ORIGINAL ARTICLE

Mite-Specific Immunotherapy Using Allergen and/or Bacterial Extracts in Atopic Patients in Brazil

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Abstract

Objective: This study aimed to evaluate the clinical efficacy and antibody response changes after specific immunotherapy (SIT) using *Dermatophagoides pteronyssinus* (Dpt) allergens with or without bacterial extracts in Brazilian mite-atopic patients.
Methods: One-hundred patients with allergic rhinitis were selected for a randomized double-blind, placebo-controlled trial and distributed into 4 groups: Dpt (Dpt allergen extract), Dpt+MRB (Dpt allergen plus mixed respiratory bacterial extracts), MRB (MRB extract only) and placebo. Rhinitis symptom and medication scores; skin prick test (SPT) to Dpt extract; and serum immunoglobulin (Ig) E, IgG4, and IgG1 levels to Dpt, Der p 1, and Der p 2 allergens were evaluated before and after a year of treatment.
Results: After 1 year, the SPT response was reduced in the Dpt group ($P = .03$), whereas IgE levels to Der p 2 decreased only in the Dpt ($P = .048$) and Dpt+MRB ($P = .005$) groups. IgG4 and IgG1 levels to Dpt and Der p 1 increased in the Dpt group ($P < .05$), whereas in the Dpt + MRB group the IgG1 level only increased to Dpt ($P = .001$) and the IgG4 only increased to Der p 1 ($P = .049$). IgE levels to Dpt decreased only in the MRB ($P = .005$) and Dpt + MRB ($P = .001$) groups. Rhinitis symptom and medication scores fell in all groups, including the placebo group ($P < .001$).
Conclusions: SIT using Dpt extract alone was effective in reducing SPT response and IgE levels to Der p 2 allergen, while bacterial extracts induced decreases in IgE levels to whole Dpt extract. However, only groups receiving Dpt allergen had higher levels of IgG1 and IgG4 to Dpt and Der p 1 after a year of treatment.

Key words: Allergen-specific immunotherapy. *Dermatophagoides pteronyssinus*. Der p 1. Der p 2. Immunoglobulin (Ig) E antibody. IgG1 and IgG4 subclasses.

Resumen

Objetivo: El objetivo de este estudio fue determinar la eficacia clínica y los cambios en la producción de los anticuerpos tras la inmunoterapia específica (ITE), utilizando alérgenos *Dermatophagoides pteronyssinus* (Dpt) con o sin extractos bacterianos, en pacientes brasileños alérgicos a los ácaros.
Métodos: Se seleccionaron cien pacientes con rinitis alérgica para participar en un estudio aleatorizado, doble ciego, controlado con placebo y se distribuyeron en 4 grupos: el de Dpt (extracto alérgeno Dpt), el de Dpt + BVRM (extractos alérgenos Dpt, más extractos bacterianos de las vías respiratorias mixtos), el de BVRM (sólo extracto de BVRM) y el de placebo. Se analizaron los resultados de los síntomas y fármacos; la prueba cutánea (PC) para el extracto Dpt; y la inmunoglobulina (Ig) E, IgG4 séricas y las concentraciones de IgG1 para los alérgenos Dpt, Der p 1 y Der p 2, antes y después de un año de tratamiento.

Resultados: Después de 1 año, se redujo la respuesta de la PC en el grupo Dpt ($P = 0,03$), mientras que las concentraciones de IgE para Der p 2 sólo disminuyeron en los grupos Dpt ($P = 0,048$) y Dpt + BVRM ($P = 0,005$). Las concentraciones de IgG4 e IgG1 para Dpt y Der p 1 aumentaron en el grupo Dpt ($P < 0,05$), mientras que en el grupo de Dpt + BVRM la concentración de IgG1 sólo aumentó para Dpt ($P = 0,001$) y la IgG4 sólo aumentó para Der p 1 ($P = 0,049$). Las concentraciones de IgE para Dpt sólo disminuyeron en los grupos de BVRM ($P = 0,005$) y Dpt + BVRM ($P = 0,001$). Los resultados de los síntomas y fármacos disminuyeron en todos los grupos, incluso en el grupo de placebo ($P < 0,001$).
Conclusiones: La ITE que utilizaba sólo extracto Dpt fue eficaz para reducir la respuesta de la PC y las concentraciones de IgE para el alérgeno Der p 2, mientras que los extractos bacterianos indujeron disminuciones en las concentraciones de IgE para el extracto Dpt completo. No obstante, sólo aquellos grupos que recibieron alérgeno Dpt tuvieron unas concentraciones más altas de IgG1 e IgG4 para Dpt y Der p 1, tras un año de tratamiento.

