

Hev b 7 is a *Hevea brasiliensis* protein associated with latex allergy in children with spina bifida

Birgit Wagner, BSc,^a Dietke Buck, MD,^b Christine Hafner, MD,^a Slawomir Sowka, PhD,^a Bodo Niggemann, MD,^b Otto Scheiner, PhD,^a and Heimo Breiteneder, PhD^a

Vienna, Austria, and Berlin, Germany

Background: In addition to their disease-associated handicaps, patients with spina bifida (SB) are at high risk of developing latex allergy. Individuals with SB represent a special group of latex-allergic patients, inasmuch as their IgE-binding patterns differ from those of other populations of latex-allergic individuals. Two allergens strongly associated with latex allergy in patients with SB—Hev b 1 and Hev b 3—have already been identified.

Objective: We intended to identify a predominant IgE-binding band—in addition to Hev b 1 and 3—at 43 kDa in a study population of 38 latex-allergic (IgE antibodies to latex and symptoms on provocation with latex gloves) and 15 latex-sensitized (IgE antibodies to latex but no symptoms on provocation) children with SB (mean age, 12.3 years) and to determine its frequency of recognition.

Methods: Sera of latex-sensitized or latex-allergic patients with SB were tested on latex C extract containing natural Hev b 1, Hev b 3, and Hev b 7 and with the recombinant 43-kDa Hev b 7 in immunoblot and inhibition studies.

Results: Natural Hev b 1 was recognized by 82% and natural Hev b 3 by 79% of the latex-allergic children with SB. In addition to some other proteins, 15 (39.5%) of 38 latex-allergic and 2 (13%) of 5 latex-sensitized children with SB revealed IgE binding to a 43-kDa band in the latex protein extract. We identified this 43-kDa IgE-binding band as natural Hev b 7 by immunoblotting and inhibition experiments using recombinant Hev b 7.

Conclusion: From these data, we conclude that Hev b 7, the patatinlike *Hevea* latex protein, is the third SB-associated latex allergen. Future immunotherapy for latex-allergic individuals with SB will have to include Hev b 7 in addition to Hev b 1 and Hev b 3. (*J Allergy Clin Immunol* 2001;108:621-7.)

Key words: Hev b 7, latex allergy, spina bifida, children, immunoblots

Spina bifida (SB) is a congenital disease that affects 0.4 to 1.0 of every 1000 newborns in the United States.^{1,2} Research studies have shown that 29% to 72% of children and adolescents with SB are sensitized to latex, as deter-

Abbreviations used

SB: Spina bifida

HCW: Health care worker

n: Natural

NRL: Natural rubber latex

r: Recombinant

mined by case history, skin prick testing, or blood tests.³⁻⁶

Latex is the milky sap produced by the laticiferous cells of the tropical *Hevea brasiliensis* tree. It is widely used in the manufacture of medical devices used for examination and surgery.

Sensitization to latex proteins in children with SB has been suggested to result from early, intense, and frequent exposure to natural rubber latex (NRL) products in the course of multiple surgeries, examinations, and diagnostic tests beginning in the first days of life.^{5,7,8}

Atopic disposition and the number of surgical interventions are the most important risk factors for developing latex allergy.^{5,7,8} Although health care workers (HCWs) and individuals without SB who have had multiple surgical interventions show a high incidence of latex allergy, their risk is much lower than the risk for children with SB (up to 17% vs up to 72%).^{6,9}

Although the incidence of SB has been reduced^{10,11} and might decline further in the future, individuals with SB who were sensitized to latex years ago are required to handle their allergy as part of their SB-associated handicaps. Therefore, research must focus on identifying all of the proteins causing latex allergy in patients with SB, standardizing reagents for improved testing, and perhaps finding ways to desensitize people with severe latex allergy.

Of the 11 officially designated *H brasiliensis* latex allergens, two—the 14-kDa Hev b 1 and the 22-kDa Hev b 3—have been found to be associated with latex allergy in patients with SB.¹²⁻¹⁴ Alenius et al¹³ found that 73% of their population of latex-allergic children with SB showed specific IgE against both Hev b 1 and Hev b 3, whereas only 1 (7%) of these 15 patients with SB showed IgE against neither Hev b 1 nor Hev b 3. Of latex-allergic patients with SB, 81% have been reported to show IgE binding against Hev b 1¹⁵ and 83% have been reported to show IgE binding against Hev b 3.¹⁶

From ^athe Department of Pathophysiology, University of Vienna, and ^bChildren's Hospital, Virchow Clinic, Berlin.

Supported by Austrian Science Fund grant P12838-GEN.

