Arch. Immunol. Ther. Exp. (2010) 58:459–465
DOI 10.1007/s00005-010-0104-z

ORIGINAL ARTICLE

Validation of Basophil CD164 Upregulation for Pollen Allergy Diagnosis

Anna Wolanczyk-Medrala · Wojciech Barg · Jerzy Liebhart · Bernard Panaszek · Grazyna Nadobna · Marta Litwa · Grzegorz Gogolewski · Wojciech Medrala

Received: 26 February 2010 / Accepted: 23 July 2010 / Published online: 28 September 2010

Abstract The aim of the study was to evaluate the sensitivity and specificity of allergen-induced basophil CD164 upregulation in patients with seasonal allergic rhinitis caused by allergy to grass pollens. This study was performed in 24 patients with allergy to grass pollens, and in 25 healthy controls. The protocol for allergen-induced basophil CD164 upregulation consisted of whole blood samples processing and staining with anti-CCR3/anti-CD164 antibodies added to a buffer at the beginning of stimulation. We observed dose-dependent allergen-induced basophil CD164 upregulation with 100% of specificity in both used allergen concentrations (12 and 1.2 ng/ml). Higher allergen concentration resulted in 100% and lower concentration in only 70.83% sensitivity. We have observed in the patients statistically significant correlations between anti-IgE stimulation and both allergen concentrations (for 12 ng/ml, $r = 0.71$, $p < 0.0001$; and for 1.2 ng/ml, $r = 0.64$, $p < 0.001$). We conclude that assessment of allergen-induced basophil CD164 upregulation is a very useful method for in vitro determination of allergy to grass pollens. This method seems to be a very promising tool in laboratory testing of allergies to other allergens.

Keywords Allergy · Basophil · CD164 · Flow cytometry · Basophil activation test

A. Wolanczyk-Medrala · J. Liebhart · B. Panaszek · G. Nadobna · M. Litwa · G. Gogolewski · W. Medrala (✉)
Department of Internal Diseases, Geriatrics and Allergology, Medical University of Wroclaw, Traugutta 57, 50-417 Wroclaw, Poland
e-mail: wojmed@wp.pl

A. Wolanczyk-Medrala · W. Medrala
Department of Clinical Research, Medical School of Legnica, Legnica, Poland

W. Barg
Department of Physiology, Medical University of Wroclaw, Wroclaw, Poland

Introduction

Making an allergy diagnosis sometimes requires laboratory support in order to avoid potentially dangerous systemic reactions during traditional clinical testing. Determination of allergen-specific IgE is the method most widely used, but it is well known that sometimes the results are false negative and need additional verification. Discovery of basophil activation markers such as CD63 and CD203c led to the development of the first generation of flow cytometric basophil activation tests (BATs) (Bühring et al. 1999; Kahlert et al. 2003; Sainte-Laudy et al. 1994; Sanz et al. 2002). These tests were successfully used as in vitro diagnostic tools in different clinical situations including allergy to inhalants, food, Hymenoptera, latex and drug allergens (Ebo et al. 2005,2006,2007; Erdmann et al. 2003; Gamboa et al. 2003; Moneret-Vautrin et al. 1999; Ocmant et al. 2007; Sanz et al. 2001,2006; Scherer et al. 2008). Some unsuccessful results, especially in drug allergy testing, recently encouraged investigators to introduce some improvements, but their diagnostic usefulness still requires validation in comparative studies (Boumiza et al. 2005; Monneret et al. 2002; Sainte-Laudy et al. 2007).

Newly discovered basophil activation markers such as CD13, CD107a and CD164 open new possibilities in the development of new BATs, but at present the diagnostic usefulness of these markers remains unknown and requires

precise determination (Hennersdorf et al. 2005; Kneidinger et al. 2008).

The aim of the study was to evaluate the sensitivity and specificity of allergen-induced basophil CD164 upregulation in patients with seasonal allergic rhinitis caused by allergy to grass pollens.

Materials and Methods

Patients and Controls

Twenty-four persons (13 males and 11 females) aged from 19 to 38 years (Me 23 years) with seasonal allergic rhinitis with positive skin prick tests to 6-Grass allergen mix (Allergopharma Joachim Ganzer KG, Reinbek, Germany) entered the study. Twenty-five healthy persons (8 males and 17 females) aged from 19 to 28 years (Me 19 years) with no symptoms and negative skin prick test results served as the controls. All the procedures used were in accordance with the ethical standards of the Helsinki Declaration and its amendment in Tokyo and Venice and were accepted by the Ethics Committee of the local Medical University. Informed consent was obtained from all patients and controls.

