

area is the possibility that groups of cytokines stimulate specific patterns of expression of receptors and that this pattern is responsible for behavior of the cell and can be diagnosed by MAb.

Third, the possibility of having clinically relevant antagonists is real with the development of powerful blocking MAb and, perhaps more important, of analogues of growth factors that act as antagonists by specifically binding one of the chains of the receptors for these factors (Tables V and VI). Although each of these reagents may have significant drawbacks in clinical use (the MAb is murine and the antagonist must be used in excess over ligand of approximately 100 times), each points the way for the specific design of smaller molecules that may be more potent and more easily administered.

Finally, the effect of cyclosporine on cells other than T lymphocytes also points the way for the inhibition of transcription of cytokine genes in non-T cells. The particularly attractive possibility is that there exist several pathways of activation only some of the activation pathways are sensitive to cyclosporine. If this were to be the case, it may be possible to target activation pathways specific for allergy.

We thank Mari Walker for typing the manuscript.

REFERENCES

1. Cockerill PN, Shannon MF, Bert AG, Ryan GR, Vadas MA. The granulocyte-macrophage colony-stimulating factor/interleukin-3 locus is regulated by an inducible cyclosporin A-sensitive enhancer. *Proc Natl Acad Sci USA* 1993;90:2466-79.
2. Braun RK, Franchini M, Erard F, et al. Human peripheral blood eosinophils produce and release interleukin-8 on stimulation with calcium ionophore. *Eur J Immunol* 1993; 23:956-60.
3. Korpelainen EI, Gamble JR, Smith WB, et al. The receptor for interleukin-3 is selectively induced in human endothelial cells by tumour necrosis factor- α and potentiates interleukin-8 secretion and neutrophil transmigration. *Proc Natl Acad Sci USA* 1993;90:11137-41.
4. Moser R, Fehr J, Bruijnzel PLB. IL-4 controls the selective endothelium-driven transmigration of eosinophils from allergic individuals. *J Immunol* 1992;149:1432-8.
5. Kita H, Ohnishi T, Okubo Y, Weiler D, Abrams JS, Gleich GJ. Granulocyte/macrophage colony-stimulating factor and interleukin 3 release from human peripheral blood eosinophils and neutrophils. *J Exp Med* 1991;174:745-8.
6. Cockerill GW, Gamble JR, Vadas MA. Angiogenesis: models and model vectors. *Ann Rev Cytol* 1994;154(2).
7. Khew-Goodall Y, Butcher CM, Litwin MS, et al. Chronic expression of P-selectin on endothelial cells stimulated by T cell cytokine, interleukin-3 (submitted).
8. Smith WB, Noack L, Vadas MA, Gamble JR. Transforming growth factor- β inhibits production of interleukin-8 and the transmigration of neutrophils through activated endothelium. *Proc Natl Acad Sci USA* (in press).
9. Hercus T, Bagley C, Cambareri B, et al. Specific human granulocyte-macrophage colony-stimulating factor antagonists. *Proc Natl Acad Sci USA* 1994;91:5838-42.

The clinical relevance of basophil releasability

Gianni Marone, MD, Giuseppe Spadaro, MD, Vincenzo Patella, MD, and
Arturo Genovese, MD Naples, Italy

In contrast to many words used in allergy (e.g., *atopy*, *asthma*, *apoptosis*) that derive from ancient Greek, the term *releasability*, recently introduced in the English-language literature, does not exist in most dictionaries. It was first

From the Division of Clinical Immunology, University of Naples Federico II, School of Medicine.

Supported in part by grants from the CNR (Project FATMA; subproject Prevention and Control of Disease Factors, No. 94.00607. PF41) and the MURST, Rome, Italy. Dr. Patella is recipient of a fellowship from the Associazione Italiana Ricerca sul Cancro.

Reprint requests: Gianni Marone, MD, Cattedra di Immunologia Clinica e Allergologia, Facoltà di Medicina e Chirurgia, Università di Napoli Federico II, Via Sergio Pansini 5, 80131 Napoli, Italy.

J ALLERGY CLIN IMMUNOL 1994;94:1293-303.
Copyright © 1994 by Mosby-Year Book, Inc.
0091-6749/94 \$3.00 + 0 1/059929

Abbreviations used

BAL:	Bronchoalveolar lavage
Con A:	Concanavalin A
D ₂ O:	Deuterium oxide
Der p I:	<i>Dermatophagoides pteronyssinus</i> p I antigen
FMLP:	<i>N</i> -Formyl-methionyl-leucyl-phenyl-alanine
IL:	Interleukin
LTC ₄ :	Leukotriene C ₄
PMC _E :	Lung parenchymae mast cells isolated enzymatically
PMC _M :	Lung parenchymae mast cells isolated mechanically
SCF:	Stem cell factor
Staph A:	Protein A from <i>Staphylococcus aureus</i> Cowan I

used by Lichtenstein and Conroy¹ in 1976 to define an event that many investigators and clinicians had probably implicitly already recognized. Initially the concept of releasability was essentially applied to the in vitro study of the release of chemical mediators from human basophils, and this has been reviewed.²⁻⁷ The concept of basophil and mast cell releasability is of practical importance because under appropriate conditions these cells synthesize several proinflammatory mediators^{6, 8} and cytokines,⁹⁻¹² thus playing a central role in the pathogenesis of immune disorders. This article focuses on the concept of basophil and mast cell releasability, mostly from the clinical viewpoint.

DOES RELEASABILITY EXIST IN VIVO?

The need to invoke releasability derives from a number of clinical observations. Every clinician is aware of persons with positive skin test reactions to various inhalants, who have no symptoms during the appropriate season, whereas other patients with similarly positive skin reactions show a wide spectrum of allergies. This apparent discrepancy has been carefully documented in patients allergic to insect venoms. Patients with identical serum concentrations of IgE and/or IgG anti-venom antibodies and similar skin test reactions can be stung, with opposite clinical results: one patient can have anaphylaxis whereas the other is completely unaffected.¹³ A similar condition is found in persons with positive skin reactions to food allergens. Approximately 50% of subjects with positive skin reactions have no reaction when the culprit food is ingested, whereas the other 50% have local or systemic reactions.¹⁴

These observations suggested that the serum concentrations of IgE and IgG antibodies against various allergens and the in vivo sensitivity of skin mast cells to allergens were not sufficient to predict the extent of an allergic response in vivo. Thus the releasability of primary effector cells (basophils and mast cells) was proposed to explain the complex array in symptoms in allergic persons.

