

Original article

Validation of a two-color flow cytometric assay detecting *in vitro* basophil activation for the diagnosis of IgE-mediated natural rubber latex allergy

Background: IgE-dependent triggering of basophils not only elicits the release of different mediators but also the up-regulation of certain markers, e.g. CD63, which can be detected by flow cytometry. We intended to investigate if flow cytometric analysis of basophil activation could be a valuable tool in the diagnosis of latex allergy, and to evaluate if the basophil activation test (BAT) could be helpful in determining the clinical significance of a positive latex IgE in individuals with negative history and negative latex skin test. Additionally we aimed to determine the role of cross-reactive carbohydrate determinants (CCDs) in causing positive latex IgE without apparent clinical significance.

Methods: Twelve healthy controls without a history of latex hypersensitivity with a negative latex IgE and skin test (group 1), 24 individuals without a history of latex hypersensitivity with a negative latex IgE and skin test but with other inhalant allergies (group 2), and 29 latex allergic patients with a compelling history of latex allergy with a positive latex IgE and prick test (group 3) were enrolled. The diagnostic performances of the BAT were further evaluated in 13 individuals with a history of latex allergy but with negative specific IgE and/or skin test (group 4). Twenty-four individuals with positive latex IgE without apparent clinical relevance, i.e. without history of latex hypersensitivity and negative latex skin tests, were also analyzed (group 5). The putative role of CCDs causing positive latex IgE results without apparent clinical significance was evaluated by quantification of IgE for bromelain.

Results: According to the receiver operating characteristics (ROC)-generated threshold value of 17% between latex allergic patients and the pooled group of nonlatex allergic individuals, the sensitivity and specificity of the basophil activation test was 93.1% and 91.7%, respectively. In healthy controls, allergic patients without latex hypersensitivity and latex allergic patients the number of positive BATs was 0/12, 3/24 and 27/29, respectively. In the individuals with an evocative history of latex allergy but with negative latex IgE and/or skin test the BAT was positive in all 13 cases. Twenty of 24 individuals without apparent latex allergy but with positive latex IgE had a negative BAT. IgE for bromelain was positive in 1/19 sera from group 2, 1/24 sera from group 3, none of the 8 sera from group 4, but in 16/18 sera from group 5, respectively.

Conclusion: Flow cytometric analysis of activated basophils seems a highly sensitive and specific tool for diagnosing latex allergy. In addition, the technique might help to determine the clinical relevance of positive IgE quantification in the absence of overt latex allergy. CCDs of natural rubber latex allergens were confirmed to mimic latex sensitization.

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Key words: allergy diagnosis; basophil activation; bromelain; carbohydrate determinant; CD63; flow cytometry; latex.

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Accepted for publication 26 March 2002

Abbreviations. BAT: basophil activation test; CCD: cross-reactive carbohydrate determinant; PE: phycoerythrin; ROC: receiver operating characteristics

IgE-mediated latex allergy has been recognized as an increasingly prevalent health problem and correct diagnosis is a prerequisite for effective management. Diagnostic methods consist of personal history, skinprick tests, quantification of latex IgE and,

eventually, appropriate cutaneous or inhalation challenge tests (1–3). Functional *in vitro* assays in latex allergy have generally focused on T-lymphocyte transformation (4–8), basophil histamine and sulfidoleukotriene release (4, 9, 10). Technical difficulties (e.g. the small number of circulating basophils, the necessity of leukocyte isolation and the difficulty of quantifying very low levels of sometimes labile mediators), cost and time-cost have limited their applications, and their clinical benefit remains controversial. As a result, during the last years several authors have focused on a new approach to study allergen-dependent basophil activation and to identify relevant allergic sensitization *in vitro* (for reviews see 11, 12).

Basophils activated with specific allergen that is recognized by surface receptor *FcεRI*-bound IgE not only secrete and generate quantifiable bioactive mediators but also up-regulate the expression of certain markers (e.g. CD45 and CD63) which can easily be detected by multicolor flow cytometry (13).

CD63 (gp53) is member of the transmembrane-4 superfamily that can be detected in a wide variety of cell types, e.g. leukocytes and platelets (14, 15). In resting basophils CD63 is anchored in the basophilic granule membrane and is only weakly expressed on the surface membrane (generally less than 5% in healthy controls (16)). In contrast, as a result of fusion between the granule and plasma membrane, CD63 is expressed with a high density on activated basophils and mirrors histamine release (9, 17).

Cross-reactive carbohydrate determinants (CCDs) are antigenic structures shared by taxonomically distant allergenic sources such as pollen from unrelated botanical families, food, latex and arthropods (e.g. *Hymenoptera*) (18, 19). Since their first description in the early 1980s (20) IgE antibodies against these nonmammalian CCDs have been reported to be very prevalent in atopics, but are generally believed to be clinically irrelevant as they fail to induce positive skin tests or elicit clinical reactions (21, 22).

