

THE JOURNAL OF
ALLERGY
AND
CLINICAL IMMUNOLOGY

VOLUME 99

NUMBER 4

Updates on cells and cytokines

Guest editors: David M. Essayan, MD, Baltimore, Md., Charity C. Fox, MD, Columbus, Ohio, Francesca Levi-Schaffer, PhD, Jerusalem, Israel, and Rafeul Alam, MD, PhD, Galveston, Texas (Supported by a grant from Hoechst Marion Roussel, Inc., Kansas City, Mo.)

New concepts: The basophil

John T. Schroeder, PhD, and Donald W. MacGlashan, Jr., MD, PhD *Baltimore, Md.*

A number of studies support the belief that human basophils play a unique role in allergic inflammation, particularly that associated with chronic disease.¹ This concept is very different from early thought, which suggested that the basophil might simply serve as a surrogate with which to study mast cell function. In fact, it is now apparent that these two cell types differ significantly from each other. Basophils release different mediators, respond to a greater variety of secretagogues, use different mechanisms of signal transduction, and have different modalities of pharmacologic control. These findings are supported, not only by *in vitro* experiments, but also by many studies showing the direct involvement of basophils at sites of experimentally induced inflammation and in natural disease. Basophils also differ from mast cells with respect to development. Although mast cell precursors are released from bone marrow and require stem cell factor to mature in tissue, basophils are thought to be released from bone marrow as mature cells, with their development being dependent on the effects of IL-3 and granulocyte-macrophage colony-stimulating factor. Of great significance, we and

Abbreviations used

HrHRF:	Human recombinant histamine-releasing factor
PKC:	Protein kinase C
PMA:	Phorbol myristate acetate

others have more recently shown that human basophils generate messenger RNA and secrete cytokines, particularly IL-4, that are known to modulate their own response and the immune responses of other cell types (i.e., eosinophils and lymphocytes) that participate in allergic reactions. This article, in fact, focuses on some of the more recent facets of cytokine secretion by basophils with the emphasis that this activity supports the concept that these cells play an active role in the pathogenesis of allergic disease.

Studies of basophil cytokine secretion actually stem from observations showing that IL-3-dependent murine mast cell lines, as a result of FcεRI cross-linking, generated a variety of cytokines originally thought to be made only by activated Th2 lymphocytes and included IL-3, IL-4, IL-5, IL-1, and IL-6.^{2,3} To date, several laboratories have reported the secretion of IL-4 by basophils,⁴ with more recent studies showing that IL-13 and the chemokine, macrophage inflammatory protein-1α,⁵⁻⁸ are made by these cells. Our discussion, however, focuses primarily on the generation of IL-4 by human basophils, because much of the information, although in its infancy, is in reference to this protein.

Human basophils secrete relatively high levels of IL-4 protein when activated by IgE-dependent stimuli and, interestingly, in a time frame consistent with the late-phase response that is often associated with both natural

From Johns Hopkins University Department of Medicine, Division of Clinical Immunology at the Johns Hopkins Asthma and Allergy Center.

Supported in part by the National Institutes of Health Grants AI27906 and AI07290.

Received for publication Jan. 13, 1997; accepted for publication Jan. 13, 1997.

Reprint requests: John T. Schroeder, Johns Hopkins University Department of Medicine, Division of Clinical Immunology at the Johns Hopkins Asthma and Allergy Center, 5501 Hopkins Bayview Circle, Baltimore, MD 21224.

J Allergy Clin Immunol 1997;99:429-33.

Copyright © 1997 by Mosby-Year Book, Inc.

