

Basophil Histamine Release and Leukotriene Production in Response to Anti-IgE and Anti-IgE Receptor Antibodies

Comparison of Normal Subjects and Patients with Urticaria, Atopic Dermatitis or Bronchial Asthma

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Abstract

The IgE receptor-dependent in vitro mediator release in basophils is characterized by a large interindividual variability both in normal and atopic subjects. The mechanism and the clinical impact of this finding, however, is largely unclear. The aim of the present study was to examine the role of surface-bound IgE and of response-modifying cytokines such as interleukin 3 (IL-3) as possible factors determining basophil releasability in atopic patients and normal controls. Cells from 30 individuals (6 with urticaria, 7 with asthma, 7 with atopic dermatitis, and 30 healthy controls) were isolated and stimulated for mediator release by IL-3 and different triggering antibodies directed against IgE or IgE receptor. Our data suggest that serum IgE levels and basophil receptor occupancy with IgE are not involved in the mechanism of basophil releasability. Furthermore, IL-3-induced similar effects on mediator release in almost all individuals, rather excluding the possibility that releasability is regulated by cytokine priming of basophils. Interestingly, we found that patients with atopic disease have a reduced capacity of releasing mediators upon activation, the mechanism of which is unclear. In conclusion, our findings support the hypothesis that basophil releasability is dependent on cell-immanent mechanisms in basophils, which may be altered in selected atopic patients.

Key Words

Basophils
 IgE receptor
 Interleukin 3
 Asthma
 Urticaria
 Atopic dermatitis

Introduction

The crossing-linking of membrane-bound IgE on basophils and mast cells by allergens or by anti-IgE antibodies triggers for release of mediators such as histamine and de novo synthesized sulfidoleukotriens. This 'anaphylactic reaction' has been supposed to be a key step in the immediate phase of type I hypersensitivities [1, 2]. Several in vitro studies have shown that the spontaneous or allergen-in-

duced mediator release in basophils is altered in patients with atopic disease compared to controls [3–9]. Moreover, a considerable interindividual variability of IgE-dependent mediator release (termed 'releasability') was observed in healthy subjects [3, 10, 11]. Some reports suggested that the extent of the release reaction depends on the number of specific IgE molecules bound to the cell surface which is proportional to the amount of specific IgE circulating in the blood [12]. However, it is well-known that peripheral blood

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IgE levels do not correlate well with the severity of allergic disease. Moreover, an individual variability of mediator release was also observed after basophil triggering with IgE antibodies, rather excluding the possibility that releasability is allergen-dependent [13].

Cell-derived factors other than specific IgE, such as cytokines or chemokines, may also influence the releasability of inflammatory effector cells [2, 14]. Indeed, we could show in previous studies that the growth factors interleukin 3 (IL-3), IL-5, granulocyte-macrophage colony-stimulating factor (GM-CSF) and nerve growth factor strongly modulate basophil mediator release in response to IgE-dependent and IgE-independent triggering agents [15–18]. After a short preincubation, these cytokines prime basophils for an enhanced mediator release upon activation by allergen or anti-IgE antibodies. On the other hand, the releasability could reflect a cell-immanent feature of basophils which may be different between allergic and nonallergic individuals [7, 11]. Thus, releasability may depend on extracellular factors modulating the release by binding to membrane receptors on basophils, or on intracellular factors, which could be genetically determined or acquired during inflammatory processes [11, 19].

The basophil degranulation test has been used for *in vitro* testing of allergic individuals [20, 21]. In this test, unfractionated leukocytes are challenged with different allergens and histamine release is measured in the supernatants. Not only the extent of sensitization but also the *in vivo* priming by IgE-independent factors such as IL-3 can affect the results of the basophil degranulation test. To elucidate the mechanisms of basophil releasability, we examined the stimulatory effect of different anti-IgE and anti-IgE receptor antibodies, and the modulatory activity of IL-3 in an *in vitro* basophil mediator release assay previously established in our laboratory. To compare the release reaction in atopic and nonatopic individuals, we isolated basophils from patients with urticaria, atopic dermatitis, bronchial asthma and from normal controls. In these patients, skin tests and IgE measurements are known to be of limited value for diagnostic and in particular for determining the severity of disease. Therefore, the basophil degranulation test could be of particular interest for both understanding the mechanism of disease and improvement of diagnostic methods.

Materials and Methods

Patients

All participants of the study were volunteers and gave written consent. 20 patients selected from the allergy outpatient clinic of the Inselspital, University of Bern, and 10 healthy individuals were included

in the study. The patients were divided into three groups: (1) 6 patients with urticaria (3 with chronic urticaria defined as an illness lasting for more than 6 weeks, 3 with recurrent acute urticarial lesions), (2) 7 patients with atopic dermatitis (all had typical eczematous lesions on typical localizations, 3 had negative skin prick tests, 4 had positive skin prick tests, 2 of the 4 with positive prick tests had a concomitant rhinoconjunctivitis), (3) 7 patients with bronchial asthma (4 of them had negative skin prick tests, 3 had positive skin prick tests and documented allergic triggering factors). For each patient, a routine diagnosis including history, physical examination, skin prick test, determination of total and specific IgE in serum was performed. Inclusion criteria for the control group were negative skin prick tests, normal serum IgE (<100 U/ml), absence of specific IgE, no seasonal symptoms, normal peripheral blood count and differential count. Medical therapy with anti-allergic or anti-inflammatory drugs was terminated in all patients at least 3 days before the experiments were performed. Further patient characteristics are summarized in table 1.