Basophil CD164 Upregulation

Blood specimens were collected into K_3 EDTA venipuncture tubes (Sarstedt AG & Co, Nümbrecht, Germany) and immediately used for cell stimulation in BD Falcon™ Round Bottom Tubes. Each patient's sample was tested in separate tubes for background values, and two positive stimulation controls: with Rabbit polyclonal anti-Human IgE antibody at the final concentration of 10 μg/ml (Dako Denmark A/S, Glostrup, Denmark), and with fMLP at the final concentration of 1 μM (Sigma, St. Louis, USA) and with the 6-Grass allergen mix (Bühlmann Laboratories AG, Schönenbuch, Switzerland) at the two final concentrations of 12 and 1.2 ng/ml. This allergen mix contains Orchard Grass, Meadow Fescue, Rye Grass, Timothy Grass, Meadow Grass, and Velvet Grass, and is identical to the grass mix used in skin prick tests provided by Allergopharma Joachim Ganzer KG.

Cell stimulation and Staining Protocol

In the first step 20 μl of allergen solution was mixed with—150 μl of PBS enriched in calcium and magnesium (final concentration 1 mM), 5 μl of anti-CCR3 antibody (PE Mouse anti-Human CD193-BD Biosciences Pharmingen, San Diego, USA—for basophil identification) and 20 μl of anti-CD164 antibody (FITC Mouse anti-Human CD164, BD Biosciences Pharmingen, San Diego, USA—to determine the degree of basophil activation). In the second step 50 μl of patient's whole blood was added to each tube, gently mixed, covered and incubated for 20 min at 37°C in a water bath. In other tubes instead of allergen solution, 20 μl of PBS (for determination of patient background) or 20 μl of positive controls was added (anti-IgE or fMLP). The stimulation process was terminated by the addition of 2 ml of diluted, pre-warmed (20–25°C) FACS Lysing Reagent 10X Concentrate (BD Biosciences Pharmingen, San Diego, USA). After mixing, the tubes were incubated at room temperature for 10 min and centrifuged (5 min, $500\times g$). Supernatants were decanted and cell pellets were resuspended with 300 μl of PBS with 1% paraformaldehyde and gently vortexed. The total amount of 100 000 events was acquired per sample using a FACScan flow cytometer (BD Biosciences). In the initial part of the study we performed observations concerning the influence of presence of staining antibodies on basophil activation degree. For this purpose incubations were performed with or without staining antibodies at the same time in separate tubes. The incubations without staining antibodies were terminated by the addition of EDTA Solution (BD Biosciences Pharmingen, San Diego, USA). After centrifugation ($200\times g$, 10 min) the cells were resuspended in 100 μl PBS and incubated for 20 min with the mentioned antibodies. The lysis of red blood cells was performed with Lysing Solution (BD Biosciences Pharmingen, San Diego, USA) and after centrifugation they were resuspended with 300 μl of PBS with 1% paraformaldehyde, gently vortexed and acquired. In these experiments the time of stimulation was modified (range 5–30 min).

Data Analysis

The data analysis was performed by CellQuest flow cytometry analysis software (BD Biosciences). In the first step the basophil population was gated as the $CCR3^{high}$ and SSC^{low} cells. In the second step, calculation of the percentage of CD164 positive cells (detected as brightly fluorescent FITC) was performed by comparison of its number to the total amount of cells gated in R1 (Fig. 1). Results of the stimulation were presented in percentages after subtraction of the background values. The cut-off values for allergen stimulation positivity and specificity were determined on the basis of two-graph receiver-operating characteristics analysis (Ebo et al. 2007; Greiner et al. 2000; Plebani et al. 1996). For both positive controls cut-off values were determined by adding to the mean value the double standard deviation value of CD164 positive basophils observed in patients' background tubes (Mean + 2 × SD). Persons with negative results in anti-IgE stimulation were excluded from the presentation and statistical analysis.

