

Cross-reacting allergens in natural rubber latex and avocado

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Background: An association between allergy to latex and avocado has been reported but the responsible cross-reacting allergens have not been identified or characterized.

Methods: Immunoblotting, immunoblot inhibition, and RAST inhibition methods were used to study cross-reactive proteins between natural rubber latex (NRL) and avocado. Sera from 18 patients with previously verified latex allergy were used as the source of IgE antibodies, and 11 of the patients underwent skin prick testing with fresh avocado.

Results: Fourteen of the 18 sera (78%) had IgE antibodies that bound to a total of 17 avocado proteins with apparent molecular weights ranging from 16 to 91 kd. Ten most strongly reacting sera were used for immunoblot inhibition studies. When NRL proteins were used as soluble inhibitors, binding of IgE antibodies to solid-phase avocado proteins was inhibited in a dose-dependent manner: 100 µg of NRL proteins inhibited IgE binding to 15 of the 17 avocado proteins, and 10 µg caused inhibition to 13 protein bands. Comparably, soluble avocado proteins were able to inhibit IgE binding to solid-phase NRL. Corresponding RAST inhibitions were performed with two patient sera; in both, avocado inhibited IgE binding to NRL and in one NRL proteins inhibited IgE binding to avocado. Skin prick test responses to fresh avocado were positive in seven of the 11 patients with latex allergy who were tested.

Conclusions: The large number of inhibitable proteins in immunoblot experiments and clinical observations from skin prick tests suggest considerable immunologic cross-reactivity between NRL and avocado. The observed cross-reacting protein components may be responsible for the recently reported type I hypersensitivity reactions to NRL and avocado in patients with a preexisting allergy to either allergen. (*J ALLERGY CLIN IMMUNOL* 1995;96:167-73.)

Key words: IgE, natural rubber latex, avocado, cross-reactivity, immunoblotting, immunoblot inhibition, RAST inhibition, prick tests

Allergy to natural rubber latex (NRL) has been recognized as an important medical problem among health care workers and patients who have undergone multiple operations because of their exposure to surgical latex gloves and other NRL-containing medical devices.^{1,2} However, the wide

Abbreviations used

NRL: Natural rubber latex
SPT: Skin prick test
TBS: Tris-buffered saline

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use of NRL products such as household gloves, condoms and balloons has made this allergy also common among other groups of people.³⁻⁷ The immediate hypersensitivity reactions in latex allergy are caused by water-soluble proteins eluting from the rubber products.^{1,5,8-12} These allergenic proteins obviously derive from the NRL of the rubber tree *Hevea brasiliensis* in which they have been identified, as well as in manufactured products.¹⁻¹² Recently, cross-reacting allergenic pro-

teins have been identified between NRL and banana,^{13,14} and clinical evidence suggests that patients with latex allergy may have allergic reactions after eating banana.^{13,15} As demonstrated by two recent case reports,^{16,17} another fruit capable of eliciting such reactions seems to be avocado, the fruit of the tropical tree, *Persea americana*. By using serum from one patient allergic to avocado Lavaud et al.¹⁶ succeeded in showing RAST inhibition when avocado extract, but not NRL extract, was used as an inhibitor allergen.

In this study we used immunoblot and RAST inhibition methods to detect cross-reactivity between NRL and avocado proteins. We found several cross-reacting proteins, and the results of avocado skin prick tests (SPTs) also suggested that immunologic cross-reactions can occur in patients allergic to NRL.

METHODS

Subjects

Sera were obtained from 18 patients with latex allergy (3 male and 15 female) who ranged in age from 7 to 42 years (mean age, 30 years) and from 14 nonallergic control subjects (2 male and 12 female) who ranged in age from 22 to 73 years (mean age, 50 years). Serum from one additional patient (a 34-year-old woman) who had experienced an anaphylactic reaction to avocado was available for a RAST inhibition experiment. The patients had positive SPT responses and the control subjects had negative SPT responses to two surgical latex glove extracts (Triflex; Baxter, Lessines, Belgium and Exona; Semperit, Vienna, Austria). These extracts were prepared and SPTs were performed as previously described by Turjanmaa et al.¹⁸ Sixteen of the 18 patients showed positive (≥ 0.4 kU/L) and all control subjects showed negative latex RAST results with the CAP method (Pharmacia, Uppsala, Sweden).

