

Absence of IgE neosensitization in house dust mite allergic patients following sublingual immunotherapy

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Abstract

Background The impact of sublingual immunotherapy (SLIT) on IgE neosensitization remains to be evaluated in large cohorts of patients.

Objectives The aim of this study was to assess the dynamics of antibody responses induced in patients with allergic rhinitis during a 12-month treatment with sublingual tablets of house dust mites (HDM) allergen extracts.

Methods Antibody responses were assessed in relationship with neosensitization and clinical benefit in sera from 509 European house dust mite-allergic patients before and after 1 year of daily sublingual immunotherapy, using tablets containing *Dermatophagoides pteronyssinus* plus *D. farinae* extracts or placebo (ClinicalTrials.gov NCT00674700).

Patients were followed for one additional year after treatment cessation. IgE and IgG4 antibodies specific for mite extracts or purified group 1, 2 and 10 allergens were assessed using Immulite, Immunocap and ISAC assays.

Results After 1 year of SLIT, mite-specific IgE and IgG4 titres increased by 1.5-fold and fourfold, respectively, in the active, but not in the placebo group. A strong IgG4 induction occurred in a subgroup (i.e. 10–15%) of “immunoreactive” patients, without any correlation with improvement in the average adjusted symptom score. Pre-existing IgE levels to purified mite allergens were not impacted during immunotherapy, and no *de novo* IgE responses to group 1, 2, 10 allergens were induced in patients who were unsensitized prior to immunotherapy. Similarly, no IgE neosensitization to wheat germ or yeast components used in the mite culture medium was observed.

Conclusions and Clinical Relevance We document in a cohort of 509 patients followed over a 2-year period that SLIT does not induce any IgE neosensitization to allergens contained in the vaccine, such as groups 1, 2 as well as the food-related group 10 allergen. This observation further corroborates the safer safety profile of SLIT over SCIT.

Keywords allergen, antibody, house dust mite, IgE, IgG, immunotherapy, neosensitization, sublingual tablets

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Introduction

Sublingual immunotherapy (SLIT) conducted with drops, and more recently with tablets, has been successfully used to treat patients with allergic rhinitis to grass pollen allergens [1–4]. SLIT is also broadly applied to patients with allergic rhinoconjunctivitis to common house dust mites (HDM) belonging to *Dermatophagoides pteronyssinus* and *D. farinae* species [5–14]. Various epidemiology studies conducted in Europe, North

America and Asia revealed that most (i.e. 90%) patients are co-exposed, and thus co-sensitized to allergens from the two species [15–20]. As a consequence, immunotherapeutic products used for desensitization purposes rely on a mix of extracts from both *D. pteronyssinus* and *D. farinae* species [5–7]. The advantage of such a combination vaccine is to offer a broad coverage to the patients by extending the repertoire of allergens and epitopes derived thereof, thereby mimicking natural exposure conditions [21].

One theoretical drawback to this approach, however, is a potential risk of *de novo* sensitization to allergens present in the vaccine in patients who have no specific IgEs to some of those allergens prior to treatment [22, 23]. Such a risk of neosensitization to mite allergens has indeed been reported in the course of subcutaneous immunotherapy [24]. Specifically, one concern related to immunotherapy with house dust mite extracts is to induce IgE sensitization to food-related allergens cross-reactive with Der p 10 and Der f 10 tropomyosin-like minor allergens [24–29]. For example, some cases of sensitization to snail or shrimp allergens have been reported following immunotherapy with mite extracts administered via the subcutaneous route [24, 30]. Thus, the question arises as to whether or not such IgE neosensitization can occur as well in the context of SLIT, i.e. when the allergen is administered in an immune environment prone to tolerance induction [31, 32].

To address this issue, we took advantage of a 2-year Phase II/III study conducted in a cohort of 509 European HDM allergic patients to evaluate the efficacy of a *D pteronyssinus*/*D farinae* sublingual tablet in reducing mite-related rhinitis symptoms and rescue medication usage [6]. We document herein the dynamics of mite-specific IgE and IgG4 (the latter being used as a control for immune exposure to mite allergens) antibody responses during 1 year of sublingual immunotherapy, with one additional year of treatment-free follow-up. We conclude that sublingual immunization does not induce any IgE neosensitization to various allergens present in the vaccine.

