



Component-resolved immunologic modifications, efficacy, and tolerance of latex sublingual immunotherapy in children

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ABSTRACT

Background: As the frequency of natural rubber latex (NRL) allergy has increased, attempts have been made to diminish exposure in high-risk patients. Despite some good results, complete NRL avoidance was not possible, so latex immunotherapy was developed.

Objective: To examine variations in immunologic parameters, clinical efficacy, and safety of NRL sublingual immunotherapy (SLIT).

Methods: This prospective, observational, open, case-control study included 23 patients (18 patients receiving NRL SLIT and 5 controls). Skin prick, conjunctival provocation, and in-use tests with NRL, specific IgE and specific IgG4 to NRL, specific IgE to recombinant NRL allergens, and basophil activation test (BAT) with whole latex, natural, and recombinant allergens were performed before immunotherapy (T0) and at 6 (T1) and 12 months (T2) of treatment.

Results: Patients were sensitized to Hev b 5, Hev b 6.01, and Hev b 6.02 proteins, optimal for SLIT. Changes in specific IgE were not significant. Increases in specific IgG4 between T1 and T2 were larger in the active group. BAT determinations showed significant decreases in recombinant Hev b 6.01 and natural Hev b 6.02 in the active group at T1 but not at T2. Both groups had new sensitizations at T1 but not at T2. The active group had significant increases in the response threshold in the in vivo tests at T1 and T2. Adverse effects were limited to local reactions.

Conclusions: NRL SLIT is effective and safe in children with latex allergy. Our results suggest that specific IgE determinations and BAT measurements to natural and recombinant latex allergens may allow obtaining an allergen-based diagnosis to help determine specific immunotherapy.

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Introduction

Natural rubber latex (NRL) allergy cases were first identified in the 1980s. As the frequency of these cases rapidly increased, protocols,¹ recommendations,² and screening programs³ were published to diminish exposure in high-risk patients (health care or latex industry workers⁴ and children with spina bifida or those undergoing multiple operations⁵). Despite some good results,⁶ complete NRL avoidance was not possible, so latex immunotherapy was developed.

The efficacy of subcutaneous immunotherapy (SCIT) with NRL has been demonstrated, but SCIT causes an unacceptable rate of

local and systemic reactions.^{7,8} Sublingual immunotherapy (SLIT) is an accepted alternative to SCIT in respiratory allergy.⁹

The efficacy and safety of SLIT with NRL extracts have been demonstrated,^{10,11} as well as treatment failures¹² and severe and recurrent adverse reactions.^{13,14} SLIT with NRL has also been proven to be effective and safe in children.¹⁵

An accurate diagnosis of latex allergy is crucial for a successful therapeutic approach. Component-resolved diagnosis has provided allergists with specific tools, leading to the identification of precise patient sensitization profiles. Hev b 1 through Hev b 14 allergens have been isolated, offering better reproducibility and stability than complete extracts. These new techniques have improved the diagnosis of latex allergy¹⁶⁻¹⁸ and the immunotherapy indication in certain pollinosis.¹⁹ Regarding latex allergy, health care workers and children with spina bifida or other congenital

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Table 1
Characteristics of the patients with natural rubber latex allergy^a