0091-6749/97 \$5.00 + 0 1/1/80414

TABLE 1. The relationship between IL-4 secretion and histamine release by human basophils activated with various stimuli

Stimulus	IL-4 secretion	Histamine release
Anti-IgE/antigen		
100 ng/ml	+	++
10 ng/ml	++	+
Costimulation w/IL-3	+++	+++
Costimulation w/HrHRF	+++	+++
HrHRF (IgE ⁺)	++	++
FMLP	-/+	++
C5a	-/+	+
Costimulation w/IL-3	++	++
Ca ⁺⁺ ionophore	++++	++
PMA	-	+++

Plus sign (+) represents relative response.

and experimental allergen exposures. In fact, changes in the levels of IL-4 mRNA in human basophils can be detected by 30 minutes after activation; and protein for this cytokine is detectable in culture supernatants by 1 hour, with levels peaking at 4 to 6 hours. The generation of IL-13 by basophils appears to be somewhat slower than that for IL-4. Protein levels for this cytokine peak at approximately 24 hours, with half maximal levels detected at about 6 to 8 hours.^{5,7,8} Unlike the release of histamine or leukotriene C₄, which occurs on degranulation or minutes after stimulation, basophils do not seem to secrete much, if any, IL-4 protein (or IL-13) within this period, suggesting that the generation of this cytokine occurs *de novo* on activation. This finding was first supported by the fact that cycloheximide, when added along with stimulus, does not block histamine release but completely ablates the secretion of IL-4. As outlined below, several additional observations now support the notion that the intracellular signals that control IL-4 protein secretion dissociate from those regulating mediator release.

There is a remarkable correlation between the amount of IL-4 mRNA and protein generated in 4-hour cultures and the purity of basophils, with no apparent contribution by the contaminating cells in these suspensions.⁹ These findings, in fact, have recently been confirmed by a study showing that basophils are the major source of IL-4 in antigen-stimulated peripheral blood mononuclear cell cultures.¹⁰ Furthermore, basophils appear to be the sole source of IL-4 protein, even during the early hours, for cultures activated with calcium ionophores, which are known to activate many different cell types. As a result, this has established the use of basophil-enriched suspensions (5% to 50%) that are rapidly prepared by using density centrifugation to study several parameters of cytokine secretion by human basophils.¹¹ Basophils prepared in this manner are relatively more responsive to IgE-dependent stimulation and are not subjected to an extensive protocol of puri-

fication techniques that likely affect their response to various stimuli. In fact, studies in which basophil-enriched suspensions are used indicate a correlation between the amount of IL-4 protein generated and the magnitude of histamine released, with donors whose basophils show a high percentage of histamine release also secreting the highest levels of IL-4 protein (up to 500 to 1000 pg/10⁶ basophils).

Several observations support the belief that releasability to IgE-specific stimulation is the most important factor in the ability of basophils to secrete IL-4 (Table I). First, the IgE-independent secretagogues, f-met peptide and C5a, when used alone, are potent activators of basophil histamine release. However, they show little to no ability to induce IL-4 secretion from basophils isolated from most donors. Second, there is no difference between the amount of IL-4 protein generated by basophils obtained from allergic and nonallergic individuals when cells are activated by anti-IgE antibody (10 ng/ml). As expected, specific antigen does trigger IL-4 secretion only by basophils from donors who are allergic and whose cells normally release histamine in response to the challenge allergen. Finally, we have recently shown that basophils expressing so-called "IgE⁺," which normally release histamine in response to the human recombinant histamine-releasing factor (HrHRF), also generate IL-4 with kinetics and protein levels that are essentially identical to those induced by anti-IgE antibody. In contrast, monocyte chemotactic protein-1, which is thought to induce histamine release from basophils independent of IgE expression, was not a potent stimulus for IL-4 in these studies.¹²

Although there are cytokines, chemokines, and growth factors that upregulate IgE-dependent mediator release, it was hypothesized that some of these same proteins (e.g., granulocyte-macrophage colony-stimulating factor, IL-5, and IL-1) would also modulate the generation and secretion of cytokines by basophils. However, to date only two cytokines, IL-3 and HrHRF, appear to significantly affect cytokine secretion by basophils. Thus the pretreatment of basophils for 15 minutes with IL-3 enhances IL-4 secretion induced by anti-IgE some 30% to 150%. In fact, there is even evidence that IL-3, when used as a costimulus in cultures challenged with the IgE-independent secretagogue C5a, promotes the production of IL-4 but at a much slower rate (18 to 24 hours) than the rate that occurs in cultures activated with IgE-dependent stimuli.¹³ Preliminary data show that IL-13 secretion is also significantly affected by IL-3 and, in many instances, will induce the secretion of this cytokine in the absence of IgE-dependent stimulation. Like IL-3, HrHRF also enhances the secretion of IL-4 and IL-13 induced by FcεRI cross-linking. Most important, this protein, which was previously thought to affect only the response of basophils expressing "IgE⁺," also modulates cytokine secretion by basophils obtained from all donors tested thus far (i.e., basophils expressing "IgE⁻"). This finding, in fact, suggests that HrHRF interacts through a specific receptor