DEFINITION OF RELEASABILITY

The term *releasability* implies that several biochemical events, not only the surface density of IgE molecules, control the release of mediators from basophils and mast cells in response to different activating stimuli, independent of the IgE molecules themselves. The first evidence of this was provided in the basophil system by Conroy et al.,¹⁵ who reported a significant correlation between the serum IgE level and the number of IgE mol-

ecules per basophil. However, they found no relationship at all between the serum IgE level and the concentration of anti-IgE required for 30% histamine release. In fact, basophils with 5000 IgE molecules responded to anti-IgE just as well as cells with 500,000 molecules. However, in basophils from different patients with 100,000 IgE molecules per cell, the maximum histamine release induced by anti-IgE ranged widely, from 10% to 75%. Thus it was concluded that the response of these different cells to anti-IgE was influenced by something in addition to the antigen-antibody interaction. Although the concept of releasability has remained biochemically elusive,⁷ information obtained during the last few years has confirmed the existence of this biologic parameter.²⁻⁶

Basophil and mast cell releasability can be assessed by (1) measuring spontaneous histamine release in normal buffers or in buffers containing deuterium oxide (D₂O) in place of water, (2) stimulating Fc ϵ RI⁺ cells with increasing concentrations of secretagogues, or (3) measuring the density of antigen-specific IgE required to release of 30% of the total cellular histamine content on challenge with an optimal concentration of antigen or anti-IgE.

It is now clear that releasability must be defined in relation to the stimulus employed.^{16, 17} Different stimuli induce the release of histamine either by interacting with cell-bound IgE or by acting on surface or intracellular targets independent of the IgE receptor. IgE-mediated secretagogues may in turn induce degranulation of basophils and mast cells either by direct anaphylaxis, that is, by inducing IgE crosslinking at the F(ab')₂ regions (specific antigen), or by "reverse" anaphylaxis, that is, crosslinking IgE by polyclonal anti-IgE antibodies or a monoclonal antibody against the α chain of Fc ϵ RI. The latter mechanisms, frequently exploited for experimental purposes, are of clinical relevance because anti-IgE autoantibodies^{18, 19} and anti- α -chain autoantibodies²⁰ have been detected in a certain percentage of atopic patients. Some bacterial immunoglobulin-binding proteins, such as protein A from *Staphylococcus aureus* Cowan I (Staph A) and protein L from *Peptostreptococcus magnus*, also induce the release of mediators from basophils and mast cells by interacting with IgE molecules.²¹⁻²³

Among non-IgE-mediated secretagogues, the formylated tripeptide, N-formyl-methionyl-leucyl-phenylalanine (FMLP), complement-derived anaphylatoxins C3a and C5a, and platelet-activating factor activate basophils by interacting with specific cell-surface receptors, independent of the IgE receptor.²⁴⁻²⁶ Ca²⁺ ionophores such as com-

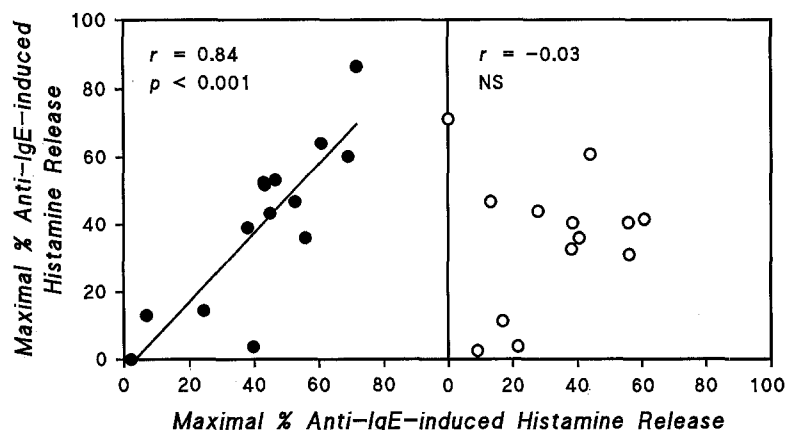


FIG. 1. Correlation between maximal percentage of anti-IgE-induced histamine release from basophils from 14 sets of monozygotic (●) and 13 dizygotic (○) twins. Each point represents mean of duplicate determinations. NS, Not significant. (From Marone G, Poto S, Celestino D, Bonini S. *J Immunol* 1986;137:3588-92. Copyright 1986, The Journal of Immunology.)

pound A23187, and tumor-promoting phorbol esters such as 12-*o*-tetradecanoylphorbol-13-acetate do not interact with surface receptors, therefore bypassing some of the early stages of signal transduction, and induce degranulation by allowing transmembrane Ca^{2+} influx or by directly activating protein kinase C, respectively.^{27, 28} Another interesting stimulus is D_2O which in vitro potentiates the release of mediators induced by other stimuli but causes little release by itself from cells of normal donors.^{29, 30}

In general, there is no correlation between the sensitivity and reactivity of the cells to release with any of these stimuli, and basophil and mast cell releasability must therefore be defined with respect to each stimulus.

AGE-RELATED CHANGES IN HUMAN BASOPHIL RELEASABILITY

We studied IgE (anti-IgE) and non-IgE-mediated (FMLP and Ca^{2+} ionophore A23187) releasability of basophils from 63 normal subjects ranging in age from 1 to 86 years. The maximum histamine release induced by anti-IgE was significantly age related.¹⁶ The percent histamine secretion of the group less than 20 years of age was significantly lower than that of the older groups at all concentrations of anti-IgE. The groups older than 20 years released the same percentage of histamine release for all concentrations of anti-IgE. These results indicate that when crosslinking of the IgE receptor is the signal for basophil histamine release, the cell donor's age significantly influences the amount of histamine secretion. Studies of IgE-mediated release should therefore take into account the fact that basophil releasability is age related.

In the groups examined, we found no correlation between the donor's age and the maximum percentage of histamine release induced by FMLP. Histamine release tended to decline slightly with age and in fact was significantly lower in the group more than 60 years old. The decreased in vitro response to FMLP in these older subjects might reflect long-term in vivo exposure and desensitization to bacterial products.

No correlation was found between the age of donors and the maximum A23187-induced histamine release. Finally, we compared the maximum percentage of histamine release with optimal concentrations of these three agents (anti-IgE, FMLP, and A23187). There was no correlation at all between any pair of the three releasing agents, suggesting again that basophil releasability must be defined for each separate stimulus.

GENETIC CONTROL OF BASOPHIL RELEASABILITY

Heredity has long been suspected of playing a role in the development of allergic disorders,³¹ and several early observations report allergy as "running in families."^{32, 33}

We assessed the influence of genetic and environmental factors on basophil releasability in 26 sets of twins.³⁴ Zygotic status was established by direct observation, detailed examination of various anthropologic markers, and questionnaire answers, with a reliability estimated at 98% through control blood typing on about 15% of sets.³⁵ The total sample consisted of 14 monozygotic and 12 dizygotic sets.

