

Monoclonal IgE antibodies against birch pollen allergens: Novel tools for biological characterization and standardization of allergens

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Background: IgE antibodies are key players in immediate hypersensitivity reactions. Allergen characterization and standardization is usually based on the sera of allergic patients, whereas monoclonal IgE antibodies specific for clinically relevant allergens are very rare.

Objective: The aim of this study was to establish IgE mAbs specific for birch pollen allergens, because these are important inhalant allergens.

Methods: IgE-producing hybridomas were identified by using the highly sensitive rat basophilic leukemia cell mediator release assay with enhanced allergen stimulation by additional cross-linking with birch pollen-specific IgG antibodies. The obtained IgE mAbs were characterized by immunologic methods and by cDNA sequencing.

Results: Seven IgE mAbs specific for the birch pollen allergens Bet v 1 or Bet v 6 were obtained and were all biologically active in mast cell-based assays. Mediator release experiments with mAb combinations indicated that 2 different epitope regions were recognized on Bet v 1, whereas the 2 Bet v 6-specific mAbs bound to the same epitope region. After sensitization of rat basophilic leukemia cells with IgE mAbs, different amounts of Bet v 1 or Bet v 6 were detected in commercial diagnostic allergen reagents, whereas sensitization with polyclonal IgE resulted in similar allergenic potency of all products.

Conclusions: IgE mAbs represent promising novel tools for allergen characterization and component-resolved standardization of allergen extracts. (*J Allergy Clin Immunol* 2003; 111:1262-8.)

Key words: Allergen standardization, monoclonal IgE antibodies, RBL cells, birch pollen allergens, mediator release

IgE antibodies are key players in immediate hypersensitivity reactions. Identification, characterization, and standardization of allergens are usually based on immunoblotting or ELISA with sera from allergic patients. These tests depend on the used sera due to individual IgE antibody binding patterns.¹ Since these sera recognize multiple allergens, they cannot be used for accurate determination and quantification of single aller-

Abbreviations used

AP: Alkaline phosphatase
BPE: Birch pollen extract
CDR: Complementarity determining regions
Cl ab: Co-cross-linking antibodies
MRA: Mediator release assay
PTS: Prick test solution
RBL: Rat basophilic leukemia

gens. Immunoassays that are based on monoclonal allergen-specific IgG antibodies can overcome this problem. However, these tests do not consider that biologically active allergens must be able to cross-link Fcε-receptor-bound IgE molecules. Thus, allergen concentrations measured by ELISA may not correlate with biological allergenic potency because of, for example, the different potency of allergen isoforms.² The biological allergenic potency can be measured by skin prick test or by histamine release of human basophils but depends on the tested patients.¹ Determination of biological allergenic potency is also possible in animal models, such as passive cutaneous anaphylaxis (PCA), or in vitro with mediator release assays (MRAs) that simulate the IgE-dependent activation of mast cells using, for example, RBL cells.^{3,4} However, since the RBL-MRA is currently based on IgE-containing murine sera, only the overall allergenic potency of extracts can be measured. Thus, it is desirable to use allergen-specific IgE mAbs to study the contribution of defined allergens and their epitopes to the biological activity of allergen preparations.

Until now, only a few mouse IgE mAbs have been produced. Immunoblotting or allergen-specific ELISA or RIA was applied for detection of IgE-producing hybridomas. Most of the established IgE mAbs are specific for artificial allergens such as dinitrophenyl⁵⁻⁸ or trinitrophenyl.⁹ So far, IgE mAbs were raised against a few clinically relevant allergens such as trichosanthen protein,¹⁰ ragweed pollen,¹¹ or ovalbumin.^{12,13}

The aim of our study was to establish IgE mAbs specific for the clinically relevant allergens of birch pollen. In combination with the RBL-MRA, such IgE mAbs would allow precise determination of allergens in extracts in regard to their biological activity and investigation of the importance of the mAb-defined epitopes for

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the cross-linking process. To select hybridomas producing allergen-specific IgE antibodies, mediator release from RBL cells was used as a sensitive and functional screening method.

METHODS

MRA with RBL cells

All RBL-MRA followed a general protocol according to Hoffmann et al⁴ with slight modifications. RBL-2H3 cells (DSMZ, Braunschweig, Germany) plated in microtiter plates (Nunc, Wiesbaden, Germany) (1.5×10^5 cells/well) were passively sensitized with IgE-containing mouse serum or IgE mAbs. After washing, the cells were stimulated with antigen or H- or L-chain-specific antibodies. Cells sensitized with IgE mAbs were stimulated with antigen followed by additional cross-linking of IgE-bound allergens with polyclonal allergen-specific IgG antibodies (termed co-cross-linking throughout this article). The specific release was quantified by measuring β -hexosaminidase activity and was expressed as percent of the total β -hexosaminidase content that was obtained by lysing the cells with Triton X-100. For measurement of spontaneous release and possible unspecific effects, RBL cells were incubated with Tyrode's buffer, MEM, antigens, antibodies, or CI ab.

Allergens and antibodies

Allergen preparations were BPE (stock solution 1 mg/mL) (Allergopharma, Reinbek, Germany), rBet v 1 (stock solution 1 mg/mL) (Biomay, Vienna, Austria), rBet v 6 (stock solution 1 mg/mL) prepared according to Karamloo et al,¹⁴ and birch pollen-specific PTSs of 4 manufacturers.

H- or L-chain-specific antibodies were goat anti-mouse IgE heavy chain (Nordic, Tilburg, The Netherlands), goat anti-mouse IgG γ -chain, monoclonal rat anti-mouse κ -light chain, and monoclonal rat anti-mouse λ -light chain (all Sigma, Taufkirchen, Germany).

