

Glove-Derived Foreign Proteins Induce Allergen-Specific IgE in a Mouse Model

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Currently, most medical gloves are produced with a low content of natural rubber latex (NRL) protein. However, they may be substituted by proteins of foreign origin to maintain specific properties of the material. The aim of this study was to investigate the allergenicity and immunogenicity of unexpected proteins (i.e., soy and casein) compared with NRL proteins in a murine model in BALB/c mice. All respective allergen sources (extracts from three brands of NRL gloves, soy, and casein) were able to induce significant allergen-specific IgE and IgG₁ responses. On average, the highest IgE induction occurred after immunization with NRL, followed by soy and casein. Certain individuals from each treatment group exhibited levels of specific IgE as high as due to NRL. To analyze further specific IgE responses on a single allergen level, we established a microarray based on recombinant allergens for allergen-specific murine IgE detection. Besides specific IgE against rHev b 3, -6, -7, -8, and -11, specific IgE against κ -casein could be detected in mice immunized with NRL glove extract, indicating a sensitization potential of the contained foreign protein. The substitution of genuine latex proteins by proteins of foreign origin may lead to a shift and *de novo* increase in sensitization to the finished products.

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INTRODUCTION

Owing to an HIV-associated increase in the use of medical gloves for single use, the prevalence of individuals allergic to natural rubber latex (NRL) allergens has significantly increased among health-care workers (HCWs) during the last decade of the previous century (Turjanmaa, 1987; Arellano *et al.*, 1992). NRL allergens bound to powder particles constituted occupational inhalative allergens that were easily propagated by air and were accessible to airway sensitization by inhalation.

By regulations (approved code of practice (TRGS) 540 (sensitizing substances)) powdered latex gloves were banned from the work environment in December 1997 and the amount of leachable protein had been limited to <30 μ g/g glove (recommendation by the Accident Prevention & Insurance Association for Health Care Workers; BGW) in Germany. Consequently, a decrease in annual incidence rates of sensitization to NRL and a decrease in suspected and

proved cases of occupational contact urticaria caused by NRL was observed (Allmers *et al.*, 2004; Mahler, 2007).

Unlabeled foreign proteins (e.g., casein) could be found in NRL gloves (Ylitalo *et al.*, 1999) and may improve the material properties of the finished product. However, substitution of genuine latex proteins and exposure to unexpected proteins of foreign origin may lead to a shift in sensitization profiles and an increase in allergic reactions elicited by the finished products.

To evaluate the allergenicity and immunogenicity of unexpected proteins compared with NRL proteins, we established a murine model in BALB/c mice.

RESULTS

Protein contents of NRL gloves

Total protein content of the analyzed glove brands ($n=16$), casein, and soy content are displayed in Table 1. The total protein content varied widely (4–1,018 μ g/g glove) among the different brands. Casein could be detected in the range between 1 and 10 μ g/g glove and was not in parallel to the total protein content. In certain gloves (e.g., no. 14 and 15), casein seems to be the relevant contributor to the total protein. Soy protein was only detected in traces.

IgE responses and time course of IgE reactivity to immunizations with NRL glove extracts, casein, and soy extract

To determine the murine-specific IgE to NRL, casein, and soy, we first used a conventional *in vitro* detection system (ELISA) to the natural proteins. All measurements of all serum samples of each animal ($n=10$ per group) were performed in duplicate and are graphically presented as means

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Abbreviations: HCW, health-care worker; NRL, natural rubber latex; PBS, phosphate-buffered saline; RT, room temperature

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Table 1. Total protein, casein, and soy content (µg/g glove) of different glove brands (no. 1–16) as determined by Lowry assay or ELISA, respectively

Glove no.	Type	Total protein (µg/g glove)	Casein content (µg/g glove)	Soy content (µg/g glove)
1	S	648	3.31	0.19
2	E	198	9.24	0.14
3	E	421	1.95	0.1
4	E	252	4.43	0.17
5	S	103	3.03	0.12
6	E	118	3.02	0.05
7	E	43	4.85	0.06
8	E	22	1.68	0
9	S	31.8	2.86	0.01
10	E	20	1.96	ND
11	E	737	3.48	0.23
12	E	120	4.58	0.06
13	E	1,018	5.62	0.3
14	S	4	4.34	ND
15	S	7	4.13	ND
16	E	22	1.4	ND

E, examination glove; ND, not done; S, surgical glove.

