

Patients with severe atopic dermatitis have activated circulating basophils

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Background: The purpose of this study was to define the potential role of the basophil in the pathogenesis of atopic dermatitis by comparing basophil releasability in patients with food allergy and either mild or severe atopic dermatitis.

Methods: A clinical scoring system was used to differentiate patients into either a low clinical score (LCS) group, 10 patients, or a high clinical score (HCS) group, 14 patients. Basophil histamine release was measured with a radioenzymatic assay, and leukotriene C₄ (LTC₄) was determined with a radioimmunoassay. Histamine and LTC₄ released from basophils spontaneously or after incubation with deuterium oxide, an IgE-dependent histamine-releasing factor, anti-IgE, and C5a were measured.

Results: Basophils from the HCS group released more histamine than those from the LCS group when stimulated with deuterium oxide (median percent total release: 85% vs 68%, $p < 0.05$), with 10 and 100 ng/ml of anti-IgE (58% vs 26% and 62.5% vs 37%, both $p < 0.05$) and 5 and 50 ng/ml of C5a (50.5% vs 11.5, $p < 0.05$; 49% vs 11%, $p < 0.01$, respectively). The spontaneous release of LTC₄ from basophils was greater in the HCS group (median release: 34 vs 3.5 pg/0.1 ml, $p < 0.05$). Basophils stimulated with deuterium oxide, with 100 ng/ml anti-IgE, and 50 ng/ml C5a released more LTC₄ in the HCS group (111.5 vs 39 pg/0.1 ml, $p < 0.05$; 64.5 vs 10 pg/0.1 ml, $p < 0.01$; 80 vs 1 pg/0.1 ml, $p < 0.05$, respectively).

Conclusions: These data indicate that patients with more severe atopic dermatitis have "activated" circulating basophils in vivo and increased basophil releasability, in particular in response to C5a. These studies may serve as markers for more severe atopic dermatitis. (*J ALLERGY CLIN IMMUNOL* 1993;91:1155-62.)

Key words: Atopic dermatitis, food hypersensitivity, basophil releasability, histamine, leukotriene C₄, D₂O, histamine-releasing factor, anti-IgE, C5a, basophil activation

Atopic dermatitis is an inflammatory skin disease characterized by extreme pruritus, a typical distribution and a long-term relapsing course.^{1, 2} Onset in infancy is common, with frequent improvement in symptoms with increasing age. However, persistent symptoms may be experienced by

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Abbreviations used:

LCS:	Low clinical score group
HCS:	High clinical score group
BHR:	Basophil histamine release
LTC ₄ :	Leukotriene C ₄
D ₂ O:	Deuterium oxide (heavy water)
HEPES-ACM:	Hepes-buffered saline solution that contained albumin, 2 mmol/L calcium, and 1 mmol/L magnesium

teenagers and adults.³ Approximately 10% to 15% of the general pediatric population is affected with this disorder, which has a strong familial predisposition. The diagnosis depends on the identification of a constellation of symptoms in the context of established, standardized criteria.^{4, 5} IgE-mediated hypersensitivity mechanisms have been implicated in the pathogenesis of this disorder, but the specific underlying defect re-

mains unknown.⁶ The factors that determine why some patients clearly have more severe skin involvement and experience more prolonged courses are also poorly defined.

Our laboratory previously reported that high spontaneous *in vitro* basophil histamine release (BHR) was present in a group of patients with atopic dermatitis and food hypersensitivity.⁷ These high rates of histamine release were shown to depend on continued ingestion of offending food allergens identified in double-blind, placebo-controlled food challenges. Normalization of this increased histamine release could be accomplished with institution of an appropriate food allergen-elimination diet. In addition, the production of a histamine-releasing factor *in vitro* was demonstrated in these patients with high spontaneous BHR. This increased releasability of basophils may contribute to the degree of severity of cutaneous symptoms in certain patients with atopic dermatitis and may be helpful in the clinical identification of those patients who are prone to have more severe, recalcitrant atopic dermatitis.

