



IL-33/s-ST2 ratio, systemic symptoms, and basophil activation in Pru p 3-sensitized allergic patients

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Abstract

Background Although *IL-33/ST2* axis is involved in the development of allergic diseases, its contribution in food allergy is still unknown.

Methods In this study, we assessed the serum levels of *IL-33* and its s-ST2 receptor in 53 control patients (without allergic diseases), 47 peach (Pru p 3)-sensitized allergic patients (SAP), and in 68 non-Pru p 3-SAP. Basophil activation test (BAT) was used to assess the basophil activation due to allergen exposure before and after the addition of s-ST2 to the blood samples from 5 Pru p 3-SAP.

Results *IL-33* levels in Pru p 3-SAP were higher than in non-Pru p 3-SAP and in normal controls. Lower s-ST2 levels were found in Pru p 3-SAP than in non-Pru p 3-SAP. *IL-33/s-ST2* ratio was higher in Pru p 3-SAP than in both non-Pru p 3-SAP and controls. Higher *IL-33/s-ST2* ratio was observed in Pru p 3-SAP with severe than in those with mild systemic symptoms. BAT analysis in Pru p 3-SAP showed a decrease in basophil activation due to Pru p 3 exposure after the addition of s-ST2 to the blood samples.

Conclusions An imbalance in the baseline levels of *IL-33/ST2* pathway is present in Pru p 3-SAP. The measurement of this pathway might be helpful to detect patients at a higher risk of developing severe systemic symptoms.

Keywords *IL-33* receptor · sST2 · Food allergy · Non-specific lipid-transfer protein · Peach allergy · sIgE to Pru p 3 · Anaphylaxis · Basophil activation test · Panallergens

The present study is dedicated to the memory of Giuseppe Albeggiani who suddenly died on November 7th, 2016.

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Giuseppe Albeggiani: Deceased.

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Abbreviations

IL-33	Interleukin 33
s-ST2	Soluble IL-33 receptor
Pru p 3-SAP	Peach lipid-transfer protein (LTP)-sensitized allergic patients

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Non-Pru p 3-SAP	Non-Pru p 3-sensitized allergic patients
sIgE	Specific immunoglobuline E
BAT	Basophil activation test
SPT	Skin prick test
OAS	Oral allergic syndrome
ELISA	Enzyme-linked immunosorbent assay
nsLTP	Non-specific lipid-transfer protein
IL-1RL	IL-1 receptor or like 1 gene

Background

IL-33 is a cytokine belonging to IL-1 family (IL-1 α , IL-1 β , IL-18) with a dual function, acting both as a traditional cytokine through activation of the ST2 receptor complex and as an intracellular nuclear factor with transcriptional regulatory properties [1]. The nuclear *IL-33* is released by necrotic cells after tissue injury and/or trauma, and subsequently provokes local inflammation as an alarmin.

IL-33 exerts its function through the ST2, an IL-1 receptor-related protein highly and specifically expressed on Th2 cells [2, 3], of which two isoforms have been identified: T1/ST2, a trans-membrane form, and s-ST2, a secreted soluble form. T1/ST2 and sST2 have a common extracellular domain, but sST2, lacking the trans-membrane and intracellular domains, can serve as a decoy receptor of IL-33 [4]. This receptor is a critical component of Th2 response [3] and triggers the production of additional pro-inflammatory cytokines in allergic diseases [5].

In addition, excessive production and/or inappropriate activation of *IL-33* is also considered to be involved in the development of allergic disorders including rhinitis [4], atopic dermatitis [6], and asthma [7, 8]. Murine studies have shown the presence of this alarmin in food allergy, particularly in peanut allergy [9, 10].

It is well known that Pru p 3, a nsLTP, is the major peach allergen and the most frequent cause of food allergy in adults living in the Mediterranean area [11, 13]. Sensitization to this nsLTPs is associated with a broad spectrum of symptoms ranging from oral allergy syndrome (OAS) to anaphylactic shock [14].

At the moment, nobody has studied the *IL-33*/s-ST2 pathway in peach allergic patients who experienced systemic symptoms after the consumption of peach. Therefore, we decided to assess the serum levels of *IL-33* and of the soluble receptor (s-ST2) in Pru p 3-sensitized allergic patients (SAP), non-Pru p 3-SAP, and in normal controls. Furthermore, to test whether s-ST2 counteracted the inflammatory effects of the released *IL-33*, basophil activation test was performed in the absence and presence of a specific allergen (Pru p 3) and with the addition of s-ST2 receptor.

Methods

Study population

168 adult patients, resident in South Italy, were recruited from the Allergy Diseases Center “Prof Giovanni Bonsignore” (IBIM—CNR) and from the Allergy Unit, Buccheri La Ferla Hospital, Palermo, Italy. The study was performed with the approval of the ethics committee of Policlinico-Giaccone Hospital-Palermo Italy (7/2013–12.06.2013) and was in agreement with the Helsinki Declaration. Informed written consent from adult patients were obtained. Neither children nor mentally impaired individuals were enrolled. Patients were grouped as follow: 53 control patients (without a clinical history of allergic diseases and with negative skin prick test (*Alk Abello, Italy*) and/or negative sIgE to common food/aero-allergens), 68 non-Pru p 3-SAP (with mild persistent asthma and or rhinitis and positive skin test and/or sIgE to common aero-allergens; negative clinical history of food allergy), and 47 Pru p 3-SAP with a clear history of an allergic reaction after being in physical contact (i.e., skin) with/or after the consumption of a peach, confirmed by SPT to peach extract (standardized extract from *Alk Abello* industry, Italy) and by molecular diagnosis. Measurement of sIgE (ImmunoCAP system, Termofisher/Phadia Diagnostic, Uppsala, Sweden) to peach, apple, corn, tomato, rice, kiwi, hazelnut, peanut, walnut, almond, soy, and wheat was done in all groups. In addition, sIgE (ImmunoCAP system, Termofisher/Phadia Diagnostic) to fish, shrimp, milk, egg, and *Anisakis* was measured in all groups to exclude other food sensitivities.

