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Latex allergy: characterization of major allergens and isotypic expression

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Latex allergy is now known to be related to type I hypersensitivity. In latex products, several reports have identified at least 10 allergens. However, 56 polypeptides have been shown to react to IgE by two-dimensional gel electrophoresis and immunoprints. All studies using immunoprints have analyzed a relatively small number of sera. The aim of the present study was to analyze the specificity and the isotypic expression of a wide sample of latex-sensitive patients. Latex proteins were separated by SDS-PAGE in an 8–18% gel gradient, transferred onto a nitrocellulose sheet by Western blot, and revealed by serum from latex-sensitive patients. The presence of IgE, IgA, IgG1, IgG2, IgG3, and IgG4 was analyzed for each of the 54 sera. The results showed Hev b 3 to be the most frequently recognized protein, followed by Hev b 1, Hev b 7, and molecules of 94 kDa and 6–8 kDa. The most common isotypic association was IgE-IgG1, frequently with IgA. IgG4 was also present in a little less than half of the IgE-positive sera. IgG3 was also expressed but frequently recognized nonallergenic molecules. IgG2 was present in only two cases. Thus, as several authors have proposed a protective role for IgG in the allergic reaction, it may be of interest to compare these results with the course of the disease.

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Type I hypersensitivity to natural rubber latex proteins is a well-known health problem. Multioperated children – especially those with spina bifida – health-care workers, and rubber-industry workers are high-risk populations (1–7). However, a study of volunteer blood donors has shown that about 6% of the blood samples contained anti-latex IgE (8). Previous studies have described the various allergens recognized by spina bifida or health-care worker patients

(1, 9–14), and at least 10 proteins or polypeptides have been identified and characterized as latex allergens (15). Recently, using two-dimensional gel electrophoresis and immunoprints, Kurup et al. (16) have found that 79 polypeptides from latex were recognized by the IgG from different latex-sensitive patients, while IgE reacted to only 56 allergens. All the studies using Western blot techniques were performed on a limited number of patients, rendering difficult the identification of major allergens.

In studying the allergenic response to different allergens, some authors have proposed a protective role for IgG antibodies (17–19), while others have mentioned the possible anaphylactic role of IgG4 (20). As latex sensitivity results mostly from mucosal contact or inhalation of airborne proteins, the IgA response could also be of interest in this case. In order to clarify the development of the immune allergenic response to latex proteins, this work aimed to study the individual isotypic response of 54 latex-allergic patients selected according to their RAST class. For each individual, the pattern of latex allergens and antigens was compared.

Material and methods

Sera

Fifty-four sera of latex-allergic patients were selected on the basis of a positive RAST (RAST class of 3 to 6).

SDS-PAGE and Western blotting

The standardized latex extract was generously donated by Stallergènes (France). Sixty micrograms of latex proteins on a single 20-cm-wide deposit was separated by SDS-PAGE in an 8–18% gel gradient (ExcelGel, Amersham-Pharmacia Biotech, Sweden), with a flat bed apparatus, according to the manufacturer's instructions. The proteins were then electroblotted onto a CNBr-activated nitrocellulose (NC) sheet (Schleicher & Schuell, Basel, Switzerland), according to Demeulemester et al. (21), with a semidry Novablot apparatus (Amersham-Pharmacia Biotech, Sweden), according to the manufacturer's instructions (1 h, 1 mA/cm²). After blotting, the NC was dried and cut into 3-mm-wide strips. Each strip was blocked with PBS containing 1% BSA and 0.1% Tween 20 (PBS-BSA-Tw) for 1 h at 22°C in an Octaline disposable tray (Intermed, Denmark). Then, the strips were incubated overnight at 22°C with 0.5 ml of a single serum diluted 1:10 in PBS-BSA-Tw. After extensive

washing, the various isotypes were detected by incubation for 2 h at 22°C with anti-human isotype antibodies: alkaline-phosphatase-conjugated goat anti-human IgE (Sigma Biochemical, USA); alkaline-phosphatase-conjugated goat anti-human IgA (Institut Pasteur, France); biotinylated monoclonal mouse anti-human IgG₃ (generously donated by Prof. R.G. Hamilton, DACT, Johns Hopkins University School of Medicine, Baltimore, MD, USA); and peroxidase-conjugated sheep anti-human IgG₁, IgG₂, or IgG₄ (Binding Site, Birmingham, UK).