Palabras clave: Inmunoterapia antígeno específica. *Dermatophagoides pteronyssinus*. Der p 1. Der p 2. Anticuerpo Inmunoglobulina (Ig)E. Subclases IgG1 y IgG4.

Introduction

Allergen specific immunotherapy (SIT) is currently the only allergy treatment for the modulation of immune responses and the reduction of symptoms that works by mediating changes in the natural course of allergic diseases, resulting in long-term relief of symptoms after discontinuation [1,2]. SIT has been used most successfully in reducing disease severity from natural exposure, especially in the treatment of Hymenoptera hypersensitivity, allergic rhinitis, and asthma [3]. The role of allergen exposure to house dust mites, particularly *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, in the sensitization and development of allergic rhinitis and asthma has been recognized worldwide [4,5].

The complex mechanisms of immunotherapy differ depending on the allergen and the route of immunization [2]. Previous studies have suggested that immunotherapy works by driving the T helper (T_H) type 2 response to T_H0/T_H1 or inducing T-cell tolerance by decreasing T_H0/T_H2 responses or probably both [2]. Recently, studies have suggested a crucial role for regulatory T cells in the inhibition of allergic inflammation by inducing T-cell tolerance through secretion of interleukin (IL) 10 and transforming growth factor ß [6, 7]. Although the precise immunological mechanism involved in SIT is uncertain, an initial transient increase in serum specific immunoglobulin (Ig) E antibodies followed by a gradual decrease over months or years of treatment without abolishing totally the immediate cutaneous reactivity has been reported [2]. However, changes in serum allergen-specific IgE are poorly correlated with clinical improvement, especially with regard to respiratory symptoms [8]. On the other hand, a significant increase in allergen-specific IgG antibodies, particularly IgG4 and IgG1, has been reported during SIT [9-11].

For preventing or treating allergic diseases, some recent studies have used bacterial extracts from common pathogenic bacterial species involved in upper and lower airway tract infections as adjuvants [12]. In support of this approach, results from in vitro cell stimulation with a recombinant fusion protein of a bacterial cell surface protein and the birch pollen allergen Bet v 1 (rSbsC-Bet v 1) showed a shift of the immune response to allergens from a T_H2 to a T_H1 cytokine profile through production of interferon-γ along with IL-10 [13].

The present study is a preliminary randomized double-blind, placebo-controlled trial to evaluate the clinical efficacy and antibody response changes to *D pteronyssinus* (Dpt) and its major allergens (Der p 1 and Der p 2) in mite allergic patients after specific immunotherapy using Dpt allergens with or without bacterial extracts as adjuvants.

Material and Methods

Patients

Subjects with allergic rhinitis were recruited from the Program of Asthma and Rhinitis Control of the Public Health System of Itumbiara, Goiás, Brazil. The diagnosis of allergic rhinitis was based on international guidelines [14,15] and included a detailed clinical history and a complete physical examination. Subjects were selected on the basis of the following inclusion criteria: males and females aged 6 to 50 years, clinical history of respiratory symptoms related to house dust exposure, and aeroallergen mono- or polysensitized subjects with a positive skin prick test (SPT) to Dpt allergen extract. The exclusion criteria were previous allergen immunotherapy; smoking; pregnancy or breast feeding; cardiovascular, autoimmune, or malignant diseases; and treatment with chemotherapeutic or immunosuppressor agents or oral corticosteroids within the previous 2 to 3 weeks or use of antihistamines within the previous week.

The study was approved by the Ethics Committee on Human Research at the Federal University of Uberlandia and informed consent was obtained prior to study entry from all subjects (and parents when appropriate).

Mite Allergen Preparation

Dpt whole extract was obtained from mite bodies provided by Dr Federico Montealegre (Ponce School of Medicine, Puerto Rico) as described elsewhere [16]. Briefly, mite powder was triturated in liquid nitrogen and allergens were extracted in 5 mM borate-buffered saline (pH 8.0) containing protease inhibitors and protein concentration was determined by the Lowry method. For SPT, Dpt extract was prepared at

protein concentration of 2 mg/mL in phosphate buffered saline containing 0.4% phenol plus 50% glycerol and stored at 4°C until use. For immunotherapy, Dpt extract was prepared on the basis of the Der p 1 allergen content measured by a 2-site monoclonal antibody enzyme-linked immunosorbent assay (ELISA) [17]. Different dilutions (10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}) were prepared starting from a stock solution at 170 µg of Der p 1/mL in sterile saline containing 0.4% phenol and stored at 4°C throughout the treatment period.