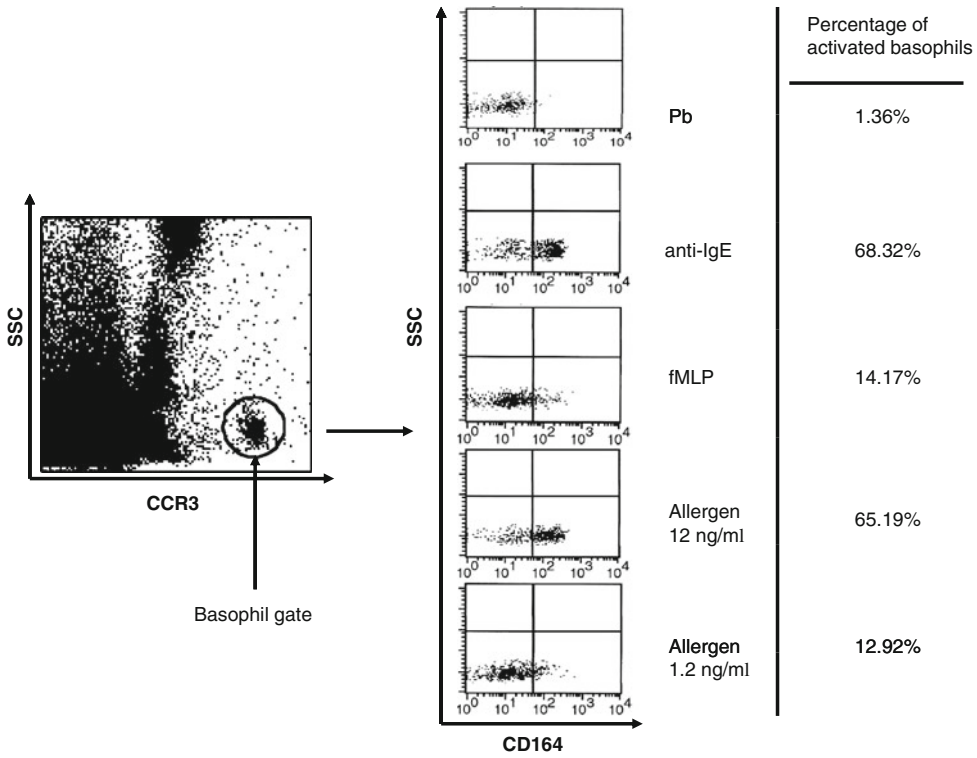

Fig. 1 Gating strategy. *pb* patient background

Statistics

The data distributions were checked for normality using the Chi-square test. According to the results obtained the nonparametric Wilcoxon test was used for further analysis. All results were expressed as median (range). Correlations were analyzed with Spearman's rank test.

Results

Basophil Number

The number of basophils ranged from 181 to 790 cells (Me 390) and the difference between the two tested groups was not statistically significant ($p = 0.501$).

Patient Background

In all tested individuals background values were at very low levels: Me 1.625%, Min 0.23%, Max 2.86%.

Time-dependent CD164 Upregulation and Possible Influence of Staining Antibodies on Basophil Activation

In order to observe the impact of time on basophil CD164 upregulation and the influence of staining antibodies during the activation process we performed experiments in five patients. These initial experiments showed a bell-shape response with maximal intensity in the 20th minute. Moreover, presence of staining antibodies during activation did not influence the final results of CD164 upregulation ($p = 0.0678$; Fig. 2).

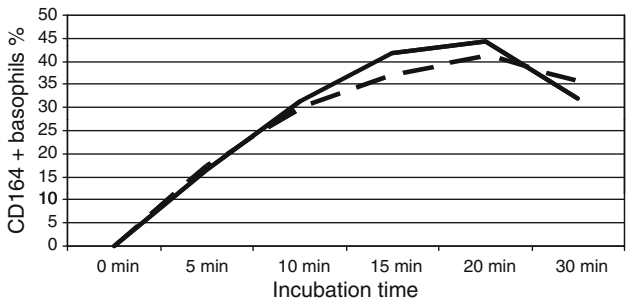

Fig. 2 Time-dependent basophil CD164 Upregulation. Blood Specimens were incubated with anti-IgE antibody for different time periods with (*solid line)* and without (*dashed line*) staining antibodies

Positive Controls

On the basis of patient background values the cut-off point for both positive controls was calculated at the level of 2.94% ($X + 2 \times SD$). In the healthy controls anti-IgE stimulation resulted in moderate basophil CD164 upregulation. The median percentage of CD164 positive basophils is 27.89% (Min 3.78%, Max 67.66%). Positive results of the stimulation were observed in 20 persons (80%). In 5