Bromelain (EC 3.4.22.4) is a thiol protease isolated from pineapple stem that possesses a single asparagine-linked oligosaccharide that is highly immunogenic mainly because it contains a β 1→2 xylose (on the β -linked mannose of the core) and α 1→3 fucose (on the proximal GlcNAc residue) not found in mammalian glycoproteins (23, 24).

This study was initiated to evaluate the diagnostic efficiency of the basophil activation test in the diagnosis of IgE-mediated latex allergy. In addition, we evaluated whether the BAT could be useful to find out the clinical relevance of a positive latex IgE quantification in an individual without overt latex allergy. Finally, we determined the contribution of CCDs as a possible cause for latex IgE antibodies

without apparent clinical significance. For this purpose quantification of IgE for bromelain was performed (22).

Material and methods

Subjects

As a first step, experiments were performed in order to validate the basophil activation test (BAT). Twelve healthy volunteers without latex-induced symptoms with negative latex IgE and latex skin test served as controls (group 1). Twenty-five individuals without latex hypersensitivity but with other inhalant allergies were also analyzed (group 2). Inhalant allergy was defined by the presence of a history of atopic dermatitis, allergic rhinoconjunctivitis or asthma corroborated with a positive specific IgE antibody and skin test for at least one classical aeroallergen. Thirty-one highly selected latex-allergic patients (12 monosensitized for latex and 17 suffering from concomitant inhalant allergies) were included (group 3). All had an evocative history of latex allergy with urticaria, rhinitis, conjunctivitis, angioedema, bronchospasm, generalized anaphylaxis (25) with hypotension, or shock, corroborated by positive latex IgE and latex skin test. Whole blood samples of all of these individuals were analyzed in the BAT. Three samples were excluded because of "nonresponsiveness" of the basophils to positive control stimulation with *N*-formyl-Met-Leu-Phe (fMLP) or another relevant allergen, i.e. grass pollen. As a result, 24/25 samples from group 2 and 29/31 samples from group 3 were eligible for effective validation of the test.

Sensitivity and specificity of the BAT was calculated between latex-allergic patients and a group of individuals without latex hypersensitivity, which was composed of healthy controls as well as individuals without latex hypersensitivity but with other inhalant allergies (group 1 + 2). Sensitivity and specificity was also calculated including the three nonresponders, i.e. one individual without latex allergy was considered as positive in the BAT whereas the two latex allergic patients were considered as negative in the BAT.

Another group (group 4) comprised 13 individuals with a history of latex allergy but with negative latex IgE and/or latex skin test were analyzed.

Finally, 24 individuals with a positive latex IgE quantification but without clinical history of latex allergy and a negative latex skin test were studied (group 5).

Specific IgE

Quantification of total IgE and specific IgE for *Dermatophagoides pteronyssinus*, birch, timothy grass, mugwort, cat, dog, *Cladosporium herbarum*, *Aspergillus fumigatus*, *Hevea* latex and bromelain was performed according to the manufacturer's instructions (Immuno-CAP, Pharmacia CAP System, Brussels, Belgium). The assay is a solid-phase immunofluorometric assay in which IgE antibody is bound to allergen allergosorbent (cellulose sponge matrix) and detected with rabbit polyclonal antihuman IgE and 4-methylumbelliferyl β -D galactosidase substrate. Because of insufficient serum quantity, not all samples could be evaluated for the presence of bromelain IgE. Values of allergen-specific IgE below 0.35 kUa/l were considered negative.

Skin prick tests

Skin prick tests were done with *Dermatophagoides pteronyssinus*, birch, timothy grass, mugwort, cat, dog, *Cladosporium herbarum*, *Aspergillus fumigatus* (Haarlem Allergen Laboratories, Haarlem, the Netherlands) and *Hevea* latex (Stallergenes, Fresnes, France). Skin test responses were considered positive only when the wheal reaction exceeded a diameter of 3 mm.

Basophil activation by latex

Three hours after sampling, aliquots of 100 μ l endotoxin-free heparinized (Vacuette, Greiner Labortechnik GmbH, Kremsmünster, Austria) whole blood were preincubated for 10 min at 37°C with 20 μ l stimulation buffer containing IL-3 (10 ng/ml, Pepro Tech, Inc Rocky Hill, NJ). IL-3 leads to a preactivation of the basophils, and therefore in an increased mediator release or CD63 expression for a given stimulus concentration or to significant release of these mediators for much lower stimulus concentrations (26–32).

Preactivated blood samples were stimulated for 20 min at 37°C in a water bath with 100 μ l nonammoniated latex (10 μ g/ml) purchased by Diagnostic Products Corp. (Brussels, Belgium), 100 μ l (2 μ mol/l) fMLP (Sigma-Aldrich Chemic GmbH, Steinheim, Germany) as a positive control, and 100 μ l washing solution to measure spontaneous CD63 expression (negative control). The optimal stimulation times and concentrations of IL-3, allergen, fMLP and latex used in this study were determined in preliminary investigations, and the latex extract was clinically validated (7). Cooling the samples on ice for 5 min stopped basophil activation and degranulation.

