

# Human basophil activation measured by CD63 expression and LTC4 release in IgE-mediated food allergy

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**Background:** IgE-dependent basophil activation induced by an allergen elicit the release of LTC4 and the expression of the CD63 membrane marker.

**Objective:** The aim of this study was to check if flow cytometric analysis of basophil activation could be applied to food allergy diagnosis and if this method paralleled LTC4 release.

**Methods:** Patients were selected by the clinical history, skin tests, and provocation tests. Basophil activation induced by food extracts was studied in 24 control subjects and in 27 patients having a food allergy by LTC4 release test (LRT) and by flow cytometric anti-IgE+, CD63+ cell counting (BAT = basophil activation test). In case of negative anti-IgE response a passive blood donor basophil passive sensitization step was added to LRT and BAT. Leucocyte histamine release test was performed in 11 patients.

**Results:** Basophil activation test was positive in 18/31 cases and LRT in 22/34 cases for food-allergic patients and, respectively, in 1/33 and 1/35 cases for the controls. A correlation was observed between specific IgE, BAT, and LRT. Basophil activation test and LRT performed after passive sensitization had an excellent sensitivity only for specific IgE levels ranging between 3.5 and 35 KU/L.

**Conclusion:** The present study shows that allergen-induced LTC4 release and anti-IgE, antiCD63 bicolor flow cytometric analysis of basophil activation may be used for food allergy diagnosis. Both tests have a good sensitivity and specificity. Basophil activation test and LRT are more efficient than histamine release test in case of high spontaneous histamine release, frequently observed in case of food allergy.

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## INTRODUCTION

The vast majority of clinical reactions due to a food allergy are IgE mediated. Diagnosis of food allergen sensitization is based mainly in vivo on clinical history and skin tests and in vitro on the determination of specific IgE or histamine release from blood leuko-

cytes. Allergy is then confirmed by simple or double-blind oral provocation tests or by labial provocation tests.<sup>1,2</sup>

Basophil activation induced by the bridging of specific IgE present on mast cell and basophil membrane elicit the release of preformed and newly formed mediators, mainly histamine and peptido-leukotriene C4 (LTC4).<sup>3</sup> The in vitro release of LTC4 has been recently used for the diagnosis of food allergy.<sup>4</sup> Further, it has also been demonstrated that anti-IgE-induced basophil activation lead to the upregulation of several membrane markers such as CD45 and CD63. CD63 marker is present on the membrane of cytoplasmic granules, is weakly expressed on

the outside membrane of resting basophils, and is expressed with a high density on activated basophil membrane after the fusion of these granules with the cytoplasmic membrane.<sup>5–7</sup> On the basis of these findings, different methods have been described for the flow cytometric analysis of basophil activation.<sup>8–10</sup> A preliminary study has confirmed that a bicolor (anti-IgE FITC and anti-CD63 PE) flow cytometric method was capable of quantifying basophil activation from a total blood leukocyte suspension.<sup>10</sup> Another study has shown that the flow cytometric method paralleled histamine release for rubber latex allergen and hymenoptera venoms.<sup>11,12</sup>

The aim of this study was to check if this flow cytometric method could be applied to food allergy diagnosis and if this method paralleled LTC4 release.

## MATERIALS AND METHODS

### Selection of Patients

Selection of the patients included in this study was performed by the clinician and concerned: 24 control subjects (10 men and 14 women, 7 subjects <15 years old, 10 from 15 to 40 years old, and 7 >40 years old, 14 nonatopic and 10 atopic) who had no history of an adverse food reaction and negative prick tests.