The purpose of the present study was to determine whether there were any measurable differences in the releasability of basophils from patients with food allergy and either mild or severe atopic dermatitis. We used a variety of standard secretagogues⁸⁻¹² to characterize different patterns of basophil activation in relation to the clinical severity of atopic dermatitis and to determine whether there is an identifiable pattern of basophil releasability that is characteristic of those patients with more active clinical disease. The preformed mediator, histamine, and the newly generated sulfidopeptide leukotriene mediator, leukotriene C₄ (LTC₄), were analyzed because these are two of the main mediators of clinical significance released by activated basophils.¹³

METHODS

Subjects

Twenty-four patients with histories of food hypersensitivity and atopic dermatitis were enrolled in the study. These patients were part of a larger group admitted to the Pediatric Clinical Research Unit for evaluation of the role of food hypersensitivity in atopic dermatitis. All patients fulfilled the criteria of Hanifin and Rajka⁴ for the diagnosis of atopic dermatitis. A standardized quarterly symptom score sheet (Table I) that reviews extent and course of the rash, intensity of scratching, medication use, and disruption of daily activities was used; the highest possible score is 30. Scores greater

than 10 defined the high clinical score (HCS) group, and scores less than 10 defined the low clinical score (LCS) group. The percentage of total body involvement with atopic dermatitis was recorded with the use of a modified burn chart. Antihistamines were withheld for 2 weeks before subjects entered the study, but patients were allowed to continue using topical corticosteroids for control of their atopic dermatitis. Five nonatopic children without atopic dermatitis or food hypersensitivity and six children with mild atopic dermatitis but no accompanying food allergy were also evaluated in our unit and had BHR studies performed. Both of these groups served as controls for basophil releasability in the LCS and HCS patients. Informed consent was obtained for each subject, and all studies were approved by the Johns Hopkins University Joint Committee on Clinical Investigation.

Preparation of peripheral blood basophils

Venous blood was collected from all patients in sterile plastic syringes that contained ethylenediaminetetraacetic acid (pH 7.2) at a final concentration of 25 mmol/L. The anticoagulated samples were added to detergent-free polycarbonate tubes that contained 6% Dextran 70 (Pharmacia, Uppsala, Sweden). After the erythrocytes were sedimented for 60 to 90 minutes at room temperature, the leukocyte-containing plasma was removed and centrifuged at 300g for 10 minutes at 4° C. The cell pellet was washed three times in cold Hepes-buffered saline solution (0.01 mol/L, pH 7.4) that contained 0.3 mg of human serum albumin per milliliter. The final cell pellet was resuspended and adjusted to 10 to 15 × 10⁶ cells per milliliter in Hepes-buffered saline solution that contained albumin, 2 mmol/L calcium, and 1 mmol/L magnesium (HEPES-ACM) for use in the histamine and LTC₄ release assays.

Leukocyte histamine and LTC₄ release assays

The supernatant histamine content that resulted from the release assays was determined with a modification of a sensitive radioenzymatic assay as previously described.^{7, 14} Purified histamine methyltransferase used in this assay provides highly reproducible results within the range of 30 pg to 10 ng per milliliter. The percent total release of histamine, either spontaneously or induced by various stimuli described below, was calculated by dividing the mean of duplicate BHR values by the mean of duplicate basophil total histamine content. LTC₄ release was measured by directly assaying 0.1 ml supernatant samples from duplicate basophil suspensions with a radioimmunoassay sensitive to 3 pg/0.1 ml.¹⁵ Basophils were counted with an alcian blue staining method.¹⁶ Leukocyte suspensions, 50 µl, which contained approximately 1.5 to 2 × 10⁴ basophils were incubated in duplicate in polystyrene tubes that con-

TABLE 1. Quarterly symptom score sheet for patients with atopic dermatitis

Score	Symptom
	Extension
0	Absent
2	Not more than 2 predilection sites involved
4	Involvement of more than 2 but less than 6 sites
6	Dermatitis > 25% involvement
	Course
0	Absent
1	More than 2 months remissions
2	Remissions less than 2 months
3	Continuous course
	Scratching: Intensity-day pattern
0	Absent
2	Scratches when tired/anxious; easily distracted
4	Evaluated as greater than 2 but less than 6
6	Almost constant scratching to point of excoriation; school and play concentration impossible
	Scratching: Intensity-night pattern
0	Absent—sleeps through night without attention
2	Occasionally awakens scratching but back to sleep with minimal attention
4	Awakens scratching 1-2 times each night
6	Requires frequent rubbing during the night to retard scratching; sleeps very little
	Antihistamine use
0	None or occasional use as needed
1	Daily bedtime use
2	Daily bedtime use plus 1-2 daytime doses usually
3	Daily bedtime use plus daytime usage
	Antibiotic/prednisone use
0	None
1	1 course with noninflamed periods
2	2 courses with noninflamed periods
3	3 or more courses or no clearing
	School missed or parent's missing work
0	None
1	Average 1 day missed per quarter
2	Average 2 days missed per quarter
3	Average 3 or more days missed per quarter