Avocado extract

Twenty grams of fresh avocado fruit was homogenized in a Waring blender (Waring Products, New Hartford, Conn.) in 200 ml of phosphate-buffered saline (pH 7.4) containing 0.01% sodium azide and incubated overnight at 4° C with constant stirring. The eluate was centrifuged at 40,000 g for 1 hour, and the supernatant was filtered through a 0.45 μ m filter membrane (Millex HV; Millipore, Molsheim, France). The supernatant was then dialyzed against 20 mmol/L ammonium bicarbonate buffer at 4° C for 2 days with dialysis tubing (cutoff, 3500 d), filtered again through a 0.45 μ m HV filter, and finally freeze-dried. The lyophilized powder was stored at 4° C until use. The extract was restored for use by solubilizing 10 mg of lyophilized powder in 1 ml of 20 mmol/L ammonium bicarbonate buffer.

Protein assays

The total protein concentration of the avocado extract was determined by Lowry's method with bovine serum albumin as the standard.¹⁹

After sodium dodecylsulfate-polyacrylamide gel electrophoresis and transfer to nitrocellulose sheet (as described below for immunoblotting), total protein staining was performed with a commercial kit (Blotting Detection Kit for Total Protein; Amersham, Amersham, U.K.) with molecular weight protein markers of 14.3 to 200 kd (Amersham). The protein concentration of the sample was adjusted to 450 μ g/ml before total protein staining and immunoblotting were performed.

Inhibitor solutions

The NRL extract was prepared as previously described by Alenius et al.¹⁰ The amounts of NRL and avocado proteins used as inhibitors were 1, 10, and 100 μ g in the final volume of 750 μ l of incubation solution (concentrations 1.3 to 133 μ g/ml).

Immunoblotting

For electrophoresis, the sample was diluted 1:3 into electrophoresis sample buffer containing 2% sodium dodecylsulfate and 5% 2- β -mercaptoethanol and kept in boiling water for 2.5 minutes. The dissolved sample, 45 μ l/cm of gel sample well width, was applied to 12.5% acrylamide gels (0.75 mm) containing 0.1% sodium dodecylsulfate, with a 4% stacking gel. Molecular weight protein markers, the same as those used in protein staining, were included in the gel. Electrophoresis was carried out for 45 minutes at a constant voltage of 200 V (Mini Protean II Cell; Bio-Rad, Richmond, Calif.).

After electrophoresis, the proteins were transferred from the gel to a 0.2 μ m pore size nitrocellulose filter (Trans-Blot, Bio-Rad) in an electroblotting buffer containing 25 mmol of Tris-HCl, 150 mmol of glycine, and 20% methanol, pH 8.3, for 1 hour at 100 V (Mini Trans-Blot Cell, Bio-Rad). After blotting, the membrane was briefly stained with Ponceaus S red (BDH, Poole, U.K.) to demonstrate successful transfer of proteins. The membrane was then cut into 3 mm wide strips, washed twice for 10 minutes in 0.1% Tween-Tris-buffered saline (TBS) and incubated for 45 minutes in a blocking buffer containing 5% non-fat dry milk powder in TBS. Sera diluted 1:5 in the blocking buffer were added, and the strips were incubated overnight at 4° C with continuous shaking. Two strips were left without sera to serve as negative reagent controls. The strips were then rinsed twice and washed three times for 5 minutes in 0.1% Tween-TBS. Then, biotinylated goat anti-human IgE antibody (KPL, Gaithersburg, Md.) diluted 1:1000 in blocking buffer was added and incubated for 30 minutes at room temperature. One of the strips without serum was also left without anti-human IgE to serve as a control for the conjugate. The strips were then washed as described above, and an additional blocking step in the blocking buffer was performed for 5

