



Basophil biomarkers as useful predictors for sublingual immunotherapy in allergic rhinitis

Massimo Caruso^{a,*}, Fabio Cibella^b, Rosalia Emma^a, Davide Campagna^a, Giovanni Tringali^{c,1}, Maria Domenica Amaradio^a, Riccardo Polosa^a

^a Department of Clinical and Experimental Medicine, Azienda Ospedaliero-Universitaria "Policlinico-V. Emanuele", University of Catania, Via S. Sofia 78, 95123 Catania, Italy

^b National Research Council of Italy, Institute of Biomedicine and Molecular Immunology, via Ugo La Malfa 153, 90146 Palermo, Italy

^c Istituto Ricerca Medica ed Ambientale (I.R.M.A.) Srl, Via Paolo Vasta, 158/C, 95024 Acireale (CT), Italy

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ABSTRACT

Prevalence of allergic diseases is increasing worldwide. Allergen-specific immunotherapy (ASIT) is potentially the only curative treatment for allergy, but there is a lack of reliable methods to monitor the immune responses to ASIT and to predict clinical efficacy. Recently, the definition of allergen sensitivity threshold (CD-Sens) by Basophil Activation Tests has been suggested as potential method in this context.

The aim of this study was to compare trends of CD-Sens, measured by the markers CD63 and CD203c, and clinical symptoms in subjects with allergic rhinitis receiving Sublingual Immunotherapy (SLIT). 26 rhinitis patients allergic to *Parietaria* were selected and matched into two groups; a SLIT treated group (SG) and a reference group (RG) treated by traditional anti-allergic medications. Visual Analogue Scale (VAS) score for the four cardinal symptoms of rhinitis and peripheral blood was collected before the first dose of SLIT (T₀) and after 12 months (T₁₂) to define the severity of the symptoms and the sensitivity of basophils to *Parietaria*. The comparison between T₀ and T₁₂ in SG patients showed a significant decrease of symptom severity (VAS score) and an increased tolerability of basophils to *Parietaria* (CD-Sens) both by CD63 and CD203c. But, only CD203c seems to be correlated with the clinical symptoms. These data corroborate the hypothesis that SLIT could change the immunological course of allergic sensitization already in the first year, and that an immunological parameter as CD-Sens measured by CD63 and CD203c expression on stimulated basophils could be useful to monitor the changes in the immune system.

1. Introduction

Prevalence of allergic diseases is increasing worldwide [1]. Allergen-specific immunotherapy (ASIT) is potentially the only curative treatment for allergy, due to its desensitizing ability resulting in clinical tolerance [2]. The ASIT exact mechanisms of action remain a matter of research and debate. However, changes in memory cells, dendritic cells polarization [3,4], modification in allergen-specific T- and B-cells behavior, as well as modulation of effector inflammatory cells (e.g. eosinophils, basophils and mast cells) activation has been proposed as major mechanisms involved in the desensitizing effects [5].

ASIT has been shown to be safe and effective for allergic rhinoconjunctivitis, Hymenoptera venom allergy, and allergic asthma [6,7]. Also, there is now emerging evidence that ASIT may be useful in the management of food allergies [8].

Currently ASIT can be administered either subcutaneously (SCIT) or sublingually (SLIT). In both cases a similar strategy, consisting in the gradual administration of increasing doses of allergen extracts in order to reach the maintenance dose of the offending allergen effective at reducing disease severity, is applied [5]. The maintenance dose differs for each patient and for each allergen, and often it is difficult to forecast the right dose because of the lack of reliable methods to monitor the immune response to ASIT and to predict its clinical efficacy.

Recently the Basophil Activation Test (BAT) has been suggested as a useful method for this purpose [9]. BAT is performed on fresh peripheral blood samples, and it aims to reproduce the allergic reaction of basophils in vitro. A particular application of BAT, defined as CD-Sensitivity or CD-Sens and performed by Nopp and colleagues [9], appoints to establish the "minimum dose" able to activate basophils at 50% of their maximum activation rate through the stimulation of these cells

* Corresponding author.

E-mail addresses: mascaru@unict.it (M. Caruso), fabio.cibella@ibim.cnr.it (F. Cibella).

¹ Centro Servizi Medici (CSM) SCRL Corso Delle Province, 218 - 95127 Catania (CT), Italy.

with increasing doses of allergen. By establishing the patient's allergen-specific sensitivity with CD-Sens, it should be possible to predict the clinical efficacy of ASIT, and potentially CD-Sens could be a useful method to predict the right maintenance dose in order to obtain an effective desensitization.

The surface markers most widely used for measuring basophil activation are the granule-associate membrane protein CD63 (Glycoprotein 53 or gp53), and the basophil-specific ecto-enzyme CD203c (ectonucleotide pyrophosphatase/phosphodiesterase 3 or ENPP-3). CD63 is a granule-associated mediators with an increased expression on basophils surface characteristically associated to the release of histamine, whereas up-regulation of CD203c expression on the basophil cell surface is the result of an early FcεRI-mediated event and it occurs very quickly following the bridging of these receptors [10].