Methods

VO57.07 mite SLIT study design

The detailed study protocol, as well as inclusion criteria will be reported elsewhere. Local Regulatory Authorities and Independent Ethics Committees approved the protocol. The study complied with Good Clinical Practice guidelines and patients provided their written informed consent before study entry. Briefly, this randomized double blind placebo-controlled phase II/III study was conducted from October 2007 to January 2010 in European patients with HDM-related allergic rhinitis [6] (ClinicalTrials.gov NCT00674700). After screening (Visit 1 or V1), 509 patients (18–50 year old, with HDM-related allergic rhinitis since at least 1 year) were enrolled in the study. Patients were selected based upon mite-specific IgE levels ≥ 0.7 kU/L, a positive skin prick test to *D pteronyssinus* or *D farinae* with a weal diameter > 3 mm, and a baseline average rhinitis total symptom score (ARTSS) ≥ 5 (on a 0–12 scale). Patients were randomized into one of three treatment groups, receiving a placebo or an active tablet at either a

300 IR (index of reactivity) or 500 IR dose on a daily basis for 12 months, with an additional treatment-free follow-up year [6]. Patients attended bimonthly scheduled visits between enrolment up to Visit 9 (V9, after 12 months of treatment), and then Visit 14 (V14, month 24, after one additional year of follow-up). The primary efficacy variable was the average adjusted symptom score (AAdSS) encompassing the daily score reported by the patient regarding the severity of four rhinitis symptoms (sneezing, rhinorrhoea, nasal pruritus and nasal congestion), adjusted for the patient rescue medication usage. Venous blood samples were collected at each of V1, V9 and V14 to assess antibody responses. All biological assays were performed on coded samples blinded to the operators.

Mite extracts

Aqueous extracts from *D pteronyssinus* and *D farinae* mites used for SLIT were from Stallergenes SA (Antony, France) [33]. Wheat and yeast proteins contained in the pharmaceutical-grade culture medium (StalmiteTM) used to grow both *D pteronyssinus* and *D farinae* were assessed by chromatography and mass spectrometry, and found to be undetectable in mite extracts [33]. Allergen content measured using commercial ELISAs (Indoor Biotechnologies VA, USA) was for a 300 IR tablet: 15 µg Der p 1, 60 µg Der f 1, 25 µg group 2 (i.e. Der p 2 + Der f 2). The presence of Der p 10 or Der f 10 allergens was confirmed in mite extracts by dot blot analysis using a specific antibody, and estimated to be in the microgram range per 300 IR tablet [33].

Measurement of mite-specific seric antibody responses

Der p 1, Der p 2, Der f 1, Der f 2 and Der p 10 specific IgE and IgG4 were assessed using purified natural or recombinant allergens from Phadia (Vienna, Austria). The ImmunoSolid-Phase Allergen Chip (ISAC) assays were performed as described elsewhere [34], with results expressed as standardized units (ISU). Seric IgEs to mite extracts were assessed with the Immulite 2000 Immunoassay system (Siemens, Munich, Germany). IgEs to wheat and/or yeast allergens and IgG4s to mite extracts were measured at V1, V9 and V14 with the ImmunoCAP 250 (Phadia). A patient was considered to be sensitized when his/her specific IgEs were ≥ 0.7 kU/L (Immulite and ImmunoCAP) or ≥ 0.3 ISU (ISAC assay).

Statistical analyses

AAdSS data were submitted to an analysis of covariance with treatment and pooled study centre as main effects, and age, gender, asthma status, sensitisation status and baseline average rhinitis total symptom score

as covariates. IgE and IgG4 results were described using geometric mean and 95% confidence intervals.

Results

Clinical improvement during the VO57.07 study

Clinical results will be reported in detail elsewhere. Briefly, the 500 IR group showed a significant improvement in AAdSS vs. placebo ($P = 0.0066$) with a LS Means difference of -0.78 , (95% CI $[-1.34, -0.22]$), corresponding to -20.2% . The LS Means difference of -0.69 , (95% CI $[-1.25, -0.14]$) between the 300 IR and placebo groups was also significant ($P = 0.0150$) and corresponded to -17.9% . Collectively, these results confirm that mite tablets are clinically efficacious, and furthermore, allow investigation at an individual patient level of correlations between changes in mite-specific antibody levels and clinical

benefit as well as the potential occurrence of IgE neo-sensitization.

