



Agreement of skin test with IL-4 production and CD40L expression by T cells upon immunotherapy of subjects with systemic reactions to Hymenoptera stings



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ABSTRACT

Venom immunotherapy is the only curative intervention for subjects with Hymenoptera venom allergy who suffering systemic reactions upon bee or wasp stings. Venom immunotherapy can restore normal immunity against venom allergens, as well as providing to allergic subjects a lifetime tolerance against venoms. Nevertheless, it is necessary using safety assays to monitoring the development of tolerance in the VIT protocols to avoid fatal anaphylactic reactions. The purpose of this study was to assess the modifications in several markers of tolerance induction in subjects with Hymenoptera venom allergy undergoing immunotherapy. The studies were performed at baseline time and after six month of VIT. Intradermal skin tests, basophil activation tests, specific IgE levels; and the T-cell markers (IL-4 and IFN- γ producing cells; and expression of the surface activation markers CD40L and CTLA-4) were assayed. At six month of immunotherapy all parameters studied had significant alterations. All decreased, except the IFN- γ producing cells. In addition, modifications in intradermal skin test showed a significant correlation with both, CD40L expression on CD4 T lymphocytes ($p = 0.043$) and IL-4 producing T lymphocytes ($p = 0.012$). Neither basophil activation test nor serum levels of sIgE demonstrated any correlation with the immunological parameters studied nor among them. These results suggest that both IL-4 production and CD40L expression could be two good indicators of the beneficial effects of venom immunotherapy which translate into skin tests.

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1. Introduction

Hymenoptera venoms are important allergens that can elicit both local and systemic allergic reactions, including life-threatening anaphylaxis. Venom immunotherapy (VIT) remains the most effective treatment, reducing the risk of systemic reactions in individuals with Hymenoptera venom allergy. Today, VIT is the only curative treatment for venom allergy in humans and protects against systemic reactions such as life-threatening anaphylaxis by exposure to bee or wasp venom (Boyle et al., 2012). VIT can restore normal immunity against venom allergens, and

provide patients with a lifetime of tolerance to venoms. Further VIT also represents a very useful tool to study the plasticity of the immune system in humans. For example, it has been demonstrated the switch between allergen-specific T helper 2 (Th2) and Treg cells as well as venom specific IgG4 in absence of IgE in VIT and in tolerant high-dose venom-exposed beekeepers (Ozdemir et al., 2011) (Varga et al., 2013). Systemic reactions due to Hymenoptera stings are mainly initiated by the IgE-mediated release of mediators from mast cells and basophils upon exposure to venom allergens. Despite of the progress in diagnostic methods of IgE-mediated mechanisms of Hymenoptera venom allergy, the cells involved in the systemic reactions are not yet fully known (Akkoc et al., 2011). Many different molecules intervene in the immune response, and an increasing interest is currently devoted to the activation pathways of T-lymphocytes. The surface expression of lymphocyte markers is disturbed in subjects with Hymenoptera venom allergy in response to immunotherapy (Bellinghausen et al., 1997). For example serum soluble CTLA-4 reduction following VIT induction is

Abbreviations: VIT, Hymenoptera venom immunotherapy; BAT, basophil activation test; IDT, intradermal test; sIgE, specific IgE antibodies.

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a very early marker of VIT effectiveness, and it is possible to envisage that the persistence of lower sCTLA4 levels indicates sustained protection against clinical reactions to field-resting (Riccio et al., 2012). Cytokines produced by T cell also regulate the release of basophil and mast cell mediators. The main central factor to Th2 type immunity is the IL-4 cytokine which has been linked to virtually all major hallmarks associated with Th2 type inflammation (Bao and Reinhardt, 2015). For instance, IL-3, IL-5, and GM-CSF increase the IgE-dependent activation of basophils.