Total possible score = 30; Total > 10 defined HCS group.

tained 50 μ l of prewarmed HEPES-ACM. All basophil suspensions were run in duplicate. Mean total histamine content was determined by boiling duplicate cell suspensions for 10 minutes and then measuring supernatant histamine. Spontaneous histamine and LTC₄ release from basophils was measured by incubating similar duplicate leukocyte suspensions for 45 minutes at 37° C, quantitating supernatant histamine as a percent of total cell content, or determining LTC₄ content in the supernatants. Histamine and LTC₄ release induced by a histamine-releasing factor, isolated from combined pools of nasal washes from allergic and nonallergic donors (supplied by Susan MacDonald, MD, Johns Hopkins University, Baltimore, Md.), was measured by addition of 50 μ l of the histamine-releasing factor to 50 μ l of the leukocyte preparation and incubation for 45 minutes at 37° C. Release of hista-

mine and LTC₄ were also determined after addition of 50 μ l of the leukocyte preparation to an equal volume of the following reagents and incubation for 45 minutes at 37° C: D₂O (88%), (Sigma, St. Louis, Mo.), anti-human IgE (kindly provided by N. Franklin Adkinson, MD, Johns Hopkins University) at concentrations of 1 ng/ml, 10 ng/ml, 100 ng/ml, and 1 μ g/ml; and C5a (provided by Henry Showell, Pfizer, Groton, Conn.) at both concentrations of 5 ng/ml and 50 ng/ml. After incubation, all of the cell suspensions were centrifuged, and the supernatants were removed and stored at -70° C until histamine and LTC₄ determinations were performed.

Statistical analysis

All of the clinical data were managed and analyzed with CLINFO provided by the General Clinical Re-

TABLE II. Clinical characteristics of atopic dermatitis patient groups

	LCS Group (n = 10)	HCS Group (n = 14)
Age (yr)	8 (1.5-21)	8 (2.5-20)
Sex	5-F 5-M	6-F 8-M
Onset of eczema (mo)	6 (7 d-24 mo)	2 (7 d-48 mo)
Clinical score	5 (0-10)	21 (10-28)
Body rash (%)	4.8 (5)	37.8 (41)
Medications for eczema	TC-50% HC-10% HY-20%	TC-93% HC-78% HY-71%
Asthma (%)	60	78
Allergic rhinitis (%)	70	78

Values given are medians; ranges are indicated in parentheses.
TC, Triamcinolone; HC, hydrocortisone; HY, hydroxyzine.

search Centers Program, National Institutes of Health. Comparisons of basophil mediator release between patients with mild and those with severe atopic dermatitis were performed with the nonparametric Wilcoxon rank sum test for nonpaired samples. A *p* value of <0.05 was considered statistically significant.

RESULTS

With the use of the clinical scoring system described, 24 patients with food hypersensitivity and atopic dermatitis were divided into two groups to determine whether the clinical severity of their disease correlated with basophil releasability. Five female and five male patients with a median age of 8 years (range, 1.5 to 21 years) had a median clinical score of 5 (range, 0 to 10) and were classified as the LCS group. The median onset of eczema in this group was 6 months (range, 7 days to 24 months) and the mean percent of body involvement was 4.8% (Table II). In contrast, six female and eight male patients with a median age 8 years (range, 2.5 to 20 years) had a median clinical score of 21 (range, 10 to 28) and comprised the HCS group. The mean percent body involvement was 38% and the median age of onset of eczema was 2 months (range, 7 days to 48 months). Overall, six patients in the LCS group and 11 patients in the HCS group used topical corticosteroids for control of their rash at the time of the study. All study patients had discontinued antihistamines for at least 2 weeks before evaluation. The majority of both groups also had asthma and allergic rhinitis (Table II). The five nonatopic control subjects with no atopic dermatitis or food allergy had a median age of 6 years (range, 3 to 13 years), and the six control patients with atopic dermatitis but without food allergy had a median age of 7.5 years (range, 6 to 10 years) and a median clinical score of 14 (range, 1 to 25).