Fig. 1 shows the significant intraset correlation coefficient of the maximum percentage of anti-IgE-induced histamine release in the 14 monozy-

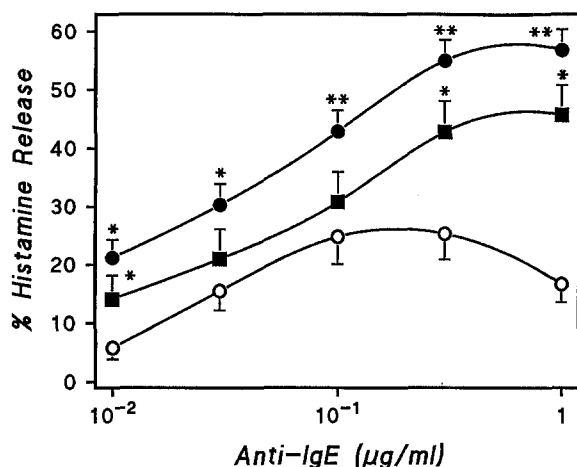


FIG. 2. Histamine release induced by anti-IgE from basophils of 29 patients with bronchial asthma (●), 17 with allergic rhinitis (■), and 31 normal donors (○). Each point represents mean $\pm$ SEM. Asterisk, Significantly different from control subjects. Double asterisks, Significantly different from patients with rhinitis and control subjects. (From Casolaro V, Spadaro G, Marone G. *Am Rev Respir Dis* 1990;142:1108-11.)

gotic twins ($r = 0.84$; $p < 0.001$) but not in the dizygotic twins. When the mean intraset variance of anti-IgE-induced histamine release was calculated in the monozygotic (VMZ = 85) and dizygotic twins (VDZ = 327) we found an F value of 3.84 ($p < 0.01$) and a heritability value of 0.74. These results suggest that IgE-mediated releasability is controlled by genetic factors and underline the importance of basophil releasability. No correlation was found between serum IgE levels and anti-IgE-induced histamine release, suggesting that the two parameters (serum IgE level and basophil IgE-mediated releasability) are under separate genetic control.

FMLP-induced histamine release from basophils appeared to be influenced mainly by environmental factors. The intratwin correlation coefficient of A23187-induced histamine release was 0.62 ($p < 0.01$) in the monozygotic sets and 0.38 (not significant) in the dizygotic sets. The difference between monozygotic and dizygotic sets was significant, suggesting that the biochemical steps explored by the Ca^{2+} ionophore A23187 are at least partly under genetic control. This evidence strongly favors a genetic control of basophil releasability.^{34, 36}

DIFFERENCES BETWEEN BASOPHIL AND MAST CELL RELEASABILITY

Human basophils differ from mast cells morphologically, ultrastructurally, biochemically, immunologically, and pharmacologically.³⁷⁻³⁹ It is therefore not surprising that many aspects of

releasability in basophils differ from those of mast cells isolated from different anatomic sites. The evidence for different control of releasability in these two primary effector cells derives from the clinical observation that patients with urticarial syndromes have increased releasability of skin mast cells,⁴⁰ whereas IgE-mediated releasability of basophils is markedly reduced.⁴¹⁻⁴³

Casolaro et al.¹⁷ compared basophil and lung mast cell releasability from individual donors. The response of basophils from 52 donors to anti-IgE, Staph A, concanavalin A (Con A), FMLP, and A23187 was compared with that of mast cells isolated enzymatically (PMC_E) or mechanically (PMC_M) from lung parenchyma. The maximum percentage of anti-IgE-induced histamine secretion was higher in basophils than in PMC_E and PMC_M . The correlation between the maximum percentage of histamine release induced by anti-IgE and Staph A was positive in basophils, whereas PMC_E and PMC_M were essentially unresponsive to Staph A and Con A. Similarly, lung mast cells were unresponsive to FMLP.

Even more dramatic are the differences between basophils and mast cells from bronchoalveolar lavage (BAL). BAL mast cells do not release histamine in response to FMLP, and anti-IgE induces a significantly higher maximum histamine release in basophils than in BAL mast cells. Striking differences also have been clearly demonstrated between basophils and mast cells and between mast cells from different anatomic sites in response to opioids.³⁹

BASOPHIL RELEASABILITY IN ALLERGIC SKIN DISORDERS

The observations discussed so far provide some insight into the concept of basophil and mast cell releasability. However, the study of releasability probably has practical implications, and releasability has been investigated in a variety of allergic disorders.

We have carried out experiments to ascertain whether there was any difference in the releasability of basophils from young patients with atopic dermatitis and normal donors.⁴⁴ Basophils from the young patients released significantly more histamine both spontaneously and in response to D_2O , and were significantly more responsive to anti-IgE and A23187. No correlation was found between histamine release with optimal and suboptimal concentrations of the stimuli and the serum IgE level. Interestingly, in a recent study in adult patients with atopic dermatitis, anti-IgE-induced histamine release was the same in patients with atopic dermatitis and in control

subjects.⁴⁵ This is a further reminder that basophil releasability is affected by the cell donor's age for normal persons and for atopic patients.

Basophil releasability is not always increased in patients with allergic disorders. In patients with chronic urticaria, basophils were significantly less responsive to anti-IgE than the cells of control subjects.⁴¹⁻⁴³ It was suggested that in vivo desensitization had occurred in response to the same stimulus tested in vitro. Interestingly, in patients with chronic urticaria the de novo synthesis of an eosinophil chemotactic factor was also lower than in normal donors.⁴⁶ We have confirmed and extended these findings, showing that basophils of patients with chronic urticaria released significantly less histamine than did control subjects and synthesized less leukotriene C₄ (LTC₄) de novo in response to anti-IgE.⁴³ The maximum release of histamine and LTC₄ from control subjects and from patients with chronic urticaria presented a significant linear correlation. Thus it appears that releasability of preformed mediators such as histamine correlates with that of de novo synthesized metabolites such as eosinophil chemotactic factor and LTC₄.

BASOPHIL AND MAST CELL RELEASABILITY IN RESPIRATORY ALLERGY

There is compelling evidence that basophils and mast cells play a role in the pathogenesis of respiratory allergy. Metachromatic cells are increased in the sputum before an asthmatic attack,⁴⁷ and basophils are increased in bronchoalveolar lavage (BAL) fluid after antigen challenge.⁴⁸ The presence of basophils in lung tissues obtained from asthma patients who died⁴⁹ and the correlation between the annual changes in methacholine airway responsiveness and basophil count⁵⁰ support the view that basophils play a major role in the pathogenesis of bronchial asthma.

Spontaneous release of histamine from basophils in the presence of D₂O is markedly greater than normal in approximately 30% of patients with allergic rhinitis or bronchial asthma.^{5, 29, 30} Studies of basophil releasability in response to different IgE-mediated or non-IgE-mediated stimuli in patients with respiratory allergies have yielded apparently contradictory results. Basophil releasability in patients with asthma was increased,⁵¹ unchanged,¹⁷ and even decreased.⁵² In one study in patients with allergic rhinitis, basophil releasability was increased in response to the anti-IgE-related stimulus Con A.⁵³

We compared basophil releasability in young adults with severe bronchial asthma or with aller-

