

Letter to the Editor

Clinical relevance of the *Hevea brasiliensis* lipid transfer protein Hev b 12

To the Editor:

Natural rubber latex (NRL) from *Hevea brasiliensis* (Euphorbiaceae) constitutes an important cause of allergy with distinct phenotypes depending on the sensitization route and profile of the patient.¹ In health care workers NRL allergy is dominated by sensitization to the proteins Hev b 5, 6.01, 6.02, and 6.03.² Because of the ubiquity of these proteins in the plant kingdom, patients frequently display cross-reactivity to various fruits and vegetables. In patients who have undergone multiple operations, such as those with spina bifida, cross-reactivity is rare because NRL allergy mainly results from sensitization to components without homologues in edible plants, namely Hev b 1 and Hev b 3.¹

However, NRL allergy has recently been confirmed in patients without clear sensitization to the components Hev b 1, 3, 5, 6, 7, 8, 9, 10, and 11.¹ The exact explanation for this observation probably relates to the fact that the panel of recombinant proteins currently available for diagnostics does not cover the entire NRL IgE response. Although Beezhold et al³ identified the nonspecific lipid transfer protein (nsLTP) Hev b 12 from NRL as a potential cross-reactive panallergen more than a decade ago, literature about the clinical significance of Hev b 12 sensitization is limited, and no Hev b 12 sensitization was demonstrable in 25 children with latex allergy.⁴ Rihs et al⁵ observed Hev

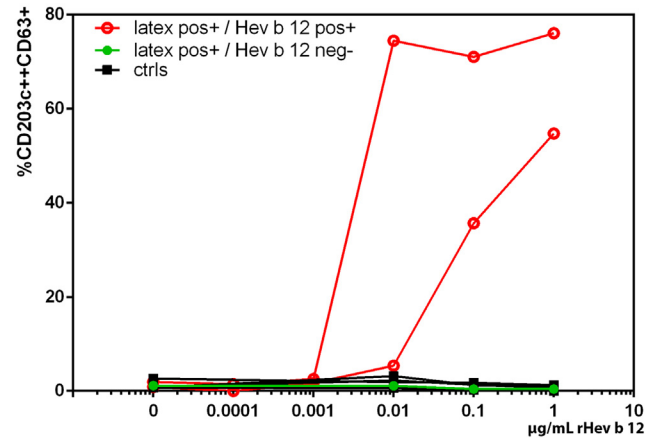


FIG 1. Basophil activation with rHev b 12. Percentages of CD63⁺ and CD203c⁺⁺ basophils after stimulation with 5 different concentrations of rHev b 12 in patients with latex allergy and Hev b 12 sensitization (n = 2, red line), patients with latex allergy without Hev b 12 sensitization (n = 3, green line), and 3 healthy control subjects (black line).

b 12-specific IgE (sIgE) reactivity in only 6 (12.5%) of 48 patients with fruit allergy but without symptoms on latex exposure. In these patients Hev b 12 sensitization seemed attributable to cross-reactive nsLTPs from different fruits and apparently of limited role in NRL allergy.

TABLE I. Demographic and clinical data for the study population and results of skin prick tests with NRL and sIgE determination (ImmunoCAP or CBA)

Patient no.	Sex/ age (y)	Clinical symptoms on NRL	Allergic symptoms to:	SPT (NRL)	Total IgE (kU/L), ImmunoCAP	sIgE (kU _A /L), NRL extract (ImmunoCAP)	Sensitization profile to commercially available NRL components, (ImmunoCAP)	sIgE (MFI)		
								rHev b 12 (CBA)	nCan s 3 (CBA)	sIgE (kU _A /L), rPru p 3 (ImmunoCAP)
1	F/26	U, AE	<i>Cannabis sativa</i> , cherry, tree nuts, peanut, apple, tobacco, peach, tomato, pineapple, raspberry, cucumber, fennel, red wine, wheat	+	212	3.23	None	2.19	12.57	34.40
2	M/11	U	Peanut, tree nuts, beans, carrot, pumpkin	NT	604	0.64	None	0.97	<0.10	2.88
3	F/39	D, Wh	Soy, apple, kiwifruit, tree nuts, potato, tomato, melon, paprika, peanut	NT	26	3.92	Hev b 8	<0.10	<0.10	<0.10
4	F/53	U, Rh	Chestnut, passion fruit, kiwifruit	+	41	6.36	Hev b 5/Hev b 6.01/Hev b 6.02	<0.10	<0.10	<0.10
5	F/52	AE	Kiwifruit, peanut, almond	+	6	0.75	Hev b 5	<0.10	<0.10	<0.10

AE, Angioedema; D, dyspnea; F, female; M, male; NT, not tested; Rh, rhinitis; SPT, skin prick test; U, urticaria; Wh, wheezing.