Changes in group 1, 2 and 10-specific antibody responses induced during SLIT

The dynamics of IgE and IgG4 seric antibody responses during sublingual immunotherapy (SLIT) were first assessed at a cohort level using purified or recombinant Der p 1, Der p 2, Der f 1, Der f 2, Der p 10 spotted on ImmunoSolid-Phase AllergenChip (ISAC). As shown in Fig 1a, for most group 1 and group 2 allergens, no changes beyond a slight decrease in IgE responses were observed in all groups of patients, whether they received the placebo or active tablets. Those changes are likely due to variations in allergen exposure occurring in the natural environment in the course of the study. Noteworthy, IgE responses measured with extracts were slightly increased (by 1.3- to 1.5- fold)

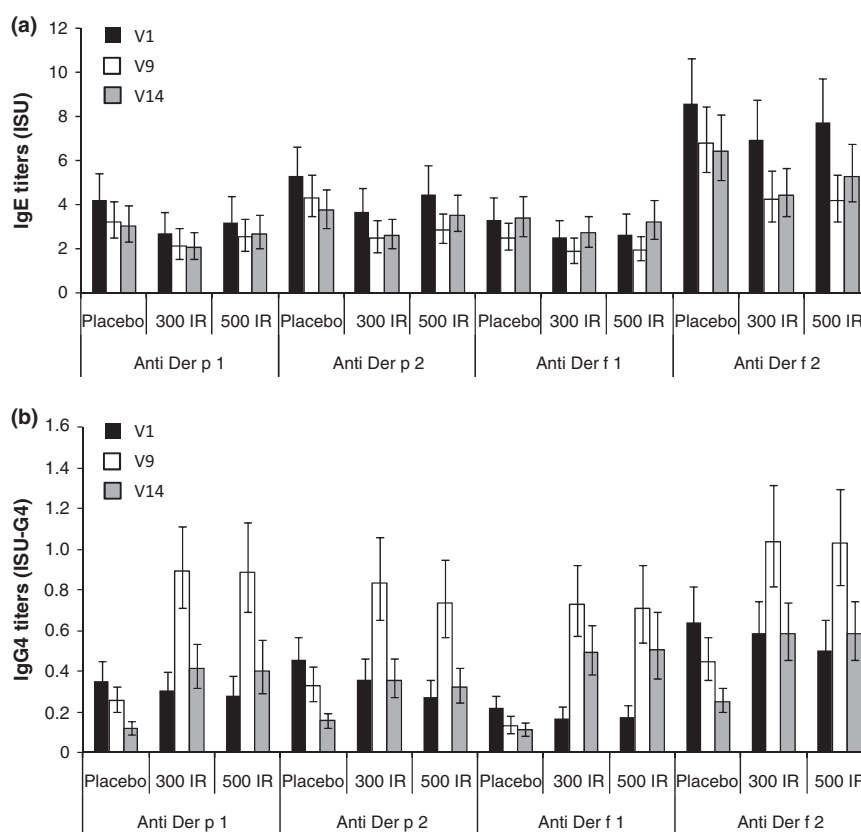


Fig. 1. IgE and IgG4 responses to purified group 1 or group 2 mite allergens (a) IgE antibodies reactive to purified/recombinant allergens were measured by the ImmunoSolid-Phase Allergen Chip (ISAC) method. Sera were harvested before (V1), and after 1 year (V9) of sublingual immunotherapy (SLIT) with a daily tablet (containing either placebo, 300 or 500 IR of a *D pteronyssinus/D farinae* mix), as well as one additional year of follow-up after stopping treatment (V14). Results are expressed as geometric mean values of ISAC Units (ISU) $\pm$ 95% confidence intervals. (b) IgG4 antibodies reactive to purified/recombinant allergens were measured by ISAC immunochip methods. Results are expressed as geometric mean values of $\pm$ ISU-G4 95% confidence intervals.

after 1 year in the active groups receiving either 300 or 500 IR tablets respectively (data not shown).

For group 1 and group 2-specific IgG4 (Fig 1b), an increase in seric antibody titres by threefold to fourfold for Der p 1 and Der f 1 and 1.8- to 2.7-fold for Der p 2 and Der f 2, was observed in patients receiving either the 300 or 500 IR tablets for 1 year. These up-regulations were not observed in the placebo group (Fig 1b). Furthermore, for patients receiving active tablets, IgG4 titres reversed back to pre-treatment levels at V14 for all mite allergens, with the exception of Der f 1 for which some specific IgG4 persisted. IgG4 levels rather decreased in the placebo group. For all time points, specific IgE or IgG4 levels detected in the sera of patients from 300 and 500 IR groups were never different. Only 21 and 39 patients had specific IgE or IgG4, respectively, directed to Der p 10 prior to immunotherapy. No variations in antibody responses to Der p 10 were observed during the 2 years, irrespective of the treatment group (data not shown).