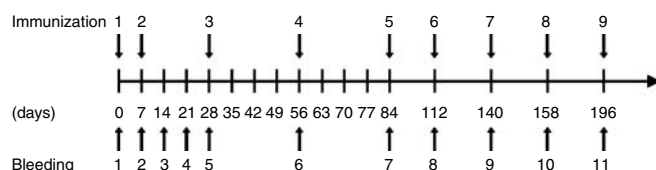


Figure 1. Scheme of immunization. Six groups of mice ($n = 10$ per group) were subcutaneously immunized in the neck with either aluminiumhydroxid-adsorbed latex, casein, soy extracts, or adjuvant only.

(Figure 1). As reported previously (Mahler *et al.*, 2000), latex-specific IgE responses were detectable 4 weeks after the first latex immunization (i.e., fifth bleeding, day 28 (Figure 1)). The increase continued until the ninth bleeding (day 140) and varied among the different brands (Figure 2). Statistical significance ($P < 0.001$) of latex-specific IgE antibody induction in comparison to the adjuvant control group was detectable in mice immunized with extracts from glove no. 3 and 13 from day 56 (sixth bleeding, i.e., 4 weeks after the third immunization), in mice immunized with glove no. 2 not earlier than day 140. The latex-specific IgE response induced by glove no. 13, containing a high protein content, was at the same time statistically significant ($P < 0.001$) over the response induced by the two other glove extracts. In the casein- and soy-treated groups, no latex-specific or cross-reacting IgE antibodies could be determined. Latex-specific IgG₁ induction was statistically significant ($P < 0.001$) from day 14 (glove no. 3 (maximum at day 84) and day 56 (glove

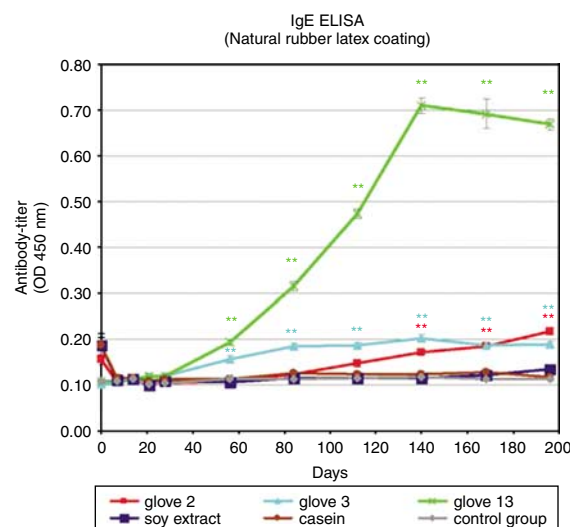


Figure 2. ELISA for latex-specific murine IgE. ELISA plates were coated with extracts of glove no. 3. Mean values of each treatment group are displayed. Level of significance ($P < 0.001$) of latex-specific IgE induction is displayed as **.

no. 13 (maximum at day 140) and glove no. 2 (maximum at day 112), respectively (data not shown).

Immunization with casein from bovine milk led to a significant allergen-specific IgE induction ($P < 0.001$) at day 84 as well as significant IgG₁ induction from day 84 to the end of study, with the highest antibody levels at the end of the study (Figure S1). None of the other treatment groups mounted casein-specific IgE or IgG antibodies.

In the active treatment group immunized with soy extract, statistically significant soy-specific IgE induction ($P < 0.001$) was observed from day 28 to the end of study with a maximum of the antibody level at the end of the study (Figure S2). Soy-specific IgG₁ antibodies were detectable from day 56 to the end of study, with a maximum of antibodies at day 84. None of the other treatment groups developed soy-specific IgE or IgG antibodies, which demonstrates that there was no cross-contamination (i.e., in the food of mice).

Determination of total serum IgE in the distinct treatment groups

Induction of total serum IgE as determined by BD OptEIA™ in murine-pooled blood of each treatment group is in parallel to specific immunizations (Figure 3). Owing to the lack of other influence factors, it can be assumed that this increase of total IgE is mainly caused by the rise of specific IgE antibodies induced by the specific immunizations. The earliest and most predominant rise in total IgE can be observed in the pooled sera of mice immunized with gloves no. 3 and 13, whereas total IgE levels in other treatment groups increase more slowly, but constantly, to reach comparable levels at the end of study (day 196). At day 196, the total IgE level is highest in the treatment group that had received extracts from glove no. 13. Identical IgE levels are found in the pooled sera of mice treated with gloves no. 2 and 3 as well as those treated with soy extract, followed by the total IgE level of casein-treated mice.

