

Determination by Flow Cytometry of Activated Basophils in Patients Sensitized to *Dermatophagoides pteronyssinus* and *Lolium perenne*

A.L. de Weck M.L. Sanz G. Sánchez-López C. Uasuf M. Chazot

Department of Allergology and Clinical Immunology, School of Medicine, University of Navarra, Pamplona, Spain

Key Words

Flow cytometry · Activated basophils · CD63 ·
Dermatophagoides pteronyssinus · *Lolium perenne* ·
In vitro diagnosis

Introduction

Blood basophils are particularly important in IgE-mediated allergic diseases, since they carry allergen-specific IgE immunoglobulins bound to high affinity IgE receptors. For that reason, detection of basophil interaction with allergen in vitro, such as microscopic or flow-cytometric [1] follow-up of basophil degranulation, has already been used in earlier times for diagnoses of allergies.

The CD63 is a protein molecule of 53 kD expressed at rest on the cytoplasmic granules of various cells. It has been shown that human basophils activated in vitro by allergen or anti-IgE antibody through their high affinity receptor for IgE newly also express the CD63 marker on their membrane [2]. In this study, we used a newly optimized flow-cytometric procedure to assess CD63 expression and specific IgE on basophils isolated from patients sensitized to house dust mites (Der p) and timothy pollen (Lol p). We analyzed the reliability of this basophil activation test, establishing the cutoff level that offers the greatest sensitivity and specificity.

Finally, we compared the results of flow cytometry with those of other in vitro diagnostic tests such as specific IgE determination (RAST), histamine release test and Ag-specific sulfidoleukotriene production (Cellular Assay Stimulation Test, CAST).

Material and Methods

54 patients suffering from asthma and/or allergic rhinitis following sensitization to *Dermatophagoides pteronyssinus* and 51 patients sensitized to *Lolium perenne* were investigated. 40 atopic patients and 30 healthy subjects were also selected as controls. Diagnosis was obtained by means of prick and/or intradermal tests, specific IgE determination (CAP-FEIA, Pharmacia, Uppsala), histamine release test by the Siraganian technique modified by us [3] and allergen-specific sulfidoleukotriene production (CAST, Bühlmann, Switzerland). The basophil activation test originally developed by Sabbah and Sainte-Laudy [4] determines the percentage of basophils which expresses CD63 as an activation marker, by means of flow cytometry, after in vitro stimulation with allergen and using double labeling with monoclonal antibody anti-CD63-PE and anti-IgE FITC.

We used aqueous ultrafiltered extracts devoid of any preservatives of *D. pteronyssinus* and *L. perenne* (Bial Aristegi, Bilbao, Spain) at final concentrations of Lol p 0.175 and 0.043 mg/ml and of Der p 1.4 and 0.35 mg/ml protein for histamine release test, Ag-specific sulfidoleukotriene production by CAST and Ag-induced basophil activation test. The basophils from the pellet were double-labeled by adding 20 µl of anti-CD63 PE-labeled antibody (Caltag Laboratories, Burlingame, Calif., USA), in tubes cytometer Falcon diluted 1:80 and anti-IgE FITC-labeled antibody (Caltag Laboratories) diluted 1:60. After incubation for 30 min at 4 °C (protected from light exposure), the cell suspensions were washed and centrifuged again at 1,000 g for 10 min. Then, we added 4 ml of erythrolytic reagent (Or-

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2001 S. Karger AG, Basel
1018–2438/01/1243–0299\$17.50/0

Accessible online at:
www.karger.com/journals/iaa

Correspondence to: Dr. María Luisa Sanz
Department of Allergology and Clinical Immunology
University Clinic of Navarra, PO Box 4209
E-31080 Pamplona (Spain)
Tel. +34 948 25 54 00, Fax +34 948 29 65 00, E-Mail mlsanzlar@unav.es

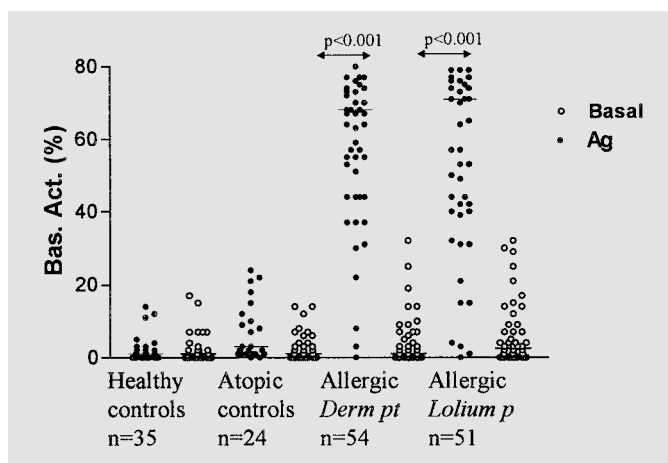


Fig. 1. Flow-Cytometric Allergen Stimulation Test (FAST) in patients allergic to inhalants and controls. Bas. Act. = Basophil activation.

