

IgE reactivity to latex allergens among sensitized healthcare workers before and after immunotherapy with latex

Background: New IgE sensitizations to proteins in allergen extracts have been shown to occur during allergen-specific immunotherapy (IT).

Methods: Twenty-four healthcare workers (HCWs) – patients included in a latex IT study – were analysed, 16 in active treatment and eight in placebo. Sera were obtained at baseline and after 6 months of IT and analysed with immunoblotting and CAP System with eight single recombinant latex allergens (rHev b 1, 3, 5, 6.01, 8, 9, 10, 11, and a mix of rHev b1, 5, 6.01 and 8).

Results: After IT with latex, three patients in the active treatment group had new IgE sensitizations, one to Hev b 5, one to Hev b 11 and another to Hev b 6.01. No other significant variation in mean of specific IgE to latex or recombinant allergens were observed in patients who received placebo or active treatment. A significant ($P = 0.012$) negative correlation (-0.72) was observed between maximal tolerated dose and specific IgE to Hev b 6.01 at baseline. After IT, immunoblot analysis demonstrated a significant increase in IgE binding in a band of approximately 22 kDa ($P = 0.032$) that may correspond to Hev b 6.01. New or more intense bands appeared in seven patients of the active group, while in three subjects a reduction was observed.

Conclusions: Hev b 6.01 seems to be the most relevant latex allergen in HCWs. New or more intense IgE binding to latex allergenic components occurs during latex immunotherapy. However, the levels of specific IgE against these new components are low and do not seem to have clinical relevance.

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An issue that has recently gained attention is whether the administration of allergen extracts during immunotherapy (IT) can induce new IgE sensitizations to the allergens contained in the extract, as well as the potential clinical significance of this phenomenon. Although previous studies have shown that *de novo* induction of specific IgE sensitizations to the allergenic components of the extracts used for IT can occur (1–6), the kinetics of these changes and their clinical relevance are not well known. We studied the IgE-reactivity profile to individual allergenic components during a latex IT study (7) using both immunoblotting and specific IgE determinations (CAP System) to several recombinant latex allergens to explore the possible induction of new IgE reactivities.

Materials and methods

Twenty-four patients included in a double-blind, placebo-controlled, natural rubber latex IT study were analysed (7). Of these, 16 were in active treatment and eight in the placebo group. The extract was administered for 6 months. All patients had a positive skin-prick test (SPT) to latex extract and positive cutaneous or respiratory provocation tests to latex gloves as previously described (7).

The latex extract used for IT and variables measured during the study are detailed in another study (7). Patients did not modify their exposure to latex during the study.

IgE-immunoblots were performed as previously described (7) using nonammoniated latex extract. Serum samples from each patient were drawn both at baseline (T0) and 6 months after the onset of IT (T6) and were assayed simultaneously with the same serum dilution. As controls, immunoblotted latex proteins were incubated with dilution buffer (negative) instead of patient serum or pooled sera from patients with a high titre of latex-specific IgE (positive). The intensity of IgE-binding bands in immunoblots before and after IT as well as their variation was evaluated by an investigator in a 'blinded' fashion using an ordinal scale with three levels.

Specific IgE and IgG4 to whole-latex extract (CAP k82, spiked with rHev b 5; Pharmacia Diagnostics, Uppsala, Sweden) was prepared following manufacturer's instructions before and after IT. A panel of single recombinant latex allergens was coupled to ImmunoCAPs. This panel comprised rHev b 1, rHev b 3, rHev b 5, rHev b 6.01, rHev b 8, rHev b 9, rHev b 10, rHev b 11 and a mix ImmunoCAP containing rHev b 1, 5, 6.01 and 8. All of them were produced in *Escherichia coli* in fusion with a maltose-binding protein (MBP as a fusion component) as previously described (8). The mix ImmunoCAP and rHev b 10 were produced as experimental research products. The MBP coupled on ImmunoCAPs served as a negative control. Results over 0.35 kU/l were considered positive as recommended by the manufacturer.

Statistical analysis was performed using SPSS (SPSS Inc., Chicago, IL, USA). Absolute and relative frequencies were calculated for each allergen and immunoblot band. The change in reactivity to the different allergens after IT was assessed by the chi-square test and the correlation between IT outcomes and allergen reactivity was evaluated by the Spearman's rank test.