To estimate activated basophils, cells were stained with 20 μ l monoclonal biotinylated antibodies to human IgE (Sigma-Aldrich Chemic GmbH, Steinheim, Germany) and 10 μ l monoclonal phycoerythrin(PE)-conjugated antihuman CD63 (PharMingen, BD Biosciences Erembodegem, Belgium), or PE-conjugated irrelevant-control antibody with identical isotype (PharMingen) during 20 min in the dark, on ice. After washing, 20 μ l of Streptavidin Alexa 488 (Molecular Probes, Leiden, the Netherlands) was added for 15 min at room temperature. Red blood cells were lysed and white blood cells were fixed (FACS Lysing solution, BD) during 10 min at room temperature. After centrifugation (5 min, 250 g, 4°C) and washing, 200 μ l of washing solution was added to the cell pellets which were used for flow cytometric analysis of basophil activation (FACScan flow cytometer (BD Immunocytometry Systems)). Green fluorescence (FL1 channel) and side scatter (SSC) were employed to gate on at least 500 basophils that expressed a high density of surface IgE (Fig. 1A). Subsequently within this gate the percentage of activated basophils, i.e. those coexpressing CD63 (FL2 channel), was measured (Fig. 1B). For this purpose, the marker (M1) was set on the value obtained with an irrelevant isotype-control antibody (Fig. 1B). Less than 1% of basophils were stained with the irrelevant-control antibody. Percentages of activated basophils were corrected by subtracting spontaneous CD63 expression (negative control) from the values obtained with latex stimulation.

Provocation test

Powdered nonsterile natural rubber latex examination gloves (Conform, Ansell Medical, Bangkok, Thailand, LOT 909007801) were donned and the patient was then observed for 15–60 min for skin and respiratory symptoms and signs. For safety reasons, every glove provocation test was started with a finger-piece provocation. Spirometry was performed before and 60 min after exposure. Polyvinyl chloride gloves served as controls.

Statistical analysis

Results were expressed as median (range). The Mann–Whitney *U*-test and Spearman's rank correlation test were applied where appropriate. Chi-squared analysis was used to compare frequencies. Differences were considered significant at a *P*-value of less than 0.05. Receiver operating characteristics (ROC) curve analysis was performed to calculate the optimal cut-off value that corresponds to the best specificity and sensitivity (33).

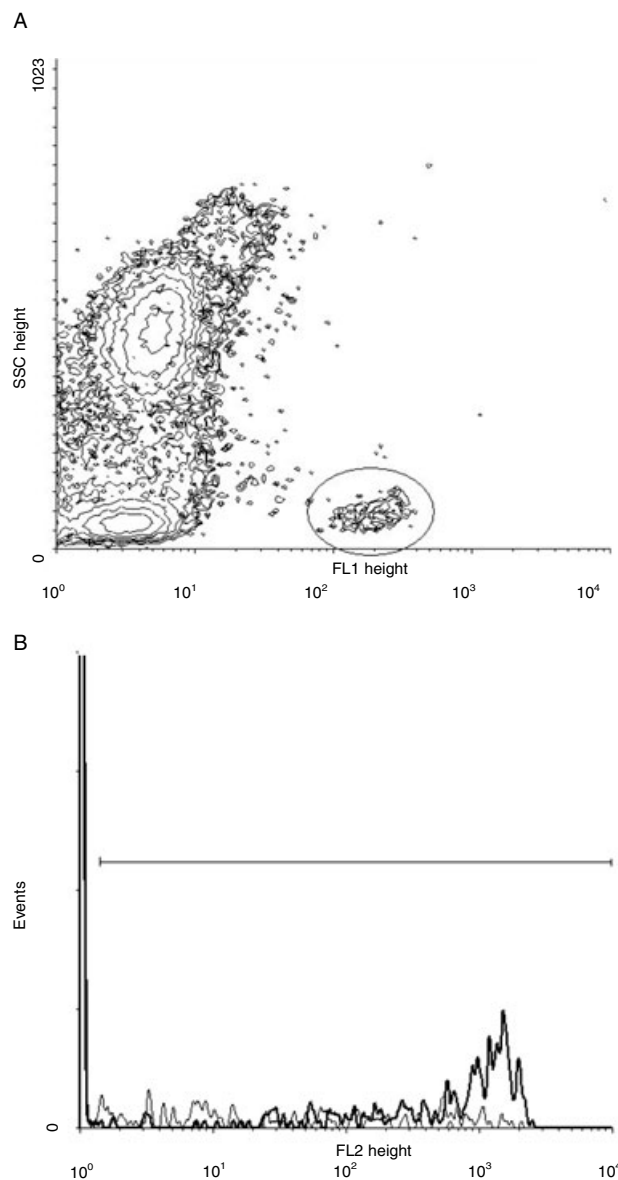


Figure 1. (A) Flow cytometric analysis using a density plot of side scatter (SSC) and fluorescence (FL1). Alexa 488-conjugated anti-IgE positive basophils are gated out in an ellipsoid region. (B) CD63 expression after challenge with washing solution (fine line) and nonammoniated latex extract (bold line). Marker M1 denotes CD63 positive cells and was set on phycoerythrin-conjugated irrelevant-control antibody.

