R. Bernardini[1], S. Pecora[2], M. Milani[3], S.E. Burastero[3]

Natural rubber latex allergy in children: clinical and immunological effects of 3-years sublingual immunotherapy

[1] Paediatric Allergy and Pneumology Centre, Anna Meyer Children's Hospital, Florence, Italy;
[2] ALK-Abellò S.p.A., Milan, Italy; [3] San Raffaele Scientific Institute, Milan, Italy

Key words

Allergy, latex, sublingual immunotherapy, children

Summary

Background: *We previously demonstrated that one year of sublingual immunotherapy (SLIT) with natural rubber latex (NRL) was safe and efficacious in paediatric patients with NRL allergy.* ***Research Design and Methods:*** *We studied 12 NRL-allergic children (age 4-15), previously assigned to the treated arm of a double-blind placebo controlled study, who received a commercial latex SLIT for three years. Adverse reactions were monitored. The primary end-point was the NRL glove-use test. As secondary end-points, skin prick test with NRL and NRL serum specific IgE were used.* ***Main outcomes measures:*** *No SLIT-related side effects were observed. A significant reduction of the glove-use score was observed after one-year treatment (5.1 ± 4.2 vs. 14.8 ± 5.7, p=0.0031). This parameter was further reduced in the second year since SLIT start (2.0 ± 2.7, p=000007). After 3 years of SLIT all patients had a negative glove-use test (p<0.0001). Baseline wheal areas of skin prick test (6.8 ± 2.5 mm^2) were significantly reduced after 2 (5.3 ± 1.8 mm^2) and 3 years (4.0 ± 1.8 mm^2) of SLIT (p=0.039 and 0.027, respectively). Baseline values of serum specific IgE (23 ± 34 KU/l) were significantly reduced after 3 years since SLIT start (6.4 ± 5.0, p=0.0371).* ***Conclusions:*** *Three years of latex SLIT is safe and consolidates the efficacy previously observed after one year of treatment in paediatric patients.*

Introduction

Natural rubber latex (NRL) causes allergy worldwide in healthcare workers (5-17% incidence in exposed subjects) as well as outside of the healthcare environment (about 1% incidence in the general population) (1, 2), mainly in kitchen personnel (3), workers at latex manufacturing plants (4), gardeners (5), hairdressers (6) and subjects who were subjected to multiple surgeries (7).

The preventive measures to reduce latex exposure taken in the last decade by removing powered latex gloves from hospitals have significantly reduced both new sensitizations and the occurrence of severe reactions following latex exposure of sensitized subjects (8). However, the situation remained critical, since NRL is used alone or combined with other substances in the manufacturing of more than 40,000 different objects for technical, professional and everyday-life use (9).

Encouraging results have been obtained with NRL specific immunotherapy by subcutaneous (10, 11), percutaneous (12) and sublingual routes (13-16). We recently demonstrated the safety and efficacy of sublingual im-

Presentation at meetings: the present work was presented as a poster at the 2007 EAACI meeting (Gøteborg, Sweden, 9-13 June 2007), and published as a supplement in Allergy [*Allergy*, 62 (suppl. 83):167-551, abstract number 1175]

munotherapy (SLIT) with a standardized NRL extract with a double blind, placebo controlled study in a population of paediatric patients sensitized to latex who had cutaneous and, in some cases, respiratory symptoms (17).
Here, we report the results of the clinical and immunological follow-up of children who were recruited in that study and show that the efficacy of SLIT further improved and consolidated after three years of treatment, in the absence of any relevant adverse event.

Methods

Study design

This was a open, observational study on 12 patients (age 6-17 years) with clinical signs of allergy to NRL that were found eligible for sublingual immunotherapy (SLIT) with a commercial NRL extract. These patients were previously recruited for a one-year double-blind placebo controlled study (17). After the first year of treatment, the study was opened and the twelve subjects assigned to the active arm were offered to enter the present study, which lasted two more years after the end of the double blind-placebo controlled phase of the study. The parents gave their informed consent after being informed of the possible alternatives, such as allergen avoidance or symptomatic medication.