The absence of allergy to the tested food allergens was established by negative prick tests to food allergens that were usually ingested without incident: peanut (11), egg (9), milk (3), fish (3), crab (2), french peas (2), soya bean (1), lentil (1), brazil nut (1), goat cheese (1), and almond (1). Atopic subjects presented a past or present history of

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atopic dermatitis, hay fever, allergic rhinitis or asthma or positivity of one or more prick tests to common aeroallergens. Twenty-seven patients had one or more than one food allergy (19 men and 8 women, 21 subjects <15 years old, 5 from 15 to 40 years old, 1 > 40 years old). All subjects were atopic except an isolated sensitization to sesame and poppy seeds. The main clinical reactions were anaphylactic shock (6), atopic dermatitis (6), urticaria and/or angioedema (8) and asthma (7). Eight of twenty-seven patients presented more than one symptom. Sensitization to food allergens was documented by a prick test (wheal >3 mm) performed on a skin normally reactive to codeine 9% including a negative saline control.<sup>13</sup> Biologic exploration was based on specific IgE determination (CAP system RIA, Pharmacia, Sweden) (Table 1) and in 11 cases on histamine release test (HRT) (RIA Immunotech, France) performed according to the previously described method.<sup>14,15</sup> Further, the histamine content of the commercial food allergen extract (Allerbio, Varennes-en-Argonne, Stallergènes, Fresnes, France) or of the fresh food diluted from  $10^{-1}$  to  $10^{-5}$  mg/mL from a first 1 mg/mL solution in saline was determined. All these cases of food allergy were selected according to the proposal of the European Food Allergies Study Group (Bruijnzeel-Koomen C et al 1995). The diagnosis is confirmed according to one or more of the following criteria: either a positive standardized provocation test, or the occurrence of an anaphylactic shock to the sensitizing food (the provocation test being, in this case, not necessary). Food allergy diagnosis was established on the basis of a labial test or a simple or double-blind oral provocation test performed according to previously published results.<sup>1,2,13</sup> The labial provocation test (LT) consists of placing one drop of the food on the outer edge of the lower lip. The positivity criteria are the appearance of one of the following symptoms in less than fifteen minutes following a scale of intensity:

- (1) A labial edema at the site of application
- (2) Erythema or urticaria extending onto the chin.
- (3) An extension of the urticaria on one side of the face with the possibility of conjunctivitis or unilateral rhinorrhea.
- (4) Remote eruptions: rash in the flexion folds in case of atopic dermatitis.

In brief, for double-blind oral provocation tests, increasing doses of fresh food were given disguised in mash potatoes or apple compote in order to reach within 3 hours accumulated dose of 965 mg (first step) or 7,110 mg (second step).<sup>13</sup> Two patients having experienced an anaphylactic reaction were not provoked (Table 1). Tested food allergens were peanut (14), egg (9), fish (3), lentil (2), wheat flour (2), brazil nut (1), goat cheese (1), sesame seed (1) and poppy seed (1) (Table 1).

#### *Experimental Design*

Blood samples taken on EDTA in Nancy were sent without any bioclinical information to the laboratory in Paris.

#### *Basophil Activation by Food Extracts*

Whole blood was sedimented in the presence of dextran (Pharmacia, Sweden) and leukocyte rich plasma was centrifuged (500 g/10 minutes, 4°C), washed, and resuspended in an HEPES-EDTA buffer (HEPES 20 mM, NaCl 130 mM, KCl 5 mM, EDTA Na3 0.5 mM, pH 7.4).

Washed leukocyte suspension was mixed vol/vol with a 2 ng/mL solution of rIL3 diluted in an HEPES-calcium buffer and incubated 15 minutes at 37°C. Thirty microliters of the cell suspension was distributed in microtiter plate wells and mixed in parallel with 30  $\mu$ L of HEPES-calcium buffer (HEPES 20 mM, NaCl 130 mM, KCl 5 mM, Ca ++ 2 mM, Mg ++ 1 mM, pH 7.4) (negative control), 30  $\mu$ L of anti-IgE diluted to 10:3 in the same buffer (positive control) and 30  $\mu$ L of 3 dilutions of the tested allergen extract diluted in the same buffer to 100  $\mu$ g/mL, 20  $\mu$ g/mL and 4  $\mu$ g/mL.

