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A novel tool for clinical diagnosis of allergy operating a microfluidic immunoaffinity basophil activation test technique

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ABSTRACT

The Basophil Activation Test (BAT) is a valuable allergy diagnostic tool but is time-consuming and requires skilled personnel and cumbersome processing, which has limited its clinical use. We therefore investigated if a microfluidic immunoaffinity BAT (miBAT) technique can be a reliable diagnostic method. Blood was collected from allergic patients and healthy controls. Basophils were challenged with negative control, positive control (anti-FcεRI), and two concentrations of a relevant and non-relevant allergen. CD203c and CD63 expression was detected by fluorescent microscopy and flow cytometry. In basophils from allergic patients the CD63% was significantly higher after allergen activation as compared to the negative control ($p < .0001$–$p = .0004$). Activation with non-relevant allergen showed equivalent CD63% expression as the negative control. Further, the miBAT data were comparable to flow cytometry. Our results demonstrate the capacity of the miBAT technology to measure different degrees of basophil allergen activation by quantifying the CD63% expression on captured basophils.

1. Introduction

Allergy is a global health problem caused by an exaggerated immunological reaction affected by several factors e.g. the propensity to form IgE-antibodies (IgE-ab) as a response to allergen exposure (e.g. pollen, dust mites, mold spores, pet dander and foods), genetic constitution and environmental factors [1,2]. The incidence of allergy, mainly against food allergens, is increasing in young people and the prevalence of allergic diseases is estimated to be 25–30% [3]. Basophils have a major role in the immune responses of IgE-mediated allergic reactions provoked by re-exposure to a specific allergen. The IgE-ab produced by plasma cells predominantly bind to high affinity receptors (FcεRI) on the surface of mast cells and basophils. Cross-linking of the allergen to IgE-ab on basophils and mast cells causes activation, degranulation, and release of a variety of immune-modulators e.g. histamine [4,5]. These events mediate a response that can result in mild to very severe symptoms (anaphylaxis) and occasionally to a fatal systemic reaction [6].

Allergy diagnostic methods have been developed overtime. Today, IgE-ab are routinely analyzed in blood and/or indirectly in the skin with skin prick test (SPT) in the diagnosis of allergy [7,8]. To distinguish sensitization from true food allergy, a double-blind placebo-controlled food challenge (DBPCFC) is “gold standard”. However, this is time-consuming, expensive and rarely performed in routine care [9]. For allergens from the plant kingdom e.g. peanuts, the introduction of analyses of IgE-ab against individual proteins (components) has revolutionized the diagnostics with both high sensitivity and specificity [10]. Similar concepts have been tested for egg, milk and other animal allergen, but since these have a more complex sensitization pattern and the proteins a more complex structure, well-functioning components with high sensitivity and specificity have not yet been produced. This deficiency has led to the development of other diagnostic tests which are based on analysis of basophils. One example is the introduction of a flow cytometric technique for the detection of the surface expression of CD63 to quantify basophil activation, the Basophil Activation Test (BAT). BAT has successfully been used to diagnose IgE-mediated

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allergies e.g. in food, hymenoptera venom, latex, and inhalant allergies [11]. Furthermore, BAT can also be used to monitor patients undergoing allergen immunotherapy (AIT) and treatment with monoclonal anti-IgE-ab (omalizumab) by assessing the basophil allergen threshold sensitivity (CD-sens) [12]. However, flow cytometry-based BAT is time consuming, expensive and the technical requirements for the operation have all together limited it's use in the clinic.

Microfluidics is a technology that handle small volume of fluids with high precision, generally on the microliter to nanoliter scale. Microfluidics present solutions to overcome the limitations of conventional methods to perform BAT. The technology enables high throughput automated analysis, low cost, integration of several steps in one device and reduces the required amounts of reagents and biological samples, [13,14]. Microfluidic immunoaffinity cell separation allows a specific method for cell enrichment, where antibodies directed against the cells of interest are immobilized onto the microfluidic surface for cell capture followed by optical detection. The affinity-capture technique has been previously used to isolate leukocytes from whole blood such as neutrophils and lymphocytes [15,16].

We have recently developed a miBAT device capable of capturing CD203c positive cells directly from whole blood for in vitro activation using anti-FcεRI-ab followed by detection of CD203c and CD63 expression on captured basophils (Aljadi et al., JALM, In Press, DOI: https://doi.org/10.1373/jalm.2018.026641). Here, to further validate the miBAT technology to be used as a reliable diagnostic method for allergy, we assessed the CD63 expression of allergen exposed captured basophils in the microfluidic chip in allergic patients compared to healthy controls. An established flow cytometry analysis of basophil activation was run in parallel.

2. Material and methods

2.1. Study population

Venous blood samples from healthy donors (Blood Bank, Stockholm, Sweden) and allergic patients ($n = 10$) (Sachs' Children and Youth Hospital, Stockholm, Sweden) were collected in 10 ml Na + Heparin vacutainer tubes (Vacutainer, Becton Dickinson, UK), stored in 4°C and analyzed within 24 h. The inclusion criteria for allergic patients were a medical history of allergic symptoms and IgE-ab to at least one of the allergens; birch, timothy, dog, cat or horse (relevant allergen) but without IgE-ab to the control allergens (non-relevant allergen) dust mite or bee. The healthy donors were non-allergic and had no IgE-ab to birch, timothy, dog, cat, horse, dust mite or bee. Clinical characteristics of the study population are illustrated in the demographic table (See Table 1).

