

Short communication

Cross-reacting IgE antibodies recognizing latex allergens, including Hev b 1, as well as papain

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The cross-reactivity of IgE antibodies recognizing epitopes of latex allergens and papain was studied in sera of 36 latex-exposed subjects and 22 papain workers. Eight out of 24 latex-sensitized persons also showed a positive reaction to papain in the CAP assay (mostly of low or moderate degree). On the other hand, six out of the 12 sensitized papain workers also revealed IgE binding to latex allergen(s). Reciprocal inhibition experiments confirmed that groups monosensitized to one of the two allergens can be separated from a group showing partial or nearly complete immunologic cross-reactivity. Papain inhibited IgE binding by 20–33% in these subjects, whereas IgE binding to papain was strongly blocked in most cases. Comparison between the primary sequences of Hev b 1, a major latex allergen, and papain suggests that the cross-reactivity may be due to several identical trimers and tetramers.

X. Baur, Z. Chen, P. Rozynek, M. Düser, M. Raulf-Heimsoth

Professional Associations' Research Institute
for Occupational Medicine (BGFA),
Ruhr-University Bochum, Bochum, Germany

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X. Baur, MD
Professional Associations' Research Institute
for Occupational Medicine (BGFA)
Ruhr-University Bochum,
Bürkle-de-la-Camp-Platz 1
D-44789 Bochum
Germany

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Immediate-type sensitization to latex proteins presents a growing problem among health-care workers who frequently wear latex gloves (3–11). There is evidence of increased sensitization to fruit such as bananas, avocados, figs, and passion fruit (6) among affected subjects (see Slater's overview (9)). The cross-reactivity between latex components and these fruits implies the presence of common antigens or antigenic determinants. Yagami et al. (12) reported that one of the allergenic proteins, a 27-kDa protein, in latex gloves has lysozyme activity. They also found lysozyme activity in avocados, papayas, and figs which was attributed to mucopeptide *N*-acetylmuramoylhydrolase (EC 3.2.1.17). It remains to be clarified whether structural similarities of these enzymes are associated with cross-reactivity between latex and several fruit species.

Recent reports by Fisher (4) and Quarre et al. (7) assumed immediate-type allergic reactions to latex as well as to papain and IgE antibodies to both allergens in a few subjects and in one papain worker. Similar antibody patterns were observed in two latex-allergic subjects (7). The authors assumed immunologic cross-reactivity between these allergens.

Finding corresponding skin prick test results in three out of five examined latex-allergic subjects, we decided to investigate cross-reactivity by direct IgE antibody estimation as well as by EAST inhibition in workers clinically exposed and sensitized to latex or papain.

Material and methods

Twenty-two papain workers, 12 of whom suffered from immediate-type hypersensitivity, as well as 36 latex-exposed health-care workers including 23 clinically sensitized subjects, underwent detailed clinical investigations. Parts of the results obtained in individual groups have already been published (1, 2, 5). The control group consisted of three asthmatic bakers sensitized to flour. Table 1 summarizes exposure levels and clinical data on the examined subjects. Sera were frozen in aliquots at -20°C until investigation.

All sera were reinvestigated for IgE antibodies to latex, papain, and rye flour using the CAP-system (Pharmacia, Uppsala, Sweden) within one assay.

Reciprocal cross-reactivity was studied in inhibi-

Table 1. Demographic data on the three study groups

	Nonsensitized asymptomatic (n)	Immediate-type symptoms induced by		IgE antibodies to		
		Latex (n)	Papain (n)	Latex (n)	Papain (n)	Rye flour (n)
Health-care workers, latex-exposed (n=36)	12	23	—	24	8	1
Papain workers (n=22)	10	—	12	6	12	—
Controls, rye flour-sensitized bakers (n=3)	0	—	—	—	—	3