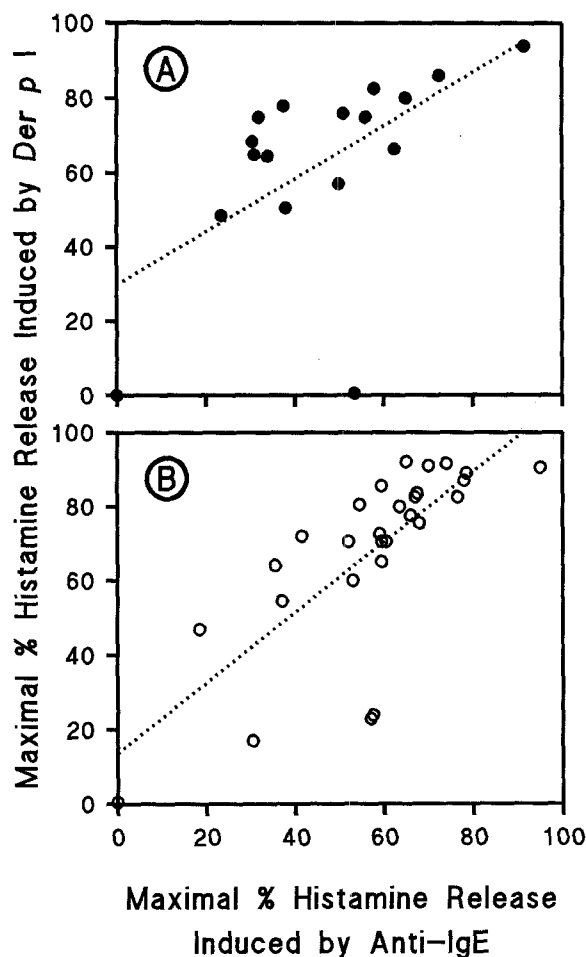


FIG. 3. Correlation between maximal percentage of histamine release induced by anti-IgE and *Der p I* from basophils of 17 patients with allergic rhinitis (●, A) and 29 with bronchial asthma (○, B). (From Casolaro V, Spadaro G, Marone G. *Am Rev Respir Dis* 1990;142:1108-11.)

gic rhinitis, all of whom were skin test-positive for *Dermatophagoides pteronyssinus* p I antigen (*Der p I*), and age-matched control subjects.⁵⁴ Basophils were challenged with increasing concentrations of anti-IgE, *Der p I*, FMLP, and A23187. Basophil reactivity (i.e., the maximal percentage histamine release) to *Der p I* was similar in asthma and allergic rhinitis. However, basophil reactivity to anti-IgE was significantly higher in patients with asthma than in subjects with allergic rhinitis and was significantly higher in both groups of patients than in control subjects (Fig. 2).

A significant correlation was found between basophil reactivity to anti-IgE and to *Der p I* in patients with bronchial asthma and in those with allergic rhinitis (Fig. 3). This is a further indication that anti-IgE may be an excellent in vitro probe of IgE-mediated releasability in vivo. Moreover, in patients with asthma,¹⁸ as in patients with other atopic conditions,^{19, 55} high serum levels of

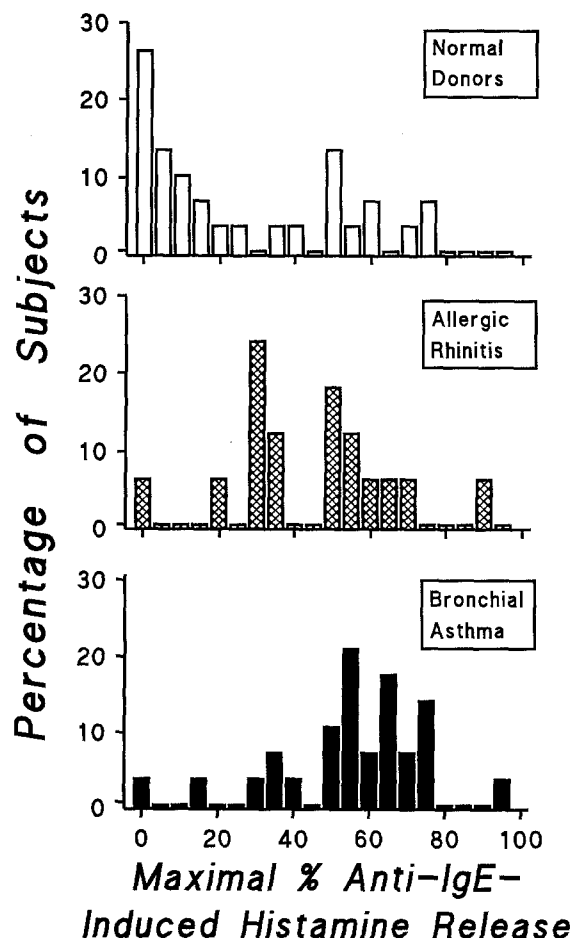


FIG. 4. Frequency distribution of maximal percentage anti-IgE-induced histamine release from basophils of 31 normal donors (open bars), 17 patients with allergic rhinitis (shaded bars), and 29 patients with bronchial asthma (dark bars). (From Casolaro V, Spadaro G, Marone G. *Am Rev Respir Dis* 1990;142:1108-11.)

anti-IgE autoantibodies are, at least in some cases, capable of inducing mediator release from human basophils and mast cells.⁵⁶

The frequency distribution of the maximal percentage of anti-IgE-induced histamine release from basophils of control subjects and of patients with bronchial asthma or allergic rhinitis showed two peaks for the control subjects, the first corresponding to donors whose basophils released between 40% and 80% of their histamine content, and the second corresponding to donors whose basophils consistently failed to release histamine on challenge with anti-IgE (nonreleasers) (Fig. 4). The latter amounted to approximately 25% of control subjects. In patients with respiratory allergies the nonreleasers' peak was much lower, and the peak for the basophils releasing of about 50% of their content was larger. This may account for the differences in the mean maximal anti-IgE-

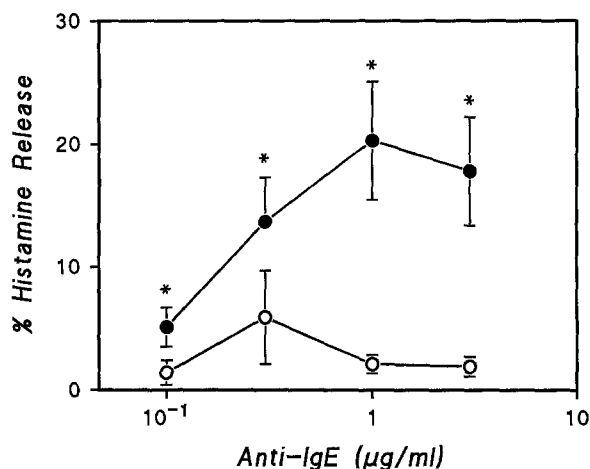


FIG. 5. Histamine release induced by anti-IgE from BAL mast cells of 19 patients with mild asthma (●) and 17 normal donors (○). Each point represents mean $\pm$ SEM. Asterisk, Significantly different from control subjects. (From Casolaro V, Galeone D, Giacommo A, Sanduzzi A, Melillo G, Marone G. *Am Rev Respir Dis* 1989;139:1375-82.)

induced histamine release between control subjects and the two groups of atopic donors.

Significant differences were also observed between basophil reactivity to FMLP in patients with bronchial asthma and control subjects, but basophil reactivity to A23187 was the same as in control subjects.