The study was approved by the regional ethics committee in Stockholm, Sweden Dnr. 2014/1630–31/4.

2.2. Serological analyses

Serum samples were sent to the diagnostic laboratory at the Karolinska University Laboratory. Specific IgE-antibodies (IgE-ab) to airborne allergens (timothy, birch, cat, dog, horse, mite and bee) were analyzed using ImmunoCAP® (Thermo Fisher Scientific, Uppsala, Sweden), according to the instructions of the manufacturer. The cut off for positive test was set to ≥ 0.35 kU_A/l.

2.3. Analysis of absolute number of basophils in allergic patients and healthy controls

The absolute number of basophils was analyzed before microfluidic chip experiments in allergic patients and healthy controls. One hundred µl of whole blood was treated with the Immunoprep reagent system (Beckman Coulter, USA) according to manufacturer's instructions. Thereafter the pre-treated whole blood was mixed with 100 µl Flow-Count beads (Beckman Coulter, USA), and samples were analyzed by flow cytometry. The total number of leukocytes was counted and the number of basophils was calculated using information regarding the percentage of CD203c positive basophils.

Table 1
Demographic characteristics of study population.

Category	Allergic patients n = 10	Healthy controls n = 10
Clinical symptoms, n		
Asthma	6	0
Rhinitis	10	0
Conjunctivitis	10	0
Medical treatment, n		
Oral steroids	0	0
Allergen immunotherapy (AIT)	0	0
Air-borne allergens, n (Relevant allergens)*		
Cat	3	5
Birch	5	1
Timothy grass	2	3
Dog	0	1
Horse	0	0
*Control allergens, n** (Non-relevant allergens)*		
House dust mite	9	10
Bee	1	0

(n) *Number of samples treated with each relevant allergen.
(n) **Number of samples treated with each non-relevant allergen.

2.4. Microfluidic chip device microfabrication and surface modification

The microfluidic chip fabrication and preparation was according to our previous work (Aljadi et al., JALM, In Press, DOI: 10.1373/jalm.2018.026641). Briefly, the microfluidic devices were fabricated in polydimethylsiloxane (PDMS, Dow Corning, US) using standard soft lithography techniques. The PDMS replica was bonded to a glass slide (70 mm × 30 mm) after brief oxygen plasma treatment. To immobilize capturing antibody (anti-CD203c biotin), the chips were chemically treated with 3-mercaptopropyl trimethoxysilane (Sigma Aldrich, Germany) for 1 h. This was followed by washing with ethanol and incubated with 0.01 µmol/ml of 4-Maleimidobutyric acid N-hydroxysuccinimide ester (GMBS) in ethanol for 20 min at room temperature. The devices were washed again first with ethanol and then with PBS, and 10 µg/ml Neutravidin (Sigma Aldrich, Germany) solution in PBS was added and incubated overnight at 4° C. Before experiments, the devices were injected and incubated with biotinylated anti-CD203c (MACS, Miltenyi Biotech, Germany) incubated for 60 min for basophil capture.

2.5. Basophil capture in microfluidic chip

Fabricated and modified chips were washed and blocked with 1% bovine serum albumin (BSA) in phosphate buffer saline (1XPBS) to remove the un-bound (anti-CD203c biotin) antibody at 20 µl min^{-1}. Two hundred µl of whole blood flowed through the chips channels. The flow condition of the straight channel has been optimized in our previous paper (Aljadi et al., JALM, In Press, DOI: https://doi.org/10.1373/jalm.2018.026641); initially, using basophil cell line (KU812), followed by whole blood. Blood samples flowed in the chip at 3 µl min^{-1}, followed by washing out of the un-wanted cells (non-basophil cells) with 1%BSA at 20 µl min^{-1}.

2.6. Allergen challenge of captured basophils in microfluidic chip

Initially, experiments were performed to optimize the allergen

concentration used to challenge the chip captured basophils. Captured basophils were stimulated with different allergen concentrations (birch, timothy, cat, dog, horse, dust mite and bee) (Aquagen, ALK, Copenhagen, Denmark) (Final concentration: 5; 50, 500 and 5000 Standard Quality Unit (SQU)/ml diluted in stimulation buffer) at 37° C for 25 min, washed with 1% BSA, and fixed with 4% paraformaldehyde (PFA) for 10 min at RT before washing of chips. The cells were then incubated with a primary anti-CD203c-ab (Abcam, UK) for 1 h at RT followed by staining with a secondary anti-mouse antibody fluorescently conjugated with Alexa-488 (Abcam, UK) for 1 h at RT. CD63 activation was detected using an Alexa-647 conjugated anti-CD63 antibody (Abcam, UK) incubated for 30 min at RT and cell's nucleus stained with (Hoechst stain). Finally, chips were washed with 1% BSA and scanned by Nikon Ti Eclipse microscope; images were acquired by Zyla 5.5 sCMOS Andor camera, and transferred using the MicroManager Version 1.4 software, plug-in and processed using Imagej software. Flow cytometric experiments were performed in parallel using the same allergen concentrations. Two concentrations (5000 and 50 SQU/ml) were chosen for subsequent experiments. In addition, anti-FcεRI antibody (Bühlmann Laboratories, Schönenbuch, Switzerland) was used as positive control and stimulation buffer (Bühlmann Laboratories) as negative control.