Absence of correlation between mite-specific IgG4 antibody responses and SLIT clinical efficacy

Seric mite-specific IgG4 (directed to whole *D pteronyssinus*, *D farinae* extracts or purified allergens) were subsequently analysed in individual patients, in relationship with clinical responses (Fig 2 and 3). For such analyses, patients with a decrease of average adjusted symptom score (AAdSS) during immunotherapy superior to 80% (i.e. corresponding to the 75th percentile of the distribution of AAdSS percentage improvement compared to baseline) were arbitrarily considered as responders, and those lower as non-responders. At a cohort level, fold increases in mite-specific IgE and IgG4 after 1 year of treatment were not different between responders and non-responders, within both placebo and active groups (data not shown). Interestingly, when plotting individual data, we consistently observed that a subset of 10–15% patients within 300 or 500 IR active groups, but not in the placebo group, exhibit a strong (i.e. superior to fivefold) induction of IgG4 during SLIT. Those patients, subsequently termed “immunoreactive”, mount mite-specific IgG4 responses detectable using both *D pteronyssinus* and *D farinae* extracts, as well as group 1 and group 2 purified mite allergens, but none of those antibody responses correlate with clinical improvement (Fig 2a and b). Specifically, a majority of clinical responders in the active groups has no detectable or low (i.e. < fivefold increase) IgG4 responses to mite allergens induced during SLIT. Conversely, strong IgG4 responses are not always associated with clinical benefit. Similar observations regarding a lack of correlation between seric

IgG4 levels and clinical responses were made at V14 (Fig 3a and b).

Absence of IgE neosensitization to allergens during SLIT

To address the issue of a potential neosensitization induced during SLIT, we focused on patients without any detectable IgEs prior to immunotherapy directed against (i) Der p 1, Der f 1, Der p 2, Der f 2, all present in tens of microgram quantities per daily tablet, (ii) Der p10, estimated to be in microgram quantities per daily tablet, (iii) yeast and wheat-derived allergens, undetectable in mite extracts.

For group 1 and group 2 major allergens, 29 and 18% of patients, respectively, had no detectable IgEs (< 0.3 ISU) at V1. Within the 2-year follow-up, including a daily sublingual exposure to mite allergens for 1 year, we only observed minor variations in a limited number of those patients, belonging to either placebo or active groups (Fig 4). Consequently, we interpret those changes as responses to natural variations in exposure to mite allergens. Similarly, no significant *de novo* IgE responses were elicited to Der p 10 in non-sensitized patients with only minor variations detected for two patients within the 300 IR group (undetected IgEs to Der p 10 at V1 and levels of 0.5 and 2.4 ISU and 0.5 and 0.6 ISU, at V9 and V14, respectively).

To investigate the theoretical risk of IgE sensitization due to components from the culture medium used to grow mites, we subsequently measured wheat and yeast-specific IgE levels in patients' sera using the threshold of ≥ 0.7 kUA/L as a positive value for sensitization. At inclusion, 94 and 98% of patients were unsensitized to wheat and yeast allergens, respectively, and this pattern was unaffected by treatment for 98% of them. Only 12 patients ($n = 6$ in the placebo group, $n = 6$ in the active 500 IR group and none in the 300 IR group) had negative IgE values for both allergen sources at inclusion, but became positive to either wheat and/or yeast allergens after 1 year of treatment, without any clear trend during the follow-up phase (Fig 5). Although not shown, four additional patients out of 20 who were IgE positive to wheat and/or yeast allergens at V1 became negative for both allergen sources at V9. Collectively, given the small magnitude of those changes and the fact that some of them are observed in the placebo group, we conclude that they are unrelated to the treatment.