Study Design

The study was conducted as a randomized, double-blind, placebo-controlled trial during the period of November 2003 through March 2005. A total of 100 subjects were randomly distributed into 4 groups of 25 patients each, as follows: Dpt a group receiving Dpt allergen extract alone; Dpt+MRB group receiving Dpt allergen extract plus mixed respiratory bacterial (MRB) extract containing cell wall antigens from different respiratory microbes (*Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Moraxella catarrhalis*; total count of 200-500 million bacteria/mL) (FDA Allergenic Ltda, Rio de Janeiro, Brazil); MRB group receiving MRB extract alone; and placebo group receiving aqueous diluent only. A single batch of all preparations was used throughout the study. The demographic and clinical characteristics of the patients who completed the study and laboratory parameters at baseline are presented in Table 1.

All treatments were given subcutaneously according to the schedule of the European Academy of Allergology and Clinical Immunology (EAACI) [18], with some modification as shown in Table 2. Patients received an updosing injection once weekly and reached a maintenance dose of 3.4 µg of Der p 1 (17 µg/mL in a volume of 0.2 mL) within approximately 3 months. Adjustments in the schedule were made on an individual basis according to standard guidelines for specific immunotherapy [19]. The doctor performing the immunotherapy (M.G.J.Q.) had no knowledge of the randomization code, which was created and locked by another doctor (E.A.T.) in the study. The code was only broken after all laboratory data had been collected and prepared for statistical analysis.

SPT and Blood Samples

Subjects underwent SPT with Dpt allergen extract prepared as described above before and after a year of treatment according to EAACI guidelines [20]. A histamine solution at 10 mg/mL and the glycerol buffered diluent of the allergen preparation were used as positive and negative controls, respectively (IPI/ASAC, São Paulo, Brazil). Skin reactions were read after 15 minutes and a mean wheal diameter 3 mm greater than the negative control was considered a positive

Table 1. Demographic, Clinical Characteristics, and Laboratory Findings at Baseline for the Subjects Who Completed the Study

	Groups, by Extracts Used for SIT			
	Dpt	Dpt + MRB	MRB	Placebo
Number of subjects	15	20	23	15
Age, y				
Mean (SD)	22 (14)	20 (13)	23 (15)	21 (13)
Range	10-47	6-50	8-49	6-48
Sex, M:F	9:6	10:10	12:11	6:9
SPT wheal, mean (SEM), mm	8.5 (0.9)	7.6 (0.6)	9.1 (0.8)	9.4 (1.1)
Specific IgE, mean (SEM) EI				
Dpt	7.6 (2.0)	5.5 (1.1)	6.9 (1.3)	3.6 (0.9)
Der p 1	5.6 (1.4)	6.5 (1.2)	6.2 (1.2)	5.2 (1.4)
Der p 2	7.7 (2.0)	7.5 (1.5)	7.8 (1.4)	6.7 (1.7)
Specific IgG4, mean (SEM) EI				
Dpt	1.9 (0.7)	1.8 (0.5)	2.0 (0.5)	1.3 (0.3)
Der p 1	1.7 (0.4)	1.6 (0.3)	1.7 (0.3)	1.0 (0.1)
Der p 2	2.2 (0.7)	3.4 (0.9)	2.2 (0.4)	2.3 (0.7)
Specific IgG1, mean (SEM) EI				
Dpt	7.5 (0.7)	5.3 (0.8)	8.3 (0.8)	5.5 (0.9)
Der p 1	1.9 (0.5)	2.8 (0.7)	2.4 (0.5)	2.4 (0.6)
Der p 2	2.4 (0.5)	3.3 (0.9)	2.4 (0.5)	2.4 (0.5)

Abbreviations: Dpt, *Dermatophagoides pteronyssinus*; EI, enzyme-linked immunosorbent assay index; MRB, mixed respiratory bacterial extracts; Ig, immunoglobulin; SIT, specific immunotherapy; SPT, skin prick test.

SPT result. In parallel, blood samples were collected from all patients at baseline and after a year of treatment and sera were stored in aliquots at –20 °C until serological testing.