Results

Total serum IgE and latex-specific IgE

Total IgE was 36 kU/l (5–172) in group 1, 204 kU/L (12–4727) in group 2, 195 kU/L (34–5000) in group 3, 166 kU/L (23–1320) in group 4 and 473 kU/L (36–4238) in group 5. Latex IgE in group 3 was 7.52 kUa/L (0.51–100.00), in group 4 <0.35 kUa/L (<0.35–7.40) and in group 5 1.28 kUa/L (0.40–21.70).

Basophil activation test

In toto 105 samples were enrolled for analysis in the

BAT. Basophils could be clearly identified in all the samples. Fourteen out of the 105 samples (13.3%) were nonresponders to fMLP (positive control). However, 11 out of these 14 showed stimulation with another relevant allergen, i.e. Timothy grass pollen (data not presented), indicating that the basophils in these samples could not be considered as “unresponsive”. As a result, three samples were classified as not interpretable (one in group 2 and two in group 3) and were withdrawn from further analysis.

ROC curve analysis was performed between individuals without latex hypersensitivity (group 1+2) and latex allergic patients (group 3) and resulted in a sensitivity 93.1% and specificity 91.7% for a threshold value of 17% ($P < 0.0001$) (Fig. 2).

The individual percentages of CD63 up-regulation and numbers of positive BATs are shown in Fig. 3. In healthy controls, allergic patients without latex hypersensitivity and latex allergic patients the number of positive BATs was 0/12, 3/24 and 27/29, respectively.

When the nonresponders were included, the number of positive BATs was 4/37 in the pooled group of individuals without latex allergy and 27/31 in the patients with latex allergy. This resulted in a specificity of 89% and sensitivity of 87%.

In the individuals with an evocative history of latex allergy but with negative latex IgE and/or latex skin test, the BAT could confirm suspected latex allergy in all 13 individuals of this group.

Finally, 20/24 individuals with positive latex IgE but with a negative history of latex hypersensitivity and latex skin test had a negative BAT. To further unravel the discrepant findings, the four individuals with a positive BAT underwent a glove provocation test that turned out to be negative in all cases.

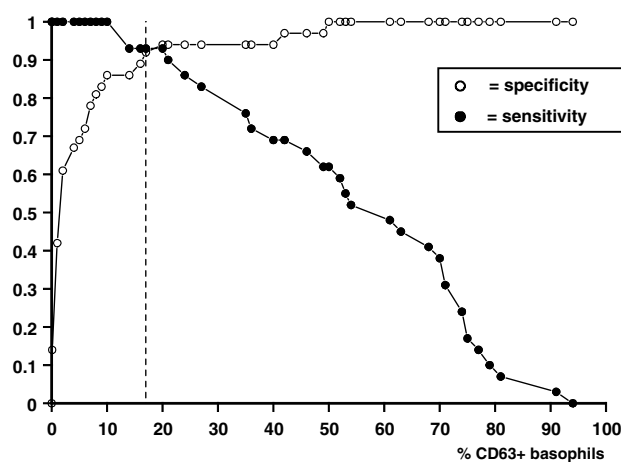


Figure 2. Two-graph ROC curve for latex-induced CD63 expression generated between individuals without latex allergy (group 1+2) and latex-allergic patients (group 3). For a threshold value of 17% (dotted line) the BAT attains a sensitivity of 93% and specificity of 92%.

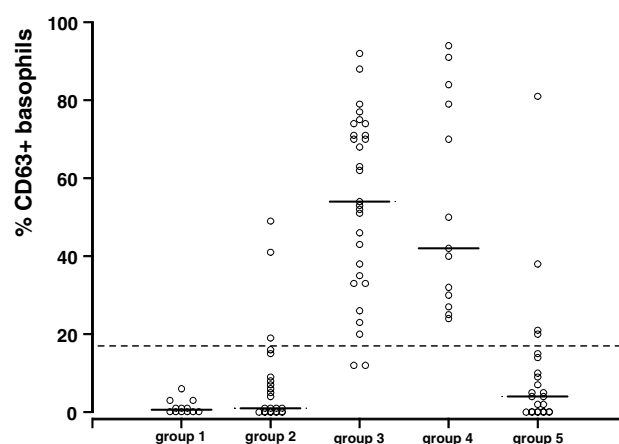


Figure 3. Latex-induced CD63 expression in healthy volunteers (group 1); individuals without latex allergy but allergic for at least one aeroallergen (group 2); patients with latex allergy (group 3); individuals with a history of latex allergy but with negative latex IgE and/or skin test (group 4); and individuals with a positive latex IgE but with a negative history and latex skin test (group 5). Results are expressed as percentages of CD63 up-regulation (i.e. percentage latex-induced CD63 expression minus spontaneous CD63 expression). The dotted line represents the threshold of 17% between latex allergic patients and individuals without latex allergy. The bold line denotes the median.

