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Basophil biomarkers as useful predictors for sublingual immunotherapy in allergic rhinitis

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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 (T_0) and after 12 months (T_{12}) 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

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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 (Allergenicity Kit; Beckman Coulter Inc.) in two different series of tubes. The $CD\text{-}Sens_{CD63}$ was performed stimulating basophils in vitro by an interleukin-3 (IL-3)-enriched buffer, whereas $CD\text{-}Sens_{CD203c}$ 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