Clinical Evaluation

Clinical efficacy was evaluated before and after a year of treatment through symptom and medication scores as previously described [21], with some adaptations. Rhinitis symptoms were scored on the basis of a questionnaire eliciting information on nasal itching or obstruction, sneezing, rhinorrhea, watery or red eyes, palatal pruritus, and interference with either normal daily activity or sleep. Each rhinitis symptom was evaluated on the following scale: 0 = absent, 1 = mild, 2 = moderate, 3 = severe. The rhinitis medication scoring was evaluated on the basis of oral antihistamine and nasal corticosteroid use, on the following scale: 0 = not at all, 1 = occasionally, seldom; 2 = occasionally, often, 3 = almost daily.

Serological Assays

All sera were assessed by ELISA for measuring specific serum IgE, IgG1, and IgG4 levels to Dpt, Der p 1, and Der p 2 allergens as previously described [22], with some modifications.

For the Dpt allergen, a conventional ELISA was used. High-binding microtiter plates were coated with Dpt crude extract and serum samples diluted 1:2 (IgE) or 1:5 (IgG1 and IgG4) were incubated for 2 hours (IgE) or 1 hour (IgG1, IgG4) at 37 °C. Subsequently, biotinylated secondary anti-human IgE (1:1000; Kirkegaard & Perry Laboratories, Gaithersburg, Maryland, USA), IgG1 (1:500; Sigma Chemical Co., St Louis, Missouri, USA) or IgG4 (1:3000; Sigma) antibodies were incubated for 1 hour at 37 °C followed by incubation with streptavidin-peroxidase conjugate (1:500; Sigma). The assay was developed by adding 2,2´-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) and H_2O_2. Optical density (OD) values were determined at 405 nm. Antibody levels were expressed as ELISA index (EI) as described earlier [23] according to the following formula: EI = OD of the test sample / cutoff, where the cutoff was established as the mean OD values of negative control sera plus 3 SDs. EI values of more than 1.0 were considered positive.

For Der p 1 and Der p 2 allergens, a reverse ELISA (rELISA) was used [22], with minor modifications. Briefly, high-binding microtiter plates were coated with mouse monoclonal antibody to Der p 1 (clone 5H8) or to Der p 2 (clone 1D8) at 1.0 µg/well and then incubated subsequently with Dpt extract, serum samples, biotinylated secondary antibody, streptavidin-peroxidase conjugate, and enzyme substrate as described above for conventional ELISA. Levels of IgE, IgG1, and IgG4 to Der p 1 and Der p 2 allergens were expressed as EI as established for conventional ELISA.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism version 4.0 (GraphPad Software Inc, San Diego, California, USA). Differences in evaluated parameters at baseline and after 1 year of treatment were analyzed within the groups by *t* test (SPT results) or Wilcoxon signed-rank test (symptom and medication scores and ELISA results). Differences between the groups were analyzed by analysis of variance (ANOVA) using the Tukey multiple comparison test (SPT results) or by Kruskal-Wallis test using the Dunn multiple comparison test (symptom and medication scores and ELISA results). Differences in the rate of adverse reactions were evaluated by χ^2 test. Values of $P < .05$ were considered statistically significant.

Results

Patient Data

From a total of 100 patients who started SIT, 73 completed the study after 1 year. All groups were comparable at baseline (Table 1). The main reasons why patients dropped out were the inconvenience of regular, frequent (weekly) visits to the medical centers, the need for absence from work or school, relocation to another city, and the concern for systemic allergic reactions. Adverse reactions related to SIT, such as generalized urticaria, hypotension, cough, wheezing and/or dyspnea were observed in 7/100 (7%) patients. Of these, 6/25 (24%) patients were from the Dpt group and 1/25 (4%) was from the Dpt + MRB group; all occurred when 6.8 µg of Der p 1/dose had been reached. The rate of adverse reactions found in the DPT group was significantly higher than in Dpt + MRB ($P = .042$) and MRB or placebo ($P = .009$) groups. Due to those adverse reactions, the maintenance dose was adjusted to 3.4 µg of Der p 1/dose for all patients as demonstrated in the SIT schedule (Table 2).

Clinical Evaluation and SPT

Clinical results demonstrated that after a year of treatment there was a significant decline in rhinitis symptom ($P < .001$;

Table 2. SIT Schedule for the Updosing and Maintenance Phases

Phase	Concentration		Volume, mL	Interval, wk
	Dpt extract, µg/mL of Der p 1	MRB extract, (million bacteria/mL)		
1	0.017	20-50	0.1–0.2–0.4–0.8	1
2	0.17	20-50	0.1–0.2–0.4–0.8	1
3	1.7	20-50	0.1–0.2–0.4–0.8	1
4	17	20-50	0.1–0.2–0.4	1
Maintenance	17[a]	20-50	0.2	4

Abbreviations: Dpt, *Dermatophagoides pteronyssinus* allergen extract; SIT, specific immunotherapy.
[a] The maintenance dose corresponded to 3.4 µg of Der p 1.