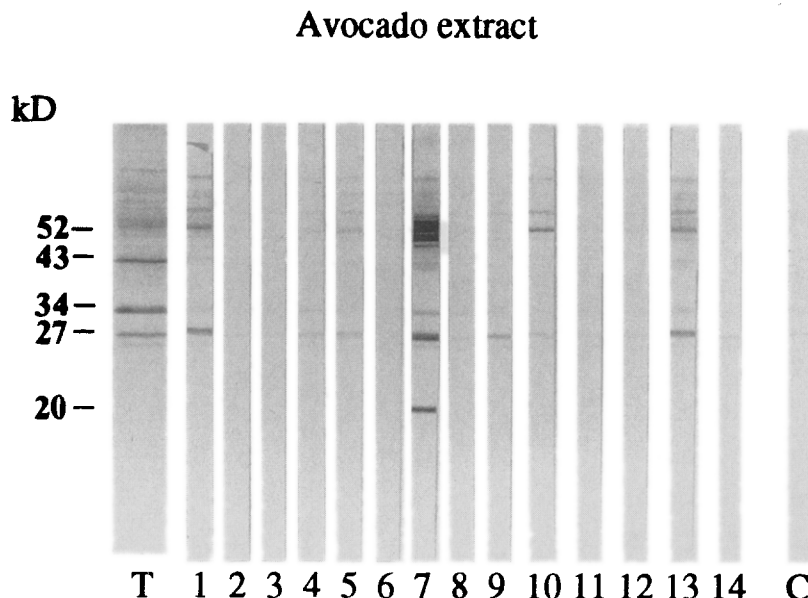


FIG. 1. Total protein staining (*T*) and immunoblot analysis of avocado extract. IgE antibodies from sera of 14 patients allergic to surgical latex gloves bound to a total of 16 avocado proteins. Fourteen control sera were used, of which one is shown (*C*). Note that the 27 kd band shows only faintly on the control strip, whereas on other strips it shows more intensely. Apparent molecular weights of some of the most prominent allergens are given on the left. Note also that although the total protein staining was performed separately, it is shown here with immunoblot strips to allow comparison of the proteins detected by these two methods. Essentially all of the protein bands detected by total protein staining have also been detected by the sera.

minutes, after which the strips were washed once for 5 minutes in 0.1% Tween-TBS. Thereafter, streptavidin-conjugated alkaline phosphatase (Bio-Rad) diluted 1:24,000 in TBS was added and incubated for 30 minutes at room temperature. The strips were then rinsed twice and washed twice for 5 minutes in 0.1% Tween-TBS and once for 5 minutes in TBS and placed in a color development solution (Alkaline Phosphatase Conjugate Substrate Kit, Bio-Rad). The reaction was stopped after 9.5 minutes by rinsing the strips in distilled water.

Immunoblot inhibition

The avocado and NRL extracts were electrophoresed, and the proteins were transferred to a nitrocellulose membrane as described above. Before they were added to the strips, the sera were incubated for 2 hours at room temperature in a solution containing 1, 10, and 100 μ g of NRL or avocado proteins. An amount of TBS corresponding to the inhibitor solution was added to the uninhibited and control sera. The immunologic detection was then performed as described above.

RAST inhibition

RAST inhibition studies were performed as described by Yman et al.²⁰ Two patient sera were tested, one that was also used in the immunoblot experiments (serum no. 7) and one from a patient with latex allergy who had experienced an anaphylactic reaction after ingesting avocado. Soluble avocado proteins (2, 0.2, and 0.02

mg/ml) were used to inhibit IgE antibody binding to solid-phase latex (ImmunoCAP, Pharmacia). When inhibiting IgE binding to avocado disks, 10, 1 and 0.1 mg/ml of soluble latex proteins were used as inhibitor. An extract from *Candida albicans* (76/513, NIBSC, London, U.K.) was used as a control inhibitor.

RESULTS

Total protein staining

Fourteen protein bands with apparent molecular weights ranging from 16 to 91 kd were detectable in the avocado extract (Fig. 1). The most intense bands were seen at about 27, 34, 43, and 52 kd.

Immunoblotting

Of the 18 sera from patients with latex allergy that were tested, 14 had IgE antibodies capable of binding to a total of 16 avocado proteins (Fig. 1). Four sera (from patients 1, 7, 10, and 13) showed "strong" binding patterns. IgE-binding proteins were seen at about 20, 26, 27, 34, 41, 43, 46, 49, 52, 54, 58, 63, 67, 70, 80, and 91 kd. The 14 control sera and two conjugate control strips all showed a faint band at 27 kd (Fig. 1). The same band was also seen with all the patient sera, but with a much greater intensity. Thus the intense 27 kd bands were considered positive reactions, whereas the