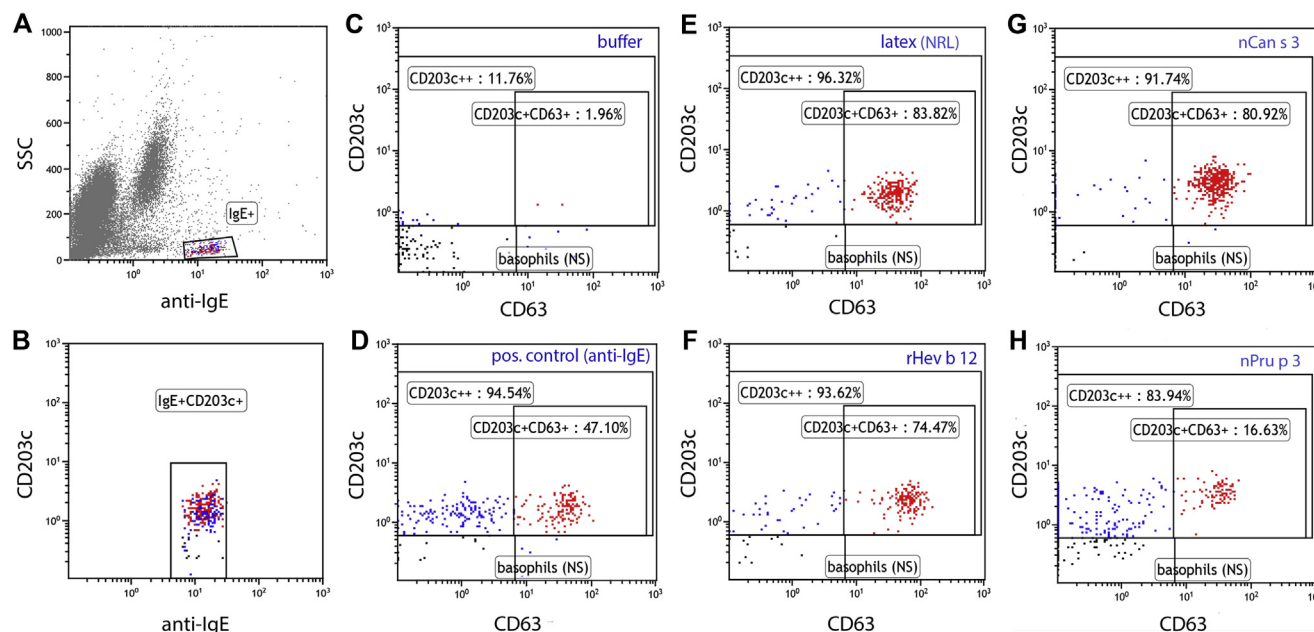


FIG 2. Basophil selection and activation: BAT plot in a patient with latex allergy and sensitization to rHev b 12 and nCan s 3. **A** and **B**, Detection of basophils by using side scatter (SSC) and gating out IgE^{high} and CD203c⁺ cells. **C**, Within this gate, numbers of activated basophils expressing CD63 were measured. Stimulation with activation buffer served as a negative control for spontaneous CD63 expression. **D**, Stimulation with anti-human IgE (10 μ g/mL, BD Biosciences) was used as a positive control. **E–H**, Basophil stimulation with NRL extract (1 μ g/mL), rHev b 12 (0.1 μ g/mL), nCan s 3 (0.1 μ g/mL), and nPru p 3 (0.1 μ g/mL), respectively.