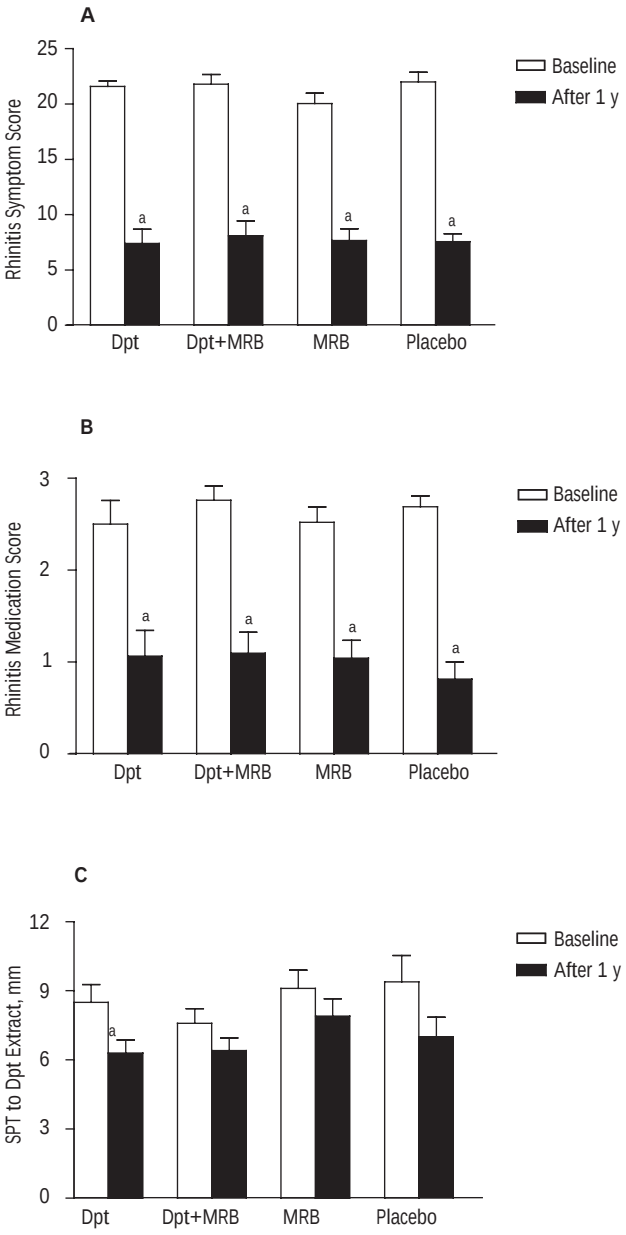

Figure 1. Rhinitis symptom (A) and medication (B) scores and skin prick test (C) results in patients who completed 1 year of treatment in 4 randomly assigned treatment groups: Dpt (*Dermatophagoides pteronyssinus* allergen extract; n = 15), Dpt+MRB (Dpt allergen plus mixed respiratory bacterial extracts; n = 20), MRB (MRB extract alone; n = 23), and placebo (n = 15). Bars represent the mean and error bars the SEM. Significant differences between baseline and after 1 year of treatment within the groups were determined by *t* test (skin prick test [SPT] results) or Wilcoxon signed-rank test (symptom and medication scores). [a] $P < .05$.

Figure 1A) and medication ($P < .001$; Figure 1B) scores in all groups, including the placebo group. There were no significant differences at baseline ($P = .369$ and $P = .515$) or after 1 year ($P = .918$ and $P = .908$) in symptom and medication scores, respectively, in intergroup analyses as determined by Kruskal-Wallis test and Dunn multiple comparison test

For evaluating the treatment effects on SPT response, the mean wheal sizes in response to Dpt extract were analyzed at baseline and after a year of immunotherapy in each group (Figure 1C). A significant decrease in mean wheal size was found for patients from the Dpt group ($P = .03$), with no significant change in the Dpt + MRB ($P = .161$), MRB ($P = .278$) or placebo ($P = .102$) groups. Also, no significant SPT response differences were found at baseline ($P = .424$) or after a year ($P = .309$) in intergroup analyses by ANOVA and Tukey multiple comparison test.

