HLA-DQ8 and the HLA-DQ8-DR4 haplotype are positively associated with the hevein-specific IgE immune response in health care workers with latex allergy

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Background: Hevein is one of the most important latex allergens affecting health care workers (HCWs).
Objective: Because the genetically determined susceptibility is one important factor regulating type I allergy, the association between the hevein-specific IgE immune response and HLA class II alleles of DQB1 and DRB1, DRB3, DRB4, and DRB5 was studied.
Methods: The distribution of HLA-DQB1 and DRB1, DRB3, DRB4, and DRB5 in 269 HCWs with latex allergy, 56 latex-sensitized patients with spina bifida (SB), and 90 nonatopic control subjects under special consideration for hevein-specific IgE was examined.
Results: Seventy percent (189/269) of the HCWs with latex allergy and 39% (22/56) of the latex-sensitized patients with SB had increased hevein-specific IgE antibody concentrations (>0.35 kU/L). HLA data analysis revealed significantly increased phenotype frequencies for DQB1*0302 (DQ8; 91/189 [48%]) and DRB1*04 (DR4; 102/189 [54%]) in hevein-positive HCWs with latex allergy compared with the 80 hevein-negative HCWs with latex allergy (DQB1*0302: 16/80 [20%], corrected *P* value $[P_c] = 7.1 \times 10^{-4}$; DRB1*04: 23/80 [29%], $P_c = .01$) and with control subjects (DQB1*0302: 16/89 [18%], $P_c = 1 \times 10^{-4}$; DRB1*04: 22/90 [24%], $P_c = 3.2 \times 10^{-4}$). The DQ8-DR4 haplotype frequency was significantly elevated in HCWs with hevein-specific IgE antibodies when compared with that in HCWs without hevein-specific IgE antibodies (47% vs 18%, $P_c = 5.3 \times 10^{-4}$) or control subjects (47% vs 18%, $P_c = 9.6 \times 10^{-4}$). In contrast, latex-sensitized patients with SB with hevein-specific IgE antibodies showed an increased but not significant DQB1*0302 frequency (7/22 [32%] vs 2/34 [6%], $P = .02$, P_c = not significant) compared with that seen in those without hevein-specific IgE antibodies.
Conclusion: The DQB1*0302 (DQ8) alone, the DQB1*0302 (DQ8)-DRB1*04 (DR4) haplotype, or both are significantly involved in the hevein-specific IgE immune response in HCWs with latex allergy. (J Allergy Clin Immunol 2002;110:507-14.)

Key words: *Latex allergy, MHC class II association, hevein, HLA-DRB1*04, DR4, HLA-DQB1*0302, DQ8*

Several natural rubber latex proteins from *Hevea brasiliensis* are responsible for a wide spectrum of clinical symptoms, ranging from rhinoconjunctivitis to severe anaphylaxis, in susceptible individuals.[1] Health care workers (HCWs) and children with spina bifida (SB) have close and repeated contacts with latex products and are considered high-risk groups.[2,3]

The frequent health problems caused by natural rubber latex products led to the identification, purification, and characterization of the allergens Hev b 1,[4] Hev b 3,[5] Hev b 5,[6,7] and Hev b 6.02.[8] Although Hev b 1 and Hev b 3 were found to be important allergens for children with SB,[5,9] the majority of HCWs were sensitized to hevein (Hev b 6.02), representing the posttranslationally processed and IgE-reactive 4.7-kd N-terminal domain of prohevein (Hev b 6.01) in natural rubber latex.[10] Approximately 74% of the HCWs with latex allergy but only 27% of the latex-sensitized patients with SB had hevein-specific IgE antibodies.[10]

The intensity of latex exposure, the route of sensitization, the genetically determined susceptibility, or the combination of all 3 factors might have significant influence on the pathogenesis of type I reactions to latex allergens. Recently, we found a strong association in a small group of individuals with latex allergy (HCWs and patients with SB) between the IgE responsiveness to hevein and the HLA class II antigens DR4 and DQ8.[11] In this study we examined 35 individuals with latex allergy (33 HCWs and 2 patients with SB) with hevein-specific IgE responsiveness and 16 individuals with latex allergy

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Abbreviations used
HCW: Health care worker
P_c: Corrected *P* value
SI: Stimulation index
SPT: Skin prick test
SSOP: Sequence-specific oligonucleotide probes
SSP: Sequence-specific primers