In latex allergy there was no significant correlation between the results of the BAT and serum latex IgE.

Bromelain IgE

Seventy-one bromelain IgE determinations were available, and was positive in 1/19 individuals with an inhalant allergy but without latex hypersensitivity, 1/24 latex allergic patients, none of 8 individuals with a compelling history of latex allergy but with a negative latex IgE and/or skin test, and 16/20 individuals with a positive latex IgE without apparent latex allergy ($P \leq 0.0001$). In this latter group bromelain IgE was positive in 3/4 individuals with a positive BAT.

Discussion

Activation by relevant allergen, through cross-linking of FcεRI-bound IgE, induces fusion of cytoplasmatic granules with the plasma membrane and the successive release of bioactive mediators. This fusion phenomenon, however, also alters the cell surface phenotype with up-regulated expression of different membrane markers, such as the pan leukocyte antigen CD45 and the tetraspan molecule CD63. These changes can easily be demonstrated by multicolor flow cytometry, a technique that utilizes characteristics of cellular light scatter and immunofluorescence for analysis of cell surface phenotype and quantification of cellular viability and activity (34–36).

Flow cytometric analysis of basophilic up-regulated expression of CD63 has already been reported in

allergies to pollen (17, 27, 37–39), house dust mite (27, 39, 40), *Hymenoptera* venoms (40, 41), foods (40, 42, 43), and drugs (44–46).

To our knowledge, data on flow cytometric analyses of basophilic up-regulated expression of CD63 in latex allergy are restricted to the preliminary results presented by Sánchez-López et al. (47), an isolated case described by Monneret et al. (40) and 10 patients evaluated by passive basophil sensitization (9).

The first aim of this study was to evaluate whether the BAT could be applied and validated in IgE-mediated latex allergy. In the absence of a gold standard, as with any study of this nature, a major difficulty was to accurately establish a group of patients with clinically significant allergy and to compose an appropriate “control” group. Patient histories, specific IgE quantification, skin tests, and provocation tests have been used in previous studies; although each method can provide useful information, all methods *in se* have limitations. In a recent study, 15% of the 304 adult individuals studied with a well-documented questionnaire provided a false-positive history of latex allergy (1). Skin tests performed with different commercially available extracts or extemporaneous glove extracts have already been demonstrated to be quite efficient for confirming latex allergy. At present, to our knowledge, only one standardized skin test extract is available commercially (48). In addition, skin tests bear a risk for anaphylaxis (49). Hamilton and co-workers reported an immediate systemic reaction rate for latex skin testing of 16.1% (1). As a result, physicians frequently rely on *in vitro* IgE quantification to confirm the diagnosis of latex allergy. In a recent study comparing three assays for latex-specific IgE, the CAP System and microplate AlaSTAT technique showed comparable sensitivity (respectively 76.3% and 73.3%) and specificity (respectively 96.7% and 97.2%) whereas the HY-TEC assay produced a sensitivity of 90% and specificity of 67.5% (2). The glove challenge has been thoroughly described (50) but the reduced allergenicity of gloves, lack of uniformity and risk for anaphylaxis diminishes its usefulness.

In other words, defining sensitivity and specificity for a new test remains a clinical problem in the sense that there is no single “gold standard” or reference test. To circumvent this limitation, in this study, latex-allergy was defined by the combined presence of a compelling history of latex-mediated hypersensitivity and positive latex IgE as well as latex skin test. Results were compared in individuals with a combined absence of history of IgE-mediated latex hypersensitivity and negative latex IgE and latex skin test. The BAT was observed to be a highly efficient instrument to diagnose IgE-mediated latex allergy. According to the ROC-generated threshold limit value of 17% CD63 up-regulation above spontaneous expression between latex

allergic patients and the composite group of individuals without latex allergy, a sensitivity and specificity exceeding 90% was obtained.

From our data it is evident that according to a maximal latex-induced CD63 expression of 6% in healthy controls, we would have attained a sensitivity and specificity of 100%. However, we believe that for latex allergy, a condition more prevalent in atopics than in nonatopics (51), an adequate control group should not be restricted to healthy volunteers (1, 2).

In addition, the BAT was able to confirm the diagnosis in all the 13 individuals with a compelling history of latex allergy but who had a negative specific IgE and/or latex skin test (group 4). This observation suggests that in such patients the BAT could be a useful additional tool to diagnose IgE-mediated latex allergy, making potentially hazardous challenge tests less necessary.