Patient No.	Age, y/sex	Atopy	Pollinosis	Symptoms	Latex-fruit syndrome ^b	SI	Conjunctival provocation threshold dose, $\mu\text{g/mL}$	In-use test time, min	Surgery, No. of patients	Latex immunotherapy
1	6/M	Yes	No	U/AE	No	0	1.6	10	0	Yes
2	7/F	Yes	No	U/AE	No	0	Not done	10	0	Yes
3	8/F	No	No	U/AE	No	2	40	30	≥ 3	Yes
4	14/F	No	No	U/AE/RC/BA	Yes	3	1.6	20	≥ 3	Yes
5	12/M	No	No	Anaphylaxis	Yes	2	1.6	10	≥ 3	Yes
6	14/F	Yes	Yes	U/AE	Yes	3	Not done	Not done	≥ 3	Yes
7	15/F	No	No	Anaphylaxis	Yes	1	8	10	≥ 3	Yes
8	5/F	No	No	U/AE	No	3	Not done	20	≥ 3	Yes
9	9/M	No	No	U/AE/RC/BA	No	2	Negative	30	≥ 3	Yes
10	14/M	No	No	RC/BA	No	1	8	10	< 3	Yes
11	15/M	No	No	U/AE	No	2	8	30	≥ 3	Yes
12	15/M	Yes	Yes	RC/BA	No	1	Negative	30	0	Yes
13	11/M	Yes	Yes	U/AE/RC/BA	No	4	40	10	≥ 3	Yes
14	17/M	Yes	No	U/AE/RC/BA	No	1	40	20	< 3	Yes
15	15/F	Yes	No	Anaphylaxis	Yes	0	40	30	0	Yes
16	16/M	No	No	RC/BA	Yes	2	8	30	≥ 3	No
17	13/M	No	No	U/AE/RC/BA	Yes	1	1.6	10	< 3	Yes
18	10/F	No	No	U/AE/RC/BA	No	5	Negative	10	≥ 3	Yes
19	7/F	Yes	No	U/AE	Yes	0	8	10	0	Yes
20	7/M	No	No	U/AE/RC/BA	No	4	Negative	30	≥ 3	No
21	17/F	Yes	Yes	U/AE	No	0	Negative	20	0	No
22	6/M	No	No	U/AE/RC/BA	No	6	8	10	≥ 3	No
23	15/F	No	Yes	RC/BA	No	7	40	20	≥ 3	No

Abbreviations: AE, angioedema; BA, bronchial asthma; RC, rhinoconjunctivitis; SI, number of surgical interventions; U, urticaria.

^aPatients 15, 18, 19, 21, and 23 did not received immunotherapy (controls); the other patients made up the active group.

^bAllergic to fruits (chestnut, kiwi, avocado) determined by clinical symptoms and positive skin prick test or positive specific IgE test results.

malformations requiring multiple surgical interventions have different patterns of sensitization.^{16,20,21}

To our knowledge, this is the first study to measure in vitro parameters, such as specific IgE to recombinant latex allergens, and basophil activation test (BAT) measurements to natural and recombinant latex allergens. These techniques may allow achieving an allergen-based diagnosis, which will improve immunotherapy indication and follow-up. These techniques may also be useful in evaluating the possible correlation of in vitro modifications, clinical efficacy, and SLIT mechanisms. In addition, this is the first study, to our knowledge, that includes both children who have undergone multiple surgical interventions and those without previous operations, allowing us to examine differences and treatment tolerance in both these groups.

Methods

Study design

This was a prospective, observational, open, case-control study of 23 children during a 12-month period.

Study participants

All patients aged 5 to 18 years diagnosed as having latex allergy during a 1-year period in our allergy department and for whom immunotherapy was indicated according to European Academy of Allergy and Clinical Immunology (EAACI) criterion²² were invited to participate in the study. Immunotherapy indication was based on the severity of the symptoms and the impossibility of successful latex avoidance. The children who were designated to receive SLIT formed the active group and the rest served as controls. Demographic characteristics are detailed in Table 1.

Latex allergy diagnosis was based on the following: a clinical history of NRL-induced allergic reaction, positive skin prick test (SPT) results, NRL specific IgE (SPT result > 3 mm and NRL specific IgE > 0.35 kU/L), and a positive conjunctival provocation test (CPT) result or a positive in-use test (UT) result with NRL.

The following exclusion criteria were used: absence of indication for immunotherapy or contraindication for immunotherapy

according to EAACI recommendations. Those patients who could not remain in the clinic for the established period after each SLIT dose was administered were also excluded. Sensitization of other inhalants was not considered an exclusion criterion.