Received for publication March 8, 2001; revised May 21, 2001; accepted for publication June 27, 2001.

Reprint requests: Heimo Breiteneder, PhD, Department of Pathophysiology, AKH-EBO-3Q, Währinger Gürtel 18-20, A-1090 Vienna, Austria.

Copyright © 2001 by Mosby, Inc.

0091-6749/2001 \$35.00 + 0 1/86/118289

doi:10.1067/mai.2001.118289

In a previous study that evaluated the percentage of latex-allergic patients with SB showing IgE against Hev b 3, we observed a 43-kDa band present in immunoblots of a latex protein extract that was recognized by approximately half of the latex-allergic patients with SB within that study population.¹⁶ The only 43-kDa latex allergen that is known from the literature is Hev b 7, an NRL protein with sequence similarity to patatins, the potato tuber storage proteins.¹⁷ Hev b 7 was described as an important allergen for adults (49%) but not for children without SB.¹⁸ Thus far, IgE binding against Hev b 7 has not been evaluated among latex-allergic children with SB.

In this study, we examined the IgE reactivity against the 43-kDa natural (n) Hev b 7 present in a latex protein extract and recombinant (r) Hev b 7 in a population of 53 latex-sensitized children with SB. Hev b 7-specific IgE was found more frequently in latex-allergic children with SB than in any latex-allergic individuals without SB, illustrating that the patatinlike latex protein Hev b 7 is the third SB-associated *Hevea* allergen.

METHODS

Study population

Fifty-three latex-sensitized patients with SB, aged 4 to 23 years (median, 12.3 years), were included in this study. These patients were recruited from Charité, a children's hospital in Berlin, Germany. Informed consent was obtained either from patients or from parents of patients aged younger than 18 years of age. A questionnaire that was completed by patients or their parents inquired about clinical symptoms on contact with latex products in the daily environment and with medical equipment. Clinical symptoms after consumption of avocado, banana, and kiwi were also inquired about in the questionnaire. In addition, blood samples were taken and skin prick tests and provocation tests were performed.

Total IgE and latex-specific IgE were determined from patients' sera through use of the Pharmacia CAP system (Pharmacia Upjohn, Uppsala, Sweden).¹⁹ Skin prick tests were performed with low ammoniated raw and unfinished NRL milk (Ansell, Munich, Germany). NaCl served as a negative control and histamine (10 mg/mL) as a positive control. Skin test responses were assessed as positive if the maximum wheal diameter was 3 mm or greater without reaction of negative control and the skin index was greater than 0.6. The skin index was calculated as the ratio of the diameter of the allergen wheal to the histamine wheal.

Provocation tests with latex gloves were performed as previously described.^{7,20} Briefly, after handwashing, the patient put one wet hand into a latex glove (Sempermed Classic, lot YE5F7; Semperit, Vienna, Austria) and, as a control, put the other wet hand into a neoprene glove (Dermaprene; Ansell, Munich, Germany). After the patient had worn the gloves for 30 minutes (or on showing clinical symptoms), the gloves were removed and clinical reactions were assessed by a physician. The single-blinded test response was assessed as positive if typical immediate-type clinical reactions (eg, urticaria or Quincke's edema) occurred. Care was taken under continuous observation that the patient did not rub the glove on other parts of the body.

Five atopic donors with high IgE levels for birch pollen but no latex-specific IgE were used as negative controls.

Allergens

Recombinant Hev b 7 and latex C extract were prepared as described.²¹ Briefly, the full-length cDNA encoding Hev b 7 was

cloned into the *Pichia pastoris* expression vector pPIC9 and purified from the culture supernatant with anion exchange and hydrophobic interaction chromatography.²¹

To obtain latex C extract, fresh *Hevea* latex was collected from rubber trees (*H brasiliensis* RRIM 600 clone) into chilled containers and then centrifuged at 44,000g at 4°C for 1 hour. The aqueous phase—the C extract—was collected, and the centrifugation was repeated. The clear aqueous C extract was freeze-dried and stored at -20°C.²¹ This extract contained (among several other latex proteins) nHev b 1, nHev b 3, and nHev b 7.

Immunoblot analysis and inhibition experiments

Immunoblotting was performed as previously described.¹⁶ Briefly, 1 µg rHev b 7 or 75 µg latex C extract per lane was separated on 12% SDS PAGE gels and blotted onto a nitrocellulose membrane. The membrane was treated with blocking buffer (40 mmol/L Na₂HPO₄, 7 mmol/L NaH₂PO₄, 0.5% w/v BSA, 0.05% w/v sodium azide, 0.5% w/v Tween-20, pH 7.5) and incubated with patients' sera overnight at 4°C. For inhibition experiments, patients' sera were preincubated with 20 µg rHev b 7 overnight at 4°C. After washing with blocking buffer, the membrane was incubated with a ¹²⁵I-labeled antihuman IgE antibody (IBL, Hamburg, Germany) and bound IgE was visualized by autoradiography.