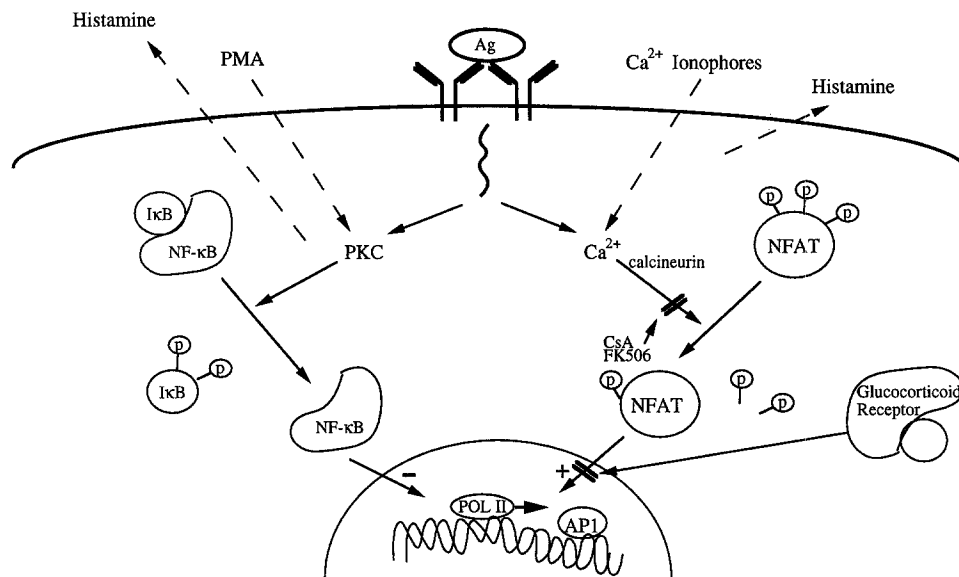


FIG. 1. Oversimplified scheme of intracellular signals regulating IgE-dependent generation of IL-4 in human basophils. Antigen binds specific IgE on the cell membrane, resulting in FcεRI cross-linking. Low-level cross-linking causes a prolonged calcium response that is likely important in activating calcium-dependent calcineurin, resulting in dephosphorylation and activation of cytosolic NFAT. Calcium ionophores, such as ionomycin, also activate NFAT independent of receptor signaling. Cyclosporine and FK-506 prevent this activation by blocking activation of calcineurin. Translocation of dephosphorylated NFAT to the nucleus has a positive effect on transcription of IL-4. Rapid inhibitory effects of glucocorticoids on generation of IL-4 may occur by affecting this pathway. In contrast, high-level cross-linking causes a short-lived calcium response but results in activation of PKC, which is associated with histamine release. PKC is also important in activation of cytosolic NFκB by promoting dissociation of its inhibitory component, IκB. Phorbol esters, such as PMA, bypass membrane signaling to directly activate PKC. Activated NFκB translocates to the nucleus and has an inhibitory role in generation of IL-4.

found on the surface of basophils, which may also be expressed by other cell types that participate in allergic lesions.¹⁴

Of therapeutic interest, it seems apparent that the secretory mechanisms controlling the generation of cytokines in basophils are very different from those important for histamine release. Once again, this is readily seen with univalent stimuli, such as f-met peptide and C5a, which are inconsistent activators of IL-4 secretion compared with the signals delivered in response to cross-linking stimuli (anti-IgE antibody). Furthermore, there is preliminary evidence that the intracellular mechanisms controlling both mediator and cytokine secretion also diverge from one another in response to cross-linking stimuli alone. In support of this belief are data showing that anti-IgE antibody, at concentrations that are suboptimal for histamine release, is actually a very potent stimulus of IL-4 secretion by basophils. Our own studies investigating the cytosolic calcium events occurring in basophils after activation may partially explain the differences in IL-4 generation seen with these stimuli. First, calcium ionophores (e.g., ionomycin or A23187) are the most potent stimuli tested for IL-4 secretion by human basophils, suggesting that this ion has an important role in the generation of this cytokine in these cells. Second, studies show that the cytosolic calcium responses induced by C5a and f-met peptide are