The release of the preformed mediator histamine was first compared in the two patient groups. There were no significant differences in the total histamine content (medians: 13.4 µg/ml in the LCS group and 9.5 µg/ml in the HCS group) and circulating basophils (median number per cubic millimeter: 38, LCS and 27 HCS) in the two study groups. Overall, BHR was higher after all stimuli in the HCS group compared with the LCS group. The spontaneous release of histamine from basophils in both groups was high (median percent total release: HCS, 41.5%; LCS, 31.5%), but no significant differences were observed (Fig. 1). After stimulation with D₂O, the percent total BHR was enhanced and significantly higher (85% vs 68%, *p* < 0.05) in the HCS group. No significant difference in the percent total BHR was observed in the two study groups after stimulation with a histamine-releasing factor. There was a significantly higher percent total BHR after the incubation with anti-IgE (58% vs 26% and 62.5% vs 37%, both *p* < 0.05) in the HCS group at both the 10 µg/ml and 100 µg/ml concentrations of anti-IgE. Finally, the effect of C5a on the percent total BHR was observed in eight of the patients in the HCS group and four of the patients in the LCS group. The basophils from the patients in the HCS group released significantly more histamine in the presence of both concentrations of C5a, 5 ng/ml (50.5% vs 11.5%, *p* < 0.05) and 50 ng/ml (49% vs 11%, *p* < 0.01), than the patients in the LCS group.

Because of a limited amount of venous blood from the control patients, only an abbreviated panel of basophil releasability studies were available. The five nonatopic control patients had significantly less BHR both spontaneously (1.9%) and after stimulation with D₂O (4.6%) and a histamine-releasing factor (3.4%) when compared

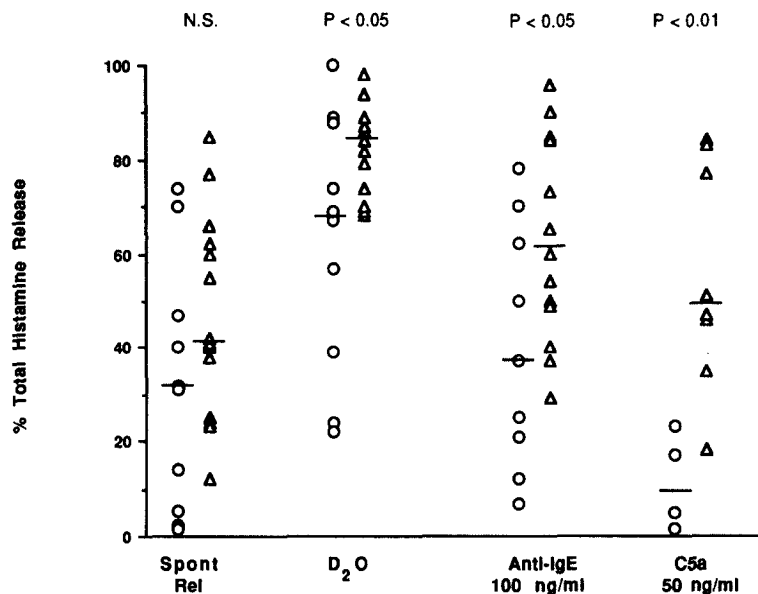


FIG. 1. BHR results after four different stimuli in two groups of patients with atopic dermatitis. Circles represent patients in the LCS group, and triangles represent patients in the HCS group. Individual plotted values represent the mean of duplicate histamine release values as a percentage of the mean of duplicate total histamine content. Horizontal bars represent median values. Differences in release from the two groups were compared statistically with the Wilcoxon rank sum test for nonpaired, nonparametric data. The actual numbers of patients tested in the LCS and HCS groups after each stimulus are as follows: spontaneous release and D₂O release, 10 and 14 patients, respectively; anti-IgE, 9 and 14 patients; C5a, 4 and 8 patients.

with the LCS patients (all $p < 0.01$). There was not a significant difference in the BHR after the concentration of 1 $\mu\text{g/ml}$ anti-IgE (control group: 17.4%, LCS group: 28.9%). In addition, the six children with atopic dermatitis but no accompanying food allergy had significantly decreased spontaneous BHR (2.74%) and decreased histamine release in response to D₂O (8.5%) when compared with the patients in the LCS group (both $p < 0.01$), but release in response to a histamine-releasing factor was not significantly different in the two groups (control group: 20.5%, LCS group: 53%).