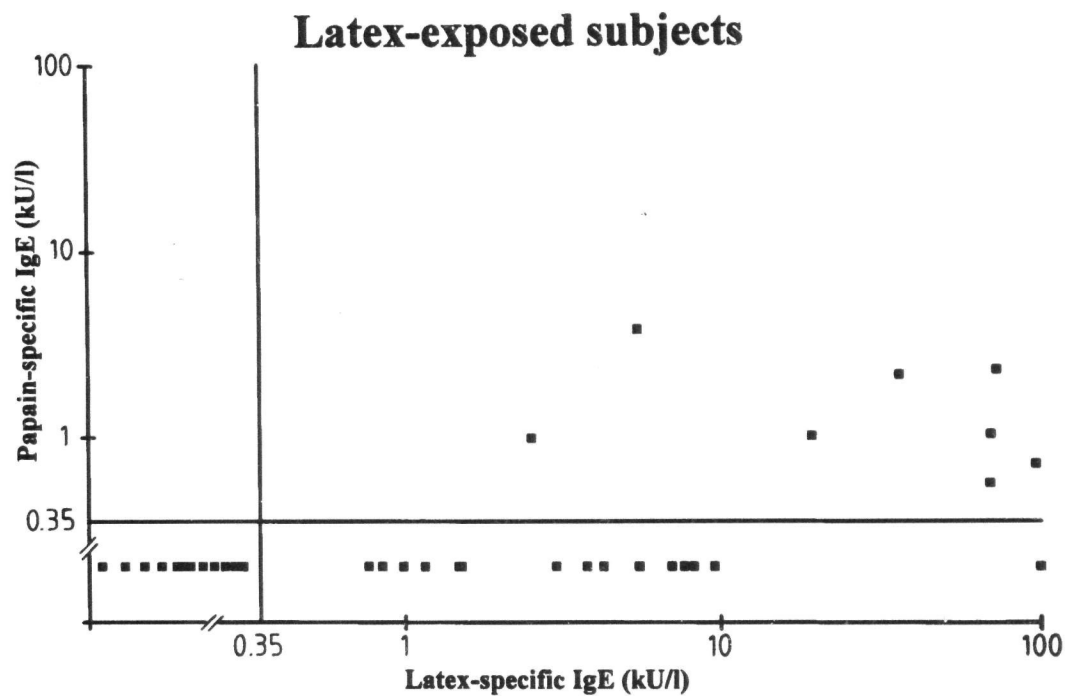


Fig. 1. Relation of IgE antibodies to latex and papain in 36 health-care workers (23 symptomatics).

tion experiments by preincubating patients' sera separately overnight with the two allergens in liquid phase. On the next day, IgE was measured as usual with the other allergen as solid-phase component.

Autoinhibition was shown to be always above 90% ($n = 5$).

The liquid-phase allergens were papain from Calbiochem (enzyme activity: 1000 milk clotting units/

