histamine secretion from human basophils.

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