The presence of concomitant respiratory allergic diseases (rhinitis and/or asthma) was also evaluated following the Allergic Rhinitis and its Impact on Asthma (ARIA) and Global Initiative for Asthma (GINA) guidelines. The Pru p 3-SAP with concomitant respiratory diseases (mild persistent asthma and or rhinitis) were 24, while the Pru p 3-SAP without concomitant respiratory diseases were 23. Patients with mild persistent asthma were treated with inhaled corticosteroids and long-acting beta 2 agonists, while patients with chronic rhinitis were treated with nasal corticosteroids. In addition, patients were grouped into Pru p 3-SAP with mild systemic reactions (Mueller classification grade 1–2; $n=27$) and Pru p 3-SAP with severe systemic reactions (Mueller classification grade 3–4; $n=20$).

All diagnostic tests were performed in stable patients for at least 2 months after disease exacerbations (for rhinitis and asthma) or after the acute allergic reactions.

Skin prick test

SPTs were performed by the same investigator, on the volar surface of the forearm using a standard 1-mm tip lancet,

according to the recommendations of the European Academy of Allergy and Clinical Immunology group. Histamine hydrochloride (10 mg/mL) and saline (ALK Abello, Milan, Italy) were used as positive and negative controls, respectively.

SPT with mix of *Lolium perenne*, *Phleum pratense* and *Cynodon dactylon* (grass pollen), *Olea europaea* (olive), *Artemisia vulgaris* (mugwort), *Parietaria judaica* (Parietaria), *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* (house dust mites), *Alternaria tenuis* (alternaria), *Canis lupus familiaris* (dog), *Felis catus* (cat) and *Prunus persica* (peach) was performed in all study participants with standardized extracts from Alk Abello industry, Italy. The SPT results were considered positive if the difference between the mean diameter of the wheal and the negative control was at least 3 mm.

Molecular diagnosis

sIgE anti-rPru p 1, anti-rPru p 3, and anti-rPru p 4 were determined using the ImmunoCAP system (ThermoFisher/Phadia Diagnostics, Uppsala, Sweden) in all groups. Results were expressed in kUA/L and values > 0.15kUA/L were considered positive.

IL-33, s-ST2, and 25-OH vitamin D serum concentrations

Blood samples were collected from the enrolled patients. IL-33 concentrations were measured in the serum of all 168 patients using an ELISA kit (Duoset; R&D Systems, Minneapolis, MN) (range 23.4–1,500 pg/mL) according to the manufacturer's instructions. Results were expressed in pg/mL.

s-ST2 receptor concentrations were measured in the serum of all 168 patients using an ELISA kit (Duoset; R&D Systems, Minneapolis, MN) (range 31.2–2000 ng/mL) according to the manufacturer's instructions. Results were expressed in ng/mL. For s-ST2 receptor evaluation, sera concentrations were above the dynamic range and were appropriately diluted and re-analyzed.

25-OH vitamin D concentrations were measured in the serum of controls and Pru p 3-SAP (Duoset; R&D Systems, Minneapolis, MN) (range 5.3–133 ng/mL) according to the manufacturer's instructions. Results were expressed in ng/mL.

Basophil activation test (BAT) by flow cytometry

To better understand the biological relevance of the altered balance of IL-33 and sST2 in Pru p 3-SAP, BAT analyses were performed in five ($n = 5$) Pru p 3-SAP without rhinitis and asthma characterized by higher systemic IL-33/s-ST2

ratio (mean 0.68 ± 0.4) in the absence of corticosteroid treatment. Peripheral blood was collected into K-EDTA venipuncture tubes as suggested by Buhlmann Manual. A basophil activation test, measured as expression of CD63 (FlowCAST™; Buhlmann Laboratories AG®, Switzerland), was performed following the manufacturer's procedure, incubating 50 μ L of whole blood cells in 50 μ L of BAT buffer with Pru p 3 allergen (Buhlmann Laboratories AG®, Switzerland) at the concentration of 22.5 ng/mL, undiluted and diluted 1:2, as suggested by FlowCAST instructions. Moreover, to determine the effect of s-ST2 on allergen-induced basophil activation, 50 μ L of whole blood cells plus 50 μ L of BAT buffer were pre-incubated with 50 ng/mL of s-ST2 receptor (R&D Systems, Minneapolis, MN, USA) for 30 min at 37 °C; cells were then stimulated with Pru p 3 allergen as described above.

To evaluate basal degranulation values in the absence of any stimulations, the stimulation buffer was used as negative control. FlowCAST™ kit included two different positive controls: (1) anti-Fc ϵ RI mAb (no specific concentration was reported in the manufacturer's instructions), which mimics the bridging of the receptor in vivo caused by the allergen, and (2) fMLP (no specific concentration was reported in the manufacturer's instructions), which is a tripeptide causing basophil activation in a non-immunologic way. In each tube, the cells were stained with a mixture of monoclonal antibodies to human CD63-FITC and CCR3-PE for 30 min at 37 °C in a water bath. After red blood cell lysis, the cells were washed and analyzed by FACSCalibur supported with CellQuest acquisition and data analysis software (Becton Dickinson, Mountain View, CA, USA). Basophilic cells were gated by including the entire basophil population CCR3^{pos} with low Side Scatter SSC^{low}, and after acquisition of 500–600 basophilic cells the procedure was stopped. Basophil activation was defined as the percentage of CD63 positive cells within the total population of identified basophilic cells. Stimulation index (SI) was calculated from the ratio of CD63 expression post-stimulus minus the basal CD63 expression divided for the basal CD63 expression. For Pru p 3, an increase of $\geq 15\%$ of CD63⁺ cells compared to background was considered positive, as suggested by the manufacturer's recommendations.

Data analysis

All data are expressed as median (with interquartile ranges). Differences among groups were tested by non-parametric tests (Kruskal–Wallis and by Mann–Whitney tests). Results were considered significant when $p < 0.05$.