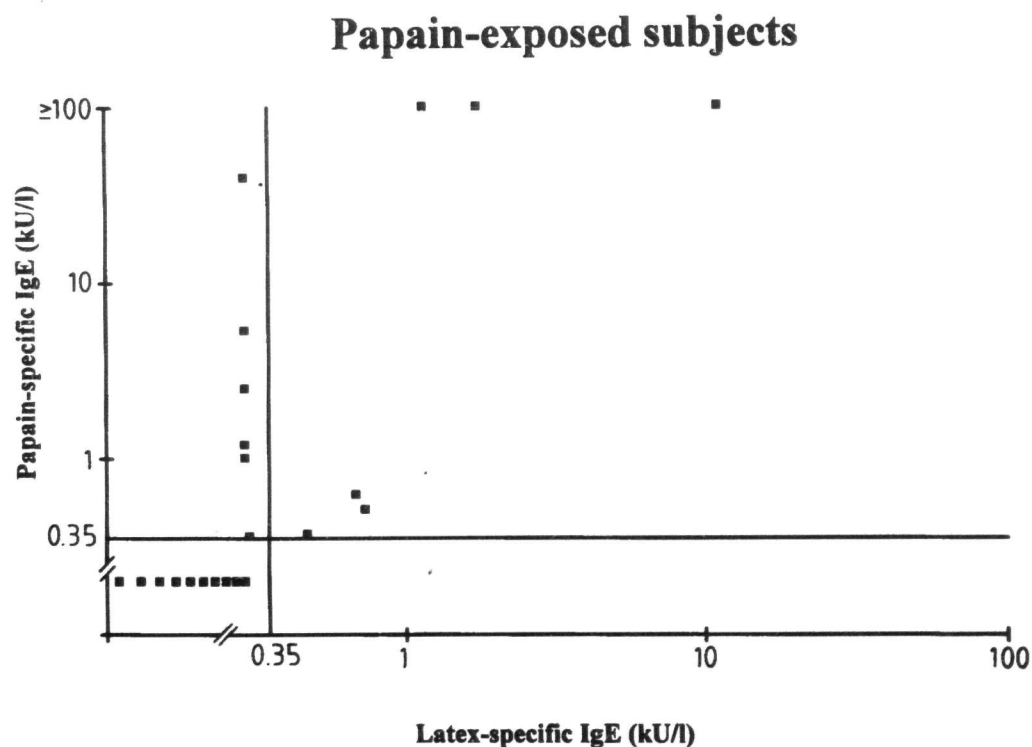


Fig. 2. Relation of IgE antibodies to latex and papain in 22 papain workers (12 symptomatics).

g; Bad Soden, Germany) and a protein extract from ammoniated Thailand latex sap prepared in our laboratory. If not otherwise stated, we used 10 µg of these inhibitors (on the basis of the protein content), solubilized in 10 µl PBS buffer which was incubated with 40 µl of undiluted or diluted patient's serum.

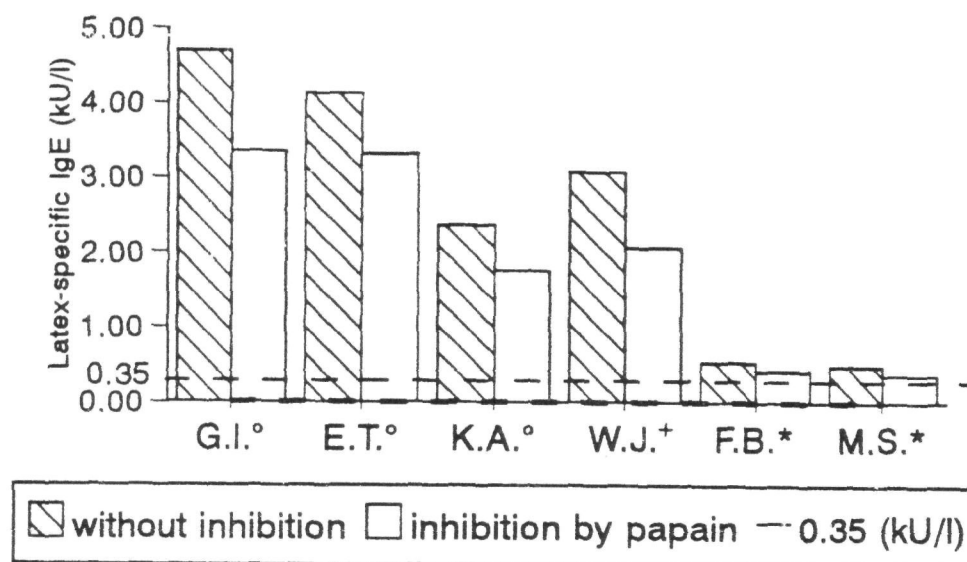
Reproducibility of CAP results was always $\geq 90\%$ and in CAP inhibition experiments $> 85\%$. Therefore, an inhibition of more than 15% was regarded as positive.