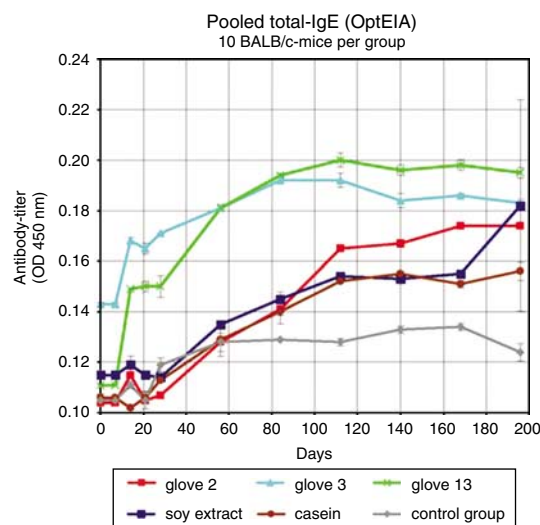


Figure 3. Determination of total serum IgE in the distinct treatment groups.

Induction of total serum IgE in parallel to specific immunizations is demonstrated in murine pooled blood of each treatment group.

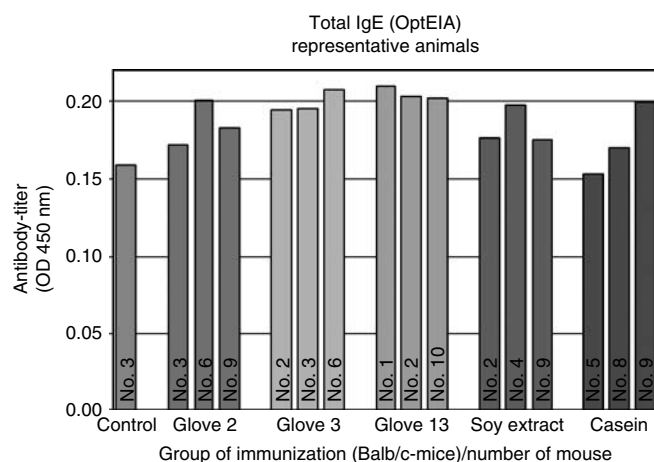


Figure 4. Total IgE of representative animals. Although the soy and casein immunized groups showed lower amounts of total IgE antibodies in pooled blood, certain mice achieve similarly high IgE levels as induced by NRL latex extracts.

Certain individuals of each group exhibited stronger IgE levels than their peers from the same treatment group. These high responders of all immunization groups showed comparable IgE levels (Figure 4).

Microarray analysis of allergen-specific murine IgE binding

To determine the individual component-resolved sensitization profile (Heiss *et al.*, 1999; Wagner and Breiteneder, 2005) in the respective treatment groups, a recombinant allergen-based microarray for the detection of murine IgE antibodies was established. Recombinant latex allergens and native casein, as well as α -, β -, and κ -casein were bound to the solid phase of the chip. Representative animals of each treatment group were examined for their component-resolved sensitization (Table 2). Depending on the glove brand used

for immunization, specific murine IgE against rHev b 3, rHev b 6, rHev b 7, rHev b 8, and rHev b 11 could be detected (Table 2). Furthermore, a relevant amount of casein-specific IgE could be detected in the serum of an individual (IV-1) who had exclusively received NRL glove extract (glove no. 13).

DISCUSSION

During the last decade of the previous century, the predominant route of sensitization to NRL in HCW used to be the inhalant route due to powder-bound latex components, whereas Spina bifida patients were sensitized during surgical interventions. However, due to regulatory interventions in Germany (and other European countries) including the ban of powdered gloves and production of disposable gloves of low NRL protein content, production modalities, composition of glove material (including unexpected proteins to maintain material properties) as well as the exposure of HCWs have changed.