Outcomes

The outcomes of this study were the safety and efficacy of immunotherapy.
Safety was evaluated clinically by recording any adverse event that could be related to SLIT. To this aim, parents and patients were interviewed at every control visit, which was scheduled every 3 months.
Efficacy was evaluated by a quantitative structured use and rubbing test with NRL-containing gloves, based on the technique first described by Turjanmaa and co-workers (18), which was previously described in great detail (17). Briefly, patients were asked to put on one latex glove (Touchy gloves, International PBI, Milan, Italy) for 15 minutes. Then the glove was removed and the face was rubbed twice with the external part and twice with the internal part of the glove. Local (itching, erythema, wheals) and general symptoms (rhinitis, asthma) were evaluated every fifteen minutes for a period of two hours. The test was blocked by the oral administration of oxatomide and betametaxone. Each symptom scored according to previously reported values, which did not take into account the severity of each symptom but attributed to the symptom itself an absolute value incorporating the assessment of severity (17).
The following secondary outcomes were also considered:
1) Conventional skin prick tests with a NRL extract with a skin prick test solution containing a NRL extract standardized at 500 μg/ml of total protein and corresponding to 30 Histamine Equivalent Prick test Units (HEP) (ALK Abellò, Milano, Italy), prepared as previously described from ammoniated NRL (19).
The skin prick test for NRL was performed and interpreted according to the EAACI guidelines (20). Briefly, the test was performed on the volar area of the forearm by introducing the tip of a lancet with a 1-mm tip (Allergy pricker, Bayer DHS, Milan, Italy) into the skin through the allergenic or the control solution, with gentle pressure and without causing any bleeding. After thirty minutes the areas of the wheal and erythema were marked with a fine-tipped ballpoint pen and transferred onto paper with adhesive tape (Scotch Tape, 3M Italia, Italy) for subsequent planimetric determination of the wheal area. Wheals with an area of less than 7 square mm (i.e. less the 3 mm in diameter) were considered negative.
2) Specific IgE, which were measured with the Phadia Immunocap method (Phadia, Uppsala, Sweden) and expressed in kU/l

Ethics

The procedures followed were in accordance with the ethical standards of the responsible Institutional Committee on Human Experimentation and with the Helsinki Declaration of 1975, as revised in 1983.

Treatment

SLIT-LATEX (ALK-Abellò), a commercially available NRL extract for sublingual administration was used. The extract was prepared by neutralization, semi-purification and concentration of an ammoniated NRL suspension and biologically standardized, as described elsewhere (19).
The build-up phase of the treatment was previously described as part of the first-year double-blind, placebo-controlled study (17) and was completed in 4 days according to a rush schedule. After the build-up phase patients followed the maintenance schedule, consisting of 2 drops of the maximum concentration (resulting in 40 μg of

NRL per administration) every day for a total of 36 months.
Maintenance administrations were performed at home by each patient, whose parents had been instructed on how to proceed in case of adverse effects and specifically asked to immediately report any adverse reaction or discomfort to the allergologists.

Statistical analysis

Comparisons of the results observed in different groups were then performed with Mann-Whitney two sample statistics for non-parametric data. All statistical analyses were done with the PRISM statistical software package (Graphpad Inc., San Diego, CA, USA). All statistical tests were two-sided with a significance level of 0.05.

Results

Safety of SLIT-LATEX treatment

All patients included in the active and placebo group well tolerated the treatment. There was no sign of local (buccolingual) or systemic side effects, including gastrointestinal symptoms and anaphylactic shock.