2.7. Measurement of CD63 MFI in microfluidic chip

Six allergen activated basophils and six non-activated basophils from two allergic patients were analyzed. The analyzed basophils were chosen from different imaging positions in activated and non-activated microfluidic chips. Fluorescence intensity measurements were made directly over the cells at multiple spots. The far dark bottom of the image was used as background measurement. The fluorescence intensity measurement was taken as the numerical difference between the background and target measurements. Mean fluorescence intensity (MFI) was measured on the activated and non-activated basophils of each patient using ImageJ software.

2.8. Flow cytometry analysis of allergen activated basophils

The flow cytometry analysis were performed on basophils from allergic patients and healthy controls in parallel to microfluidic chip analysis of activated basophils as previously described [17,18]. One hundred µl of whole blood was incubated with 100 µl of relevant airborne allergen (birch, timothy, cat, dog and horse) and non-relevant allergens (dust mite and bee) (Final concentrations: 5, 50, 500 and 5000 SQU/ml diluted in stimulation buffer). Anti-FcεRI antibody was used as positive control (Bühlmann Laboratories) and stimulation buffer (Bühlmann Laboratories) as negative control. Samples were incubated at 37° C for 20 min, followed by staining using anti-CD203c-PE and anti-CD63-FITC (Beckman Coulter, Paris, France) for 25 min at 4° C. The RBCs were then lysed with 2 mL cold isotonic solution (154 mM NH4Cl, 10 mM KHCO3 supplemented with 0.1 mM EDTA, pH 7.2), samples were centrifuged for 5 min at 300 × *g* at 4 C, cells were then washed with PBS before being re-suspended in 300 µL of cold PBS and subsequently analyzed. The surface expression of CD203c and CD63% on basophils was analyzed by flow cytometry (Navios, Beckman Coulter, Hialeah, FL, USA).

2.9. Flow cytometric gating strategy of basophils

Basophils were gated according to their granularity on side scatter and expression of CD203c and 200–300 basophils were analyzed (Fig. 2a). The percentage of CD63 positive cells (CD63%) within the total basophil population was calculated as shown in (Fig. 2b). Data were analyzed by the Kaluza Analysis Software (Beckman Coulter Inc.).

2.10. Statistical analysis

The graphs were prepared by GraphPad Prism 8.0.1 (GraphPad Software, Inc., La Jolla, CA, USA). Statistical analysis was done in GraphPad Prism 8.0.1. Since the study population was not normally distributed, the results were presented as median and interquartile range and the significant differences between groups were analyzed using the non-parametric test Mann Whitney test. A p value of < 0.05 was considered significant. The Bland-Altman method was used to compare the data measurements from both microfluidic chip and flow cytometry. The average difference between measurements and difference between measurements of the two methods were used to make a Bland-Altman plot. The average bias and agreement limits were analyzed.

3. Results

3.1. Number of basophils in allergic patients and healthy controls

The absolute number of basophils in whole blood was analyzed by flow cytometry before capture experiments in the microfluidic chip in allergic patients and healthy control samples. The number of basophils in allergic patients were 21 (12 − 31) basophils/µl (median and range) and in healthy controls 25 (20 − 33) basophils/µl blood.

3.2. IgE-ab in serum from allergic patients and healthy controls

The IgE-ab level for relevant allergens was 6.3 in median (range: 2.1–71.8) kU_A/l in allergic patients while no detectable IgE-ab were found to non-relevant allergens (data not shown). In healthy controls no specific IgE-ab were detected (data not shown).

3.3. Optimization of basophil allergen activation analyzed in microfluidic chip

Basophils from a number of allergic patient samples were stimulated by various concentrations (5, 50, 500 and 5000 SQU/ml) of the selected airborne allergens in parallel to flow cytometry analysis, and the activation measured as the proportion of activated basophils (%CD63 +). The highest CD63% expression on basophils captured in a microfluidic chip based analysis was noted at 50 SQU/ml (data not shown).

3.4. Optimization of basophil allergen activation analyzed by flow cytometry

Basophils from allergic patients were stimulated by various concentrations (5, 50, 500 and 5000 SQU/ml) of the selected airborne allergens and the activation measured as the proportion of activated basophils (%CD63 +). The flow cytometry analysis on basophils from allergic patients was done in parallel to the microfluidic chip analysis. The basophil reactivity (CD63%) reached a plateau within the range 50–5000 SQU/ml and consequently, both the higher concentration 5000 SQU/ml and the lower concentration 50 SQU/ml were chosen for subsequent experiments (data not shown).

3.5. Basophil activation in microfluidic chip in healthy and allergic patients

The proportion of CD63 expression in anti-FcεRI and allergen activated captured basophils were compared with non-activated basophils and analyzed in microfluidic chip ($n = 10$) (Fig. 3a-b). Basophils from allergic patients stimulated with anti-FcεRI (positive control) showed a significantly higher proportion of activated basophils (%CD63 +) as compared to the stimulation buffer (negative control) ($p < .0001$). Furthermore, basophils from allergic patients, activated with relevant allergen at two different concentrations; 50 or 5000 SQU/ml, showed significantly higher proportion of activated basophils (%CD63 +) as

compared to the stimulation buffer (negative control) ($p = .0001$, $p = .0004$, respectively) (Fig. 3a). Basophils from allergic patients stimulated with non-relevant allergen in either allergen concentration (50 and 5000 SQU/ml) showed comparable proportion of activated basophils (%CD63 +) as the stimulation buffer (negative control) ($p = .42$ and $p = .18$, respectively).