Before immunotherapy our patients were more frequently sensitized to Hev b 5, Hev b 6.01, and Hev b 6.02 allergens according to specific IgE and BAT measurements.²³ Sensitization to Hev b1 and Hev b 3 were only detected in patients who had undergone past operations.

Ethical aspects

The study was accepted by the Clinical Trials Committee of Navarra. An informed written consent from the patients or their legal representative was obtained.

Allergenic extract

A glycerinated NRL extract (*Hevea brasiliensis*) for sublingual administration (SLIT-LATEX; ALK-Abelló, S.A., Madrid, Spain) was used. Immunotherapy extract was distributed in 5 vials containing dilutions of glycerinated NRL solution, with phenol (0.4% wt/vol) as

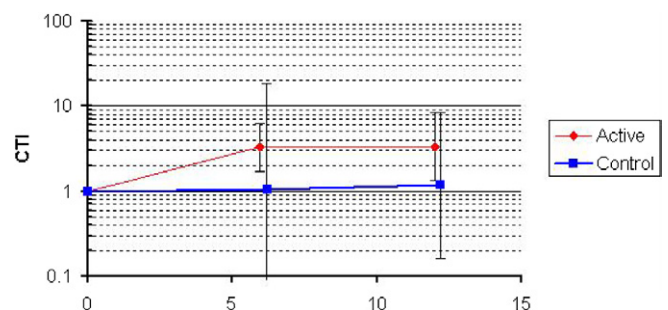


Fig. 1. Parallel line assay in the active and control groups where the cutaneous tolerance index (dilutions of the extract provoking the same cutaneous answer) is determined. This figure shows how the active group needs higher concentrations of the natural rubber latex extract than the control group to elicit the same cutaneous tolerance index.

Table 2
Median (interquartile range) provocation test results

Test	Active group			Control group		
	T0	T1	T2	T0	T1	T2
CPT, mg/mL	8.00 (1.60–40.00)	120.00 (40.00–200.00)	200.00 (40.00–200.00)	40.00 (8.00–200.00)	40.00 (8.00–200.00)	104.00 (8.00–200.00)
UT for pruritus, min	5.00 (5.00–5.00)	15.00 (5.00–25.00)	25.00 (15.00–35.00)	5.00 (5.00–15.00)	15.00 (5.00–25.00)	20.00 (10.00–30.00)
UT for urticaria, min	5.00 (5.00–25.00)	15.00 (5.00–35.00)	35.00 (25.00–35.00)	15.00 (15.00–25.00)	25.00 (15.00–25.00)	20.00 (10.00–30.00)

Abbreviations: CPT, conjunctival provocation test; T0, before the administration of immunotherapy; T1, after 6 months of immunotherapy; T2, after 12 months of immunotherapy; UT, in-use test.

the conservative agent and human serum albumin (0.04% wt/vol) in every vial except the most concentrated. The vial with the maximum concentration contained 20 µg/mL of NRL protein, containing Hev b 6.01, Hev b 1, Hev b 5, Hev b 7, Hev b 2, and hevamine.⁷

Doses and duration of the different phases of immunization

The immunotherapy administration was performed according to the ultrarush guidelines recommended by the manufacturer. Every build-up phase dose was administered at the immunotherapy unit under medical supervision. Emergency equipment was available for the treatment of potential adverse reactions. The initial doses were administered every 15 minutes, and the patients were supervised for 60 minutes after the last dose. The administration of the maintenance phase was performed at the patient's residence. Patients were instructed to maintain the allergen solution under the tongue for at least 3 minutes and then to spit it out.

Efficacy assessment

In vitro tests and the SPT with different dilutions of the NRL extract, CPTs, and UTs were performed before the administration of immunotherapy (T0) and at 6 (T1) and 12 months (T2) of treatment. SPTs with food and other inhalant allergens were performed at T0.

Tolerance assessment

Every adverse event was recorded. Investigators evaluated the nature and severity of the events, determining the casual relation with the immunotherapy.

