

Cytokine-induced protein tyrosine phosphorylation is essential for cytokine priming of human eosinophils

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Background: Human eosinophils are strongly modulated by the eosinophilotropic cytokines IL-5, IL-3, and granulocyte-macrophage colony-stimulating factor (GM-CSF). A clear intracellular effect of these cytokines is the induction of tyrosine phosphorylation of multiple cellular substrates. However, the relevance of tyrosine phosphorylation for eosinophil functioning has not been established.

Objective: In this study we have investigated dose-response and time curves of IL-5-, IL-3-, and GM-CSF-induced tyrosine phosphorylation in eosinophils. Moreover, we have evaluated the importance of IL-5-induced tyrosine phosphorylation for priming of human eosinophils.

Methods: Cytokine-induced tyrosine phosphorylation was monitored on western blot with an antiphosphotyrosine antibody (4G10). To probe the relevance of tyrosine phosphorylation for priming, eosinophils were primed with IL-5 in the presence of the tyrosine kinase inhibitor herbimycin A. Platelet activating factor (PAF) was used as a control priming agent. Subsequently, the eosinophils were incubated with serum-treated zymosan (STZ) to activate the respiratory burst. Binding of STZ was determined by FACS analysis.

Results: IL-5-, IL-3-, and GM-CSF-induced tyrosine phosphorylation was found at concentrations that primed eosinophil effector mechanism (median effective dose values: $\approx 5.10^{-11}$ mol/L, $\approx 5.10^{-10}$ mol/L, and $\approx 5.10^{-12}$ mol/L for IL-5, IL-3, and GM-CSF, respectively). Cytokine-induced tyrosine phosphorylation was transient with an optimum value at 15 minutes. IL-5 priming of STZ-induced activation of the respiratory burst was blocked by herbimycin A, whereas PAF still primed this response. In fact, herbimycin A inhibited IL-5 priming of STZ binding to human eosinophils. On the other hand, PAF priming of STZ binding was not affected by herbimycin A. Both IL-5-induced and PAF-induced tyrosine phosphorylation were inhibited by herbimycin A.

Conclusion: These data demonstrate for the first time that IL-5 priming of opsonized particle-induced responses is mediated by tyrosine kinase activity in human eosinophils. (J Allergy Clin Immunol 1998;101:103-9.)

Key words: Eosinophils, IL-5, platelet activating factor, signal transduction, tyrosine phosphorylation, priming, serum-treated zymosan, herbimycin A, respiratory burst, binding

Eosinophils play an important role in the pathogenesis of allergic diseases such as allergic asthma and atopic dermatitis.^{1,2} Eosinophils are present at allergic sites and contribute to inflammation by releasing inflammatory mediators and toxic proteins.^{2,3} Importantly, eosinophils are very sensitive to priming by cytokines such as granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-5, and IL-3.⁴ Primed eosinophils react much more vigorously when they are activated by subsequent stimuli. For instance, GM-CSF, IL-3, and IL-5 increase (prime) degranulation,⁵⁻⁷ superoxide production,⁸ the synthesis and release of lipid mediators,^{9,10} and chemotaxis.¹¹⁻¹³

Eosinophil priming also occurs in vivo. Eosinophils obtained from the peripheral blood of allergic asthmatic individuals respond chemotactically to 1000-fold lower concentrations of platelet activating factor (PAF) compared with eosinophils from healthy donors.¹⁴ This primed phenotype can be mimicked in vitro by preincubation with GM-CSF, IL-3, and IL-5,^{11,12} suggesting that eosinophils from allergic asthmatic patients are primed in vivo by these cytokines. Patients with eosinophilia have elevated levels of IL-5 in the peripheral blood,^{15,16} which might account for the in vivo primed phenotype of eosinophils.

Human eosinophils express high-affinity receptors for GM-CSF, IL-3, and IL-5¹⁷⁻²⁰ that are heterodimers composed of a cytokine-specific α -subunit and a common β -chain (β_c).²¹ The β_c by itself does not bind cytokine but forms a high affinity receptor in combination with the α -chain and is indispensable for signal transduction.²¹ The central role of the β_c for signal transduction can explain the many overlapping biologic effects of GM-CSF, IL-3, and IL-5.