In patients with asthma there was a significant correlation between total serum IgE levels and basophil reactivity to anti-IgE ($r = 0.44$; $p < 0.05$), whereas no such correlation was seen in patients with allergic rhinitis or in control subjects. This agrees with a previous report that the prevalence of asthma is closely related to serum IgE levels whereas allergic rhinitis appears to be independent.⁵⁷ Interestingly, these two groups of patients showed no correlation between basophil sensitivity to *Der p* I (i.e., the concentration that induced 50% of maximal in vitro histamine release) and skin mast cell sensitivity to the same allergen (i.e., the concentration that induced the same wheal area on skin prick testing in each patient as did 10 mg/ml of histamine). This is further evidence that basophil releasability is controlled differently from skin mast cell releasability in patients with respiratory allergy.

IgE-mediated basophil releasability appears to be correlated with the pathogenesis of airway reactivity in asthma: an inverse correlation was found between IgE-mediated releasability and the provocative dose of inhaled histamine required to produce a 20% decrease in the forced expiratory volume in 1 second.⁵¹

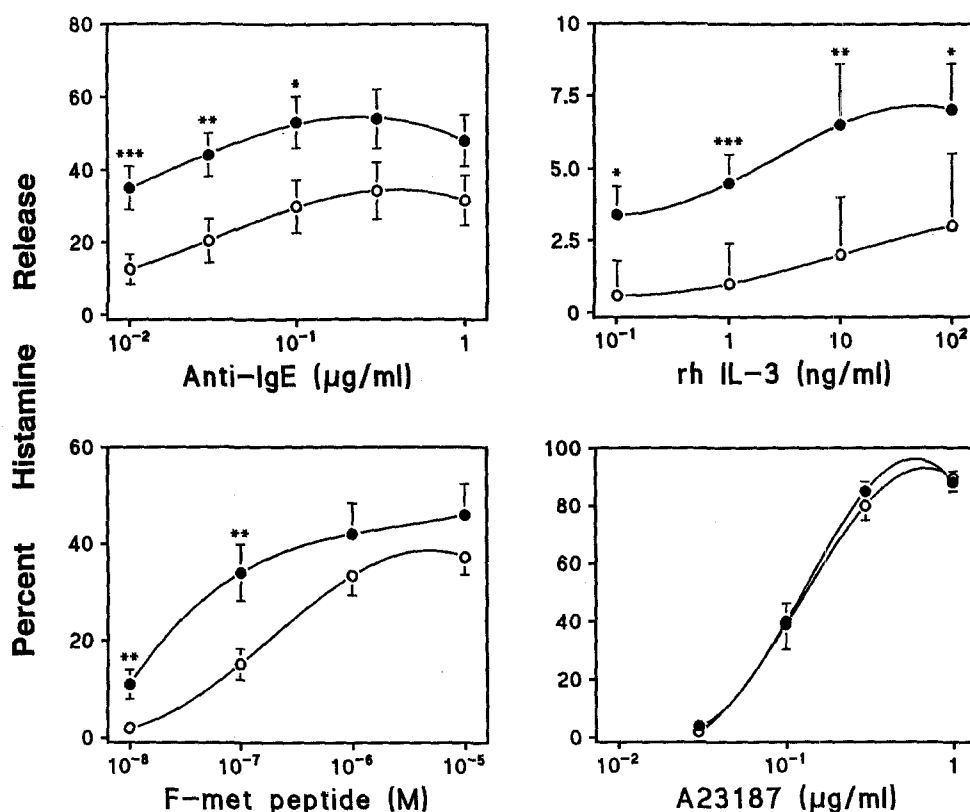


FIG. 6. Histamine release induced by anti-IgE, recombinant human IL-3 (*rhIL-3*), FMLP, and A23187 from basophils of 16 women with scleroderma (●) and 16 normal women (○). Each point represents mean $\pm$ SEM. Asterisk, $p < 0.05$; double asterisks, $p < 0.01$; triple asterisks, $p < 0.05$ versus control subjects. (From de Paulis A, Valentini G, Spadaro G, Lupoli S, Tirri G, Marone G. *Arthritis Rheum* 1991;34:1289-96, by permission.)

In another study we compared the releasability of basophils and BAL mast cells from patients with mild asthma and from control subjects.¹⁷ Interestingly, the patients with mild asthma had basophil IgE-mediated and non-IgE-mediated releasability similar to that of control subjects, whereas their BAL mast cells released more histamine when challenged with anti-IgE (Fig. 5). The histamine concentration in BAL from patients with asthma (1628 ± 632 pg/ml) was significantly higher than in control subjects (371 ± 65 pg/ml; $p < 0.05$), suggesting that patients with mild asthma have a selective alteration of BAL mast cell releasability, which is presumably responsible for the increased histamine levels in BAL. Furthermore, because this group of adults with mild asthma had normal IgE-mediated and non-IgE-mediated basophil releasability, evaluation of effector cells that would come into immediate contact with inhaled allergens (e.g., BMC) might be a more sensitive basis than basophil releasability for distinguishing donors with mild asthma. This observation might partly explain the apparently contradictory results of ear-

lier studies in which the severity of asthma was not always considered.

ALTERATION OF BASOPHIL RELEASABILITY IN NONALLERGIC DISORDERS

Basophil and mast cell releasability has been studied so far primarily in patients with allergic disorders. However, there is evidence that these cells and their mediators play a role in an increasing number of immune and inflammatory disorders.⁶ Scleroderma is a multisystem disease characterized by vascular and microvascular injury and fibroblast activation leading to fibrosis.⁵⁸ Although the cause of the disease is unclear, there is increasing evidence that the immune dysfunction also involves the skin^{59, 60} and lung mast cells.⁶¹ Although the importance of skin mast cell releasability in scleroderma has been demonstrated experimentally⁵⁸ and clinically,^{59, 60} skin mast cell and basophil releasability appear to be independently controlled.¹⁷

We evaluated basophil releasability in 16 female patients with scleroderma and in 16 normal

TABLE I. Factors that influence releasability of basophils and mast cells

-
- IgE-mediated basophil releasability increases with age of cell donors
 - Basophils and mast cell releasability must be defined with respect to each stimulus (anti-IgE, FMLP, A23187, D₂O, C5a, etc.)
 - IgE-mediated and, to a lesser extent, A23187-mediated basophil releasability are influenced by genetic factors
 - FMLP-mediated basophil releasability is influenced by environmental factors
 - IgE-mediated basophil releasability is increased in atopic dermatitis, allergic rhinitis, and bronchial asthma, and decreased in chronic urticaria
 - In mild asthma IgE-mediated BAL mast cell releasability, but not basophil releasability, is increased
 - Basophil releasability is increased not only in patients with allergic disorders but also in systemic sclerosis
-

age- and sex-matched donors.⁶² Cells from patients with scleroderma showed enhanced releasability when challenged in vitro with recombinant human interleukin (IL)-3. Releasability induced by anti-IgE and FMLP at the lower concentrations was higher in the patients with scleroderma than in the control subjects (Fig. 6). This finding is presumably clinically relevant because anti-IgE autoantibodies have been found in some patients with systemic sclerosis.⁶³ Basophil reactivity and sensitivity to A23187 were similar in the two groups. These results show that basophil releasability may be altered not only in patients with allergic disease but presumably in other immune disorders also.