Cell Isolation

From each patient, 150 ml venous blood anticoagulated with 10 mM EDTA (Fluka, Buchs, Switzerland) was taken and mixed with 0.25 vol of 6% dextran (Pharmacia, Uppsala, Sweden) to allow erythrocytes to sediment at room temperature. As described previously [14–18], leukocytes were pelleted by centrifugation and fractionated by Ficoll-Hypaque (Pharmacia) density centrifugation. The basophil-rich mononuclear cell layer was harvested, washed 3 times in Hepes/albumin buffer [20 mM Hepes (Calbiochem-Behring, La Jolla, Calif., USA), 125 mM NaCl, 5 mM KCl, 0.5 mM glucose, 0.025% bovine serum albumin, fatty acid-free (Boehringer, Mannheim, Germany)] and finally suspended in Hepes/albumin buffer supplemented with 1 mM CaCl_2 and 1 mM MgCl_2 . The cell suspension containing 1–3% basophils was distributed over 2×18 tubes (duplicates).

Cell Stimuli

Cells were triggered with four different antibodies directed against IgE (mAb LE 27 [22], polyclonal sheep antiserum from Serotec, lot A5158) or IgE receptor (mAb 29C6 and mAb 6F7 against the α -chain of the high-affinity IgE receptor; kindly provided by Hoffmann-La Roche, Nutley, N.J., USA [23]). Each antibody was used at a suboptimal and an optimal concentration as determined in previous studies in normal individuals [13, 16, 24]. The characteristics of the antibodies are summarized in table 2. Recombinant human IL-3 (Sandoz Pharmaceuticals, Basel, Switzerland) was always used at a maximally effective concentration of 10 ng/ml [15, 16].

Mediator Release Assay

Cell stimulation was performed in a shaking water bath at 37°C exactly as described [15, 16]. After a warming-up period of 10 min, buffer or IL-3 was added. 10 min later, buffer or antibodies were added. The reaction was stopped by placing the tubes in ice-cold water. 20 min after addition of the triggering antibodies, a time sufficient for a complete release reaction as determined in previous studies. Histamine release was measured fluorimetrically [25], sulfidoleukotrienes were determined by radioimmunoassay [18]. Histamine data are generally expressed as percent of total cellular histamine content before stimulation minus the spontaneous release (= 'specific histamine release' except in fig. 1a), and leukotriene release is shown as picograms of leukotrienes per nanograms of total cellular histamine in order to correct for different basophil numbers.

Table 1. Clinical data

	Urticaria	Dermatitis	Asthma	Normals
Patients	6	7	7	10
Male/female	3/3	3/4	4/3	6/4
Age ^a , in years	31 (20–43)	27 (19–39)	37 (16–61)	28 (25–43)
Other chronic diseases	0	0	4 ^b	0
Smokers	0	3	1	3
Tonsillectomy	2	3	3	2
Family: atopy ^c , % of patients	67	86	57	20
Symptoms				
Rhinitis/conjunctivitis	2	2	5	1
Lower airways symptoms	1	2	7	0
Atopic dermatitis/eczema	0	7	5	1
Food intolerance	4	3	1	0
Drug allergy	1	0	0	0
Perennial symptoms	5	4	4	0
Duration				
<1 year	2	1	0	0
1–5 years	4	2	3	2
>5 years	0	4	3	0
Allergens ^d				
No sensibility	3	3	4	10
Pollens	2	3	3	0
House dust	0	1	1	0
Moister	0	1	0	0
Food	3	2	1	0
Animals	0	1	1	0
Others	1 ^e	0	0	0
RAST-negative	5	5	4	10
Therapy				
No therapy	0	2	1	0
β_2 -Mimetics	0	1	6	0
Antihistamines	4	3	1	0
Cromoglycate	1	0	2	0
Steroids, local	2	3	2	0
Steroids, oral	2	1	0	0
Immunotherapy	0	0	1	0

^a Values represent mean with the range in parentheses.
^b 1 $\times$ elevated blood pressure, 3 $\times$ chronic obstructive lung disease.
^c 'Atopy' = presence of asthma or atopic eczema in at least one of the first grade relatives of the patients.
^d History and skin test or specific IgE-positive.
^e Fly.

Statistical Analysis

Each experiment was performed in duplicates. Data are shown as means ($\pm$ standard error) if not indicated otherwise. Mean values of groups were compared by one-way analysis of variance (ANOVA). Paired data were analyzed using the two-tailed paired *t* test. Correlation analyses were performed by calculating the Spearman correlation coefficient *r* and the rank correlation probability *p*.