Both markers are up regulated upon basophil activation, but their usefulness in monitoring ASIT has not been clearly defined. In fact, CD-Sens has been studied only by means of the marker CD63, and the results obtained on its predictive ability in monitoring the allergic sensitivity are very encouraging [9,11–13].

The purpose of the present study was to investigate the variation of basophils threshold sensitivity, measured by the markers CD63 and, for the first time, CD203c in allergic rhinitis patients treated with SLIT or standard medication (e.g. antihistamines, topical steroids).

Head-to-head comparison between changes in CD-Sens by CD63/CD203c and in perception of allergic rhinitis symptoms, measured by a clinical symptoms score as the Visual Analogue Scale (VAS) during 12 months of therapy, were also carried out. Association of CD-sens variation and changes in clinical symptoms scores was investigated to establish the aptitude of these two markers as clinical efficacy predictors.

2. Materials and methods

2.1. Subjects

Participants were recruited during a screening visit from our outpatient clinic. Between [January 2015] and [September 2015], we consecutively enrolled 26 patients with allergic rhinitis sensitized to *Parietaria* from at least two years, confirmed by a positive Skin Prick Test or positive IgE antibodies and with a VAS score ≥ 5 for each of the four symptoms. Subjects who were currently taking ASIT to *Parietaria* and/or other allergens or biologic drugs (e.g. Omalizumab) or had taken ASIT to *Parietaria* in the past were not included, along with pregnant or lactating women. All the subjects were monosensitized to *Parietaria*. The study was performed after approval of the local ethics committee and all the participants signed a written informed consent.

2.2. Study design

The 26 patients were randomized into two groups and the randomization sequence was computer generated by using blocks size of 3 with an allocation ratio of 2 (for the SLIT Group): 1 (for the Reference Group): 16 patients were included in the SLIT Group (SG) receiving a formulation of SLIT (Stallergenes; Staloral 300® *Parietaria officinalis*), and 10 patients were included in the Reference Group (RG) receiving only the standard medications (i.e. Antihistamines, topical steroids) (Fig. 1). All the selected patients had a diagnosis of allergy to *Parietaria* spp. and performed specific IgE dosage in the last 6 months. During the screening visit, skin prick tests for *Parietaria* (BIAL Industrial Farmacéutica S.A.) were performed on the flexor aspect of the forearm, and the early skin response at 15–30 min was recorded in the clinical report forms. Patients enrolled had a positive skin prick test (wheal diameter > 3 mm), and specific IgE (> 0.70 kU/L) to *Parietaria* spp.

Patients selected during the screening visits were invited to sign the informed consent and to attend the baseline visit (T_0) within 30 days. During the T_0 visit, the first dose of SLIT treatment was administered:

SLIT was followed daily for 12 months self-administered by the patients themselves. Peripheral blood samples were collected at both baseline visit (T_0) and after 12 months of treatment, during the follow-up visit (T_{12}).

At both T_0 and T_{12} patients recorded their symptom score severity on VAS ranging from 0 to 10. Furthermore patients were interviewed monthly by phone in order to report both eventually missed doses and/or any occurrence of adverse events or worsening of allergic symptoms.

2.3. Clinical symptoms score by visual analogue scale (VAS)

During both the baseline (T_0) and the follow-up (T_{12}) visits participants were asked to assess the severity of each of the four cardinal symptoms of allergic rhinitis: nasal congestion (NC), nasal itching (NI), rhinorrhea (Rh), and sneezing (Sn). The score of each symptom varies from 0 (i.e. no symptoms) to 10 (i.e. most severe symptoms). Therefore the maximum total score was 40. Participants reported the severity of their symptoms at both T_0 and T_{12} , placing a cross on a 10 cm line divided into 10 sections, each of 1 cm, in order to indicate their perception of each symptom. The scores were recorded and the % values at T_{12} were computed with respect to those at T_0 .

