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Characterization of T Cell Responses to Hev b 3, an Allergen Associated with Latex Allergy in Spina Bifida Patients

Barbara Bohle,* Birgit Wagner,* Ute Vollmann,* Dietke Buck,§ Bodo Niggemann,§ Zsolt Szépfalusi,‡ Gottfried Fischer,‡ Otto Scheiner,* Heimo Breiteneder,* and Christof Ebner2*  

The prevalence of type I allergy to Hevea brasiliensis latex is particularly high among individuals with frequent exposure such as health care workers and patients with spina bifida (SB). Due to a birth defect of the spinal canal and the resulting neurological and orthopedic defects, these patients require multiple surgeries during childhood. SB patients display a unique pattern of sensitization: IgE-reactivity is preferentially directed against Hev b 3 and Hev b 1, two latex allergens with high sequence similarity. In this study, we analyzed the T cell response to Hev b 3 in latex-allergic SB patients using poly-, oligo-, and monoclonal T lymphocyte cultures. All T cell clones (TCC) were CD3/CD4-positive and expressed the αβ TCR. According to their cytokine production pattern (IL-4 vs IFN-γ), 12 of 21 TCC were classified as Th2-like, 2 of 21 were Th1-like, and 7 of 21 belonged to a Th0-like subset. Using 11 T cell lines and 21 TCC, nine T cell stimulating fragments were determined out of 52 overlapping 12-mer peptides representing the complete amino acid sequence of Hev b 3. Ag presentation of one dominant T cell epitope could be associated with a four-amino acid binding motif (YSTS, position 11–13) in the β1 chain of HLA-DR molecules expressed by the respective patients. No reactivity was observed when Hev b 3-reactive T cell lines or TCC were incubated with peptides representing homologous parts of the Hev b 1 molecule, i.e., no cross-reactivity between Hev b 3 and Hev b 1 at the T cell level was evident. The Journal of Immunology, 2000, 164: 4393–4398.

Due to the increasing use of natural rubber latex (NRL) products, type I allergy to Hevea brasiliensis latex has become an important and increasing health problem worldwide. Especially persons who are frequently exposed to NRL products suffer from hypersensitivity to latex allergens, causing allergic reactions including contact-urticaria, rhinoconjunctivitis, asthma, and even anaphylaxis (1–3). It has been reported that 2.8–8.8% of all health care workers and 29–72% of patients suffering from spina bifida (SB) are allergic to Hevea latex proteins (1–7). In the latter case, multiple surgeries and the frequent use of NRL-containing instruments like catheters have been regarded as the cause for the sensitization. However, recent reports indicate the contribution of SB-specific susceptibility factors, because multioperated children with other diseases displayed significantly lower sensitization rates (8). Another argument indicating a special situation in SB patients is evident by the fact that these individuals typically produce IgE Abs preferentially directed against two allergens in the latex extract: Hev b 1 and Hev b 3. IgE binding to these molecules is much less frequently observed in other latex-allergic patients (9–11). According to its sequence, Hev b 1, the rubber elongation factor, is a 14.6-kDa protein (12). Hev b 3, the small rubber particle protein of 204 aa and a molecular mass of 22.3 kDa, has recently been cloned and sequenced (13, 14). Concurrent IgE binding to Hev b 1 and 3 indicated common B cell epitopes on these allergens, which are obviously due to the high degree of sequence identity (15). Indeed it has been shown that preincubation with rHev b 3 does not only inhibit IgE binding to the corresponding natural counterpart but also in part to natural Hev b 1 present in latex C-serum extract (13).

Though much work has been done concerning the characterization, purification, and cloning of latex allergens in the recent past, practically no information about the underlying T cell responses is available (16). Allergen-specific T lymphocytes determine the quality of the subsequent Ab response by their pattern of cytokine production in response to specific activation. To understand the sensitization process, it is therefore necessary to analyze the immunoreactivity to Ags at the level of specific T cells. Specific immunotherapy (SIT) is the treatment of choice in many forms of type I allergy. Recently the mechanisms operative in SIT have been elucidated. It has been shown that during the administration of increasing doses of allergen, a shift from a typical allergic Th2-response to Th1 could be detected (immunodeprivation) and that allergen-specific T cell responses were suppressed (tolerance induction) (17–21). It was concluded that strategies aiming in the correction of the allergic immune response should target allergen-specific T lymphocytes. Therefore, it is of interest to identify T cell-reactive fragments of allergens to design peptides or to produce recombinant molecules with high T cell reactivity (22–24).