As a confirmation of those results, the detection of IgEs specific for wheat and yeast allergens at inclusion was not linked with any specific side effects during SLIT. Among patients sensitized prior to immunotherapy ($n = 3$ in placebo group, $n = 4$ and 2 in the 300 and 500 IR groups, respectively), local adverse events reported in year 1 in the various groups included as

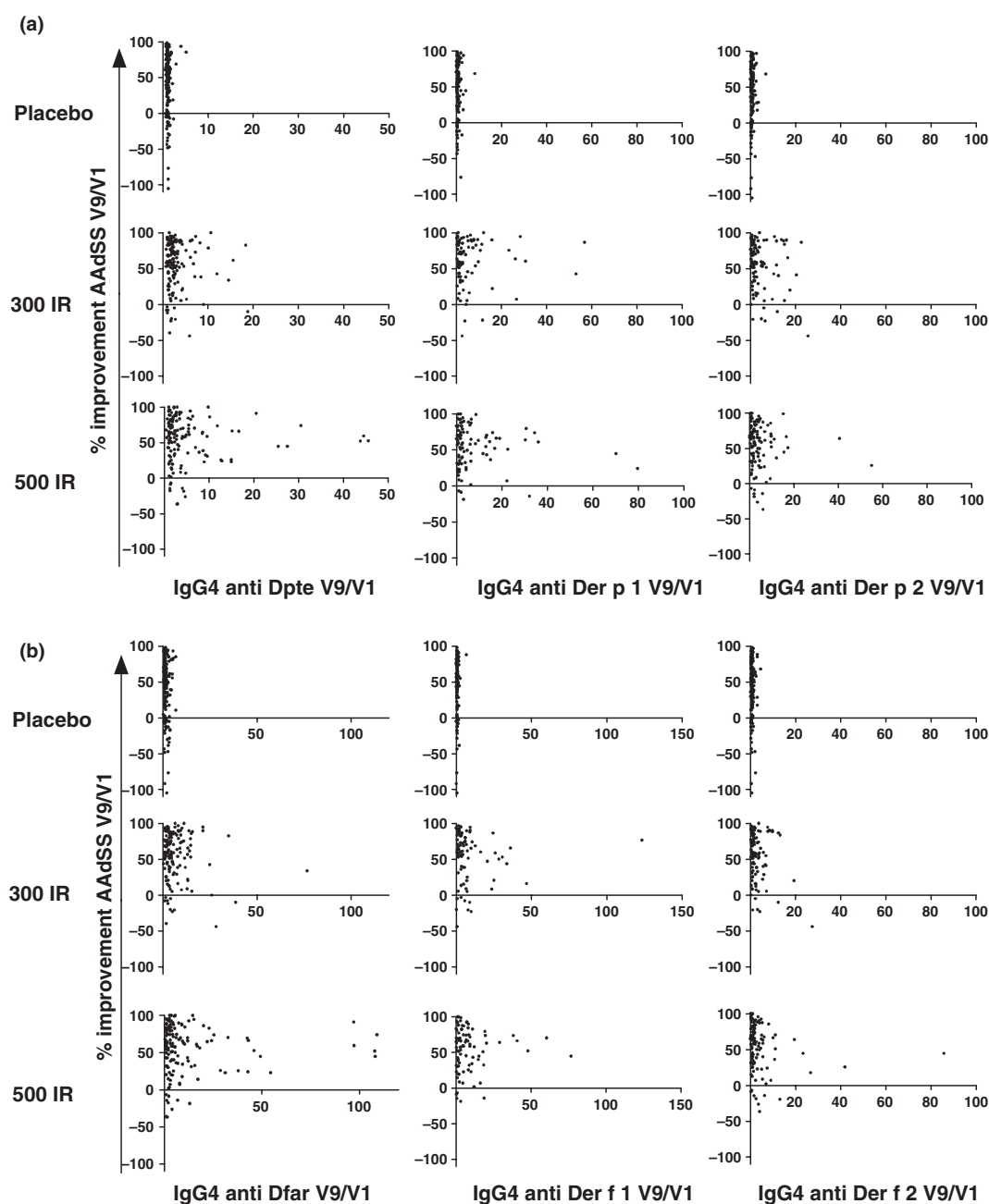


Fig. 2. Absence of correlation between symptom improvement and induction of mite-specific IgG4s after 1 year of sublingual immunotherapy (SLIT). Clinical responses are expressed for individual patients as percentages of improvement in average adjusted symptom scores (AAdSS) measured at V9 in comparison to V1. IgG4 responses against mite extracts or purified allergens were measured by ImmunoCAP and ImmunoSolid-Phase Allergen Chip (ISAC), respectively, and results are expressed for each patient as ratios between IgG4 levels at V9 vs. V1. Patients with an AAdSS percentage improvement $\geq 80\%$ and $< 80\%$ are considered as responders and non-responders, respectively.

expected oral pruritus, throat irritation, pharyngolaryngeal pain but no food allergy symptoms. Systemic adverse events were reported in only four of those patients, including asthma ($n = 1$ in placebo group, $n = 1$ in 500 IR group), urticaria ($n = 1$ in placebo group, $n = 1$ in 300 IR group) and cough ($n = 1$ in 500 IR group). We conclude that SLIT with mite tablets does not induce IgEs to wheat and/or yeast allergens, nor

any particular specific side effects in those patients previously sensitized to those allergens.