GM-CSF, IL-3, and IL-5 clearly induce tyrosine phosphorylation of multiple cellular substrates in neutrophils^{22,23} and cell lines.²⁴⁻²⁶ Tyrosine phosphorylation

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Abbreviations used

fMLP:	N-formyl-methionyl-leucyl-phenylalanine
GM-CSF:	Granulocyte-macrophage colony-stimulating factor
HEPES:	N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid
PAF:	Platelet activating factor
STZ:	Serum-treated zymosan

has also been documented in eosinophils,⁸ although no kinetic data are currently available. Subsequent studies have demonstrated that many of the tyrosine phosphorylated substrates are part of the signaling pathways that regulate differentiation and proliferation.²⁷ However, no link has been established between tyrosine phosphorylation and priming of eosinophils by IL-5, IL-3, or GM-CSF.

In this study the kinetics of IL-5-, IL-3-, and GM-CSF-induced tyrosine phosphorylation in human eosinophils were analyzed. Such kinetic data are useful for subsequent signal transduction studies with eosinophils and could support the hypothesis that tyrosine phosphorylation is coupled to priming when dose-response relationships are comparable. Furthermore, we have probed the relevance of tyrosine phosphorylation for IL-5 priming of eosinophils by using herbimycin A, a specific and widely used tyrosine kinase inhibitor.

METHODS**Materials**

The murine antiphosphotyrosine monoclonal antibody (4G10, IgG2bk) was obtained from UBI (Lake Placid, N.Y.), and the peroxidase-conjugated rabbit anti-mouse antibody (P260) was obtained from Dakopatts (Denmark). Both antibodies were stored at 4°C. Recombinant human IL-5 was a kind gift of Dr. Dilnya Fattah (GLAXO group research, Greenford, U.K.). Recombinant human IL-3 and GM-CSF were obtained from Genzyme (Cambridge, Mass.). PAF (1-O-hexadecyl-2-acetyl-sn-glycero-3-phosphorylcholine) was obtained from Sigma (St. Louis, Mo.). Herbimycin A was purchased from Biomol (Plymouth Meeting, Pa.). Serum-treated zymosan was prepared as described before.²⁸ All other chemicals were reagent grade.

Isolation of eosinophils

Blood from healthy volunteers was kindly provided by the blood bank of Utrecht, The Netherlands. Mixed granulocytes were isolated from the buffy coat of 500 ml blood anticoagulated with 0.4% trisodium citrate (pH 7.4) as described before.²⁹ The granulocyte fraction was centrifuged for 20 minutes at room temperature over 4 ml Percoll (density, 1.084 gm/ml, layered on 1 ml Percoll with a density of 1.1 gm/ml) to reduce the number of neutrophils. The eosinophil-enriched fraction was collected from the Percoll 1.084/1.1 interface. Eosinophils were subsequently isolated essentially by the method as described by Hansel et al.³⁰ This isolation procedure is based on the fact that, in marked contrast to neutrophils, eosinophils lack the epitope on FcγR III³¹ recognized by the monoclonal antibody CLB-FcR-gran 1.³² As a result, highly purified eosin-

ophils can be isolated by removing neutrophils coated with FcR-gran-1 by using immunomagnetic beads. In short, neutrophils were coated with a CD16 monoclonal antibody (CLB FcR-gran 1; 2 µg/10⁷ cells/ml) for 20 minutes at 4°C. Thereafter, the cells were washed twice and subsequently coincubated head over head with dynabeads coated with sheep anti-mouse antibodies at a ratio of 1:2 (cells:beads) for 20 minutes at 4°C. Neutrophils were subsequently removed by a magnetic particle concentrator (MPCTH-1, Dynal). Eosinophil purity was always higher than 95%, with a viability of more than 98% as judged by trypan blue exclusion.