RESULTS

Evaluation of the study population

In this study, we evaluated a population of 53 latex-sensitized individuals with SB; they had a median age of 12.3 years (Table I). Total IgE ranged from 4.0 kU/L to 1865.0 kU/L (median, 300.9 kU/L). Latex-specific IgE in serum ranged from 0.4 kU/L to 98.6 kU/L (median, 14.1 kU/L). Thirty-eight of the 53 tested patients showed a positive response to the glove provocation test (latex-allergic SB group, Table I), whereas 15 patients with SB were sensitized but provocation-negative (latex-sensitized SB group, Table I).

After eating avocado, banana, or kiwi—3 fruits described as contributing to the latex fruit syndrome^{22,23}—none (0%) of the latex-sensitized and 2 (5%) of the 38 latex-allergic children with SB had a history of localized type I symptoms, as determined by the questionnaire. No systemic allergic reactions were described in the questionnaire; patient 2 complained about perioral contact urticaria after eating kiwi, and patient 22 complained about a localized pruritus and perioral urticaria after eating banana (Table I).

The 5 patients who were allergic to atopic birch pollen showed no specific IgE to latex with the Pharmacia CAP system and no reaction to skin prick testing (data not shown).

Immunoblot and inhibition studies of Hev b 7

By immunoblot analysis, all 53 sera described in Table I were screened for specific IgE against latex C extract proteins, which included the 43-kDa nHev b 7. This extract also contains nHev b 1 (14 kDa) and nHev b 3 (23 kDa). Only sera that recognize Hev b 7 among other latex allergens are shown in Fig 1. Natural Hev b 7-positive sera were then tested for their IgE binding to rHev b 7

TABLE I. Characterization of the study population, which consisted of 38 latex-allergic and 15 latex-sensitized children with spina bifida

Patient no.	Age (y)	Sex	SPT result	Total IgE (kU/L)	Latex-specific IgE (kU/L)	Latex CAP class	IgE to Hev b 7*	IgE to Hev b 1*	IgE to Hev b 3*	Fruitst
Latex-allergic children with spina bifida										
1	17	M	+	194	0.7	2	—	—	—	—
2	12	F	+	117	2.7	2	+	+	+	K
3	18	M	+	107	4.2	3	—	+	+	—
4	10	F	+	44	2	2	—	—	—	—
5	11	M	+	182	5.3	3	—	+	+	—
6	8	M	+	970	24	4	—	+	+	—
7	15	M	+	43	2	2	+	+	+	—
8	13	F	+	33	1.1	2	—	+	+	—
9	8	F	+	68	12.4	3	—	+	+	—
10	11	M	+	691	13	3	—	—	+	—
11	10	M	+	85	26	4	—	+	+	—
12	6	M	+	458	21.7	4	—	+	+	—
13	10	F	+	53	6.8	3	—	+	+	—
14	11	F	+	112	35.9	4	—	+	+	—
15	18	M	+	1318	18.9	4	+	+	+	—
16	7	M	+	286	45.1	4	—	—	+	—
17	8	M	+	46	1	2	—	+	+	—
18	22	M	+	114	6.6	3	+	+	+	—
19	12	M	+	121	1.1	2	+	—	+	—
20	11	M	+	176	46.4	4	+	+	+	—
21	8	M	+	84	7	3	—	+	+	—
22	11	M	+	926	14	3	—	—	—	B
23	6	M	+	318	6	3	—	+	—	—
24	4	M	+	140	4.8	3	—	+	+	—
25	20	M	—	75	0.5	1	—	+	—	—
26	10	F	+	58	22.8	4	+	+	+	—
27	10	M	+	1865	98.6	5	+	+	+	—
28	7	M	+	221	19.5	4	—	+	+	—
29	21	F	+	218	42.3	4	+	+	+	—
30	7	M	+	113	25.8	4	+	+	+	—
31	23	M	+	238	22.1	4	—	—	+	—
32	11	M	+	353	9.4	3	—	+	—	—
33	13	F	+	449	16.7	3	—	+	+	—
34	14	M	+	69	6	3	+	+	+	—
35	14	M	+	1770	31.7	4	+	+	+	—
36	8	M	+	175	33.4	4	+	+	+	—
37	9	F	+	860	35	4	+	+	—	—
38	8	F	+	226	13.6	3	+	+	—	—
Latex-sensitized children with spina bifida										
1	14	M	+	244	0.4	—	—	1	+	—
2	6	F	+	24	2.5	2	—	—	—	—
3	21	M	—	4	1.9	2	—	—	—	—
4	15	F	+	1181	14.3	3	—	+	—	—
5	7	F	—	35	0.9	2	—	—	—	—
6	15	F	—	122	0.4	1	—	—	—	—
7	13	F	+	26	4.1	3	—	—	—	—
8	12	F	+	404	1.8	2	—	—	—	—
9	15	F	+	60	2.2	2	—	—	—	—
10	23	F	+	132	1.7	2	—	+	+	—
11	11	M	—	105	0.9	2	—	+	—	—
12	14	F	+	24	1.6	2	+	+	—	—
13	12	M	+	178	26.5	4	+	+	+	—
14	16	F	—	8	0.9	2	—	+	+	—
15	16	M	+	27	0.6	1	—	+	—	—