Results

Histamine Content of the Blood

For each experiment, the number of isolated mononuclear cells was counted, and cellular histamine content was determined after cell lysis [15, 16]. No significant differences between the patient groups and controls were found concerning the number of isolated mononuclear cells per milli-

Table 2. Triggering antibodies

	Antiserum	LE 27	29C6	6F7
Specificity	polyclonal	monoclonal	monoclonal	monoclonal
Origin	sheep	mouse	mouse	mouse
Subclass	—	IgG1	IgG1	IgG1
Affinity (K_D , nM)	—	1.9	3.2	3.2
Binding site	IgE (F_c portion)	IgE (F_c portion)	IgE receptor ($F_{c\epsilon}RI$, α -chain)	IgE receptor ($F_{c\epsilon}RI$, α -chain, IgE-binding epitope)
Characteristics	requires cell-bound IgE	requires cell-bound IgE	binds to all IgE receptors	cross-competes with IgE
Suboptimal concentration ^a	dilution 1:10 ⁴	30	10	10
Optimal concentration ^a	dilution 1:10 ³	300	100	100
References	(Serotec)	22	23, 24	23, 24

^a Values of antibodies represent ng/ml.

liter blood, the cellular histamine content, and the calculated amount of cellular histamine in blood (table 3). It should be noted that, compared to the *in vivo* situation, the calculated amount of cellular histamine per milliliter blood shown in table 3 is most likely lower, because approximately 40% basophils are lost during the cell preparation procedure [17, 18]. Provided that the histamine content per basophil is similar in different individuals (approximately 1 pg/cell), the number of basophils did not differ between the groups (49–72 basophils/ml blood).

'Spontaneous' Histamine Release

Previous publications reported that the spontaneous release of histamine was increased in patients with allergic disease [20, 21]. In our study, we found no significant differences between the spontaneous release in patients with allergic diseases and healthy controls (fig. 1a). This discrepancy may be due to the different kinds of disease examined in the studies and, as shown by Weyer et al. [26], the results may depend on the season in which the studies were performed [27].

Histamine Release by IL-3

In previous studies, we showed that IL-3 by itself induces no or only a marginal histamine release in basophils, but strongly enhances the IgE-dependent release [15, 16]. However, some other studies showed that IL-3 alone is capable of triggering for histamine release at least in some selected individuals [28]. Therefore, we measured basophil his-

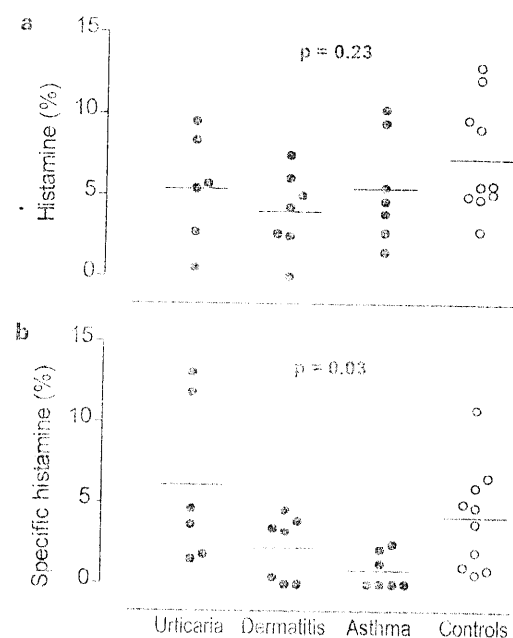


Fig. 1. Spontaneous (a) and IL-3-induced (b) histamine release in peripheral blood mononuclear cells. After a warming-up period of 10 min, cells from patients with urticaria ($n = 6$), atopic dermatitis ($n = 7$), asthma ($n = 7$) and normal controls ($n = 10$) were incubated at 37°C in buffer, or were challenged with IL-3 at 10 ng/ml for 30 min. Histamine was then measured in supernatants and expressed as % of total cellular histamine (a) or as % specific release (= % release minus % spontaneous release, (b)). Each point represents the mean of duplicate determinations in one individual, the bars indicate mean values for the groups. The means were compared by analysis of variance.