Table 1 Demographic characteristics of all study patients

Characteristics	Control patients (n = 53)	Non-Pru p 3-SAP (n = 68)	Pru p 3-SAP (n = 47)
Age (years, mean with range)	33.5 (20–50)	33.1 (18–54)	29.2 (18–46)
Gender (female/male; number)	25/28	31/37	25/22
Rhinitis and/or asthma (yes/no; number)	0/53	68/0	24/23
Sensitization (poli/mono; number)	0/0	45/23	30/17
Peach F.A./other F.A (number)	0/0	0/0	47/0
Positive SPT to peach extract (number)	0	0	47
Positive sIgE to Pru p 3/Pru p 1/Pru p 4 (number)	0/0/0	0/0/0	47/0/0

Table 2 Clinical characteristics of all study patients

Clinical manifestations (number)	Control patients (n = 53)	Non-Pru p 3-SAP (n = 68)	Pru p 3-SAP (n = 47)
<i>Mild systemic reactions</i> generalized urticaria, angioedema	0	0	27
<i>Severe systemic reactions</i> generalized urticaria, angioedema, vomiting, diarrhea, dyspnea, hypotension	0	0	20

Results

Patient demography

Demographic and clinical characteristics of the recruited subjects are reported in Table 1 and Table 2, respectively. All Pru p 3-SAP were only positive for both SPT to peach and sIgE to Pru p 3. All non-Pru p 3-SAP and control subjects were negative for both SPT to peach and sIgE to all peach allergens.

Serum IL-33 levels in controls, Pru p 3-SAP, and non-Pru p 3-SAP

IL-33 serum levels were significantly higher in Pru p 3-SAP ($p < 0.0001$) as well as in non-Pru p 3-SAP ($p < 0.0001$) than in normal controls. IL-33 serum concentrations were significantly higher in Pru p 3-SAP than in non-Pru p 3-SAP ($p < 0.0001$) [Fig. 1].

Serum s-ST2 levels in controls, Pru p 3-SAP, and non-Pru p 3-SAP

s-ST2 receptor serum levels were significantly higher ($p < 0.0001$) in non-Pru p 3-SAP than in normal controls. Surprisingly, significantly lower ($p < 0.0001$) s-ST2 receptor serum levels were found in Pru p 3-SAP than in non-Pru p 3-SAP. No difference was found between normal controls and Pru p 3-SAP [Fig. 2].

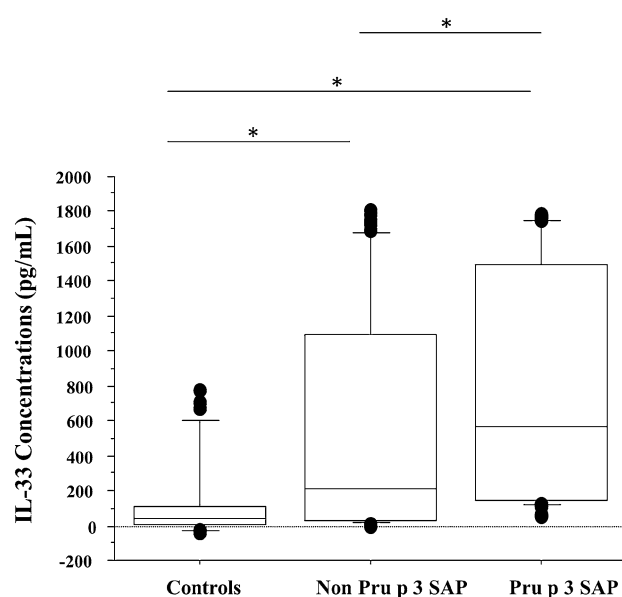


Fig. 1 IL-33 serum levels in normal controls, non-Pru p 3-SAP, and Pru p 3-SAP. Sera from normal controls ($n = 53$), non-Pru p 3-SAP ($n = 68$), and Pru p 3-SAP ($n = 47$) were assessed for IL-33 levels by ELISA method. Data were expressed as median (with interquartile ranges) * $p < 0.05$

IL-33, s-ST2 levels, and respiratory diseases

Interestingly, there was a significant difference ($p < 0.03$) in the levels of IL-33 [Fig. 3A] but not in the levels of s-ST2 [Fig. 3 B] between Pru p 3-SAP with respiratory diseases (rhinitis/asthma) and Pru p 3-SAP without respiratory diseases (rhinitis/asthma). Within Pru p 3-SAP, no significant

differences were observed between poli-sensitized (median 504.2 pg/mL) and mono-sensitized (median 718.7 pg/mL) Pru p 3-SAP. No correlation was found between *IL-33* serum levels and the severity of the clinical reaction as well as the serum levels of sIgE to Pru p 3 (data not shown).

IL-33/s-ST2 ratio

Furthermore, the IL-33/s-ST2 ratio was assessed in the recruited subjects. IL-33/s-ST2 ratio was significantly higher in Pru p 3-SAP than in normal controls ($p < 0.0001$) and in non-Pru p 3-SAP ($p < 0.0001$) [Fig. 4a]. Within Pru p 3-SAP, IL-33/s-ST2 ratio was significantly higher in patients with severe systemic reactions ($p < 0.02$) [Fig. 4b].