short-lived in basophils relative to those initiated by IgE-dependent stimulation. In fact, supraoptimal anti-IgE antibody also induces a briefer calcium response than suboptimal anti-IgE.¹⁵ Therefore although mediator release and cytokine secretion both require changes in cytosolic calcium, it is apparent that the latter response is very much dependent on stimuli that induce elevated calcium changes.

Protein kinase C (PKC) activation also appears to have modulatory effects on the generation of cytokines by human basophils. However, most of the evidence at this time clearly shows that activation of this kinase has an antisecretory role.¹⁶ For example, phorbol myristate acetate (PMA), a potent activator of PKC activity that causes nearly complete degranulation and histamine release, does not induce the secretion of IL-4 protein from basophils. This dissociation alone suggests that basophils use different intracellular mechanisms for the release of preformed mediators compared with those involved in cytokine secretion. Most astonishing, however, is the observation that PMA, without blocking calcium influx, downregulates the secretion of protein from basophils induced by calcium ionophore; this effect is completely reversed with the simultaneous addition of a PKC inhibitor (either staurosporin or bisindolylmaleimide). This finding is quite surprising, because calcium influx and PKC activation are implicated in many secre-

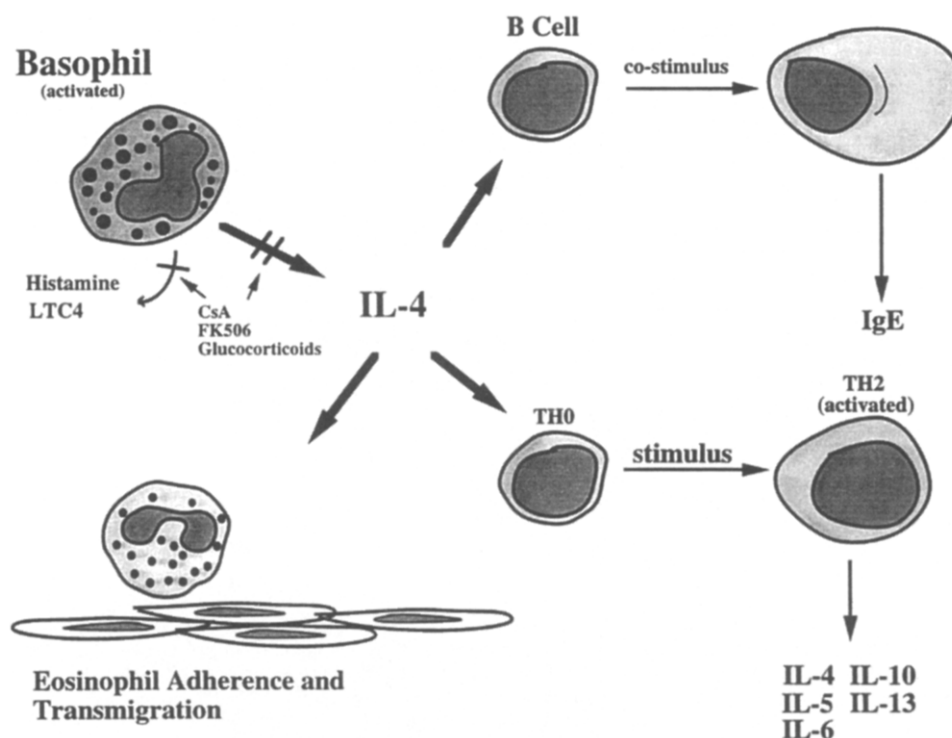


FIG. 2. Diagrammatic representation of how basophil-derived IL-4 could potentially influence immune responses of other cell types that participate in allergic inflammation. Isotype switching to IgE in B cells is mediated by effects of IL-4. Development of Th2-like lymphocytes from Th0-like cells is also influenced by IL-4 activity. Finally, IL-4 has an important role in upregulating expression of adhesion molecules, such as vascular cell adhesion molecule-1, which help facilitate selective transendothelial migration of several cell types, particularly eosinophils. Glucocorticoids, cyclosporine, and FK506 are all potent inhibitors of IL-4 secretion by basophils, although they have modest inhibitory activity against histamine release and leukotriene formation.