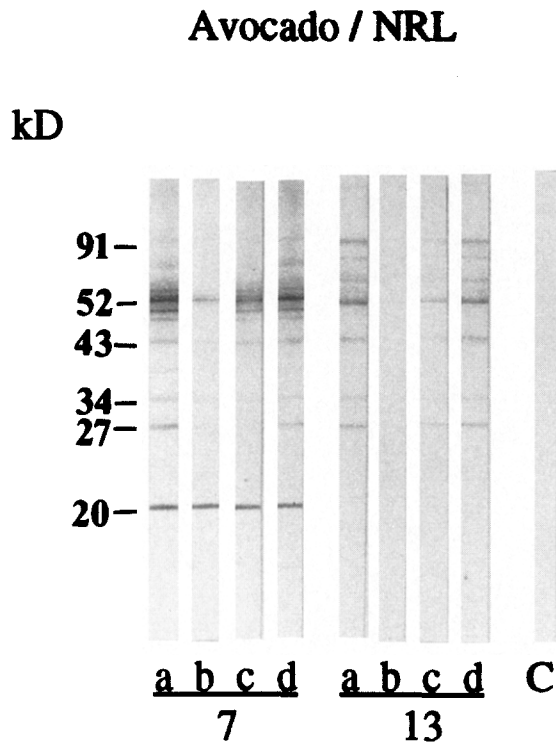


FIG. 2. Immunoblot inhibition analysis with avocado extract as solid-phase antigen, NRL extract as soluble inhibitor and two sera from patients with latex allergy (no. 7 and no. 13) as source of IgE antibodies. The binding of IgE antibodies to almost all the avocado proteins (*lane a*) could be prevented by using 100 µg of inhibitor proteins (*lane b*). However, there was no detectable inhibition at the 20 and 52 kD bands with serum no. 7. The amount of 10 µg of inhibitor (*lane c*) was sufficient for partial inhibition, as indicated by the stepwise reappearance of the avocado bands. One microgram of inhibitor (*lane d*) was not able to inhibit IgE binding. C, Control serum.

faint bands were interpreted as negative results, possibly caused by the streptavidin conjugates. Other frequently detected proteins were those at about 34, 63, and 52 kD (detected by >50% of the reacting sera). The most intense reactions appeared with 27 and 52 kD proteins, followed by 63, 34, 41, 43, 70, 80, and 91 kD proteins. One serum (no. 7) also reacted strongly with a 20 kD protein (Fig. 1).

Immunoblot inhibitions

Of the sera tested above, the 10 most strongly reacting ones (nos. 1, 4, 5, 6, 7, 8, 9, 10, 11, and 13) were used for inhibition studies. Thirteen avocado protein bands were detected by these 10 sera: 20, 27, 34, 41, 43, 46, 49, 52, 54, 60, 68, 72, and 91 kD. Two control sera were used in the inhibition experiment, and both produced negative results.

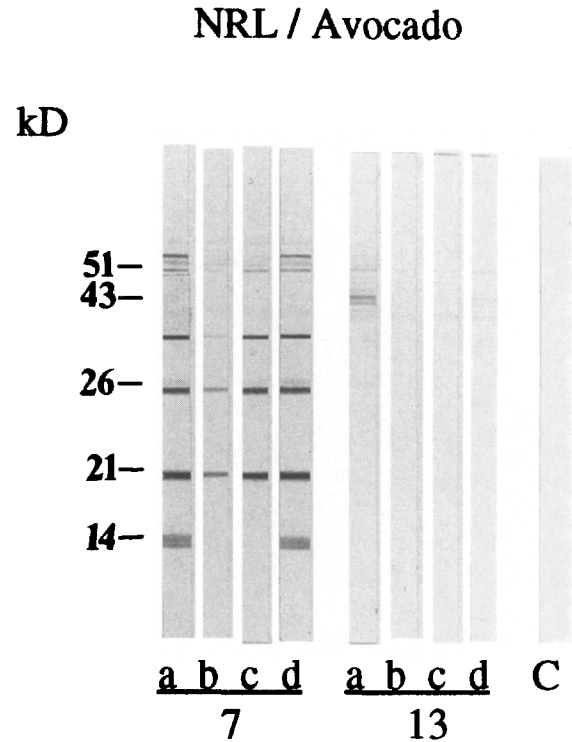


FIG. 3. Immunoblot inhibition analysis with NRL proteins as solid-phase antigen and avocado extract as soluble inhibitor. Avocado proteins in amounts of 100 µg (*lane b*) and 10 µg (*lane c*) clearly inhibit IgE binding to the NRL 14 kD band, whereas inhibition at the 21 kD band is less obvious. The strips are denoted as in Fig. 2.