(8 HCWs and 8 patients with SB) without hevein-specific IgE antibodies. On the basis of these observations and the improved knowledge about characterized latex allergens, we have extended these investigations and now distinguish strictly between HCWs with latex allergy (n = 269) and latex-sensitized patients with SB (n = 56). Patients with latex allergy were defined as all subjects showing specific IgE antibodies to latex or one of its characterized allergens and having at least one additional clinical symptom (rhinitis, conjunctivitis, asthma, urticaria, edema, or anaphylactic shock) after exposure to latex devices. All 269 HCWs in this study fulfilled both criteria. In contrast, latex-sensitized patients were defined as having specific IgE against latex or one of its characterized allergens. All 56 patients with SB in this study fulfilled this criterion; if they presented one of the clinical symptoms described above, they were also identified as allergic. Twenty-nine (52%) of 56 patients with SB fulfilled this second criterion.

The aim of this study was to investigate the association between the specific IgE response to the major latex allergen hevein (Hev b 6.02) in a large cohort of HCWs with latex allergy and latex-sensitized patients with SB. A group of 90 nonatopic individuals (defined on the basis of negative reactions to commercial skin prick test [SPT] solutions, excluding latex) served as a control group to display the HLA-D distribution in a nonallergic white population.

Environmental and occupational disorders

METHODS

Patients

Between July 1996 and September 1999, we investigated the HLA-DRB1, DRB3, DRB4, DRB5, and DQB1 distribution of 269 HCWs with latex allergy and 56 latex-sensitized patients with SB by using PCR–sequence-specific oligonucleotide probe (SSOP) and PCR-SSP typing methods. The study was approved by the local ethics committee of the Ruhr-University Bochum. All subjects, patients, or parents of patients were fully informed and provided written or verbal consent for their blood samples to be tested. The subjects and patients were examined by experienced physicians (D.-A. M-V., F.R., X.B., and R.C.), and a detailed clinical history referring to, for example, contact with latex articles, allergic symptoms after latex exposure, and number of operations was taken. The characteristics of the HCWs, patients with SB, and control subjects are summarized in Table I.

Skin prick tests

SPTs were performed either with our own natural rubber latex SPT solution (0.01 mg of protein/mL)[10] or Regent latex milk (undiluted). In addition, up to 19 allergens, including grass pollen mixture (ALK-Scherax and Bencard), tree pollen I, II, and III (HAL), mugwort pollen (HAL), rye flour (ALKScherax), cat dander (ALK-Scherax and Bencard), horse hair (ALK-Scherax), hen's egg (ALK-Scherax), cow's milk (ALK-Scherax), feathers (Allergopharma), animal hairs I (Allergopharma), *Chiromomus thummi* (BGFA), *Blaterius germanica* (Hycor Biomedical), *Dermatophagoides pteronyssinus* (ALK-Scherax and Bencard), *Dermatophagoides farinae* (ALK-Scherax), *Alternaria alternata* (tenuis; ALK-Scherax), *Aspergillus fumigatus* (ALK-Scherax), *Cladosporium herbarum* (ALK-Scherax), and appropriate SPT controls (histamine 0.1%, 0.4% phenol in 0.9% NaCl solution; ALK-Scherax) were used in the SPTs. Reactions were evaluated after 20 minutes. Only those individuals who exhibited a wheal-and-flare reaction to latex milk were regarded as reacting specifically to natural rubber latex. SPT responses were not considered if there was a reaction to the control or no reaction to the positive control.

Control subjects included 90 local white subjects (46 female and 44 male subjects; mean age, 32.6 years) with negative SPT reactions when tested with the different commercial SPT solutions, excluding latex extracts (wheal-and-flare reactions <50% of that induced by 0.1% histamine), as described above.

Atopy

Atopy was diagnosed if there was a history of any atopic disease (rhinitis, conjunctivitis, asthma, or atopic eczema), at least one positive SPT reaction to one of the allergens described above, or both.

Determination of total IgE level

Total IgE levels were determined by using the Pharmacia CAP System (Pharmacia AB), according to the manufacturer's instructions. The measurement range is 2 to 2000 kU_A/L.