Plates were incubated 45 minutes at 37°C and 150  $\mu$ L of HEPES-EDTA buffer was added in every well. Plates were centrifuged (500 g, 10 minutes 4°C). Supernatants were used immediately for LTC4 determination or stored at -20°C in polycarbonate microtiter plates if LTC4 determination has to be delayed in order to avoid LTC4 adsorption on polystyrene solid phase.<sup>16</sup> Cell pellets were used for the flow cytometric analysis of basophil activation.

#### *LTC4 Determination*

For the LTC4 release test (LRT), the amount of LTC4 released in the supernatants were measured by RIA (Amersham, England) using a monoclonal antibody showing a negligible cross reaction, according to the manufacturer, with LTD4 and LTE4. The sensitivity of the test was 50 pg/mL.

According to our experience of the LRT the positivity threshold was set at 100 pg/mL of LTC4 released for, at least, 2 successive dilutions of the allergen extract and the anti-IgE positive control and a spontaneous release less than 100 pg/mL.

#### *Basophil Activation Test*

After the allergen stimulation step and removal of 150  $\mu$ L of the supernatants, 40  $\mu$ L of HEPES-EDTA were added to the cell pellets and plates were centrifuged (500 g, 5 minutes 4°C). Forty microliters of supernatants was discarded and 20  $\mu$ L of cell suspensions was mixed with the two antibodies (anti-IgE FITC and anti-CD63 PE). After 20 minutes at room temperature in the dark, 100  $\mu$ L of lysing solution (Ortho diagnostics, USA) were added in the wells in order to lyse the remaining erythrocytes. After exactly 3 minutes at room temperature, 100  $\mu$ L of HEPES-EDTA were added and plates were centrifuged (500 g, 5 minutes, +4°C). Leukocytes were resuspended in 250  $\mu$ L of HEPES-EDTA and analyzed by flow cytometry using a bi-color flow cytometer (Cyturon absolute, Ortho diagnostics, USA). Basophils were gated within the lym-

Table 1. Clinical and Biologic Parameters Related to 27 Patients Having Experienced a Food Allergy