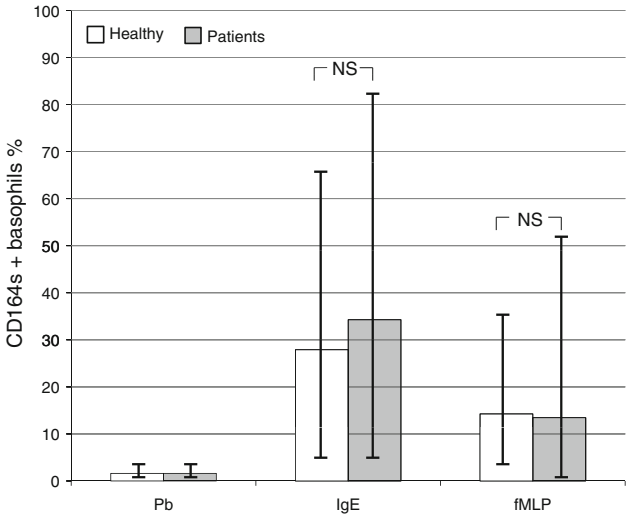

Fig. 3 Percentages of CD164 positive basophils in nonstimulated and stimulated by anti-IgE and fMLP blood samples in patients and control group. *Pb* patient background; *NS* not significant

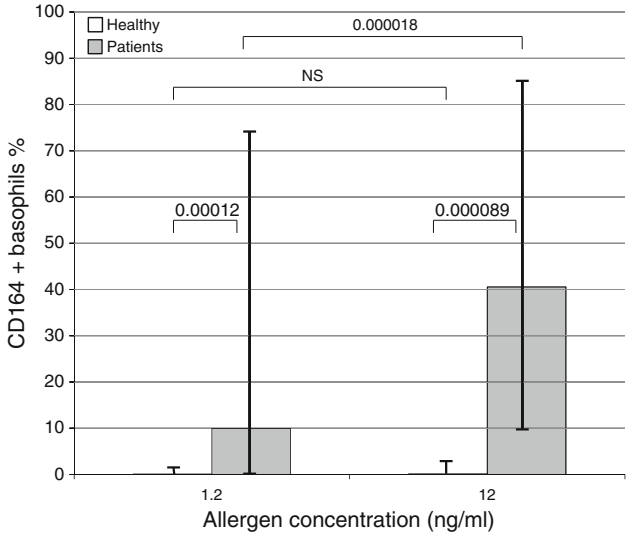

Fig. 4 Allergen-induced CD164 positive basophils after allergen stimulation in patients and control group. *NS* not significant

controls (20%) anti-IgE stimulation was negative and all their results were excluded from the presentation and further statistical analysis. Stimulation with fMLP was positive in all participating persons (Fig. 3). The median percentage of CD164 positive cells was 14.44% (Min 3.07%, Max 35.98%).

In the patients, anti-IgE stimulation also resulted in moderate basophil CD164 upregulation and was positive in each individual patient (Me 32.95%, Min 3.62%, Max 82.17%). After fMLP stimulation the median percentage of CD164 positive cells was 13.11% (Min 0%, Max 51.45%) and was positive in 23 patients (95.83%), and negative in 1 patient (4.17%). The results after anti-IgE stimulation were slightly higher in the patients than in the controls ($p = 0.327$). After fMLP stimulation we observed slightly stronger effects of stimulation in healthy persons than in the patients, but again the difference was not statistically significant ($p = 0.077$; Fig. 3).

Allergen-induced Basophil CD164 Upregulation

In the controls, basophil stimulation with higher allergen concentration of 12 ng/ml resulted in the median of CD164 positive basophils at the level of 1.51% (Min 0%, Max 3.84%). The lower allergen concentration of 1.2 ng/ml resulted in the same low level of CD164 positive basophils (Me 0%, Min 0%, Max 2.3%). These results were not statistically different ($p = 0.059$; Fig. 4). In the patients, the median percentage of CD164 positive basophils after stimulation with higher allergen concentration was 40.55% (Min 9.84%, Max 86.59%). These results were significantly higher than in the controls ($p = 0.000089$).

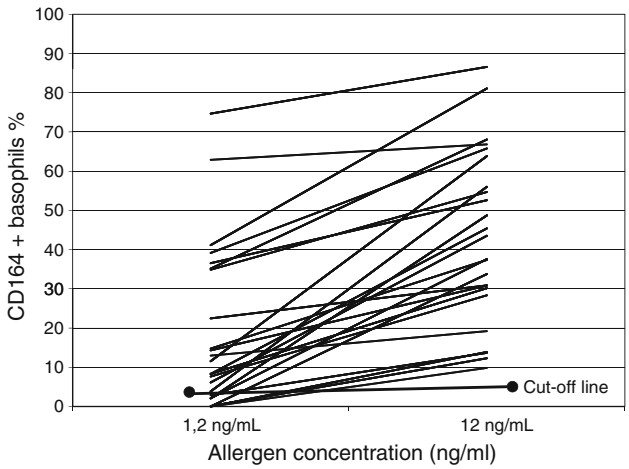

Fig. 5 Individual data of dose-dependent allergen-induced CD164 upregulation in the patients