Discussion

Sublingual immunotherapy is a well established treatment for type I respiratory allergies to common allergens originating from either grass or tree pollens [1–4].

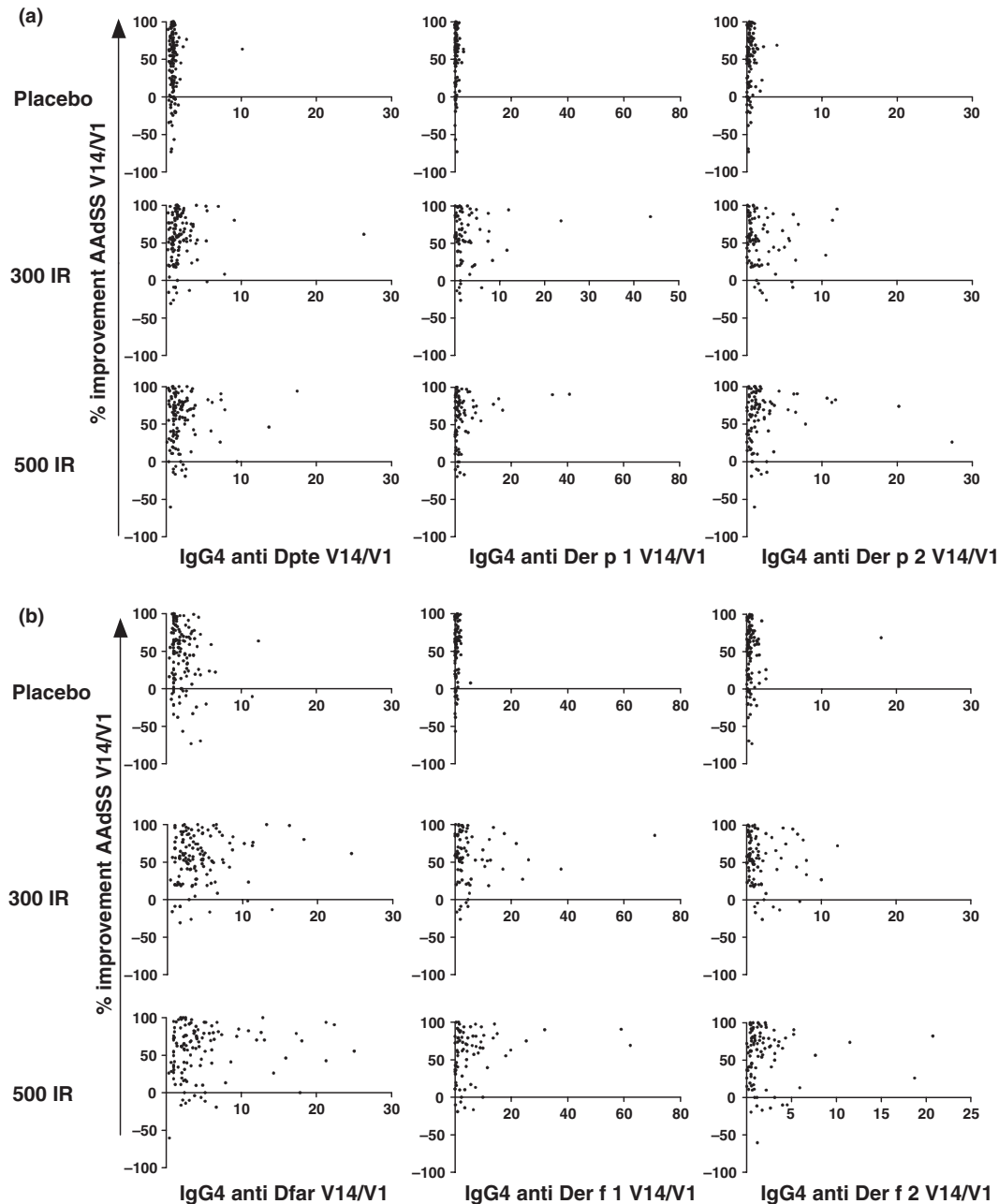


Fig. 3. Absence of correlation between symptom improvement and induction of mite-specific IgG4s after 1 year of treatment-free follow-up. Clinical responses are expressed for individual patients as percentages of improvement in average adjusted symptom scores (AAdSS) measured at V14 in comparison to V1. IgG4 responses against mite extracts or purified allergens were measured by ImmunoCAP and ImmunoSolid-Phase Allergen Chip (ISAC), respectively, and results are expressed for each patient as ratios between IgG4 levels at V14 vs. V1. Patients with an AAdSS percentage improvement $\geq 80\%$ and $< 80\%$ are considered as responders and non-responders, respectively.