Results obtained from the healthy control group ($n = 10$) (Fig. 3b), showed that the proportion of anti-FcεRI activated basophils (%CD63 +) was significantly higher ($p < .0001$) compared to the stimulation buffer (negative control). In addition, basophils, from the healthy control group, stimulated with allergen showed comparable results as the stimulation buffer (negative control) ($p = .38–0.81$).

3.6. Basophil activation by flow cytometry in healthy and allergic patients

The proportion of CD63 expression in anti-FcεRI and allergen activated basophils were compared with non-activated basophils and analyzed by flow cytometry ($n = 10$) (Fig. 4a-b). Basophils from allergic patients stimulated with anti-FcεRI (positive control) showed a significantly higher proportion of activated basophils (%CD63 +) as compared to the stimulation buffer (negative control) ($p < .0001$). Furthermore, basophils from allergic patients, activated with relevant allergen at two different concentrations; 50 or 5000 SQU/ml, showed significantly higher proportion of activated basophils (%CD63 +) as compared to the stimulation buffer (negative control) (p<0.0001, p<0.0001, respectively) (Fig. 4a). Basophils from allergic patients stimulated with non-relevant allergen in either allergen concentration (50 and 5000 SQU/ml) showed comparable proportion of activated basophils (%CD63 +) as the stimulation buffer (negative control) ($p = .54$, $p = .52$, respectively).

Results obtained from the healthy control group ($n = 10$) (Fig. 4b), showed that the proportion of anti-FcεRI activated basophils (%CD63 +) was significantly higher ($p < .0001$) compared to the proportion of stimulation buffer incubated basophils (negative control). In addition, basophils, from the healthy control group, stimulated with allergen showed comparable results as the stimulation buffer (negative control) ($p = .27–0.87$).

3.7. Measurement of CD63 MFI in microfluidic chip

Further characterization of the CD63 expression on basophils was done in order to detect the difference of CD63 expression level on activated and non-activated basophils. CD63 MFI of basophils activated with relevant allergen or the stimulation buffer (negative control) was measured. The difference in CD63 MFI was significantly higher in allergen activated basophils than in non-activated captured cells ($p = .0001$). The MFI ratio of analyzed basophils (MFI of activated basophils/non-activated basophils) was 3.1 (Suppl. Fig. 1).

3.8. Basophil activation measured in microfluidic chip versus flow cytometry

Allergen activation analyzed with microfluidic chip and flow cytometry showed comparable results and we found no significant difference in the proportion of activated basophils (+ CD63%) following activation with 50 or 5000 SQU/ml of relevant allergen ($p = .52$, $p = .20$, respectively).

The Bland-Altman comparison plot revealed the average bias between the measurements obtained using microfluidic chip and flow cytometry for 50 and 5000 SQU/ml to be −11.5 and −6.2 respectively and the agreement limits −46 to 23 and −51 to 39, respectively.

4. Discussion

In the present study, we aimed to evaluate if the microfluidics-based immuno-affinity approach (miBAT) could be a consistent method to achieve a basophil activation test for allergy diagnosis. We demonstrate that the CD63% in anti-FcεRI and allergen activated captured basophils in allergic patients is significantly higher compared to non-activated captured basophils (negative control) and non-relevant allergen. The obtained results of basophil activation in microfluidic chip are comparable to flow cytometry analysis.

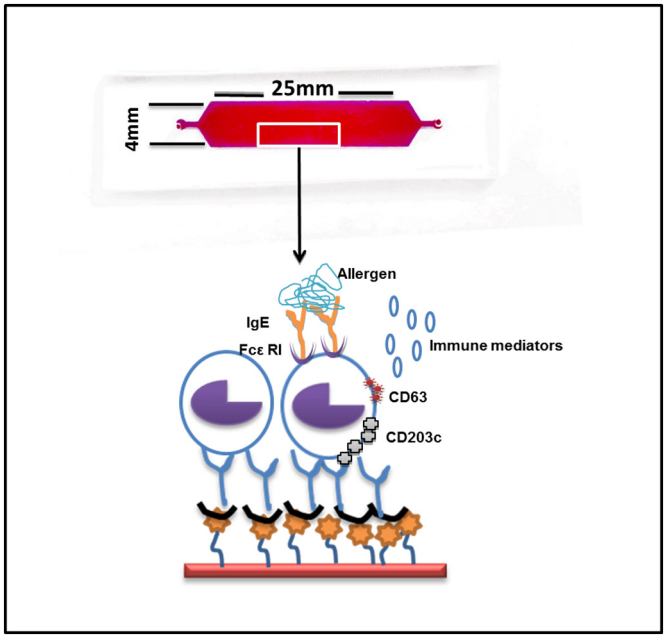

Fig. 1. Microfluidic chip design and functionalization. The figure illustrates the chip geometry and surface functionality of the microfluidic chip.

