

Activation markers of human basophils: CD69 expression is strongly and preferentially induced by IL-3

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Background: The biological functions of basophils are precisely regulated by various cytokines *in vitro*, but little is known about surface markers that are upregulated during the cytokine-mediated activation process.

Objective: It has been well established that CD69, CD44, and CD54 represent "activation markers" for cytokine-mediated eosinophil activation. The objective of this study was to elucidate the expression and regulation of these molecules in human basophils *in vitro* as well as *in vivo*.

Methods: Basophils were purified from venous blood by means of density gradient centrifugation followed by negative selection. Surface expression was analyzed by means of flow cytometry. We also studied the expression of CD69, CD44, and CD54 on basophils in bronchoalveolar lavage fluid and blood specimens from patients with asthma.

Results: CD44 and CD54 were constitutively expressed on basophils and moderately upregulated by IL-3. On the other hand, CD69 expression was only weakly observed in freshly isolated basophils, but IL-3 induced extremely high levels of expression. Surface CD69 appeared rather slowly in comparison with CD63 and CD11b, and the induction of expression was completed within 24 hours. Basophil CD69 had no functional relevance, but it did have biological relevance. Whole blood basophils from asthmatic individuals expressed significantly higher levels of CD69 than did those from normal individuals. Furthermore, bronchoalveolar lavage fluid basophils showed higher levels of CD69 expression than did blood basophils from the same donors.

Conclusion: CD69 expression on basophils was preferentially and strongly upregulated by IL-3. CD69 on basophils might be useful as an *in vitro* as well as *in vivo* marker of activation of these cells by IL-3. (*J Allergy Clin Immunol* 2002;109:817-23.)

Key words: Basophils, eosinophils, allergy, bronchial asthma, cytokines, bronchoalveolar lavage fluid, CD69, IL-3, human

Basophils account for only 0.5% to 1% of peripheral leukocytes. However, through the release of granule-associated and newly synthesized mediators, these cells are actively involved in the pathogenesis of allergic disorders in concert with eosinophils.¹ The role of basophils in allergic reactions has become more apparent with the recognition and understanding of allergic late-phase reactions. In fact, both basophils and basophil-derived mediators have been identified in a number of late-phase reactions induced by experimental antigen challenge of various organs.²⁻⁴

In allergic disorders, pivotal roles of cytokines have also been established: these autacoids participate in allergic inflammation by modulating or amplifying immune responses of inflammatory cells. In fact, the biological functions of basophils are precisely regulated by various cytokines *in vitro*. For example, a basophilopoietin, IL-3, not only promotes proliferation of immature basophils but also amplifies various aspects of the biological functions of mature basophils. IL-3 acts on peripheral basophils, causing increased mediator release, prolonged survival, enhanced locomotion, and increased adhesiveness to the endothelium.⁵ The same effects are also observed with IL-5 and GM-CSF, which share a receptor β -chain in common with IL-3.⁵ Although the functional modulation of basophils by cytokines is becoming increasingly well understood, little is known about the protein markers induced on the surface of basophils during the cytokine-mediated activation process. On the other hand, substantial evidence to date has clearly demonstrated that eosinophils express several proteins during cytokine-mediated activation; these include CD69, CD44, and CD54 (ICAM-1).⁶ Because eosinophils and basophils represent 2 major classes of effector cells in the inflammation associated with allergic disorders, we investigated the expression and regulation of eosinophil "activation markers" on basophils.

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Abbreviations used

BALF: Bronchoalveolar lavage fluid
 CEP: Chronic eosinophilic pneumonia
 fMLP: Formyl-methionyl-leucyl-phenylalanine
 MESF: Molecules of equivalent soluble fluorochrome units