Results

CAP results at baseline are described in Table 1. In one patient from the active group, specific IgE to k82 and to Hev b 5 varied from negative to positive after IT (patient 9; k82 = 12.9 and Hev b 5 = 6.42 kU/l), and more intense IgE binding was evident in the immunoblot. In two patients from the active group CAP results varied from negative to slightly positive (patient 2, Hev b 6.01 from <0.35 to 0.37 kU/l, and patient 23 rHev b 11 from <0.35 to 0.38 kU/l). No other significant variation in specific IgE to k82 or latex recombinants allergens was observed in patients who received placebo or active treatment. Overall, the levels of CAP-specific IgE did not show significant changes during IT either in the active or in the placebo group. A significant ($P = 0.012$) negative correlation ($r = -0.72$) was observed between the maximal tolerated dose of IT and specific IgE to Hev b 6.01 at baseline. Mean IgG4 to latex in active group at T0 ($n = 11$) was 0.59 ± 0.54 mgA/l and at TF 1.41 mgA/l ($P = 0.033$). In the placebo group ($n = 3$) IgG4 did not change significantly (Fig 1 and Table 1).

Immunoblot analysis (Fig. 2 and Table 2) showed bands at 15 kDa in eight of 24 (33%) patients, at 22 and 35 kDa in 18 of 24 (75%), and high-molecular-weight bands (> 35 kDa) were observed in 21 of 24 patients (88%). New IgE-binding bands were detected in two patients (patients 2 and 36) of the active treatment group (patient 2 with CAP values to Hev b 6.01 from <0.35 to 0.37 kU/l, and patient 36 CAP k82 from 0.51 to 0.47; no serum available for other determinations). These bands could correspond to the allergens Hev b 6.01 (approximately

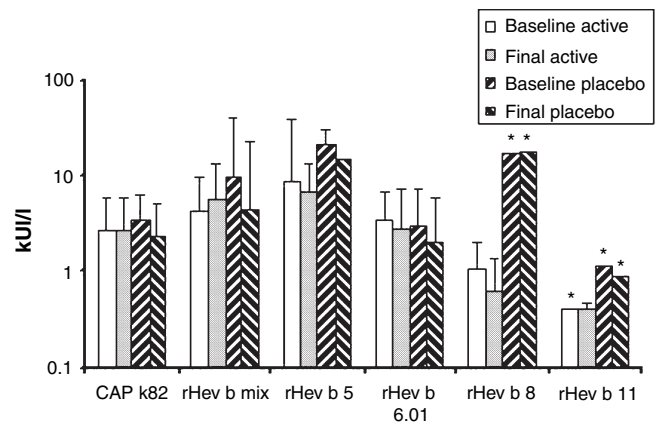


Figure 1. Results of specific IgE to latex and latex recombinant allergens at baseline and after immunotherapy (geometric means and upper 95% confidence intervals). Bars with "*" correspond to only one serum.

22 kDa) and Hev b 2 (approximately 35 kDa). IgE-binding bands disappeared in two patients of the active group (patients 26 and 27) (for patient 26 CAP k82 from 1.75 to 0.36, and for patient 27 from 1.77 to 0.61), and in one patient of the placebo group (for patient 24 CAP k82 from 1.60 to 0.80). The bands involved may also correspond to Hev b 6.01 and Hev b 2. More intense IgE-binding bands appeared in patients 5, 6, 9, 23 and 28 of the active treatment group. Interestingly, patient 9 had detectable IgE levels at T0 in contrast to the CAP results. A decrease in band intensity was present in patients 12, 26 and 27 of the active treatment group and patients 3, 8, 18 and 24 of placebo group. As a whole, immunoblot analysis demonstrated a significant increase in IgE-binding after IT to the 22 kDa band ($P = 0.032$, chi-square test), that very likely corresponds to Hev b 6.01, in patients of the active treatment group vs placebo group.

Patients in the active group had a statistically significant reduction in skin reactivity to latex extract by SPT, a significant improvement in rubbing and glove use test and a significant reduction in nasal and bronchial symptoms during the controlled inhalation challenge (7). Nevertheless, none of the immunological changes were associated with these clinical outcomes of efficacy.

Discussion

This study shows that Hev b 6.01 and Hev b 5 are important allergens in this population of healthcare workers (HCWs) allergic to latex from Spain. CAP System with the latex recombinant allergens tested identified newly developed IgE sensitizations after latex IT in one patient in the active group (k82 and Hev b 5 varied from negative to positive), who also showed more intense IgE-binding bands in the immunoblot analysis. In other two patients of the active treatment group, Hev b 6.01 and Hev b 11 varied to

Table 1. Specific IgE results at baseline

Allergen	Positive CAP results/tested, median and range of the positive responders
k82 (latex extract)	18/24 (75%), 2.5 (0.4–29.5)
mix rHev b 1, 5, 6.01, 8	18/21 (86%), 4.4 (0.5–49.0)
rHev b 1 (rubber elongation factor)	1/17 (6%), 0.5 (0.5)
rHev b 3 (small rubber particle protein)	0/9
rHev b 5 (acidic structural protein)	6/17 (35%) 19.5 (2.0–41.3)
rHev b 6.01 (prohevein)	14/17 (82%), 2.3 (0.8–36.3)
rHev b 8 (profilin)	3/17 (18%), 1.5 (0.8–17.6)
rHev b 9 (enolase)	0/9
rHev b 10 (superoxide dismutase)	0/9
rHev b 11 (class I chitinase)	2/9 (22%) 0.8 (0.4–1.0)
MBP (fusion protein)	0/24

Only one serum was negative to all determinations although skin-prick test and challenge tests with latex was positive in this patient.