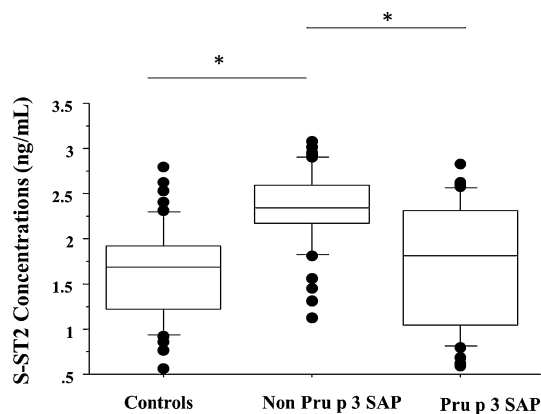


Fig. 2 s-ST2 Serum levels in normal controls, non-Pru p 3-SAP, and Pru p 3-SAP. Sera from normal controls ($n=53$), non-Pru p 3-SAP ($n=68$), and Pru p 3-SAP ($n=47$) were assessed for s-ST2 levels by the ELISA method. Data were expressed as median (with interquartile ranges) * $p < 0.05$

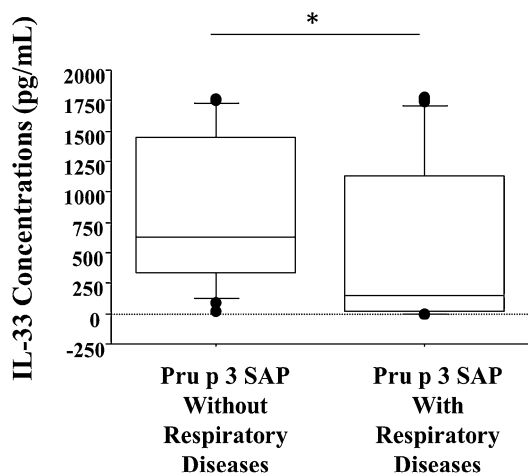


Fig. 3 Influence of concomitant allergic respiratory diseases in the measurement of *IL-33* and s-ST2 serum levels. Pru p 3-SAP recruited patients were stratified and compared for *IL-33* (a) and for s-ST2 (b)

Serum 25-OH vitamin D in controls and Pru p 3-SAP

Since vitamin D can limit *IL-33*-mediated responses up-regulating the expression of mRNA for the soluble decoy receptor sST2, the levels of 25-OH vitamin D in sera from Pru p 3-SAP were assessed and compared with the levels of 25-OH vitamin D in sera from controls. Higher levels of 25-OH vitamin D were observed in Pru p 3-SAP than in normal controls [Fig. 5].

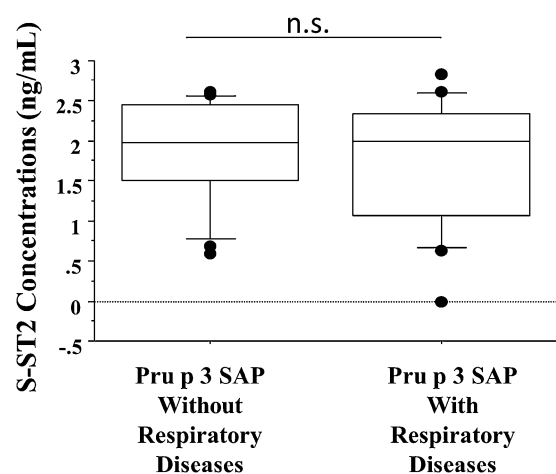
Basophil activation test (BAT)

BAT analysis demonstrated that the addition of 50 μ L of rPru p 3 to Pru p 3-SAP blood samples significantly increased ($p < 0.0001$) the percentage of activated CD63+ cells, while the addition of s-ST2 significantly decreased ($p < 0.002$) the percentage of activated CD63+ cells (Fig. 6a,b). All the tested patients positively responded to anti-Fc ϵ RI mAb and to FMLP used as positive controls (Fig. 6a).

Discussion

Dysregulation of ST2/*IL-33* signaling and sST2 production have been implicated in a variety of inflammatory diseases including allergic diseases. This study, for the first time, demonstrates an imbalance between increased levels of *IL-33* and reduced levels of its soluble extracellular receptor ST2 in the serum of peach sensitized patients, particularly Pru p 3-sensitized allergic patients. *IL-33*/s-ST2 ratio is increased in Pru p 3-SAP with severe systemic reactions.

IL-33, originally identified as a nuclear factor protein in endothelial cells, can be produced by structural cells in



serum levels on the basis of the presence or absence of rhinitis and asthma. Data were expressed as median (with interquartile ranges) * $p < 0.05$

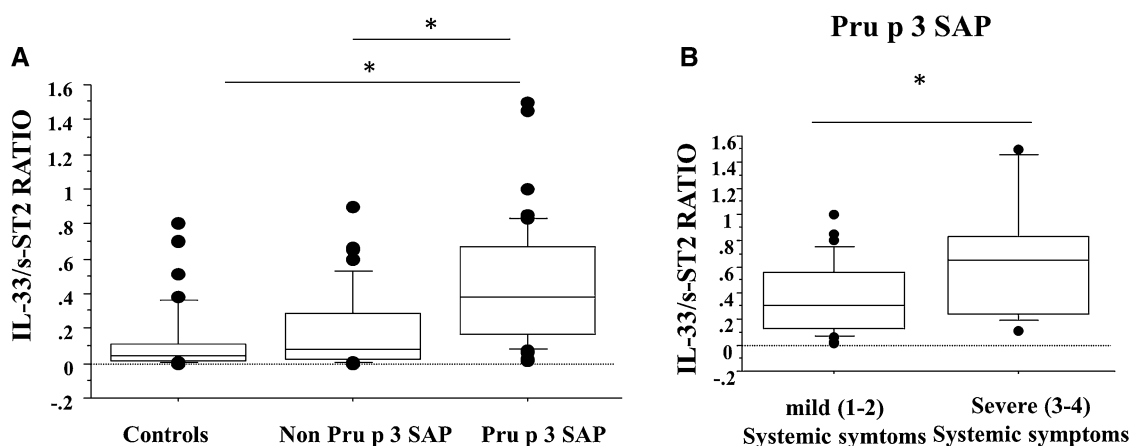


Fig. 4 IL-33/s-ST2 ratio in sera from normal controls, non-Pru p 3-SAP, and Pru p 3-SAP. **a** Sera from controls, non-Pru p 3-SAP, and Pru p 3-SAP were assessed for IL-33 and s-ST2 levels by the ELISA method and IL-33/s-ST2 ratio was calculated. Data were expressed as median (with interquartile ranges) * $p < 0.05$. **b** Pru p 3-SAP recruited

patients were stratified and compared for IL-33/s-ST2 ratio on the basis of the presence or absence of the severity of systemic symptoms (mild or severe). Data were expressed as median (with interquartile ranges) * $p < 0.05$