The diagnosis of Hymenoptera venom allergy is usually performed through skin tests and *in vitro* assays such as specific IgE and IgG4 (Biló et al., 2005). Skin tests are performed by skin prick and intradermal testing, with a sensitivity for the skin prick tests lower than the intradermal test (Hamilton, 2010). Venom immunotherapy induces an initial rise of venom-specific IgE antibodies followed by a decline after a few months, with a large individual variation (Kemeny et al., 1989). There is no clear correlation between the concentration of venom-specific IgE and the reactivity status of the individual patients (Korošec et al., 2015). Furthermore the basophil activation test shows the effect of the induced desensitization after completing VIT (Eržen et al., 2012). There are not conclusive data about the behavior of the tests used to assess Hymenoptera venom allergy in venom immunotherapy.

We have recently described a downregulation induced by VIT in activation biomarkers of T cell activation as CD40L, CTLA-4 and IL-4 as well as an upregulation of IFN- γ (Cabrera et al., 2014).

The purpose of this work is to assess the induction of tolerance in subjects with Hymenoptera venom allergy undergoing immunotherapy. For this purpose parameters such as intradermal skin tests, basophil activation tests and specific IgE levels, besides immune activation markers as cytokines production (IL-4 and IFN- γ) and surface T-cells expression of CD40L and CTLA4 were assayed. In addition the possible relationships between both types of assays was determined in the course of a VIT protocol.

2. Material and methods

2.1. Subjects

Fourteen patients with a history of systemic reactions to Hymenoptera stings from the Allergy Outpatient Clinic of the General University Hospital of Ciudad Real (Spain) were enrolled in the study. Three patients were female and eleven were male, with a mean age of 47.2 ± 16.2 years (mean $\pm$ SD). Most patients had

venom allergy to *Polistes dominulus* ($n = 10$), the resting to *Vespula* spp. ($n = 2$), *Apis mellifera* ($n = 1$), and a final patient presented allergy to both *P. dominulus* and *Vespula* spp. simultaneously ($n = 1$). The sensitization state was determined through intradermal skin tests (IDT) and venom specific IgE (sIgE) levels according to the diagnostic recommendations (Biló et al., 2005). IDT was quantified by measuring the major (d_1) and minor (d_2) diameter of the wheal in mm to the lowest dose of venom with a positive reaction. The wheal area in mm^2 was estimated in accordance to the following formula $(d_1/2) \times (d_2/2) \times \pi$. The clinical characteristics of the venom allergic patients are summarized in Table 1.

2.2. Venom immunotherapy protocol

Fourteen venom allergic patients who, within the last 2 years, presented a history of anaphylaxis caused by Hymenoptera stings were eligible for venom immunotherapy (VIT) according to the criteria of the European Academy of Allergy and Clinical Immunology (Bonifazi et al., 2005). These patients were treated with a conventional VIT protocol of subcutaneous injections of the venom administered in the induction phase at doses increasing up to 100 μg by the 30th day as previously described (Cabrera et al., 2014). Maintenance injections of 100 μg were then administered at monthly intervals. Blood samples of the active patients were collected at baseline time before the start of VIT (T0), and after the sixth month of therapy (T6). The corresponding venom extracts were supplied by ALK-Abelló, S.A., (Madrid, Spain). All patients gave their informed consent for the immune intervention. The study was approved by the ethics committee of the Hospital General Universitario de Ciudad Real.

2.3. Mononuclear cell isolation

Cells were isolated from heparinized venous blood by density gradient centrifugation (Ficoll Hypaque, Sigma–Aldrich, St. Louis, MO, USA). Mononuclear cells were then washed twice in phosphate buffered saline (PBS) and resuspended in 2 mL of RPMI-1640 cell culture medium (Gibco, Scotland, UK) supplemented with streptomycin (100 UI/mL) and penicillin (100 mg/mL). For surface staining conditions we used 1 mL of diluted lymphocytes obtained immediately after cell isolation. Cells from allergic patients were collected just before venom immunotherapy (T0) and after 6 months of treatment (T6).

Table 1
Characteristics of venom Allergic patients and baseline data before VIT.