The alkaline-phosphatase activity was detected with 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium (Sigma Biochemicals, USA) in 0.1 M Tris buffer, pH 9.5. The peroxidase activity was detected by di-aminobenzidine in the presence of H₂O₂. Biotinylated antibodies were revealed by incubation with alkaline-phosphatase-conjugated streptavidine (Amersham, UK) followed by the detection of the alkaline-phosphatase activity.

ELISA

The reactivity of the various isotypes of the latex-sensitive patient sera was tested by the ELISA method. Briefly, the microtest plates were coated with 10 µg/well of the standard latex extract in a 50 mM sodium carbonate buffer, pH 9.6, overnight at 22°C. After blocking with PBS-BSA-Tw, the wells were successively incubated for 2 h at 37°C with 100 µl of the human serum dilution (1:10 in PBS-BSA-Tw) and 100 µl of one of the enzyme-conjugated anti-human antibodies used for the immunoblots. The alkaline-phosphatase activity was detected with *p*-nitrophenyl phosphate disodium (Sigma Biochemicals, USA) in a 0.1 M Tris buffer, pH 9.5. The peroxidase activity was detected with *o*-phenylene diamine (Sigma Biochemicals, USA) in a 0.1 M citrate buffer, pH 5.0, in the presence of H₂O₂, and stopped with 2 N HCl. The wells were extensively rinsed with 0.9% NaCl-Tw after each incubation.

Results

Fifty-four sera were individually tested by RAST. The specificity and isotypic expression of each individual serum were tested on nitrocellulose strips after SDS-PAGE separation of the standardized latex extract.

Of the 54 sera studied, five did not have any detectable Ig response to latex proteins, with the Western blot technique. The five negative sera had a RAST class of 3. Out of the 49

Table 1. Summary of isotypic expression of each individual serum with positive Ig response in Western blot

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
IgE	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
IgA	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
IgG1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
IgG2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
IgG3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
IgG4	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
RAST	6	6	6	6	6	6	5	5	5	5	5	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3		
Asterisk (*) indicates that this isotype is expressed without indication of recognized proteins. Serum numbers are indicated in first line and their RAST class in last line.																																																	

positive sera, 46 had a clear IgE response to various latex allergens, with various isotypic expressions. The isotypic expression of each of the 49 sera is summarized in Table 1. The most common isotypic association was IgE-IgG1 (30/46) with an association of IgA in 19 out of the 30 cases. In 10 cases, IgG4 was added, giving the isotypic association IgE-IgA-IgG1-IgG4. IgG4 was associated with IgE in only 21 of the 46 cases.

The relative quantification of the various isotypes by ELISA showed that the sera which had the highest IgE titers were also those having the highest IgA, IgG1, or IgG4 titers. But, frequently, the sera which had the highest IgG3 values also had a very low IgE value (Fig. 1, sera 18 and 43). In a very few cases, the antibodies detected by ELISA could not reveal antigens by Western blot (Fig. 1, serum 4).

Individual analysis of the specificity of the various antibodies clearly showed that the major allergens were usually recognized by the other isotypes when various isotypes were expressed. However, in some cases, allergens were recognized by IgE but not, or only partly, by the other isotypes. For example, in serum 10 (Fig. 1), one allergen was recognized by IgE, IgA, and IgG1, whereas the other three were recognized only by IgE. Another example is serum 3 (Fig. 1): a doublet around 6–8 kDa was recognized only by IgE and IgG1, whereas the isotypic association of this serum was IgE-IgA-IgG1-IgG4 with traces of IgG2 and IgG3.