| Patient | Sex | Age, yr | Symptoms   | Food*       | LPT or SB(DB)PCFC (mg) | Prick (mm)C:codeine | SIgE (kU/L) | LHR (% max) |
|---------|-----|---------|------------|-------------|------------------------|---------------------|-------------|-------------|
| C.T.    | M   | 8       | CA         | Goat's Milk | nd                     | 6.5 (c:4)           | 70          | 22%         |
| J.H.    | F   | 2 1/2   | AO         | Egg         | FC: +7 110 (D, AP)     | 8 (c:4)             | 1,5         |             |
|         |     |         |            | Peanut      | FC: +965 (U, A)        | 6 (c:4)             | >100        |             |
| B.L.    | M   | 5 1/2   | AO, C      | ARA         | LPT+                   | 10 mm (c:2,5)       | 9.5         | 23%         |
| S.F.    | M   | 5       | CA         | Fish        | FC: 10 000+ (V)        | 0                   | 0           |             |
| B.A.    | M   | 2       | AD         | Egg         | LPT+++                 | 20 (4)              | 26          |             |
|         |     |         |            | Peanut      | LPT+++                 | 7.5 (4)             | 19          |             |
| T.V.    | M   | 2       | AD, AO     | Egg         | +15 LPT+++             | 15 mm (c:3)         | 12          |             |
|         |     |         | CA         | Lentil      |                        | nd                  | 3.4         |             |
| S.V.    | M   | 3       | A          | Lentil      | LPT+                   | 8 mm (c:2)          | 24.6        |             |
|         |     |         | V          | Fish        | LPT+++                 | 10 mm               | 2.6         |             |
| J.A.    | F   | 1 1/2   | AD         | Egg         | LPT+                   | 5.5 (c:8)           | 1.60        |             |
| W.      | F   | 15      | AO         | Brasil Nut  | LPT+++                 | 7 (C:2,5)           | 16.6        | sp. LHR     |
| C.C.    | M   | 8       | A, V       | Peanut      | FC: +110 (AP, LP)      | 8.5 (c:4,5)         | >100        |             |
| N.L.    | F   | 33      | AD         | Wheat       | FC: +5 000 (AD)        | 1.5 (C:2)           | 1.43        |             |
| C.J.    | M   | 6       | U          | Egg         | FC: +610 (A, AP, D)    | 4 mm (C:3)          | nd          |             |
| D.T.    | M   | 18      | CA         | Sesame      | nd                     | 14 mm               | 4.6         |             |
|         |     |         |            | Poppy seed  |                        | 12.5 mm             | 25          |             |
| L.A.    | M   | 5       | A          | Peanut      | FC: +10 mL oil         | 4 mm (C:3)          | >100        | sp. LHR     |
| R.M.    | F   | 3 1/2   | OQ         | Egg         | FC: 610 (AP)           |                     | 4.9         | sp. LHR     |
|         |     |         |            | Peanut      | FC: 965 mg (U)         |                     | 13.7        |             |
| K.G.    | M   | 3       | AD         | Peanut      | LPT+                   |                     | >100        |             |
| S.T.    | M   | 10      | CA         | Peanut      | FC: +600 (V)           | 7 mm                | 84          |             |
| C.T.    | M   | 4       | AO, C      | Peanut      | FC: 2 110 mg (AP)      | 7.5                 | 3           |             |
| T.H.    | F   | 43      | A + AO + C | Egg         | FC: 6 000              | 10 mm               | 7.24        | sp. LHR     |
| G.A.    | M   | 9       | A          | Peanut      | FC: + oil 5 ml (A)     | 11.5 mm             | >100        |             |
| M.A.    | M   | 16      | AO, W      | Peanut      | FC: + 265 (A, AP)      | 9 mm (C:3)          | >100        | 24%         |
| O.S.    | M   | 9       | A          | Peanut      | LPT++                  | 5 mm (C:3)          | >100        | sp. LHR     |
| G.A.    | M   | 20      | CA, A      | Peanut      | FC: +200 mg (AP, R)    | 11 mm (c:1,5)       | 32          | sp. LHR     |
| A       | F   | 15 1/2  | A          | Peanut      | FC: +700 mg (V, A)     | 8 mm (c:4)          | >100        |             |
| D.E.    | M   | 8       | D, U       | Fish        | LPT+                   | 4 mm (c:4)          | 1           | sp. LHR     |
| M.J.    | M   | 2       | AD         | Egg         | FC: +965 (U, V, Co)    | 10 mm               | 71          |             |
|         |     |         |            | Wheat       |                        | 7 mm                |             |             |
| M.O.    | F   | 2       | AD         | Egg         | FC: +995 (A, D)        | 5                   | 13          |             |

\*Abbreviations:

A: Asthma

AD: Atopic dermatitis

AO: Angioedema

nd = not done.

C: Conjunctivitis

CA: Anaphylactic shock

Co: Cough

D: Diarrhea

LP: Laryngeal edema

LPT: Labial Provocation Test

SB (DB) PCFC: single (double) blind, placebo controlled food challenge

SIgE: Specific IgE

Sp. LHR: Spontaneous Leucocyte Histamine Release

U: Urticaria

V: Vomiting

W: Wheezing

AP: Abdominal pain

phocyte region of the small (FSC) and large angle (RT-SC) scatter pattern. The anti-IgE FITC brightly fluorescent population (comprising a vast majority of basophils) was gated and anti-CD63 positive and negative cells were separated by an electronic threshold. One hundred fifty to 500 basophils were systematically counted. Results were expressed in percentage activation calculated according to the formula:

$$\text{Percentage activation} = \frac{\text{Number of IgE FITC}^+, \text{CD63 PE}^+ \text{ cells}}{\text{Number of IgE FITC}^+ \text{ cells.}}$$

According to our experience based on previous statistical results (unpublished results) the positivity threshold was 5% over the spontaneous activa-

tion for at least 2 successive allergen dilutions and for the positive control.