It is not commonly known that cow's milk casein can be added as a stabilizer during glove manufacturing (Ylitalo *et al.*, 1999). In the analyzed set of 16 different gloves brands, we found casein contents from 1 to 10 $\mu\text{g/g}$ glove (Table 1) and could confirm the earlier findings of casein additives in the finished products (Ylitalo *et al.*, 1999). In the previous report from Finland (Ylitalo *et al.*, 1999), the clinical relevance of the addition of casein could be demonstrated by positive *in vivo* IgE reactivity to the finished NRL glove product in cow's milk allergic and NRL skin prick test- and radioallergosorbent test-negative patients.

Although according to European norm 1041 (DIN EN 1041, 1998) information has to be supplied by the manufacturer, a complete labeling of all ingredients contained in finished latex products is not given. However, complete labeling constitutes a prerequisite for allergy prevention.

The substitution of genuine latex proteins by proteins of foreign origin may lead to a shift and an increase in sensitization to extraneous allergenic components contained in NRL gloves.

In our mouse model, we could demonstrate the *in vivo* allergenicity of unexpected protein sources of foreign origin (e.g., casein and soy) compared with NRL glove extracts. All protein preparations of the different allergen sources were capable of inducing highly significant levels ($P < 0.001$) of allergen-specific IgE. No crossreactivity of latex-induced IgE (Figure 2), casein, or soy antibodies nor cross-contamination (Figures S1 and S2) was observed. Although the dynamics of the IgE induction as well as of the IgG1 induction varied from allergen source to allergen source, in the end of the study, comparable amounts of total IgE were observed in all treatment groups (Figure 3) with the lowest IgE levels in the casein-treated group. Casein (Bos d8) is composed of four proteins (α s1-, α s2-, β -, and κ -casein) (Elsayed *et al.*, 2004). Although the four proteins do not share many structural similarities, most patients with cow's milk allergy exhibit specific IgE to all of them (Wal, 2001). Compared with casein, the composition of latex and soy extracts is more

Table 2. Microarray analysis of allergen-specific murine IgE binding

Mice	Adjuvant control	Glove no. 2	Glove no. 2	Glove no. 2	Glove no. 2	Glove no. 3	Glove no. 13	Glove no. 13	Glove no. 13	Casein	Casein	Buffer
	I-2	II-1	II-2	II-3	II-6	III-2	IV-1	IV-3	IV-4	IV-8	IV-9	
α -Casein	0	0	0	0	0	0	0	0	0	0	1,546	0
β -Casein	0	0	0	0	0	0	0	0	0	0	1,433	0
κ -Casein	0	0	0	0	0	0	3,168	0	0	5,670	14,163	0
Casein	0	0	0	0	0	0	0	0	0	738	4,829	0
rHev b 3	0	15,225	11,325	1,595	14,471	0	24,587	5,981	31,777	0	0	0
rHev b 5	0	0	0	0	0	0	0	0	0	0	0	0
rHev b 6	0	0	0	2,268	0	9,717	0	0	0	0	0	0
rHev b 7	0	0	2,331	0	0	0	0	0	0	0	0	0
rHev b 8	0	0	0	0	0	0	2,231	0	5,524	0	0	0
rHev b 9	0	0	0	0	0	0	0	0	0	0	0	0
rHev b 10	0	0	0	0	0	0	0	0	0	0	0	0
rHev b 11	0	0	0	1,780	0	1,263	0	0	0	0	0	0

FI, fluorescence intensity.

Component-resolved sensitization profiles of representative animals examined by microarray analysis are displayed. The respective immunization is displayed above the identification of the individual animal. Depending on the glove brand used for immunization, rHev b 3, rHev b 6, rHev b 7, rHev b 8, rHev b 11, and casein are recognized by the specific murine IgE antibodies. FI values ≥ 500 are displayed.

complex, containing multiple IgE-binding components that may contribute to the total allergenicity of the allergen source. For soy, at least 28 soy IgE-binding proteins have been described (Awazuha *et al.*, 1997; Cordle, 2004), and for NRL, at least 13 identified allergens and more than 200 polypeptides (Posch *et al.*, 1998; Wagner and Breiteneder, 2005) with a predominance of nHev b 6 in *Hevea brasiliensis* serum have been described (Yeang *et al.*, 2006).