IgG2 was very rarely expressed and, when present, reacted very slightly (Fig. 1, serum 3). IgG3 was present in 18 of the 49 Ig-positive cases in Western blot. However, if, in some cases, IgG3 was directed toward allergens (Fig. 1, serum 14 or 18), in most cases, the IgG3 was directed toward nonallergenic molecules (Fig. 1, serum 17), and, in two cases, IgG3 was the only isotype expressed (Fig. 1, serum 43). In these two cases, the molecules recognized by IgG3 were not related to allergens but represented a particular IgG3 pattern expressed in six of the 18 IgG3-positive cases.

If we define “allergogram” as the panel of proteins recognized by IgE (Fig. 2), it is obvious that the major allergen had a molecular mass of 23–26 kDa. Some patients recognized a wide band ranging from 23 to 26 kDa (Fig. 1, serum 1 or 22), whereas others presented two thin bands, corresponding to distinct molecular masses (Fig. 1, serum 4, 9, or 18). In some cases, only one of these two bands was recognized (Fig. 1, serum 13 or 17). If we consider its frequency, the second allergen appeared as a wide band of a molecular mass of 14–15 kDa. Other molecules with molecular masses of 94, 43 and 6–8 kDa were also recognized by IgE. All these molecules could be recognized by other isotypes (Fig. 2).

In addition to these allergens, a few latex proteins, recognized not by IgE but by other classes of antibodies, could be identified. For example, a protein of approximately 34 kDa was recognized by IgG4 or IgG3, but not by IgE (Fig. 1, sera 13 and 18 [arrows]).

Discussion

The present work describes the isotypic response of patients to latex allergens and antigens. The most commonly recognized allergen was a protein of 23–26 kDa, followed by a protein of approximately 14–15 kDa. Proteins of 94, 43, and 6–8 kDa were also identified as common allergens.

Hev b 3, a molecule of 23 kDa, was recognized as an allergen closely associated with rubber particles, presenting homologies with Hev b 1 and particularly recognized by spina bifida patients (15, 22). From similarities in amino-acid sequences, Yeang et al., (23) have identified the 23- and 26-kDa molecules as rubber-particle protein Hev b 3. In the present work, molecules ranging from 23 to 26 kDa were the most frequently recognized allergens. However, the sera did