For priming experiments, eosinophils were isolated with the N-formyl-methionyl-leucyl-phenylalanine (fMLP) method as previously described.^{29,33} Basically, the granulocyte fraction was incubated with 10 nmol/L fMLP for 10 minutes at 37°C, layered on Percoll 1.082/1.1, and centrifuged for 15 minutes (1000× g).^{29,33} Highly purified eosinophils can be collected from the 1.082/1.1 interface. The characteristics of eosinophils isolated with the fMLP method or the CD16 method have been compared extensively.³³

Western blotting

Eosinophils (1.10⁶ cells/ml) were incubated with cytokine in N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES) medium (20 mmol/L HEPES, 132 mmol/L NaCl, 6 mmol/L KCl, 1 mmol/L MgSO₄, 1.2 mmol/L KH₂PO₄, 1 mmol/L CaCl₂, 5 mmol/L glucose, and 0.5% human serum albumin (wt/vol); pH 7.4) at 37°C. After incubation, cells (250.10³ cells/sample) were washed in human serum albumin-free HEPES buffer (37°C) and boiled for 2 minutes in sample buffer (4% sodium dodecylsulfate, 4% β-mercaptoethanol, and 10% glycerol; pH 6.8). Cell pellets were immediately resuspended after sample buffer was added. Samples were run on a 7.5% polyacrylamide gel, transferred to a nitrocellulose membrane (0.45 µm), and probed with an antiphosphotyrosine monoclonal antibody (4G10) as the primary antibody and peroxidase-conjugated rabbit-anti-mouse as the secondary antibody (1:4000) as described before.⁸ Immunoprecipitated proteins were visualized with enhanced chemiluminescence (ECL; Amersham, Buckinghamshire, U.K.).

Films (X-omat AR; Kodak, Rochester, N.Y.) of the western blots were imaged with a densitometer (Molecular Dynamics, Sunnyvale, Calif.), and subsequently signal intensities and molecular weights were analyzed with Image Quant software (Molecular Dynamics).

Culture of eosinophils

Eosinophils (10⁶ cells/ml) were cultured overnight (37°C, 5% CO₂) with herbimycin A (0.1 µg/ml) or 0.5% dimethyl sulfoxide as a control in RPMI 1640 (Gibco, Paisley, U.K.) supplemented with 10% fetal calf serum, 2 mmol/L L-glutamine, 100 U/ml penicillin, and 100 U/ml streptomycin. Eosinophils were collected after 16 hours and used to measure viability (trypan blue exclusion), IL-5- or PAF-induced tyrosine phosphorylation, priming of STZ binding, and the STZ-induced respiratory burst.

Measurement of STZ binding

Binding of STZ particles to human eosinophils was analyzed in a flow cytometer (Becton Dickinson) as described before.³⁴ In short, eosinophils were cultured for 16 hours in the presence of herbimycin A (0.1 µg/ml) or 0.5% dimethyl sulfoxide as a control. Biotinylated STZ particles (30 µg biotin/mg STZ) were labeled with streptavidin-fluorescein isothiocyanate to make

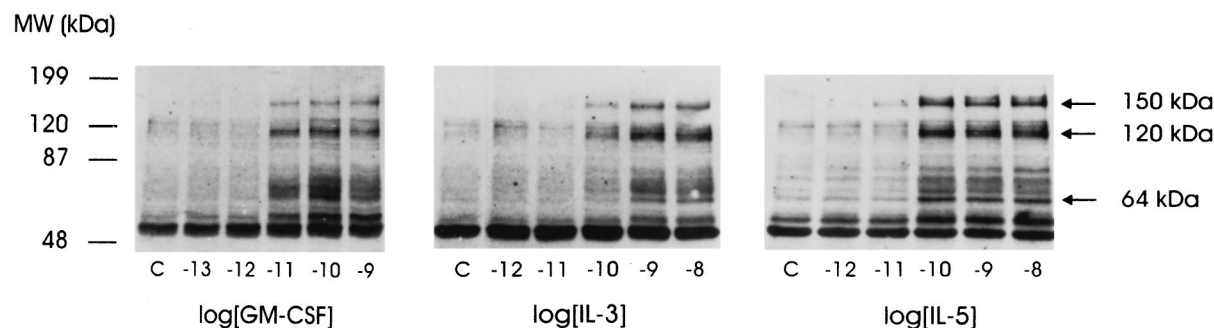


FIG. 1. Concentration-dependency of GM-CSF-, IL-3-, and IL-5-induced tyrosine phosphorylation in human eosinophils. Cells were incubated (10^6 cells/ml) with buffer (C) or various concentrations of the indicated cytokine for 30 minutes at 37°C . Thereafter, protein samples were prepared and used for a western blot with an antiphosphotyrosine antibody. *MW*, Molecular weight.