Several clinical studies including the one reported herein, further document SLIT efficacy in treating house dust mite allergies [5–14]. Commonly, such treatments are performed using a mixture of *D pteronyssinus* and *D farinae* extracts, in order to offer a broad range of allergens and specific epitopes, thus mimicking natural exposure conditions [5–7]. A potential risk of *de novo* induction of IgEs in non sensitized patients was pre-

sented as a drawback to immunotherapy [22, 23]. The latter has been most particularly documented during subcutaneous immunotherapy conducted with mite extracts, with the appearance of IgEs cross-reacting with snail and shrimp allergens [24, 30], although other studies have not confirmed this potential issue [35]. A recent SLIT study conducted in a small cohort of mite-allergic patients failed to document any neosensitiza-

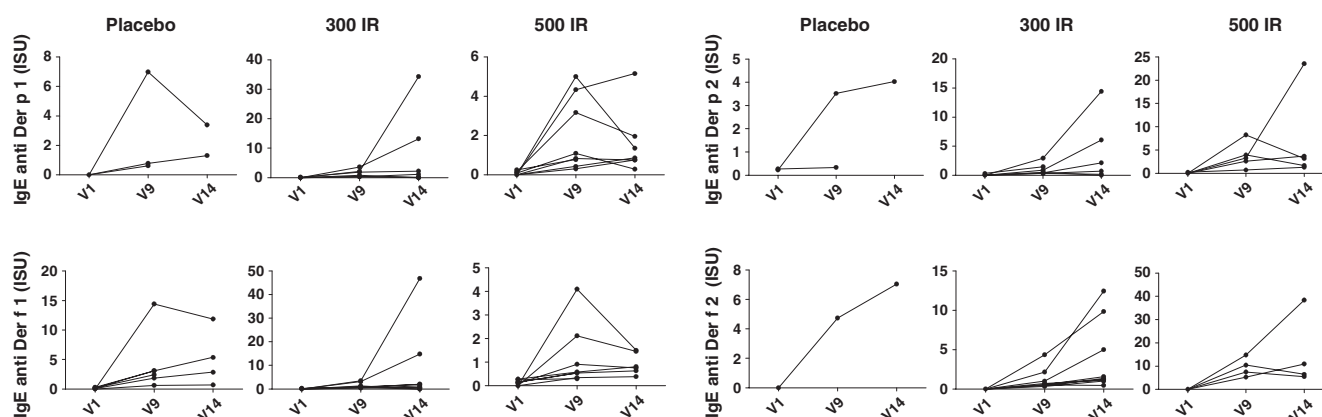


Fig. 4. Absence of *de novo* induction of IgE responses to purified mite allergens. Seric IgE levels were measured by ImmunoSolid-Phase Allergen Chip (ISAC) at V1, V9, V14. Each line represents a patient with negative values at enrolment, for which a positive level (i.e. ≥ 0.3 ISU) was observed for at least one time point.