Stimulation with lower allergen concentration resulted in decreased CD164 basophil upregulation. Median percentage was 9.94% (Min 0%, Max 74.67%). These results were significantly higher than in controls ($p = 0.00012$; Fig. 4). In every patient we observed allergen-induced basophil CD164 upregulation in a dose-dependent fashion and the difference between stimulation with both allergen concentrations used was statistically significant ($p = 0.000018$; Fig. 5).

The positivity and specificity of the cut-off values after allergen stimulation were determined on the basis of two-graph receiver-operating characteristics analysis. An absolute sensitivity and specificity for allergen stimulation with the higher concentration of 12 ng/ml were observed at the level of 4% of CD164 positive basophils (Fig. 6). At

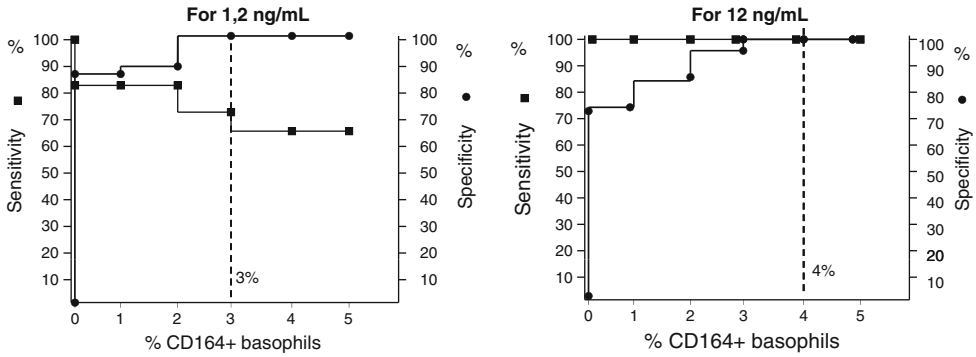

Fig. 6 Sensitivity and specificity of allergen-induced CD164 upregulation determined by receiver-operating characteristic curves for different allergen concentrations

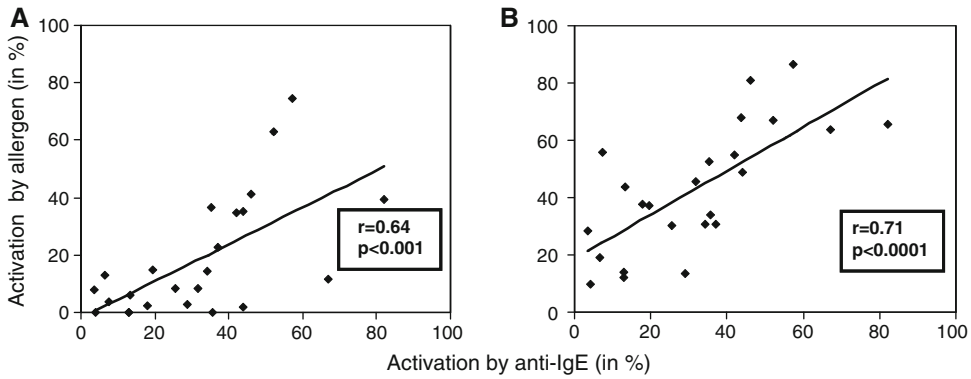

Fig. 7 Correlations between CD164 upregulation after stimulation by anti-IgE and both allergen concentrations (**a** 1.2 ng/ml, **b** 12 ng/ml)

the lower allergen concentration an absolute specificity was observed at the cut-off value of 3%, but sensitivity was only 70.83% (Fig. 6).

In the patients correlations between anti-IgE stimulation and both allergen concentrations were statistically significant (for 12 ng/ml, $r = 0.71, p < 0.0001$; and for 1.2 ng/ml, $r = 0.64, p < 0.001$; Fig. 7). Contrary to these data we did not observe statistically significant correlations between fMLP and IgE stimulation ($r = 0.26$, $p = 0.9$) and in both allergen concentrations (for 12 ng/ml, $r = 0.3$, $p = 0.27$; and for 1.2 ng/ml, $r = 0.14$, $p = 0.73$).