tho-Mune lytic reagent, Ortho Diagnostic System, San Fernando de Henares, Madrid) into each tube and left them at room temperature for 5 min. The reaction was stopped with 1 ml of buffer Hepes, pH 7.3 without calcium or magnesium and containing EDTA (0.1%). After centrifuging for another 5 min at 1,000 *g* the supernatant was decanted and 500 μ l of the Hepes buffer was added into each tube, which was gently shaken before flow-cytometric analysis. Flow-cytometric analysis of surface markers was performed at 488 nm on a FACScan flow cytometer equipped with a 15-nW argon ion laser (Becton Dickinson) and analyzed by CellQuest software.

On the histogram defined by forward scatter and side scatter, the initial cell gate was defined by a bit map around lymphocytes. The second gate was defined around cells showing high density anti-IgE label, identifying them as basophils. In each assay, we assessed at least 500 basophils. The other parameter analyzed on the identified basophils was CD63.

Results and Discussion

No differences in basal values (nonactivated control) were found between sensitized patients, atopic controls and healthy controls. On the other hand, sensitized patients showed a significantly higher percentage of activated basophils after stimulation by allergen *in vitro* than both control groups ($p < 0.001$) (fig. 1). We found a significant correlation between the skin test and basophil activation test ($r = 0.6$, $p < 0.001$). We also found a positive and significant correlation between the basophil activation test and histamine release test ($r = 0.76$, $p < 0.001$), allergen-specific sulfidoleukotriene production ($r = 0.72$, $p < 0.001$) and serum allergen-specific IgE ($r = 0.6$, $p < 0.001$). Flow cytometry is an analytic technique suitable to quantify the expression of cellular membrane markers, such as CD63 on basophils. This technique was previously described and developed by Sainte-Laudy et al. [6–8] for allergy diagnosis in 1994–1996. Some technical aspects have been modified by us, such as the use of allergens in the fluid phase (with dilutions prepared right before use), and the adjustment of the first stimulation phase to the technique described for the CAST assay (Bühlmann Labs), making it possible to run simultaneously both the determination of CD63 expression and evaluation of sulfidoleukotriene production.

The basophil activation test is a highly reliable technique in the diagnosis of allergy to inhalant allergens. The sensitivity of the basophil activation test was 93.5%, and the specificity 98.4%, with a cutoff of 15% for positive results. The good correlation observed between this technique and the test of the *in vivo* and *in vitro* diagnostic techniques makes us consider the basophil activation test a highly reliable procedure for the detection of IgE-mediated allergy to inhalant allergens.

References

- 1 Gane P, Pecquet C, Crespeau H, Lambin P, Abuaf N, Leynadier F: Flow cytometric evaluation of human basophils. *Cytometry* 1993;14: 344–348.
- 2 Gane P, Pecquet C, Crespeau H, Lambin P, Abuaf N, Leynadier F, Rouger P: Flow cytometric monitoring of allergen induced basophil activation. *Cytometry* 1995;19:361–365.
- 3 Castillo JG, Gamboa PM, Oehling A, Wong E, De la Cuesta CG: Variations in antigen-specific histamine release related with immunotherapeutic treatment. *Allergol Immunopathol* 1989;7:4872–4877.
- 4 Sabbah A, Sainte-Laudy J: Flow cytometry applied to the analysis of lymphocyte and basophil activation. *ACI Int* 1996;8/4:116–120.
- 5 Sabbah A, Lauret MG, Maillard H: Preliminary study of basophil activation test for drug allergy using anti-membrane antibodies and flow cytometry. *Allerg Immunol (Paris)* 1995; 27:274–276.
- 6 Sainte-Laudy J, Vallon C, Guérin JC: Diagnosis of latex allergy: Comparison of histamine release and flow cytometric analysis of basophil activation. *Inflamm Res* 1996;45(suppl 1):35–36.
- 7 Sainte-Laudy J: Application of flow cytometry to the analysis of activation of human basophils. *Immunologic validation of the method. Allerg Immunol (Paris)* 1998;30:41–43.
- 8 Sainte-Laudy J, Drouet M, Lauret MG, Loiry M, Sabbah A: Test d'activation des basophiles par cytométrie en flux dans l'allergie aux venins d'hyménoptères. *Allerg Immunol (Paris)* 1999;31:11–14.

Copyright: S. Karger AG, Basel 2001. Reproduced with the permission of S. Karger AG, Basel. Further reproduction or distribution (electronic or otherwise) is prohibited without permission from the copyright holder.