Determination of latex-specific IgE levels

All 269 HCWs and the 56 patients with SB had positive test results for having latex-specific IgE antibodies, as determined by using the Pharmacia CAP System (k 82, Pharmacia AB). Values of greater than 0.35 kU_A/L were regarded as positive.

Determination of hevein-specific IgE levels

The presence of hevein-specific IgE antibodies in sera was determined by using EAST, as previously described.[10] Briefly, the purified hevein (0.5 mg/mL) was coupled onto CNBr-activated cellulose discs in 0.1 mol/L sodium bicarbonate buffer (pH 8.4). After washing and treatment with diethylamine, the hevein discs were incubated with 50 μL of each patient serum for 3 hours at room temperature. A Phadezym RAST test kit (Pharmacia AB) was used to detect specific IgE antibodies. Values of greater than 0.35 kU_A/L were regarded as positive.

Genetic analysis of HLA-DRB1, DRB3, DRB4, DRB5, and DQB1 alleles

Genomic DNA was isolated from frozen white blood cells by using the DNA Easy-Prep Kit (Lifecodes) or the genome DNA isolation kit PUREGENE (Biozym).

PCR amplification for HLA-D loci DRB1, DRB3, DRB4, DRB5, and DQB1 were performed for each sample. DRB-specific exon 2 amplification was carried out with the primer pair DRB5′-I (ACCGGATCGTTCITGTCCCCICAGCA)/DRB3′-I (CTCGCCITGCACGTIAAGC).[12] For DQB1-specific exon 2 amplification, we used the primer pair DQ1 (AGGGATCCCCGCAGAGATTTCGTTGT(A,T)CC/DQ2 (GAGCTGCAGGTA-GTTGTGTCTGCA(C,T)AC) (Lifecodes).

HLA-DRB1, DRB3, DRB4, and DRB5 alleles were determined with 28 nonradioactive labeled SSOPs, as previously described.[11-

TABLE I. Characteristics of HCWs and patients with SB with and without hevein-specific IgE antibodies and control subjects*

Phenotype	HCWs with hevein-specific IgE antibodies (n = 189)	HCWs without hevein-specific IgE antibodies (n = 80)	Patients with SB with hevein-specific IgE antibodies (n = 22)	Patients with SB without hevein-specific IgE antibodies (n = 34)	Control subjects (n = 90; negative SPT reactions to commercial extracts, excluding latex)
Sex (female/male)	157/32	68/12	12/10	12/22	46/44
Age (y)	31.6 ± 9.4†; 16-63‡	32.8 ± 9.8†; 20-64‡	12.6 ± 4.9†; 5-21‡	10.5 ± 5.1†; 2-22‡	32.6 ± 10.2†; 20-64‡
Atopy (%)	104/120 (87)	35/48 (73)	12/22 (55)	15/34 (44)	0/90 (0)
Total IgE (kU/L [% of tests performed])	129/189 (68); 405†; 9.9-2000‡	48/80 (60); 350†; 18-2000‡	18/22 (82); 579†; 23-2000‡	34/34 (100); 169†; 8-614‡	87/90 (97); 31.4†; 2-188‡
Latex-specific IgE (kU/L)	17.43†; 0.56-100‡	5.62†; 0.50-100‡	58.54†; 1.77-100‡	14.23†; 0.49-79.8‡	ND
Hevein-specific IgE (kU/L)	4.49†, 0.39-17.5‡	<0.35	6.74†; 0.50-17.5‡	<0.35	ND
Positive latex SPT reaction/SPTs performed	106/110	50/51	5/5	2/14	ND
Asthma (%)	104/189 (55)	26/80 (33)	5/22 (33)	0/34 (0)	0/90 (0)
Urticaria (%)	137/189 (73)	44/80 (55)	8/22 (36)	8/34 (24)	0/90 (0)
Rhinitis (%)	110/189 (58)	35/80 (44)	1/22 (5)	1/34 (3)	0/90 (0)
Conjunctivitis (%)	74/189 (39)	24/80 (30)	8/22 (36)	8/34 (24)	0/90 (0)
Eczema (%)	25/189 (13)	16/80 (20)	0/22 (0)	0/34 (0)	0/90 (0)
Edema (%)	6/189 (3)	1/80 (1)	1/22 (5)	1/34 (3)	0/90 (0)
Anaphylactic shock (%)	3/189 (2)	1/80 (1)	5/22 (23)	1/34 (3)	0/90 (0)

ND, Not done.
*Not all items could be tested in all patients.
†Mean or mean ± SD as shown.
‡Range.

