

Patterns of latex allergen recognition in children sensitized to natural rubber latex

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Single recombinant latex allergens permit the study of the pattern of sensitization to individual allergens. We aimed to quantify the IgE-response to individual latex allergens in children sensitized to latex. The study group included 31 latex-sensitized children: 26 operated at least twice, 20 of them with spina bifida; two children with one operation and three atopic non-operated children. IgE antibodies to rHev b 1, rHev b 3, rHev b 5, rHev b 6.01, rHev b 7.02 and rHev b 8, coupled to ImmunoCAPs, were measured in each serum. IgE responses to rHev b 1, rHev b 5 and rHev b 6.01 were found in 17 children each, and their mean $\pm$ s.d. levels were 5 ± 7.4 , 16.8 ± 14 and 10 ± 18 kU/l, respectively. IgE responses to rHev b 3 (4 ± 5.4 kU/l) were found in eight children. Two children had IgE to rHev b 7 (1.7 and 3.2 kU/l), and none to rHev b 8. Four sera were negative to all tested recombinant allergens. We divided the patients in three groups: sensitized only to rHev b 1, sensitized only to rHev b 5 and/or rHev b 6.01, and sensitized to both rHev b 1 and to rHev b 5 and/or rHev b 6.01. The three groups had the same profile of clinical features. Hev b 5 induces the quantitatively higher IgE responses in children with multiple surgeries sensitized to latex. Responses to Hev b 6.01 equal those of Hev b 1.

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Children with multiple surgeries, especially with spina bifida (SB), are at the highest risk for developing sensitization to natural rubber latex (NRL). The number of operations and atopy are the main factors implicated in this process (1, 2). Avoiding contact with NRL devices since birth has proved to be efficacious as primary prophylaxis, yet some children still become sensitized (3, 4).

Thirteen NRL proteins with allergenic properties have been registered by the International Union of Immunologic Societies (IUIS) so far (5). Most have been cloned and synthesized, and are available for *in vivo* and *in vitro* tests (6–8), allowing studies of IgE-recognition with isolated single allergens.

Distinct allergen recognition patterns have been found for children with multiple surgeries and for adults (9–14), but there has been no simultaneous study of a large panel of latex allergens in children, to compare responses to

different recombinant allergens. This study aimed to quantify the IgE response to certain recombinant latex allergens (rHev b 1/3/5/6.01/7.02/8) in children sensitized to NRL, to determine and measure specific patterns of sensitization. Through the study of these, important conclusions can be drawn concerning the therapeutic approach to high risk and sensitized patients.

Patients and methods

Patients

Sera of 31 children in whom sensitization to latex was confirmed by latex specific IgE CAP RAST (Pharmacia CAP® > 0.35 kU/l) were studied. Twenty children suffered from SB, they had been operated between two and nine times, and 16 of them had symptoms related to latex; the remaining 11 children, without SB, had been studied regarding NRL sensitization due to symptoms

related to latex: three of them had never been operated, two had been operated once, while the other six had between two and eight operations. All the operations had been performed using NRL devices, before we could implement NRL avoidance measures.

Methods

Six recombinant NRL allergens were coupled to individual ImmunoCAPs: rHev b 1, rHev b 3, rHev b 5, rHev b 6.01, rHev b 7.02 and rHev b 8. All of them were produced in *E. coli* in fusion with a maltose binding protein (MBP), as described elsewhere (6, 7). The MBP was also coupled on ImmunoCAPs and served as a negative control. The individual NRL allergen profile was determined with sera and the ImmunoCAPs using the Pharmacia UniCAP-System. Results > 0.35 kU/l were considered as positive.

Statistical analysis

The analysis of results was performed with the SPSS 10.0 software program. As the values of specific IgE had a non-normal distribution, the non-parametric Kruskal–Wallis test was used for comparisons.