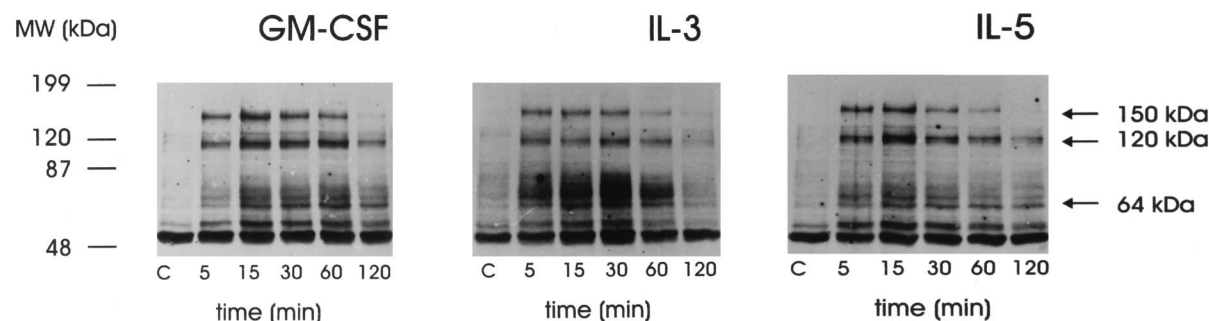


FIG. 2. Time-dependency of GM-CSF-, IL-3-, and IL-5-induced tyrosine phosphorylation in human eosinophils. Eosinophils (10^6 cells/ml) were incubated with buffer (C) for 2 hours or 10^{-11} mol/L GM-CSF, 10^{-9} mol/L IL-3, or 5.10^{-10} mol/L IL-5 for the indicated times at 37°C . Subsequently, proteins samples were prepared and used for a phosphotyrosine western blot. *MW*, Molecular weight.

them green-fluorescent. Eosinophils were incubated for 30 minutes with IL-5 (5.10^{-10} mol/L) or 2 minutes with PAF ($1 \mu\text{mol/L}$) in HEPES medium (containing $0.1 \mu\text{g/ml}$ herbimycin A) at 37°C . The cells were then transferred to a vessel that was stirred and maintained at 37°C , and fluorescent STZ (1 mg/ml) was added. Samples taken at different time points were suspended in ice-cold phosphate-buffered saline containing paraformaldehyde (1% wt/vol) and analyzed in the flow cytometer. Eosinophils that bound STZ were scored and expressed as percentages of the whole cell population (both binding and nonbinding cells).

Measurement of the respiratory burst

The respiratory burst was measured as described before.⁸ In short, the respiratory burst was assayed as oxygen consumption of eosinophils transferred in HEPES medium to an air-tight vessel that was stirred and maintained at 37°C . Oxygen consumption ($\text{nmol O}_2/\text{min} \times 10^6$ cells) was calculated from the decline of the O_2 concentration of the cell suspension, measured with an oxygen probe (Yellow Springs Instrument, Yellow Springs, Ohio). Eosinophils ($1.6 \times 10^6/\text{ml}$) were activated with 1 mg/ml STZ.