Results

Measurement of latex- and papain-specific IgE antibodies

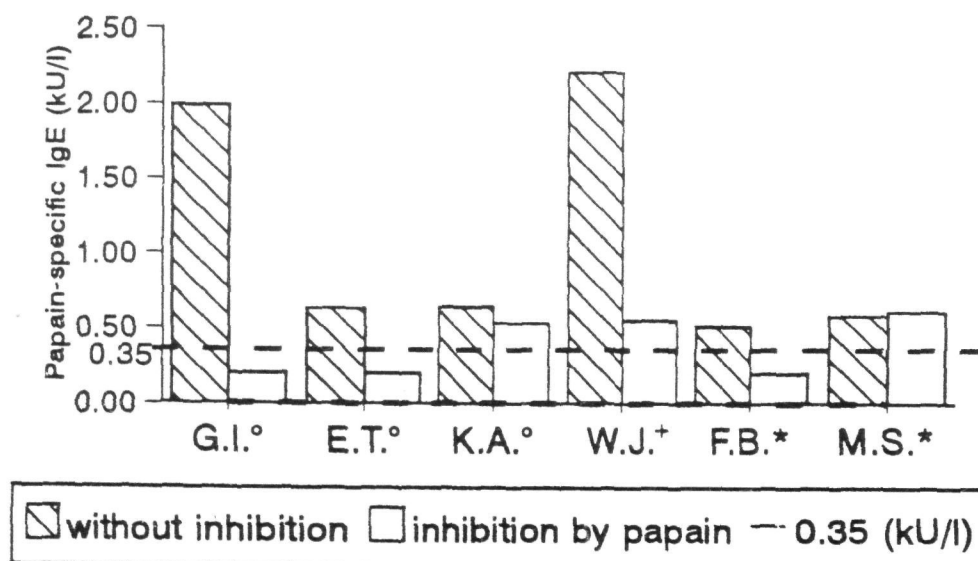
The latex-exposed group consisted of 36 subjects. Twenty-four of them were sensitized to latex, and 23 out of these 24 subjects showed clinical hypersensitivity to latex with preponderance of contact urticaria (5). One IgE-positive subject frequently wearing latex gloves, as well as the 12 IgE-negative

a) Inhibition of IgE binding to latex by 10 µg of papain Group: Latex- as well as papain-sensitized patients



- ° clinically sensitized to latex
- + atopic subject frequently wearing latex gloves without symptoms of latex hypersensitivity
- * clinically sensitized to papain

b) Inhibition of IgE binding to latex by 3.3 µg of latex protein Group: Latex- as well as papain-sensitized patients



- ° clinically sensitized to latex
- + atopic subject frequently wearing latex gloves without symptoms of latex hypersensitivity
- * clinically sensitized to papain

Fig. 3. Reciprocal inhibition of IgE binding (patients GI, ET, KA) to latex (a) or papain (b) in six individual sera from subjects sensitized to both allergens.

subjects, was asymptomatic. Eight persons of the latex-positive group ($n = 24$) also demonstrated IgE antibodies to papain and one to rye flour (Fig. 1 and Table 1).

Out of the 22 papain workers, the 12 symptomatic subjects revealed IgE antibodies to papain. Six were also sensitized to latex. However, their IgE levels were frequently much lower than those to papain (Fig. 2).

Investigation of IgE cross-reactivity to latex and papain by EAST inhibition

Subjects whose sera were available were assigned to four separate groups corresponding to their latex and/or papain IgE antibodies.

- 1) Patients with antibodies to latex and to papain: in all six investigated subjects (three latex-allergic, one with latex contact but without symptoms, and two papain-sensitized), the binding of IgE antibodies to latex was inhibited by papain in the range of 20–33% (Fig. 3a).

On the other hand, binding of IgE antibodies to papain was totally or almost totally blocked by latex in four out of these six cases; once there was a 17% blockage and once no inhibition was observed (Fig. 3b).

- 2) Latex-sensitized subjects without antibodies reacting to papain: these five latex-allergic subjects whose IgE antibodies did not directly bind to papain in the CAP assay showed no significant inhibition of IgE binding to latex by papain (Fig. 4).
- 3) Papain-sensitized subjects without antibodies reacting to latex: three workers exclusively sensitized to papain did not demonstrate a significant inhibition of IgE binding to papain by latex (Fig. 5).
- 4) Rye flour-sensitized bakers (latex- and papain-

Inhibition of IgE binding to latex by 10 μ g of papain
Group: Latex-sensitized patients

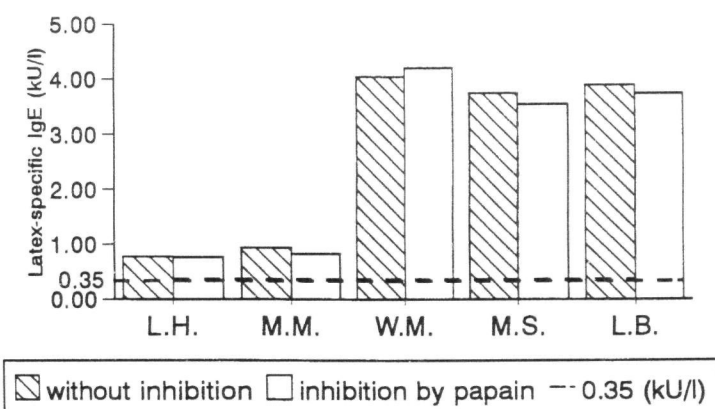


Fig. 4. Inhibition of IgE binding to latex by papain in sera of five latex-sensitized subjects.