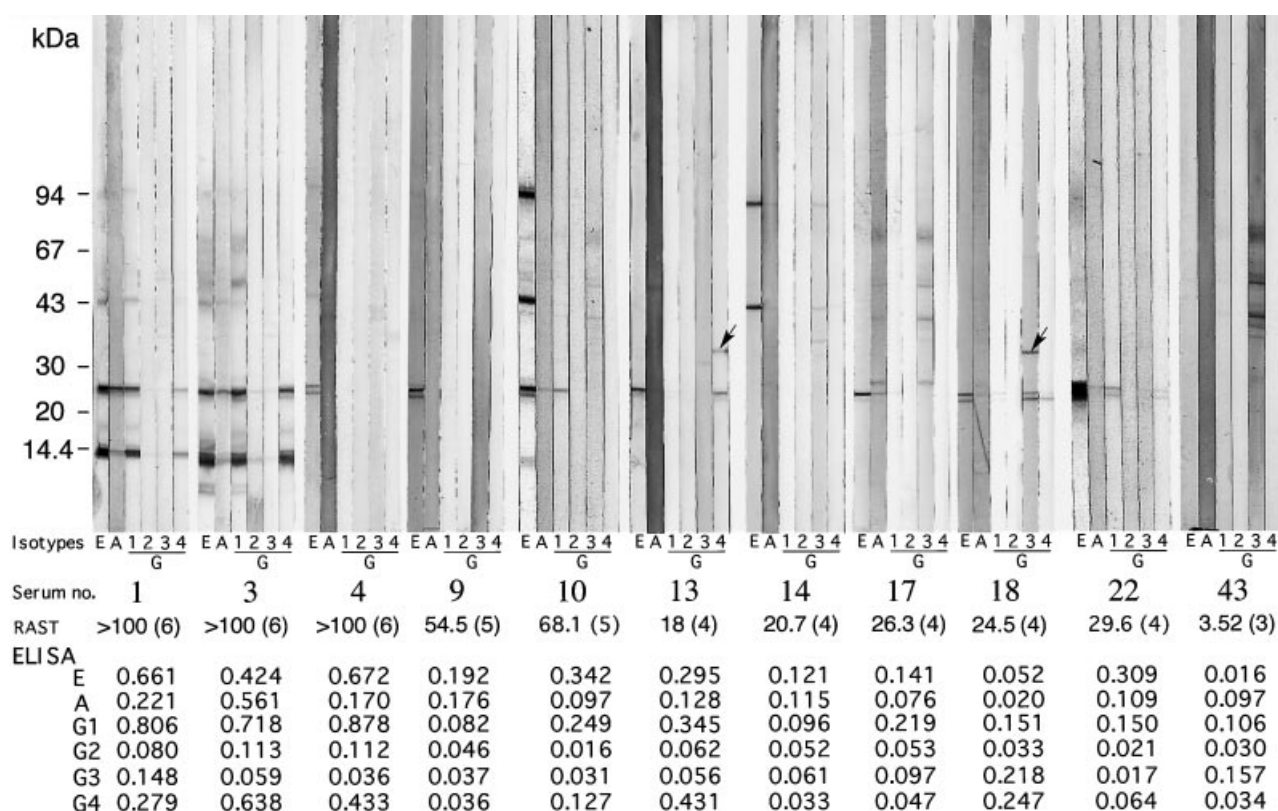


Figure 1. Individual isotypic response to latex proteins. Latex proteins were separated by SDS-PAGE in 8–18% gel gradient and transferred by Western blot onto CNBr-activated nitrocellulose (NC) sheet. Each NC strip was incubated with serum of latex-allergic patient, and with one of anti-human isotypes IgE, IgA, IgG1, IgG2, IgG3, or IgG4. For each individual serum, six isotypes were sought on neighboring strips. On this photomontage, isotype is indicated under each strip, as well as serum number, RAST value (and class), and ELISA value of each isotype. ELISA values are given in optical densities. Molecular masses are indicated on left. Example of nonallergenic molecule is shown by arrows.

not always recognize the two molecules: some sera recognized both molecules as a unique wide band or as two separated thin bands, whereas others recognized only the 26-kDa molecule. This result suggests that if some epitopes are shared by both molecules, some others are specific to the 26-kDa molecule.

A molecule of 20 kDa, identified as prohevein (Hev b 6.01), has been found to be a major allergen in latex-allergic adults (1, 24–26). We did not identify any band of 20 kDa recognized by IgE. However, prohevein is a protein which is processed to an N-terminal domain of 4.7 kDa (hevein, Hev b 6.02) and a C-terminal domain of 14 kDa (Hev b 6.03), and we obtained a strong IgE reaction to a protein of approximately 14–15 kDa. If we cannot exclude the possibility that, at least in some cases, this molecule was Hev b 6.03, the most important allergen of this molecular mass range to be recognized was the rubber elongation factor (Hev b 1), which is recognized by 50% of the health-care workers and 80% of the spina bifida patients allergic to latex (11, 13). In 1997, Chen et al. (11) identified some bands of 8–10 kDa as degradation products of Hev b 1. This result fits well with

our work, in which a doublet of approximately 6–8 kDa was sometimes observed with the Hev b 1-positive sera. Moreover, Chen et al. (27) have demonstrated that the minimal IgE-binding epitope of Hev b 1 is almost identical with the IgG-binding epitope. This agrees with our results in