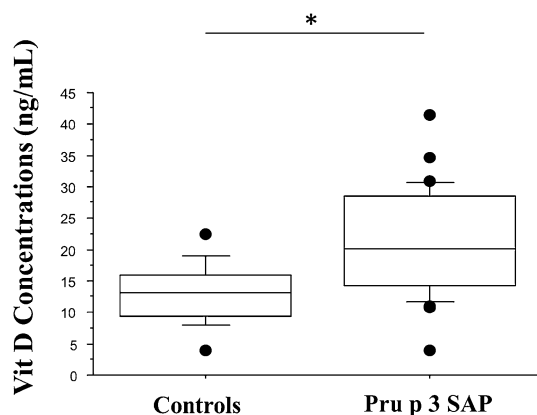


Fig. 5 25-OH vitamin D in sera from normal controls and Pru p 3-SAP. Sera from controls and Pru p 3-SAP were assessed for 25-OH vitamin D levels by the ELISA method. Data were expressed as median (with interquartile ranges) * $p < 0.05$

various tissues (epithelial, smooth muscle, and fibroblasts) and by hematopoietic cells, especially macrophages. IL-33 dysregulation is present in different pathological contexts including chronic airway and allergic diseases [17, 18].

IgE antibodies signaling through the high affinity FcεR1 induce the transcription of IL-33 mRNA and the synthesis of the IL-33 protein [19]. So, anaphylaxis and anaphylactic shock, characterized by higher levels of IgE could be associated to higher levels of pro-inflammatory mediators including IL-33. Here, the highest IL-33 serum levels were found in the group IL-33 of peach allergic patients. Two possible mechanisms could explain this finding: (1) nsLTP is a heat and enzymatic breakdown-resistant protein that can be potentially very dangerous, with the

ability to promote the activation of several cytokines generating the development of systemic reactions; (2) all Pru p 3-SAP were only sensitized to Pru p 3 and not to other molecular allergens of the peach, such as PR 10 or profilin. The influence of different co-factors in modulating the severity of allergic reactions to LTP food in patients suffering from a concomitant pollen allergy is of great interest. Co-sensitization to labile plant-food allergens, namely allergens from the PR-10 family and profilin, seems to reduce the severity of LTP allergy. This has been recently demonstrated in a large population of peach allergic patients with and without concomitant pollen allergy in which the simultaneous reactivity to Pru p 3 and Pru p 1 and/or Pru p 4 can determine significantly different clinical situations in comparison to mono-sensitized patients. In addition, patients positive to Pru p 3 and simultaneously positive to Pru p 1 and/or Pru p 4 could be protected against the development of severe symptoms induced by Pru p 3 [20]. Recently, Uasuf et al. have published a study showing that this type of antibody pattern is present only in patients from North-East Italy, but absent in patients from South Italy, which might explain the development of more severe symptoms [21]. The Pru p 3-SAP included in the present study are all resident in South Italy and all are exclusively positive to Pru p3. Future evaluations are needed to clarify whether the presence or absence of Pru p 1 and/or Pru p 4 in different cohorts of patients is associated with increased serum levels of IL-33.

IL-33 is involved in the development of allergic disorders such as rhinitis and asthma. Evidences from experimental models strongly support a functional role for IL-33/IL-1RL1 signaling in asthma pathogenesis [7]. Furthermore, it has been shown that IL-33 levels in the sera of patients with allergic rhinitis were significantly higher than that in