Scores of symptoms triggered by the glove use and rubbing test

A significant reduction of the symptom score of the glove use and rubbing test was observed after one-year treatment as compared to baseline (p=0.0031) (figure 1). A further reduction was measured in the second year since SLIT start (p=0.0010). After 3 years of SLIT all patients had a negative glove-use test (p<0.0001)

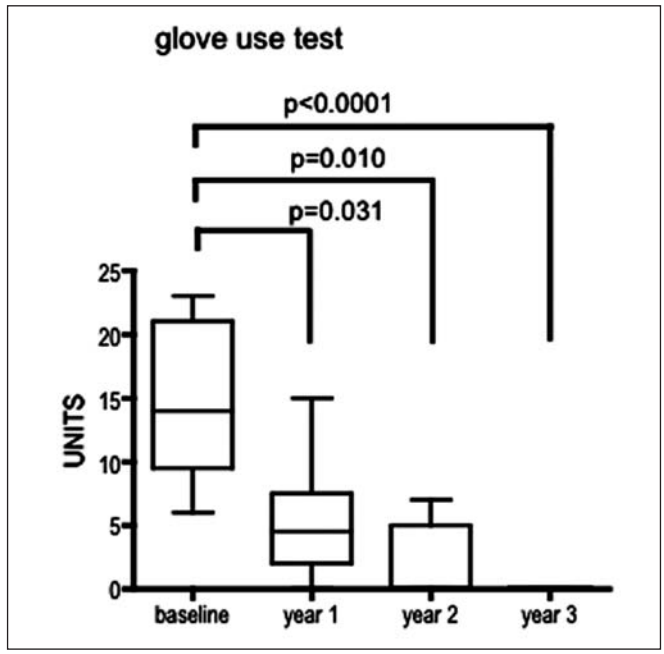
Skin reactivity to NRL

Baseline values of wheal areas measured with skin prick test with NRL extract remained unchanged after the first year of treatment (figure 2, top panel). Wheal areas were significantly reduced as compared to baseline after two and three years since treatment start (p=0.027 and 0.039, respectively).

NRL specific IgE in serum

Although a trend toward reduction was observed, values of NRL specific IgE remained unchanged as compared to baseline after the one and after two years of treatment (figure 2, bottom panel). A significant reduction of NRL specific IgE as compared to values measured at study enter was observed after three years of treatment (p=0.0371) (*ibidem*).

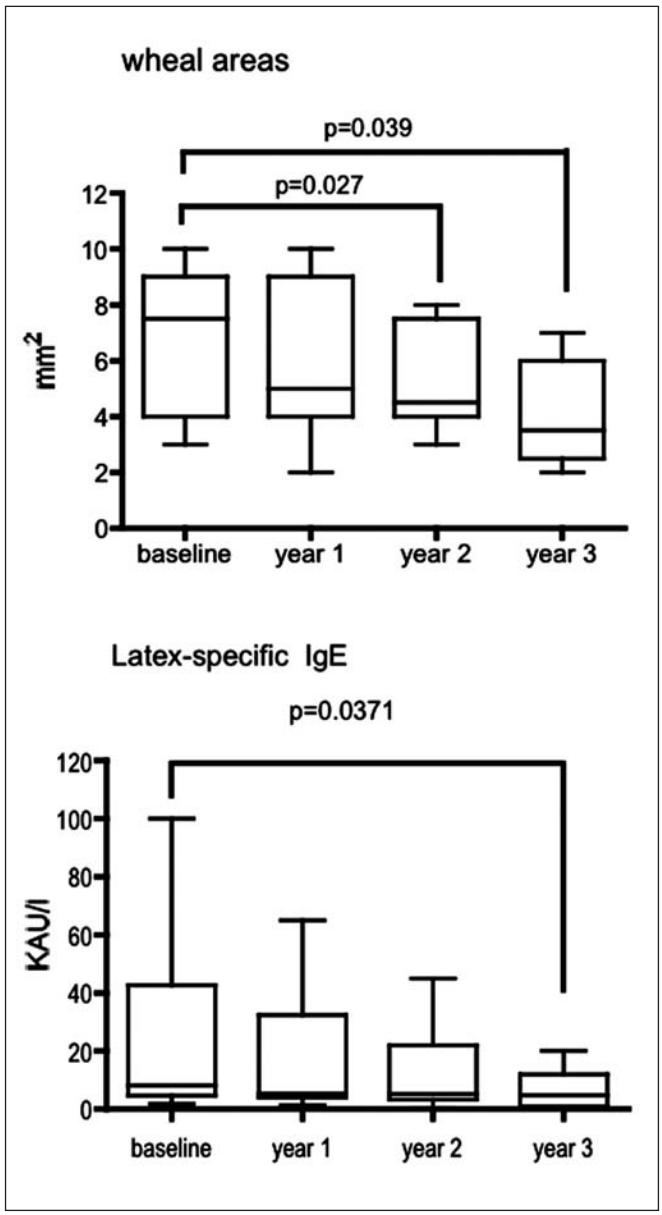
Figure 1 - Whisker-plot representation of the distribution of values of results of "Glove use test" (units on the *y*-axis) at baseline and at the indicated times of follow up (*x*-axis). Lines indicate minimum, maximum, median and interquartile ranges of the distribution of values. Results of statistical analysis for score reduction as compared to baseline are indicated