SPT, Skin prick test; K, kiwi; B, banana.

*As determined by immunoblot.

†Allergic symptoms after eating fruits.

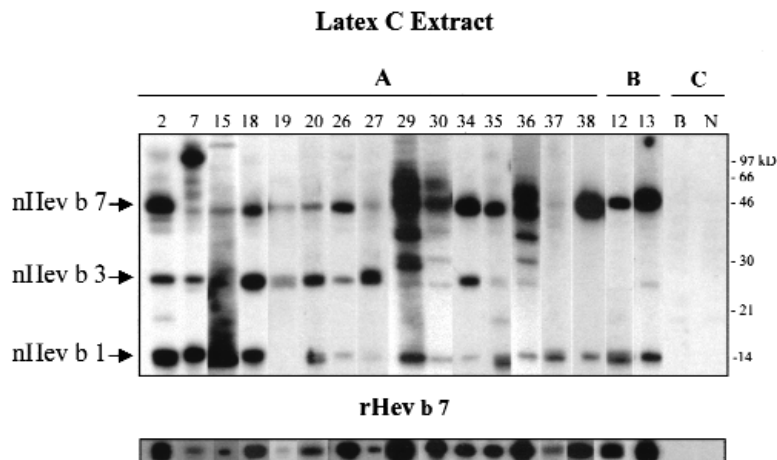


FIG 1. Immunoblot analysis of 38 latex-allergic (A) and 15 latex-sensitized (B) patients with SB with latex C extract and rHev b 7. Only the Hev b 7-positive results are shown. Patient numbers refer to Table I. Controls (C) consist of a negative control serum pool of 5 atopic donors without latex allergy (lane N) and a buffer control (lane B).

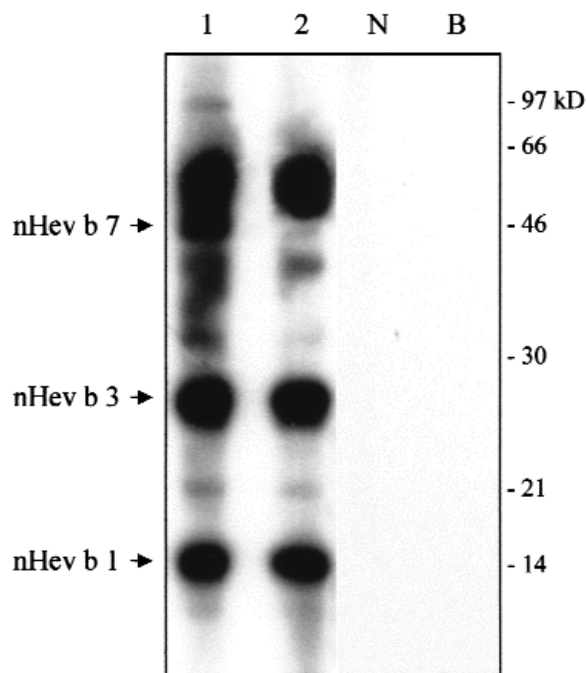


FIG 2. Inhibition experiment. A pool of the 17 Hev b 7-positive sera from latex-sensitized or latex-allergic patients with SB (as listed in Table I) was tested on latex C extract without pretreatment (lane 1) and after preincubation with rHev b 7 (lane 2). Lane N, negative control serum pool of 5 atopic donors without latex allergy. Lane B, Buffer control.