[16] This procedure permits us to identify the broad DRB and DQB1 alleles equivalent to the serologic specificities and, in some cases, a further subdivision.

Alternatively, the commercially available HISTO TYPE DNA-DR kit (BAG), based on the PCR-SSP method, was used for a part of the DNA samples.

Individuals with latex-specific IgE antibodies with or without hevein-specific IgE antibodies, as well as control subjects showing DR4 positivity after oligotyping, underwent DR4 subtyping with the DRB1*04 PCR-SSP-subtyping kit (Dynal) or the HISTO TYPE/DNA-DRB1*04 Subtyping kit (BAG), allowing the determination of alleles DRB1*0401 through DRB1*0424.

Inhibition experiments

For testing the HLA class restriction of hevein-induced PBMC proliferation response, mAbs against HLA-DR, HLA-DP, or HLA-DQ (Becton Dickenson) were added (200 ng/mL) to the PBMC suspension (5×10^5 cells/mL). PBMCs incubated in the absence of antibodies served as controls. After incubation for 2 hours at 37°C, the cells were washed twice with culture medium and stimulated in 6 parallel determinations with hevein (5 μg/mL) or medium for 5 days. The tritium-labeled thymidine incorporation of PBMCs in the absence and presence of different HLA class II–specific mAbs was compared and expressed as a percentage of inhibition.

Statistical analysis

The Fisher exact test (2-tailed when necessary) was used in all cases and carried out with the StatXact program (Cytel Software). Significant *P* values were corrected *(P_c)* by multiplying the number of comparisons (ie, ×13 for DRB1 and ×15 for DQB1) with the number of subgroups.

RESULTS

Typing of HLA-DRB1, DRB3, DRB4, and DRB5

HLA class II alleles of DRB were determined by using the PCR-SSOP method with 28 SSOPs, which allowed us to differentiate between 13 HLA DRB1 types (ie, 1*01, 1*15/16, 1*03, 1*04, 1*11, 1*12, 1*13/14, 1*1401, 1*1404, 1*0701, 1*08, 1*0901, and 1*1001), 3 DRB3 types (ie, 3*0101, 3*02, and 3*0301), 1 DRB4 type (ie, 4*0101), and 2 different DRB5 types (ie, 5*0101 and those who had a DRB5 allele other than 5*0101).

The distribution of the phenotype frequencies of DRB alleles in HCWs with latex allergy are summarized in Table II, and those of the latex-sensitized patients with SB are shown in Table III.

Regarding the phenotype frequencies of DRB1 alleles in the 189 HCWs with hevein-specific IgE antibodies, we observed significantly elevated frequencies for DRB1*04 (54%) when compared with the corresponding frequencies in the 80 HCWs without hevein-specific IgE antibodies (29%, $P = .0002$ and $P_c = .01$), as well as in the 90 control subjects (24%, $P = 3.06 \times 10^{-6}$ and $P_c = 3.2 \times 10^{-4}$). Influenced by the high DRB1*04 frequency in this group, the frequency for DRB4*0101 (61%) in the hevein-positive HCW group was also increased. In contrast, the comparison between the hevein-positive and the hevein-negative group showed a decreased frequency for DRB1*0701 (15% vs 28%, $P =$

TABLE II. Phenotype frequencies of DRB1, DRB3, DRB4, and DRB5 alleles (DR antigens) in HCWs with latex allergy with and without hevein-specific IgE antibodies and in control subjects

	HCWs with hevein-specific IgE antibodies (n = 189)		HCWs without hevein-specific IgE antibodies (n = 80)		Control subjects (n = 90)	
DRB allele (DR antigen)	No.	%	No.	%	No.	%
DRB1*01 (1)	30	16	24	30	15	17
DRB1*15/16 (2)	35	19	24	30	25	28
DRB1*03 (3)	35	19	11	14	18	20
DRB1*04 (4)	102*†	54	23	29	22	24
DRB1*11 (5)	39	21	10	13	10	11
DRB1*12 (5)	6	3	2	3	4	7
DRB1*13/14 (6)	56	30	25	31	33	37
DRB1*0701 (7)	29	15	22	28	15	17
DRB1*08 (8)	5	3	4	4	5	6
DRB1*09 (9)	0	0	1	1	3	3
DRB1*1001 (10)	2	1	0	0	0	0
DRB3*0101 (52a)	50	27	15	19	31	34
DRB3*02 (52b)	66	35	24	30	27	30
DRB3*0301 (52c)	6	3	7	9	7	8
DRB4*0101 (53)	115	61	42	53	38	42
DRB5*01/02 (51)	35	19	24	30	25	28