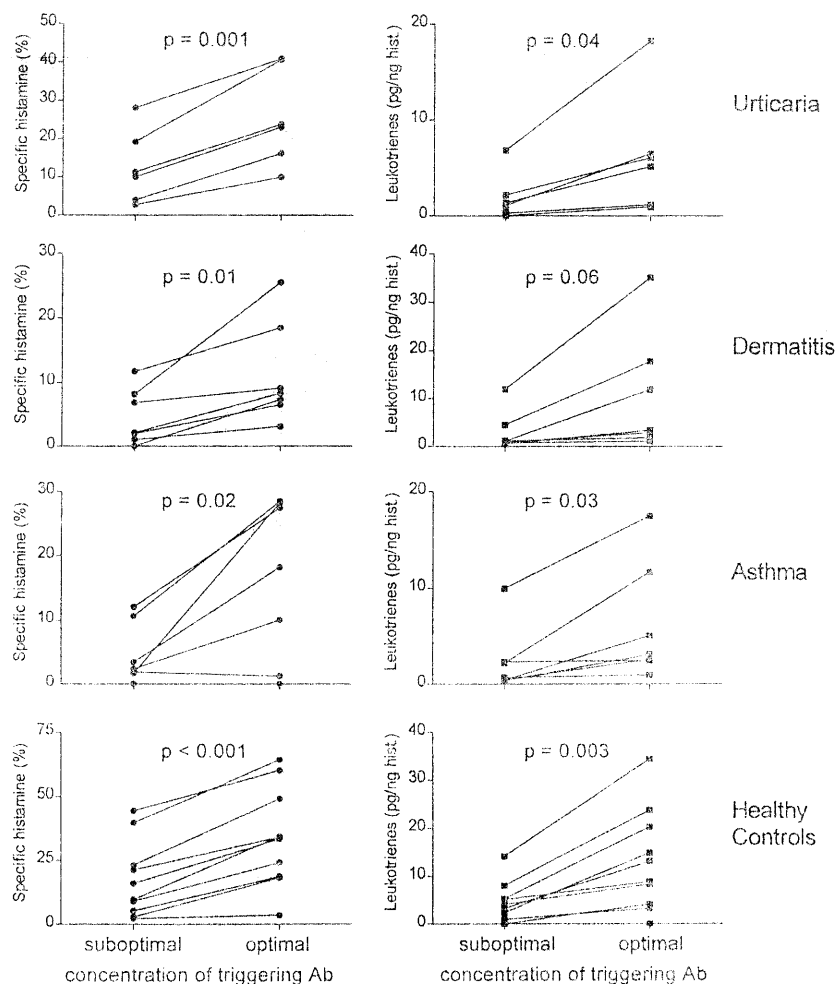


Fig. 2. Histamine and sulfidoleukotriene release in response to mAb 29C6. After a warming-up period of 20 min cells were stimulated with an suboptimal (10 ng/ml) and optimal (100 ng/ml) concentration of the antibody for 20 min. The results were compared by the two-tailed paired t test. Patient groups as in figure 1; the data from the same patient are connected by lines. Analogous results were obtained in response to other triggering antibodies [mAb 6F7, mAb 1E27, polyclonal antiserum; data not shown].

mine release after challenge with 10 ng/ml of IL-3 without addition of further agonists (fig. 1b). In most individuals, IL-3 caused a weak histamine release slightly higher than the buffer control. However, leukotriene synthesis in response to IL-3 alone could not be detected. The lowest IL-3-induced histamine release was found in patients with bronchial asthma. In 2 of the patients with urticaria, but none of the other patient groups, the IL-3-induced histamine release was $> 10\%$ of total cellular histamine. Interestingly, both of them had acute urticarial lesions and were the only ones of the urticaria group who had an additional history of seasonal rhinoconjunctivitis and other extrinsic allergies.

Concentration Dependency of Mediator Release

All cell preparations were challenged with at least two different concentrations of antibodies, a suboptimal and a

maximally effective concentration as determined in previous studies. In all patients and in healthy controls, the release reaction induced by antibodies occurred in a concentration-dependent fashion (fig. 2). The absolute amount of released mediators was highly variable within the experiments performed with cell preparations from different individuals, whereas the relative and absolute differences between the effect by the two antibody concentrations were similar in almost all individuals. In a few patients with weak responses even to the high antibody concentrations, the concentrations were enhanced by one order of magnitude. Interestingly, this enhancement failed to cause a further increase of mediator release suggesting that the concentrations assumed to be optimal for mediator release were also the maximally effective ones in atopic 'low responders' [data not shown].

Table 3. Histamine content of the blood

	Urticaria	Dermatitis	Asthma	Normals
10^6 MNC/ml blood	1.43 ± 0.51	1.31 ± 0.30	1.45 ± 0.20	1.30 ± 0.36
Histamine/ 10^6 MNC, ng	18.9 ± 13.4	15.7 ± 6.4	19.8 ± 7.4	17.0 ± 6.3
Histamine/ml blood, ng	23.8 ± 10.8	19.5 ± 6.91	28.8 ± 11.6	21.3 ± 8.67
Total IgE in serum, ng/ml	196 ± 166	878 ± 1149	159 ± 252	82 ± 81

Values represent mean $\pm$ SD. MNC = Mononuclear cells.