In vivo tests

Cutaneous tests. The SPTs were performed on the volar surface of the forearm and duplicated with 4 different dilutions of the NRL extract with protein concentrations of 4, 20, 100, and 500 µg/mL in 50% of glycerinated solution, as previously described.¹¹ Glycerosaline solution and histamine (10 µg/mL) were used as negative and positive controls, respectively. Wheal areas were measured by planimetry. Differences in skin reactivity were expressed by the cutaneous tolerance index (CTI) as the dilutions of the extract eliciting the same cutaneous response. We also performed SPTs with the most common food and inhalant allergens in our environment.

Patients with anaphylaxis also underwent SPTs. No systemic adverse events related to SPTs were observed.

Conjunctival provocation tests. CPTs were performed with NRL extract (ALK-Abell6) at increasing concentrations: 0.32, 1.6, 8, and 40 µg/mL. Control tests were performed with saline solution. The concentration eliciting symptoms was considered the threshold dose. Symptoms were scored by the scale of Möller et al.²⁴

In-use tests. UTs were performed by placing a latex glove (Fave-sam, Toledo, Spain) on the patient's previously moistened hand. The test ended when local or systemic manifestations were observed or 30 minutes after the beginning of the test. Positive UT results were considered according to 2 variables: time of appearance of pruritus and time of appearance of wheals. Both results were independently considered independent for analysis.

In vitro tests

Specific IgE tests were performed for the following recombinant NRL allergens: rHev b 1, rHev b 2, rHev b 3, rHev b 5, rHev b 6.01, rHev b 6.02, rHev b 8, rHev b 9, and rHev b 11 (ImmunoCAP-Phadia, Uppsala, Sweden). In addition, tests of specific IgE and IgG4 to latex (ImmunoCAP Pharmacia) were performed in all patients. BAT was performed with the whole latex extract and latex natural and recombinant allergens rHev b 5, rHev b 6.01, and nHev b 6.02 according to a previously described technique.²³

Statistical analysis

Immediate cutaneous reactivity was analyzed by a parallel lines trial after logarithmic transformation. Qualitative data were compared by the Fisher exact test for binomial variables and the χ^2 test for variables with more than 2 categories. The BAT results, being categorical (positive or negative), were assessed by the Sign and McNemar tests. Quantitative data were compared by nonparametric tests (Mann-Whitney for intergroup and Wilcoxon for intra-group comparisons) and parametric (*t* test and paired *t* test). The comparisons between the active patients and controls were made by analysis of variance for repeated measures. $P < .05$ was considered statistically significant for all tests.

Table 3
Median (interquartile range) specific ige test results to natural rubber latex allergens

Test	Active group			Control group		
	T0	T1	T2	T0	T1	T2
Natural rubber latex, kU/L	17.00 (2.76–47.20)	18.25 (3.83–26.00)	20.70 (6.08–58.00)	5.04 (1.66–8.08)	4.22 (2.41–5.14)	5.58 (1.63–8.37)
rHev b 1, kU/L	0.10 (0.10–0.10)	0.10 (0.10–0.10)	0.10 (0.10–0.10)	0.10 (0.10–0.10)	0.10 (0.10–0.35)	0.10 (0.10–0.10)
rHev b 2, kU/L	0.10 (0.10–0.10)	0.10 (0.10–0.10)	0.10 (0.10–0.10)	0.10 (0.10–0.10)	0.10 (0.10–0.10)	0.10 (0.10–0.10)
rHev b 3, kU/L	0.10 (0.10–0.10)	0.10 (0.10–0.10)	0.10 (0.10–0.10)	0.10 (0.10–0.10)	0.10 (0.10–0.10)	0.10 (0.10–0.10)
rHev b 5, kU/L	0.10 (0.10–11.10)	0.10 (0.10–10.80)	0.10 (0.10–13.70)	0.10 (0.10–2.90)	0.10 (0.10–2.87)	0.10 (0.10–2.73)
rHev b 6.01, kU/L	1.79 (0.10–7.57)	3.15 (0.10–6.14)	2.82 (0.10–6.73)	0.79 (0.10–1.41)	0.70 (0.10–0.88)	0.79 (0.10–1.23)
rHev b 6.02, kU/L	2.28 (0.10–7.01)	2.92 (0.10–9.28)	2.54 (0.10–9.00)	0.73 (0.10–2.95)	0.88 (0.10–2.13)	0.78 (0.10–13.19)
rHev b 8, kU/L	0.10 (0.10–0.10)	0.10 (0.10–0.10)	0.10 (0.10–0.10)	0.10 (0.10–0.10)	0.10 (0.10–0.10)	0.10 (0.10–0.10)
rHev b 11, kU/L	0.10 (0.10–0.10)	0.10 (0.10–0.10)	0.10 (0.10–0.10)	0.10 (0.10–0.10)	0.10 (0.10–0.10)	0.10 (0.10–0.10)