*HCWs with hevein-specific IgE versus those without hevein-specific IgE: $P = .0002$ and $P_c = .01$.
†HCWs with hevein-specific IgE versus control subjects: $P = 3.06 \times 10^{-6}$ and $P_c = 3.2 \times 10^{-4}$.

TABLE III. Phenotype frequencies of DRB1, DRB3, DRB4, and DRB5 alleles (DR antigens) in patients with SB with and without hevein-specific IgE antibodies and in control subjects

	Patients with SB with hevein-specific IgE antibodies (n = 22)		Patients with SB without hevein-specific IgE antibodies (n = 34)		Control subjects (n = 90)	
DRB allele (DR antigen)	No.	%	No.	%	No.	%
DRB1*01 (1)	2	9	9	26	15	17
DRB1*15/16 (2)	11	50	7	21	25	28
DRB1*03 (3)	3	14	10	29	18	20
DRB1*04 (4)	7	32	6	18	22	24
DRB1*11 (5)	9	41	8	24	10	11
DRB1*12 (5)	1	5	0	0	4	7
DRB1*13/14 (6)	6	27	10	29	33	37
DRB1*0701 (7)	5	23	11	32	15	17
DRB1*08 (8)	0	0	2	6	5	6
DRB1*09 (9)	0	0	1	3	3	3
DRB1*1001 (10)	0	0	0	0	0	0
DRB3*0101 (52a)	4	18	11	32	31	34
DRB3*02 (52b)	13	59	10	29	27	30
DRB3*0301 (52c)	3	14	0	0	7	8
DRB4*0101 (53)	12	55	15	44	38	42
DRB5*01/02 (51)	11	50	7	21	25	28

.027), which was not significant after performing the appropriate correction.

In the SB group the distribution of DRB alleles revealed no remarkable findings when comparing patients with hevein-specific IgE antibodies and patients without hevein-specific IgE antibodies or control subjects.

Typing of DQB1 alleles

Twenty SSOPs from the 11th Histocompatibility Workshop or commercial SSP kits were used to determine the different DQB1 alleles, allowing us to differentiate among 15 DQB1 alleles: *0201, *0301, *0302, *0303, *0401/2, *0501, *0502, *05031/05032, *0504, *0601, *0602, *0603, *0604, and *0605. The distribution of the phenotype frequencies of the DQB1 alleles in HCWs with latex allergy are summarized in Table IV. The corresponding distribution for latex-sensitized patients with SB is shown in Table V.

Regarding the HLA class II alleles in HCWs with hevein-specific IgE antibodies, a remarkably high frequency of the DQ3 antigen (80%), which comprises the 3 DQB1 alleles *0301-*0303, was observed. This anti-

TABLE IV. Phenotype frequencies of DQB1 alleles (DQ antigens) in HCWs with latex allergy with and without hevein-specific IgE antibodies and in control subjects

	HCWs with hevein-specific IgE antibodies (n = 189)		HCWs without hevein-specific IgE antibodies (n = 80)		Control subjects (n = 89)	
DQB1 allele (DQ antigen)	**No.**	**%**	**No.**	**%**	**No.**	**%**
DQB1*05+*06 (DQ1)	90	48	51	64	65	73
DQB1*0201 (DQ2)	43	23	29	36	30	34
DQB1*0301-3 (DQ3)	151*†	80	40	50	40	45
DQB1*0401/2 (DQ4)	4	2	4	5	2	2
DQB1*0501-05031/2 (DQ5)	42	22	29	36	26	29
DQB1*0601-5 (DQ6)	65	34	31	39	44	49
DQB1*0301 (DQ7)	69	37	24	30	27	30
DQB1*0302 (DQ8)	91‡§	48	16	20	16	18
DQB1*0303 (DQ9)	9	5	3	4	6	7

*HCWs with hevein-specific IgE versus those without hevein-specific IgE: $P = 1.76 \times 10^{-6}$ and $P_c = 1.06 \times 10^{-4}$.
†HCWs with hevein-specific IgE versus control subjects: $P = 1 \times 10^{-8}$ and $P_c = 1.2 \times 10^{-6}$.
‡HCWs with hevein-specific IgE versus those without hevein-specific IgE: $P = 1.2 \times 10^{-5}$ and $P_c = 7.14 \times 10^{-4}$.
§HCWs with hevein-specific IgE versus control subjects: $P = 8.6 \times 10^{-7}$ and $P_c = 1.03 \times 10^{-4}$.