2.4. Ex vivo basophil threshold sensitivity (CD-Sens) measured by CD63 and CD203c

We collected blood samples in EDTA pre-treated tubes from each patient at T_0 and T_{12} . Patients were instructed to avoid taking antihistamines and corticosteroids within 10 days prior to the visits. If a patient has taken an antiallergic drug during the 10 days preceding the visits (T_0 and T_{12}), the latter were rescheduled. In SLIT treated patients (SG) the blood sampling at T_0 was performed before the first administration of SLIT. The blood samples were divided into two aliquots in order to perform the CD-Sens by the two activation markers CD63 (Flow-CAST; Buhlmann Labs) and CD203c (AllergenCity Kit; Beckman Coulter Inc.) in two different series of tubes. The CD-Sens_{CD63} was performed stimulating basophils in vitro by an interleukin-3 (IL-3)-enriched buffer, whereas CD-Sens_{CD203c} was executed by an IL-3-free buffer. Two different gating strategies for each test were used to select basophils. For CD63 detection, basophils were identified as CCR3^{pos}/SS^{low} cells (Fig. 2a), due to the fact that CCR3 (eotaxin receptor) is expressed only on basophils (SS^{low}) and eosinophils (SS^{high}). Whereas for CD203c detection, basophils were identified as CD3^{neg}/CRTH2^{high}/CD203c^{high} and SS^{low} cells (Fig. 3a), since CRTH2 (Prostaglandin D2 receptor) is expressed on TH2 cells, eosinophils and basophils, in our gating strategy we excluded eosinophils as SS^{high} cells and TH2 cells as CD3^{pos} cells. CD63-activated basophils were detected by monoclonal antibodies (mAb) to human CD63 labelled with fluorescein isothiocyanate (anti-CD63-FITC) (Fig. 2b), while CD203c-activated basophils were recognized by mAb to human CD203c labelled with R-Phycoerythrin (CD203c-PE) (Fig. 3b). In both sets of test tubes basophils were stimulated with decreasing allergen doses dissolved in a Calcium-enriched buffer. The maximum activation rate for each marker was established by stimulating basophils (a test tube for each experiment set) with an anti-FcεRI-mAb. Therefore decreasing concentrations of allergens were useful to establish the “minimum allergen concentration” needed to reach the 50% of maximum activation rate of basophils for each marker. These “minimum allergen concentration” is utilized to calculate the threshold sensitivity (CD-Sens), for each patient, multiplying by 100 its inverse value.

Data were acquired by a flow cytometer equipped with an argon laser (Epics XL-MCL; Beckman Coulter Inc.) and analysed by the Kaluza® software (Beckman Coulter Inc.).

2.5. Statistical analysis

It was estimated that, to detect at least a 50% change in CD203c at

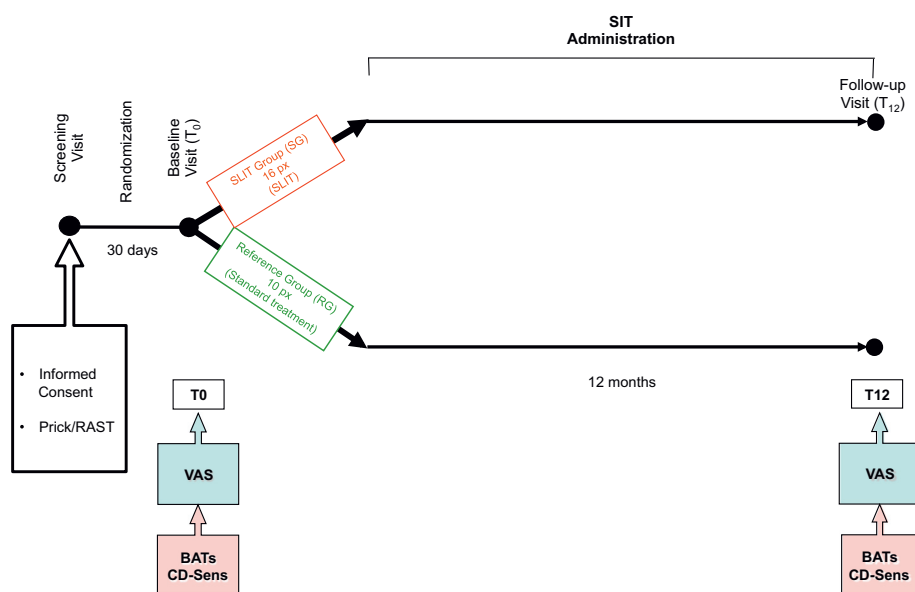


Fig. 1. Study design: patients selected in the screening visits attended baseline visit within 30 days. Subjects were randomized into two groups: SLIT group (SG), treated by Sublingual Immunotherapy (SLIT) and Reference Group (RG), treated by standard medications. At each visit, CD-Sens analysis by BATs (CD63 and CD203c) and VAS (Visual Analogue Scale) questionnaires for the cardinal symptoms of rhinitis were performed.

T12 with respect to T0 in subjects treated with SLIT, a sample of 15 subjects would have been adequate for the study, with a type I error of 0.05 and a statistical power ($1-\beta$) of 0.50.

Symmetric continuous data were summarized using the mean (standard deviation; SD); continuous data exhibiting skewness were summarized using the median (and inter-quartile range - IQR). Differences between normally and not normally distributed continuous variables were investigated by means of one-way analysis of variance (ANOVA) and Mann-Whitney *U* test, respectively. Wilcoxon signed-rank test was used for testing differences between matched variables. Differences in frequency distribution of categorical variables were evaluated by χ^2 test. Correlation between non parametric variables was investigated by means of Spearman's rank correlation.

The analyses were carried out using Statistical Package for Social Sciences (SPSS Inc., Chicago, IL) for Windows version 20.0 and *p* values $< .05$ were considered significant.