CYTOKINE-INDUCED MODULATION OF BASOPHIL AND MAST CELL RELEASABILITY

Human basophils and mast cells have membrane-bound receptors for various cytokines. Human basophils express receptors for IL-2, IL-3, IL-4, granulocyte-macrophage colony-stimulating factor,⁶⁴ and for the *c-kit* receptor ligand.⁶⁵ Human lung and skin mast cells do not have the same array of membrane receptors for cytokines. For instance, mast cells isolated from human lung and skin tissues express surface receptors for the *c-kit* receptor ligand and IL-4.^{65, 66}

Cytokines have a profound influence on basophil function; IL-3 is the most potent upregulator of the basophil response yet found, although IL-5, IL-1, and interferon- γ also significantly enhance

IgE-mediated basophil releasability.⁶⁷⁻⁶⁹ Granulocyte-macrophage colony-stimulating factor enhances the release of mediators from basophils challenged with FMLP.⁶⁹ The enhancing effect of IL-3 is evident after short (≈ 10 minutes) or prolonged (≈ 18 hours) incubation before addition of the releasing stimulus.⁶⁷ The effects of IL-3 are global, the responses to all stimuli are increased, and all mediators (histamine, LTC₄, and IL-4) are affected.^{12, 67}

Stem cell factor (SCF), the ligand of the *c-kit* receptor, is the only cytokine known that activates human mast cells, and recombinant human SCF enhances the release of histamine from lung and skin mast cells and basophils.^{65, 66}

In view of the in vitro and in vivo^{70, 71} effects of various cytokines on human basophil and mast cell functions, it is tempting to suggest that these are the cause of all the differences in releasability. However, the decreased basophil releasability,^{42, 43} the genetic control of the response of basophils,³⁴ and the lack of correlation between the response of basophils to IgE-mediated and other univalent stimuli suggest that a complete explanation of IgE- and non-IgE-mediated basophil and mast cell releasability may involve more than circulating or local exposure to cytokines.

CLOSING THOUGHTS

Cellular releasability is an important parameter, not yet biochemically defined, that controls the release of proinflammatory mediators and cytokines from Fc ϵ RI⁺ cells. Basophil and mast cell releasability are clearly distinct parameters that are just beginning to be characterized. Table 1 summarizes a series of factors that influence releasability of these two cells.

It is now evident that releasability must be defined with respect to each stimulus in light of the profound differences between IgE- and non-IgE-mediated releasability in normal donors and in atopic patients. Our observation that IgE-mediated releasability in normal donors is influenced by an age-dependent factor is of practical importance because this might be important in atopic disorders as well.^{44, 45} Therefore studies of basophil and probably mast cell releasability should take into account the fact that the cell donor's age might influence the results.

Our studies of twins and of subjects of different ages indicate that basophil releasability is controlled by a complex interaction between genetic and environmental factors. Basophil histamine release in response to anti-IgE appears to be primarily controlled by genetic factors.³⁴ Non-IgE-

mediated releasability such as responses to FMLP is presumably controlled by environmental factors and follows an age-related pattern different from anti-IgE-induced secretion.¹⁶ A23187-induced release of mediators from basophils is also at least partly under genetic control but is not influenced by the age of the cell donor.

IgE-mediated and non-IgE-mediated basophil releasability may be either increased or reduced. It is enhanced in patients with respiratory allergy^{51, 54}; the severity of allergic symptoms roughly correlates with the degree of basophil responsiveness in vitro. Basophils from patients with asthma release more histamine than basophils from those with rhinitis on challenge with anti-IgE.⁵⁴ When examining the frequency distribution of basophil reactivity to anti-IgE, a certain percentage of nonreleasers may account for some of the differences observed in average basophil releasability between populations.

There appears to be marked immunologic and functional heterogeneity between basophils and mast cells, so that most results with basophils cannot be extrapolated to mast cells. In vitro studies of human mast cell releasability are still hampered by the technical problems of obtaining these cells from different tissues in large amounts and sufficiently purified. BAL mast cells from patients with mild asthma show enhanced releasability even in the absence of increased basophil releasability.¹⁷ Unfortunately, however, the influence of environmental or genetic factors on human mast cell releasability has not yet been analyzed.

Preliminary evidence suggests that basophil releasability may be altered not only in allergic diseases but also in other immune disorders such as systemic sclerosis. Much work remains to be done to define the molecular and genetic bases of basophil and mast cell releasability and its relevance in different pathophysiologic conditions.

REFERENCES

- Lichtenstein LM, Conroy MC. The "releasability" from human basophils and granulocytes. In: Mathov E, Sindt T, Naranjo P, eds. *Allergy and clinical immunology*. Amsterdam: Excerpta Medica, 1976;109-15.
- Marone G, Poto S, Giugliano R, Bonini S. Studies on human basophil releasability. *Int Arch Allergy Appl Immunol* 1985;77:103-6.
- Marone G. Modulation of basophil/mast cell functions in vivo: the 'releasability' concept. In: Reed CE, Bellanti J, Davies RJ, Friedlander S, Oehling A, Slavin RG eds. *Progress in allergy and clinical immunology*. St Louis: CV Mosby, 1985:175-81.
- Plaut M, Kazimierzczak W, Lichtenstein LM. Abnormalities of basophil 'releasability' in atopic and asthmatic individuals. *J ALLERGY CLIN IMMUNOL* 1986;78:968-73.
- Marone G, Poto S, Giugliano R, Celestino D, Bonini S. Control mechanisms of human basophil releasability. *J ALLERGY CLIN IMMUNOL* 1986;78:974-80.
- Marone G, Casolaro V, Cirillo R, Stellato C, Genovese A. Pathophysiology of human basophils and mast cells in allergic disorders. *Clin Immunol Immunopathol* 1989;50:S24-S40.
- MacGlashan DW Jr. Releasability of human basophils: cellular sensitivity and maximal histamine release are independent variables. *J ALLERGY CLIN IMMUNOL* 1993;91:605-15.
- Stellato C, de Paulis A, Ciccarelli A, et al. Anti-inflammatory effect of cyclosporin A on human skin mast cells. *J Invest Dermatol* 1992;98:800-4.
- Brunner T, Heusser CH, Dahinden CA. Human peripheral blood basophils primed by interleukin 3 (IL-3) produce IL-4 in response to immunoglobulin E receptor stimulation. *J Exp Med* 1993;177:605-11.
- Bradding P, Feather IH, Howarth PH, et al. Interleukin 4 is localized to and released by human mast cells. *J Exp Med* 1992;176:1381-6.
- Möller A, Lippert U, Lessmann D, et al. Human mast cells produce IL-8. *J Immunol* 1993;151:3261-6.
- MacGlashan DW Jr, White JM, Huang S-K, Ono SJ, Schroeder JT, Lichtenstein LM. Secretion of IL-4 from human basophils: the relationship between IL-4 mRNA and protein in resting and stimulated basophils. *J Immunol* 1994;152:3006-16.
- Hunt KJ, Valentine MD, Sobotka AK, Benton AW, Amadio FJ, Lichtenstein LM. A controlled trial of immunotherapy in insect hypersensitivity. *N Engl J Med* 1978;299:157-61.
- Sampson HA, Albergo R. Comparison of results of skin tests, RAST, and double-blind, placebo-controlled food challenges in children with atopic dermatitis. *J ALLERGY CLIN IMMUNOL* 1984;74:26-33.
- Conroy MC, Adkinson NF Jr, Lichtenstein LM. Measurement of IgE on human basophils: relation to serum IgE and anti-IgE-induced histamine release. *J Immunol* 1977;118:1317-21.
- Marone G, Poto S, di Martino L, Condorelli M. Human basophil releasability. I. Age-related changes in basophil releasability. *J ALLERGY CLIN IMMUNOL* 1986;77:377-83.
- Casolaro V, Galeone D, Giacommo A, Sanduzzi A, Melillo G, Marone G. Human basophil/mast cell releasability. V. Functional comparisons of cells obtained from peripheral blood, lung parenchyma, and from bronchoalveolar lavage in asthmatics. *Am Rev Respir Dis* 1989;139:1375-82.
- Nawata Y, Koike T, Yanagisawa T, et al. Anti-IgE autoantibody in patients with bronchial asthma. *Clin Exp Immunol* 1984;58:348-56.
- Quinti I, Brozek C, Wood N, Geha RS, Leung DYM. Circulating IgG autoantibodies to IgE in atopic syndromes. *J ALLERGY CLIN IMMUNOL* 1986;77:586-94.
- Hide M, Francis DM, Grattan CEH, Hakimi J, Kochan JP, Greaves MW. Autoantibodies against the high-affinity IgE receptor as a cause of histamine release in chronic urticaria. *N Engl J Med* 1993;328:1599-640.
- Marone G, Poto S, Petracca R, et al. Activation of human basophils by staphylococcal protein A. I. The role of cyclic AMP, arachidonic acid metabolites, microtubules and microfilaments. *Clin Exp Immunol* 1992;50:661-8.
- Marone G, Tamburini M, Giudizi MG, Biagiotti R, Almerigogna F, Romagnani S. Mechanism of activation of