TABLE V. Phenotype frequencies of DQB1 alleles (DQ antigens) in latex-sensitized patients with SB with and without hevein-specific IgE antibodies and in control subjects

	Patients with SB with hevein-specific IgE antibodies (n = 22)		Patients with SB without hevein-specific IgE antibodies (n = 34)		Control subjects (n = 89)	
DQB1 allele (DQ antigen)	**No.**	**%**	**No.**	**%**	**No.**	**%**
DQB1*05+*06 (DQ1)	11	50	20	59	65	73
DQB1*0201 (DQ2)	10	41	19	56	30	34
DQB1*0301-3 (DQ3)	16	73	16	47	40	45
DQB1*0401/2 (DQ4)	1	5	1	3	2	2
DQB1*0501-05031/2 (DQ5)	3	14	12	35	26	29
DQB1*0601-5 (DQ6)	11	50	11	32	44	49
DQB1*0301 (DQ7)	10	45	14	41	27	30
DQB1*0302 (DQ8)	7*	32	2	6	16	18
DQB1*0303 (DQ9)	2	9	1	3	6	7

*Patients with SB with hevein-specific IgE versus those without hevein-specific IgE: $P = .021$ and P_c = not significant.

gen was significantly increased in comparison with levels in hevein-negative subjects (50%, $P = 1.76 \times 10^{-6}$ and $P_c = 1.06 \times 10^{-4}$), as well as with levels in control subjects (45%, $P = 1 \times 10^{-8}$ and $P_c = 1.2 \times 10^{-6}$). Further analysis of the typing data revealed that DQB1*0302 (DQ8) alone is responsible for this high frequency because the distribution of *0301 and *0303 yielded comparable frequencies in hevein-positive and hevein-negative subjects, as well as in control subjects. The DQB1*0302 frequency in hevein-positive HCWs was 48% and was significantly increased when compared with that seen in hevein-negative HCWs (20%, $P = 1.2 \times 10^{-5}$ and $P_c = 7.14 \times 10^{-4}$) and control subjects (18%, $P = 8.6 \times 10^{-7}$ and $P_c = 1.03 \times 10^{-4}$).

Also in the hevein-positive patients with SB, increased frequencies for DQ3 (73%), as well as for DQB1*0302 (32%), were found, but after comparing these frequencies with the frequencies of patients with SB without hevein-specific IgE antibodies (DQ3: 47%, DQB1*0302: 6%) or control subjects (DQ3: 45%, DQB1*0302: 18%), the P values did not withstand correction.

DRB1*04 (DR4) subtyping

Because the DRB1*04 phenotype frequency was significantly increased in HCWs with hevein-specific IgE antibodies, we addressed the question of whether a specific DRB1*04 suballele was responsible for the association with the hevein-specific IgE response. Therefore we performed DR4 subtyping, allowing the determination of suballeles DRB1*0401 through *0424 in all study groups. The results for HCWs are shown in Table VI. In HCWs with hevein-specific IgE antibodies, we found 12 different DR4 subtypes, and in HCWs without hevein-specific IgE antibodies, we found only 4 subtypes. The most prominent suballele in the first group was *0401, with a frequency of 39%, followed by *0404 (9%), *0403 (3%), and *0402 (3%). In comparison with the *0401 frequency found in HCWs without hevein-specific IgE antibodies (22%), the *0401 frequency in HCWs with hevein-specific IgE antibodies was increased ($P = .01$) but did not reach the significance level after P value correction. In contrast, the analogous comparison with

TABLE VI. DR4 subtypes (*0401-0424) found in HCWs with and without hevein-specific IgE antibodies, as determined by means of PCR-SSP typing