The aim of this study was to characterize the T cell response to Hev b 3 in latex-allergic SB patients. For this purpose, we stimulated T lymphocytes with recombinant Hev b 3 and overlapping synthetic peptides representing the amino acid sequence of Hev b...
3 and (to analyze possible T cell cross-reactivities) Hev b 1. Established Hev b 3-reactive T cell cultures were characterized evaluating cytokine patterns in response to allergen-specific stimulation, and T cell epitopes of Hev b 3 were determined. Moreover, we performed HLA typing and analyzed MHC restriction patterns of Hev b 3-reactive T cell cultures.

Materials and Methods

Patients
Eleven patients suffering from SB were included in this study. NRL allergy was proven by positive radioallergosorbent test (RAST), positive skin prick test, and provocation tests (25). HLA typings for HLA-DRB and -DQB alleles were performed according to methods described (26).

Allergens
rHev b 3 and latex C-serum were prepared as described (13).

Immunoblots
Immunoblotting of patients’ sera was performed as previously described (13). Briefly, rHev b 3 (2 μg/ml) and latex C serum (75 μg/ml) were separated by 12% SDS-PAGE and blotted onto a nitrocellulose membrane. The membrane was incubated with patients’ sera or plasma overnight at 4°C. After incubation with a 125I-labeled anti-human IgE Ab (IBL, Hamburg, Germany), bound IgE was visualized by autoradiography. A serum pool obtained from five donors with birch pollen allergy (RAST, ≥4) served as a negative control.

Proliferation assays
PBMC (2 × 10^6) were cultured in duplicates in 96-well plates (Nunclonal; Nunc, NaK Orfeo, Rochester, NY) in 200 μl serum-free Ultra Culture Medium (BioWhittaker, Walkersville, MD) supplemented with 2 mM l-glutamine and 2 × 10^-3 M 2-ME in the presence of rHev b 3 for 6 days at 37°C and 5% CO2 in a humidified atmosphere. rHev b 3 was titrated in the concentration range from 0.78 to 100 μg/ml. During the last 16 h of culture, 3H-thymidine (0.5 μCi/well) was added, and the incorporated radioactivity was measured by scintillation counting.

Allergen-specific T cell lines (TCL)
Allergen-specific short-term TCL were obtained by a technique previously described (27). Briefly, 1.5 × 10^6 PBMC were stimulated with 20 μg/ml purified rHev b 3 in 24 well flat-bottom culture plates (3524; Costar, Cambridge, MA) under conditions as described above. After 5 days, suboptimal doses of human rIL-2 (10 U/ml; Boehringer Mannheim, Mannheim, Germany) were added, and cultures were continued for an additional 7 days. Thereafter, monoclonal T cell cultures were established by limiting dilution, and remaining T cell blasts were used for epitope-mapping experiments.

Allergen-specific T cell clones (TCC)
TCC were established according to published protocols (27). Briefly, T cell blasts from Hev b 3-specific TCL were seeded in limiting dilution (0.3 cells/well) in 96-well round-bottom plates (Nunclone) in the presence of 2 × 10^3 irradiated (50 Gy) allogeneic PBMC as “feeder cells,” 0.5% v/v PHA (Life Technologies, Grand Island, NY), and rIL-2 (4 U/well) in the above-described medium. Growing microcultures were expanded at weekly intervals with fresh allogeneic irradiated feeder cells and rIL-2. The specificity of TCC was assessed in proliferation assays as soon as the cell number reached 2 × 10^5. Thereafter, Hev b 3-specific TCC were expanded by alternating turns of stimulation with autologous irradiated APC and rHev b 3 or with allogeneic feeder cells and rIL-2.

Analysis of the phenotype of TCC
The phenotype of TCC was analyzed by flow cytometry using a FACSscan (Becton Dickinson, Mountain View, CA). For the analysis the FITC-labeled mAbs, anti-Leu 4/CD3, anti-Leu 3a/CD4, anti-Leu 2a/CD8, anti-TCR αβ WT 31, and anti-TCR αβ (Becton Dickinson) were used as described (27).

Measurement of cytokines
TCC were washed and incubated with irradiated (50 Gy) autologous APC in the presence of rHev b 3 (5 μg/ml) for 24 h. Cytokine levels in supernatants were measured in ELISA using matched Ab pairs (Endogen, Woburn, MA) according to instructions by the manufacturers (sensitivity limits: IL-4, 9 pg/ml; IFN-γ, 9.5 pg/ml). Cultures containing TCC and APC alone served as negative controls.

Epitope mapping
A panel of 52 peptides was synthesized according to the Hev b 3 amino acid sequence by Abimed (Langenfeld, Germany) (13). To analyze possible cross-reactive T cell epitopes, a panel of 42 peptides was synthesized according to the amino acid sequence of Hev b 1 (Swiss Prot data base, accession no. P15252). Peptides were 12 residues long and overlapped for 3 aa, i.e., neighboring peptides shared 9 aa. A total of 5 × 10^4 T cells of TCL or TCC were directly tested with all 94 peptides (5 μg/ml) in the presence of 1 × 10^5 autologous irradiated APC. A stimulation index (SI; ratio between cpm obtained in cultures with TCC plus autologous APC plus Ag and cpm obtained in cultures containing TCC and APC alone) >5 was considered as positive.