The release of the newly formed mediator LTC₄ was evaluated in the two study groups after the same stimuli. As was observed with histamine, the LTC₄ release was higher in the HCS group after all stimuli when compared with that of the LCS group. The spontaneous release of LTC₄ was significantly higher (median release: 34 vs 3.5 pg/0.1 ml, $p < 0.05$) in the HCS group (Fig. 2), a finding in contrast to spontaneous histamine release. The release of LTC₄ after incubation with D₂O was also significantly higher (111.5 vs 39 pg/0.1 ml, $p < 0.05$) in the HCS group. Again, no significant difference in the release of LTC₄ was observed in the two study groups after stimulation with a histamine-releasing factor. LTC₄ release

was significantly higher after the incubation with anti-IgE (64.5 vs 10 pg/0.1 ml, $p < 0.01$) in the HCS group at the 100 $\mu\text{g/ml}$ concentration of anti-IgE. Finally, the effect of C5a on LTC₄ release was studied with the same eight patients in the HCS group and four patients in the LCS group mentioned above. At the 5 ng/ml concentration of C5a there was no significant difference between the two groups. However, with a concentration of 50 ng/ml of C5a, significantly more (80 vs 1 pg/0.1 ml, $p < 0.05$) LTC₄ was released from basophils in the HCS group.

DISCUSSION

These studies demonstrate that patients with food allergy and clinically more severe atopic dermatitis had increased spontaneous basophil release of histamine and a significantly increased spontaneous release of LTC₄. Significant increases in basophil releasability in response to D₂O suggest that the circulating basophils in the severe atopic dermatitis group are more activated.⁹ Furthermore, significant increases in LTC₄ releasability after C5a stimulation, which normally only stimulates the release of histamine,¹⁷ suggest a "primed" or "activated" circulating basophil in vivo with increased releasability in patients with more severe disease.

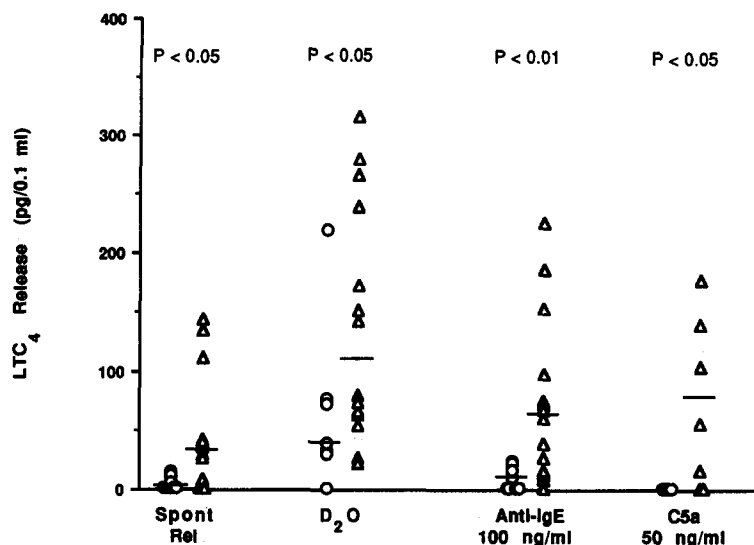


FIG. 2. LTC₄ release results after four different stimuli in two groups of patients with atopic dermatitis. Circles represent patients in the LCS group and triangles represent patients in the HCS group. Individual plotted values represent the mean of duplicate LTC₄ release values. Horizontal bars represent median values. Differences in release from the two groups were compared statistically with the Wilcoxon rank sum test for nonpaired, nonparametric data. The actual numbers of patients tested in the LCS and HCS groups after each stimulus are as follows: spontaneous release, 10 and 14 patients, respectively; D₂O release, 9 and 14 patients; anti-IgE, 9 and 14 patients; C5a, 4 and 8 patients.