METHODS**Reagents**

The following reagents were purchased: IL-4, GM-CSF, and monocyte chemoattractant protein 1 (MCP-1/CCL2, PeproTech, London, United Kingdom); eotaxin/CCL11 (R&D Systems, Minneapolis, Minn); IFN- γ (Shionogi Pharmaceutical Co, Osaka, Japan); and C5a and formyl-methionyl-leucyl-phenylalanine (fMLP; Sigma Chemicals, St Louis, Mo). IL-3 and IL-5 were kindly donated by Kirin Brewery (Tokyo, Japan) and Suntory (Osaka, Japan), respectively. The following antibodies were purchased: FITC-conjugated mouse IgG1 and FITC-conjugated anti-CD44 mAb (IgG1, clone J-173, Coulter Immunotech, Marseille, France); FITC-conjugated anti-CD63 mAb (IgG1, clone CLB-gran12, Coulter Immunotech); FITC-conjugated anti-CD11b mAb (IgG1, clone Bear1, Coulter Immunotech); FITC-conjugated anti-CD69 mAb (IgG1, clone FN50, PharMingen, San Diego, Calif); FITC-conjugated anti-CD54 mAb (IgG1, clone 8.4A6, Biosource, Camarillo, Calif); anti-CD69 mAb (IgG2a, clone P8.1, Chromaprobe, Aptos, Calif); mouse IgG2a (PharMingen); and goat polyclonal anti-human IgE Ab (Medical & Biological Laboratories, Nagoya, Japan). Anti-Fc ϵ RI α -chain mAb (CRA-1)⁷ and basophil-specific mAb (BB1)⁸ were prepared as previously described.

Determination of CD69, CD44, CD54, CD63, and CD11b in vitro

Basophils and eosinophils were purified from venous blood obtained from consenting volunteers with no histories of atopic diseases. Briefly, basophils were semipurified by means of Percoll density gradient centrifugation; this was followed by negative selection through use of a MACS Basophil Isolation Kit (Miltenyi BioTech, Bergisch-Gladbach, Germany), as previously described.⁹ The purity of the basophil preparations was $99.2\% \pm 0.1\%$ ($n = 31$). Approximately 60% of basophils were recovered after this selection, and $2.1 \pm 0.2 \times 10^4$ ($n = 31$) cells were obtained from 1 mL venous blood. Eosinophils were purified from peripheral blood by means of Percoll density gradient centrifugation, as previously described.¹⁰ Cells ($2\text{--}10 \times 10^4/200 \mu\text{L}$) were cultured in RPMI 1640 (Gibco, Grand Island, NY) supplemented with 10% FCS and antibiotics (100 U/mL penicillin and 100 $\mu\text{g}/\text{mL}$ streptomycin) at 37°C in 5% CO_2 in flat-bottomed, 96-well culture plates (Iwaki, Chiba, Japan).

For flow cytometry, cells were incubated first with human IgG (5 mg/mL) and then with 10 $\mu\text{g}/\text{mL}$ of FITC-labeled anti-CD mAb. An isotype-matched FITC-labeled mouse IgG was used as a negative control. Stained cells were analyzed through use of an EPICS XL SYSTEM II (Coulter, Miami, Fla). The median values of fluorescence intensity were converted to the numbers of molecules of equivalent soluble fluorochrome units (MESF), as described previously.¹¹ Surface receptor levels were calculated through use of the following formula:

$$\Delta\text{MESF} = (\text{MESF of cells stained with anti-CD mAb}) - (\text{MESF of cells stained with control IgG}).$$

Intracellular cytometric analysis was performed after fixing in 4% paraformaldehyde and permeabilization with 0.1% Tween 20, as previously described.¹²

RT-PCR analysis of CD69 mRNA

Total RNA was extracted from purified basophils through use of a QIAGEN Total RNA Isolation Kit (Invitrogen NV, Leek, The Netherlands) and treated with DNase I to remove contaminating DNA. Reverse transcription for the first strand cDNA and hot-start PCR amplification were performed as previously described.¹¹ Direct and reverse oligo primers for CD69 (5'TCCTCCCAAGTTCCT-GTCCT3' for sense and 5'GCACCATGATCAGAACAAAGC3' for antisense) were constructed on the basis of the published sequence.¹³ PCR products were electrophoresed through a 2% agarose.