HLA restriction
Blocking Abs directed against HLA-DP (B7/21, IgG1), -DQ (Leu 10, IgG1), and -DR (L243, IgG2a) were purchased from Becton Dickinson. The Abs were used in a final concentration of 10 μg/ml in the presence of 2 μg/ml Hev b 3, 30-114 peptide, autologous APC, and Hev b 3, 30-114 specific TCC. Proliferation assays were performed as described (28). Furthermore, class II exon 2 sequences of DR and DQ molecules expressed by the patients were compared so that motifs possibly involved in peptide binding and presentation could be determined.

Results

IgE immunoblots
IgE binding patterns of the 11 SB patients are depicted in Fig. 1. All sera displayed IgE Abs reacting with the 23-kDa latex allergen, i.e., natural Hev b 3 present in latex C serum (Fig. 1A), as well as with the rHev b 3 (Fig. 1B). IgE binding to the 14-kDa latex allergen Hev b 1 was evident in 10 of 11 patients (Fig. 1A). Negative
controls revealed no IgE binding to latex proteins (Fig. 1, lanes N and B).

Proliferation assays

PBMC were stimulated with titrated concentrations of rHev b 3. Results are summarized in Fig. 2. Lymphoproliferative responses varied considerably between the individuals. The maximal stimulatory concentrations of rHev b 3 were 25 μg and 50 μg/ml. Proliferative responses from three birch pollen-allergic and two non-allergic individuals are shown as negative controls (Fig. 2).

Hev b 3-specific TCC

In total, 21 TCC from 7 of 11 latex-allergic individuals were established (Table I). All TCC belonged to the Th subset, i.e., were CD3/CD4-positive and expressed the αβ TCR. According to their cytokine production pattern in response to stimulation with rHev b 3, 12 of 21 were attributed to the Th2-like subset (pg IL-4 more than five times pg IFN-γ), 2 of 21 were Th1-like (pg IFN-γ more than five times pg IL-4), and 7 of 21 produced similar levels of IL-4 and IFN-γ and were classified as Th0.

T cell epitopes

We were able to identify nine T cell epitopes of Hev b 3 by stimulating 11 TCL and 21 TCC with 52 overlapping peptides. Epitope specificities of 17 of 21 TCC are shown in Table I; 4 of 21 TCC did not react with any of the peptides, in spite of a strong reactivity with rHev b 3. In general, short-term rHev b 3-induced TCL reacted with the same peptides as the TCC established from the respective line. T lymphocytes from four patients reacted with the peptide sequence PRVLDVASSVF (Hev b 3103–114). Three individuals harbored T cells specific for TAVYFSEKYNDV (Hev b 3160–171). The peptides Hev b 310–21, Hev b 313–24, Hev b 355–69, and Hev b 3178–189 were recognized by two patients each. Peptides Hev b 349–60, Hev b 3136–147, and Hev b 3184–195 were recognized by one individual each. No reactivity was observed when TCL or TCC were incubated with peptides synthesized according to the amino acid sequence of Hev b 1. Epitope recognition patterns of the different individuals are shown in Fig. 3.

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<th>IFN-γ (pg/ml)</th>
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HLA typing and MHC restriction of T cell recognition of Hev b 3 peptides

Results of HLA-DRB and HLA-DQB typing of patients are shown in Table I. Because Hev b 3103–114-induced lymphoproliferative responses in 4 of 8 individuals, MHC class II-exon 2 sequences of the HLA-DR and -DQ molecules were compared. Sequence alignment revealed that these four individuals shared a 4-aa motif (YSTS) at position 10–13 of HLA-DRB, whereas individuals not reactive with Hev b 3103–114 did not display this distinctive feature (Fig. 4). Blocking experiments of four Hev b 3103–114-specific TCC using anti-HLA-DP, -DQ, and -DR framework Abs confirmed the HLA-DR-restricted presentation of this peptide (Fig. 5A). HLA-typed cells from latex allergic SB patients and from healthy controls were used to present the Hev b 3103–114 epitope to specific TCC G23 (Table I). Fig. 5B shows that APC were only able to present this epitope provided that one of their MHC class II molecules contained the YSTS motif at position 10–13. Individuals expressing HLA-DR-specificities that did not include this polymorphism were not capable of activating Hev b 3103–114-specific T lymphocytes.