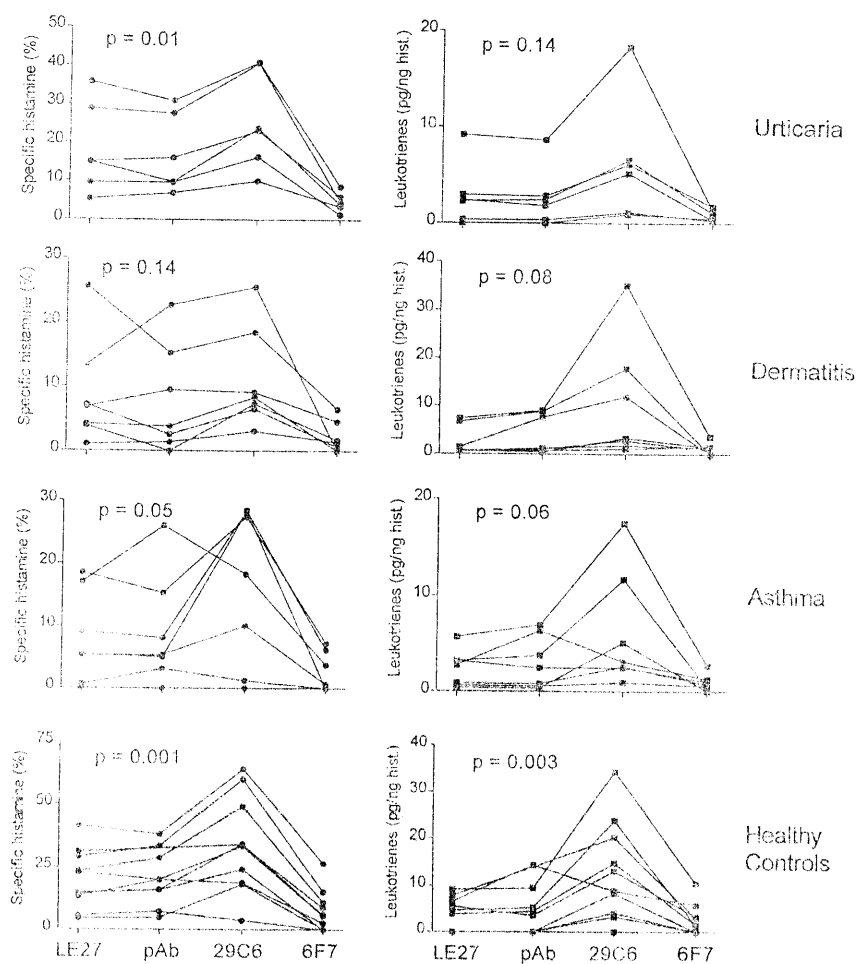


Fig. 3. Mediator release in response to four different triggering antibodies. Antibody characteristics are summarized in table 2. Cells were stimulated for 20 min with maximally effective concentrations of the antibodies, and histamine and leukotrienes were measured as described. The data from the same patient are connected by lines. Similar results were obtained with cell preparations pretreated with IL-3 [data not shown]. The effect of the different antibodies was compared by analysis of variance.

Comparison of Different Triggering Antibodies

The mAb 29C6 directed against the IgE receptor, which triggers basophils independently of whether surface-bound IgE is present or not, was the most efficient triggering antibody in almost all individuals. In comparison, the mAb 6F7, which binds only to IgE-free IgE receptors, induced a weak release reaction both in normal and atopic individuals. The

efficacy of the anti-IgE antibodies (mAb LE27, pAb) was higher than that of mAb 6F7, but clearly lower than that of mAb 29C6. The pattern of response towards the four different triggering antibodies was similar in atopic patients and healthy controls. The differences in efficacy of the antibodies was more pronounced with regard to leukotriene production than to histamine release (fig. 3).