Functional evaluation of CD69

Anti-CD69 mAb (clone P8.1) was immobilized onto plates; we used the method described by Walsh et al¹⁴ with slight modification. Briefly, flat-bottomed, 96-well microtiter plates (Nunc, Roskilde, Denmark) were treated with 0.1 mg/mL poly-L-lysine (MW: 84000); this was followed by 2.5% glutaraldehyde. After washing, the plates were incubated with 0.1 mg/mL protein A (Sigma) for 5 to 8 hours. The plates were then further treated with 2 mg/mL casein overnight. After washing, the treated wells were incubated with anti-CD69 mAb (P8.1), control IgG, or anti-Fc ϵ RI α mAb (CRA-1) as a positive control, at 4°C overnight at a final concentration of 25 $\mu\text{g}/\text{mL}$. Finally, the wells were blocked with FCS. Basophils were cultured overnight with IL-3 (300 pmol/L) before addition to the wells. Live cell counts were determined after 48 hours, as previously described.¹⁵ Histamine release experiments were performed as previously described.⁹

Determination of CD69, CD44, and CD54 on whole blood basophils

Fifteen patients with atopic asthma were recruited from outpatients of the Department of Medicine, University of Tokyo Hospital. The studied asthmatic subjects were diagnosed on the basis of the reported criteria,¹⁶ and atopy was demonstrated by a positive skin test result and/or detection of allergen-specific IgE for 1 or more aeroallergens. The control subjects were 13 healthy volunteers with no histories of asthma or other allergic diseases. After written informed consent was obtained, venous blood was drawn and anticoagulated with EDTA. Buffy-coat cells were obtained by sedimenting with dextran T 500. After the cells were collected by means of centrifugation, erythrocytes were hemolyzed by hypotonic shock. After washing with PBS supplemented with 3% FCS and 0.1% NaN_3 , the cells were incubated for 60 minutes at 4°C with FITC-conjugated anti-CD mAb (10 $\mu\text{g}/\text{mL}$) and biotin-conjugated anti-Fc ϵ RI α -chain mAb (CRA-1; 5 $\mu\text{g}/\text{mL}$). The cells were further incubated with PE-conjugated avidin (10 $\mu\text{g}/\text{mL}$) for 30 minutes at 4°C . Finally, cells stained strongly with the anti-Fc ϵ RI α -chain mAb were analyzed for the expression of CD69, CD44, and CD54.

Determination of CD69, CD44, and CD54 on bronchoalveolar lavage fluid cells

Six asthmatic patients were recruited from inpatients of the Department of Pulmonology, International Medical Center of Japan, the Department of Internal Medicine, Teikyo University (Tokyo, Japan), and the Department of Medicine, University of Tokyo Hospital. After written informed consent was obtained, bronchoalveolar lavage fluid (BALF) and blood were collected as described previously.¹⁷ The interval between bronchoalveolar lavage and blood collection was always ≤ 30 minutes, and both specimens were processed simultaneously for cytometric analyses. Flow cytometric analysis of basophils from the BALF and whole blood was performed as described above. BALF basophil populations were identified on the basis of a forward scatter/side scatter distribution characteristic of peripheral blood basophils, in addition to strongly positive

staining for FcεRIα-chain (CRA-1). These cells were further confirmed not to be mast cells on the basis of apparent expression of CD11b¹⁸ and absence of c-kit expression. Although several reports have revealed that activated eosinophils express FcεRIα on their surface,^{19,20} their characteristic forward scatter/side scatter distribution was obviously different from the distribution of basophils. Furthermore, these CRA-1–strongly positive cells were also positive for a basophil-specific BB1 mAb (data not shown).

Statistics

All data are expressed as means ± SEMs. The differences between values in the *in vitro* experiments were analyzed by means of 1-way ANOVA. When this test indicated significant differences, the Fisher protected least significant difference (PLSD) test was used to compare individual groups. Differences in the expression between asthmatic and normal individuals were analyzed by means of the Mann-Whitney *U* test. Differences in the expression between peripheral basophils and tissue basophils from the single donors were analyzed by means of the Wilcoxon signed-ranks test.

RESULTS

In the first series of experiments, we examined the effects of various cytokines on the expression of CD69, CD44, and CD54 by using basophils purified from nonatopic individuals. As shown in Table I, only a marginal level of surface CD69 expression was detected on freshly isolated cells. No significant increase in the expression was observed on basophils cultured for 24 hours in medium alone, but addition of IL-3 induced high levels of CD69 expression. Moreover, IL-5, GM-CSF, and IFN-γ slightly increased the level of CD69 in all cases, but the differences did not reach statistical significance. On the other hand, none of the other tested cytokines, including IL-4, eotaxin/CCL11, and MCP-1/CCL2, exerted any significant effects at all. In contrast to CD69, considerable levels of CD44 and CD54 were detected on freshly isolated basophils. Compared with eosinophils, freshly isolated basophils expressed a higher level of CD54 ($P < .01$) and CD44 ($P < .01$), both of which were significantly upregulated by IL-3 ($P < .01$). The effects of secretagogues on the expression of CD69, CD44, and CD54 are summarized in Table II. We found no significant increase in the expression of these molecules on cells stimulated with any of the secretagogues—ie, anti-IgE, C5a, and fMLP.