In our microarray for component-resolved detection of specific murine IgE, we were able to analyze the induced *in vivo* allergen-specific immune responses individually on the single-molecule level (Table 2). Individual animals exhibit individual sensitization profiles to the glove-derived allergenic components. Depending on the glove brand used for immunization, specific IgE against rHev b 3, rHev b 6, rHev b 7, rHev b 8, rHev b 11 as well as κ -casein-specific IgE could be detected in the murine sera (Table 2). Specific IgE raised against rHev b 5, rHev b 9, and rHev b 10 were not detected. Hev b 1 and b 3 are known as major allergens in latex-allergic *Spina bifida* patients, whereas Hev b 5 and b 6 have been recognized as major allergens in HCW. However, with a prevalence of 7–32% in HCW and latex-allergic (not SB) patients, Hev b 3 also constitutes a minor allergen in these patients (Wagner and Breiteneder, 2005). Influences of the route of exposure on sensitization profiles to allergens have been described in BALB/c mice (Woolhiser *et al.*, 2000). However, in contrast to the results of this report based on immunization with a single non-ammoniated latex extract and immunoblots of pooled sera ($n=5$), by microarray we could demonstrate an individual and more complex IgE induction to several allergenic components including rHev b 6 depending on the allergen source administered by subcutaneous route.

The induction of comparable amounts of specific IgE to NRL, casein, and soy (Figures 3 and 4) as well as the induction of specific IgE to κ -casein (individual IV-1 Table 2) after immunization with a glove-derived extract (glove no. 13) demonstrates the risk of *de novo* sensitization against foreign protein sources added to NRL during the manufacturing process. The amount of κ -casein-specific IgE ranges between the levels of specific IgE induced against certain genuine latex allergens such as Hev b 3 and Hev b 8 (individual IV-1, Table 2).

This is in concordance with our finding that the amount of total IgE induced in parallel with the specific immunizations in certain individuals is higher than in their peers and in high responders similar IgE levels can be observed after immunization with the NRL extracts, casein, or soy, respectively (Figure 4).

On the basis of our findings, we conclude that unlabelled substitution of NRL products with proteins from foreign allergen sources constitutes a health threat with regard to *de novo* sensitization as well as elicitation of pre-existing immediate-type allergies against these independent allergen sources.

As a contribution to primary and secondary allergy prevention, a substitution of allergenic NRL proteins by other allergenic proteins from foreign allergen sources should be avoided. However, if this substitution cannot be entirely avoided due to material properties, complete information on the composition of the glove material, including these unexpected proteins, should be mandatory.

MATERIALS AND METHODS

Protein extraction of NRL gloves and protein quantification

Sixteen lots of different medical gloves were examined by extraction of eight randomly sampled gloves from each lot according to European norm 455-3 (DIN EN 455-3, 2000-02).

The protein contents of the extracts were measured by the modified Lowry assay (Lowry *et al.*, 1951) following precipitation of interfering substances. In brief, for precipitation, 100 µl of 0.15% (w/v) sodium desoxycholate was added to 1 ml of each extract sample incubated at room temperature (RT) for 30 minutes. Subsequently, 100 µl of 72% (w/v) trichloroacetic acid and 100 µl of 72% (w/v) phosphotungstic acid were added and the samples incubated at RT for 30 minutes, thereafter centrifuged for 10 minutes at 20,800 U/minute, the supernatant discarded, and the pellet dissolved in 200 µl of 0.1 M NaOH (yielding a fourfold concentration of each sample). Protein was determined using the DC protein assay (Bio-Rad Laboratories, Hercules, CA). Each sample was tested in duplicate using four twofold serial dilutions. The optical density was read at 620 nm and the concentration of protein determined referring to the ovalbumin protein standard.

Casein and soy quantification in NRL glove extracts and protein preparation for immunization

Casein content in NRL glove extracts was determined using the Casein Assay Kit (Tepnel, Biosystems, Flintshire, UK) according to the manufacturer's instructions. In brief, 100 µl of diluted glove extracts were incubated in microwells coated with anti-casein antibody at RT. Plates were washed four times with 400 µl Assay Diluent between the respective incubation steps with 50 µl casein-biotin reagent, 50 µl avidin-horseradish peroxidase, and 50 µl tetramethyl-benzidine substrate, and then read at 450 nm (Titertek; Labsystems, Frankfurt a. M., Germany).

The intensity of the blue color reaction inversely correlates to the amount of casein present in the glove sample. Comparison with a set of casein standards allows the calculation of the casein level in the original glove sample.