We have recently developed a miBAT technique that isolates basophils directly from whole blood to diagnose allergy (Aljadi et al., JALM, In Press, DOI: https://doi.org/10.1373/jalm.2018.026641). The microfluidic device captures CD203c positive cells (basophils) in a single step directly from whole blood without pre-labeling and processing of the sample. The captured basophils are thereafter activated with anti-FcεRI-ab followed by detection of the basophil CD63 expression level by fluorescent microscopy. Over all, the results are consistent with an established flow cytometric method. The optimized microfluidic chip captured > 200 basophils, which mean that this platform is capable to capture significant number of basophils for CD63 quantification. The purity of captured cells was approximately 40%. The non-specific binding of other leukocytes can be reduced by optimizing the device design and flow conditions. Among the captured non-basophil cell populations, monocytes can express a low level of FcεRI and CD63. However, the activation mechanism of the FcεRI pathway in monocytes is different and requires a very high concentration of stimuli to cross link FcεRI and longtime incubation compared to in basophils [19]. Furthermore, only CD203c/CD63 double positive cells were selected for further analysis which exclude a potential significant contamination of monocytes within the cell population selected for analysis.

The basophil activation reactivity to various concentrations of airborne allergens varied between allergic patients, an observation previously described [20]. The underlying mechanism of heterogeneity of basophil reactivity between individuals is not clearly identified [21]. Differences in basophil sensitivity might be due to the variation in allergen extracts in each individual allergen and between allergens, which might affect the IgE reactivity [22]. The seasonal changes of exposure to allergens such as birch and timothy as well as house dust mite allergens have an effect on the level of allergic patients sensitivity [23]. Moreover, it has been reported that basophils have two distinct subpopulations according to the cytokine milieu, i.e. Thymic stromal lymphopoietin (TSLP)-elicited basophils and IL-3-elicited basophils.

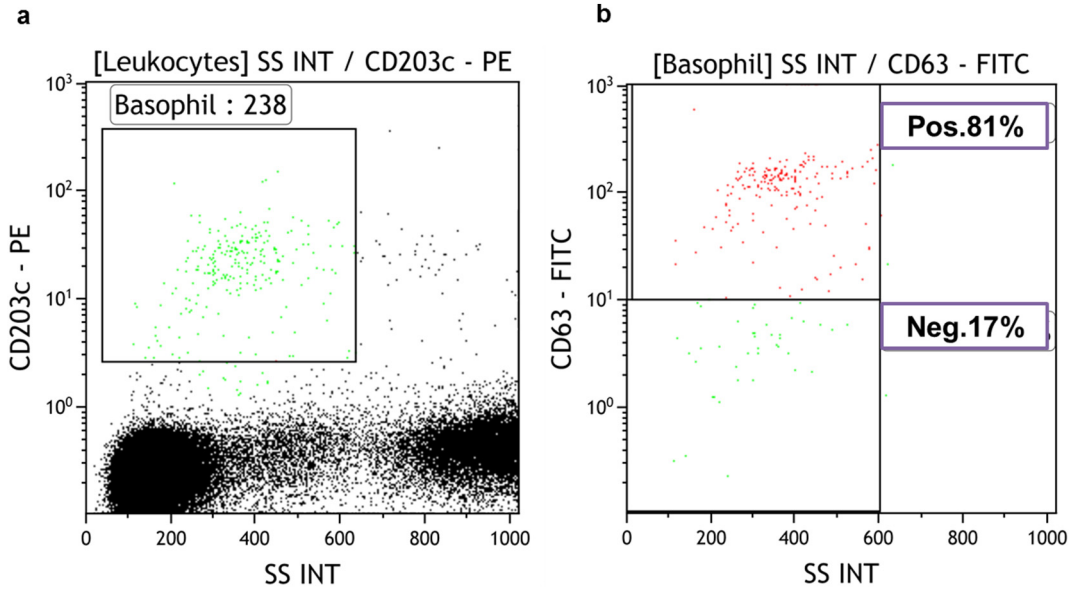

Fig. 2. Basophil gating strategy (a) Basophils were gated according to their granularity on side scatter and expression of CD203c. (b) CD63 positive cells were detected within the total basophil population (CD203c positive cells).

TSLP-elicited basophils display higher expression of receptors for IL-3, IL-18 and IL-33, which implies an increased IL-4 release in response to those cytokines. In contrast, TSLP elicited basophils display lower degranulation capacity in response to IgE dependent activation compared to IL-3 elicited, which suggest that the individual basophil subpopulation composition impacts the global basophil responsiveness in allergic inflammation [24]. An additional plausible explanation for the intra individual variation is the density of FcεRI on cell surface, since the Syk protein signaling interferes with the degree of cell activation [25].

Our data demonstrate that the CD63 expression in anti-FcεRI activated captured basophils in allergic and healthy individuals was significantly higher compared to non-activated captured basophils (negative control). In line with our previous study (Aljadi et al., JALM, In Press, DOI: https://doi.org/10.1373/jalm.2018.026641) we noticed a relatively high basal in vitro value in the negative control samples. The reason for this could be manifold. Present pyrogens and endotoxins could contaminate the materials used in the technique such as plastic tubes or syringes [26]. It is therefore important to work in a sterile environment and to re-design a chip with possibility to minimize the assay process time that might reduce the negative background. The mechanical stress formed during cell capturing process in the chip may also prime the spontaneous activation of captured basophils and induce degranulation [27,28]. Nevertheless, the CD63 MFI value in allergen activated basophils on a single cell level was higher than that on negative control captured cells, which support our assumption that the microfluidic chip method discriminates efficiently between non-activated and activated cells. We are now also in progress to include a computerized imaging compensation step that will facilitate the automatic discrimination between non-activated and activated cells.