The preincubation with 100 µg of NRL proteins (in a final volume of 750 µl) totally inhibited IgE antibody binding from all sera to nearly all avocado proteins. The only exceptions were the 68 kD avocado protein with one serum (no. 6), the 20 kD protein with another serum (no. 7), and the minor decrease in intensity of the 52 kD band with all sera. The preincubation with 10 µg of NRL proteins was sufficient to totally inhibit antibody binding to 34, 41, 43, 60, and 91 kD avocado proteins, whereas the 27, 52, 54, and 72 kD proteins showed varying degrees of loss of band intensity. There was no detectable inhibition at 20, 46, 49, and 68 kD bands. Results for two of the tested sera are shown in Fig. 2.

IgE antibodies of the same 10 sera from patients with latex allergy bound to a total of 14 NRL proteins with molecular weights ranging from 14 to 81 kD. Antibody binding to the band at about 14 kD was totally inhibited by 100 and 10 µg of avocado proteins, whereas inhibition at all the other bands occurred heterogeneously. Results for two patient sera are shown in Fig. 3.

RAST inhibition

In RAST inhibition, the binding of IgE antibodies to solid-phase NRL was shown to decrease when the sera were preincubated with soluble avocado proteins. The inhibition observed with both of the tested sera showed a clear dose-dependency (Fig. 4). Similarly, IgE binding to avocado disks was inhibited with NRL proteins in the first serum (no. 7) in a dose-dependent way (Fig. 4). In the second serum the level of IgE binding to avocado was too low to be detectable in inhibition studies. No significant inhibition (1% in latex RAST and 4% in avocado RAST inhibition, respectively) occurred with the control *Candida* extract.

Avocado prick test

Eleven patients underwent skin prick testing with fresh avocado fruit (prick-prick test).²¹ Seven patients had positive results (15-minute reaction compared with histamine [10 mg/ml] control), and four patients had negative results (Table I). The four patients with negative SPT results, however, had demonstrated crosswise inhibition of their IgE in immunoblotting.

DISCUSSION

Avocado (the fruit of the tropical tree, *Persea americana*, belonging to the family Lauraceae) and NRL from the Brazilian rubber tree (*Hevea brasiliensis*, family Euphorbiaceae) are not botanically related. Thus the observed immunologic cross-reactions between these two plants have been somewhat unexpected. Anaphylactic reaction to avocado has been reported in a patient with pre-existing allergy to NRL¹⁶ and vice versa.¹⁷ Lavaud et al.¹⁶ used a RAST inhibition technique to study cross-reactions between NRL and avocado proteins with serum from a patient with an assumed primary allergy to avocado and banana. The patient had experienced severe reactions to NRL, and IgE binding to a latex disk was inhibited by avocado extract. The inverse RAST inhibition did not reveal cross-reactivity, yet Ceuppens et al.¹⁷ reported an episode of anaphylaxis in a patient with latex allergy after ingesting avocado.

In this study, we demonstrated with the immunoblot and the RAST inhibition methods cross-reactivity between NRL and avocado proteins. Sera from 18 patients with latex allergy were used to detect cross-reacting components in immunoblot inhibition experiments. Fourteen (78%) sera had IgE antibodies that bound to 17 different avocado proteins with molecular weights ranging from about 20 to 91 kd. Soluble NRL proteins

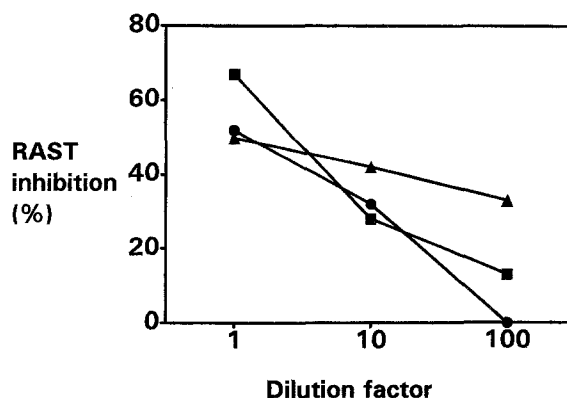


FIG. 4. Inhibition of latex RAST with avocado in serum number 7 (—■—) and serum number 15 (—●—) and inhibition of avocado RAST with latex in serum number 7 (—▲—).

inhibited, in a dose-dependent manner, binding of these antibodies to several of these avocado proteins. One hundred micrograms of NRL (in a final volume of 750 μ l) inhibited IgE binding, in all sera, to 15 of the 17 avocado proteins; and 10 μ g was able to detectably inhibit the binding to 13 of the proteins. The major cross-reacting proteins seemed to be the 27, 34, 43, and 91 kd proteins. When NRL was used as the solid-phase antigen, IgE binding to the 14 kd NRL protein was totally inhibited by avocado proteins at amounts of 100 and 10 μ g. The inhibition to other NRL protein bands occurred more heterogeneously. In agreement with these findings, a cross-wise RAST inhibition between NRL and avocado proteins was also demonstrated by using IgE antibodies from one patient with latex allergy.