For quantification of soy content, ELISA plates were coated in duplicate with 10 µg protein in 100 µl glove extract (10 µg per well) and a serial dilution of a soy standard (1:16–1:512) overnight at 4°C.

After two washes with phosphate-buffered saline (PBS)/0.05% Tween 20 and a 2 hour-blocking step with PBS containing 1% BSA (200 µl per well) at RT, a rabbit anti-soy antibody (S 2519; Sigma-Aldrich, Steinheim, Germany) at a dilution 1:2,000 in PBS/0.05%/Tween 20/0.5% BSA was added overnight at 4°C.

Thereafter, a peroxidase-labeled donkey anti-rabbit antibody (NA 934, Amersham, Freiburg, Germany) was added at a dilution 1:5,000 in PBS/0.05% /Tween 20/0.5% BSA overnight at 4°C. Next day, 100 µl 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) substrate (Sigma, St Louis, MO) was added. The optical density corresponding to the amounts of bound antibodies were determined at 450 nm (Titertek; Labsystems).

For immunization, casein from bovine milk (purified powder, Sigma-Aldrich) was suspended in aqua bidest, under shaking for 3 hours at 4°C. Soy extracts were gained from isolated soybean protein (ICN Biomedicals Inc. Aurora, OH) suspended in aqua bidest and shaken for 3 hours at 4°C and centrifuged at 4,000 × g (30 minutes at 4°C) to remove insoluble particles. For analysis of protein content, protein extracts (125 µg per cm gel) were separated by analytical 12.5% SDS polyacrylamide gel (Fling and Gregerson, 1986) and visualized by Coomassie Blue staining (Bradford, 1976). A Rainbow Marker (Amersham, Buckinghamshire, UK) was used as molecular weight standard. Protein content was further analyzed by Lowry assay (Lowry *et al.*, 1951). Aliquots of 1 ml were stored at –20°C until use.

Immunization of mice

Sixty 8-week-old female BALB/c mice were purchased from Charles River (Kislegg, Germany). The animals receiving a soy-free diet were maintained in the animal care unit of the Department of Dermatology of the University Hospital Erlangen according to the local guidelines for animal welfare. As a sensitization protocol for the mouse model a subcutaneous route was chosen, since due to ban of powdered gloves (in Germany and other European countries) the inhalant sensitization route especially in HCW no longer represents the predominant sensitization route. Six groups consisting of 10 mice each were subcutaneously immunized in the neck with adjuvant adsorbed extracts obtained from three different glove brands (glove no. 2, 3, and 13), soy, or casein on days 7, 14, and 28, consecutively in 4-week intervals. For immunization representative glove brands were chosen: no. 2 with an intermediate content of total protein and a high content of casein; no. 3 with an intermediate content of total protein and a low content of casein and no. 13 with a high content of total protein and an intermediate content of casein.

Each injection contained 10 µg protein adsorbed to 200 µl 2% Al(OH)₃ (AluGel-S; Serva, Heidelberg, Germany). As a negative control, one group obtained the adjuvant Al(OH)₃ containing 20 µl of aqua bidest instead of protein extract. Blood samples were obtained from the eye veins shortly before the first immunization (preimmune serum) and subsequently before each consecutive immunization (Figure 1).

Total serum IgE assay (BD OptEIA™)

For measurement of total murine IgE, the OptEIA™ mouse IgE Kit (BD Biosciences PharMingen, Heidelberg, Germany) was used according to the manufacturer's instructions.

Briefly, wells of 96-well Nunc-Maxisorb microtiter plates (Nunc, Roskilde, Denmark) were coated with 100 µl of anti-mouse IgE mAb at a 1:250 dilution in 0.1 M carbonate buffer (pH 9.5) overnight at 4°C. Plates were washed with PBS/0.05% Tween 20 and blocked with PBS with 10% fetal bovine serum (Assay Diluent, BD PharMingen, Heidelberg, Germany). Serial dilutions of murine IgE standard and serum samples in 10% fetal bovine serum in PBS were added to the wells in duplicate. After 2 hours at RT, the plates were washed and incubated with biotinylated anti-mouse IgE mAb and avidin-horseradish peroxidase reagent for 1 hour at RT. The plates were read at 450 nm (Titertek; Labsystems).