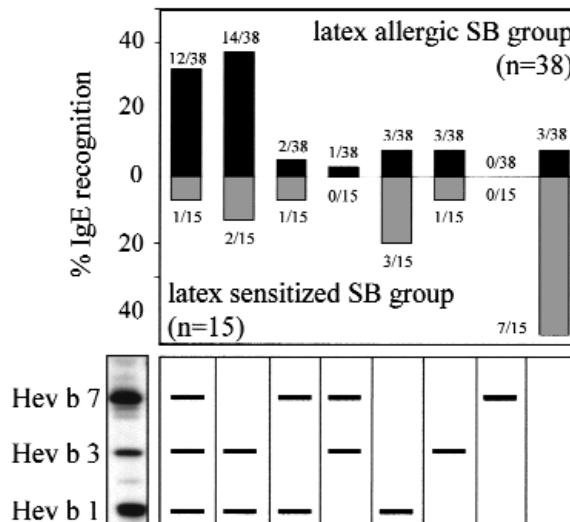


FIG 3. IgE recognition patterns of latex-sensitized or latex-allergic children with SB. Lower panel shows the different possible combinations of IgE binding to the 3 latex allergens associated with latex allergy in children with SB. Upper panel shows the percentage of IgE recognition of the possible combinations shown in the lower panel. Black bars indicate data for the latex-allergic SB group (n = 38); gray bars indicate data for the latex-sensitized SB group (n = 15). The number of individuals recognizing the different combinations of allergens in relation to the total number of individuals is indicated over or under each bar.

expressed in *P pastoris* (Fig 1). With respect to IgE-binding capacity, nHev b 7 in latex C extract and rHev b 7 were comparable; in other words, rHev b 7 expressed in *P pastoris* is immunologically equivalent to nHev b 7 present in latex C extract, as has been asserted by Sowka et al.²¹ Fifteen (39.5%) of 38 latex-allergic and 2 (13%) of 15 latex-sensitized children with SB (Table I; Fig 1) revealed IgE binding to natural as well as rHev b 7. The

negative control serum pool of 5 atopic birch pollen-allergic donors showed no IgE binding either to nHev b 7 or rHev b 7 or to any other latex C extract proteins. In Table II, these findings are summarized and compared with previously published data illustrating that latex-allergic children with SB are especially prone to sensitization against Hev b 7 in comparison with latex-allergic children without SB.

TABLE II. IgE binding to Hev b 7: a comparison

Population/study (year)	Allergen source	IgE binding and study population	IgE binding Assay
Latex-allergic children with SB Present study (2001)	nHev b 7c and rHev b 7 (yeast)	15/38 (40%) latex-allergic children with SB 2/15 (13%) latex-sensitized children with SB	Immunoblot
Latex-allergic children Seppälä et al ¹⁸ (2000)	nHev b 7c	1/35 (3%) latex-allergic children (including 7 with SB)	Immunoblot, ELISA, SPT
Latex-allergic adults, mostly HCWs Beezhold et al ²⁴ (1994)	Ammoniated and non-ammoniated latex extract	10/40 (25%) latex-allergic adults (including 34 HCWs and 1 patient with multiple urogenital surgeries)	Immunoblot
Kostyal et al ¹⁷ (1998) Yip et al ²⁵ (2000)	rHev b 7 as GST fusion (<i>E coli</i>) rHev b 7 as GST fusion (<i>E coli</i>)	Pool of 5 latex-allergic HCWs 12/31 (39%) latex-allergic patients (including 20 HCWs)	Immunoblot SPT
Seppälä et al ¹⁸ (2000)	nHev b 7c	17/35 (49%) latex-allergic adults	Immunoblot, ELISA, SPT
Latex-allergic adults Sowka et al ²¹ (1998)	nHev b 7c as well as rHev b 7 (yeast, isoform S1)	4/36 (11%) latex-allergic patients	Immunoblot
Sowka et al ²⁶ (1999)	Latex C extract as well as rHev b 7 (yeast, isoforms S1 and S2) and rHev b 7/GST fusion (<i>E coli</i> , isoform D1 and D2)	Only positive patients are shown	Immunoblot, ELISA, SPT
Yeang et al ²⁷ (2000)	nHev b 7c (isoform of latex C extract) and nHev b 7b (isoform of latex B extract)	3/36 (8%) latex-allergic patients with Hev b 7c 22/36 (61%) latex-allergic patients with Hev b 7b	Immunoblot

SB, *Spina bifida*; SPT, skin prick test; HCW, health care worker; *E coli*, *Escherichia coli*.