#### *Passive Sensitization of Blood Donor Basophils*

If the anti-IgE positive control was negative, a passive sensitization step was performed prior to LRT and BAT. This method has been described elsewhere.<sup>17-19</sup> Briefly, after stripping the IgE bound to their specific receptors

on the basophil membrane by a short incubation at low pH, a leukocyte suspension pool obtained from at least 5 different donors was incubated (90 minutes, 37°C) with the plasma of the tested patients. Sensitized leukocytes were then ready to be tested by LRT or BAT. For each experiment a negative control (leukocyte pool alone) and a positive control (grass pollen-specific IgE-rich serum between 5 to 10 IU/mL measured by Pharmacia CAP system) were systematically added for each series of tests.

This method measures the capacity of specific IgE contained in the serum of the tested patient to bind to blood donor basophils and not the intrinsic property (releasability) of the patients basophils.

#### Reagents

The reagents used were rIL3 (Pepro- tech, USA), anti-IgE FITC, anti-CD63 PE (Caltag USA), and unlabelled polyclonal anti-IgE (ATAB, USA). All chemicals were purchased from Sigma and all plastic material was disposable.

Leukocyte histamine release test was performed in 11 patients according to the previously published method.<sup>14</sup>

## RESULTS

### *Ex Vivo Variation of Basophil Reactivity with Time*

The average delay between the moment when samples were taken in Nancy and the moment when they were analyzed by LRT and BAT in Paris was 18 hours, but in some cases it was more than 24 hours. In order to determine the evolution of basophil reactivity with time we tested, by BAT, 11 blood samples taken from anti-IgE responders at T = 0 and T = 24 hours. Percentage activation obtained at T0, T24 hours and T48 hours in the absence and in the presence of 0.2 µg/mL anti-IgE were, respectively, 5.3 ± 5.9% and 51.4 ± 18%, 8.2 ± 6.1% and 43.1 ± 21.7%, 9.45 ± 9.3% and 35.7 ± 14%. No statistical difference (Wilcoxon distribution paired test) was observed between T0 and T24 hours (Fig 1) whereas the differences between the tests performed in the absence and in the presence of anti-

IgE were significant at 48 hours as compared with T0.

### *Anti-IgE Induced Basophil Activation*

#### Control Subjects

Thirty-three BAT and 35 LRT were performed. Eleven of twenty-four samples tested positive to anti-IgE respectively for BAT and LRT in the absence and in the presence of anti-IgE 9.5 ± 4.5% and 39 ± 17%, 129 ± 76 pg/mL, and 374 ± 123 pg/mL, leading to 16/33 and 18/33 direct BAT and LRT.

#### Allergic Subjects

Thirty-one BAT and 34 LRT were performed. Ten of twenty-seven tests showed a positive response to anti-IgE, respectively for BAT and LRT in the absence and in the presence of anti-IgE 7.5 ± 4.7% and 38 ± 13%, 362 ± 179 pg/mL, and 641 ± 229 pg/mL. No statistical difference of spontaneous activation and anti-IgE-induced response was observed by BAT (Fig 2) and LRT (Fig 3) between controls and allergic patients.

### *Food Allergen-Induced Basophil Activation*

#### Control Subjects

One of thirty-three BAT and 1/35 LRT were positive.

#### Allergic Patients

Eighteen of thirty-one positive BAT (8/10 direct and 10/21 after passive

sensitization) and 22/34 positive LRT (11/13 direct and 11/24 after passive sensitization) were observed (Table 2) with a total correlation between the two tests. No correlation was observed between maximum percentage activation and maximum LTC4 release, and, on the other hand, the degree of patients' sensitization measured by prick tests or the clinical sensitivity measured by the cumulated dose given for the oral provocation test.

As compared with the clinical history, sensitivity, specificity, positive predictive value, and negative predictive value of BAT and LRT performed by direct stimulation of basophils were respectively 80% and 85%, 100% and 100%, 100% and 100%, and 89% and 90%.

We observed a relationship between specific IgE levels and results of BAT and LRT (Table 2) the method of passive sensitization of blood donor basophils lacking sensitivity for specific IgE levels under 3.5 kU/L and over 35 kU/L. For specific IgE levels ranging between 3.5 and 35 kU/L the sensitivity of the tests was very high (12/13 positive tests for the allergic patients) without any decrease of the specificity.