Discussion

This study is the prosecution, in the form of an open observational phase, of a previously published double-blind, placebo controlled study on specific SLIT in paediatric patients allergic to NRL (17). We found that the clinical and immunological improvement obtained after one year of NRL-specific SLIT was consolidated after three years of treatment. Moreover, no relevant adverse effects were observed. Our data confirm and strengthen the conclusions of a recent, short-term open study on paediatric patients (21).
Overall, specific immunotherapy was reported to yield successful desensitization in trials involving adult patients allergic to NRL (11, 13, 16, 22, 23). However, a relatively

Figure 2 - *Top panel.* Whisker-plot representation of the distribution of values of wheal areas (in square mm, on the y-axis) measured with NRL skin prick test at baseline and at the indicated times of follow up (x-axis). Lines indicate minimum, maximum, median and interquartile ranges of the distribution of values. P-values of comparison analysis of areas measured during follow up, as compared to baseline, are indicated.
Bottom panel. Whisker-plot representation of the levels of NRL-specific IgE (in kU/l, on the *y*-axis) at baseline at the indicated times of follow up (on the *x*-axis). Lines indicate minimum, maximum, median and interquartile ranges of the distribution of values. P-values of comparison analysis of IgE titres measured during follow up, as compared to baseline, are indicated



high frequency of systemic adverse event was observed with the subcutaneous route of administration (e.g., 46% and 8% of administered doses in ref. (11) and (24), respectively). In contrast, SLIT with NRL extracts has proven efficacious and safe in the seminal works from Patriarca's group (13, 22, 23, 25), which were recently confirmed by other investigators (16). Only one case of anaphylaxis with latex SLIT has been reported until now (26).

Our data extend these results by showing in a three-years follow-up that clinical and immunological parameters are consistently consolidated by the prosecution of NRL SLIT up to an extent of time, which is considered suitable to establish the results in SLIT protocols with other airborne allergens (27).

The rubber use test we performed mimicked real-life exposure to this allergen. Such a challenge test was necessary, since data on clinical symptoms following spontaneous NRL exposure are hardly obtained in paediatric patients, for whom allergen avoidance is more easily achieved and maintained as compared to adult individuals.

Our data clearly demonstrate that the significant reduction of symptom scores, achieved in the first year since immunotherapy start, was confirmed and extended in the following two years.

It cannot be excluded that the reduction of environmental allergen exposure could *per se* improve the reactivity to NRL in our patients. However, our data indicate that specific immunotherapy, which after one year was already capable of reducing the glove use score in treated but not in control subjects (17), was associated in the following two years to the virtual disappearance of any measurable reactivity to NRL.

Moreover, the modification of two biological parameters, which were considered in the follow up of patients included in the present study, were consistent with the clinical scores. Namely:

i) Skin reactivity to NRL was lower as compared to baseline after two (p=0.027) and three years (p=0.039) of SLIT, a result which is in agreement with observations reported in previous trials with NRL subcutaneous (24) and sublingual (16) immunotherapy;

ii) NRL specific IgE levels tended to progressively decrease in the first and second since SLIT start, and were indeed significantly reduced after 3 years of immunotherapy. Allergen specific IgE levels are not usually considered useful in the evaluation of immunotherapy in general and of SLIT in particular. Recently, *Nettis* et al. (16) reported that specific IgE did not change after SLIT with NRL (16). Similarly, NRL IgE specific levels did not change

following specific subcutaneous immunotherapy (24). However, to our knowledge this is the first time that specific IgE have been monitored in a three-year follow up of NRL SLIT.