To verify that the proposed 43-kDa band recognized in the latex C extract is indeed nHev b 7, all Hev b 7–positive sera were pooled and preincubated with 20 µg rHev b 7. The pretreated serum pool was used for inhibition experiments on latex C extract. Recombinant Hev b 7 was able to abolish IgE binding to nHev b 7 (Fig 2, lane 2). The recognition intensities of the 14-kDa nHev b 1 band and the 23-kDa nHev b 3 band were not reduced after preincubation of the pooled sera with rHev b 7 (Fig 2). In addition, preincubating the pooled sera with rHev b 1 and rHev b 3 had no effect on the IgE binding to nHev b 7 present in latex C serum (data not shown).

IgE recognition patterns of latex-allergic or latex-sensitized children with SB

The immunoblots of sera of all of the 53 patients on latex C extract also produced data on the IgE recognition of Hev b 1 and Hev b 3 (Table I). Thirty-one (82%) of 38 latex-allergic and 7 (47%) of 15 latex-sensitized children with SB revealed IgE binding to Hev b 1 (Table I). Hev b 3 was recognized by 30 (79%) of 38 latex-allergic and 4 (27%) of 15 latex-sensitized children with SB (Table I).

In Fig 3, the possible combinations of the 3 SB-associated latex allergens—Hev b 1, Hev b 3, and Hev b 7—as recognized by IgE of latex-sensitized or latex-allergic children with SB are illustrated. Only 8% of the latex-allergic but 47% of the latex-sensitized patients with SB did not reveal IgE binding to Hev b 1, Hev b 3, or Hev b

7 (Table I; Fig 3). The frequency of recognition of only 1 of the 3 allergens was 16% among the latex-allergic patients and 27% in the latex-sensitized SB group (Table I; Fig 3). None of the patients showed IgE against Hev b 7 alone (Table I; Fig 3). Of the latex-allergic children with SB, only 8% displayed IgE against Hev b 1 and only 8% displayed IgE against Hev b 3 (Table I A; Fig 3). Latex-allergic patients who recognized 2 allergens reacted mainly with Hev b 1 and Hev b 3 (37%), the most frequent combination of IgE recognition among latex-allergic children with SB. Only 3 latex-allergic children with SB reacted with a combination of either Hev b 1 and Hev b 7 or Hev b 3 and Hev b 7 (Table I; Fig 3). Thirty-two percent of the latex-allergic patients displayed IgE against Hev b 1, Hev b 3, and Hev b 7 together (Table I; Fig 3).

DISCUSSION

Hev b 7 has been identified as an important allergen for latex-allergic adults^{17,18,21,24-27} but not for latex-allergic children without SB.¹⁸ In our study, we focused on a population of 53 patients with SB—38 latex-allergic and 15 latex-sensitized children—and their IgE binding to Hev b 7. In all, 15 (39.5%) of 38 latex-allergic and 2 (13%) of 15 latex-sensitized children with SB had serum IgE that bound to Hev b 7. The high percentage of IgE recognition by sera from latex-allergic patients with SB establishes Hev b 7 as the third SB-associated latex allergen.

Several groups have evaluated the IgE binding to the 43-kDa latex allergen Hev b 7.^{17,18,21,24-27} These data are summarized and compared with our data in Table II. Because of different study designs, data on the percentage of IgE binding to Hev b 7 varied from 8% to 61% (Table II). The IgE binding to Hev b 7 was examined through use of different methods, including immunoblot, ELISA, and skin prick testing. The study populations consisted mainly of adult latex-allergic patients, including a high percentage of HCWs. Only Seppälä et al¹⁸ focused on latex-allergic children and their IgE-binding pattern to Hev b 7. Allergen sources ranged from ammoniated and nonammoniated latex extracts to purified nHev b 7 and rHev b 7 produced in *Escherichia coli* or *P. pastoris*. The immunologic equivalence of the rHev b 7 preparations produced in *E. coli* or yeast and their isoforms (S1, S2, D1, and D2) was shown to be identical to that for nHev b 7 present in latex C extract.²⁶

In the investigation of Seppälä et al,¹⁸ which was the only study to examine latex-allergic children, the population consisted of 35 individuals, among whom were 7 children with SB. Only 1 of the 35 tested sera of latex-allergic children showed IgE against Hev b 7¹⁸; this was not a child with SB (U. Seppälä, personal communication). In contrast, we observed a significantly higher percentage (39.5%) of IgE binding to Hev b 7 in our study population of latex-allergic children with SB.