3. Results

3.1. Participants demographic characteristics

A total of 26 patients (17 male and 9 female subjects) mean age 41 years [range, 24–54 years] with a positive skin prick test were recruited, but only 22 completed the 12 months of treatment, 10 subjects in RG (5 male and 5 female) and 12 subjects in SG (7 male and 5 female). Four subjects enrolled in SG were excluded because they discontinued SLIT treatment. The baseline (T_0) characteristics of the 22 subjects who completed the study are shown in Table 1, separately for different treatments. No statistical differences were evident between the two groups.

3.2. CD-Sens measured by CD63 and CD203c

Medians (IQR) relative to both markers are presented in Table 2. Decreases of basophils sensitivity between T_0 and T_{12} were significant for SLIT Group (SG) both by CD-Sens_{CD63} ($\Delta = -33.32\%$; $p = .009$) and CD-Sens_{CD203c} ($\Delta = -43.56\%$; $p = .006$). There was a slight, but significant, increase of sensitivity in the RG for CD-Sens_{CD203c} ($\Delta = +14.39\%$; $p = .04$). CD-Sens_{CD63} showed a marked increase between T_0 and T_{12} in the RG, but we did not observe any statistical significance ($\Delta = +144.75\%$; $p = .07$).

3.3. Clinical symptoms score by visual analogue scale (VAS)

Changes from T_0 to T_{12} in nasal symptom scores were also reported in Table 2. Significant improvements were shown for each of the four investigated symptoms in the SG. Indeed, we observed a 37.5% decrease in the median of perception for nasal congestion (NC) ($p = .003$), 33.33% for nasal itching (NI) ($p = .003$), 28.57% for rhinorrhea (Rh) ($p = .008$) and 46.67% for sneezing (Sn) ($p = .005$). Conversely, no significant changes over the time were reported in the RG.

3.4. Correlation analysis

Correlation analyses were performed between clinical symptom scores (VAS) at T_{12} (expressed as % of T_0) and CD-Sens for both CD63 and CD203c basophil markers at T_{12} (in % of T_0). CD-Sens_{CD203c} changes were strongly correlated with changes in NC ($p < .0001$), NI ($p = .0001$), Rh ($p = .0003$), and Sn ($p < .0001$) (Fig. 4). Conversely, weak or not significant correlations were observed between CD-Sens_{CD63} and VAS changes (Fig. 5).

4. Discussion

In this randomized controlled longitudinal pilot study we corroborated the observation by Nopp and colleagues [9] that BATs, through the application of CD-Sens_{CD63}, can be useful to monitor the immunological changes induced by ASIT in well-characterized AR patients. Additionally to Nopp and colleagues [9], we showed the ability of the immunological marker CD203c to predict the clinical efficacy of sublingual immunotherapy on rhinitis subjects allergic to *Parietaria*.

The usefulness of basophil activation tests (BATs) on allergy diseases (such as asthma, allergic rhinitis, food allergy, allergic rhinoconjunctivitis) have been well-documented [14,15]. However, unlike the traditional application that indicates if the patient is allergic or not to an allergen, we evaluated the basophil sensitivity threshold (CD-Sens) to determine whether the basophils, and therefore the AR patients, become more tolerant to the allergen during ASIT treatment. This new application of BATs was developed by Nopp and colleagues, which used the basophil degranulation marker CD63 to establish how sensitive the patient is to the allergen, providing an objective measure of tolerance/sensitivity of the basophil from AR patients and therefore of the effectiveness of the therapy [9]. In addition to CD63, for the first time, we aimed to evaluate the ability of the emerging immunological marker CD203c, expressed on activated basophils during allergic