Beneficial effects of allergen-specific immunotherapy on oral allergy symptoms have been reported (28). However, this advantage was reversible, and symptoms reappear at immunotherapy end. Also in patients included in the present study, we observed an overall trend towards improvement of food allergy to cross-reacting foods, which was already evident one year of SLIT with NRL and further increased in the second and third year (not shown). These data suggest that oral allergy can be partially improved when immunotherapy is performed with allergen components, which are immunologically cross-reactive (29).

In the NRL allergy field, it is well established that prevention from allergen exposure can induce a reduction in the incidence of sensitization. However, this environmental measure is not sufficient to warrant in single subjects re-sensitization or adverse reaction on re-exposure (30). Moreover, although the peak of the epidemic of NRL allergy was passed at least for health care workers (8), the question arises whether the history of latex allergy will repeat itself in fast developing Countries, which are increasing the use of latex products (31, 32). Thus, we believe that research on latex allergy, including accurate diagnosis (33) and specific immunotherapy should not decrease due to this partial epidemiological improvement.

Our data support the notion that NRL specific immunotherapy should enter clinical practice and no longer be utilized as an experimental therapeutic approach in paediatric patients with severe symptoms for whom allergen avoidance cannot be warranted. SLIT for NRL allergy is a safe a treatment for allergic children as it was previously reported for adults and should be extended for three years to achieve full efficacy.

Acknowledgements

ALK Abellò supported this study by providing placebo and immunotherapy extract. S.P., and M.M. are employed by ALK AbellòItaly as Medical Service Manager and Medical Director, respectively.

References

1. Charous BL. The puzzle of latex allergy: some answers, still more questions. Ann Allergy 1994; 73: 277-81.
2. Nettis E, Assennato G, Ferrannini A, Tursi A. Type I allergy to natural rubber latex and type IV allergy to rubber chemicals in health care workers with glove-related skin symptoms. Clin Exp Allergy 2002; 32: 441-7.
3. Sussman GL, Lem D, Liss G, Beezhold D. Latex allergy in housekeeping personnel. Ann Allergy Asthma Immunol 1995; 74: 415-8.
4. Tarlo SM, Wong L, Roos J, Booth N. Occupational asthma caused by latex in a surgical glove manufacturing plant. J Allergy Clin Immunol 1990; 85: 626-31.
5. Carrillo T, Blanco C, Quiralte J, Castillo R, Cuevas M, Rodriguez de Castro F. Prevalence of latex allergy among greenhouse workers. J Allergy Clin Immunol 1995; 96: 699-701.
6. Nettis E, Dambra P, Soccio AL, Ferrannini A, Tursi A. Latex hypersensitivity: relationship with positive prick test and patch test responses among hairdressers. Allergy 2003; 58: 57-61.
7. Nettis E, Dambra P, Soccio AL, Loria MP, Ferrannini A, Tursi A. Type I allergy to natural rubber latex and Type IV allergy to rubber chemicals in children with risk factors. Contact Dermatitis 2001; 44: 181-2.
8. Reunala T, Alenius H, Turjanmaa K, Palosuo T. Latex allergy and skin. Curr Opin Allergy Clin Immunol 2004; 4: 397-401.
9. Thurlow KL. Latex allergies: management and clinical responsibilities. Home Healthc Nurse 2001; 19: 369-75; quiz 76.
10. Pereira C, Rico P, Lourenco M, Lombardero M, Pinto-Mendes J, Chieira C. Specific immunotherapy for occupational latex allergy. Allergy 1999; 54: 291-3.
11. Leynadier F, Herman D, Vervloet D, Andre C. Specific immunotherapy with a standardized latex extract versus placebo in allergic healthcare workers. J Allergy Clin Immunol 2000; 106: 585-90.
12. Nucera E, Schiavino D, Pollastrini E, et al. Desensitization to latex by percutaneous route. J Investig Allergol Clin Immunol 2002; 12: 134-5.
13. Patriarca G, Nucera E, Pollastrini E, et al. Sublingual desensitization: a new approach to latex allergy problem. Anesth Analg 2002; 95: 956-60, table of contents.
14. Cistero Bahima A, Sastre J, Enrique E, et al. Tolerance and effects on skin reactivity to latex of sublingual rush immunotherapy with a latex extract. J Investig Allergol Clin Immunol 2004; 14: 17-25.
15. Savi E, Maffini I, Burastero SE. A latex-containing hepatitis-B vaccine administered in a severely latex allergic paediatric patient after specific sublingual immunotherapy: a case report. Allergy 2004; 59: 1014-5.
16. Nettis E, Colanardi MC, Soccio AL, et al. Double-blind, placebo-controlled study of sublingual immunotherapy in patients with latex-induced urticaria: a 12-month study. Br J Dermatol 2007; 156: 674-81.
17. Bernardini R, Campodonico P, Burastero S, et al. Sublingual immunotherapy with a latex extract in paediatric patients: a double-blind, placebo-controlled study. Curr Med Res Opin 2006; 22: 1515-22.
18. Turjanmaa K, Reunala T, Rasanen L. Comparison of diagnostic methods in latex surgical glove contact urticaria. Contact Dermatitis 1988; 19: 241-7.
19. Morales C, Basomba A, Carreira J, Sastre A. Anaphylaxis produced by rubber glove contact. Case reports and immunological identification of the antigens involved. Clin Exp Allergy 1989; 19: 425-30.