### *Comparison of BAT, LRT, and HRT*

Eleven patients were tested by the three methods, HRT was performed in Nancy (7/11 tests showing a high spontaneous histamine release) and LRT and BAT in Paris on the same

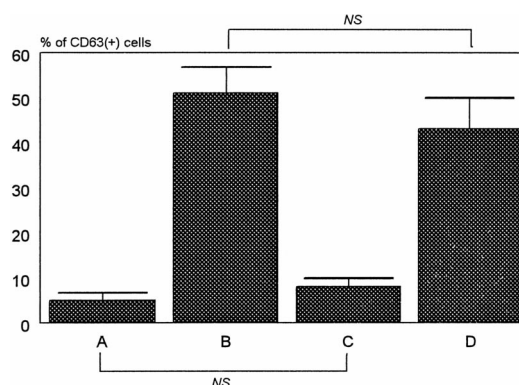


Figure 1. Mean percentage of CD63(+) cells ± SEM observed in the absence (A and C) and in the presence of a target concentration (0.2 pg/mL) of an anti-IgE (B and D) at T = 0 (A and B) and T = 24 hours (C and D) for 11 anti-IgE responders, healthy donors. NS = not significant.

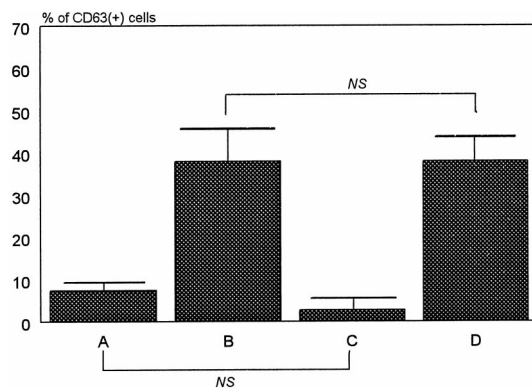


Figure 2. Mean percentage of CD63(+) cells, observed in the absence (A and C) and in the presence (B and D) of an anti-IgE target concentration (0.2 pg/mL) for 10 allergic patients (A and B) and 11 non-allergic controls (C and D). NS = not significant.

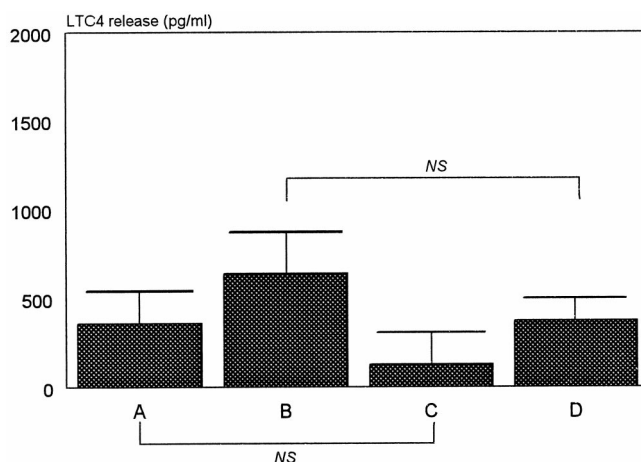


Figure 3. Mean leukotriene release + SEM observed in the absence (A and C) and in the presence (B and D) of an anti-IgE target concentration (0.2 pg/mL) for 10 allergic patients (A and B) and 11 non-allergic controls (C and D). NS = not significant.

blood samples and only 5 BAT and LRT were performed by the direct method and could be compared. Three of five tests showed a high spontaneous histamine release (>10%) while LTC4 spontaneous release and CD63 spontaneous expression were normal (respectively <100 pg/mL and <5%).