Abbreviations: rHev, recombinant Hev; T0, before the administration of immunotherapy; T1, after 6 months of immunotherapy; T2, after 12 months of immunotherapy.

Table 4
Median (interquartile range) of specific igg4 to natural rubber latex

Time	Active group	Control group
T0	0.35 (0.22 to 0.86)	0.36 (0.22 to 0.44)
T1	0.34 (0.25 to 0.86)	0.34 (0.27 to 0.59)
T2	0.51 (0.22 to 1.07)	0.25 (0.21 to 0.27)
T1-T0	0.03 (−0.04 to 0.08)	0.12 (0.02 to 0.15)
T2-T0	0.06 (−0.04 to 0.15)	−0.08 (−0.11 to 0.01)
T2-T1	−0.02 (−0.03 to 0.19)	−0.13 (−0.27 to −0.10)

Abbreviations: T0, before the administration of immunotherapy; T1, after 6 months of immunotherapy; T2, after 12 months of immunotherapy.

Results

Study participants

Twenty-three patients with latex allergy (12 boys and 11 girls) aged 5 to 18 years were included. Eighteen accepted and received SLIT (50% boys; mean $\pm$ SD age, 12 $\pm$ 4 years). Five patients (60% boys; mean $\pm$ SD age, 12 $\pm$ 5 years), who refused SLIT, remained as controls. No significant differences in demographic and clinical data, except some detected in the BAT results, were found between either group at baseline (Table 1).

Clinical efficacy evaluation

Cutaneous tests. Changes in skin reactivity were detected after 6 months (CTI, 3.27; 95% confidence interval [CI], 1.7–6.27; $P < .05$) and maintained until the end of the study. Cutaneous reactivity to the allergen measured by the CTI increased 3.34 times (95% CI, 1.33–8.38) in the active group after 12 months of SLIT ($P < .05$) (Fig 1). In the control group, cutaneous reactivity remained unchanged.

Conjunctival provocation tests. CPTs were performed in 20 patients; 3 patients refused skin tests. CPTs revealed significant increases in the response threshold in the active group compared with the initial assessments (mean $\pm$ SD, 53.23 $\pm$ 77.54 μ g/mL). This increase was observed 6 (mean $\pm$ SD, 113.14 $\pm$ 77.54 μ g/mL; Wilcoxon $P = .01$) and 12 months after SLIT (mean $\pm$ SD, 140.57 $\pm$ 83.12 μ g/mL; Wilcoxon $P = .05$). CPT results in controls remained unchanged. Intergrout results were not significant (Table 2).

In-use tests. All patients but 1 underwent UTs. The exposure time needed to elicit pruritus or urticaria in the UT showed a significant increase in the active group at 6 months (mean $\pm$ SD, 15 $\pm$ 11.73 minutes and 19.12 $\pm$ 13.26 minutes, respectively) and 12 months (mean $\pm$ SD, 25 $\pm$ 11.18 and 27.35 $\pm$ 10.91, respectively) compared with basal status (mean $\pm$ SD, 10.29 $\pm$ 10.07 minutes and 12.65 $\pm$ 9.03 minutes, respectively) (Wilcoxon $P = .02$ and $P = .06$ at T1 and $P = .002$ and $P = .001$ at T2 for pruritus and urticaria, respectively). Control group results did not reach statistical significance (Table 2).