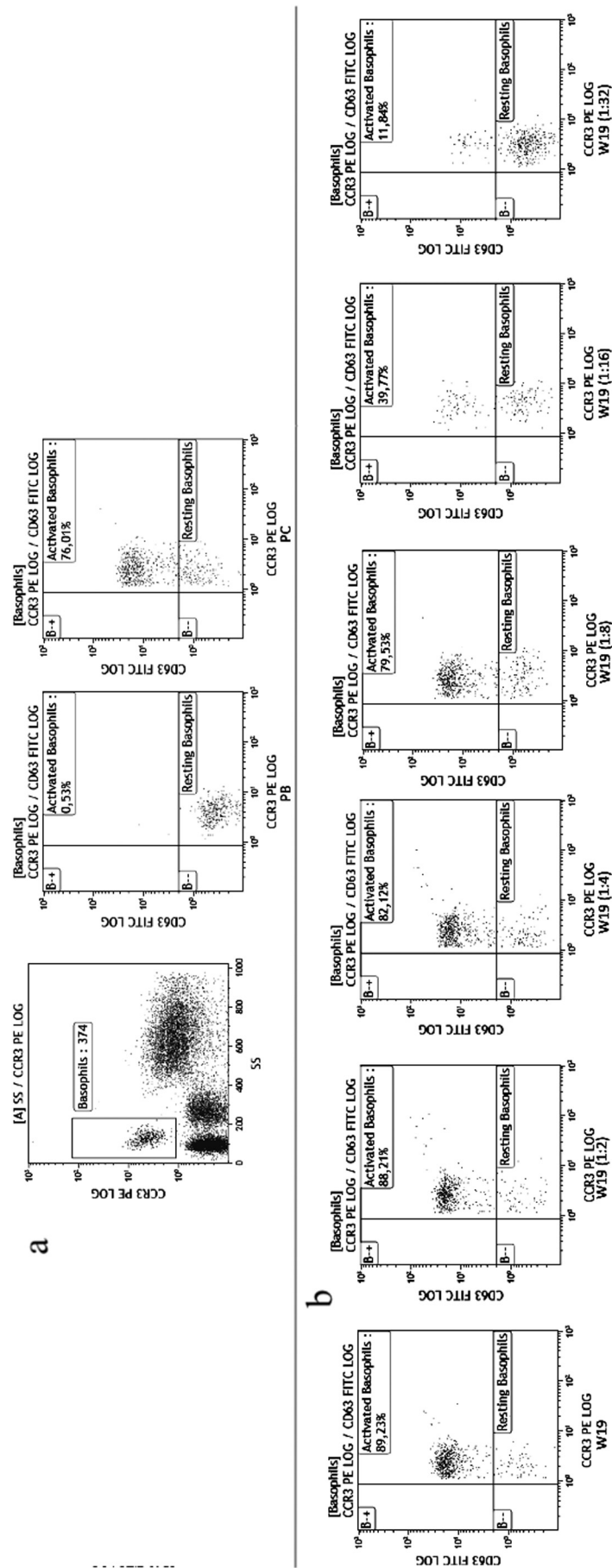


Fig. 2. Dot plot of CD63 detection from a patient. Gating strategy of basophils and establishment of CDmax (a): basophils were selected in the first plot as CCR3^{pos}/SSLow cells, and then the expression of CCR3^{pos}/CD63^{pos} was quantified as percentage of activated basophils in the third plot, that is 0.53% in patient background (PB) and 76.01% in positive control (PC). The PC value is considered as the CDmax. Therefore the threshold of sensitivity by CD63 for this patient (CDmax/2) was considered 38,005% (or the value that comes closest to this number), and thus the dilution of allergen that causes a percentage of activated basophils closest to 38% is considered as the threshold of basophils activation. As can be seen, the dilution that activates the percentage of basophils closest to the threshold is the 1:16 dilution (b). The concentration of the *Parietaria* allergen (W19) on this dilution was used to calculate the CD-SensCD63 for the patient, as described in the method section.



Fig. 3. Dot plot of CD203c detection from a patient. Gating strategy of basophils and establishment of CDmax (a): after a negative selection of Leucocytes (plot 1) with low SS not expressing CD3 (T⁺ lymphocytes), basophils were selected in the third plot as CD3^{neg}/CRTH2^{high}/CD203c^{high} cells, and then the expression of CD203c was quantified as percentage of activated basophils, that is 1.36% in patient background (PB) and 38.05% in positive control (PC). The PC value is considered as the CDmax in that case. Therefore the threshold of sensitivity of basophils by CD203c for this patient (CDmax/2) was considered as 19.025% (or the value that comes closest to this number), and thus the dilution of allergen that causes a percentage of activated basophils closest to 19%. As can be seen, the dilution that activates the percentage of basophils closest to the threshold is the 1:2 dilution (b). The concentration of the *Parietaria* allergen (W119) on this dilution was used to calculate the CD-SensCD203c for each patient, as described in the method section.

Table 1
Demographic and clinical characteristics of the study groups at baseline visit (T0).

	Standard medication (RG) (N = 10)	SLIT (SG) (N = 12)	P value
Age (yrs, mean $\pm$ SD)	41.7 $\pm$ 9.5	41.2 $\pm$ 8.5	0.89†
Sex (N., M/F)	5/5	7/5	0.70‡
CD-sens CD63 (median and IQR)	214.29 (142.9–571.4)	220.98 (119.0–619.4)	0.95§
CD-sens CD203c (median and IQR)	14.04 (12.0–96.2)	18.02 (8.3–96.2)	0.74§
Nasal congestion (score, median and IQR)	7.0 (6.0–8.0)	8.0 (7.0–8.0)	0.19§
Nasal itching (score, median and IQR)	7.0 (6.0–8.0)	7.5 (6.5–9.0)	0.28§
Rhinorrhea (score, median and IQR)	7.0 (7.0–8.0)	7.0 (7.0–7.0)	0.60§
Sneezing (score, median and IQR)	7.0 (6.0–7.0)	7.5 (6.5–8.0)	0.06§

P values were calculated by applying †one-way ANOVA, ‡ χ^2 test or §Mann-Whitney U test.