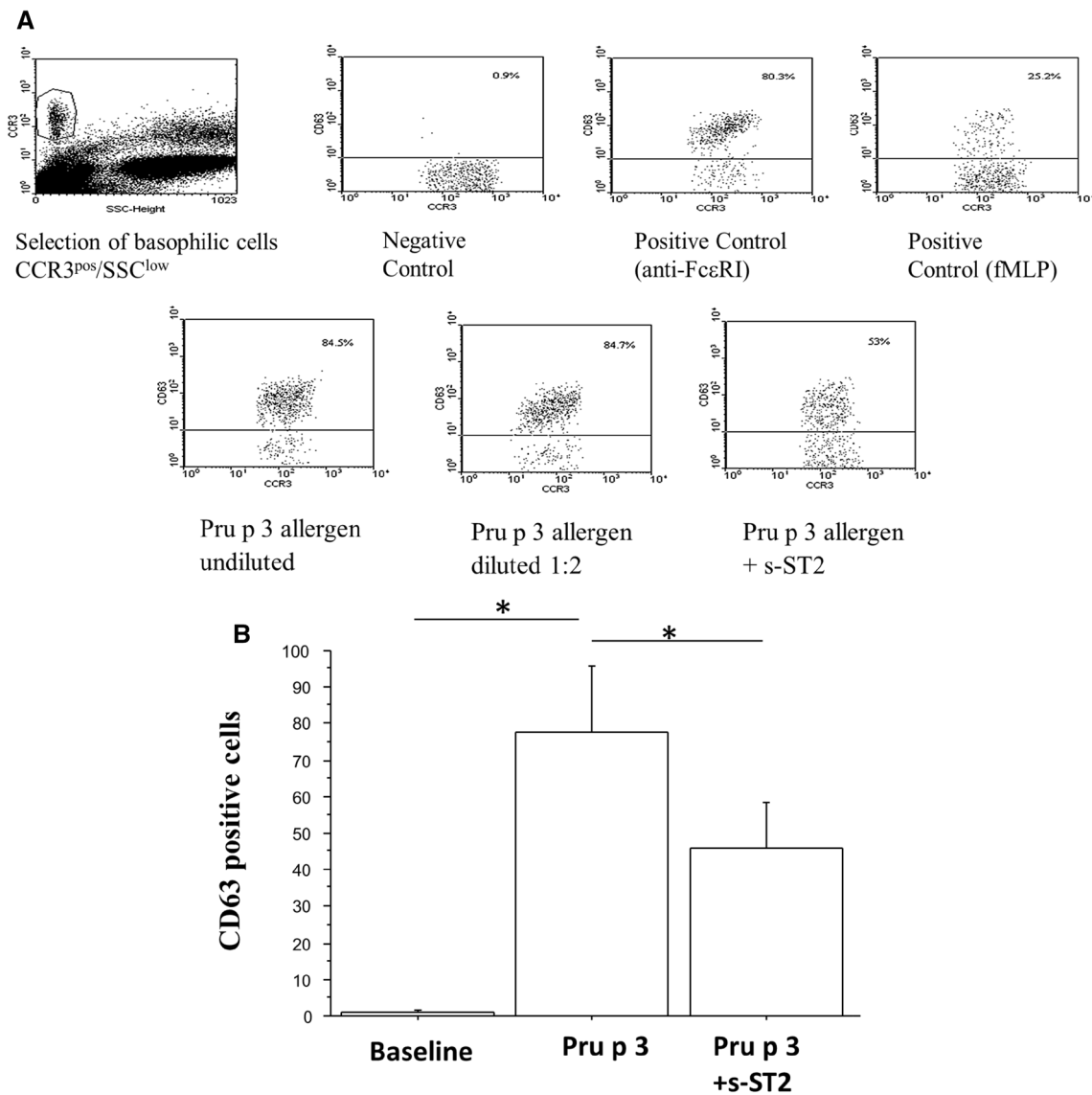


Fig. 6 Effects of s-ST2 on basophil activation in Pru p 3-SAP. Basophil activation test (BAT) was performed on peripheral blood basophils of Pru p 3-SAP patients ($n=5$), assessing CD63 expression by flow cytometry. **a** Representative dot plots from a basophil activation test experiment showing the strategy used to gate cell population to

analyze and showing positive and negative controls, Pru p 3 allergen (undiluted or 1:2 diluted), and undiluted allergen with s-ST2. **b** Data from $n=5$ patients were expressed as percentage (mean $\pm$ SD) of positive cells. Only data on undiluted allergen were included in the graph. * $p < 0.05$

normal controls [2]. In the view of these observations, we investigated whether the presence of concomitant respiratory diseases could influence *IL-33* serum levels in Pru p 3-SAP. For this purpose, we also included non-Pru p 3-SAP with concomitant respiratory diseases. We found significantly lower *IL-33* serum levels in non-Pru p 3-SAP and in Pru p 3-SAP who suffer from rhinitis and/or asthma when compared with those without concomitant respiratory diseases. This could be related to the fact that both non-Pru p 3-SAP and Pru p 3-SAP with rhinitis and/or asthma recruited in the present study were under treatment with inhaled steroids (nasal and/or oral), drugs able to modulate the production

of inflammatory cytokines [22, 23] including *IL-33*. A previous study has demonstrated that the production of *IL-33* due to allergen challenge is modulated by glucocorticoid therapy [24].

The biological activities of *IL-33* are mediated by the binding of *IL-33* to the ST2 expressed on the membrane of hematopoietic cells (basophils, mast cells, Th2 lymphocytes, and eosinophils) and of structural cells (endothelial and epithelial cells and fibroblasts). Upon the binding to the membrane receptor, *IL-33* in lymphocytes contributes to amplify Th2 responses promoting *IL-4*, *IL-5*, and *IL-13* synthesis [24]. In mast cells, *IL-33* acts through its membrane-bound

receptor, ST2 receptor, and induces degranulation following IgE-sensitization [16]. In this regard, it has been recently demonstrated that *IL-33* potentiates IgE-mediated mast cell responses by both increasing the number of responding cells and enhancing the magnitude of the responses of individual mast cells [25].

The receptor for *IL-33* is encoded by *IL1RL1*, and a differential splicing of the gene can produce a functional membrane-bound receptor (ST2L) or a soluble decoy receptor (sST2).

To limit *IL-33*-mediated responses, mast cells respond to *IL-33* and allergen challenge releasing increasing concentrations of soluble ST2 that acts as a decoy receptor [26]. Taking into account these concepts, we further investigated the imbalance of the *IL-33* pathway in the recruited subjects, assessing s-ST2 receptor. Interestingly, the present study revealed significantly lower s-ST2 receptor serum levels in Pru p 3-SAP than in non-Pru p 3-SAP. s-ST2 receptor serum levels were not statistically different in Pru p 3-SAP with and without concomitant respiratory diseases suggesting that, differently from *IL-33*, s-ST2 levels are not susceptible to the effects of corticosteroids. ST2 receptor serum levels were similar in Pru p 3-SAP and in controls although *IL-33* levels were higher in Pru p 3-SAP suggesting that a dysregulation in the mechanisms leading to the release of s-ST2 occurred in Pru p 3-SAP. Moreover, we assessed *IL-33*/s-ST2 ratio in the recruited subjects demonstrating that this ratio is specifically increased in Pru p 3-SAP who experienced severe systemic symptoms.

It is important to underline that the *IL-33*/s-ST2 ratio values showed here were obtained in stable conditions at least 2 months after the acute allergic reactions. It is possible that other concomitant inflammatory pathologies or conditions such as cardiac disease, inflammatory bowel disease, and type 2 diabetes [27], not investigated in this study, could also influence the *IL-33*/ST2 axis in Pru p 3-SAP. We could hypothesize that the higher *IL-33*/s-ST2 ratio might contribute to generate a minimal persistent inflammation that predisposed our patients to develop systemic multi-organ involvement when they were exposed to a lipid-transfer protein's source. It is well known that in allergic rhinitis and asthma, even in the absence of symptoms, a minimal level of persistent inflammation is found [28]. In this regard, patients with dust mite allergy (i.e., continuously exposed to allergen) present a minimal persistent inflammation both at nasal and conjunctiva levels. This inflammation is characterized by the presence of leukocyte infiltration and ICAM-1 expression in epithelial cells and by a relationship between specific and non-specific hyperactivity in the absence of clinical symptoms [29]. As in house dust mite allergy, where the natural exposure to allergen is continuous and its avoidance is almost impossible, the absence of nsLTP from diet is very difficult because most of the food used in the Mediterranean

cuisine contains this protein. Therefore, we believe that minimum but continuous exposure to nsLTP may result in a persistent inflammation even when subjects are asymptomatic.