Adverse effects. Six patients (33%) developed adverse effects during the initiation phase: 5 presented with oral pruritus and 1 with exacerbation of atopic dermatitis. During the maintenance phase 5 patients (28%) had oral symptoms during the first weeks and good tolerance afterward. Four of them had had adverse effects during

the initiation phase. No patient left the study or required specific treatment or immunotherapy dose adjustments due to adverse effects.

Immunologic changes assessment

Specific IgE. Specific IgE measurements to NRL at the beginning of the study in the active and control groups (mean $\pm$ SD, 25.28 $\pm$ 25.34 and 6.48 $\pm$ 6.64 kU/L, respectively) were not homogeneous ($P < .05$). The differences between both groups were maintained during all follow-up, but IgE levels within groups remained unchanged without intragroup variation (Table 3).

Specific IgG4. We observed greater increases in the specific IgG4 between T1 and T2 in the active group than in the control group. This difference reached statistical significance (Mann-Whitney $P = .01$) (Table 4).

Basophil activation test. The BAT results detected different baseline values for active patients and controls. Significant decreases were observed in the percentage of basophil activation in the active group in the first 6 months of SLIT with NRL, rHev b 6.01, and nHev b 6.02 (t for paired data $P = .001$ and $P = .032$ for 0.125 and 0.031 mg/mL of NRL, respectively; $P = .03$ for 0.05 μ g/mL of rHev b 6.01 and 0.11 for 0.5 μ g/mL of nHev b 6.02, respectively). This significance disappeared at 12 months of immunotherapy (Table 5).

New sensitizations detected by in vitro tests after immunotherapy. New sensitizations were detected by specific IgE determinations to recombinant allergens along the treatment. In the control group, 2 patients had new sensitizations to rHev b 1 at T1 that disappeared at T2. In the active group 6 patients had new sensitizations: 3 to rHev b 11 (2 at T1 and T2 and 1 at T2), 1 to rHev b 3 (at T2), 1 to rHev b 6.01 (at T1 that disappeared at T2), and 1 to rHev b 6.02 (at T1 and T2).

The BAT results also showed new sensitizations. Positive results to new allergens were found in 2 patients in the active group (Hev b 5 and Hev b 6.02) and 1 patient in the control group (Hev b 6.02). These new sensitizations only remained at T2 in 1 patient in the active group.

Discussion

We performed a prospective, observational, open, case-control study of 23 patients. Eighteen of the patients underwent SLIT with an NRL extract. Because this treatment efficacy and security had already been established by means of a double-blind, placebo-controlled trial^{11,15} and SLIT with an NRL extract was an already commercialized treatment, SLIT was offered to all patients for ethical reasons. Because only 5 patients refused SLIT, the control group remained small. This small control group explains the lack of consistency in the differences observed between the groups. Our aim was to evaluate the immunologic changes induced by effective SLIT. Therefore, the inclusion of a control group allowed us to distinguish between the changes induced by SLIT from the spontaneous ones.

Table 5
Median (interquartile range) basophil activation test results

Component	Active group					
	T0		T1		T2	
	C1	C2	C1	C2	C1	C2
Natural rubber latex	49.9 (28.5–65.5)	54.1 (41.9–61.3)	29.2 (5–35.1)	36.2 (2.5–47.4)	37.6 (33.2–55.6)	50.1 (26.6–64.9)
rHev b 5	13.7 (0.0–43.4)	13.7 (0.0–54.6)	17.5 (0.0–34.4)	4.0 (0.0–37.7)	2.5 (0.0–32.8)	1.9 (0.0–60.2)
rHev b 6.01	0.2 (0.0–18.0)	0.7 (0.0–15.1)	0.0 (0.0–2.0)	0.0 (0.0–0.2)	0.0 (0.0–0.4)	0.0 (0.0–0.3)
nHev b 6.02	44.3 (8.6–52.6)	51.5 (14.3–64.9)	23.1 (2.1–42.0)	25.9 (2.2–50.9)	9.2 (2.3–65.7)	9.6 (0.0–69.7)