Patient	Age (years) and sex	Culprit	Tryptase (mg/L) ^b	Systemic reaction ^a	IDT ($\mu\text{g}/\text{mL}$ of venom)	sIgE (KU/L) ^b	BAT-A	BAT-B
1	67, M	<i>P. dominulus</i>	7.3	Grade IV	15 $\times$ 12 mm (1.0)	7.11	30.0	23.6
2	59, F	<i>P. dominulus</i>	4.31	Grade IV	11 $\times$ 12 mm (0.1)	29.9	84.9	28.6
3	67, M	<i>P. dominulus</i>	5.1	Grade IV	10 $\times$ 15 mm (0.1)	48.3	35.6	16.8
4	10, M	<i>P. dominulus</i>	3.7	Grade II	9 $\times$ 7 mm (0.01)	48.8	26.8	17.6
5	49, M	<i>P. dominulus</i>	6	Grade III	10 $\times$ 11 mm (1.0)	2.01	63.9	17.4
6	78, F	<i>P. dominulus</i>	8.8	Grade II	8 $\times$ 9 mm (0.01)	96.0	88.4	17.2
7	67, M	<i>Vespula</i> spp.	4	Grade IV	11 $\times$ 11 mm (0.1)	11.5	57.6	42.7
8	70, M	<i>P. dominulus</i>	22.5	Grade III	7 $\times$ 7 mm (1.0)	0.96	100.0	22.3
9	33, F	<i>Apis mellifera</i>	4.26	Grade II	7 $\times$ 6 mm (0.1)	39.6	3.1	2.4
10	60, M	<i>P. dominulus</i>	5	Grade IV	12 $\times$ 13 mm (0.1)	51.8	40.3	17.3
		<i>Vespula</i> spp.			12 $\times$ 13 mm (0.01)	30.9	31.4	20.5
11	54, M	<i>P. dominulus</i>	4.7	Grade III	11 $\times$ 10 mm (0.01)	28.7	51.5	19.2
12	33, M	<i>P. dominulus</i>	4.39	Grade III	10 $\times$ 9 mm (1.0)	49.3	25.6	1.7
13	70, M	<i>P. dominulus</i>	5.66	Grade II	9 $\times$ 10 mm (0.1)	6.43	95.5	92.4
14	36, M	<i>Vespula</i> spp.	4.12	Grade IV	9 $\times$ 11 mm (0.001)	20.9	87.5	80.0

Abbreviations: F, female; IDT, intradermal test; M, male. (a) According to the classification of systemic reactions published by Mueller (1966). (b) Levels of tryptase and sIgE were measured by ImmunoCAP (Phadia AB, Uppsala, Sweden). BAT-A high dose at 1 $\mu\text{g}/\text{mL}$ of venom, BAT-B low dose at 0.1 $\mu\text{g}/\text{mL}$ of venom. Intradermal tests were performed with 0.02 mL of solution with concentrations ranging from 0.001 to 1.0 $\mu\text{g}/\text{mL}$ of the corresponding venom. The lowest concentration yielding a positive reaction was considered as the endpoint.

2.4. Intracellular T cell cytokine assay

Lymphocyte cells at a concentration of 1×10^6 cells/mL (1 mL per tube) were stimulated for 16 h with 50 ng/mL of phorbol myristate acetate (PMA) (Sigma–Aldrich, Steinheim, Germany) and 1 μ M of ionomycin (Sigma, Steinheim, Germany) in 5% CO₂ at 37 °C. ABD GolgiPlug™ protein transport inhibitor (Becton Dickinson, San Jose, USA) was added for the last 5 h of the incubation period, for purposes of the intracellular cytokine staining protocol.

2.5. Antibodies used in flow cytometry

The following anti-human conjugated monoclonal antibodies were employed in the surface expression of CD4+ T-cells: fluorescein isothiocyanate (FITC) anti-CD4, phycoerythrin (PE) anti-CD154, and PE anti-CD152. The intracellular cytokine staining were performed in CD3+ T-cells using the following monoclonal antibodies: PE anti-IL-4, PE anti-IFN γ , and PerCP-Cy5 anti-CD3. All the monoclonal antibodies were purchased from Becton Dickinson (BD, San Jose, USA). SimulTest™ Control $\gamma 1/\gamma 1$ (IgG1/IgG1) (BD) was used as a negative control to estimate the amount of non-specific staining.