The cross-reactivity between Hev b 7 and the potato seed storage protein patatin was described by Seppälä et al.¹⁸ Patatin homologs are found not only in *H. brasiliensis* latex and Solanaceae but also in cucumber (*Cucumis sativus* [EMBL database: Y12793]) and possibly other plants and fruits as well. Accordingly, we wanted to know whether latex-allergic children with SB are also especially prone to sensitization against fruits associated with the latex fruit syndrome—eg, avocado, banana, and kiwi. Möller et al²³ illustrated with immunoblot inhibition studies that a 43-kDa protein in avocado, banana, and kiwi extracts showed cross-reactivity with a 43-kDa protein present in the latex extract. In the present study, only 2 patients displayed allergic symptoms after eating kiwi or banana (Table I), and none did so after eating avocado. We conclude that allergy against fruits contributing to the latex fruit syndrome is not a significant problem for latex-allergic children with SB, though atopy itself has been described as one of the 2 most important risk factors for latex allergy in patients with SB.^{6,28}

Hev b 1, Hev b 3, and now Hev b 7 are associated with latex allergy in children with SB. No disposition for a Hev b 7 sensitization could be deduced from the patient's age, CAP class, or latex-specific IgE (Table I). However, it is interesting that most patients were sensitized either to Hev b 1 and Hev b 3 (37%) or to Hev b 1, Hev b 3, and Hev b 7 (32%; Fig 3). No monosensitization to Hev b 7 could be observed (Table I; Fig 3). Nevertheless, it is noteworthy that 3 of 38 latex-allergic children with SB who showed positive reactions after provocation with latex gloves recognized Hev b 1, Hev b 3, or Hev b 7 (Table I, Fig 3); one of these children reacted with type I allergic symptoms after eating banana (Table I, patient

22). These 3 patients displayed an IgE-binding pattern completely different from that which is common for latex-allergic children with SB (data not shown).

Our data indicate that 4 of 10 latex-allergic patients with SB show IgE directed against Hev b 7, representing a much higher percentage of IgE binding to Hev b 7 than is seen in groups of latex-allergic adults and children without SB. Therefore, we conclude that the patatinlike latex protein Hev b 7 is the third SB associated *Hevea* allergen. Future immunotherapy for latex-allergic individuals with SB will need to include Hev b 7 in addition to Hev b 1 and Hev b 3.

In summary, our data confirm once again that latex-allergic children with SB are a distinct group of latex-allergic patients and that their IgE-binding patterns differ from those of other populations of latex-allergic individuals.

We are grateful to Hong Yeet Yeang for the provision of the latex C extract and to Nyu Ping Chew for technical assistance with *Hevea* RNA isolation.

REFERENCES

- Noetzel MJ. Myelomeningocele: current concepts of management. *Clin Perinatol* 1989;16:311-29.
- Kirpalani HM, Parkin PC, Willan AR, Fehlings DL, Rosenbaum PL, King D, et al. Quality of life in spina bifida: importance of parental hope. *Arch Dis Child* 2000;83:293-7.
- Cremer R, Hoppe A, Korsch E, Kleine-Diepenbruck U, Bläker F. Natural rubber latex allergy: prevalence and risk factors in patients with spina bifida compared with atopic children and controls. *Eur J Pediatr* 1998;157:13-6.
- Nieto A, Estornell F, Mazon A, Reig C, Garcia-Ibarra F. Allergy to latex in spina bifida: a multivariate study of associated factors in 100 consecutive patients. *J Allergy Clin Immunol* 1996;98:501-7.
- Michael T, Niggemann B, Moers A, Seidel U, Wahn U, Scheffner D. Risk factors for latex allergy in patients with spina bifida. *Clin Exp Allergy* 1996;26:934-9.
- Konz KR, Chia JK, Kurup VP, Resnick A, Kelly KJ, Fink JN. Comparison of latex hypersensitivity among patients with neurologic defects. *J Allergy Clin Immunol* 1995;95:950-4.
- Niggemann B, Buck D, Michael T, Wahn U. Latex provocation tests in patients with spina bifida: who is at risk of becoming symptomatic? *J Allergy Clin Immunol* 1998;102:665-70.
- Niggemann B, Breiteneder H. Latex allergy in children. *Int Arch Allergy Immunol* 2000;121:98-107.
- Yassin MS, Lierl MB, Fischer TJ, O'Brien K, Cross J, Steinmetz C. Latex allergy in hospital employees. *Ann Allergy* 1994;72:245-9.
- Mazon A, Nieto A, Linana JJ, Montoro J, Estornell F, Garcia-Ibarra F. Latex sensitization in children with spina bifida: follow-up comparative study after two years. *Ann Allergy Asthma Immunol* 2000;84:207-10.
- Niggemann B, Buck D, Michael T, Haberl H, Wahn U. Latex allergy in spina bifida: at the turning point? [letter]. *J Allergy Clin Immunol* 2000;106:1201.
- Czuppon AB, Chen Z, Rennert S, Engelke T, Meyer HE, Heber M, et al. The rubber elongation factor of rubber trees (*Hevea brasiliensis*) is the major allergen in latex. *J Allergy Clin Immunol* 1993;92:690-7.
- Alenius H, Palosuo T, Kelly K, Kurup V, Reunala T, Makinen-Kiljunen S, et al. IgE reactivity to 14-kD and 27-kD natural rubber proteins in latex-allergic children with spina bifida and other congenital anomalies. *Int Arch Allergy Immunol* 1993;102:61-6.
- Yeang HY, Cheong KF, Sunderasan E, Hamzah S, Chew NP, Hamid S, et al. The 14.6 kD rubber elongation factor (Hev b 1) and 24 kD (Hev b 3) rubber particle proteins are recognized by IgE from patients with spina bifida and latex allergy. *J Allergy Clin Immunol* 1996;98:628-39.
- Chen Z, Cremer R, Posch A, Raulf-Heimsoth M, Rihs HP, Baur X. On the allergenicity of Hev b 1 among health care workers and patients with spina bifida allergic to natural rubber latex. *J Allergy Clin Immunol* 1997;100:684-93.