Having observed that CD69 expression was highly and preferentially inducible by IL-3, we further examined the kinetics of IL-3–mediated CD69 expression. Dose-response analyses showed that apparent CD69 expression was observed at an IL-3 concentration of 30 pmol/L and reached the plateau levels at 300 pmol/L of IL-3 (ED₅₀, approximately 50 pmol/L; $n = 5$; data not shown). Previous studies by others demonstrated that β₂ integrins²¹ and CD63²² are rapidly upregulated by IL-3 and secretagogues, respectively. In comparison with these molecules, the surface CD69 appeared rather slowly. We compared the expression of CD69, CD63, and CD11b at both early (1 hour) and late (24 hours) time points. CD11b expression was upregulated by IL-3 at 1 hour and decreased at 24 hours. Although IL-3 was unable to

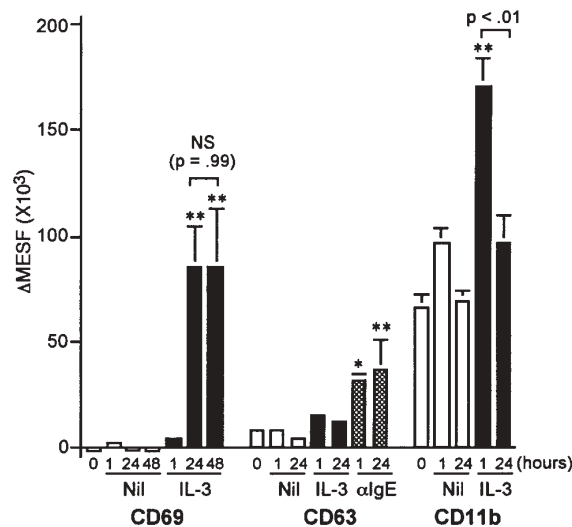


FIG 1. Effects of IL-3 on surface CD69, CD63, and CD11b expression at early and late time points. Purified basophils were cultured in medium alone (*Nil*) or in the presence of 300 pmol/L IL-3 or anti-IgE (1:3000) for the indicated times. Surface CD molecules were analyzed by means of flow cytometry. Data are expressed as ΔMESF values, as described in the Methods section ($n = 3$). ** $P < .01$, * $P < .05$ versus Nil at the same time point. NS, No significant difference.

enhance CD63 expression, stimulation with anti-IgE resulted in significant upregulation of the expression at 1 hour (Fig 1). On the other hand, maximal CD69 surface expression was observed after 24 hours of stimulation (Figs 1 and 2, A), indicating rather slow time kinetics for the expression.

When permeabilized basophils were analyzed by means of flow cytometry, we found that intracellular CD69 was virtually undetectable in freshly isolated basophils (Fig 2, B). Furthermore, pretreatment with cycloheximide (10 μmol/L for 24 hours) almost completely blocked the IL-3–mediated expression of CD69 ($15.7\% \pm 4.0\%$ of controls; $n = 3$), indicating that *de novo* protein synthesis is involved in the process leading to surface CD69 expression. As expected, surface CD69 protein expression paralleled mRNA accumulation. RT-PCR experiments showed that freshly isolated basophils expressed only negligible amounts of CD69 mRNA (Fig 2, C). Although apparent CD69 transcripts were readily detected in cells cultured for 4 hours even in the absence of exogenous factors, CD69 mRNA accumulation was markedly upregulated in the presence of IL-3 (Fig 2, C).