Discussion

The prevalence of allergy to NRL is high in certain risk groups (1–3). The sensitization rate is exceptionally high in patients with SB, and these individuals display a typical IgE binding profile, i.e., specific IgE to the latex allergens Hev b 3 and Hev b 1 (4–11). Hev b 3 shares 47% sequence identity with Hev b 1 on the amino acid level (13, 14). The proteins also possess structural similarity, consequently leading to IgE cross-reactivity (13). The immune recognition of these two allergens is associated with SB as every patient (sensitized or clinically allergic to latex) displays IgE binding to these allergens (Fig. 1). In this context it has been suggested that all latex-allergic SB patients could be diagnosed by using Hev b 1 and Hev b 3 alone (9–13). The high prevalence of latex allergy in SB patients is due to the early and high degree of exposure through repeated surgery in childhood. However, a genetic predisposition can also be suspected because latex allergy is not common in children undergoing comparable operations due to other diseases (8).

During allergic sensitization, the recognition of peptide fragments (in context with MHC class II molecules) by allergen-specific T cells is decisive. In case of type I allergy, characteristically a Th2 response with high levels of IL-4 and IL-5 (consequently leading to IgE production and eosinophilia) is induced (29, 30). Analyzing the T cell subsets specific for rHev b 3 in SB patients with latex allergy reflected this situation. PBMC proliferated in response to stimulation with rHev b 3 (Fig. 2), and the majority of established rHev b 3-specific TCC belonged to the Th2-like subset (57%; Table I). We tested TCL and TCC with overlapping peptides representing the complete amino acid sequence of Hev b 3. Nine T cell epitopes were identified, i.e., the respective peptides induced significant proliferation (Fig. 3). This result is comparable with T cell epitope mapping studies using other allergens in which multiple specificities were detected (for review see Ref. 24). However, the finding contradicts the hypothesis of a SB-associated specific immune reactivity in which a low number of T cell epitopes would have been expected. On the other hand, epitope Hev b 3103–114 can be designated dominant because 50% of the individuals showed T cell reactivity with this peptide (Fig. 3). Moreover, five of six TCC specific for this epitope belonged to the Th2 type, indicating the pathogenetical importance of this immune reactivity (Table I). Associating this T cell epitope with certain restriction molecules (HLA-DR and -DQ) revealed the involvement of a 4-aa motif (YSTS) located in the HLA-DR binding groove in the presentation of this peptide (polymorphic residues at position 10–13) (31). Blocking experiments using anti-HLA framework Abs confirmed that the presentation of this peptide was HLA-DR restricted (Fig. 5A). In addition, TCC recognized this peptide in connection with numerous DR haplotypes, provided that the respective motif was present (32–34). However, the relevance of this HLA association (i.e., the possible use as a diagnostic marker) can only be evaluated...
in a study analyzing a high number of SB patients with latex allergy. Recently, a population study investigating HLA association with Hev b 1 reported an elevated frequency of the DRB1*0701 phenotype in Hev b 1 IgE-positive SB patients. However, the authors came to the conclusion that HLA-D alleles do not play a major role in the pathogenesis of latex allergy (35). The DR7 sequence does not contain the above-mentioned sequence motif.

Hev b 3-specific TCL and TCC were tested with peptides synthesized according to the sequence of Hev b 1 and representing the corresponding homologous sequence sections of this protein. TCC reacting with defined Hev b 3 T cell epitopes revealed no response corresponding homologous sequence sections of this protein. TCC synthesized according to the sequence of Hev b 1 and representing the corresponding peptides of Hev b 1, although the sequence does not contain the above-mentioned sequence motif.

Amino acid sequences of HLA-DRB-exon 2: HLA-DRB1*1301, *1302, *1401, and *1101 share the sequence YSTS at position 10–13 (underlined), a motif associated with the presentation of a major T cell epitope of Hev b 3. Cons., The conserved framework sequence.

FIGURE 4. HLA typing of MHC class II molecules expressed by latex-allergic SB patients. Amino acid sequences of HLA-DRB-exon 2: HLA-DRB1*1301, *1302, *1401, and *1101 share the sequence YSTS at position 10–13 (underlined), a motif associated with the presentation of a major T cell epitope of Hev b 3. Cons., The conserved framework sequence.

FIGURE 5. A, T cell reactivity with peptide Hev b 3103–114 is blocked by preincubation of APC with anti HLA-DR Ab, whereas anti HLA-DP or -DQ do not inhibit T cell proliferation. B, Irradiated APC expressing different HLA-DR molecules were incubated in the presence of peptide Hev b 3103–114 with a TCC specific for this peptide. Only APC displaying MHC molecules possessing the sequence YSTS at position 10–13 (YSTS-positive DR specificities are underlined) are able to induce proliferation in Hev b 3103–114-specific T cells.

References