## DISCUSSION

The immunomodulation of human basophil activation by picomolar concentrations of IL3 has been well documented, especially for IgE-dependent stimuli, leading to an increase of histamine and peptido-leukotriene release for a given stimuli concentration or to

a significant release of these mediators for much lower stimuli concentrations.<sup>21-24</sup> Interleukin-3 increases also the mediator release kinetic. These effects are specific of basophils which bear high affinity receptors for IL3, in opposition with GM-CSF which is a co-activator of all the myelocyte-derived cell lines.<sup>21</sup>

CD63 is a 53-kD glycoprotein present in basophils but also in other leukocytes and in platelets.<sup>5-7</sup> The CD63 epitope is present on the granular membrane of resting basophils and expressed with a high density on the membrane of activated basophils. It has been shown that CD63 is weakly

expressed (less than 5%) on resting basophil membrane of non-atopic subjects. CD63 expression on basophil membrane is correlated with morphologic aspects of basophil degranulation and with histamine release kinetic.<sup>7</sup> One of us<sup>10</sup> has applied this method for allergen-induced basophil activation using a bicolor anti-IgE FITC, anti-CD63 PE flow cytometric method (basophil activation test or BAT). We have demonstrated (unpublished results) that the anti-IgE FITC +/CD63 + population selected by the flow cytometer expressed the phenotype CD45+, CD14-, CD16-, CD41- which is related to human basophil.<sup>10</sup> As CD63 is also expressed by platelets and as basophil activation is performed, in the conditions described here, in the presence of an overwhelming amount of platelets that may display an increase adhesiveness to basophils after stimulation, CD63 upregulation on the basophil membrane could be the result of platelet activation. As buffers used for the first technical steps contained EDTA (which inhibit an ulterior platelet activation even in the presence of calcium) and as the majority of the IgE-positive cells had the phenotype CD41-, CD45+, the involvement of platelets in the described phenomenon is unlikely, a supposition in agreement with the results obtained with purified basophils. Further, recent results obtained for hymenoptera venoms and aeroallergens, rubber latex, house dust mite, grass pollen, and cat dander showed a good correlation between BAT and histamine release.<sup>11,12</sup>

The present study concerning food-allergic patients shows that these methods have an excellent sensitivity and an excellent specificity, its use being limited by the unresponsiveness of the tested leukocytes to anti-IgE in 18/27 cases for allergic patients and in 13/24 cases for the controls. It may be hypothesized that the low frequency of anti-IgE responders observed here was due to the anti-IgE (polyclonal, affinity purified) used for this study, the variability of the anti-IgE response being demonstrated for other methods,<sup>26</sup> and

Table 2. Frequency of Positive Tests (BAT > 5% and LRT > 100 pg/mL) Obtained by Direct Stimulation of Patients Basophils (d) and After Passive Sensitization of Basophils Taken from Blood Donors (ps)

|                                 | Anti-IgE Induced<br>Positive BAT | Positive Tests with Food Extracts |       |       |       |
|---------------------------------|----------------------------------|-----------------------------------|-------|-------|-------|
|                                 |                                  | BAT                               |       | LRT   |       |
|                                 |                                  | d                                 | ps    | d     | ps    |
| Controls (n = 24)               | 11/14                            | 0/16                              | 1/17  | 0/18  | 0/17  |
| Food allergic patients (n = 27) | 10/27                            | 8/10                              | 10/21 | 11/13 | 11/21 |

\* BAT = basophil activation test and LRT = LTC4 release test.

Table 3. Relationship Between Specific IgE Levels and BAT and LRT Performed by Direct Stimulation or After Passive Sensitization.

| Positive BAT         |          |          |                          |       |
|----------------------|----------|----------|--------------------------|-------|
|                      | Direct   |          | Passive<br>Sensitization | Total |
| SIgE (KU/L)          | Anti-IgE | Allergen | Allergen                 |       |
| < 3.5 KU/L           | 1/6      | 1/1      | 0/5                      | 1/6   |
| 3.5 < SIgE < 35 KU/L | 7/13     | 6/7      | 6/6                      | 12/13 |
| > 35 KU/L            | 2/10     | 2/2      | 2/8                      | 4/10  |

BAT = basophil activation test and LRT = LTC4 release test.