ELISA measurements

Mouse IgE and IgG₁ antibodies from each individual and time of bleeding were detected by ELISA as described previously (Mahler *et al.*, 2000). In brief, ELISA plates (Nunc-Maxisorb, Nunc) were coated with 100 µl PBS containing 10 µg of the respective extracted protein (NRL, soy, or casein) per well overnight at 4°C. Plates were washed twice with 200 µl PBS/0.05% Tween 20 and incubated with 200 µl blocking solution (PBS/0.05% Tween 20/1% BSA) for 2.5 hours at RT. Plates were then incubated with 100 µl PBS-diluted mouse sera per well overnight at 4°C. For IgE measurements, sera were diluted 1:20, for IgG₁ measurements 1:1,000 in PBS/0.05% Tween 20/0.5% BSA. After five washes with 200 µl PBS/0.05% Tween 20, bound mouse IgE and IgG₁ antibodies were detected with purified monoclonal anti-mouse IgE or IgG₁ antibodies from rat (PharMingen), which were diluted 1:1,000 in PBS/0.05% Tween 20/0.5% BSA incubated overnight at 4°C. Plates were washed five times

with 200 µl PBS/0.05% v/v Tween and bound rat antibodies were detected with a peroxidase-coupled anti-rat Ig anti-serum from goat (Amersham Pharmacia Biotech Europe, Freiburg, Germany) and visualized with 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) substrate (Sigma, St. Louis, MO). The optical density corresponding to the amounts of bound antibodies was determined at 450 nm (Titertek; Labsystems). Possible plate-to-plate variabilities were excluded by including reference sera on each of the different plates. All measurements were performed in duplicate which did not differ from each other more than 5%. Statistical analysis was performed as described below. The time course of the measurements is presented graphically.

Protein microarray for allergen-specific IgE detection

A customized, commercially available allergen microarray (ISAC™ version CRD 51, VBC Genomics Bioscience Research, Vienna, Austria) was employed that has been shown to yield reliable analytical results when compared with conventional fluorescence enzyme immunoassays in other clinical and research settings (Hiller *et al.*, 2002; Jahn-Schmid *et al.*, 2003; Wöhrl *et al.*, 2006).

The device consisted of a microscopy glass slide modified with a Teflon™ mask to create four individual reaction sites coated with amine-reactive polymers allowing covalent immobilization of 12 purified natural or recombinant allergen components (rHev b 3, rHev b 5-11, native α -, β -, and κ -casein) spotted onto the microarray slide in vertical triplicates (Table 2).

Microarray immunoassays were performed according to the manufacturer's recommendations as recently published (Deinhofer *et al.*, 2004; Harwanegg and Hiller, 2005), using different secondary/tertiary antibodies to detect murine IgE. Briefly, each microarray reaction site was incubated with 20 µl of undiluted murine serum for 180 minutes to capture allergen-specific serum IgE antibodies by their corresponding allergen molecules. In a second step, the microarray slides were washed with Tris-buffered saline/Tween (TBST) buffer solution twice for 5 minutes, rinsed with deionized water, and dried under nitrogen flow. Hereafter, microarray-bound IgE was marked by co-incubation with a secondary, fluorescence-tagged rat anti-mouse IgE antibody (PharMingen) at a concentration of 1 µg ml⁻¹ for 60 minutes at RT and a fluorochrome-labeled goat anti-rat IgG antibody at a concentration of 4 µg ml⁻¹ (Alexa Fluor546; A11081; Invitrogen, Carlsbad, CA) as tertiary antibody.

After a subsequent washing procedure with TBS-T, the corresponding fluorescence signals were scanned at a 10 µm resolution using a conventional biochip reader (Scan Array Express™, Perkin Elmer Life Sciences, Boston, MA) and dimensionless fluorescence intensity values were measured.

Statistical analysis

Data entry was done using Microsoft (TM) Excel 97, the statistical evaluation of the data was performed with Statistical Analysing System (SAS Inst. Inc., Cary, NC) version 8.2. Win. The distribution of the ELISA-measured values by group and time was described using mean and standard deviation, possible differences between the groups were calculated using repeated measurement analysis of variance technique. Level of significance, α , was set to 5%. The course over time was visualized graphically.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Figure S1. ELISA for casein-specific murine IgE.

Figure S2. ELISA for soy-specific murine IgE.

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