Several authors have shown the possibility of “false” positivity in *in vitro* IgE assays (1, 7, 52). Of particular interest was the observation that in 20/24 cases of a selected group of individuals with positive latex IgE but without clinical history of latex allergy and a negative skin test, the BAT remained negative. This might suggest that the latex IgE antibodies in this group had no biologic activity. In the remaining four individuals that displayed a positive BAT, an additional provocation test was negative. Taken together, these results suggest that in this group the positive latex IgE could be considered as clinically irrelevant.

There are several mechanisms resulting in clinically irrelevant specific serum IgE, e.g. nonspecific absorption frequently associated with high total serum IgE, and cross-reactivity due to so-called panallergens and CCDs. Only two out of the 24 sera from group 5 contained total serum IgE exceeding 3000 kU/l and had potential to produce irrelevant results because of nonspecific absorption. Subsequently we sought to investigate the possible contribution of CCDs in causing positive latex IgE without apparent clinical significance. Whereas in allergic individuals without latex hypersensitivity, latex allergic patients and in patients with latex allergy but with negative latex IgE and/or skin test antibodies for bromelain were rare or even absent, the reverse was seen in individuals that displayed irrelevant latex IgE (group 5). In these cases, bromelain IgE was present in 75% of the individuals. Most of these individuals were polysensitized to pollen and some were allergic to *Hymenoptera* venom. These results confirm that systems using solid phase-coupled allergens can detect specific IgE for allergenic extracts known to bear carbohydrate moieties on their components and, as a result, mimic sensitization (22, 53). Because such misdiagnosis may affect negatively the patient’s quality of life, the clinical significance of *in vitro* assays should always be assessed carefully. Being a completely safe, rapid and easily performed technique,

the BAT seems a valuable additional tool to discriminate between clinically relevant and irrelevant IgE responses.

In conclusion, the BAT was demonstrated to be a good and safe *in vitro* correlate for *in vivo* latex hypersensitivity. In addition the BAT was observed to be an accurate tool to determine the clinical relevance of a positive IgE quantification in the absence of overt latex allergy. Cross-reactive carbohydrate determinants

of natural rubber latex allergens, tentatively assessed by quantification of bromelain-specific IgE, were confirmed to mimic latex sensitization.

Acknowledgments

We would like to thank Mrs Christel Mertens for her skilled and valuable technical assistance.