We finally tested whether the imbalance in the *IL-33*/s-ST2 ratio could affect basophil activation. BAT analysis was performed in five Pru p 3-SAP without rhinitis and asthma and without corticosteroid treatment, characterized by higher systemic *IL-33*/s-ST2 ratio. Using the basophil activation test assay, we have shown that the addition of recombinant s-ST2 receptor significantly reduced allergen-induced in vitro activation of basophils expressing surface allergen-specific IgE, thus counteracting the *IL-33* pro-inflammatory effects. This might be considered a novel mechanism, never described before, of a possible pathogenic role for *IL-33*/s-ST2 in Pru p 3-SAP. The molecular mechanisms leading to the reduced levels of s-ST2 in the sera of Pru p 3 are largely unknown. Although previous reports have demonstrated that vitamin D can limit *IL-33*-mediated responses by selectively up-regulating the expression of mRNA for the soluble decoy receptor sST2 [30, 31] and that vitamin D deficiency is associated with an increased risk of food allergy, higher vitamin D levels were observed in Pru p 3-SAP thus suggesting that other mechanisms lead to the reduced levels of s-ST2 in these patients. In this regard, it has been demonstrated that nuclear expression of *IL-33* modulates sST2 release from endothelial cells [32]. Future studies are needed to clarify whether altered nuclear expression of *IL-33* could contribute to reduce s-ST2 in Pru p 3-SAP.

Conclusions

Higher *IL-33* and lower s-ST2 receptor baseline serum levels were detected in Pru p 3-SAP. *IL-33*/s-ST2 ratio is increased in Pru p 3-SAP and more in patients who experienced severe systemic symptoms. The addition of recombinant s-ST2 receptor might contribute to a natural regulatory mechanism that could conceivably limit systemic *IL-33* responses in peach food allergy.

The imbalance of this pathway could represent in the near future a biomarker to detect high-risk patients. These data open up new therapeutic approaches for food allergy.