- human basophils by *Staphylococcus aureus* Cowan I. Infect Immun 1987;55:803-9.
23. Patella V, Casolaro V, Björck L, Marone G. Protein L: a bacterial Ig-binding protein that activates human basophils and mast cells. J Immunol 1990;145:3054-61.
24. Glovsky MM, Hugli TE, Ishizaka T, Lichtenstein LM, Erickson BW. Anaphylatoxin-induced histamine release with human leukocytes: studies of C3a leukocyte binding and histamine release. J Clin Invest 1979;64:804-11.
25. Marone G, Columbo M, Soppelsa L, Condorelli M. The mechanism of basophil histamine release induced by pepstatin A. J Immunol 1984;133:1542-6.
26. Columbo M, Casolaro V, Warner JA, MacGlashan DW Jr, Kagey-Sobotka A, Lichtenstein LM. The mechanism of mediator release from human basophils induced by platelet-activating factor. J Immunol 1990;145:3855-61.
27. Lichtenstein LM. The mechanism of basophil histamine release induced by antigen and by the calcium ionophore A23187. J Immunol 1975;114:1692-9.
28. Schleimer RP, Gillespie E, Lichtenstein LM. Release of histamine from human leukocytes stimulated with the tumor-promoting phorbol esters. I. Characterization of the response. J Immunol 1981;126:570-4.
29. Tung R, Lichtenstein LM. In vitro histamine release from basophils of asthmatic and atopic individuals in D₂O. J Immunol 1982;128:2067-72.
30. Kazimierzczak W, Plaut M, Knauer KA, Meier HL, Lichtenstein LM. Deuterium-oxide-induced histamine release from basophils of allergic subjects. I. Responsiveness to deuterium oxide requires an activation step. Am Rev Respir Dis 1984;129:592-6.
31. Marsh DG, Meyers DA, Bias WB. The epidemiology and genetics of atopic allergy. N Engl J Med 1981;305:1551-9.
32. Bazaral M, Orgel HA, Hamburger RN. Genetics of IgE and allergy: serum IgE levels in twins. J ALLERGY CLIN IMMUNOL 1974;54:288-304.
33. Hopp RJ, Bewtra AK, Watt GD, Nair NM, Townley RG. Genetic analysis of allergic disease in twins. J ALLERGY CLIN IMMUNOL 1984;73:265-70.
34. Marone G, Poto S, Celestino D, Bonini S. Human basophil releasability. III. Genetic control of human basophil releasability. J Immunol 1986;137:3588-92.
35. Parisi P, Gatti M, Prinzi G, Caperna G. Familial incidence of twinning. Nature 1983;304:626-8.
36. Casolaro V, Spadaro G, Marone G. Genetic studies of basophil releasability. In: Marsh DG, Lockhart A, Holgate ST, eds. The genetics of asthma. Oxford: Blackwell Scientific Publications, 1993:291-308.
37. Dvorak AM, Galli SJ, Schulman ES, Lichtenstein LM, Dvorak HF. Basophil and mast cell degranulation: ultrastructural analysis of mechanisms of mediator release. Fed Proc 1983;42:2510-5.
38. Marone G, Cirillo R, Genovese A, Marino O, Quattrin S. Human basophil/mast cell releasability. VII. Heterogeneity of the effect of adenosine on mediator secretion. Life Sci 1989;45:1745-54.
39. Stellato C, Cirillo R, de Paulis A, et al. Human basophil/mast cell releasability. IX. Heterogeneity of the effects of opioids on mediator release. Anesthesiology 1992;77:932-40.
40. Bédard PM, Brunet C, Pelletier G, Hébert J. Increased compound 48/80 induced local histamine release from nonlesional skin of patients with chronic urticaria. J ALLERGY CLIN IMMUNOL 1986;78:1121-5.
41. Greaves MW, Plummer VM, McLaughlan P, Stanworth DR. Serum and cell bound IgE in chronic urticaria. Clin Allergy 1974;4:265-71.
42. Kern F, Lichtenstein LM. Defective histamine release in chronic urticaria. J Clin Invest 1976;57:1369-77.
43. Casolaro V, Cirillo R, Genovese A, Formisano S, Ayala F, Marone G. Human basophil releasability. IV. Changes in basophil releasability in patients with chronic urticaria. J Immunol Res 1989;1:67-73.
44. Marone G, Giugliano R, Lembo G, Ayala F. Human basophil releasability. II. Changes in basophil releasability in patients with atopic dermatitis. J Invest Dermatol 1986;87:19-23.
45. Bull HA, Courtney PF, Bunker CB, Rustin MHA, Pearce FL, Dowd PM. Basophil mediator release in atopic dermatitis. J Invest Dermatol 1993;100:305-9.
46. Czarnetzki BM, Kern F, Lichtenstein LM. Defective release of eosinophil chemotactic factor from peripheral leukocytes in patients with chronic urticaria. J Invest Dermatol 1976;67:276-8.
47. Kimura I, Moritani Y, Tanizaki Y. Basophils in bronchial asthma with reference to reagin-type allergy. Clin Allergy 1973;3:195-202.
48. Liu MC, Hubbard CW, Proud D, et al. Immediate and late inflammatory responses to ragweed antigen challenge of the peripheral airways in allergic asthmatics: cellular, mediator, and permeability changes. Am Rev Respir Dis 1991;144:51-8.
49. Koshino T, Teshima S, Fukushima N, et al. Identification of basophils by immunohistochemistry in the airways of post-mortem cases of fatal asthma. Clin Exp Allergy 1993;23:919-25.
50. Sparrow D, O'Connor GT, Rosner B, Weiss ST. Predictors of longitudinal change in methacholine airway responsiveness among middle-aged and older men: the normative aging study. Am J Respir Crit Care Med 1994;149:376-81.
51. Gaddy JN, Busse WW. Enhanced IgE-dependent basophil histamine release and airway reactivity in asthma. Am Rev Respir Dis 1986;134:969-74.
52. Findlay SR, Lichtenstein LM. Basophil "releasability" in patients with asthma. Am Rev Respir Dis 1980;122:53-9.
53. Busse WW, Swenson CA, Sharp G, Koschat M. Enhanced basophil histamine release to concanavalin A in allergic rhinitis. J ALLERGY CLIN IMMUNOL 1986;78:90-7.
54. Casolaro V, Spadaro G, Marone G. Human basophil releasability. VI. Changes in basophil releasability in patients with allergic rhinitis or bronchial asthma. Am Rev Respir Dis 1990;142:1108-11.
55. Marone G, Casolaro V, Quinti I, Paganelli R, Spadaro G, Stellato C. Mast cell and basophil activation by human anti-IgE autoantibodies. In: Pichler WJ, Stadler BM, Dahinden C, et al. Progress in allergy and clinical immunology. Toronto: Hogrefe & Huber, 1989:125-8.
56. Marone G, Casolaro V, Paganelli R, Quinti I. IgG anti-IgE from atopic dermatitis induces mediator release from basophils and mast cells. J Invest Dermatol 1989;93:246-52.
57. Burrows B, Martinez FD, Halonen M, Barbee RA, Cline MG. Association of asthma with serum IgE levels and skin-test reactivity to allergens. N Engl J Med 1989;320:271-7.
58. Claman HN. Mast cell and fibrosis: the relevance to scleroderma. Rheum Dis Clin North Am 1990;16:141-51.
59. Pearson ME, Huff JC, Giorno RC, Panicheewa S, Claman HN, Steigerwald JC. Immunologic dysfunction in scleroderma: evidence for increased mast cell releasability