References

- HAMILTON RG, ADKINSON NF. Diagnosis of natural rubber allergy: Multicenter latex skin testing efficacy study. *J Allergy Clin Immunol*, 1998;**102**:482–490.
- HAMILTON RG, BIAGINI RE, KRIEG EF. Diagnostic performance of Food and Drug Administration-cleared serologic assays for natural rubber latex-specific IgE antibody: the Multi-Center Latex Skin Testing Study Task Force. *J Allergy Clin Immunol*, 1999;**103**:925–930.
- KURTZ KM, HAMILTON RG, ADKINSON NFJ. Role and application of provocation in the diagnosis of occupational latex allergy. *Ann Allergy Asthma Immunol*, 1999;**83**:634–639.
- TURJANMAA K, RÄSÄNEN L, LEHTO M, MÄKINEN-KILJUNEN S, REUNALA T. Basophil histamine release and lymphocyte proliferation tests in latex contact urticaria. *Allergy*, 1989;**44**:181–186.
- MURALI PS, KELLY KJ, FINK JN, KURUP VP. Investigations into cellular immune responses in latex allergy. *J Laboratory Clin Med*, 1994;**124**:638–643.
- RAULF-HEIMSOOTH M, CHEN Z, URBANSLI A, LIEBERS V, BAUR X. Lymphocyte response of latex-sensitized patients to purified allergen Hev b 1. *J Allergy Clin Immunol*, 1996;**98**:640–651.
- EBO DG, STEVENS WJ, BRIDTS CH, DE CLERCK LS. Latex-specific IgE, skin testing, and lymphocyte transformation to latex in latex allergy. *J Allergy Clin Immunol*, 1997;**100**:618–623.
- JOHNSON BD, KURUP VP, SUSSMAN GL, et al. Purified and recombinant latex proteins stimulate peripheral blood lymphocytes of latex allergic patients. *Int Arch Allergy*, 1999;**120**:270–279.
- SAINTE-LAUDY J, VALLON C, GUERIN JC. Diagnosis of latex allergy: comparison of histamine release and flow cytometric analysis of basophil activation. *Inflamm Res*, 1996;**45**:S35–S36.
- MARAIS GI, FLETCHER JM, POTTER PC. In vivo and in vitro diagnosis of latex allergy at Groote Schuur Hospital. *S Afr Med J*, 1997;**87**:1004–1008.
- CROCKARD AD, ENNIS M. Basophil histamine release tests in the diagnosis of allergy and asthma. *Clin Exp Allergy*, 2001;**31**:345–350.
- CROCKARD AD, ENNIS M. Laboratory-based allergy diagnosis: should we go with the flow? *Clin Exp Allergy*, 2001;**31**:975–977.
- BOCHNER BS. Systemic activation of basophils and eosinophils: Markers and consequences. *J Allergy Clin Immunol*, 2000;**106**:S292–S302.
- NIEUWENHUIS HK, VAN OOSTERHOUT JJG, ROZEMULLER E, et al. Studies with a monoclonal antibody against activated platelets: evidence that a secreted 53,000 molecular weight lysosome-like granule protein is exposed on the surface of activated platelets in the circulation. *Blood*, 1987;**70**:838–845.
- METZELAAR M, WIJNGAARD PLJ, PETERS PJ et al. CD63 antigen: a novel lysosomal membrane glycoprotein, cloned by a screening procedure for intracellular antigens in eukaryotic cells. *J Biol Chem*, 1991;**266**:3239–3245.
- KNOL EF, MUL FPJ, JANSSEN H, CALAFAT J, ROOS D. Monitoring human basophil activation via CD63 monoclonal antibody 435. *J Allergy Clin Immunol*, 1991;**88**:328–338.
- SAINTE-LAUDY J, SABBAH A, VALLON C, GUERIN JC. Analysis of anti-IgE and allergen induced human basophil activation by flow cytometry. Comparison with histamine release. *Inflamm Res*, 1998;**47**:401–408.
- AALBERSE RC, VAN REE R. Crossreactive carbohydrate determinants. *Clin Rev Allergy Immunol*, 1997;**15**:375–387.
- FUCHS T, SPITZAUER S, VENTE C et al. Natural latex, grass pollen, and weed pollen share IgE epitopes. *J Allergy Clin Immunol*, 1997;**100**:356–364.
- AALBERSE RC, KOSHTÉ V, CLEMENS JGJ. Immunoglobulin E antibodies that cross react with vegetable foods, pollen and *Hymenoptera* venom. *J Allergy Clin Immunol*, 1981;**68**:356–364.
- VAN DER VEEN MJ, VAN REE R, AALBERSE RC et al. Poor biologic activity of cross-reactive IgE directed to carbohydrate determinants of glycoproteins. *J Allergy Clin Immunol*, 1997;**100**:327–334.
- MARI R, IACOVACCI P, AFFERNI C et al. Specific IgE to cross-reactive carbohydrate determinants affect the in vitro diagnosis of allergic diseases. *J Allergy Clin Immunol*, 1999;**103**:1005–1011.
- ISHIHARA H, TAKAHASHI N, OGURI S, TEJIMA S. Complete structure of the carbohydrate moiety of stem bromelain. *J Biol Chem*, 1979;**254**:10715–10719.
- FAYE L, GOMORD V, FICHETTE-LAINÉ AC, CHRISPEELS MJ. Affinity purification of antibodies specific for Asn-linked glycans containing β 1 (r) 2 xylose or α 1 (r) 3 fucose. *Anal Biochem*, 1993;**209**:104–108.
- JOHANSSON SGO, HOURIHANE JO'B, BOUSQUET J et al. A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. Position paper. *Allergy*, 2001;**56**:813–824.
- KNOL EF, MUL FPJ, LIE WJ, VERHOEVEN AJ, ROOS D. The role of basophils in allergic disease. *Eur Resp J*, 1996;**9**:126–131.
- COZON GJN, FERRÁNDIZ J, PEYRAMOND D, BRUNET JL. Detection of activated basophils using flow cytometry for diagnosis in atopic patients. *Allergol Immunopathol*, 1999;**27**:182–187.
- GRANT JA, ALAM R, LETT-BROWN A. Histamine-releasing factors and inhibitors: historical perspectives and possible implications in human illness. *J Allergy Clin Immunol*, 1991;**88**:683–693.
- KURIMOTO Y, DE WECK L, DAHINDEN CA. The effect of interleukin-3 upon IgE-dependent and IgE-independent basophil degranulation and leukotriene generation. *Eur J Immunol*, 1991;**21**:361–368.
- SUGYVAMA H, EDA R, HOPP JR et al. Importance of interleukin-3 in histamine release from human basophils. *Ann Allergy*, 1993;**71**:391–395.