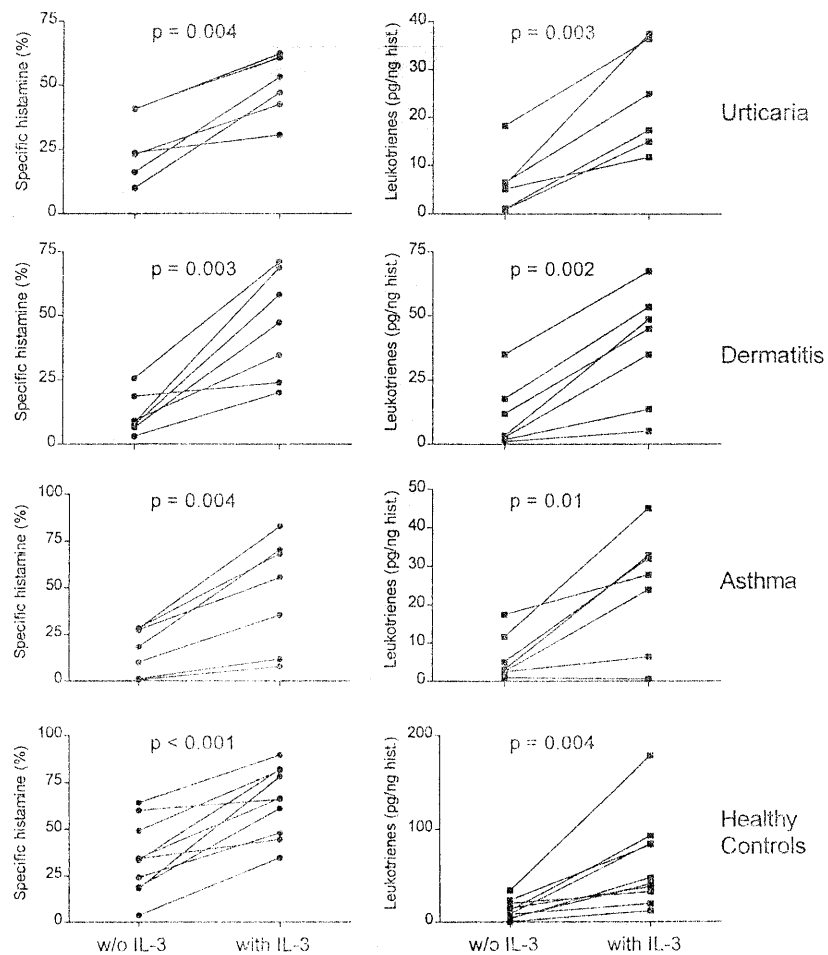


Fig. 4. Effect of IL-3 on basophil mediator release. After a warming-up of 10 min, cells were preincubated in buffer (w/o IL-3) or with 10 ng/ml of IL-3 (with IL-3) for 10 min, and then stimulated with mAb 29C6 at 100 ng/ml. The data from the same patient are connected by lines. Statistical analysis by the two-tailed paired t test. The enhancing effect of IL-3 were observed independently of which antibody was used as trigger.

Effect of IL-3 on IgE Receptor-Dependent Mediator Release

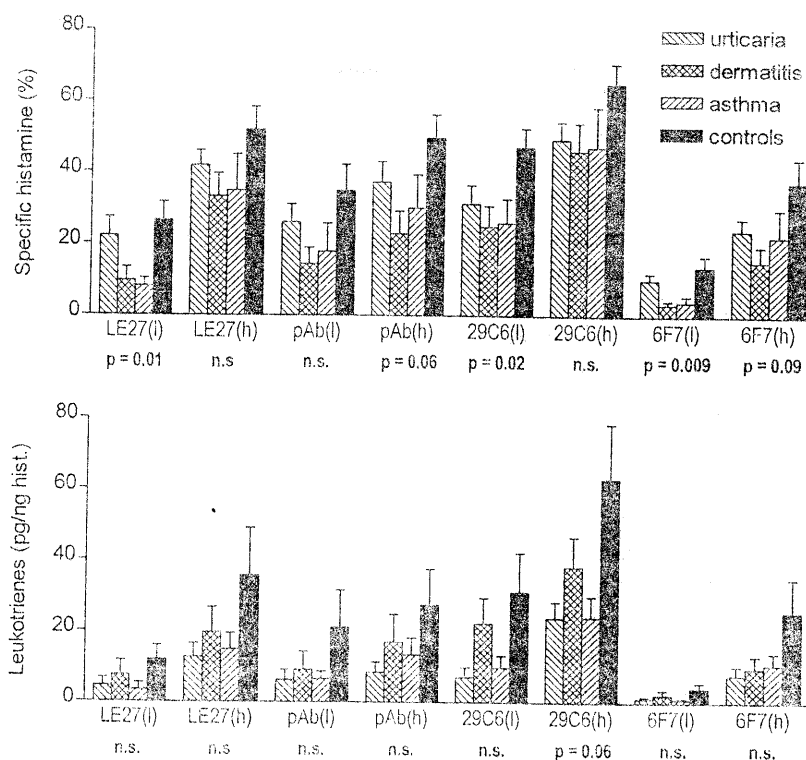
As shown in previous studies performed in nonatopic individuals, a short preincubation of the cells with IL-3 strongly enhanced the release reaction induced by suboptimal [data not shown] and maximally effective concentrations of triggering antibodies (fig. 4). In some individuals, IL-3 rendered basophils responsive to triggering antibodies which were otherwise almost ineffective. An enhancement of mediator release by IL-3 was observed both in normal and atopic individuals. The extent of the IL-3-induced enhancement was dependent on releasability to the triggering agents rather than on disease.

Comparison of Mediator Release in Different Patient Groups

Figures 2-4 show that the mean histamine and leukotriene release in response to different stimulation protocols

varied between the patient groups and normal controls. For a more detailed comparison of the mediator release in healthy and atopic subjects, we put together the mean reactions in response to all triggering agents tested in our experiments (fig. 5). The release reactions were generally less pronounced in atopic patients than in controls. The lowest histamine release was observed in patients with atopic dermatitis and bronchial asthma, respectively, whereas leukotriene production was the lowest in patients with urticaria or bronchial asthma. The number of patients within the different groups was too small to draw a final conclusion, but despite the limited numbers of patients and the marked interindividual variability of the results, the differences in mediator release reached statistical significance for some stimulation protocols.

Fig. 5. Mean ($\pm$ SE) histamine release and sulfidoleukotriene production in response to suboptimal (= low, l) and optimal (= high, h) concentrations of four different triggering antibodies. All cells were preincubated with IL-3, but a similar pattern of responses was observed without IL-3 pretreatment [data not shown]. The responses towards each antibody were separately compared between the four groups of individuals by one-way analysis of variance. No significance (n.s.) was assumed if p was >0.10 .