The two study populations with either mild or severe atopic dermatitis provide distinct clinical groups with which to investigate the potential role of the basophil in this disease. All of the patients entered this study were part of a larger group of patients who were undergoing an evaluation of the role of food hypersensitivity in the pathogenesis of atopic dermatitis. Although the median age of onset of the rash and the median age at presentation were similar in the two groups, there were significant clinical differences. For example, the HCS group had more severe clinical disease and more extensive cutaneous involvement. In addition, several of the patients in the HCS group were not yet on appropriate allergen elimination diets, and therefore differences in basophil releasability may in part be a reflection of dietary allergen avoidance. Although the specific effects of topical corticosteroids on individual basophil histamine and LTC₄ release assays are unknown, the use of these agents did not appear to blunt significantly the observed differences in basophil releasability. We would anticipate an even more significant difference if steroids had been discontinued, since patients with severe disease were receiving more topical steroids.

High spontaneous release of histamine from leukocytes in vitro from patients with food hyper-

sensitivity was originally reported by May¹⁸ and repeated by the same group several years later.¹⁹ Total BHR was greater than 25% of total basophil histamine content when leukocyte preparations from food-sensitive patients were incubated under physiologic conditions without the addition of antigen or other stimuli for BHR. Only 5% or less of normal persons without food allergies or patients with predominantly inhalant allergy had high spontaneous BHR in vitro. These findings have been confirmed in a group of patients with food hypersensitivity and atopic dermatitis.⁷ Our current studies confirm these reports of increased spontaneous histamine release from basophils in patients with food hypersensitivity and provide new evidence that patients with more severe atopic dermatitis spontaneously release more histamine and LTC₄ than similar patients with less severe disease. A disproportionate difference in compliance with food avoidance diets could account for the differences observed in the two patient groups, but this seems unlikely.

The effect of IgE-dependent basophil mediator release was measured with a specific antibody to human IgE. This cross-linking of surface-bound IgE on basophils in both patient groups did not trigger a homogenous release response. More histamine and LTC₄ were released from basophils

in the HCS group after specific concentrations of anti-IgE.

MacDonald et al.¹² initially described a histamine-releasing factor that induced release of histamine in a subset of atopic donors with a unique species of IgE (i.e., IgE⁺) bound to their basophils. Production of such a histamine-releasing factor in vivo may be responsible for the increased releasability of histamine from basophils previously reported in some patients with atopic dermatitis.⁷ This factor may also lead to increased cutaneous symptoms including pruritus and rash in these patients. In the present study, patients with more severe disease released more histamine and LTC₄ after stimulation with a histamine-releasing factor than did patients with less severe disease, but the differences did not reach statistical significance. Thus further studies in larger patient groups are needed to determine whether an increased responsiveness to histamine-releasing factors differs with the clinical severity of atopic dermatitis.

Although the mechanism of D₂O enhancement of basophil mediator release is unknown, it has been confirmed in many experimental models.^{9, 20, 21} D₂O appears to enhance release of histamine rather than actually triggering its release, which suggests in vivo activation of basophils in those who respond, the majority of whom are subjects with atopic asthma. The present study clearly demonstrates a significant difference in basophil mediator release after stimulation with D₂O in patients with severe versus mild atopic dermatitis, with significantly increased basophil mediator release after exposure to D₂O in patients with more severe atopic dermatitis. These findings support the presence of more activated circulating basophils in these patients.

Complement components have also been shown to mediate basophil histamine release.²²⁻²⁵ There are some important differences between complement-induced and IgE-mediated histamine release from basophils. Release triggered by complement is much more rapid and is not affected by IgE desensitization procedures. Another important distinction of complement-induced mediator release in contrast to IgE-mediated release involves the preferential release of histamine, and not LTC₄.¹⁷ In the present study, not only did the basophils from the patients in the HCS group release significantly more histamine in response to C5a, they also released significantly more LTC₄ with C5a. Stimulation with C5a triggered the release of both preformed and newly generated

mediators from basophils of patients in the HCS group. These latter results support the presence of circulating basophils that are "primed" in vivo and have greater releasability in patients with more severe atopic dermatitis.

In summary, to further define the role of the basophil in atopic dermatitis, basophil releasability was compared in two groups of patients with food hypersensitivity and either mild or severe forms of atopic dermatitis. Both the evidence of increased basophil releasability and activation in vivo, in particular in response to C5a, in patients with more severe disease are supported by the results of the present study. These differences may serve as markers and reliable cellular determinants of patients who are more severely affected by atopic dermatitis.

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