Allergen-Specific IgE, IgG4, and IgG1 Antibodies

To investigate the treatment effects on mite-specific antibody responses, the serum levels of IgE, IgG4, and IgG1 to whole Dpt extract and its major Der p 1 and Der p 2 allergens were analyzed at baseline and after a year of treatment in each group (Figure 2). The change between the 2 time-points (day 0 and 1 year) within each group was demonstrated for each study patient and compared by Wilcoxon signed-rank test. In the Dpt group (Figure 2A) there was a significant decrease in IgE levels to Der p 2 only (7.7 vs 7.3; $P = .048$) whereas a significant increase was found for IgG4 to Dpt (1.9 vs 4.8, $P = .003$) and Der p 1 (1.7 vs 4.1, $P = .021$), but not to the Der p 2 allergen. Similarly, IgG1 to Dpt and Der p 1 levels increased significantly after a year of treatment (7.5 vs 9.5, $P = .002$ and 1.9 vs 6.6, $P = .001$, respectively). In the Dpt + MRB group (Figure 2B) there was a significant decrease in IgE levels to Der p 2 (7.5 vs 5.9, $P = .005$) and whole Dpt extract (5.5 vs 3.5, $P = .001$) while responses of IgG4 and IgG1 were inconsistent, with a significant increase only seen for IgG4 to Der p 1 (1.6 vs 3.8, $P = .049$) and IgG1 to Dpt extract (5.3 vs 7.9, $P = .001$). In the MRB group (Figure 2C), IgE levels decreased significantly only to whole Dpt extract (6.9 vs 5.1, $P = .005$) and no significant changes were observed for IgG4 and IgG1 to Dpt or its major allergens. No significant antibody response changes were seen in the placebo group (Figure 2D) after a year of treatment ($P > .05$).

Discussion

In this year-long randomized double-blind, placebo-controlled trial, all groups were well matched at baseline for age, sex, SPT, and levels of IgE, IgG4, and IgG1 to Dpt, Der p 1 and Der p 2 allergens. After the groups were decoded, we verified that the majority of systemic adverse reactions associated with immunotherapy injection occurred predominantly in the treatment group receiving Dpt alone. Such reactions have also been reported in other studies of SIT with house dust mites and were associated with a high potency of the allergen extracts [24, 25]. In our study, the maintenance dose had to be adjusted to 3.4 µg of Der p 1 because above this dose, the risks of patients developing systemic side-effects were clinically harmful. Since these reactions were not equally observed in the Dpt + MRB group, we hypothesize that a decreased T_H2-type immune response induced by MRB