	HCWs with hevein-specific IgE antibodies (n = 189)		HCWs without hevein-specific IgE antibodies (n = 80)		Control subjects (n = 90)	
Subtype	No.	%	No.	%	No.	%
All DR4	102	54	23	29	22	24
*0401	74*†	39	19	24	15	17
*0402	6	3	1	1	0	0
*0403	5	3	2	3	1	1
*0404	17	9	2	3	7	8
*0405	4	2	0	0	0	0
*0406	1	1	0	0	0	0
*0407	4	2	0	0	1	1
*0408	4	2	0	0	0	0
*0412, *0413, *0419, *0421	6	3	0	0	0	0

*HCWs with hevein-specific IgE versus those without hevein-specific IgE: $P = .017$ and P_c = not significant.
†HCWs with hevein-specific IgE versus control subjects: $P = .0002$ and $P_c = .02$.

TABLE VII. DQB1-DRB1*04 haplotypes in HCWs with latex allergy with and without hevein-specific IgE antibodies

DQB1-DRB1*04 Haplotype	HCWs with hevein-specific IgE antibodies (n = 189)		HCWs without hevein-specific IgE antibodies (n = 80)		Control subjects (n = 89)	
	No.	%	No.	%	No.	%
*0302-*04	88*†	47	14	18	16	18
*0301-*04	19	10	7	9	5	6
*0201-*04	1	1	1	1	2	2
*0401/2-*04	0	0	0	0	1	1

*HCWs with hevein-specific IgE versus those without hevein-specific IgE: $P = 4.75 \times 10^{-6}$ and $P_c = 5.32 \times 10^{-4}$.
†HCWs with hevein-specific IgE versus control subjects: $P = 2.86 \times 10^{-6}$ and $P_c = 9.6 \times 10^{-4}$.

the control group yielded a significantly increased frequency (39% vs 17%, $P = .0002$ and $P_c = .02$)

Regarding the DR4 subtypes, in patients with SB, we found 6 different subtypes. The *0401 frequency was comparable in hevein-positive (14%) and hevein-negative (18%) patients, as well as in the control group (17%).

DQB1*0302 (DQ8)-DRB1*04 (DR4) haplotypes

It is well known that in white subjects a linkage disequilibrium exists, mainly between the different DRB1*04 alleles and alleles DQB1*0301 (DQ7) and DQB1*0302 (DQ8).[14] In our HCW group (Table VII) we observed 3 kinds of haplotypes. The most frequent in hevein-positive patients was *0302-*04 (47%), which was significantly increased when compared with levels found in hevein-negative patients (18%, $P = 4.75 \times 10^{-6}$ and $P_c = 5.32 \times 10^{-4}$) and control subjects (18%, $P = 2.86 \times 10^{-6}$ and $P_c = 9.6 \times 10^{-4}$). Also, in the SB group this haplotype was the most prominent (32%) and increased in comparison with that seen in patients with SB without hevein-specific IgE antibodies (6%). Nevertheless, in the SB group with hevein-specific IgE antibodies, the difference was not striking enough to reach statistical significance after correction of the P values ($P = .02$ and P_c = not significant).

Inhibition experiments with anti-HLA-DR, anti-HLA-DQ, and anti-HLA-DP antibodies

When the cellular stimulation activity of hevein in PBMCs was tested, only 2 (17%) of 12 of the subjects with latex allergy showed a significant induction (stimulation index [SI] ≥ 2.5) of the proliferation response.[17] Thus far, inhibition experiments with hevein as a stimulus were performed successfully only in PBMCs of these 2 HCWs with latex allergy. After preincubation with anti-HLA-DR, neither sample showed any significant inhibition of proliferation, whereas anti-HLA-DQ, as well as anti-HLA-DP, revealed a significant inhibition of the proliferation response.

The first HCW displayed an SI value of 3.3 ± 0.5 for the hevein-specific proliferation response. The inhibition frequency with anti-HLA-DR was 27.3%. In contrast, the anti-HLA-DQ and anti-HLA-DP displayed inhibition frequencies of 54.5% and 64.8%, respectively. The second HCW showed comparable results. The SI value was 5.1 ± 0.9, and absolutely no inhibition (0%) was observed with anti-HLA-DR, whereas the anti-HLA-DQ and anti-HLA-DP yielded inhibition frequencies of 82.4% and 76.5%, respectively.