The CD63 expression in activated basophils did not significantly differ between microfluidic chip method and flow cytometry analysis. This indicates a consistent sensitivity of miBAT to measure the reactivity of basophils. The minor difference in basophil reactivity observed using microfluidic chip technique might partially be explained by the fact that we have isolated the basophils from whole blood in miBAT prior to activation compared to the use of whole blood in the flow cytometric method [29,30]. It has previously been published that the dose-response curve after allergen stimulation differs in basophils

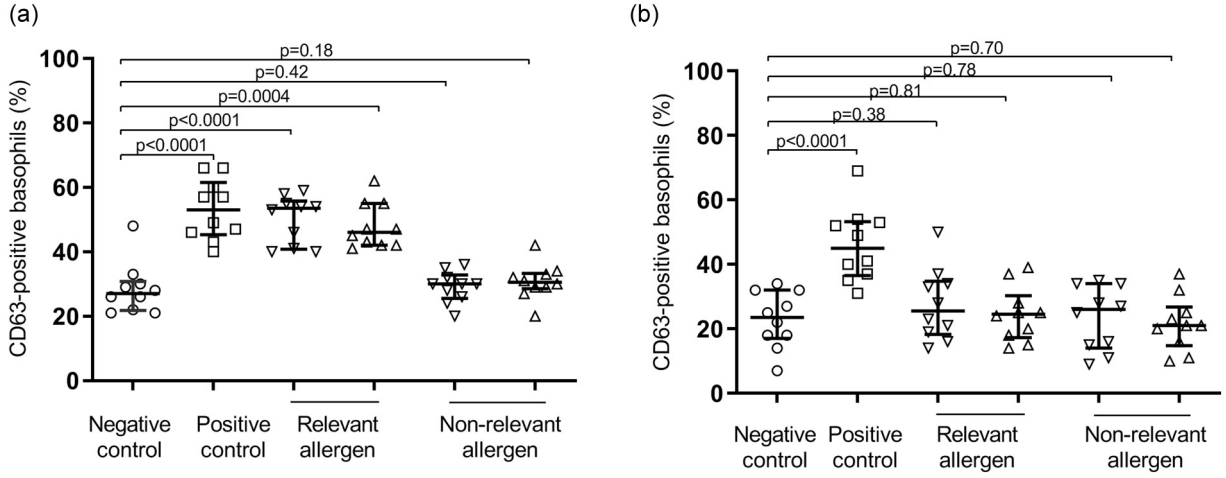

Fig. 3. Difference of CD63 expression in activated captured basophils compared to non-activated cells in microfluidic chip. (a) CD63 expression of negative control (stimulation buffer) (O), positive control (anti-FcεI) (□) and allergen (50 SQU/ml (∇) and 5000 SQU/ml (Δ) stimulated basophils in allergic patients and (b) in healthy controls. Results are presented as median and interquartile range (n = 10). A p value of < 0.05 was considered significant.

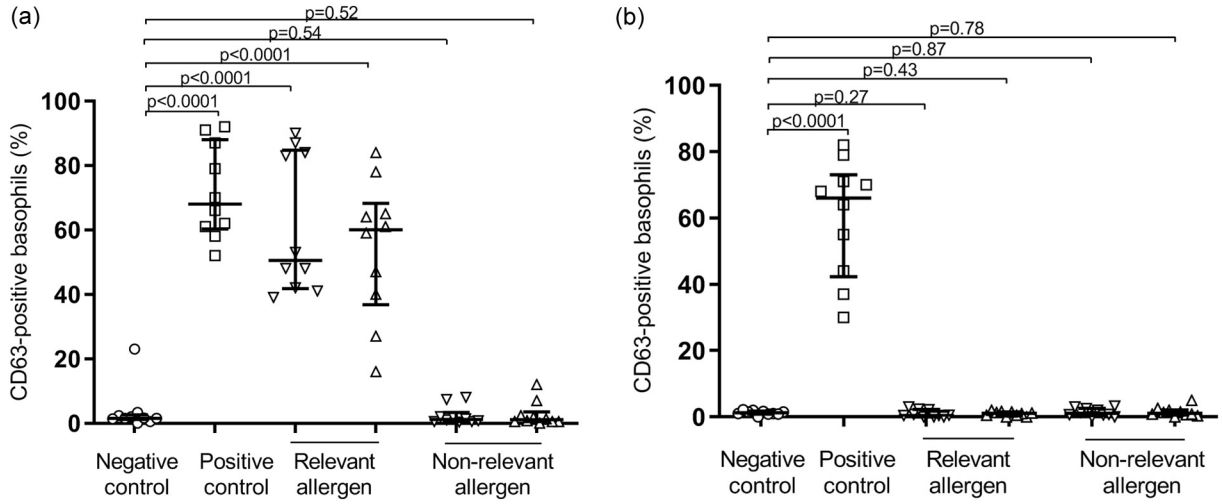

Fig. 4. Difference of CD63 expression in activated captured basophils compared to non-activated cells in flow cytometry. (a) CD63 expression of negative control (stimulation buffer) (O), positive control (anti-FcεRI) (□) and allergen (50 SQU/ml (∇) and 5000 SQU/ml (Δ) stimulated basophils in allergic patients and (b) in healthy controls. Results are presented as median and interquartile range (n = 10). A p value of < 0.05 was considered significant.

activated in whole blood and in basophils activated after removal of plasma, which is a milieu more similar to the microfluidic chip [31]. The average bias for the Bland-Altman comparison method analysis was approximately 10% and the average agreement limit was ± 37%, figures that should be interpreted with caution. The bias and agreement limit might be improved by including a larger sample size but more importantly by activating the basophils in both methods in a more similar environment.