Several patients in this series (notably, patients 4, 7, 10, 11, and 14) showed markedly elevated levels of total IgE, and it may be argued that such high levels could, in a nonspecific way, account for the binding of IgE to avocado. However, IgE immunoblot profiles of NRL and avocado were distinctly different in these sera, and there was no correlation between the total IgE levels and immunoblot positivity (Table I).

The sera used in the immunoblot and RAST inhibition studies were from patients whose clinical histories and laboratory tests were compatible with NRL allergy. A retrospective survey among 15 of these patients revealed that 12 had not experienced any adverse symptoms from contact with avocado. Nevertheless, the patients' IgE antibodies also bound strongly to both solid-phase and soluble avocado proteins, suggesting that considerable immunologic cross-reactivity between NRL and avocado allergens may exist. The great number of inhibitable protein

TABLE I. SPT and RAST results and IgE antibody binding in immunoblotting to some significant allergens in NRL and avocado in 14 patients with latex allergy

Patient No.	Sex	Age (yr)	SPT		Latex RAST (kU/L)	Total IgE (kU/L)	Immunoblot					
			Avocado	Latex			NRL			Avocado		
							14 kd	21 kd	43 kd	27 kd	34 kd	91 kd
1	F	42	2+	4+	2.3	797	—	—	+	+	+	+
2	M	7	ND	2+	0.9	282	—	—	—	(+)	(+)	—
3	F	12	ND	3+	<0.4	193	—	—	(+)	+	+	—
4	F	41	3+	3+	24.0	3,400	—	+	+	+	+	(+)
5	F	36	1+	2+	0.5	641	—	+	(+)	+	+	+
6	F	28	3+	4+	4.5	382	—	+	—	(+)	(+)	—
7	M	26	0	2+	6.3	17,200	+	+	+	+	+	+
8	F	40	0	4+	3.8	102	—	+	—	+	+	—
9	F	28	ND	3+	1.2	136	+	+	—	+	(+)	—
10	F	37	0	3+	65.6	16,040	—	+	+	+	(+)	+
11	F	27	2+	4+	21.9	7,075	—	+	—	(+)	(+)	—
12	F	33	3+	4+	20.0	396	+	+	+	(+)	(+)	—
13	M	34	0	3+	<0.4	256	—	—	+	+	+	+
14	F	28	3+	3+	13.1	2,867	—	+	—	+	—	—

SPT results are graded from the size of histamine (10 mg/ml) reaction as 4+ (twice), 3+ (the same), 2+ (half), 1+ (less than half), and 0 (negative).

Immunoblot reactions are graded as + (clear) band or as (+) weak but observable band.

ND, Not done.

bands indicates the presence of cross-reacting antigenic structures in most of the proteins and suggests that the number of strictly avocado- or latex-specific allergens may be small. These shared antigenic structures may be responsible for causing the reported type I hypersensitivity reaction in a patient with latex allergy when ingesting avocado.¹⁶ This view is also supported by one of our patients with latex allergy who had an anaphylactic reaction to avocado and showed RAST inhibition results compatible with cross-allergenicity of NRL and avocado proteins (Fig. 4). Two other patients (nos. 6 and 9, Table I) had experienced adverse oral symptoms after eating avocado. In agreement with these *in vitro* results, we could show positive avocado SPT results in seven of 11 of our patients with latex allergy. Interestingly, sera from four patients (nos. 7, 8, 10, and 13) with negative SPT responses to avocado showed crosswise inhibition of avocado-binding IgE. This discrepancy could be due to differences in the presentation of allergens in the SPT preparation and the extract used for immunoblotting.

These *in vitro* and *in vivo* results, as well as the reported case histories,^{16, 17} suggest that because of cross-allergenicity of NRL and avocado proteins, patients with latex allergy should avoid contact with avocado and banana, as we

and others have previously demonstrated.¹³⁻¹⁵ Whether immunologic cross-reactions also occur between avocado and banana remains to be shown. However, at present it seems reasonable that patients known to be allergic to avocado should be advised about the potential risks for developing allergic reactions from contact with NRL products.

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