Results

The group comprised 31 children (17 boys) with a mean age of 7.1 ± 4.3 yr (range 11 months to 15.1 yr). None was sensitized to fruits. Twenty-seven of the children had one or more latex related symptoms: asthma ($n = 2$), rhinoconjunctivitis ($n = 11$) and urticaria-angioedema ($n = 23$). The twenty SB patients had been operated 5.2 ± 2 times (range 2–9); four of them had a history of allergy other than to latex. Among the 11 children without SB, six had been operated 4.7 ± 2.3 times (range 2–8), two had

one operation each, and three had never been operated; six had also a personal history of allergy other than to latex. There were no significant differences between multiooperated children with and without SB, regarding age, number of operations, personal and family history of allergy, sensitization to the different allergens and serum total IgE, so the rest of the results are shown together for both groups.

The results of specific IgE to each allergen are shown in Table 1. Four sera were negative to all tested recombinant NRL allergens. The sera belonged to younger patients and their latex-specific IgE levels (1.4 ± 1.1 kU/l) were lower than in children with any positive result (22.4 ± 28 kU/l). Responses to rHev b 1 were only found in children with two or more operations: they were 15 of 20 SB children, and two of six non-SB patients ($p = 0.06$, SB vs. non-SB). None of the five children with one or no operation showed a specific IgE-response to rHev b 1 or rHev b 3 ($p < 0.01$, compared to those with > 1 operation), even though one of them had over 50 kU/l of both rHev b 5 and rHev b 6 IgE.

The eight children, all of them SB patients, with rHev b 3-specific IgE antibodies had also positive results for rHev b 1, and in seven of them responses were equal or higher for rHev b 1. Responses to rHev b 5 were significantly higher than for the rest of allergens, while levels of rHev b 6.01 IgE were not different from those of rHev b 1. Only two sera had rHev b 7.02-specific IgE and were from SB patients also sensitized to other recombinant NRL allergens. There was no detectable rHev b 8 IgE in any of the sera.

Four patterns of sensitization to the tested allergens were defined (Table 2): a group (G0) with negative responses to all tested allergens, another (G1) with sensitization to rHev b 1/rHev b 3, a third group (G56) sensitized to rHev b 5 and/or rHev b 6.01, and a fourth group (G156)

Table 1. Number of patients with IgE response and levels of specific IgE (kU/l) with each recombinant latex allergen

	Whole group ($n = 31$)		Patients with > 1 operation ($n = 26$)	
	Patients with specific IgE > 0.35 n (%)	Mean $\pm$ s.d. specific IgE (median; range)	Patients with specific IgE > 0.35 n (%)	Mean $\pm$ s.d. specific IgE (median; range)
RHev b 1	17 (55%)	5 ± 7.4 (1.57; 0.48–25.8)	17 (65%)	5 ± 7.4 (1.57; 0.48–25.8)
RHev b 3	8 (26%)	4 ± 5.4 (1.7; 0.9–16.9)	8 (31%)	4 ± 5.4 (1.7; 0.9–16.9)
RHev b 5	17 (55%)	$16.8 \pm 14^*$ (15.7; 0.45–52.7)	15 (58%)	$14.9 \pm 11.2^*$ (15.7; 0.45–33.3)
rHev b 6.01	17 (55%)	10 ± 18 (1.5; 0.58–73.4)	13 (50%)	6.6 ± 8.9 (1.2; 0.58–24.6)
rHev b 7	2 (6.5%)	1.7 and 3.2 (absolute values)	2 (7.7%)	1.7 and 3.2 (absolute values)
rHev b 8	0	–	0	–

No statistically significant differences among rHev b 1, rHev b 3 and rHev b 6.01.

* $p < 0.05$ for comparisons of rHev b 5 with any of rHev b 1, rHev b 3 or rHev b 6.01.