2.6. Immunofluorescent staining and FACs analysis

In the surface staining for CD4+ T-cells, the cells were first incubated in the dark with the corresponding monoclonal antibody for 20 min. They were then washed, resuspended in PBS and analyzed by flow cytometry. Cytokine intracellular staining of CD3+ T-cells was carried out according to the instructions of the BD intracellular staining kit (Cytofix/Cytoperm™ Plus Fixation/Permeabilization kit). Lymphocytes were acquired with a FACScan cytometer (Becton Dickinson) and analysed using CellQuest Pro software. At least 10,000 events were acquired and analysed in the lymphocyte gate using two-color immunofluorescence.

2.7. Basophil activation test (BAT)

The basophil degranulation, expressed as the percentage of CD63+ basophils was determined using a BASOtest kit (Glyco-type Biotechnology GmbH, Heidelberg, Germany). Heparinized whole blood samples were incubated with high dose at 1 μ g/mL (dose A) or low dose at 0.1 μ g/mL (dose B) of the corresponding venom extracts (ALK-Abelló, S.A., Madrid, Spain) according to the kit's recommendations. All determinations were assayed in parallel with positive and negative controls (fMLP chemotactic peptide and wash solution, respectively). The cells were stained with PE anti-IgE and FITC anti-CD63 monoclonal antibodies, and a minimum of at least 1000 basophils were acquired for flow cytometry.

2.8. Statistical study of venom allergic subjects

We used the non-parametric Wilcoxon matched-pairs signed rank test to compare the values of paired venom allergic subjects values at T0 and after immune intervention (T6). Pearson's correlation coefficient and regression coefficients was used to analyse the correlation between quantitative variables. The results were analysed using IBM SPSS 22.0 Software. The differences were considered statistically significant at p values of less than 0.05

3. Results

3.1. Baseline data of venom allergic subjects

Before the beginning of immunotherapy protocol all patients had positive results of IDTs (minimum diameter of the wheal

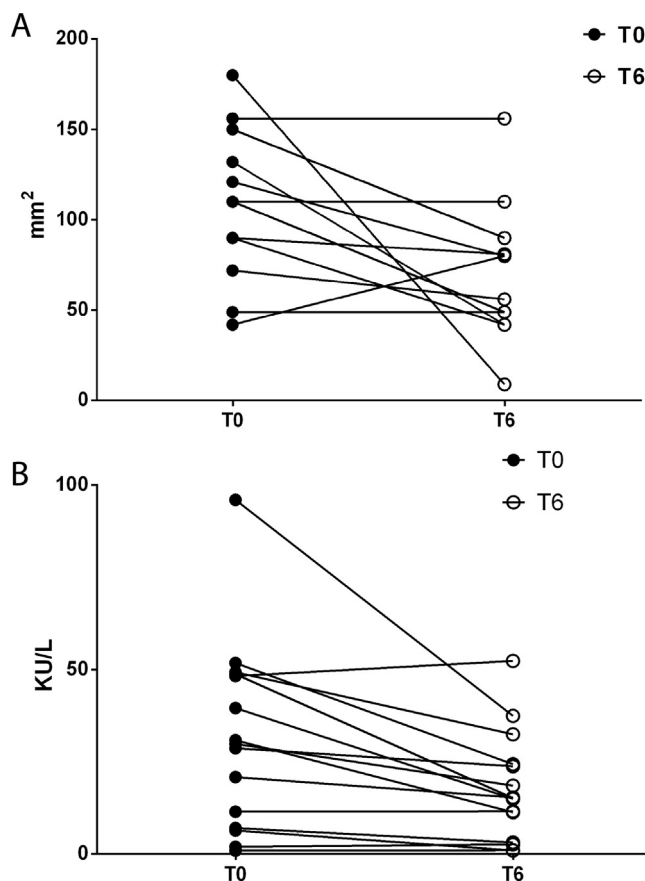


Fig. 1. (A) Areas of intradermal tests (IDTs) of the 14 subjects at the beginning of the immune intervention (T0) and after the 6th month. (B) Basal levels of sIgE (T0) and upon immunotherapy (T6).