Discussion

CD164 plays a role in hematopoiesis by facilitating the adhesion of human $CD34^+$ cells to the stroma and by negatively regulating $CD34^+CD38^{lo/-}$ cell proliferation (Chan et al. 2001), in the development and regeneration of skeletal muscle (Bae et al. 2008) and in prostate cancer metastasis (Havens et al. 2006). Its role in allergic diseases is currently unknown. The discovery of CD164 as a new basophil activation marker and some observations of its IgE-dependent upregulation were first reported by Hennersdorf et al. (2005). The next study, published in 2008, presented data concerning both anti-IgE and allergen-induced basophil CD164 upregulation, but was performed only in allergic patients without controls and mainly focused on pharmacological aspects of dasatinib (tyrosine kinases inhibitor) (Kneidinger et al. 2008). Because allergen stimulation in control persons was not presented in that study, diagnostic usefulness of CD164 upregulation upon allergen stimulation remained an open issue. This encouraged us to an attempt to develop a new in vitro method using flow cytometric determination of CD164 upregulation and to evaluate its sensitivity and specificity by comparing results from allergic and healthy persons. Time-dependent observation performed in our study confirmed rapid and significant IgE-dependent basophil CD164 upregulation, which belongs to the "CD203c group" (Hennersdorf et al. 2005). Our results showed a bell-shape response with maximal intensity in the 20th minute. Moreover, we detected no influence of staining antibodies during the stimulation stage on the final results. In other words, neither antibody stimulates nor inhibits basophil response. This important observation enabled us to perform stimulation and staining steps simultaneously, resulting in time saving and reasonable simplification of the method.

Identification of basophils by anti-IgE or CD203c is in many cases a powerful method, but incidentally may create some problems. In the case of anti-IgE, it may lead to contamination of other cells (dendritic cells and monocytes) (Abuaf et al. 2008; Ocmant et al. 2007) and in the case of CD203c to omission of up to 50% of basophils (Boumiza et al. 2005). Optimization of basophil gating by these methods requires the introduction of additional antibodies, which results in additional costs and time elongation during the interpretation procedure (Abuaf et al.

2008; Boumiza et al. 2005; Ocmant et al. 2007). Taking into account these data we used anti-CCR3 antibody, which gave the opportunity for easy gating of the entire basophil population with negligible contamination of other cells (Ducrest et al. 2005).

Control stimulations with anti-IgE antibody and fMLP gave satisfactory results, as observed in other similar studies (Ebo et al. 2005; Ebo et al. 2006; Kahlert et al. 2008; Monneret et al. 2002). All patients positively responded with anti-IgE stimulation; in contrast 20% of controls were negative. This seems to be consistent with previous observations that basophils from 10 to 20% of normal subjects do not degranulate in response to IgE- or FcεRI-dependent stimulation (Yamaguchi et al. 1996) and was also partially confirmed in the BAT technique (Sanz et al. 2001). These subjects were classified as non-responders and therefore were excluded from the presentation and statistical analysis. Stimulation by fMLP in this study design is not necessary but in the case of non-IgE-dependent basophil reactions it might be helpful, especially when the anti-IgE control response is negative.

Sensitivity and specificity of the results of allergen-induced basophil CD164 upregulation were calculated on the basis of two-graph receiver-operating characteristics analysis. Allergen-induced basophil CD164 upregulation was observed in a dose-dependent fashion and in higher allergen concentration (12 ng/ml) the results were positive in all tested patients with an absolute specificity at the cut-off value of 4%. Lower allergen concentration of 1.2 ng/ml resulted in decrease of sensitivity to 70.83% with 100% specificity at the cut-off value of 3%. The higher allergen concentration used in our study is the highest commercially available one. This and very comparable concentrations of the allergen were successfully tested in vitro by our group for basophil leukotriene C4 production (Medrala et al. 1997) and CD63 upregulation assessment (Wolanczyk-Medrala et al. 2009); therefore it was also used in this study, with excellent efficiency. Calculated statistical thresholds based on our results allow one to distinguish between healthy persons and persons with disease caused by allergy to grass pollens. However, in some healthy persons with positive skin tests to this allergen mixture in vitro results might be positive (just like skin tests and allergen-specific IgE) and apparently suggest the presence of disease. Therefore comparison between laboratory and clinical data is still necessary to establish a precise final diagnosis. Moreover, the possibility of cross-reactivity between different allergens might be a cause of some diagnostic mistakes; therefore establishing a "clinical threshold" clearly indicating cut-off values should be the next step of studies employing the BAT technique in the future. Determination of this threshold would give more information than a simple description of allergic status—establishment of allergic disease caused by a specific allergen.