- and HLA-DR positivity in the dermis. *Arthritis Rheum* 1988;31:672-7.
60. Seibold JR, Giorno RC, Claman HN. Dermal mast cell degranulation in systemic sclerosis. *Arthritis Rheum* 1990;33:1702-9.
61. Chanez P, Lacoste J-Y, Guillot B, et al. Mast cells' contribution to the fibrosing alveolitis of the scleroderma lung. *Am Rev Resp Dis* 1993;147:1497-502.
62. de Paulis A, Valentini G, Spadaro G, Lupoli S, Tirri G, Marone G. Human basophil releasability. VIII. Increased basophil releasability in patients with scleroderma. *Arthritis Rheum* 1991;34:1289-96.
63. Kaufman LD, Gruber BL, Marchese MJ, Seibold JR. Anti-IgE autoantibodies in systemic sclerosis (scleroderma). *Ann Rheum Dis* 1989;48:201-5.
64. Valent P, Bettelheim P. Cell surface structures on human basophils and mast cells: biochemical and functional characterization. *Adv Immunol* 1992;52:333-423.
65. Columbo M, Horowitz EM, Botana LM, et al. The human recombinant *c-kit* receptor ligand, rhSCF, induces mediator release from human cutaneous mast cells and enhances IgE-dependent mediator release from both skin mast cell and peripheral blood basophils. *J Immunol* 1992;149:599-608.
66. de Paulis A, Ciccarelli A, Cirillo R, de Crescenzo G, Columbo M, Marone G. Modulation of human lung mast cell function by the *c-kit* receptor ligand. *Int Arch Allergy Immunol* 1992;99:326-9.
67. MacGlashan DW Jr, Hubbard WC. IL-3 alters free arachidonic acid generation in C5a-stimulated human basophils. *J Immunol* 1993;151:6358-69.
68. Cirillo R, Triggiani M, Siri L, et al. Cyclosporin A rapidly inhibits mediator release from human basophils presumably by interacting with cyclophilin. *J Immunol* 1990;144:3891-7.
69. de Paulis A, Ciccarelli A, Cirillo R, de Crescenzo G, Columbo M, Marone G. Cytokine-induced modulation of human Fc ϵ RI⁺ inflammatory cells. In: Romagnani S, Mosmann TR, Abbas AK, eds. *New advances on cytokines*. New York: Raven Press, 1992:367-86.
70. Merget RD, Maurer AB, Koch U, et al. Histamine release from basophils after in vivo application of recombinant human interleukin-3 in man. *Int Arch Allergy Appl Immunol* 1990;92:366-74.
71. White MV, Igarashi Y, Emmerly BE, Lotze MT, Kaliner MA. Effects of in vivo administration of interleukin-2 (IL-2) and IL-4, alone and in combination, on ex vivo human basophil histamine release. *Blood* 1992;76:1491-5.

Basophils and eosinophils in allergic rhinitis

Robert M. Naclerio, MD, Fuad M. Barood, MD, Anne Kagey-Sobotka, PhD,
and Lawrence M. Lichtenstein, MD *Baltimore, Md.*

Unequivocal data support the role of the mast cell in the immediate or early-phase anaphylactoid reaction to antigen. Although the contribution of the early reaction to the pathophysiology of allergic rhinitis should not be ignored, there is clear evidence that the early response cannot fully explain the extent of the disease. One of the more important observations that led investigators to this conclusion is that systemic corticosteroids do not inhibit the early response to antigen in the nose, despite having a profound effect on the clinical course of the disease.¹ This discrepancy in the effect of systemic corticosteroids between experimental and clinical disease led to multiple

Abbreviations used

ICAM-1:	Intercellular adhesion molecule-1
IL:	Interleukin
PGD ₂ :	Prostaglandin D ₂
VCAM-1:	Vascular cell adhesion molecule-1

investigations into the inflammatory processes after the early reaction. The central hypothesis behind these efforts was that the infiltration of inflammatory cells into the site of an allergic reaction alters the reactivity of the end organ. In the nose, manifestations of altered reactivity include late-phase reactions, priming, and hyperresponsiveness to nonantigenic stimuli. We briefly describe here some of our observations on the characterization of the cellular changes that occur after nasal provocation with antigen. We will not only focus on changes in basophils and eosinophils in nasal secretions but also include some observations on cellular changes that occur within the nasal mucosa.

From the Departments of Medicine (Division of Clinical Immunology) and Otolaryngology-Head and Neck Surgery, Johns Hopkins University School of Medicine.

Reprint requests: Robert M. Naclerio, MD, Department of Surgery MC 1035, University of Chicago Medical Center, Room E-114, 5841 S. Maryland Ave., Chicago, IL 60637. *J ALLERGY CLIN IMMUNOL* 1994;94:1303-9.

Copyright © 1994 by Mosby-Year Book, Inc.
0091-6749/94/\$3.00 + 0 1/0/59930