reactions [9,10,16,17], to predict the clinical efficacy of sublingual immunotherapy on AR patients allergic to *Parietaria*, using the CD-Sens approach. Recent studies, pointed to classify the basophil activation markers, highlighted the possibility that CD63 and CD203c are derived from two distinct compartments and have markedly different kinetics of activation, given that the expression of CD203c is very rapid while expression of CD63 is fairly slow [11,12]. Indeed, the responses to specific activators and inhibitors of CD63 and CD203c antigens appear to follow different kinetics and seem to be directed through alternative signal transduction pathways [11,13,14]. For instance, the PGD2, a potent activator of basophils, is able to increase strongly the CD203c expression, but it is not effective on both histamine release and CD63 expression [14]. Furthermore, in contrast to CD63, CD203c is exclusively expressed on the outer basophil/mast cells membrane [15] and it is clearly raised on basophils from sensitized subjects following treatment with allergen [10,11]. Therefore we deemed essential to perform the measurements of CD-Sens by CD203c and CD63 in two distinct experiments. This approach is needed to exclude the potential bias of the unspecific IL-3-driven CD203c overexpression on activated basophils [18,19], whereas the pre-warming by IL-3 is essential to the optimal expression of CD63 on basophil surface [20].

Other studies aimed to investigate the usefulness of BATs in detect immunological changes in course of SLIT [21,22], but not obtained significant results. In contrast to our study, Van Overtvelt et al. [21] analysed the effect of SLIT over a period of 2–4 months, that is probably too short to observe significant responses of basophil reactivity.

Whereas, Ciepiela et al. [22] focused their attention on paediatric patients affected by AR and bronchial asthma, allergic to House Dust Mite (HDM) or grass pollen, showing no differences in basophil activation by CD203c over a 1 year of SLIT administration. Notably the researchers observed an increase in TH1 frequency ($p = .01$, $n = 19$). In consideration that allergic asthma has a predominant Th2 phenotype and that SIT leads to apoptosis/anergy of T cells and induces immune-regulatory responses and immune deviation towards Th1 [23], it could be understandable that, in this type of subjects, the first immunological effect of SLIT is observed on this class of cells and not on basophils. Moreover we have to take into account that HDM is a particular type of allergen [24], and grass pollen is a heterogeneous family of allergens quite different from *Parietaria* as biological properties [25].

Finally, the approach used in these studies, investigates the different reactivity of basophils to allergens measured by percentage or Mean Fluorescence Intensity (MFI) of activated cells. In our study we aimed to study the different concentration of allergen needed to activate basophils in order to reach a threshold of activation fixed as the 50% of the maximum activation achievable for basophils (CD_{max}) stimulated by the anti-Fc ϵ RI-mAb, also called CD-Sens. Using the CD-Sens approach, it is possible to fix a threshold of sensitivity, at any time, as an immunological parameter useful to better outline the magnitude of allergic sensitivity for each subject to a specific allergen. All these difference could contribute to the different results obtained by our study and that of Ciepiela and colleagues.

Conceptually, the sensitivity to an allergen in an allergic subject

Table 2

Comparison of CD-sens (both CD63 and CD203c) and VAS score between baseline visit (T0) and follow-up visit after 12 months of treatment (T12) for reference group (treated by antihistamines) and SLIT Group (treated by SLIT).

Reference group (RG) (N = 10)				
	T0	T12	$\Delta\%$	P value
CD-sens CD63	214.30 (142.9–571.4)	524.50 (142.9–763.4)	+144.75	0.07
CD-sens CD203c	14.04 (12.0–96.2)	16.06 (16.0–96.2)	+14.39	0.04*
Nasal congestion	7.00 (6.0–8.0)	7.00 (6.0–8.0)	0.00	0.99
Nasal itching	7.00 (6.0–8.0)	7.00 (6.0–8.0)	0.00	0.99
Rhinorrhea	7.00 (7.0–8.0)	7.50 (7.0–8.0)	+7.14	0.42
Sneezing	7.00 (6.0–7.0)	7.00 (6.0–7.0)	0.00	0.22
SLIT group (SG) (N = 12)				
	T0	T12	$\Delta\%$	P value
CD-sens CD63	220.98 (119.0–619.4)	149.56 (62.1–357.1)	–33.32	0.009**
CD-sens CD203c	18.02 (8.3–96.2)	10.17 (3.8–72.1)	–43.56	0.006**
Nasal congestion	8.00 (7.0–8.0)	5.00 (4.5–6.0)	–37.50	0.003**
Nasal itching	7.50 (6.5–9.0)	5.00 (4.0–6.0)	–33.33	0.003**
Rhinorrhea	7.00 (7.0–7.0)	5.00 (4.0/6.5)	–28.57	0.008**
Sneezing	7.50 (6.5–8.0)	4.00 (4.0/6.0)	–46.67	0.005**

Data are reported as median (IQR). P values were calculated by applying Wilcoxon signed-rank test.

*P values < .05; **P values < .01.