The significant correlations between results of stimulation by anti-IgE and both allergen concentrations observed in our study confirm the old fact that the results of allergen stimulation depend on the density of allergen-specific IgE on the basophil surface.

A direct comparison of our method with other BATs is impossible, as we did not perform a comparative parallel study. However, our earlier experiment on a comparable population with allergy to grass pollens gave almost the same excellent results in terms of sensitivity and specificity of allergen-induced basophil CD63 upregulation (Wolanczyk-Medrala et al. 2009). However, in the mentioned study the cut-off points calculated on the basis of the same receiver-operating characteristic curve analysis were higher (even 15%) but the median response was much higher and exceeded even 92% of CD63 positive basophils. The ratio between the median response and cut-off level (R/C ratio) was about 6. Such high values, never previously reported by other investigators in diagnosing allergy to inhalant allergens, most probably depend on technical differences in BAT protocols (Cozon et al. 1999; González-Muñoz et al. 2008; Paris-Köhler et al. 2000; Sanz et al. 2001). In the present study the median response to higher allergen concentration was observed at the level of 40% of CD164 positive basophils but the R/C ratio reached the value of 10 and in one case with an extremely high response exceeded the value of 21. Despite this difference all the mentioned studies showed the very high diagnostic usefulness of allergen-induced CD63 or CD203c upregulation in inhalant allergy. Our results suggest that beside these two well established activation markers, a new and powerful laboratory tool—CD164 upregulation assessment— might be used with comparable efficiency.

To conclude, in this paper we presented a very sensitive flow cytometric BAT for in vitro grass pollen allergy diagnosis based on allergen-induced basophil CD164 upregulation. This method employs the accepted technique of basophil identification, offers a simplification of technical aspects as well as low cut-off levels, and allows one to acquire data in a relatively short time. The results obtained encourage further studies on CD164 upregulation in allergy to other allergens. Experiments directly comparing the efficiency of this method to other BATs are needed.

References

Abuaf N, Rostane H, Rajoely B et al (2008) Comparison of two basophil activation markers CD63 and CD203c in the diagnosis of amoxicillin allergy. Clin Exp Allergy 38:921–928

Bae GU, Gaio U, Yang YJ et al (2008) Regulation of myoblast motility and fusion by the CXCR4-associated sialomucin, CD164. J Biol Chem 283:8301–8309

Boumiza R, Debard AL, Monneret G (2005) The basophil activation test by flow cytometry: recent developments in clinical studies, standardization and emerging perspectives. Clin Mol Allergy 30:9

Bühring HJ, Simmons PJ, Pudney M et al (1999) The monoclonal antibody 97A6 defines a novel surface antigen expressed on human basophils and their multipotent and unipotent progenitors. Blood 94:2343–2356

Chan JY, Lee-Prudhoe JE, Jorgensen B et al (2001) Relationship between novel isoforms, functionally important domains, and subcellular distribution of CD164/endolyn. J Biol Chem 276:2139–2152

Cozon G, Ferrándiz J, Peyramond D et al (1999) Detection of activated basophils using flow cytometry for diagnosis in atopic patients. Allergol Immunopathol 27:182–187

Ducrest S, Meier F, Tschopp C et al (2005) Flowcytometric analysis of basophil counts in human blood and inaccuracy of hematology analyzers. Allergy 60:1446–1450

Ebo DG, Hagendorens MM, Bridts CH et al (2005) Flow cytometric analysis of in vitro activated basophils, specific IgE and skin tests in the diagnosis of pollen-associated food allergy. Cytometry B Clin Cytom 64:28–33

Ebo DG, Bridts CH, Hagendorens MM et al (2006) Flow-assisted diagnostic management of anaphylaxis from rocuronium bromide. Allergy 61:935–939

Ebo DG, Hagendorens MM, Schuerwegh AJ et al (2007) Flow-assisted quantification of in vitro activated basophils in the diagnosis of wasp venom allergy and follow-up of wasp venom immunotherapy. Cytometry B Clin Cytom 72:196–203

Erdmann SM, Heussen N, Moll-Slodowy S et al (2003) CD63 expression on basophils as a tool for the diagnosis of pollen-associated food allergy: sensitivity and specificity. Clin Exp Allergy 33:607–614

Gamboa PM, Sanz ML, Caballero MR et al (2003) Use of CD63 expression as a marker of in vitro basophil activation and leukotriene determination in metamizol allergic patients. Allergy 58:312–317