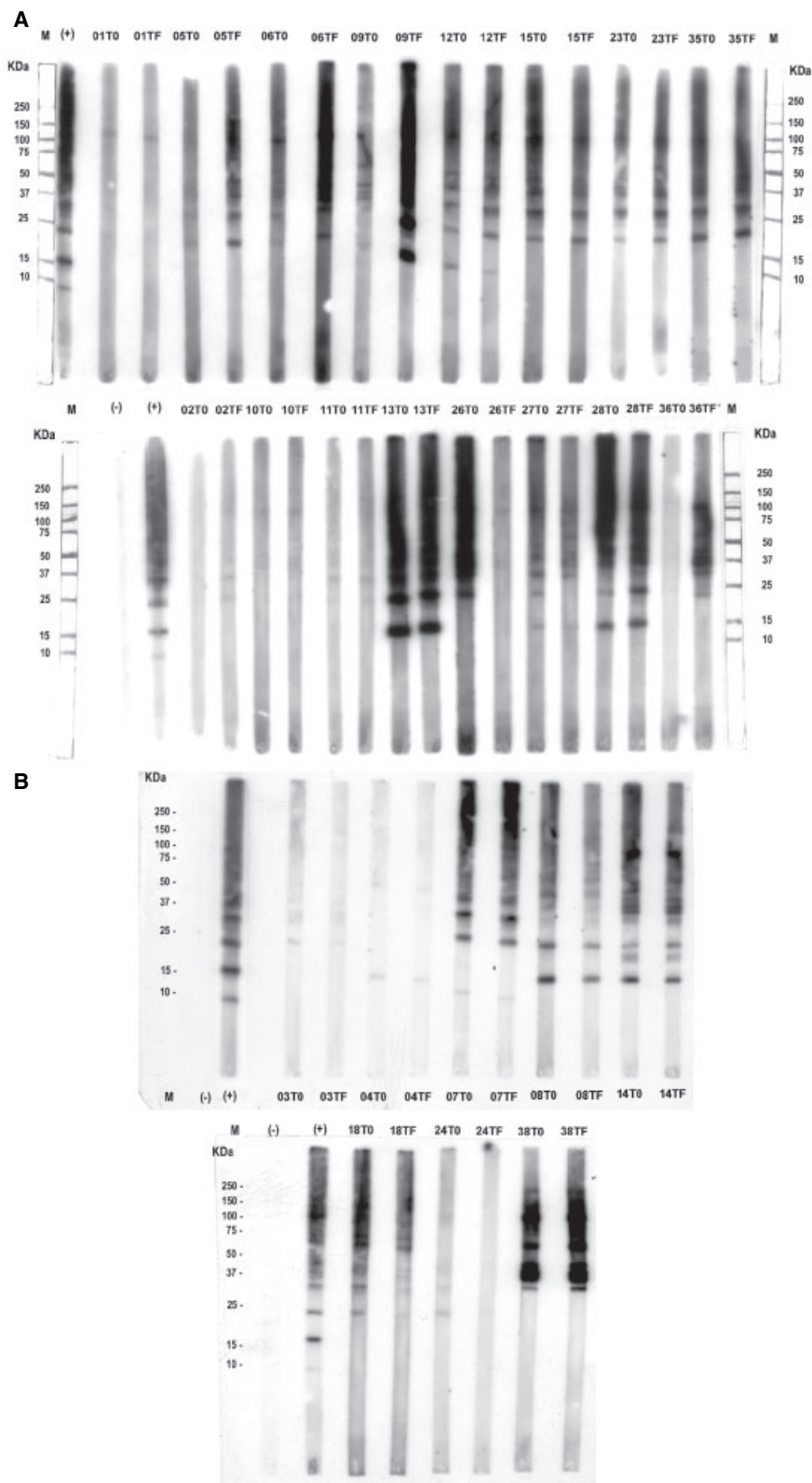


Figure 2. (A) Immunoblot analysis of patients of active group at baseline (T0) and after 6 months of immunotherapy (TF). Lane 2: latex extract; M: molecular weight markers; (–) and (+): negative and positive controls respectively. (B) Immunoblot analysis of patients of placebo group at baseline (T0) and after 6 months of treatment (TF). Lane 2: latex extract; M: molecular weight markers; (–) and (+): negative and positive controls respectively.