Correlations

Table 4 shows that the release reaction in patients was not dependent on the total serum IgE levels. However, highly significant correlations were found between the histamine release [and leukotriene release, data not shown] induced by different antibodies. Furthermore, the release reactions in unprimed and IL-3-primed basophils were highly correlated (all $p < 0.001$). Finally, a significant correlation was found between histamine and leukotriene release induced by the same triggering antibodies independent of whether the cells were primed with IL-3 or not. The correlation was significant in the whole group of atopic and nonatopic individuals, in the subgroups of patients with bronchial asthma and healthy controls, but not in the groups of patients with urticaria and atopic dermatitis [data not shown].

Discussion

The *in vitro* diagnosis of allergic diseases is mainly based on skin tests and measurement of total IgE and specific IgE in serum. However, it is well-known that these diagnostic means do not well correlate with the occurrence and

severity of allergic disease. Particularly in patients with urticaria, asthma and atopic dermatitis, the correlation between disease and diagnostic means based on IgE-dependent mechanisms is known to be weak, and therefore the diagnosis depends on typical clinical findings. The mechanisms of these forms of atopic disease are largely unclear, and there is some evidence that they are not or not only based on type I hypersensitivity reactions. Therefore, we examined selected individuals suffering from such chronic allergic disease.

Recent studies provided some new insight into the mechanisms underlying allergic diseases. There is increasing evidence that, apart from IgE and mast cells, which are the classical components of immediate allergic reactions, other cell types and molecules such as basophils, eosinophils, anaphylatoxins, chemokines and cytokines are involved [1, 2, 14]. This is particularly true for the late phase of allergic reactions, which may be more important for the clinical outcome of disease than immediate reactions [1, 2]. In addition, still unidentified cell-inherent factors determining the 'releasability' in inflammatory effector cells may be altered in allergic patients [2, 3]. The concept and study of 'releasability' was originated by Lichtenstein and colleagues in the

1970s. During that time, a number of publications appeared showing that histamine release is neither correlated to the number of IgE antibodies bound to the basophil surface nor to serum IgE levels [3-11]. However, it has been suggested that basophil releasability is age-dependent and controlled by genetic factors [10, 11]. Based on these studies it is tempting to speculate that the releasability of effector cells such as basophils, eosinophils or mast cells, which predominate at sites of allergic inflammation, may contribute to the abnormalities found in allergic patients.

In previous studies on releasability basophils were often chosen as target cells, since they are easily available from peripheral blood and can be used for studying mediator release without further purification. Basophils have been stimulated by allergens in passively sensitized cells or by anti-IgE antibodies [2-9]. The cross-linking of surface-bound IgE leads to the release of inflammatory mediators such as preformed histamine or de novo synthesized leukotrienes. Mediator release in response to anti-IgE antibodies or allergen is dependent on surface-bound IgE. It is known that many allergic patients have elevated serum IgE levels, increased numbers of IgE receptors on the surface of basophils, and thus a higher rate of cell-bound IgE [12]. It has been shown that anti-IgE-induced histamine release correlates with cellular IgE receptor density, but due to the variability of the results derived from these studies, it is tempting to speculate that IgE-dependent mediator release is also regulated by other yet unknown factors [7]. Therefore, we examined a triggering agent, which activates basophils through the IgE receptor but which is at the same time independent of surface-bound IgE. The use of anti-IgE and anti-IgE receptor antibodies allows the discrimination between IgE-dependent and IgE-independent activation of basophils through the IgE receptor.

The present data confirm previous findings showing that basophil mediator release is subjected to a large interindividual variability [3-9]. As reported by Conroy et al. [12], the release reaction was not correlated with total serum IgE levels. We found a consistent relation between the response towards anti-IgE and anti-IgE receptor in all individuals indicating that basophil releasability is independent of the level of serum IgE and the ratio of IgE-free and occupied IgE receptors. This hypothesis is additionally supported by the observation that both the mAb LE27, which requires surface-bound IgE for stimulation, and the mAb 6F7, which can only bind to IgE-free IgE receptors, were capable of inducing mediator release in almost all individuals. Finally, the interindividual differences in basophil releasability occurred independently of which antibodies were used as triggering agent. Our data also show that basophil releasability is not

Table 4. Correlations

	HR by pAb sheep	HR by mAb LE27	HR by mAb 29C6	HR by mAb 6F7
IgE, U/ml	0.12	0.26	0.07	0.07
HR by mAb LE27	<u>0.92</u>			
HR by mAb 29C6	<u>0.87</u>	<u>0.86</u>		
HR by mAb 6F7	<u>0.75</u>	<u>0.81</u>	<u>0.82</u>	
HR by IL-3 + pAb	<u>0.90</u>			
HR by IL-3 + LE27		<u>0.74</u>		
HR by IL-3 + 29C6			<u>0.66</u>	
HR by IL-3 + 6F7				<u>0.75</u>
sLT by pAb sheep	0.41			
sLT by mAb LE27		<u>0.51</u>		
sLT by mAb 29C6			<u>0.54</u>	
sLT by mAb 6F7				<u>0.57</u>
sLT by IL-3 + pAb	<u>0.65</u>			
sLT by IL-3 + LE27		<u>0.66</u>		
sLT by IL-3 + 29C6			<u>0.63</u>	
sLT by IL-3 + 6F7				<u>0.81</u>