Table 2. Patterns of sensitization to latex allergens

Group (no. of patients)	G0 (4)	G1 (4)	G56 (10)	G156 (13)	G56* (5)
No. of patients with spina bifida	3	4	2	11	2
Age (yr)	2 ± 1	7.7 ± 3.9	8 ± 4.3	7.7 ± 4.2	7.5 ± 6.1
Latex IgE CAP® kU/l (k82)	1.4 ± 1.1	7.9 ± 2.9	15 ± 23	32 ± 33	11 ± 19
Surgeries with NRL devices (n)	4.2 ± 2.5	5 ± 2.2	2.9 ± 3.3	5.3 ± 1.7	5.4 ± 2.9
Personal history of allergy (n)	0	0	7	3	2
Family history of allergy (n)	2	0	5	6	3

*Same children as in group G56 excluding the five patients with one or no operation.

NRL, natural rubber latex; G0, non-sensitized to tested allergens; G1, sensitized to rHev b 1; G56, sensitized to rHev b 5 and/or rHev b 6.01; G156, sensitized to rHev b 1 and to rHev b 5 and/or rHev b 6.01. (Sensitization to rHev b 3 is grouped with rHev b 1.)

sensitized to rHev b 1/rHev b 3 and also to rHev b 5 and/or rHev b 6.01. The number of operations was lower for children sensitized to rHev b 5/6.01. That third group included three children with no surgery, and two with just one operation. Excluding these five children, the remaining five children with multiple surgeries (G56* in Table 2) had an age and number of operations similar to the two other groups. A personal or family history of allergy was less often found in children sensitized only to rHev b 1/rHev b 3.

There were ten children, four of them with SB, who had symptoms of atopic disease not related to latex. They had one of more of the following: asthma (n = 5), rhinoconjunctivitis (n = 7), urticaria (n = 1) and atopic dermatitis (n = 1), and were sensitized to house dust mites (n = 6), pollens (n = 4), cow's milk (n = 2), *Alternaria* (n = 2) and animal dander (n = 3). Seven of these children (one with SB) belonged to group G56, and the other three (all with SB) to group G156. Just like sensitization to rHev b 1/3 was associated with operations, atopy was associated with sensitization to rHev b 5/6.01 (p = 0.03).

Only four children (all with SB) had not had symptoms related to latex. Two of them belonged to the G0 group, the third had only a weak response (0.51 kU/l) to rHev b 1, and the fourth was sensitized to rHev b 1/3/5 and 6.01, with responses between 0.95 and 3.1 kU/l. No comparison was made due to the small number of patients in this group.

Discussion

Sensitization to NRL is a health hazard for some groups such as multioperated children and health care workers, with varying prevalence depending on the geographical location, type of latex exposure and prevalence of atopy in the community. Thus, the identification and isolation of NRL proteins has become a task in order to develop strategies for prevention and treatment of NRL allergy. So far 13 allergens have been

recognized and registered by the IUIS (5). There seems to be two main patterns of allergen sensitization: that of children with multiple surgeries, and that of adults sensitized through occupational exposure, mostly health-care workers. In the latter group, Hev b 5 and Hev b 6.01 are the main allergens, while Hev b 1 acts as a minor allergen (9–11). In contrast, Hev b 1 is reputedly the most important allergen in SB patients (10, 12–14).

We found IgE responses to rHev b 1 in 17 patients, all with two or more surgeries. There was a strong association of responses to rHev b 3 with rHev b 1. All children with response to rHev b 3 had IgE antibodies to rHev b 1. This association can be only partly explained by cross-reactivity, as IgE binding to Hev b 1 is largely but not completely inhibited by Hev b 3 (15). A further explanation is sensitization to the same allergenic complex source, as the two allergens are found in the particle fraction of NRL, especially in latex sap, are rather insoluble, and the route of sensitization (mucosal) is the same for both.