RESULTS

GM-CSF-, IL-3-, and IL-5-induced tyrosine phosphorylation in human eosinophils

As shown in Figs. 1 and 2, GM-CSF-, IL-3-, and

IL-5-induced tyrosine phosphorylation was concentration- and time-dependent. The pattern of tyrosine phosphorylated proteins was similar for GM-CSF, IL-3, and IL-5; proteins with an estimated molecular mass of approximately 120 kd (sometimes visible as a doublet) and 150 kd were particularly visible. Induction of tyrosine phosphorylation by GM-CSF started between 10^{-12} mol/L and 10^{-11} mol/L (median effective dose, $\pm 5.10^{-12}$ mol/L), whereas IL-3- and IL-5-induced tyrosine phosphorylation at 10- to 100-fold higher concentrations (median effective dose, $\pm 5.10^{-10}$ mol/L for IL-3 and $\pm 5.10^{-11}$ mol/L for IL-5) (Fig. 1). Tyrosine phosphorylation was rapid and transient (Fig. 2) and maximal at 15 minutes as determined by quantitative analysis of densitometric signal intensities on the films (data not shown).

It has been previously demonstrated that ammonium chloride lysis of erythrocytes, commonly used during the isolation of eosinophils from peripheral blood, inhibits cytokine-induced survival of eosinophils.³⁵ This might be a consequence of impaired cytokine-signaling in these cells. We have tested whether the erythrocyte lysis procedure could affect IL-5-induced tyrosine phosphorylation in human eosinophils. Eosinophils that were isolated by using different erythrocyte lysis protocols

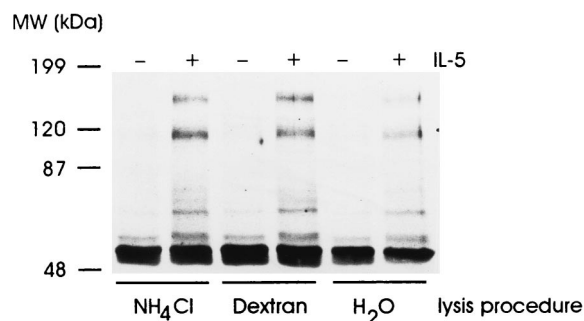


FIG. 3. Effect of different erythrocyte lysis protocols on IL-5-induced tyrosine phosphorylation in human eosinophils. Eosinophils were isolated by using different erythrocyte lysis procedures (i.e., ammonium chloride lysis, dextran sedimentation, or H_2O lysis). These eosinophils were incubated with buffer or 5.10^{-10} mol/L IL-5 for 30 minutes at $37^\circ C$ and subsequently used for a phosphotyrosine western blot. MW, Molecular weight.

(i.e., ammonium chloride lysis and dextran sedimentation) responded similarly to IL-5 with respect to induction of phosphotyrosine proteins (Fig. 3). After H_2O lysis, the signal of IL-5-induced tyrosine phosphorylation was reduced. None of these lysis procedures induced an increase in phosphotyrosine proteins by itself (Fig. 3).

We conclude that in this study ammonium chloride lysis has no inhibitory effect on IL-5-induced tyrosine phosphorylation.

Effect of herbimycin A on IL-5 and PAF priming of human eosinophils

So far, no link has been established between cytokine-induced tyrosine phosphorylation and priming, which is crucial for eosinophil functioning. Therefore we have tested the effect of herbimycin A, a potent and specific inhibitor of tyrosine kinases on priming of eosinophils. At this stage, only IL-5 was used to prime eosinophils, because GM-CSF and IL-3 have similar effects on eosinophils. PAF was administrated as a control priming agent. For priming experiments, eosinophils were used that were isolated with the fMLP method.²⁹ Unprimed eosinophils that are isolated with the fMLP method have a relatively quiescent phenotype in functional assays. Therefore these eosinophils are very suitable for priming experiments.³³

Priming of the STZ-induced respiratory burst by IL-5 (15 minutes) was abrogated when eosinophils were treated overnight with herbimycin A (Table I). In contrast, the STZ-induced respiratory burst of herbimycin A-treated eosinophils was still primed by PAF for 2 minutes (Table I). Comparable results were obtained after 15 minutes of PAF priming (results not shown). These data demonstrate that STZ-induced activation of respiratory burst per se is not sensitive to herbimycin A. The respiratory burst was not activated within 15 minutes of incubation with IL-5, PAF, or herbimycin A in the absence of STZ (data not shown). Herbimycin A (2 $\mu g/ml$) had little effect on the PMA-induced respiratory