31. MIADONNA A, SALMACO C, COTTINI M et al. Enhancement of basophil histamine release by interleukin-3: reduced effect in atopic subjects. *Allergy*, 1996;**51**:525–531.
32. YAMAGUCHI M, HIRAI K, OHTA K et al. Nonreleasing basophils convert to releasing basophils by culturing with IL3. *J Allergy Clin Immunol*, 1996;**97**:1279–1287.
33. GREINER M. Two-graph receiver operating characteristic (TG-ROC): update version supports optimisation of cut-off values that minimise overall misclassification costs. *J Immunol*, 1996;**191**:93–94.
34. GANE P, PECQUET C, CRESPEAU H, LAMBIN P, LEYNADIER F, ROUGER P. Flow cytometric monitoring of allergen induced basophil activation. *Cytometry*, 1995;**19**:361–365.
35. BOCHNER BS, STERBINSKY SA, SAINI SA, COLUMBO M, MCGLASHAN DW, JR. Studies of cell adhesion and flow cytometric analyses of degranulation, surface phenotype, and viability using human eosinophils, basophils and mast cells. *J Immunol Meth*, 1997;**13**:61–68.
36. SAINTE-LAUDY J. Application of flow cytometry to analysis of the activation of human basophils. Immunological validation of the method. *Allergy Immunol*, 1998;**30**:41–43.
37. PARIS-KÖHLER A, DEMOLY P, PERSI L, LEBEL B, ARNOUX B. In vitro diagnosis of cypress pollen allergy by using cytofluorimetric analysis of basophils (Basotest). *J Allergy Clin Immunol*, 2000;**105**:339–345.
38. SAPORTA M, KAMEI S, PERSI L, BOUSQUET J, ARNOUX B. Basophil activation during pollen season in patients monosensitized to grass pollens. *Allergy*, 2001;**56**:442–445.
39. SANZ ML, SÁNCHEZ G, GAMBOA PM et al. Allergen-induced basophil activation: CD63 expression detected by flow cytometry in patients allergic to *Dermatophagoides pteronyssinus* and *Lolium perenne*. *Clin Exp Allergy*, 2001;**31**:1007–1013.
40. MONNERET G, GUTOWSKI MC, BIENVENU J. Detection of allergen-induced basophil activation by expression of CD63 antigen using a tricolour flow cytometric method. *Clin Exp Immunol*, 1999;**115**:393–396.
41. STEVENS S, DROUET M, LAURET M-G, LOIRY M, SABBAAH A. Basophil activation test by flow cytometry in *Hymenoptera* venom allergy. *Allergie Immunologie*, 1999;**1**:11–14.
42. MONERET-VAUTRIN DA, SAINTE-LAUDY J, KANNY G, FRÉMONT S. Human basophil activation measured by CD63 expression and LTC4 release in IgE-mediated food allergy. *Ann Allergy Asthma Immunol*, 1999;**82**:33–40.
43. GARCIA-AVILLÉS C, SÁNCHEZ-LOPÉZ G, SANZ ML et al. Flow-cytometric cellular allergen stimulation test (FAST) in the in vitro diagnosis of food allergy. *Allergy*, 2000;**55**:128.
44. ABUAF N, RAJOELY B, GHAZOUANI E et al. Validation of a flow cytometric assay detecting in vitro basophil activation for the diagnosis of muscle relaxant allergy. *J Allergy Clin Immunol*, 1999;**104**:411–418.
45. EBO DG, SCHUERWEGH A, STEVENS WJ. Anaphylactic-anaphylactoid reaction to starch. *Allergy*, 2000;**55**:1098–1099.
46. UASUF C, SANZ ML, GAMBOA PM, CHAZOT M, DE WECK AL. Validation of Flow-cytometric Allergen Stimulation Test (FAST) to assess basophil activation in allergic patients to betalactam antibiotics. *Allergy*, 2000;**55**:129.
47. SÁNCHEZ-LÓPEZ G, SANZ ML, GARCÍA-AVILÉS C et al. Flow-cytometric cellular allergen stimulation test (FAST) in latex allergy. *Allergy*, 2000;**55**:22(abstract).
48. TURJANMAA K, PALOSUO T, ALENIOUS H et al. Latex allergy diagnosis: in vivo and in vitro standardization of a natural rubber latex extract. *Allergy*, 1997;**52**:41–50.
49. NETTIS E, DAMBRA P, TRAETTA PL, LORIA A, FERRANNINI A, TURSI A. Systemic reactions on SPT to latex. *Allergy*, 2001;**4**:355–356.
50. TURJANMAA K, MÄKINEN-KILJUNEN S, REUNALA T, ALENIOUS H, PALOSUO T. Natural rubber latex allergy. The European experience. *Immunol Allergy Clin North Am*, 1995;**15**:71–87.
51. WATTS DN, JACOBS RR, FORRESTER BR, BARTOLUCCI A. An evaluation of the prevalence of latex sensitivity among atopic and non-atopic intensive care workers. *Am J Ind Med*, 1998;**34**:359–363.
52. PRIDGEON C, WILD G, ASTWORTH F, EGNER W, WARD AM. Assessment of latex allergy in a healthcare population: are the available tests valid? *Clin Exp Allergy*, 2000;**30**:1444–1449.
53. BREHLER R, KÜTTIG B, RÜTTER A, FIEBIG H. IgE antibodies to carbohydrate mimic sensitization to NRL. *J Allergy Clin Immunol*, 2001;**107**:S118.