tory functions. In fact, these signals seem necessary for the expression of IL-4 mRNA and the secretion of protein by peripheral blood lymphocytes costimulated with PMA and ionophore. Furthermore, this finding suggests that the mechanisms regulating IL-4 secretion by basophils may very well differ from those controlling the generation of this cytokine in lymphocytes.

The mechanism of PMA-mediated inhibition of IL-4 synthesis in basophils is presently unknown. A recent study, however, has suggested that IL-4 generation is regulated through the competitive effects of the transcription factors, NF κ B and NF-AT.¹⁷ In this instance NF κ B binding, in response to activation by PMA, resulted in decreased IL-4 promoter activity. As noted below, human basophils contain an NF-AT-like protein that is believed to have a role in generating IL-4. Thus it is possible that PKC activation (occurring with PMA treatment) causes decreased IL-4 generation in basophils by activating NF κ B, which competes with NF-AT for binding sites within the human IL-4 promoter (Fig. 1).

Studies investigating the pharmacologic control of the basophil response function not only in showing those drugs that have therapeutic potential in allergic disease but also in providing insight into the signal transduction mechanisms that are important in mediator release and

cytokine secretion by these cells. Our own studies show that IL-4 protein secretion by basophils, as is the case with histamine release, is sensitive to the inhibitory effects of glucocorticoids. However, unlike the extended preincubation time (24 hours) necessary to inhibit mediator release, the secretion of IL-4 by basophils is immediately affected by the addition of glucocorticoids (at concentrations considered physiologic), suggesting that these drugs act on multiple pathways controlling the release of these products. Glucocorticoids may show such vast differences on these basophil responses because the generation of IL-4 occurs *de novo*, whereas histamine is preformed and released on degranulation. Indeed, our own results suggest that steroids inhibit IL-4 protein secretion from basophils by preventing the accumulation of IL-4 mRNA after IgE-dependent activation. It is likely that steroids also affect the transcription of mRNA coding for proteins involved in histamine release. However, these unknown proteins are likely expressed constitutively, given the facts that histamine is rapidly released from activated cells and that the additional time necessary to observe inhibition of this secretion may result from the natural decay of these factors within the cell after steroid pretreatment.

Finally, studies show that in lymphocytes the drugs, cyclosporine and FK506, by interfering with the activa-

tion and translocation of a calcium-calculinurin-dependent factor, nuclear factor of activated T cells (NF-AT), prevent the transcription of several cytokine genes including IL-2, IL-4, and tumor necrosis factor- α .¹⁸ As noted above, calcium plays an important role in the generation of IL-4 protein by basophils. We have recently performed experiments showing that FK506 and cyclosporine are some 100 times more potent in inhibiting IL-4 secretion than in preventing histamine release by basophils. Likewise, we have found that a protein immunologically identical to NF-AT is found in the cytoplasm of basophils and is translocated to the nucleus after IgE-dependent activation (Schroeder. Unpublished observations). As expected, this protein does not translocate, nor is IL-4 protein secreted, if the basophils are pretreated with either cyclosporine or FK506, suggesting that an NF-AT-like protein has an important role in the generation of this cytokine in basophils (Fig. 1). It is not presently known whether this NF-AT-like protein in basophils is identical to that occurring in lymphocytes.

In conclusion, it is our hypothesis that human basophils contribute to allergic disease by infiltrating tissue and secreting proinflammatory cytokines, such as IL-4 (and IL-13), that play an important role in the ongoing allergic response (Fig. 2). In fact, studies support the belief that basophils may be the sole source of IL-4 protein generated within a few hours after allergen exposure. Thus basophil-derived cytokines could potentially influence the involvement of eosinophils and lymphocytes by upregulating adhesion molecules, such as vascular cell adhesion molecule, and by promoting the development of Th2-type cells, both of which are processes that constitute the hallmark of allergic disease. A knowledge of the mechanisms regulating cytokine secretion in human basophils will therefore have important therapeutic implications.