TABLE I. Effect of herbimycin A on priming of the STZ-induced respiratory burst

	STZ-induced respiratory burst (nmol $O_2/min \cdot 10^6$ cells)	
	No inhibitor	Herbimycin A
No priming	2.5 ± 1.7	1.1 ± 0.6
IL-5 priming	5.7 ± 0.6	$1.1 \pm 0.5^*$
PAF priming	8.5 ± 0.3	7.2 ± 1.5

Eosinophils (10^6 cells/ml) were cultured in the presence of herbimycin A (0.1 $\mu g/ml$) or 0.5% dimethyl sulfoxide (untreated) for 16 hours. Subsequently, the cells were suspended in HEPES medium (+ 0.1 $\mu g/ml$ herbimycin A), incubated with IL-5 (5.10^{-10} mol/L, 30 minutes, $37^\circ C$) or PAF (1 $\mu mol/L$, 2 minutes, $37^\circ C$), and transferred to a vessel that was stirred and maintained at $37^\circ C$ before addition of STZ (1 mg/ml). The respiratory burst was measured as oxygen consumption. Results are expressed as the maximal rate of oxygen consumption (nmol $O_2/min \times 10^6$ cells) and are shown as mean values $\pm$ SEM of three independent experiments.

*A significant difference was found between herbimycin A-treated eosinophils and control cells ($p < 0.01$) as determined by Student's t test for paired samples.

burst (100 ng/ml), demonstrating the relative specificity of herbimycin A for tyrosine kinase or kinases (data not shown).

A well-established effect of IL-5 and PAF is increased binding of opsonized particles (STZ) to human eosinophils, primarily mediated by complement receptor type 3.^{34,36} As shown in Table II, herbimycin A (0.1 $\mu g/ml$) fully blocked an increase in binding of STZ particles in response to IL-5. Similar to priming of the respiratory burst, PAF still increased binding, indicating that enhanced binding can be achieved through distinct signal transduction routes.

The effect of herbimycin A on IL-5- and PAF-induced tyrosine phosphorylation in human eosinophils

Overnight culture (16 hours) of eosinophils with herbimycin A (0.1 $\mu g/ml$) resulted in a marked reduction of IL-5-induced tyrosine phosphorylation (15 minutes), demonstrating the effectiveness of herbimycin A (Fig. 4). Baseline tyrosine phosphorylation was low, but could be even further reduced by herbimycin A (data not shown). Overnight treatment with herbimycin A (up to 2 $\mu g/ml$) was not cytotoxic as judged by trypan blue exclusion ($87\% \pm 3\%$ viable cells, $n = 3$). In this study herbimycin A (2 $\mu g/ml$) had a negligible effect on IL-5-induced tyrosine phosphorylation in human eosinophils after a short-term (≤ 2 hours) incubation.

When PAF (1 $\mu mol/L$) was administrated for 2 minutes to eosinophils, a 120 kD protein was phosphorylated on tyrosine (Fig. 4). However, tyrosine phosphorylation of a 150 kD protein was not observed in PAF-treated cells, suggesting that PAF and IL-5 induce tyrosine phosphorylation of an overlapping, but distinct, set of proteins. Also, after an incubation period of 15 minutes, PAF did not induce tyrosine phosphorylation of a 150 kD protein (data not shown). This supports the finding that

TABLE II. Effect of herbimycin on IL-5 priming of STZ binding to human eosinophils

	STZ binding (%)	
	No inhibitor	Herbimycin A
No priming	100	87.7 ± 21.6
IL-5 priming	100	17.3 ± 13.8*
PAF priming	100	96.7 ± 4.1

Eosinophils (10^6 cells/ml) were treated overnight with herbimycin A (0.1 μ g/ml) or dimethyl sulfoxide (0.5%) as a control (no inhibitor), and allowed to bind fluorescein isothiocyanate-labeled STZ particles for the indicated times in HEPES medium containing 0.1 μ g/ml herbimycin A. Before addition of STZ, eosinophils were preincubated with buffer (no priming), IL-5 (5.10^{-10} mol/L, 30 minutes, 37° C) or PAF (1 μ mol/L, 2 minutes, 37° C). Eosinophils that bound STZ particles were measured by FACS analysis. Results are expressed as the percentage of eosinophils that bound STZ particles compared with the number of STZ-binding eosinophils that were not incubated with herbimycin A (100%). The percentage of STZ-binding cells as compared with the whole cell population was 13.0 ± 6.2 (no priming), 24.0 ± 7.8 (IL-5 priming), and 49.3 ± 8.1 (PAF priming). Values are means of three independent experiments $\pm$ SEM.