Evidence from in vitro and human clinical studies report that the FcεRI expression is variable between atopic and non-atopic individuals, as well as between various diseases, which is mainly correlated to circulating IgE level [32,33]. This suggests that the significant difference of CD63 expression in allergic patients compared to healthy controls might be associated with the variation of FcεRI expression level on basophils.

A limitation of the current version of the miBAT is that the analysis time is longer than for conventional BAT, it takes around four to five hours from basophil capture in microfluidic chip to final analysis. However, there are strategies to reduce the time required for the analysis procedure e.g. using prefabricated and antibody coated chips, further optimizing flow rates and incubation procedures and to include automatic reading and image analysis.

To summarize, in this report we demonstrate that miBAT can be used to detect the difference of CD63 expression on allergen activated captured basophils in microfluidic chip comparing allergic patients with healthy controls. We also show that miBAT data parallels data using an established flow cytometric method.

5. Conclusion

The results from this study demonstrate the efficacy of the miBAT technology to be used to measure the level of basophil activation after allergen exposure of whole blood from allergic patients by quantifying their CD63 expression by a microfluidic chip. The microfluidic chip discriminates between allergen activation and background noise as well as between stimulation with relevant and non-relevant allergen. This technique provides a new method for measurement of basophils activation level by a microfluidic chip, which has the potential to facilitate diagnosis and monitoring of allergic patients.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clim.2019.108268.

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Declaration of Competing Interest

The author has no conflict of interest.

References

[1] S.G. Johansson, T. Bieber, R. Dahl, P.S. Friedmann, B.Q. Lanier, R.F. Lockey, et al., Revised nomenclature for allergy for global use: report of the nomenclature review Committee of the World Allergy Organization, October 2003, J. Allergy Clin. Immunol. 113 (5) (2004) 832–836.
[2] A. Muraro, T. Werfel, K. Hoffmann-Sommergruber, G. Roberts, K. Beyer, C. Bindslev-Jensen, et al., EAACI food allergy and anaphylaxis guidelines: diagnosis and management of food allergy, Allergy. 69 (8) (2014) 1008–1025.
[3] R. Pawankar, G.W. Canonica, S.T. Holgate, R.F. Lockey, Allergic diseases and asthma: a major global health concern, Curr. Opin. Allergy Clin. Immunol. 12 (1) (2012) 39–41.
[4] B.E. Chipps, Update in pediatric anaphylaxis: a systematic review, Clin. Pediatr. 52 (5) (2013) 451–461.
[5] H. Karasuyama, Y. Tsujimura, K. Obata, K. Mukai, Role for basophils in systemic anaphylaxis, Chem. Immunol. and Allergy. 95 (2010) 85–97.
[6] H. Karasuyama, K. Obata, T. Wada, Y. Tsujimura, K. Mukai, Newly appreciated roles for basophils in allergy and protective immunity, Allergy. 66 (9) (2011) 1133–1141.
[7] D. Bremond-Gignac, N. Beydon, L. Laroche, Skin tests and cutaneous anergy in children with ocular allergy, Acta Ophthalmol. Scand. Suppl. 230 (2000) 76–77.
[8] W.J. van Veen, L.D. Dikkeschei, G. Roberts, P.L. Brand, Predictive value of specific IgE for clinical peanut allergy in children: relationship with eczema, asthma, and setting (primary or secondary care), Clin Transl Allergy. 3 (1) (2013) 34.
[9] H.A. Sampson, R.G. van Wijk, C. Bindslev-Jensen, S. Sicherer, S.S. Teuber, A.W. Burks, et al., Standardizing double-blind, placebo-controlled oral food challenges: American Academy of Allergy, Asthma & Immunology-European academy of allergy and clinical immunology PRACTALL consensus report, J. Allergy Clin. Immunol. 130 (6) (2012) 1260–1274.
[10] R. Valenta, Recombinant allergen-based concepts for diagnosis and therapy of type I allergy, Allergy. 57 (71) (2002) 66–67 Suppl.
[11] M. Steiner, A. Harrer, M. Himly, Basophil reactivity as biomarker in immediate drug hypersensitivity reactions-potential and limitations, Front. Pharmacol. 7 (2016) 171.
[12] J. Brandstrom, A. Nopp, S.G. Johansson, G. Lilja, A.C. Sundqvist, M.P. Borres, et al., Basophil allergen threshold sensitivity and component-resolved diagnostics improve hazelnut allergy diagnosis, Clin. Exp. Allergy 45 (9) (2015) 1412–1418