to the delay exceeding 24 hours for several samples. An intrinsic lack of releasability of the tested basophils is unlikely as far as this phenomenon was observed with the same frequency for both the control and the allergic patients populations. A low anti-IgE response is also unlikely as no anti-IgE negative response was observed in association with a positive response in the presence a food allergen. The LTC4 release test commercialized under the name of CAST (cellular antigen stimulation test, Buhlmann laboratory, Switzerland) is based on the measurement of peptido-leukotriene C4 release from blood leukocyte suspensions. Different human leukocytes (mainly eosinophils and monocytes) are capable of releasing LTC4 and the presence of low affinity receptors (FcERII) on monocytes and eosinophils may lead to the hypothesis of the involvement of these cells in allergen and anti-IgE LTC4 release from total leukocytes.<sup>4</sup> It has been established that peripheral blood leukocytes taken from atopic individuals exhibit in vitro an enhanced production of leukotrienes, as compared with leukocytes taken from non-atopic subjects.<sup>26</sup> Two arguments support the idea that LTC4 is mainly released by activated ba-

sophils: the experiments dealing with specific enrichment of monocytes, eosinophils, and neutrophils by density gradient performed by Kurimoto et al<sup>21</sup> showed an absence of LTC4 release by supernatants related to these three fractions stimulated either by C5a or FMLP and a good correlation observed with histamine release.

Only preliminary results were obtained with LRT for food allergy.<sup>4,27</sup> For the population of anti-IgE responders, a significant LTC4 release was observed in 11/12 cases, this high sensitivity being observed for both methods. For LRT performed after passive sensitization, the sensitivity of both methods was still excellent for patients for whom specific IgE levels ranged from 3.5 to 35 kU/L but dropped for higher or lower specific IgE concentrations (Table 3). It could be hypothesized that the poor sensitivity observed for these specific IgE concentrations ranges was due, for the low concentrations range, to a weak antibody transfer on blood donor basophils; and for the high concentrations range, to an inhibition by antibody excess. This latter interpretation was supported by further experiments performed on 2 specific IgE-rich sera (>100 kU/L) which led to a positive BAT (data not shown)

after a 3-fold dilution. These results confirm also that histamine release is often useless due to a high spontaneous release (up to 50% in 7/11 cases). This phenomenon was first described in food allergy<sup>1,28,29</sup> and is also often described, to a lesser degree, for other IgE-dependent reactions, such as for grass pollen sensitization<sup>18</sup> as it decreases with the progression of the pollen season and for house dust mite, increasing in this case after allergen exposition.<sup>18</sup> Spontaneous histamine release was also observed for hemodialyzed patients sensitized to ethylene oxide.<sup>30</sup> This phenomenon is due to the activity of HRF.<sup>31</sup> It has also been shown that spontaneous histamine release is inhibited at 4°C, in the absence of calcium at 37°C and in the presence of oxydative metabolism inhibitors.<sup>32</sup> The absence of CD63 expression demonstrates that spontaneous histamine release is not the result of a degranulation process nor that might reflect activation, as it is confirmed by the absence of LTC4 release. This phenomenon limits the use of HRT for the current diagnosis of food allergy.<sup>33,34</sup>

In clinical practice, the routine assay is still represented by the determination of circulating specific IgE. Cellular tests have to be considered as ref-

erence tests (second level tests). Nevertheless, cellular tests show several advantages as compared with the simple circulating antibody determination. They measure a cellular reactivity (releasability) closer, at least theoretically, to the physiopathologic reaction even in terms of immunomodulation by physiologic cofactors such as IL3 and C5a (priming). Further, they may pretend to a higher specificity as IgE bridging, which is a key factor of basophil and mast cell activation, is dependent of the allergen quaternary structure. As compared with cellular tests based on the measurement of a mediator release (histamine and LTC<sub>4</sub>), flow cytometry is a faster (3 hours) and a less expensive method. An excellent correlation has been observed between the three methods for hymenoptera venoms,<sup>33</sup> the intra-assay variation coefficients standing between 5% and 20%.

The results presented here are in favor of the reliability of BAT and LRT for the diagnosis of food allergy.

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