We studied the functional significance of CD69 molecules: basophils were cultured with IL-3 for 24 hours and then stimulated with immobilized anti-CD69 mAbs. Degranulation was monitored by histamine release. Immobilized anti-CD69 mAbs failed to induce significant histamine release, whereas a positive control, immobilized anti-FcεRIα mAb (CRA-1), did ($n = 4$; percent release against total histamine content, $37.3\% \pm 9.8\%$, $33.0\% \pm 9.6\%$, and $60.7\% \pm 9.3\%$, for control IgG, anti-CD69 mAb, and anti-FcεRIα mAb, respectively). In

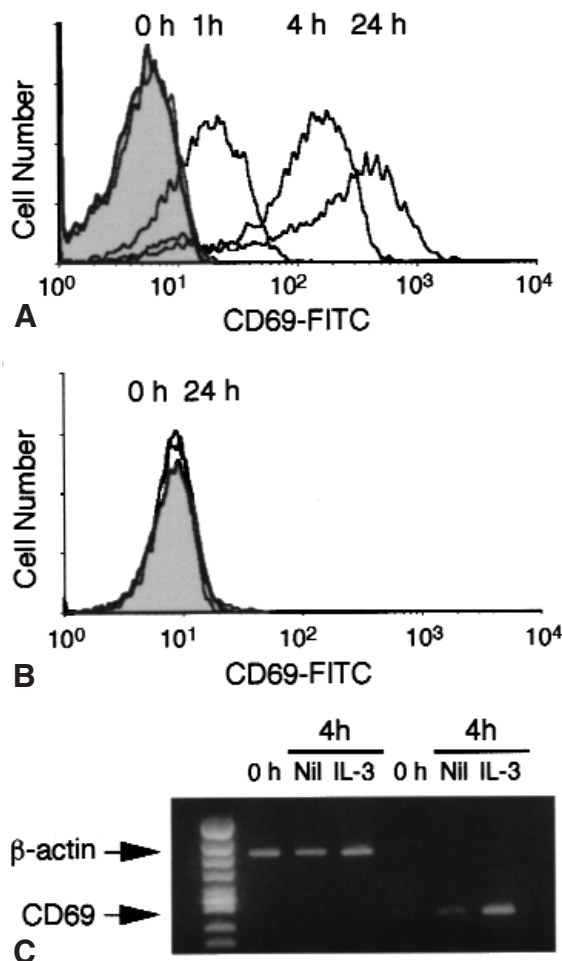


FIG 2. Surface (A) and intracellular CD69 protein (B) expression and CD69 mRNA expression (C) in purified basophils. **A**, Purified basophils were cultured in medium alone or in the presence of 300 pmol/L IL-3 for 1, 4, or 24 hours. Surface CD69 was analyzed by means of flow cytometry. Δ MESF values for freshly isolated, 1-hour IL-3-, 4-hour IL-3-, and 24-hour IL-3-cultured basophils, and basophils cultured for 24 hours with medium alone were 108, 3910, 44612, 131127, and 157, respectively. The shaded area indicates the fluorescence of cells stained with FITC-conjugated control mouse IgG. This is representative of 4 different experiments that showed similar results. **B**, Intracellular CD69 expression was analyzed as described in the Methods section for freshly isolated basophils and basophils cultured in medium alone for 24 hours. Δ MESF values for freshly isolated basophils and basophils cultured for 24 hours in medium alone were 134 and 130, respectively. The shaded area indicates the fluorescence of cells stained with FITC-conjugated control mouse IgG. This is representative of 2 different experiments that showed similar results. **C**, Purified basophils were cultured in medium alone (Nil) or in the presence of 300 pmol/L IL-3 for 4 hours. RT-PCR was performed as described in the Methods section. The PCR products were electrophoresed and then visualized with ethidium bromide. A representative example of 3 separate experiments is shown; the other experiments all showed similar results.

addition, we found that crosslinking of CD69 also failed to enhance histamine release induced by 12-o-tetradecanoylphorbol-13-acetate ($n = 3$; data not shown). The viability of basophils was also totally unaffected by immobilized CD69 ($n = 2$; data not shown), whereas the viability of eosinophils was efficiently reduced by the