Inhibition of IgE binding to papain by 3.3 μ g of latex protein
Group: Papain-sensitized patients

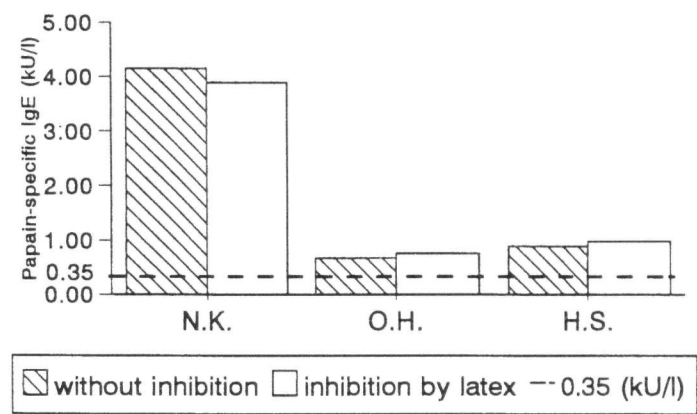
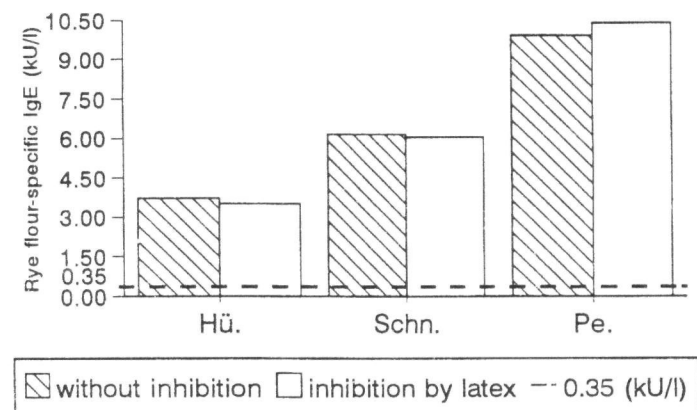


Fig. 5. Inhibition of IgE binding to papain by latex in sera of three papain-sensitized subjects.

negative control group): IgE binding to rye flour in these three asthmatic bakers was influenced by neither latex nor papain (Figs. 6a and b).

Dose-related reciprocal inhibition tests were also performed using a pool serum obtained from three patients with antibodies to latex and to papain (GI, ET, KA). The IgE binding to latex was inhibited by up to 31% with papain solution in a dose-dependent

Inhibition of IgE binding to rye flour by 3.3 μ g of latex protein
Group: Controls (rye flour-sensitized bakers)



Inhibition of IgE binding to rye flour by 10 μ g of papain
Group: Controls (rye flour-sensitized bakers)

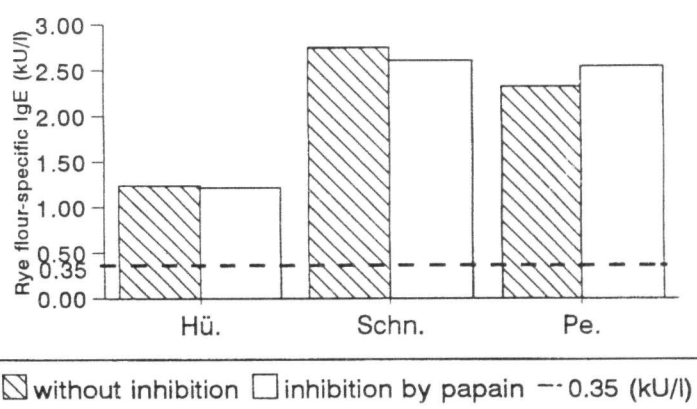


Fig. 6. Inhibition of IgE binding to rye flour by latex (a) or papain (b) in sera of three rye flour-sensitized bakers.