>5 mm) and detectable serum levels of sIgE (>0.35 KU/L) as shown in Table 1. The mean value of sIgE was of 31.47 ± 25.47 KU/L ranging from 0.96 to 96.0 KU/L and for IDTs the mean wheal area was 108.0 ± 41.7 mm² with a range of values between 42.0–180 mm² in doses of the respective venoms ranging from 0.01 to 1.0 μ g/mL. For BAT assay as shown in Table 1, at high dose (A, 1 μ g/mL), 13 out of the 14 subjects (13/14) showed percentages above 25% for the expression of CD63 (ranging from 25.6% to 100% with a mean value 54.81 ± 30.65). However, at low dose (B, 0.1 μ g/mL), all subjects except two (12/14) had values higher than 15% (ranging from 16.8% to 92.4% with a mean value of 27.98 ± 25.30). All subjects had negative control values for CD63 expression of less than 5%. The 14 patients had a different degree of systemic reactions to the Hymenoptera sting. According to the classification of systemic reactions (Mueller, 1966), six patients had grade IV, four patients grade III and finally four patients had grade II. Initially no relationship between immunological parameters and the degree of systemic reaction to the sting was found.

3.2. Changes induced by VIT in IDT sIgE and BAT

To compare homogeneously the disturbances induced by VIT at T6, these turned into percentages relative to T0 values. As shown in Fig. 1, upon immunotherapy at T6, IDTs showed a significant reduction in 9 out of the 13 subjects with a mean value in reduction of $24.9 \pm 47.4\%$ ($p = 0.015$). In two patients who did not suffer reduction the dose that produces reaction was lower than the observed in T0. The rest remained the same concentration of positive reaction. Likewise, there was a significant decrease for the serum levels of sIgE ($p = 0.009$), with a mean decline in sIgE levels of $33.9 \pm 34.7\%$.

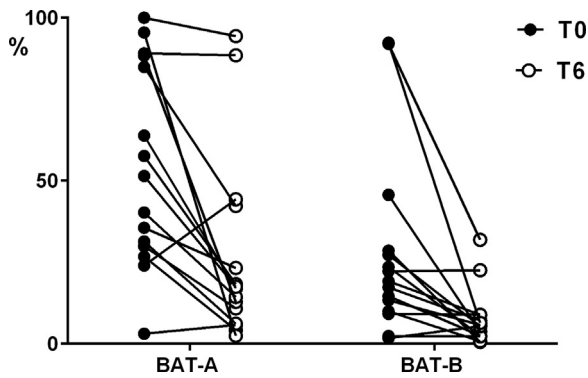


Fig. 2. Basophil activation tests (BATs) at baseline time (T0) and after VIT. Data are expressed as percentages of CD63 expression; A, high dose of venom extract (1 µg/mL); and B, low dose of venom extract (0.1 µg/mL).

Lower levels of sIgE at T6 were observed in 11 out of the 14 subjects as shown in Fig. 1. The CD63 expression on basophils was decreased both at high as low dose, and only two subject did not show reduction for BAT neither at A dose nor B dose (Fig. 2). Moreover, BATs had a mean value of decrease in the percentage of degranulated basophils of $40.2 \pm 58.1\%$ for the dose A ($p = 0.003$) and $43.4 \pm 77.9\%$ for the dose B ($p = 0.002$).