Abbreviations: C1, maximum concentration tested (natural rubber latex, 0.125 mg/mL; rHev b 5, 0.5 μ g/mL; rHev b 6.01, 0.5 μ g/mL; rHev b 6.02, 0.5 μ g/mL); C2, minimum concentration tested (natural rubber latex, 0.031 mg/mL; rHev b 5, 0.05 μ g/mL; rHev b 6.01, 0.05 μ g/mL; rHev b 6.02, 0.05 μ g/mL); nHev, natural Hev; rHev, recombinant Hev.

Despite the lack of randomization, no significant differences were found in the patient baseline demographic characteristics in either group. The differences in BAT results detected in both groups might not hamper the subsequent immunologic modifications after SLIT.

The novelty of this trial lies in the evaluation of the modifications of the immunologic parameters, including IgG4 determinations to latex, specific IgE to recombinant latex allergens, and BAT measurements to natural and recombinant latex allergens. By these techniques we tried to evaluate the correlation of *in vitro* parameters with clinical efficacy, to improve both immunotherapy indication and its follow-up, and to clarify the mechanisms of SLIT.

The availability of purified allergens improved our diagnostic ability, immunotherapy indication, and treatment monitoring, making possible the measurement of IgE and IgG to individual allergen components during the treatment, valuating its correlation with clinical efficacy. The appearance of neosensitization was also monitored by these techniques. To our knowledge, this is the first study that included children with multiple surgical interventions and those without past operations, which allowed us to study the differences between both patient types.

NRL allergy risk groups have different patterns of NRL sensitization: health care workers are predominantly sensitized to Hev b 5, Hev b 6.01, and Hev b 6.02 proteins and children with multiple operations to Hev b 1 and Hev b 3.^{16,20,21} Children with latex allergy and few or no surgical interventions follow patterns similar to adults.²⁵ Our children, with or without past operations, were more frequently sensitized to Hev b 5, Hev b 6.01, and Hev b 6.02 proteins according to specific IgE and BAT measurements. Sensitization to Hev b 1 and Hev b 3 was found only in patients who had undergone multiple operations.²³ Sensitization to these proteins has been related to clinically relevant latex allergy.²⁶ The sensitization profile of our patients (Hev b 5 and Hev b 6 proteins) allowed us to use NRL SLIT, which contains these proteins.⁷

The changes in specific IgE determinations during treatment did not reach statistical significance, as previously described with latex immunotherapy.^{8,10,27} Nevertheless, the modifications in IgE levels described during SCIT are not consistently observed in SLIT.²⁸ Some studies that include low-dose SLIT extracts have shown 2- to 30-fold increases in specific IgG4 without specific IgE modifications, demonstrating the immunologic effect of these extracts.²⁸

We found slight differences in the evolution of IgG4 measurements to complete latex allergen, but only the specific IgG4 increases between T1 and T2 reached statistical difference, being larger in the active group than in controls. These results strengthen other authors' data regarding the immunologic changes observed during SLIT.²⁹

The BAT results showed significant decreases in the percentage of basophil activation in the active group at T1 and rHev b 6.01 and nHev b 6.02 allergens. Ebo et al²⁶ described similar results studying venom immunotherapy, but in contrast to their results, our changes disappeared after 1 year of SLIT. The BAT results show the SLIT modifications in immunologic mechanisms involved in NRL

allergy, particularly the effects on circulatory effector cells. The decrease of basophil sensitivity to NRL and its allergens at T2 can be attributed to different factors, one of which could be SLIT effective maintenance doses (100 µg per NRL protein), much lower than the initial phase (500 µg per NRL protein). This finding should be analyzed in the future. Some trials have also analyzed the utility of BAT in monitoring Hymenoptera SCIT, obtaining also contradictory results.^{30,31} Because inconsistent BAT results are found, more studies are necessary to determine the real use of BAT monitoring immunotherapy.