REFERENCES

- Schroeder JT, Kagey-Sobotka A, Lichtenstein LM. The role of the basophil in allergic inflammation. *Allergy* 1995;50:463-52.
- Plaut M, Pierce JH, Watson CJ, Hanley HJ, Nordan RP, Paul WE. Mast cell lines produce lymphokines in response to cross-linkage of Fc epsilon RI or to calcium ionophores. *Nature* 1989;339:64-7.
- Wodnar-Filipowicz A, Heusser CH, Moroni C. Production of the haemopoietic growth factors GM-CSF and interleukin-3 by mast cells in response to IgE receptor-mediated activation. *Nature* 1989;339:150-2.
- Schroeder JT, MacGlashan DW Jr, Kagey-Sobotka A, White JM, Lichtenstein LM. Cytokine generation by human basophils. *J Allergy Clin Immunol* 1994;94:1189-95.
- Li H, Sim T, Alam R. IL-13 released by and localized in human basophils. *J Immunol* 1996;156:4833-8.
- Li H, Sim T, Grant J, Alam R. The production of macrophage inflammatory protein- α by human basophils. *J Immunol* 1996;157:1207-12.
- Ochensberger B, Daepf G-C, Rihs S, Dahinden CA. Human blood basophils produce interleukin-13 in response to IgE-receptor-dependent and independent activation. *Blood* 1996;88:3028-37.
- Gibbs BF, Haas H, Falcone FH, Albrecht C, Vollrath IB, Noll T, et al. Purified human peripheral blood basophils release interleukin-13 and preformed interleukin-4 following immunological activation. *Eur J Immunol* 1996;26:2493-8.
- MacGlashan DW Jr, White JM, Huang SK, Ono SJ, Schroeder J, Lichtenstein LM. Secretion of interleukin-4 from human basophils: the relationship between IL-4 mRNA and protein in resting and stimulated basophils. *J Immunol* 1994;152:3006-16.
- Kasaian M, Clay M, Happ M, Garman R, Hirani S, Luqman M. IL-4 production by allergen-stimulated primary cultures: identification of basophils as the major IL-4-producing cell type. *Int Immunol* 1996;8:1287-97.
- Schroeder JT, MacGlashan DW Jr, Kagey-Sobotka A, White JM, Lichtenstein LM. The IgE-dependent IL-4 secretion by human basophils: the relationship between cytokine production and histamine release in mixed leukocyte cultures. *J Immunol* 1994;153:1808-18.
- Schroeder J, Lichtenstein L, MacDonald S. An IgE-dependent recombinant histamine-releasing factor induces IL-4 secretion from human basophils. *J Exp Med* 1996;183:1265-70.
- Ochensberger B, Rihs S, Brunner T, Dahinden C. IgE-independent interleukin-4 expression and induction of a late phase of leukotriene C4 formation in human blood basophils. *Blood* 1995;86:4039-49.
- Schroeder JT, Lichtenstein LM, Kagey-Sobotka A, MacDonald SM. Human recombinant IgE-dependent histamine releasing factor (HrHRF) enhances IL-4 secretion by human basophils [abstract]. *J Allergy Clin Immunol* 1996;97:358.
- MacGlashan DW Jr, Guo CB. Oscillations in free cytosolic calcium during IgE-mediated stimulation distinguish human basophils from human mast cells. *J Immunol* 1991;147:2259-69.
- Schroeder JT, Lichtenstein LM, Kagey-Sobotka A, MacGlashan DW Jr. Differential control of basophil IL-4 and histamine secretion with protein kinase C (PKC) activation [abstract]. *J Allergy Clin Immunol* 1995;95:344.
- Casolaro V, Georas S, Song Z, Zubkoff I, Abdulkadir S, Thanos D, et al. Inhibition of NF-AT-dependent transcription by NF- κ B: implications for differential gene expression in T helper cell subsets. *Proc Natl Acad Sci USA* 1995;92:11623-7.
- Rao A. NF-ATp: a transcription factor required for the co-ordinate induction of several cytokine genes. *Immunol Today* 1994;15:274-81.