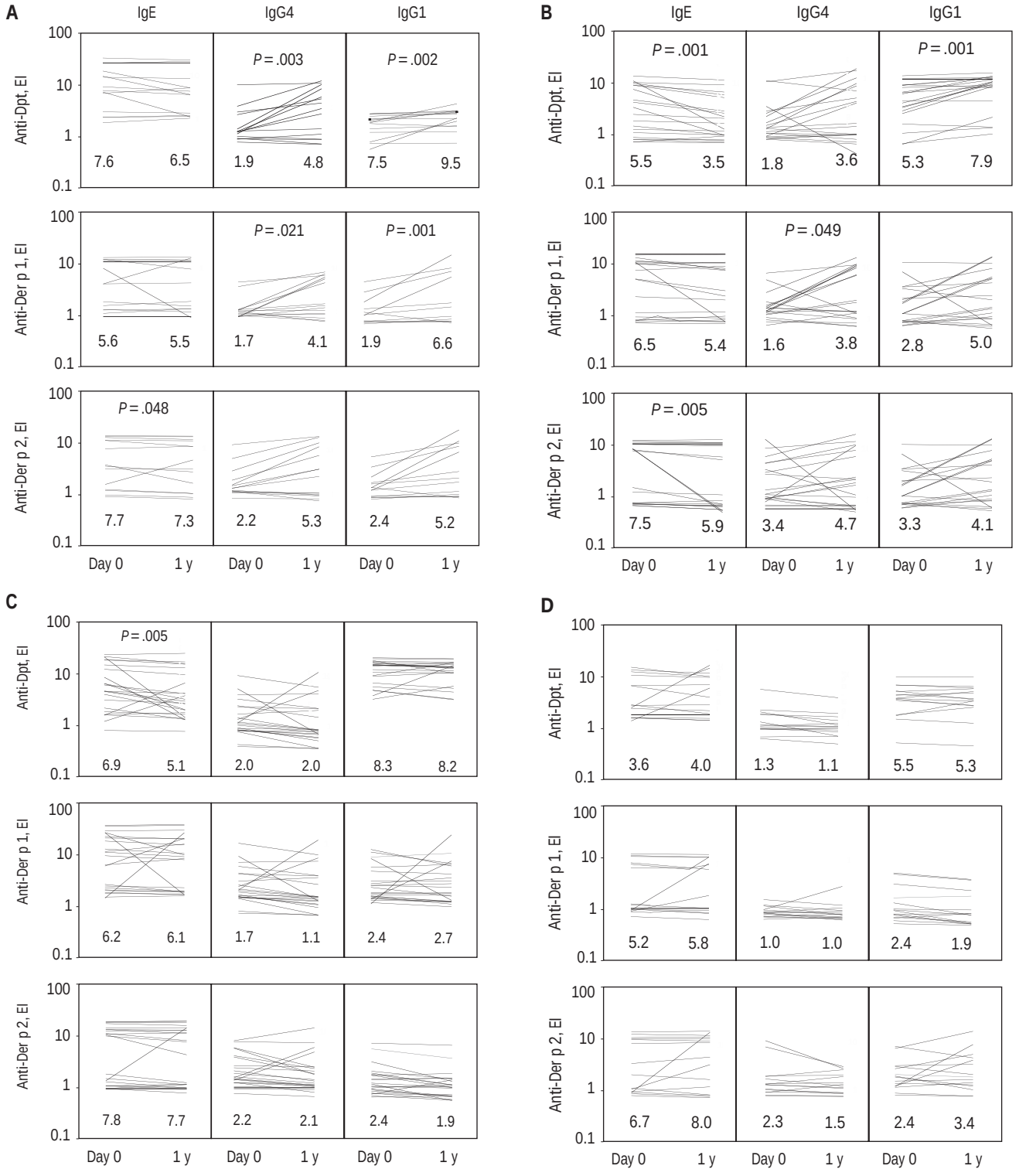

Figure 2. Levels of specific immunoglobulin (Ig) E, IgG4 and IgG1 to *Dermatophagoides pteronyssinus* (Dpt) and its major allergens (Der p 1 and Der p 2) in sera from patients randomized to 4 treatment groups: (A) DPT (Dpt allergen extract; n = 15), (B) Dpt+MRB (Dpt allergen plus mixed respiratory bacterial extracts; n = 20), (C) MRB (MRB extract alone; n = 23) and (D) placebo (n = 15). Antibody levels are expressed in enzyme-linked immunosorbent assay (ELISA) indices (EI) as values of individual patients at 2 time-points (day 0 and after 1 year of treatment) and connected with a line; the mean EI values for each of those time-points are also indicated inside the graph. Significant differences before and after treatment within the groups were determined by the Wilcoxon signed-rank test.

(bacterial antigens) extract as verified by diminishing IgE levels to Dpt whole extract may be responsible for protecting patients from these systemic adverse reactions when compared to the treatment group with whole Dpt extract alone.

Clinical results showed that there was a significant average decline after a year of treatment in both rhinitis symptom and medication scores, by 64% and 61%, respectively, considering all treatment groups, including the placebo group, but without significant differences between them. Our clinical data are in agreement with results previously reported by Varney et al [26], who demonstrated a clinically useful improvement in patients with poorly controlled rhinitis with or without mild asthma after a year of SIT with Dpt extract. These data suggest that variables other than immunotherapy may be influencing the improvement of rhinitis symptom and medication scores in the study period. As the patients visited a physician weekly, they achieved good treatment adherence with the accomplishment of inhaled allergen avoidance measures, correct use of medications, and improvement in psychosomatic aspects associated with allergic diseases.

Our results also showed that SPT response to Dpt allergen extract was reduced in all groups after a year of treatment, although the changes were significant only for the Dpt group. However, this decline in skin reactivity might be unrelated to the clinical improvement. Other studies have also reported a decrease in SPT response after SIT, even though it might be transitory and show lower correlation with clinical efficacy than has been shown for late-phase reactions [26, 27].