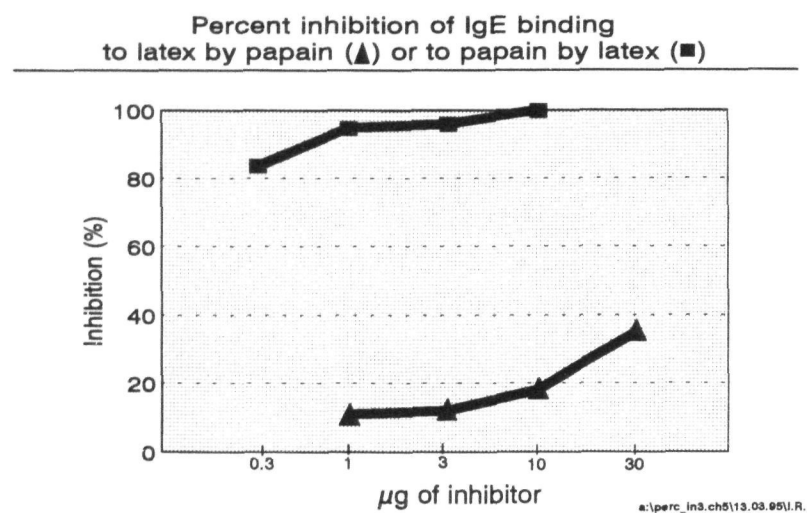


Fig. 7. Reciprocal inhibition of IgE binding to latex by papain (▲) or to papain by latex protein (■) in pool serum of three patients sensitized to both allergens. Autoinhibition by 3.3 µg of latex was 100% and by 10 µg of papain was 91% (see also Baur (2)).

manner. On the other hand, the IgE binding to papain was entirely blocked by 10 µg of latex protein (Fig. 7).

Discussion

Negative IgE results in all asymptomatic, exposed subjects, successful autoinhibition with latex or papain, and negative results in inhibition experiments with the unrelated allergen rye flour provide evidence of the specificity of IgE binding in our study.

Thirty-three percent (8/24) of patients in the group sensitized to latex and 50% (6/12) in the group sensitized to papain showed cross-reactivity with the other allergen not contacted. This was shown in direct IgE antibody estimation. Corresponding results were obtained in EAST inhibition experiments. IgE antibodies of subjects exclusively sensitized to one of the two allergens could not be blocked by the other allergen.

These results provide evidence of one or several major papain epitopes which are also present in the latex protein extract. However, latex epitopes are mainly different from papain epitopes. Additional experiments with one of the major latex allergens, Hev b 1, produced in two latex-allergic subjects results similar to those with the latex protein fraction (reciprocal inhibition with papain ranged from 37% to 42%). When we compared the primary structures of Hev b 1 (137 amino acid residues) and papain (211 amino acid residues), five identical trimers and two identical tetramers could be identified (Table 2).

The two allergens originate from different plant genera, i.e., papain from *Violales* (subclass *Dilleniidae*) and latex from *Euphorbiales* (subclass *Rosidae*). In view of several morphologic similarities, a joint pedigree is probable.

In conclusion, our results prove partial IgE cross-reactivity between latex allergens including Hev b 1 and papain which probably occurs on account of several short-sequence identities. However, subgroups of exposed subjects do not demonstrate cross-reactivity, suggesting recognition of other non-identical regions.

Our results do not clarify whether immunologically proven cross-reactivity is of clinical significance. No subject of our groups had major contact with both allergens, but minor contact with latex-made articles as well as papain-containing fruit, especially juice mixtures, cannot be excluded. However, Quarre et al. (7) argue for the clinical relevance of cross-reactivity in one case. In the future, more detailed case histories and appropriate challenge tests should be performed to clarify the clinical relevance of these experimental findings.

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Table 2. Identical tetramers and trimers in papain and Hev b 1

Hev b 1	55	PVKN	58	55	PVKN	58	27	AVT	29	52	IEG	54	73	GAL	75	113	EAA	115
Papain	15	PVKN	18	209	PVKN	212	12	AVT	14	34	IEG	36	119	GAL	121	135	EAA	137

Numbers indicate amino acid positions in primary structure of Hev b 1 and papain, respectively.

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