TABLE I. Regulation of CD69, CD44, and CD54 expression by cytokines

	CD69	CD44	CD54
Basophils			
0 hour	0.08 $\pm$ 0.03	182.41 $\pm$ 27.90	4.96 $\pm$ 0.31
24 hours			
Nil	0.15 $\pm$ 0.04	162.05 $\pm$ 40.99	4.14 $\pm$ 0.29
IL-3	56.50 $\pm$ 18.63**	420.25 $\pm$ 91.58**	10.31 $\pm$ 1.20**
IL-4	0.12 $\pm$ 0.09	153.66 $\pm$ 40.86	4.68 $\pm$ 0.26
IL-5	2.35 $\pm$ 0.98	213.10 $\pm$ 39.38	5.32 $\pm$ 0.13
GM-CSF	1.32 $\pm$ 0.30	187.34 $\pm$ 44.15	5.09 $\pm$ 0.24
IFN- γ	0.50 $\pm$ 0.11	162.23 $\pm$ 40.48	6.67 $\pm$ 0.38
Eotaxin	0.25 $\pm$ 0.07	159.88 $\pm$ 42.25	4.29 $\pm$ 0.24
MCP-1	0.30 $\pm$ 0.05	161.63 $\pm$ 41.32	4.47 $\pm$ 0.19
Eosinophils			
0 hour	0.63 $\pm$ 0.11	85.11 $\pm$ 9.60	1.87 $\pm$ 0.37
24 hours			
Nil	5.32 $\pm$ 3.16	91.30 $\pm$ 8.89	6.38 $\pm$ 1.99
IL-5	31.31 $\pm$ 4.25**	133.09 $\pm$ 12.92**	12.68 $\pm$ 3.15*

Purified basophils and eosinophils were cultured in medium alone (Nil) or in the presence of various cytokines (cytokines, 300 pmol/L; chemokines, 100 nmol/L) for 24 hours. Surface CD69, CD44, and CD54 expression was analyzed by means of flow cytometry. Data are expressed as Δ MESF values ($\times 1000$), as described in the Methods section ($n = 5$).

** $P < .01$.

* $P < .05$ versus Nil.

same treatment, as previously described by others ($n = 2$; data not shown).¹⁴

In the last series of experiments, we examined in vivo expression of CD69, CD44, and CD54 on basophils. By double-staining with mAbs against Fc ϵ RI and each marker, we examined the expression of these markers on whole blood basophils from normal and asthmatic individuals. No statistically significant difference was observed in the expression of CD44 and CD54 between the 2 groups. However, we found that atopic basophils expressed slightly but significantly higher levels of CD69 than normal basophils (Fig 3). To determine the expression profile in basophils at inflamed tissue sites, we analyzed BALF basophils from 6 asthmatic individuals. In all cases, BALF basophils showed increased levels of surface CD69 expression in comparison with blood basophils from the same donors, the difference being statistically significant. On the other hand, there was no association regarding the expression of CD44 and CD54 (Table III).

DISCUSSION

A mounting body of evidence has demonstrated that interaction of cytokines with both eosinophils and basophils initiates a series of coordinated biochemical and cellular events.⁵ Furthermore, studies on eosinophils have clearly demonstrated that cytokine-mediated activation resulted in de novo induction and/or upregulation of surface expression of several molecules. As to basophils, however, "activation markers" are less well understood, the exception being demonstration of upregulated expression of an adhesion molecule (CD11b) during the cytokine-mediated activation process.²¹