*A significant inhibition of priming by herbimycin A was found ($p < 0.05$) as determined by Student's *t* test for paired samples.

the difference between PAF- and IL-5-induced protein tyrosine phosphorylation in human eosinophils reflects a qualitative difference between PAF and IL-5. Also, PAF-induced tyrosine phosphorylation of the 120 kd protein was strongly reduced by herbimycin A (0.1 μ g/ml).

DISCUSSION

Eosinophil priming is an important step in the activation of this cell type. It is therefore relevant to gain more insight into the intracellular pathways that mediate priming. In this study we have analyzed the role of tyrosine phosphorylation induced by the cytokines GM-CSF, IL-3, and IL-5, which potently prime human eosinophils.

Analysis of phosphotyrosine blots of human eosinophils demonstrated that GM-CSF, IL-3, and IL-5 induced concentration-dependent tyrosine phosphorylation of approximately 64 kd, 120 kd, and 150 kd proteins (Figs. 1 and 2). These molecular masses deviate from previous findings, probably because of the different sets of protein markers used.⁸ Multiple sets of markers were compared in this study, and therefore we believe that the current estimated molecular weights are accurate.

The concentrations of cytokines required for induction of tyrosine phosphorylation are in line with the ligand affinity of the receptors for GM-CSF, IL-3, and IL-5.¹⁷⁻²⁰ The concentration dependency of GM-CSF- and IL-3-induced tyrosine phosphorylation superimposes the concentration-dependent priming of the respiratory burst and PAF release.^{8,10} This suggests that induction of tyrosine phosphorylation is important for priming of eosinophilic effector mechanisms. Cytokine-induced tyrosine phosphorylation was rapid and transient (Fig. 2), which was comparable to that found in

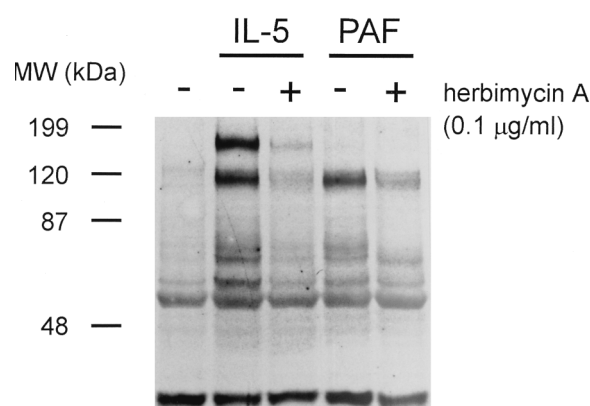


FIG. 4. Effect of tyrosine kinase inhibitors on IL-5-induced tyrosine phosphorylation in human eosinophils. Eosinophils were cultured (10^6 cells/ml) for 16 hours (37° C, 5% CO₂) with 0.5% dimethyl sulfoxide or 0.1 μ g/ml herbimycin A. After 16 hours, IL-5 (5.10^{-10} M, 15 minutes) or PAF (1 μ mol/L, 2 minutes) was added at 37° C. Protein samples were prepared from these cells and used for phosphotyrosine western blot. MW, Molecular weight.

studies with other cell types.²⁴⁻²⁶ The kinetics of cytokine-induced tyrosine phosphorylation appear to precede the time required for priming, which is about 15 to 30 minutes. This suggests that tyrosine phosphorylation is an early event of the signal transduction cascade that ultimately results in cellular priming.