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References

1. Sims JE, Smith DE. The IL-1 family: regulators of immunity. *Nat Rev Immunol*. 2010;10:89–102.
2. Kamekura R, Kojima T, Takano K, Go M, Sawada N, Himi T. The role of IL-33 and its receptor ST2 in human nasal epithelium with allergic rhinitis. *Clin Exp Allergy*. 2012;42:218–28.
3. Lee JH, Wang LC, Yu HH, Lin YT, Yang YH, Chiang BL. Type I IL-1 receptor (IL-1RI) as potential new therapeutic target for bronchial asthma. *Mediat Inflamm*. 2010 (Epub 5 2010).
4. Glück J, Rymarczyk B, Rogala B. Serum IL-33 but not ST2 level is elevated in intermittent allergic rhinitis and is a marker of the disease severity. *Inflamm Res*. 2012;61(6):547–50.
5. Ohno T, Morita H, Arae K, Matsumoto K, Nakae S. Interleukin-33 in allergy. *Allergy*. 2012;67:1203–14.
6. Tamagawa-Mineoka R, Okuzawa Y, Masuda K, Katoh N. Increased serum levels of interleukin 33 in patients with atopic dermatitis. *J Am Acad Dermatol*. 2014;70:882–8.
7. Préfontaine D, Nadigel J, Chouiali F, Audusseau S, Semlali A, Chakir J, Martin JG, Hamid Q. Increased IL-33 expression by epithelial cells in bronchial asthma. *J Allergy Clin Immunol*. 2010;125:752–4.
8. Préfontaine D, Lajoie-Kadoch S, Foley S, Audusseau S, Olivenstein R, Halayko AJ, Lemièrre C, Martin JG, Hamid Q. Increased expression of IL-33 in severe asthma: evidence of expression by airway smooth muscle cells. *J Immunol*. 2009;183:5094–103.
9. Chu DK, Llop-Guevara A, Walker TD, Flader K, Goncharova S, Boudreau JE, Moore CL, Seunghyun In T, Wasserman S, Coyle AJ, Kolbeck R, Humbles AA, Jordana M. IL-33, but not thymic stromal lymphopoietin or IL-25, is central to mite and peanut allergic sensitization. *J Allergy Clin Immunol*. 2013;131(1):187–200.
10. Tordesillas L, Goswami R, Benedé S, Grishina G, Dunkin D, Järvinen KM, Maleki SJ, Sampson HA, Berin MC. Skin exposure promotes a Th2-dependent sensitization to peanut allergens. *J Clin Invest*. 2014;124(11):4965–75.
11. Asero R, Antonicelli L, Arena A, Bommarito L, Caruso B, et al. Causes of food-induced anaphylaxis in Italian adults: a multi-centre study. *Int Arch Allergy Immunol*. 2009;150:271–7.
12. Fernandez-Rivas M, Bolhaar S, Gonzalez-Mancebo E, Asero R, van Leeuwen A, et al. Apple allergy across Europe: how allergen sensitization profiles determine the clinical expression of allergies to plant foods. *J Allergy Clin Immunol*. 2006;118:481–8.
13. Zuidmeer L, van Ree R. Lipid transfer protein allergy: primary food allergy or pollen/food syndrome in some cases. *Curr Opin Allergy Clin Immunol*. 2007;7:269–27.
14. Egger M, Hauser M, Mari A, Ferreira F, Gadermaier G. The role of lipid transfer proteins in allergic diseases. *Curr Allergy Asthma Rep*. 2010;10:326–35.
15. Bindeslev-Jensen C, Ballmer-Weber BK, Bengtsson U, Blanco C, Ebner C, Hourihane J, Knulst AC, Moneret-Vautrin DA, Nekam K, Niggemann B, Osterballe M, Ortolani C, Ring J, Schnopp C, Werfel T. Standardization of food challenges in patients with immediate reactions to foods—position paper from the European Academy of Allergy and Clinical Immunology. *Allergy*. 2004;59:690–7.
16. Chan YC, Ramadani F, Santos AF, Pillai P, Ohm-Laursen L, Harper CE, Fang C, Dodev TS, Wu SY, Ying S, Corrigan CJ, Gould HJ. “Auto-anti-IgE”: naturally occurring IgG anti-IgE antibodies may inhibit allergen-induced basophil activation. *J Allergy Clin Immunol*. 2014;134:1394–401.
17. Liew FY, Pitman NI, McInnes IB. Disease-associated functions of IL-33: the new kid in the IL-1 family. *Nat Rev*. 2010; 103–10.
18. Pace E, Di Sano C, Sciarrino S, Scafidi V, Ferraro M, Chiappara G, Siena L, Gangemi S, Vitulo P, Giarratano A, Gjomarkaj M. Cigarette smoke alters IL-33 expression and release in airway epithelial cells. *Biochim Biophys Acta*. 2014;1842:1630–7.
19. Nabe T. Interleukin (IL)-33: new therapeutic target for atopic diseases. *J Pharmacol Sci*. 2014;126:85–91.
20. Pastorello EA, Farioli L, Pravettoni V, Scibilia J, Mascheri A, Borgonovo L, Piantanida M, Primavesi L, Stafylarakis C, Pasqualetti S, Schroeder J, Nichelatti M, Marocchi A. Pru p 3-sensitized Italian peach-allergic patients are less likely to develop severe symptoms when also presenting IgE antibodies to Pru p 1 and Pru p 4. *Int Arch Allergy Immunol*. 2011;156(4):362–72.
21. Uasuf CG, Villalta D, Conte ME, Di Sano C, Barrale M, Cantisano V, Pace E, Gjomarkaj M, Gangemi S, Brusca I. Different co-sensitizations could determine different risk assessment in peach allergy? Evaluation of an anaphylactic biomarker in Pru p 3 positive patients. *Clin Mol Allergy*. 2015;13:30.
22. Pace E, Di Sano C, La Grutta S, Ferraro M, Albegiani G, Liotta G, Di Vincenzo S, Uasuf CG, Bousquet J, Gjomarkaj M. Multiple in vitro and in vivo regulatory effects of budesonide in CD4 + T lymphocyte subpopulations of allergic asthmatics. *PLoS One*. 2012;7(12):e48816.
23. Shen H, O’Byrne PM, Ellis R, Wattie J, Tang C, Inman MD. The effects of intranasal budesonide on allergen-induced production of interleukin-5 and eotaxin, airways, blood, and bone marrow eosinophilia, and eosinophil progenitor expansion in sensitized mice. *Am J Respir Crit Care Med*. 2002;166(2):146–53.
24. Nabe T, Wakamori H, Yano C, Nishiguchi A, Yuasa R, Kido H, Tomiyama Y, Tomoda A, Kida H, Takiguchi A, Matsuda M, Ishihara K, Akiba S, Ohya S, Fukui H, Mizutani N, Yoshino S. Production of interleukin (IL)-33 in the lungs during multiple antigen challenge-induced airway inflammation in mice, and its modulation by a glucocorticoid. *Eur J Pharmacol*. 2015;757:34–41.
25. Jouila R, L’Faqihi FE, Valitutti S, Espinosa E. IL-33 fine tunes mast cell degranulation and chemokine production at the single-cell level. *J Allergy Clin Immunol*. 2017;140(2):497–509.
26. Bandara G, Beaven MA, Olivera A, Gilfillan AM, Metcalfe DD. Activated mast cells synthesize and release soluble ST2—a decoy receptor for IL-33. *Eur J Immunol*. 2015. <https://doi.org/10.1002/eji.201545501>.
27. Griesenauer B, Paczesny S. The ST2/IL-33 axis in immune cells during inflammatory diseases. *Front Immunol*. 2017;8:475.
28. Storms WW. Minimal persistent inflammation, an emerging concept in the nature and treatment of allergic rhinitis: the possible role of leukotrienes. *Ann Allergy Asthma Immunol*. 2003;91:131–40.
29. Canonica GW, Ciprandi G. Minimal persistent inflammation may be controlled by cetirizine. *Ann Allergy Asthma Immunol*. 1999;83:445–8.
30. Pfeffer PE, Chen YH, Woszczek G, Matthews NC, Chevetton E, Gupta A, Saglani S, Bush A, Corrigan C, Cousins DJ, Hawrylowicz CM. Vitamin D enhances production of soluble ST2, inhibiting the action of IL-33. *J Allergy Clin Immunol*. 2015;135(3):824–7.
31. Koplin JJ, Suaini NH, Vuillermier P, Ellis JA, Panjari M, Ponsonby AL, Peters RL, Matheson MC, Martino D, Dang T, Osborne NJ, Martin P, Lowe A, Gurrin LC, Tang ML, Wake M, Dwyer T, Hopper J, Dharmage SC, Allen KJ. HealthNuts Study. Polymorphisms affecting vitamin D-binding protein modify the relationship between serum vitamin D (25[OH]D3) and food allergy. *J Allergy Clin Immunol*. 2015. (pii: S0091-6749(15)00911-2).
32. Shao D, Perros F, Caramori G, Meng C, Dormuller P, Chou PC, Church C, Papi A, Casolari P, Welsh D, Peacock A, Humbert M, Adcock IM, Wort SJ. Nuclear IL-33 regulates soluble ST2 receptor and IL-6 expression in primary human arterial endothelial cells and is decreased in idiopathic pulmonary arterial hypertension. *Biochem Biophys Res Commun*. 2014;451(1):8–14.