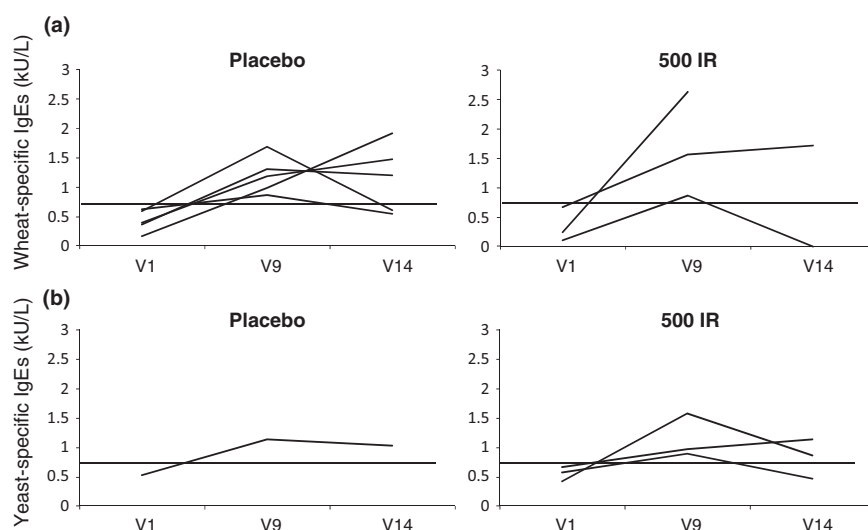


Fig. 5. Absence of *de novo* induction of IgE responses to wheat germ and yeast allergens. Seric IgE levels were measured by ImmunoCAP at V1, V9, V14. Each line represents a patient with negative values at enrolment for which a positive level (i.e. ≥ 0.7 kU/L) was observed for at least one time point.

tion to the food-related group 10 tropomyosin allergen [36]. Nonetheless, the risk of IgE neosensitization during sublingual immunotherapy has never been evaluated in large cohorts of patients.

In this context, we took advantage of a double blind placebo-controlled phase II/III study conducted over 2 years (including 1 year of treatment and 1 year of follow-up) in 509 mite-allergic patients [6] to assess in details the dynamics of antibody responses induced during SLIT. To this aim, seric mite-specific IgE and IgG4 responses were assessed using extracts and purified allergens following sublingual immunotherapy with tablets containing a mix of *D pteronyssinus* plus *D farinae* extracts at two doses (i.e. 300 and 500 IR). As expected, after 1 year of SLIT, specific IgE levels were

little impacted, whereas IgG4 titers increased by twofold to fourfold in the group receiving active tablets. Such mite-specific IgG4 antibodies decreased in the following year after stopping treatment, almost reversing to baseline levels. Importantly, SLIT resulted in a significant clinical benefit in the active groups relative to placebo, with a decrease by 17.9 and 20.2% of the average adjusted symptom score after 1 year of immunotherapy, and with such an efficacy maintained for up to 8 months following treatment cessation (manuscript in preparation).

These combined observations are in agreement with other studies reporting significant increases in specific IgG4 during sublingual immunotherapy [3, 4, 37, 38], supporting the hypothesis that the induction of anti

inflammatory antibodies could contribute to allergen immunotherapy efficacy, for example in the form of blocking antibodies capable to compete with IgEs for binding to allergens [31, 38]. However, in the present study, a strong IgG4 induction only occurred in a subgroup of "immunoreactive" patients (representing 10–15% of patients) within the active groups. Importantly, no correlation was established between changes in the rhinitis symptom scores and IgG4 induction detected using either mite extracts, or purified group 1 and group 2 allergens. Also, we observed a dampening of specific IgG4 titres within a year following treatment cessation, despite a preserved clinical efficacy in some patients, in agreement with another study documenting a similar lack of correlation between antibody titres and persistence of clinical benefit over time [39]. Altogether, we conclude that IgG4 antibody responses to mite allergens cannot be used as a biomarker for clinical efficacy.

Importantly, we document herein that in patients unsensitized to selected allergens prior to immunotherapy, no *de novo* induction of IgE responses occurs during SLIT. The latter has been confirmed using allergens present in the vaccine at various quantities, ranging from tens of micrograms (i.e. groups 1 and 2), or micrograms (group 10) per day, to undetectable levels

(i.e. yeast and wheat allergens potentially originating from the culture medium used to grow the mites). The latter results are in agreement with a recent study, also conducted in mite-allergic patients [36], as well as with our recent trial conducted in grass pollen allergic patients (manuscript in preparation), both documenting an absence of IgE neosensitization during SLIT.

In conclusion, our results obtained in 509 patients followed over 2 years establish that the sublingual route does not carry any significant risk of IgE neosensitization, likely due to the peculiar biology of the oral immune system, biased towards tolerance induction [31, 32]. When contrasted with previous reports on the risk of IgE neosensitization associated with SCIT [22, 24, 30], this observation further corroborates the safer profile of the sublingual route of immunotherapy relative to subcutaneous administration.

Conflict of interest: Véronique Baron-Bodo, Thierry Batard, Hélène Nguyen, Marine Frèreux, Stéphane Horiot, Olivier de Beaumont and Philippe Moingeon are employees at Stallergenes. Christian Harwanegg is an employee at Phadia Multiplexing Diagnostics. Karl Christian Bergmann has no conflict of interests.

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