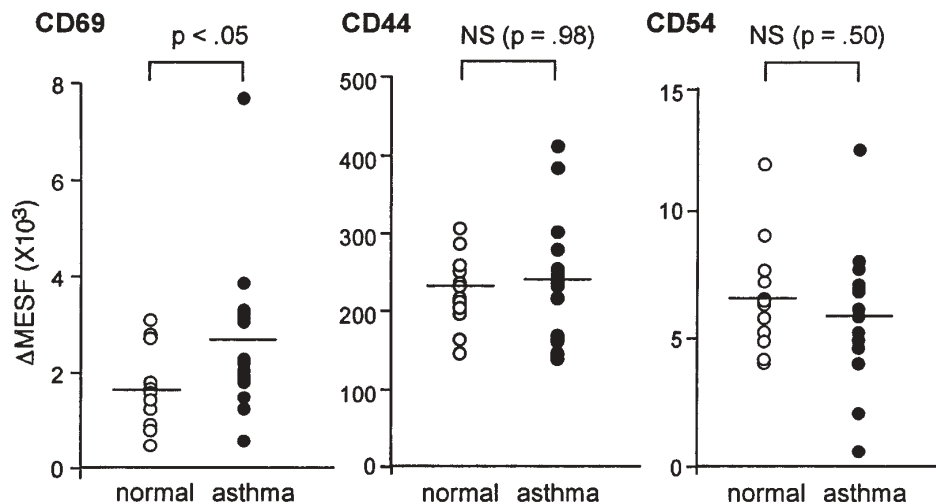


FIG 3. Expression of CD69, CD44, and CD54 on whole blood basophils of normal and asthmatic individuals. Whole blood basophils were analyzed for expression of CD69, CD44, and CD54, as described in the Methods section. Data are expressed as Δ MESF values. Statistical significance between normal ($n = 13$) and asthmatic ($n = 15$) individuals was assessed by means of the Mann-Whitney U test; P values are indicated in the figure. Horizontal bars show the mean values. NS, No significant difference.

In the present study, we for the first time investigated the regulation of the expression of CD44, CD54, and CD69 on human basophils. Constitutive expression of CD44 and CD54 has been reported in cells of basophil lineage.^{23,24} In the present study, we found that both CD44 and CD54 were significantly upregulated by cytokine-mediated activation, but the extent of the increase was rather small (2.2 ± 0.6 -fold and 1.6 ± 0.2 -fold increase by IL-3 in comparison with freshly isolated cells for CD44 and CD54, respectively). Furthermore, the increase in the expression of the latter was less pronounced than in eosinophils (10.7 ± 4.7 -fold increase in eosinophils stimulated by IL-5). On the other hand, CD69 expression was highly inducible on the basophil surface after activation by IL-3. CD69, a disulfide-linked homodimer, was originally described as an early activation marker for lymphocytes.²⁵ Subsequently, CD69 was also identified on activated leukocytes of other cell types, including neutrophils,²⁶ monocytes,²⁷ and eosinophils.²⁸ Our present study has extended these findings by providing evidence that CD69 is also an activation marker for human basophils.

In contrast to highly constitutive expression of CD44 and CD54, CD69 expression was not detected on purified basophils (Δ MESF = -60 ± 62) and only marginally detected on whole blood basophils (Δ MESF = 1652 ± 221). The difference in the CD69 expression for the 2 preparations might be a result of the different handling procedures for basophils. That is, slight activation might occur after binding of anti-Fc ϵ RI mAb in whole blood basophils, and/or a specific subset with CD69 expression was eliminated in purified preparations during the negative selections. CD69 protein was also hardly detectable inside these cells. Furthermore, appearance of the CD69 protein was accompanied by mRNA accumulation, and it was almost completely blocked by a protein synthesis

TABLE II. Regulation of CD69, CD44, and CD54 expression by secretagogues

	CD69	CD44	CD54
Basophils			
0 hour	-0.38 ± 0.08	208.42 ± 8.43	1.82 ± 0.31
24 hours			
Nil	-0.28 ± 0.11	201.75 ± 8.07	0.67 ± 0.29
IL-3	$111.05 \pm 16.50^{**}$	$479.03 \pm 15.69^{**}$	$4.05 \pm 1.20^{**}$
C5a	-0.46 ± 0.07	206.68 ± 8.32	0.86 ± 0.26
fMLP	-0.35 ± 0.10	205.86 ± 8.04	0.48 ± 0.13
α IgE	1.10 ± 0.91	232.12 ± 8.30	0.48 ± 0.91

Purified basophils were cultured in medium alone (Nil) or in the presence of various secretagogues, as indicated (IL-3, 300 pmol/L; C5a, 10 nmol/L; fMLP, 100 nmol/L; α IgE, 1:3000) for 24 hours. Data are expressed as Δ MESF values ($\times 1000$; $n = 3$).

$^{**}P < .01$ versus Nil.

inhibitor, cycloheximide. These results collectively indicate that basophil CD69 expression was induced qualitatively rather than quantitatively, being regulated mainly at the level of transcription.