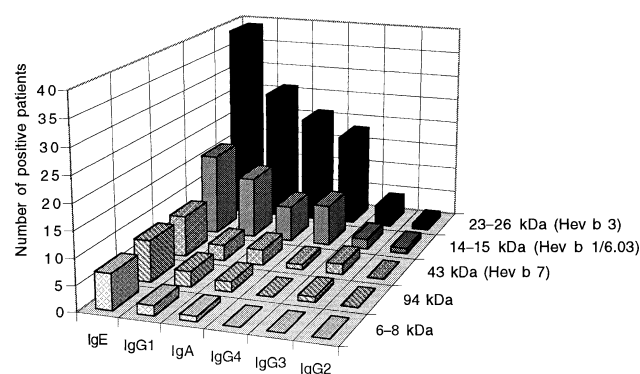


Figure 2. Identification of major latex allergens. Individual response to various latex proteins was analyzed by Western blot. Five major allergens were identified by their binding to IgE and their molecular mass. Major allergens were also recognized by other isotypes IgG1, IgA, IgG4, IgG3, and IgG2.

which the 14–15-kDa allergen was also commonly recognized by other Ig classes, especially IgG1, IgA, and IgG4.

Concerning the amino-acid sequence homologies between Hev b 1 and Hev b 3, previously described (28, 29), we found that both molecules were recognized by only 15 of the 39 sera which recognized Hev b 3, whereas Hev b 1 alone was recognized in a single case. This result suggests a possible, but limited, cross-reactivity between the two molecules, with a higher allergenicity for Hev b 3.

Proteins of 45 (26) or 46 kDa (9) have been identified as allergens for health-care workers. These molecules are now related to patatin-like protein (Hev b 7) (15) and could be related to the 43-kDa molecule we found. The molecule of 94 kDa does not seem to be characterized, but corresponded in our work to an allergen in six out of 54 cases.

In addition to the identification of these allergens, the present study showed that some proteins were antigens, but not allergens. However, if these molecules were never recognized by IgE antibodies, they can induce various isotypes such as IgG3, IgG4, or IgA. Therefore, it will be of interest in a further study to determine whether such molecules are antigenic for nonallergic individuals.

In this study, the isotypic expression of anti-latex antibodies was demonstrated to be mostly IgG1/IgE, with the usual association of IgA. IgG4 was also frequently detected but to a lesser extent than the previous ones.

The analyses of the IgE and IgG responses to various allergens demonstrated that, in addition to IgE, the most common IgG subclass expressed was IgG1, followed by IgG4; IgG2 and IgG3 were rarely detected and, if present, were mainly directed toward molecules other than allergens (30, 31). We found the same isotypic association for latex allergens, but added the fact that IgA was also usually found. The expression of IgA against an allergen acting widely by mucosal contact is not a surprise, as the mucosal secretory system is closely associated with an IgA switch. However, the physiologic and/or protective role of these IgA remains

to be elucidated. IgG2 is classically associated with an anti-carbohydrate response (32). The major latex allergens are described as proteins and possess no (or very little) carbohydrate residue, a fact which is consistent with the absence of IgG2 response. In the present study, an IgG3 response was observed in some patients presenting a RAST class 4 and below. These IgG3 usually recognized molecules that were not allergens; this phenomenon has been observed previously by Batard et al. (18).

It has been previously suggested that, with classical immunochemical techniques, autoantibodies against IgE could be mistaken for specific anti-allergen antibodies (33). The existence of such autoantibodies has been suggested in grass-pollen allergy (34). However, if the IgG1 or IgG4 antibodies were directed against IgE instead of an allergen, the allergenic pattern observed in Western blot must be the same for the three isotypes; and that was not always the case in our study. Moreover, in studying the protective role of IgG4, Dudler et al. (19) have demonstrated that IgG4 was specifically directed toward the major allergenic molecule of bee venom, phospholipase A₂. In latex, Kurup et al. (16) have also shown that not all allergens were recognized by IgG1, even in patients with an IgG1 response to latex.

In conclusion, this study established that in a wide population of latex-sensitive patients, the most common allergens were molecules of 23–26 kDa and 14–15 kDa, and that the common response to these allergens frequently associated IgE and IgG1 with IgA. In the future, it could be of interest to analyze such results in connection with the symptomatology of the disease, as well as with the response of nonallergic individuals exposed to latex.

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