3.3. Changes induced by VIT in T-cell activity markers

VIT induced modifications on the parameters tested in allergic patients. Table 2 shows the mean values of percentage of T CD4 with expression of activation markers and percentage of cytokines T lymphocytes producing cells at T0 and after 6 months undergoing immunotherapy. For the expression of the activation marker CD40L and CTLA-4 on CD4 T-lymphocytes, both showed a decreased expression with respect to baseline data. Subjects had a mean reduction induced by VIT (T6) varying from $45.15 \pm 85.5\%$ for CD40L ($p = 0.035$) to $73.03 \pm 11.8\%$, for CTLA-4 ($p = 0.012$) as shown in Fig. 3. All subjects had reduction of CTLA-4 expression while for CD40L only had reduction in seven of eight. Finally, the behavior of T cells producing cytokines was uneven. A significant decrease of the percentage of IL-4 producing T cells of 48.0 ± 21.3 was observed after the immune intervention at T6 ($p = 0.01$). In contrast, we found an upregulation of IFN-γ producing cells of 59.2 ± 44.1 after the 6th month of immunotherapy ($p = 0.02$) (Fig. 3). All subjects had reduction of IL-4 producer cells while for IFN-γ only had gain in seven of eight.

3.4. Relationship between IDT, sIgE and BAT with immunological parameters

The possible relationships between the alterations induced by VIT in the diagnostic test; IDT, BAT and sIgE and immune activation parameters were tested. As expected, a significant correlation was found in the results achieved between the used doses (A, high; B,

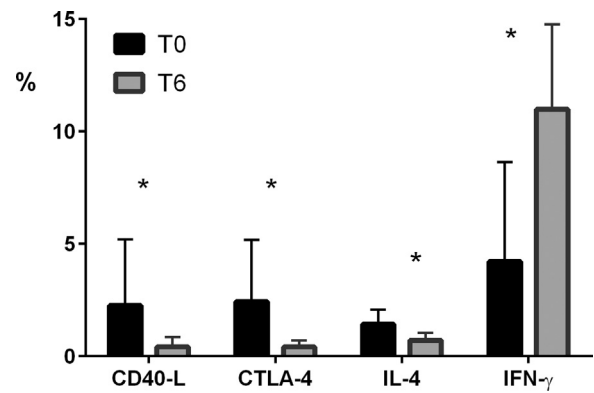


Fig. 3. Percentage of CD4 T cells with expression of CD40L and CTLA-4, and IL-4 and IFN-γ producing T cells in eight patients at baseline time (T0) and upon VIT (T6). Data in the plot are given as the mean and standard deviation. * $p < 0.05$.

low) of BAT; $p = 0.003$. Neither BAT nor serum levels of sIgE had any correlation with none of the immunological parameters studied nor among them. In contrast, the reduction in IDT wheal area, showed a significant correlation with both, IL-4 producing T lymphocytes ($p = 0.018$) and CD40L expression on CD4 T lymphocytes ($p = 0.043$). Furthermore, a significant correlation between the decrease in the percentage of T cells producing IL-4 and the decline in CD4 T lymphocytes expressing CD40L was found ($r = 0.744$; $p = 0.034$). With the remaining parameters (CTLA-4 and IFN-γ) no significant correlation was found. Fig. 4 shows the statistically significant and regression coefficients (r^2) of the expression of CD40L and IL-4 production with diagnostic test IDT, BAT and sIgE.

4. Discussion

Venom immunotherapy is a very effective and successful treatment method providing long term protection in patients allergic to Hymenoptera venom. Despite its remarkable effectiveness, the precise mechanisms of action are not fully understood. In this study, we demonstrate that immunotolerance in the course of VIT was associated with some modifications in immunological behavior of T lymphocytes which resulting into changes in the settings achieved in tests used to diagnose and evaluate the allergy like skin tests, basophil activation tests, and sIgE levels. There is large evidence about the role that T-cells play in the development of allergy as well as the modifications occurring upon immunotherapy (Akdis and Akdis, 2011). It has been previously described that VIT induces changes in the levels of soluble CTLA-4 (Riccio et al., 2012; Saverino et al., 2009) and surface expression of CD40L (Bellinghausen et al., 1997; Cabrera et al., 2014). It has been described in the present study that VIT produce abatement in the expression of both molecules on cell surface. These results are expected considering that these molecules are specific markers of T cell activation and are upregulated after antigen presentation. During allergen sensitization, cooperation between T and B cells through CD40–CD40L interaction is a basic signal for isotype class switching toward IgE (Poulsen and Hummelshoj, 2007). The reduction in CD40L expression may be an active mechanism through which treatment with VIT modulates the response to allergens. In fact in patients with hay fever, it has been found a high proportion of Th2 lymphocytes with surface expression of CD40L (Smith et al., 2013). It has been recently shown the inhibition of CD40 expression using small interfering RNA in ovalbumin-sensitized mice results in decreased production of Th2 type cytokines and increased Treg cells. (Suzuki et al., 2009). Also a reduced expression of CD40L in T CD4 lymphocytes during pollen allergy immunotherapy has been described (Urra et al., 2014). CTLA-4 is recognized as a negative