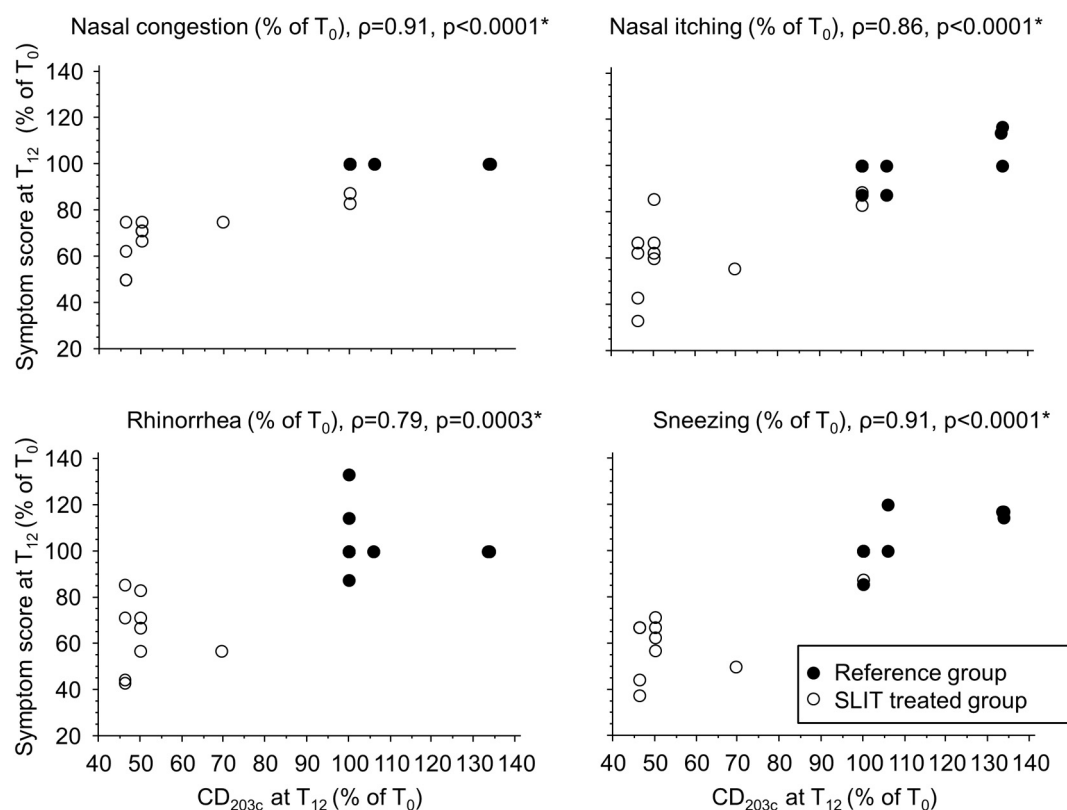


Fig. 4. Scatter plots of the correlations between different symptomatic scores at T₁₂ (VAS, in % of T₀) and CD-Sens_{CD203c} (at T₁₂, in % of T₀). *ρ and p values were computed by means of Spearman rank correlation.