Because of limited data about the clinical relevance of Hev b 12, this study aims to re-evaluate the role of Hev b 12 in patients with NRL allergy, and in addition, particular focus is put on the position of NRL allergy in patients with the nsLTP syndrome.

Five patients with a definite history of immediate allergic symptoms on exposure to NRL and an objective allergy to different plant foods were selected. All patients with latex allergy had a positive sIgE level to NRL extract, and 2 patients showed no sIgE reactivity to any of the available recombinant latex components. Demographic and detailed clinical data of the entire study population are shown in Table I. Patients provided informed consent in accordance with the Declaration of Helsinki.

NRL extract (Stallergenes, Fresnes, France) was used for skin prick tests. Results are shown in Table I.

Levels of total IgE and sIgE to NRL extract; rHev b 1, 3, 5, 6.01, 6.02, 8, 9, and 11; and the peach nsLTP (*Prunus persica*, rPru p 3) were quantified by using a single-plexed technique (ImmunoCAP; Thermo Fisher Scientific, Uppsala, Sweden), and results of 0.35 kU_A/L or greater were considered positive.

A flow cytometric bead array (CBA) (BD Biosciences, Franklin Lakes, NJ) was developed to detect sIgE to the nsLTP allergens rHev b 12 and nCan s 3 of *Cannabis sativa*.⁶ The recombinant nsLTP from *H brasiliensis* latex and the native nsLTP extract from *C sativa* were purified, as previously reported.⁷ Mean fluorescence intensity (MFI) results of 0.10 or greater were considered positive (detailed methodology is described in the Online Repository at www.jacionline.org).

The basophil activation test (BAT) was performed with NRL extract, rHev b 12, nCan s 3, and nPru p 3 and prepared as previously described.^{3,7} Flow-assisted BAT was performed with 2 concentrations of NRL extract (0.01 μ g/mL or 1 μ g/mL) and 5 concentrations of rHev b 12, nCan s 3, and nPru p 3 (0.0001,

0.001, 0.01, 0.1, and 1 μ g/mL). Three healthy subjects served as the control group.

As displayed in Table I, both patients without sIgE to any of the available latex components had sIgE antibodies to rHev b 12. In addition, both patients showed sIgE reactivity to rPru p 3, and one of the patients showed a positive sIgE result to nCan s 3. In the other allergic patients sensitized to Hev b 5, 6.01, 6.02, and/or 8, no sIgE antibodies were found to rHev b 12, nCan s 3, or rPru p 3.

Fig 1 shows a clear upregulation of the activation marker CD63 for rHev b 12 in both patients with latex allergy and rHev b 12 sensitization. In the other patients with latex allergy and the healthy control subjects, rHev b 12 did not trigger basophil activation. Both Hev b 12-sensitized patients had a positive BAT result to the nsLTP extract of peach. As demonstrated in Fig 2, the patient with sIgE antibodies to nCan s 3 also had a clear upregulation of CD63 with the nsLTP extract of cannabis. In the other Hev b 12- and Pru p 3-sensitized patient, no basophil activation to nCan s 3 was observed (data not shown).

These data indicate that sensitization to Hev b 12, the nsLTP from *H brasiliensis* latex, can be clinically relevant on exposure to products containing NRL.

Although the exact routes of exposure to Hev b 12 remain elusive, we observed that 1 patient had symptoms of latex allergy after having nsLTP-related allergic symptoms to *C sativa* and the other patient had NRL allergy after being sensitized to multiple nsLTP-containing fruits. For both patients, it seems less probable that Hev b 12 is the primary sensitizer. The female patient with positive sIgE results to rHev b 12 and rCan s 3 was notable in that her first allergic symptoms occurred while smoking marijuana. It is noteworthy that this patient did not mention latex-induced allergic symptoms at first consultation and appeared to have the new sensitization to NRL in a timeframe of 1 year,

with seroconversion of sIgE results to NRL and skin prick test responses with latex extract becoming positive.⁸ Additional studies are needed to obtain better insight into the allergenicity of Hev b 12 and to confirm the hypothesis that sensitization to Can s 3 can cause cross-reactive allergies extending beyond fruits and vegetables.⁷ In these studies BATs, along with IgE-binding studies, might be adopted to elucidate the clinical relevance of different nsLTPs. We should note that we did not determine sIgE levels to Hev b 2, an allergen of NRL also associated with the latex-fruit syndrome.⁹ Therefore we cannot exclude the possibility that our Hev b 12-sensitized patients are cosensitized to Hev b 2. In conclusion, Hev b 12 seems to be a clinically relevant allergen in patients with an nsLTP allergy, although further studies are needed in larger study populations and additional geographic areas.