Substantial evidence indicates that CD69 is associated with functional significance in various types of cells, including natural killer cells,²⁹ monocytes,²⁷ and eosinophils.¹⁴ However, we failed to observe any functional significance for basophil CD69: neither degranulation nor survival of basophils was affected by crosslinking of surface CD69. The lack of an apoptogenic effect of basophil CD69 was in contrast to the results in eosinophils, the life span of which was shortened by incubation with several mAbs against CD69.¹⁴ Apoptosis of basophils and eosinophils is prevented by IL-3, IL-5, and GM-CSF, and it is promoted by glucocorticoids.¹⁵ Although the extracellular regulation of apoptosis of

TABLE III. Expression profiles of CD69, CD44, and CD54 in peripheral blood (PB) and BALF of asthmatic patients

Subject no.	Admitting diagnosis	Age (y)	Sex		Differential count (%)		Basophils (Δ MESF)			Eosinophils (Δ MESF)		
					Basophils	Eosinophils	CD69	CD44	CD54	CD69	CD44	CD54
1	BA + drug	59	M	PB	1.0	34.0	2.25	108.44	4.57	4.20	202.60	1.72
	Induced EP			BALF	0.4	82.3	2.60	75.48	3.68	46.56	174.32	5.52
2	BA + CEP	59	F	PB	0.2	17.5	1.61	193.38	6.30	ND	ND	ND
				BALF	1.0	22.6	4.96	114.74	1.17	36.48	71.23	3.28
3	BA + ABPA	70	M	PB	2.0	13.0	1.03	334.34	6.98	ND	ND	ND
	s/o			BALF	0.1	3.3	6.65	437.42	51.55	ND	ND	ND
4	BA + CEP	67	M	PB	2.0	3.0	1.44	269.13	6.22	ND	ND	ND
				BALF	0.3	7.0	10.89	491.41	6.66	ND	ND	ND
5	BA + CEP	73	M	PB	1.5	36.0	0.39	ND	ND	ND	ND	ND
				BALF	0.1	51.7	1.98	ND	ND	ND	ND	ND
6	BA + AGA	66	M	PB	1.0	11.6	2.75	123.30	6.12	3.20	288.66	4.03
				BALF	1.0	50.4	53.11	2323.13	6.33	36.47	170.90	4.63
Mean				PB			1.58 $\pm$	205.72 $\pm$	6.04 $\pm$			
$\pm$ SEM							0.35	43.00	0.40			
				BALF			13.36 $\pm$	688.44 $\pm$	13.88 $\pm$			
							8.06*	418.20	9.47			

The data are expressed as Δ MESF ($\times 1000$) values, as described in the Methods section.

BA, Bronchial asthma; M, male; F, female; PB, peripheral blood; EP, eosinophilic pneumonia; BALF, bronchoalveolar lavage fluid; CEP, chronic eosinophilic pneumonia; ND, not determined; ABPA, allergic bronchopulmonary aspergillosis; AGA, allergic granulomatous angitis.

* $P < .05$ versus corresponding value of peripheral blood basophils in asthmatic patients.

basophils shows remarkable similarities with that of eosinophils, the intracellular events occurring during apoptosis might be distinct between these cell types.

Eosinophil CD69 expression is principally compartmentalized at inflamed tissue sites. Although CD69 expression is found on peripheral eosinophils in certain disease conditions (such as parasitic diseases³⁰), BALF eosinophils in patients with eosinophilic lung diseases show extremely high levels of surface CD69 expression.²⁸ Although circulating basophils of asthmatic patients expressed higher levels of CD69 than those of normal individuals, the difference was slight. In contrast to peripheral basophils, clear CD69 expression was observed in basophils at tissue sites. These results are consistent with those for eosinophils, but the extent of the CD69 expression in BALF basophils varied widely among patients. This wide variation in CD69 expression in tissue basophils might be attributable to the local IL-3 levels, because strong CD69 expression on basophils is preferentially induced by IL-3. The widely variable expression of CD69 on tissue basophils among our patients suggests a heterogeneous cytokine profile secreted in inflamed tissues. Factors accounting for in vivo CD69 expression on basophils might be an interesting point meriting further study.

In summary, we have for the first time reported the regulation of the expression of CD69, CD44, and CD54 on human basophils. Our results collectively demonstrate that de novo appearance of CD69 was strongly and preferentially induced by IL-3. Basophil CD69 had no functional relevance but did show biological significance. Thus, CD69 on basophils might be useful as an in vitro as well as in vivo marker of activation of these cells by IL-3.

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