Clinical and experimental allergy:journal of the British Society for Allergy and Clinical Immunology.
[13] N.M. Pires, T. Dong, U. Hanke, N. Hoivik, Recent developments in optical detection technologies in lab-on-a-chip devices for biosensing applications, Sensors (Basel). 14 (8) (2014) 15458–15479.
[14] Y. Chen, P. Li, P.H. Huang, Y. Xie, J.D. Mai, L. Wang, et al., Rare cell isolation and analysis in microfluidics, Lab Chip 14 (4) (2014) 626–645.
[15] K.T. Kotz, W. Xiao, C. Miller-Graziano, W.J. Qian, A. Russom, E.A. Warner, et al., Clinical microfluidics for neutrophil genomics and proteomics, Nat. Med. 16 (9) (2010) 1042–1047.
[16] S. Sharma, J. Zapatero-Rodriguez, P. Estrela, R. O'Kennedy, Point-of-care diagnostics in low resource settings: present status and future role of microfluidics, Biosens.(Basel). 5 (3) (2015) 577–601.
[17] S.G. Johansson, A. Nopp, M. van Hage, N. Olofsson, J. Lundahl, L. Wehlin, et al., Passive IgE-sensitization by blood transfusion, Allergy. 60 (9) (2005) 1192–1199.
[18] A. Nopp, S.G. Johansson, J. Ankerst, G. Bylin, L.O. Cardell, R. Gronneberg, et al., Basophil allergen threshold sensitivity: a useful approach to anti-IgE treatment efficacy evaluation, Allergy. 61 (3) (2006) 298–302.
[19] H. Agis, W. Fureder, H.C. Bankl, M. Kundi, W.R. Sperr, M. Willheim, et al., Comparative immunophenotypic analysis of human mast cells, blood basophils and monocytes, Immunology. 87 (4) (1996) 535–543.
[20] J.R. Konradsen, B. Nordlund, O.B. Nilsson, M. van Hage, A. Nopp, G. Hedlin, et al., High basophil allergen sensitivity (CD-Sens) is associated with severe allergic asthma in children, Pediatr. Allergy Immunol. 23 (4) (2012) 376–384 Pediatric allergy and immunology:official publication of the European Society of Pediatric Allergy and Immunology.
[21] M. Wensing, A.H. Penninks, S.L. Hefle, S.J. Koppelman, C.A. Bruijnzeel-Koomen, A.C. Knulst, The distribution of individual threshold doses eliciting allergic reactions in a population with peanut allergy, J. Allergy Clin. Immunol. 110 (6) (2002) 915–920.
[22] R. Moverare, K. Westritschnig, M. Svensson, B. Hayek, M. Bende, G. Pauli, et al., Different IgE reactivity profiles in birch pollen-sensitive patients from six European populations revealed by recombinant allergens: an imprint of local sensitization, Int. Arch. Allergy Immunol. 128 (4) (2002) 325–335.
[23] S. van der Heide, J.G. de Monchy, K. de Vries, T.M. Bruggink, H.F. Kauffman, Seasonal variation in airway hyperresponsiveness and natural exposure to house dust mite allergens in patients with asthma, J. Allergy Clin. Immunol. 93 (2) (1994) 470–475.
[24] K. Miyake, H. Karasuyama, Emerging roles of basophils in allergic inflammation, Allergol. Int. 66 (3) (2017) 382–391.
[25] D. MacGlashan Jr., FceRI density and spontaneous secretion from human basophils, PLoS One 12 (7) (2017) e0179734.
[26] M.L. Sanz, J.P. Maselli, P.M. Gamboa, A. Oehling, I. Dieguez, A.L. de Weck, Flow cytometric basophil activation test: a review, J Investig. Allergol Clin. Immunol. 12 (3) (2002) 143–154.
[27] J.J. Paszkowiak, A. Dardik, Arterial wall shear stress: observations from the bench to the bedside, Vasc. Endovasc. Surg. 37 (1) (2003) 47–57.
[28] F. Boccafoschi, M. Bosetti, P.M. Sandra, M. Leigheb, M. Cannas, Effects of mechanical stress on cell adhesion: a possible mechanism for morphological changes, Cell Adhes. Migr. 4 (1) (2010) 19–25.
[29] D.G. Ebo, C.H. Bridts, M.M. Hagendorens, N.E. Aerts, L.S. De Clerck, W.J. Stevens, Basophil activation test by flow cytometry: present and future applications in allergology, Cytometry B Clin. Cytom. 74 (4) (2008) 201–210.
[30] A.F. Santos, G. Lack, Basophil activation test: food challenge in a test tube or specialist research tool? Clin.Transl.Allergy. 6 (2016) 10.
[31] A. Nopp, L.O. Cardell, S.G.O. Johansson, H. Oman, CD-Sens: a biological measure of immunological changes stimulated by ASIT, Allergy. 64 (5) (2009) 811–814.
[32] B.S. Sihra, O.M. Kon, J.A. Grant, A.B. Kay, Expression of high-affinity IgE receptors (fc epsilon RI) on peripheral blood basophils, monocytes, and eosinophils in atopic and nonatopic subjects: relationship to total serum IgE concentrations, J. Allergy Clin. Immunol. 99 (5) (1997) 699–706.
[33] Z. Aljadi, A. Nopp, O. Winqvist, A. Russom, B. Hylander, S.H. Jacobson, et al., Altered basophil function in patients with chronic kidney disease on hemodialysis, Clin. Nephrol. 88 (8) (2017) 86–96.