Neosensitizations have been previously described during SCIT with complete extracts.^{27,31–34} Both patient groups developed new sensitizations, detected by specific IgE and BAT determinations. Other authors had described new sensitizations only in treated patients.^{27,31} These variations appeared at 6 months of SLIT and were not constant after a year of immunotherapy. The variability of these data could have been attributed to several factors, such as the low number of patients and the specificity of the techniques used or, more likely, to the scarce magnitude of the neosensitizations. Other authors have described similar results.^{33–35} In our opinion, these variations seem to be clinically irrelevant because both patient groups presented similar results.

Regarding the clinical efficacy of SLIT, the active group showed significant increases in the response's threshold in the *in vivo* tests after 12 months of SLIT. Skin sensitivity measured by SPT, UT, and CPT decreased after 6 months of SLIT and was maintained at 12-month evaluation. The median value of the concentration needed to elicit a positive result after 12 months of SLIT in CPT, UT for pruritus, and UT for urticaria were 25-fold, 5-fold, and 7-fold, respectively, comparing baseline values. We consider that these improvements in skin and mucosal responses have clinical relevance and corroborate the results described by previous studies.^{10,11,15,23} In fact, during immunotherapy 6 of the active group patients had real-life exposure to NRL in an uncontrolled setting, and only 1 of them had mild symptoms, with the other 5 (including 1 patient with previous anaphylaxis) remaining asymptomatic.

The adverse effects during the study were limited to local reactions (oral pruritus), except for a patient with an atopic dermatitis exacerbation during the initiation phase. We confirmed the good tolerance described by other authors.^{10,11,25,23}

In conclusion, our results support previous trials affirming NRL SLIT is a safe treatment for children with latex allergy. Although it has modest efficacy in reducing latex symptoms, it should be considered when systemic symptoms appear despite latex avoidance measures. Our results suggest that specific IgE determinations and BAT measurements to natural and recombinant latex allergens may allow obtaining an allergen-based diagnosis to indicate and monitor specific immunotherapy. Despite some changes, IgG4 and BAT results have failed to document consistent immunologic modifications. Further studies should search for basophil responses induced by SLIT to find a useful *in vitro* model to study the immunologic changes induced by this treatment. The mechanisms of SLIT have not been fully established yet. Basophil cells could play a role in SLIT

Table 5
(Continued) Median (interquartile range) basophil activation test results

Control group				P value							
T0		T1		T2		T0		T1		T2	
C1	C2	C1	C2	C1	C2	C1	C2	C1	C2	C1	C2
18.2 (13.0–24.0)	23.6 (13.5–27.7)	22.4 (11.5–56.6)	32.6 (10.8–35.9)	16.6 (0.1–73.5)	23.5 (2.8–73.2)	.11	.03	.65	.98	.61	.45
9.7 (0.0–11.5)	0.3 (0.1–12.0)	0.4 (0.3–4.0)	0.0 (0.0–0.0)	0.2 (0.0–6.8)	0.0 (0.0–10.3)	.39	.21	.42	.60	.94	.69
0.0 (0.0–0.7)	1.1 (0.0–2.9)	0.4 (0.0–1.1)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	.03	.28	.73	.42	.41	.44
12.4 (7.2–21.8)	17.4 (10.5–28.7)	1.4 (1.2–52.0)	2.1 (0.6–57.8)	0.0 (0.0–28.2)	0.0 (0.0–20.6)	.01	.01	.87	.81	.53	.51

mechanism by modifications of their susceptibility to histamine release after the allergen exposure.

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