DISCUSSION

In this study 269 HCWs with latex allergy and 56 latex-sensitized patients with SB of white origin were examined for having specific IgE antibodies to hevein, a 4.7-kd major allergen of *Hevea brasiliensis* latex designated as Hev b 6.02.[8,10] One hundred eighty-nine (70%) of 269 HCWs with latex allergy and 22 (39%) of 56 latex-sensitized patients with SB showed Hev b 6.02–specific IgE antibodies when tested by means of EAST.

The present HLA data showed that the specific IgE response to hevein is strongly associated with the HLA class II alleles DQB1*0302 (DQ8) and DRB1*04 (DR4). Because both types are in linkage disequilibrium, the combination of both the DQ8 and DR4 haplotype is strongly associated with the hevein-specific IgE immune response. Computer-aided prediction programs, such as PROTSHELL,[18] for the determination of HLA-DR4–binding motifs, as well as experimental hevein-specific binding studies with a DRB1*0401–specific cell line (data not shown), revealed that hevein does not have a DR4-binding motif. Furthermore, the inhibition experiments with anti-HLA-DQ, anti-HLA-DP, and anti-HLA-DR with hevein as a stimulus on PBMC proliferation response revealed that anti-HLA-DQ and anti-HLA-DP were able to inhibit the proliferation in PBMCs of 2 HCWs, whereas anti-HLA-DR had no significant influence.

The reason for the weak correlation between the hevein-specific IgE immune response to HLA class II alleles DR4, DQ8, or both in patients with SB could be due to the small number of available patients with SB with hevein-specific IgE antibodies in this study. Nevertheless, in this context it is also important to know that hevein-specific IgE antibodies in all patients with SB were accompanied by a high level of Hev b 1–specific IgE. It is well known that Hev b 1 is one of the major latex allergens in patients with SB.[9] The median of the hevein-specific IgE level in these patients was 3.52 kU/L, whereas the median of the Hev b 1–specific IgE values was 7.19 kU/L. In contrast, the level of hevein-specific IgE antibodies in HCWs was either accompanied by a low level of Hev b 1–specific IgE antibodies, or no Hev b 1–specific IgE antibodies were detectable. Among the 269 sera from the HCW sera in this study, 136 (50.6%) were tested for both hevein- and Hev b 1–specific IgE antibodies by means of EAST. Eighty-five (62.5%) sera of HCWs showed hevein-specific IgE values but displayed no measurable IgE response to Hev b 1. The median of the hevein-specific IgE values was 1.88 kU/L. The 51 (37.5%) HCWs with hevein-specific, as well as Hev b 1–specific, IgE antibodies displayed a much higher level of hevein-specific IgE (median, 4.52 kU/L) when compared with the corresponding level of Hev b 1–specific IgE antibodies (median, 0.68 kU/L). In 46 (90%) of 51 of the patients, the hevein-specific IgE value was higher than the corresponding Hev b 1–specific IgE value. As a consequence, the HLA association with the hevein-specific IgE immune response in HCWs might appear much more significant than in patients with SB.

Furthermore, there is also some evidence that a specific HLA-independent mechanism of IgE immune response regulation exists in patients with SB with latex sensitivity. Recently, Nunez et al[19] reported that adults with spinal cord injuries and repeated surgery had a latex sensitivity of only 15.6%. Also, Szepfalusi et al[20] found a disease-associated propensity for latex sensitization on contact with latex products in patients with SB (9/21 [43%]), whereas the corresponding prevalence in 32 patients with posthemorrhagic hydrocephalus was only 6%. These findings support the interpretation that SB per se represents the primary risk factor for latex sensitization after exposure, and the number of operations represents only the second risk factor. Consequently, both SB and latex exposure would be necessary to induce the IgE immune response. If this is true, it could be one explanation for why no significant association between HLA-DQ and HLA-DR alleles and the Hev b 1–specific IgE immune response in patients with SB were found in a recent study.[21]

In summary, the present data strongly indicate that in HCWs the DQB1*0302 (DQ8) alone or the haplotype DQ8-DR4 is significantly more involved in hevein-specific IgE generation than DR4 alone. Nevertheless, this hypothesis has to be confirmed by extended binding studies and inhibition experiments with specific anti-HLA-DQ8–restricted antibodies as soon as they become available.

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