Table 2

Results of immunological parameters obtained from patients allergic to Hymenoptera venom before beginning VIT (T0) and six months after the initiation of VIT (T6). Results are expressed as the mean $\pm$ standard deviation.

	CD40L (%)	CTLA-4 (%)	IL-4 (%)	IFN-γ (%)
N	8	8	8	8
T0	2.27 ± 2.9	2.42 ± 2.7	1.43 ± 0.64	4.21 ± 4.41
T6	0.41 ± 0.43	0.41 ± 0.19	0.71 ± 0.33	11.0 ± 3.78
p	0.03	0.01	0.01	0.02

N = number of patients.

p = statistical significance of the Wilcoxon test.

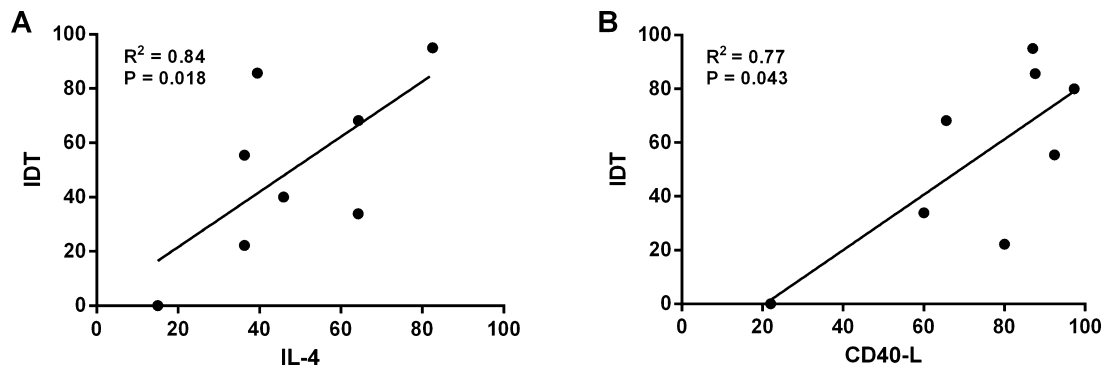


Fig. 4. Correlations between reduction in percentage of IL-4 producing T cells (A) and CD4+ T-cells with expression of CD40L (B) with reduction of IDT wheal area after six month of immunotherapy. Every plot shows the square of the correlation coefficient r^2 and their statistical significance.