20. Dreborg S, Backman A, Basomba A, Bousquet J, Dieges P, Malling HI. Skin tests used in type I allergy testing. Position paper prepared by the subcommittee on Skin Tests of the EAACI. Allergy 1989; 44 (suppl 10): 1-59.
21. Nucera E, Schiavino D, Pollastrini E, et al. Sublingual desensitization in children with congenital malformations and latex allergy. Pediatr Allergy Immunol 2006; 17: 606-12.
22. Nucera E, Schiavino D, Buonomo A, et al. Latex rush desensitization. Allergy 2001; 56: 86-7.
23. Patriarca G, Nucera E, Buonomo A, et al. New insights on latex allergy diagnosis and treatment. J Investig Allergol Clin Immunol 2002; 12: 169-76.
24. Sastre J, Fernandez-Nieto M, Rico P, et al. Specific immunotherapy with a standardized latex extract in allergic workers: a double-blind, placebo-controlled study. J Allergy Clin Immunol 2003; 111: 985-94.
25. Patriarca G, Nucera E, Buonomo A, et al. Latex allergy desensitization by exposure protocol: five case reports. Anesth Analg 2002; 94: 754-8.
26. Antico A, Pagani M, Crema A. Anaphylaxis by latex sublingual immunotherapy. Allergy 2006; 61: 236-7.
27. Marogna M, Spadolini I, Massolo A, Canonica G, Passalacqua G. Long-lasting effect according to the duration of sublingual immunotherapy: A 15-year prospective study. Word Allergy Congress. Bangkok, Thailand, 2007.
28. Asero R. How long does the effect of birch pollen injection SIT on apple allergy last? Allergy 2003; 58: 435-8.
29. Lavaud F. Cross-sensitization between latex and fruits. J Allergy Clin Immunol 1996; 98: 473-4.
30. Nettis E, Colanardi MC, Ferrannini A. Type I latex allergy in health care workers with latex-induced contact urticaria syndrome: a follow-up study. Allergy 2004; 59: 718-23.
31. Chen YH, Lan JL. Latex allergy and latex-fruit syndrome among medical workers in Taiwan. J Formos Med Assoc 2002; 101: 622-6.
32. Ozkan O, Gokdogan F. The prevalence of latex allergy among health care workers in Bolu (Turkey). Dermatol Nurs 2003; 15: 543-7, 54.
33. Wagner S, Breiteneder H. Hevea brasiliensis latex allergens: current panel and clinical relevance. Int Arch Allergy Immunol 2005; 136: 90-7.