16. Wagner B, Krebitz M, Buck D, Niggemann B, Yeang HY, Han KH, et al. Cloning, expression, and characterization of recombinant Hev b 3, a *Hevea brasiliensis* protein associated with latex allergy in patients with spina bifida. *J Allergy Clin Immunol* 1999;104:1084-92.
17. Kostyal DA, Hickey VL, Noti JD, Sussman GL, Beezhold DH. Cloning and characterization of a latex allergen (Hev b 7): homology to patatin, a plant PLA2. *Clin Exp Immunol* 1998;112:355-62.
18. Seppälä U, Palosuo T, Kalkkinen N, Ylitalo L, Reunala T, Turjanmaa K. IgE reactivity to patatin-like latex allergen, Hev b 7, and to patatin of potato tuber, Sol t 1, in adults and children allergic to natural rubber latex. *Allergy* 2000;55:266-73.
19. Axen R, Drevin H, Kober A, Yman L. A new laboratory diagnostic system applied to allergy testing. *N Engl J Allergy Proc* 1998;53:513-9.
20. Buck D, Michael T, Wahn U, Niggemann B. Ventricular shunts and the prevalence of sensitization and clinically relevant allergy to latex in patients with spina bifida. *Pediatr Allergy Immunol* 2000;11:111-5.
21. Sowka S, Wagner S, Krebitz M, Arija-Mad-Arif S, Yusof F, Kinaciyan T, et al. cDNA cloning of the 43-kDa latex allergen Hev b 7 with sequence similarity to patatins and its expression in the yeast *Pichia pastoris*. *Eur J Biochem* 1998;255:213-9.
22. Brehler R, Theissen U, Mohr C, Luger T. "Latex-fruit syndrome": frequency of cross-reacting IgE antibodies. *Allergy* 1997;52:404-10.
23. Möller M, Kayma M, Vieluf D, Paschke A, Steinhart H. Determination and characterization of cross-reacting allergens in latex, avocado, banana, and kiwi fruit. *Allergy* 1998;53:289-96.
24. Beezhold DH, Sussman GL, Kostyal DA, Chang NS. Identification of a 46-kD latex protein allergen in health care workers. *Clin Exp Immunol* 1994;98:408-13.
25. Yip L, Hickey V, Wagner B, Liss G, Slater J, Breiteneder H, et al. Skin prick test reactivity to recombinant latex allergens. *Int Arch Allergy Immunol* 2000;121:292-9.
26. Sowka S, Hafner C, Radauer C, Focke M, Brehler R, Astwood JD, et al. Molecular and immunologic characterization of new isoforms of the *Hevea brasiliensis* latex allergen hev b 7: evidence of no cross-reactivity between hev b 7 isoforms and potato patatin and proteins from avocado and banana. *J Allergy Clin Immunol* 1999;104:1302-10.
27. Yeang HY, Chow KS, Yusof F, Arif SA, Chew NP, Loke YH. Appraisal of latex glove proteins in the induction of sensitivity to multiple latex allergens. *J Invest Allergol Clin Immunol* 2000;10:215-22.
28. Szepefalusi Z, Seidl R, Bernert G, Dietrich W, Spitzauer S, Urbanek R. Latex sensitization in spina bifida appears disease-associated. *J Pediatr* 1999;134:344-8.