Overnight treatment with the tyrosine kinase inhibitor herbimycin A abrogated IL-5-induced tyrosine phosphorylation (Fig. 4) without affecting viability. IL-5 priming of the respiratory burst was blocked by herbimycin A (Table I). Also, priming of binding of STZ particles to human eosinophils was inhibited by herbimycin A (Table II). These data indicate that tyrosine kinase activity and priming by IL-5 are causally related. Herbimycin A also inhibited background priming of eosinophils (Tables I and II), which correlates with inhibition of baseline protein tyrosine phosphorylation.

Others have demonstrated that cytokine-enhanced survival of eosinophils is also mediated by tyrosine kinase or kinases.³⁷ The identity of the herbimycin A-sensitive tyrosine kinases that mediate priming remains to be determined. Possible candidates are JAK2 and lyn, which are presently identified as tyrosine kinase involved in IL-5 signaling.³⁸⁻⁴¹ IL-5-induced tyrosine phosphorylation of JAK2 in human eosinophils is inhibited by herbimycin A (data not shown). Therefore the role of JAK2 during eosinophil priming is not excluded by these experiments.

In this study we also show that PAF induced tyrosine phosphorylation in human eosinophils, particularly of a protein or proteins with a molecular mass around 120 kd, which was markedly reduced by herbimycin A. Interestingly, PAF was still able to prime STZ binding to human eosinophils and STZ-induced activation of the respiratory burst in the presence of herbimycin A (Tables I and II). This indicates that the STZ-induced respiratory burst per se is not mediated by herbimycin

A-sensitive tyrosine kinases. This observation supports the conclusion that IL-5 priming, and not the primed response, is inhibited by herbimycin A. Moreover, priming of STZ binding can be mediated by herbimycin A-sensitive tyrosine kinase or kinases and herbimycin A-insensitive routes (compare IL-5 and PAF). Previously it has been shown that the PAF-induced increase in $[Ca^{2+}]_i$ is important for the priming of human neutrophils.⁴² In addition, both human neutrophils and human eosinophils can be primed by the Ca^{2+} ionophore ionomycin.^{8, 42} On the other hand, IL-5 priming seems to be independent of $[Ca^{2+}]_i$.⁸ Therefore priming of eosinophils is also established via Ca^{2+} -dependent and Ca^{2+} -independent pathways.

In human neutrophils PAF, C5a, fMLP, and IL-8 induce tyrosine phosphorylation of multiple proteins, including a major 120 kd protein, similar to the 120 kd phosphotyrosine protein or proteins induced by PAF in human eosinophils.^{43, 44} In these studies the identity of the 120 kd protein was not clarified, but focal adhesion kinase (125 kd) and GTPase-activating protein (120 kd) were ruled out as possible candidates.⁴³ Two-dimensional analysis of tyrosine phosphorylated proteins in neutrophils showed that the GM-CSF-induced 120 kd phosphotyrosine protein was more acidic than the 120 kd phosphotyrosine protein induced by fMLP.⁴⁴ It is also possible that the IL-5- and PAF-induced 120 kd phosphotyrosine proteins differ, although the relative mobilities appear to be identical. A possible candidate for the 120 kd phosphotyrosine protein induced by GM-CSF, IL-3, or IL-5 is the β_c subunit of the GM-CSF/IL-3/IL-5 receptor. Indeed, GM-CSF induces tyrosine phosphorylation of the β_c subunit in BaF/3 cells transfected with the human GM-CSF receptor.⁴⁵ Multiple cytokines, including IL-3, induce tyrosine phosphorylation of a 145 kd protein that binds to the grb2-binding site of Shc.^{46, 47} Therefore this unknown protein of 145 kd is a potential regulator of p21^{ras}. It will be interesting to test whether or not the 150 kd protein found in this study coimmunoprecipitates with Shc from extracts of IL-5-treated eosinophils.

In conclusion, the eosinophilotrophic cytokines GM-CSF, IL-3, and IL-5 clearly induce tyrosine phosphorylation of several cellular proteins in human eosinophils in a concentration- and time-dependent fashion. Importantly, tyrosine kinase activity is indispensable for enhanced binding of opsonized particles to human eosinophils and priming of the respiratory burst in response to IL-5.

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