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METHODS

Methodology: CBA

A CBA was developed to detect sIgE to nsLTP allergens of *H brasiliensis* latex (Hev b 12) and *C sativa* (Can s 3).^{E1} The recombinant nsLTP from *H brasiliensis* latex was produced by Beezhold et al,^{E2} and the native nsLTP extract from *C sativa* was purified as previously reported.^{E3} The method was validated and standardized by using rBet v 1 (Stallergenes) from birch pollen (*Betula verrucosa*).

Conjugation procedure

Functional beads (BD CBA functional beads; BD Biosciences, Franklin Lakes, NJ) were conjugated according to the manufacturer's procedures with selected allergens, as described by Pomponi et al.^{E1} Briefly, 75 μ L of microbeads were incubated with 1.9 μ L of 1 mol/L dithiothreitol for 1 hour in the dark at room temperature, vortexing the tubes every 15 minutes. Afterward, the microbeads were washed with coupling buffer (BD Biosciences) and centrifuged at 900g for 3 minutes, and the supernatant was resuspended in 20 μ L of coupling buffer. Ninety microliters of the selected allergens at a concentration of 1 mg/mL were incubated with 2 μ L of fresh prepared sulfo-succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC) solution (2 mg/mL; Thermo Scientific, Pierce, Ill) for 1 hour at room temperature in the dark vortexing every 15 minutes. The Bio Spin columns (Bio-Rad, Milan, Italy) were prewashed with coupling buffer, and the protein/sulfo-SMCC mix was transferred to the column, followed by spinning at 1000g for 2 minutes. The modified proteins in the eluate were transferred to functional microbeads and incubated for 1 hour at room temperature and protected from light. The tubes were vortexed every 15 minutes. Afterward, 2 μ L of N-ethylmaleimide (2 mg/mL in dimethyl sulfoxide, Thermo Scientific) was added for 15 minutes, with vortexing every 5 minutes. After incubation, 1 mL of storage buffer was added, and the tubes were spun for 3 minutes at 900g. Washing with storage buffer was performed 3 times before the microbead pellet was resuspended in 500 μ L of storage buffer at a concentration of 6×10^6 microbeads/mL and stored at 2°C to 8°C and protected from light before use.

Testing procedure

One microliter of each allergen-conjugated microbead was used for each test. The microbeads were washed with 1 mL of PBS with 0.05% Tween 20 and 0.05% NaN₃ (PBS-T) and centrifuged for 5 minutes at 500g. After removing the supernatant, the microbeads were resuspended in 50 μ L of PBS-T with 1% BSA (PBS-T-BSA).

Fifty microliters of diluted serum samples (1:5 in PBS-T-BSA) was added to 50 μ L of the mixed capture beads and incubated for 1 hour at room temperature in the dark. Tubes were then washed with PBS-T and resuspended in 100 μ L of PBS-T-BSA after removing the supernatant. Then 50 μ L of diluted phycoerythrin-conjugated anti-human IgE (1:100 in PBS 0.05% NaN₃; BioLegend, San Diego, Calif) was added and incubated for 1 hour at room temperature in the dark. The samples were washed with 1 mL of PBS-T, centrifuged for 5 minutes at 200g, and resuspended in 200 μ L of PBS-T. Afterward, the samples were measured on a FACSCanto II Flow Cytometer (BD Biosciences), and results were expressed as MFI.

Validation of the CBA

Standardization of the flow cytometric immunobead array was performed with rBet v 1—positive samples compared with the ImmunoCAP technique. At least 5 samples with known rBet v 1 IgE concentrations (ImmunoCAP; in kilounits of allergen per liter) were pooled and tested. A 5-parameter logistic nonlinear standard curve was constructed to normalize the MFI. Reproducibility was tested, and a coefficient of variation of 10% was found.

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