regulator of T-cell activation. In fact, the binding of CTLA-4 to the T-cell surface initiates a cascade of biochemical events that attenuates the ongoing immune response (Keane-Myers et al., 1997). It is widely known that immunotherapy promotes regulatory mechanisms through the Treg cells and the release of regulatory cytokines (Akdis and Akdis, 2015). Just because a decreased in activation of T lymphocytes by major regulatory activity induced upon VIT, both molecular forms of CTLA-4, soluble and native reduce their expression levels. The decline of CTLA-4 also suggests that VIT promotes immune regulation through immunoregulatory cytokines such as IL-10 and TGF- β rather than through cell-to-cell contact (Tai et al., 2012). Despite the existence of conclusive evidence linking the expression of CTLA-4 in the regulation of immune response it seems that their effect is important in sensitization to the allergen but does not act on already established allergy (Linhart et al., 2007). An appreciable decrease in the production of IL-4 has been widely demonstrated in VIT and in many cases the decline in IL-4 production is associated with a rise in the production of IFN- γ (Bellinghausen et al., 1997; Jutel et al., 1995; McHugh et al., 1995). Levels of IL-4-producing T-cells by patients undergoing immunotherapy for 6 months decreased to levels similar to those of healthy individuals (Urra et al., 2013). However data for the effect of VIT on the allergen-induced production of IFN- γ are conflicting. Although it has been described that VIT inhibits both the Th1 and the Th2 type response (Müller et al., 1998), we detected a decrease in IL-4 and increase in IFN- γ production in patients undergoing VIT. It has been previously described, besides the reduced production of IL-4, increases in the production of INF- γ associated with immunotherapy (Schuerwegh et al., 2001). Functional studies on antigen-specific Treg cells revealed that the modulation of Th2 type responses depends mostly on secretion of the cytokine IL-10. In fact, IL-10 inhibits the proliferative response of peripheral cells against specific allergens (James and Durham, 2008). All these immunological changes over time directly impact on IDTs, sIgE levels and BATs as we proven in our study. These modifications are detected after 6 months following the introduction of immunotherapy. It has been detected also immunological modifications like decrease in activation markers and alteration in cytokine profile even at 4 months after the start of immunotherapy (Cabrera et al., 2014). The decline in membrane expression of CD40L as well as in the production of IL-4 by T CD4 cells are directly related to the *in vivo* skin test, and however unrelated with both sIgE levels and *in vitro* basophils activation tests. Considering that skin tests are the most physiological assay with the highest agreement with clinical in allergy (Carr and Saltoun, 2012) these results suggest that the decline in the production of IL-4 together with the fall in the expression of CD40L are the events assayed more directly involved in IgE-mediated hypersensitivity. The quantification of these parameters would be useful to assess the efficacy of immunotherapy.

The absence of correlation between levels of sIgE and basophils activation could be explained since these tests evaluate very definite areas of the immune response unlike to the skin tests where the entire immune response against the allergen into the skin is evaluated. In our study immunological molecules were analyzed in T-lymphocytes while BAT qualify the *in vitro* basophil degranulation mediated by allergen, as well as sIgE the production and circulation of IgE antibodies. In fact, the poor relationship between BAT and skin test in aeroallergens studies was firstly described (Khan et al., 2012), and later, the disagreement between skin test and sIgE levels in young people with inhalant and food allergy (Schoos et al., 2015). Finally, significant levels of sIgE were detected even in healthy individuals (Cho et al., 2014). Already earlier, looking at the susceptibility to type-1 diabetes in allergic patients a disagreement between total IgE levels and CTLA-4 gene could be observed (Maier et al., 2006). Nevertheless, previous studies have found correlation between BATs with the clinical severity of peanut allergy (Song et al., 2015). The absence of relationship between CTLA-4 and IFN- γ with the diagnostic tests suggests that modifications observed in them are brought about by both, the decline in the production of IL-4 and the low activation of T cells trigger by VIT. Five of the 14 patients included suffered field stings without systemic reaction after VIT treatment. Another one showed a well tolerated sting challenge test at the hospital. Therefore, it is not possible to know the relationship between immunological issues with the clinic. The only possibility to evaluate the clinic in patients undergoing VIT is the re-sting which is scheduled at the end of the desensitization protocol.

5. Conclusions

An important issue in Hymenoptera venom allergy which has not yet been clarified is the ability of VIT to produce desensitization of patients as well as the involved mechanisms in this course. In the present study we display the modifications observed in T cell activation markers (CTLA-4 and CD40L) and cytokine producing T-cells (IL-4 and IFN- γ) at six months of VIT. Moreover, the relationship between them with skin tests, BAT and sIgE levels were inquired. A significant decline for all molecules studied on T- lymphocytes as well as in skin test, BAT and sIgE levels was found. A direct relationship between the decrease in producing IL-4 T-cells along with surface CD40L expression with the improvement in skin tests was also seen. Conversely there was not any agreement of cytokines and T-cell activation markers nor sIgE levels neither basophil activation test. These results suggest that both IL-4 and CD40L expression could be two good markers to assess the beneficial effects of VIT which translate into the skin tests.

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