Table 2. Immunoblotting band intensity as assessed by a subjective ordinal scale

Cod	Treatment	15 kDa			22 kDa			>35 kDa		
		T0	TF	Change	T0	TF	Change	T0	TF	Change
01	Active	0	0	=	0	0	=	0	0	=
02	Active	0	0	=	0	1	+	0	1	+
03	Placebo	0	0	=	1	1	-	1	1	=
04	Placebo	1	1	=	0	0	=	0	0	=
05	Active	0	0	=	1	2	+	1	2	+
06	Active	0	0	=	1	2	+	1	1	+
07	Placebo	0	0	=	2	2	=	2	2	=
08	Placebo	2	2	-	2	2	-	0	0	=
09	Active	1	2	+	1	2	+	1	2	+
10	Active	0	0	=	0	0	=	0	0	=
11	Active	0	0	=	1	1	=	1	1	=
12	Active	1	1	-	1	1	=	1	1	=
13	Active	2	2	=	2	2	=	2	2	=
14	Placebo	2	2	=	1	1	=	1	1	=
15	Active	0	0	=	1	1	=	2	2	=
18	Placebo	0	0	=	2	1	-	2	1	-
23	Active	0	0	=	1	1	+	2	2	=
24	Placebo	0	0	=	1	0	-	1	0	-
26	Active	0	0	=	2	0	-	2	0	-
27	Active	1	1	-	1	0	-	1	1	-
28	Active	2	2	=	1	2	+	2	2	=
35	Active	0	0	=	2	2	=	2	2	=
36	Active	0	0	=	0	1	+	0	2	+
38	Placebo	0	0	=	0	0	=	1	1	=

Subjective ordinal scale (0, absent; 1, mild; 2, clear band) and change from T0 to TF (=, same; +, increase; -, decrease).

positive, although specific IgE levels were close to the cut-off value of 0.35 kU/l.

Immunoblot analysis demonstrated a significant ($P = 0.032$) increase in IgE-binding to a 22-kDa band, probably corresponding to Hev b 6.01, among patients in the active IT group. Immunoblotting also revealed a new or an increase in IgE-binding in seven of the 16 (44%) patients and a disappearance or decrease in three patients (19%) on active treatment. In patients who received placebo, there was a disappearance or decrease in IgE-binding in four of eight (50%) patients. The discrepancy between the immunoblot and CAP assays could be explained by the fact that immunoblotting included most allergenic components in the latex extract, while the CAP System included only eight recombinant latex allergens

(although CAP k82 theoretically contains all allergens). Moreover, immunoblotting is a semiquantitative technique, and the development of some new allergenic bands or the increase in IgE-binding intensity to others was not very pronounced. The cut-off value of the CAP System is 0.35 kU/l, while the sensitivity of the immunoblot method for IgE against individual allergens is <0.1 kU/l (8). This could also explain why the development of IgE antibodies against Hev b 6.01 was more frequent when analysed by immunoblotting than with CAP System. In this sense, patient 9 was negative at T0 to latex extract and the recombinant allergens by CAP but not by immunoblotting. These changes in IgE levels to different allergens as determined by CAP or immunoblotting were not associated with any outcomes of efficacy or safety described in a previous trial (7). However, a significant ($P = 0.012$) negative correlation ($r = -0.72$) was observed between the maximal tolerated dose and the serum concentration of specific IgE to Hev b 6.01 at baseline. This finding may have safety implications, as patients with lower levels of IgE Hev b 6.01 are able to tolerate higher doses of the extract during IT, and this specific IgE level might predict the allergen dose tolerated by the patient. To the best of our knowledge, no previous immunotherapy studies have addressed this issue.

In summary, Hev b 6.01 and Hev b 5 are important allergens recognized by our population of HCWs. CAP k82 shows a good diagnostic sensitivity similar to the mix of four recombinant latex allergens, but the use of a panel of allergens seems to be necessary for better performance of *in vitro* diagnosis in some HCWs allergic to latex. Levels of specific IgE to Hev b 6.01 may predict the maximal tolerated dose of allergen during IT. After IT, new IgE-binding and/or more intense bands appeared on immunoblotting in some patients and, in a few cases, new specific IgE to some allergens appeared, although at very low levels. Nevertheless, these findings do not seem to be associated with clinical outcomes of IT in terms of efficacy or safety.

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