None of the five children with one or no operation developed IgE antibodies to rHev b 1 or rHev b 3. Instead, they were sensitized to rHev b 5 and/or rHev b 6.01. This could be explained by the physical properties of the allergens: Hev b 6.01 attaches more easily to powder to become aerosolized (16), and is able to sensitize through the inhalatory route; in contrast, Hev b 1 takes much harder to aerosolize (16), and requires direct contact of latex with the blood or mucosas, in operations, to sensitize. IgE to rHev b 3 appeared only in children with SB, and IgE to rHev b 1 in 15 of 20 of these children, while it was found in only two of six non-SB multioperated children. There is a trend for a specific disease association of these two allergens with SB, but the small number of patients in the non-SB group precludes reliable conclusions. As shown in Table 2, five SB children (three in Group G0 and two in group G56) showed no IgE response

to Hev b 1 or Hev b 3. The use of just these two allergens has been suggested to be sufficient to screen patients with SB for latex sensitization (15), but this would fail to identify 25% of latex sensitized patients in our group of SB children. The selection of key or major recombinants would therefore not be sensitive enough for detecting all sensitized individuals, and a whole, or at least more ample, extract would be needed instead.

There were rHev b 5- and rHev b 6.01-specific IgE responses in 17 children. If we regard only the patients with two or more operations, the number of positive sera is 15 and 13 for these allergens respectively, which confirms that they are still major allergens in patients with multiple surgeries (12). Most of the studies on the allergenic pattern of latex conducted before have been based on qualitative techniques such as immunoblotting. Our study offers quantitative results for several allergens, and shows that specific IgE is slightly higher for rHev b 6.01, and significantly for rHev b 5, than for rHev b 1. It could be presumed, therefore, that the latter might have an intrinsically lower allergenic potential. But this difference might be due to different degree of exposure: Hev b 5 and 6.01 enter the organism both through direct contact, like Hev b 1, and also through the inhalatory route. Likewise, if Hev b 5 and 6.01 are more readily dispersible in air than Hev b 1, they would probably disperse better in blood during operations. Thus, the IgE response would be proportional to the load of allergen, and most likely not dependent on any intrinsic allergenic potential. Whatever the reason, our findings challenge the concept of Hev b 1 as the most important allergen in children with multiple surgeries.

Only two sera, of children with SB, reacted with rHev b 7.02. In contrast to the findings of other authors (14), the importance of this allergen seems to be limited among our patients. No sera showed positive results with rHev b 8, a profilin isoform. IgE antibodies to this allergen have been found in 6–12% of SB latex sensitized children in other studies (7, 17). Sensitization to this allergen is usually not clinically significant, and has been associated to sensitization to fruits (17), which was the case in none of our patients. Additionally, there were four children's sera with no response to any of the recombinant NRL allergens tested. All the children were sensitized to NRL, so they can be sensitized to other allergens present in the commercial ImmunoCAP® (k82) test, but different from the six recombinant tested ones. They might also have low IgE responses, under the threshold value of

0.35 kU/l, to several isolated recombinant allergens.

The pattern of sensitization has implications for prevention and immunotherapy treatment. Primary prophylaxis of NRL sensitization in SB is being successful, but a small proportion of patients still become sensitized (3, 4). If they become sensitized to Hev b 1, through direct contact with latex, essentially in operations, it would mean a failure in avoidance measures. This problem should be solved by improved education of the involved health care workers. In the case where they become sensitized to mainly airborne latex allergens, like Hev b 5 or Hev b 6.01, global strategies to get a hospital free from NRL contamination would be necessary.

As for immunotherapy, treatment extracts should contain the allergens to which the patient is sensitized to reach maximal efficacy and to avoid further sensitization. Therapeutic extracts with an individualized qualitative and quantitative allergenic content are not available at the moment. The current approach to estimate the pattern of sensitization is by the risk group to which the patient belongs but, as seen in Table 2, a proportion of patients would not fit in the usual pattern, and they are not identified on clinical features. Immunotherapy would probably fail in these if the therapeutic extract does not contain all allergens to which the patient is sensitized. Thus, in candidates for current or future treatment with immunotherapy, a meticulous study of the allergenic pattern might be needed, and manufacturers should provide information on the contents of their commercial extracts, to estimate the chances of success.

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