González-Muñoz M, Villota J, Moneo I (2008) Analysis of basophil activation by flow cytometry in pediatric house dust mite allergy. Pediatr Allergy Immunol 19:342–347

Greiner M, Pfeiffer D, Smith RD (2000) Principles and practical application of the receiver-operating characteristic analysis for diagnostic tests. Prev Vet Med 45:23–41

Havens AM, Jung Y, Sun YX et al (2006) The role of sialomucin CD164 (MGC-24v or endolyn) in prostate cancer metastasis. BMC Cancer 6:195

Hennersdorf F, Florian S, Jakob A et al (2005) Identification of CD13, CD107a, and CD164 as novel basophil-activation markers and dissection of two response patterns in time kinetics of IgE-dependent upregulation. Cell Res 15:325–335

Kahlert H, Cromwell O, Fiebig H (2003) Measurement of basophil activating capacity of grass pollen allergens, allergoids and hypoallergenic recombinant derivatives by flow cytometry using anti-CD203c. Clin Exp Allergy 33:1266–1272

Kahlert H, Suck R, Weber B et al (2008) Characterization of a hypoallergenic recombinant Bet v 1 variant as a candidate for allergen-specific immunotherapy. Int Arch Allergy Immunol 145:193–206

Kneidinger M, Schmidt U, Rix U et al (2008) The effects of dasatinib on IgE receptor-dependent activation and histamine release in human basophils. Blood 111:3097–3107

Medrala W, Malolepszy J, Medrala AW et al (1997) CAST-ELISA test—a new diagnostic tool in pollen allergy. J Investig Allergol Clin Immunol 7:32–35

Moneret-Vautrin DA, Sainte-Laudy J, Kanny G et al (1999) Human basophil activation measured by CD63 expression and LTC4 release in IgE-mediated food allergy. Ann Allergy Asthma Immunol 82:33–40

Monneret G, Benoit Y, Debard AL et al (2002) Monitoring of basophil activation using CD63 and CCR3 in allergy to muscle relaxant drugs. Clin Immunol 102:192–199

Ocmant A, Peignois Y, Mulier S et al (2007) Flow cytometry for basophil activation markers: the measurement of CD203c up-regulation is as reliable as CD63 expression in the diagnosis of cat allergy. J Immunol Methods 320:40–48

Paris-Köhler A, Demoly P, Persi L et al (2000) In vitro diagnosis of cypress pollen allergy by using cytofluorimetric analysis of basophils (Basotest). J Allergy Clin Immunol 105:339–345

Plebani M, Borghesan F, Basso D et al (1996) Receiver-operating characteristic (ROC) curves: a fundamental tool for improving the clinical usefulness of in vitro IgE tests. Allergy 51:407–411

Sainte-Laudy J, Vallon C, Guérin JC (1994) Analysis of membrane expression of the CD63 human basophil activation marker. Applications to allergologic diagnosis. Allerg Immunol 26:211–214

Sainte-Laudy J, Boumediene A, Touraine F et al (2007) Use of both CD63 up regulation and IgE down regulation for the flow cytometric analysis of allergen induced basophil activation. Definition of an activation index. Inflamm Res 56:291–296

Sanz ML, Sanchez G, Gamboa PM et al (2001) Allergen-induced basophil activation: CD63 cell expression detected by flow cytometry in patients allergic to Dermatophagoides pteronyssinus and Lolium perenne. Clin Exp Allergy 31:1007–1013

Sanz ML, Gamboa P, Antépara I et al (2002) Flow cytometric basophil activation test by detection of CD63 expression in patients with immediate-type reactions to betalactam antibiotics. Clin Exp Allergy 32:277–286

Sanz ML, García-Avilés MC, Tabar AI et al (2006) Basophil Activation Test and specific IgE measurements using a panel of recombinant natural rubber latex allergens to determine the latex allergen sensitization profile in children. Pediatr Allergy Immunol 17:148–156

Scherer K, Weber JM, Jermann TM et al (2008) Cellular in vitro assays in the diagnosis of Hymenoptera venom allergy. Int Arch Allergy Immunol 146:122–132

Wolanczyk-Medrala A, Gogolewski G, Liebhart J et al (2009) A new variant of basophil activation test for allergen-induced basophil CD63 upregulation. The effect of cetirizine. J Investig Allergol Clin Immunol 19:465–473

Yamaguchi M, Hirai K, Ohta K et al (1996) Nonreleasing basophils convert to releasing basophils by culturing with IL-3. J Allergy Clin Immunol 97:1279–1287