The Spearman rank correlation coefficient is shown; significant correlations are underlined ($p < 0.001$). HR = Histamine release; sLT = sulfidoleukotrienes.

due to a shift of the dose response curves of the agonists, because a further increase of the antibody concentrations determined to be maximally effective failed to augment mediator release in patients with weak release reactions. These observations suggest that the releasability is most likely due to cell-immanent changes induced by in vivo priming of basophils or by a genetically altered capacity of the cells to release mediators upon activation. Human basophils are primed for enhanced mediator release by a particular set of hematopoietic growth factors including IL-3, IL-5 and GM-CSF [15-17]. Therefore, we examined the effect of IL-3 on IgE receptor-dependent mediator release. Preincubation of the cells with IL-3 caused an enhancement of mediator release in atopic and nonatopic individuals. The enhancement by IL-3 was similar in all patient groups and normal controls, which rather excludes the fact that cytokine priming is the mechanism of basophil releasability. This hypothesis is confirmed by our finding that the release reaction in IL-3-primed and unprimed basophils triggered with any antibodies was highly correlated. Moreover, the responses towards the different triggering antibodies were correlated, which is a further indication for a cell-immanent mechanism of basophil releasability.

Since the interindividual variability of *in vitro* basophil mediator release was observed within all patient groups and normal controls, the comparison of the release reaction between the different groups of individuals is rather difficult and requires large populations. Nevertheless, our data indicate that the mean mediator release differs between patient groups and controls. Despite the limited number of experiments and the rather high standard errors of the means, the differences could be consistently observed and reached statistical significance under some experimental conditions. A particularly low histamine release was observed in patients with atopic dermatitis and bronchial asthma, respectively, whereas in patients with urticaria the release was only slightly lower compared to controls. The results were somewhat different regarding leukotriene production, which was reduced in all patient groups, particularly in patients with urticaria and atopic dermatitis.

These findings confirm previous studies showing that basophils isolated from patients with chronic urticaria release less histamine than those from nonallergic controls when stimulated with anti-IgE antibody [3]. For asthma patients, the results are rather conflicting: some reported an enhanced anti-IgE-induced histamine release in bronchoalveolar cells [6–9], whereas others found no differences in peripheral blood basophil releasability [9] except in basophils from steroid-dependent asthmatics, which released a smaller amount of mediators upon stimulation than control subjects [29]. In patients with atopic dermatitis, however, the sensitivity towards anti-IgE antibodies was found to be increased [5], an observation which cannot be confirmed by our study. Such discrepancies are unclear, but may be due to different cell isolation procedures, different mediator release assays or differences in the age of the patients, a factor which has been reported to be related to basophil releasability [10].

Sulfidoleukotrienes have rarely been measured in studies on basophil releasability. However, leukotrienes may play a particular important role in the pathogenesis of allergic inflammation, since they are released during late phase reactions [2]. Previous studies showed that they are approximately 100 times more potent on a molar basis compared to histamine in inducing weal and flare reactions [30]. It has been described that the donor variability of leukotriene release is even more pronounced than that of histamine release [31]. Basophils release only low levels of leukotrienes in response to most agonists, but the release reaction can be strongly enhanced by pretreatment of the cells with growth factors such as IL-3 [14–18]. Despite the fact that many basophil triggers including IgE receptor-dependent activation induce both the release of histamine and leu-

kotrienes, the requirements for degranulation and *de novo* synthesis of lipid mediators may be different. Some studies indicated that different intracellular signal transducing pathways are involved [32]. For example, the kinetics are different for histamine and leukotriene release [16]. Furthermore, particular basophil agonists such as C5a or monocyte-chemotactic protein-1 induce histamine release but no leukotriene synthesis [14, 15]. Finally, our previous studies showed that the IL-3-dependent enhancement of leukotriene production is more pronounced than the enhancement of histamine release [14–18]. Our observations raise the question of whether deregulation of histamine release and leukotriene production, respectively, are distinct features of atopic and related diseases. Similar findings could be made in patients with intolerance to salicylates. In these patients, the *in vitro* release of leukotrienes by basophils stimulated with C5a is enhanced, but the release of histamine is unchanged compared to controls [33]. Further studies are required to determine whether such selective alterations of mediator release can also be defined for atopic diseases.

In conclusion, the present study confirms the concept of 'basophil releasability' as an important factor in the pathogenesis of allergic disease. However, the mechanisms of basophil releasability are still unclear. Based on our and previous findings, two hypotheses which are not mutually exclusive may be envisaged to explain the mechanism of a modified basophil releasability in atopic patients: (1) an alteration of the IgE-receptor-signal-transducing pathway in atopic patients, and (2) the induction of a yet unidentified agent which is capable of modulating the IgE receptor-dependent release in atopies. Hematopoietic growth factors [2], nerve growth factor [18], chemokines [14] and anti-IgE autoantibodies [34] would be candidates for such an agent-modulating basophil function.

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