With regard to the levels of specific antibodies to Dpt and its major allergens, Der p 1 and Der p 2, the significant reduction in levels of specific IgE to Dpt only in patients of the groups receiving bacterial extracts (MRB and Dpt + MRB groups) for 1 year suggests an important role of bacterial extracts in reducing T_H2-dominated immune responses and consequently allergen-specific IgE synthesis as previously proposed [28]. Therefore, the MRB extract alone was able to reduce specific IgE response to crude Dpt extract only, but not to Der p 1 or Der p 2 allergens, indicating that the MRB extract is unable to induce IgE reduction at a molecular level. In contrast, after a year of treatment, only the groups receiving allergen (Dpt and Dpt + MRB) showed a significant reduction in levels of IgE to Der p 2, but not to Der p 1 allergen. These results were similar to the findings of Mastrandrea et al [29], who reported a significant and progressive decrease in IgE levels to Dpt and Der p 2 after just 1 year of mite-specific immunotherapy, while levels of IgE to Der p 1 showed a significant reduction only after 3 years of treatment. We can speculate that the decrease of Der p 2- but not Dpt- and Der p 1-specific IgE observed in our Dpt group may be due to a probable modulation of the T_H1/T_H2 balance at the molecular level induced by the Der p 2 allergen in the whole Dpt extract. In this context, a previous study showed that levels of IgE against Der p 2 were always higher than levels of IgE against Der p 1 in patient sera, confirming that Der p 2 is a more immunologically active molecule than Der p 1 in inducing IgE synthesis [29]. In addition, it is noteworthy that there were 2 clearly distinct patient subgroups regarding Der p 1- and Der p 2-specific IgE levels in all treatment groups, with 1 group showing negative or borderline specific IgE levels and another showing moderate to elevated IgE levels to Der p 1 or Der p 2 as already observed in our previous study [30]. After a year of immunotherapy, these reactivity profiles were not much changed, indicating that no significant induction of new mite IgE reactivities could be observed in contrast to the findings in SIT using pollen extracts [31,32].

Regarding the IgG subclass responses, the significant increase of IgG4 and IgG1 to Dpt and Der p 1, but not to Der p 2 allergen, only in the treatment groups receiving allergen (Dpt and Dpt + MRB) suggests that mite SIT was able to modulate the immune response after just 1 year of treatment. These results diverge from a previous study showing that IgG1 was dominant in the early (1 year) immune response of ragweed immunotherapy while IgG4 appears in significant quantities only after prolonged treatment (3 years) [9]. Assuming that IgG antibody responses to major allergens of Dpt were induced by SIT, we can observe that Der p 1 is more immunogenic than Der p 2 in inducing IgG synthesis. Alternatively, the content of Der p 2 allergen in the Dpt extract could have been insufficient for stimulating the production of these specific IgG antibodies. Once again, these data suggest that SIT using mite whole Dpt extract also modulates the balance of T_H1/T_H2 immune response or regulatory T cell development at a molecular level on IgG synthesis, showing increased response to Der p 1 but not to Der p 2 allergen. On the other hand, the group receiving only bacterial extract (MRB) or placebo showed no significant increase in mite-specific IgG responses, reinforcing the premise that only allergen SIT could induce such increases.

SIT has been considered useful and efficacious as demonstrated by significant positive correlations between increased specific IgG1 or IgG4 levels and clinical improvement during such therapy [11]. A possible explanation for these findings is that IgG1 and IgG4 antibodies induced by SIT act as blocking antibodies by competing with IgE for allergen binding and inhibiting IgE-dependent activation of mast cells and basophils [33]. Alternatively, IgG antibodies induced by SIT can recognize different epitopes from IgE antibodies and thus IgG might prevent aggregation of FcεRI-bound IgE through steric hindrance rather than direct competition for allergen-binding sites [34]. Therefore, specific IgG1 and IgG4 antibodies could confer long-term benefit after discontinuation of allergen immunotherapy [35].

The results of this study indicate that after a year of treatment, SIT using Dpt extract alone was effective in reducing the SPT response to Dpt allergen extract and the specific serum IgE levels to Der p 2 allergen, while SIT using bacterial extracts decreased IgE levels to whole Dpt extract and induced fewer systemic adverse reactions. However, only the allergen-containing treatment groups were able to induce increased levels of specific IgG1 and IgG4 to Dpt and Der p 1 allergens after just 1 year of treatment, suggesting that mite SIT is efficient in modulating the immune response. Taken together, although there was no clinical efficacy of SIT beyond some placebo effect after 1 year of treatment, the serological results showed significant antibody response changes that could be predicting the most favorable SIT group for inducing protection by both reducing IgE synthesis and stimulating the production of IgG blocking antibodies against exacerbation of allergic symptoms in the early period following SIT discontinuation.

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