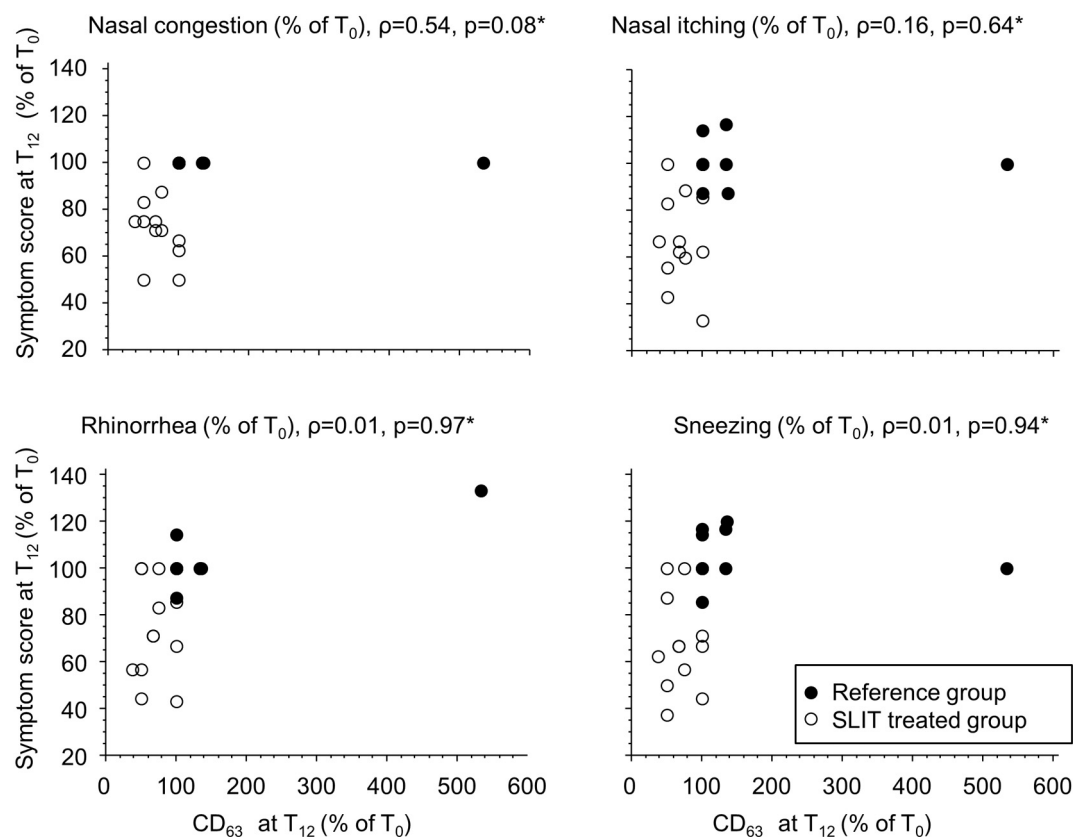


Fig. 5. Scatter plots of the correlations between different symptomatic scores at T₁₂ (VAS, in % of T₀) and CD-Sens_{CD63} (at T₁₂, in % of T₀). *ρ and p values were computed by means of Spearman rank correlation.

should be stable, or at least could worsen, over the time, as a consequence of the progression of allergic march [26]. Indeed, we observed slightly significant increase in sensitivity of subjects included in the reference group (RG) treated only by standard medications (Table 2). On the contrary, given the desensitizing ability of ASIT [2], the concentration of allergens required to activate the basophils increased in SG, proportionally to the improvement of the basophils tolerance to the allergen and therefore, inversely to their sensitivity, over the time. As expected, SLIT treated patients at 12 months showed a significant decrease in sensitivity measured by CD-Sens for both markers, CD63 and CD203c (Table 2). On the contrary, in the RG (i.e. patients treated by standard medications), we observed an increase in CD-Sens by CD63, close to the significant value, along with a significant slight increase of sensitivity by CD203c. These results confirm the ability of SLIT to reduce the sensitivity of basophils/mast cells to allergens, thus to slow down the immunologic sensitivity progression, and the inability to achieve these results by using only the standard medications. In order to establish if the outcomes on basophils sensitivity find a correspondence in clinical symptoms reported by patients, we compared the immunological results of CD-Sens, for each of the two markers, to a score that focus on the four cardinal symptoms of rhinitis, measured using a Visual Analogue Scale (VAS). By the VAS the patients reported the level of perception of their symptoms at each visit (T_0 and T_{12}). The differences observed by the changes in immunological markers (CD-Sens_{CD203c} and CD-Sens_{CD63}) in patients treated by SLIT were compared to the clinical results obtained by the VAS score, between T_0 and T_{12} . VAS highlighted a significant improvement in all the four symptoms of rhinitis (Table 2), such as nasal congestion (NC; $p = .003$), nasal itching (NI; $p = .003$), rhinorrhea (Rh; $p = .008$) and sneezing (Sn; $p = .005$); no significant changes in the VAS scores were observed in the RG (Table 2). These results indicate that SLIT is effective to reduce the severity of symptoms already from the first year of therapy, and that it is effective to reduce the sensitivity of the immune cells involved in the allergic response. The first aim of this study was to determine whether the markers investigated on basophils might predict the effects of SLIT on the immune system. Both CD63 and CD203c markers evidenced a decrease in basophils sensitization after 12 months of sublingual immunotherapy. Conversely, no improvement was observed in patients who did not receive SLIT. These data support the usefulness of basophils activation/degranulation marker to monitor the immunological changes during SLIT. In order to define the marker better correlated to clinical symptoms, we associated the clinical VAS score data with the immunological markers analysis. Again, both markers showed a trend similar to that of clinical symptoms (VAS) for both patients in the RG and SG. Current evidences suggest that CD63 is useful for this purpose [17]. But, despite the similar improving effects induced by SLIT observed on CD63, CD203c and VAS score in SG, the trend of CD-Sens_{CD63} does not correlate closely with the symptoms measured using the VAS Score (Fig. 5).

Contrariwise the data showed a better correlation with variation of the four clinical symptoms reported by VAS score and variation of CD-Sens_{CD203c} (Fig. 4).

The correlation between changes in CD-Sens_{CD203c} and symptoms reported by VAS score fosters the hypothesis that the marker CD203c better correlates with clinical data and that it could be even more useful to the monitoring and, probably, to the fine-tuning of allergen dosage during the SLIT.

A possible limitation of this study is the small sample size, composed by only 12 subjects in the study group (along with 10 subjects in the control group). Nevertheless, by evaluating both groups in the same relationship, we were able to find strong correlations between change in symptoms of allergic rhinitis and change in CD-Sens_{CD203c}. On the contrary, the relationship between symptoms and CD-Sens_{CD63} was always weaker or not significant.

In conclusion, these data provide evidences that SLIT can change the immunological course of allergic sensitization in AR adults already

in the first year of administration, as reported by patients treated by immunotherapy, and that an immunological parameter as CD-Sens measured by both CD63 and CD203c on stimulated basophils could be useful to confirm and monitor the changes in the immune system. In particular, CD-Sens_{CD203c} seems to be better correlated to clinical symptoms than CD-Sens_{CD63}, probably due to the faster response of this biomarker to allergenic stimuli.

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