Allergen-specific antibodies for co-cross-linking were mouse serum pool with birch pollen-specific IgG (diluted 1:800), supernatant mixture of Bet v 1-specific IgG mAbs (2 μ g/mL), or of Bet v 6-specific IgG mAbs (1 μ g/mL).¹⁵

Preparation of mouse serum pools containing allergen-specific IgE antibodies

Balb/c mice (Charles River, Sulzfeld, Germany) were immunized 5 times in intervals of 2 weeks with 0.5 mL BPE (8 μ g/mL of protein) adsorbed to aluminum hydroxide. The allergen-specific IgE titer of sera were tested by RBL-MRA, and the RBL cells were sensitized with mouse serum diluted 1:50 and stimulated with BPE (15 μ g/mL of protein). Mice sera eliciting more than 30% β -hexosaminidase release were pooled.

Production and selection of IgE antibody-producing hybridomas

Spleen cells of mice with high birch pollen-specific IgE serum titers (immunized and IgE titer tested as described for IgE serum pools) were harvested, and the hybridomas were raised by conventional hybridoma technology using X63Ag8.653 cells (DSMZ) as a fusion partner.¹⁶ IgE-secreting hybridomas were identified by RBL-MRA. For sensitization, hybridoma supernatants were diluted 1:4 in MEM. After washing, the RBL cells were stimulated with 50 μ L BPE (15 μ g/mL of protein), and co-cross-linking with 50 μ L allergen-specific mouse serum was applied. Induced mediator release was determined as described previously. The IgE concentration in the hybridoma cell culture supernatants was determined by a sandwich ELISA.

Determination of allergenic potency of PTSs

RBL cells were sensitized with mouse serum containing birch pollen-specific IgE (dilution 1:50), IgE mAb mP 31, or IgE mAb mP 34 (both 0.5 μ g/mL). For stimulation the 4 PTSs, BPE, rBet v 1, and rBetv6 were used. Co-cross-linking was applied with Bet v 1-specific IgG mAbs or Bet v 6-specific IgG mAbs for IgE mAb sensitized cells. A standard curve was fitted as a 4-parameter logistic curve (SigmaPlot, SPSS Science Software, Erkrath, Germany) to the rBet v 1-induced or rBet v 6-induced mediator release of mP 31 or mP 34 sensitized cells, respectively, and the Bet v 1 or Bet v 6 concentration in the PTS was calculated.

Characterization of birch pollen-specific IgE mAbs by immunoblotting

BPE (5 μ g/cm total protein), rBet v 1, and rBet v 6 (both 0.3 μ g/cm) were separated by SDS-PAGE and blotted as described previously.¹⁴ Membranes were probed with IgE mAbs, Bet v 1-specific and Bet v 6-specific IgG mAbs¹⁵ (positive control) and IGE b4 (ATCC, Manassas, Va), a TNP-specific IgE mAb without reactivity to birch pollen allergens (negative control). All mAbs were used as hybridoma supernatants, adjusted to an IgE concentration of 0.5 μ g/mL. Bound mAbs were detected with rat anti-mouse IgE AP conjugate (Southern Biotechnology Associates, Birmingham, Ala) or goat anti-mouse IgG (γ -chain-specific) AP conjugate (Sigma) using an NBT/BCIP-based AP staining kit (Biorad). Applied buffers were described previously.¹⁴

Confirmation of IgE isotype by sequencing of IgE coding cDNA

Amplification of IgE coding cDNA was performed after preparing total RNA (RNeasy Total RNA System, Qiagen, Hilden, Germany) of 1×10^7 hybridoma cells and subsequent reverse transcription using dT₁₈-oligonucleotides (First strand cDNA Kit, Amersham Pharmacia Biotech, Freiburg, Germany) and 1 μ g up to 5 μ g RNA as template. Variable domains of the immunoglobulin heavy chain (V_H) were amplified by PCR primed by immunoglobulin-specific oligonucleotides. For amplification of the V_H -cDNA, the oligonucleotides $V_H1(5)$: 5'-ATG GCC SAG GTS MAR CTG CAG SAG TCW GG-3'¹⁷ and $C_H1(188-208)(3)$: 5'-AGC CTA GGG TCA TGG AAG CTG-3' [MMIgEHC] were applied. Immunoglobulin gamma-specific reverse primers C_H1 : 5'-CTC AAT TTT CTT GTC CAC CTT GGT GC and $C_H2,3$: 5'-CTG GAT TCT CTT GAT CAA CTC AGT CT-3' were used as negative controls. IGE b4 (ATCC) producing cells and IgG producing hybridoma cells (of our laboratory) served as further controls.

PCR products of the V_H -coding cDNA were purified by the Gel Extraction Kit or PCR Purification Kit (Qiagen) and cloned into pCRII-TOPO using the TA cloning kit (Invitrogen, Groningen, The Netherlands). Positive transformed *Escherichia coli* TOP10 cells (Invitrogen) were screened by colony PCR. Plasmids containing a PCR fragment of the expected size were purified by the Plasmid Miniprep Kit (Qiagen) and subsequently sequenced by M13 forward and reverse primers (ABI 373 "stretch" automated fluorescent sequencer, Applied Biosystems, Weiterstadt, Germany).

RESULTS

Birch pollen-specific IgE mAbs

By use of BPE in the RBL-MRA screening procedure, 7 birch pollen-specific IgE mAbs (mP 31, mP 33, mP 34, mP 36, mP 37, mP 39, and mP 40) were obtained. Stimulation of RBL cells sensitized with an IgE mAb with allergen alone did not lead to mediator release, or only weak release was obtained by sensitizing with a high

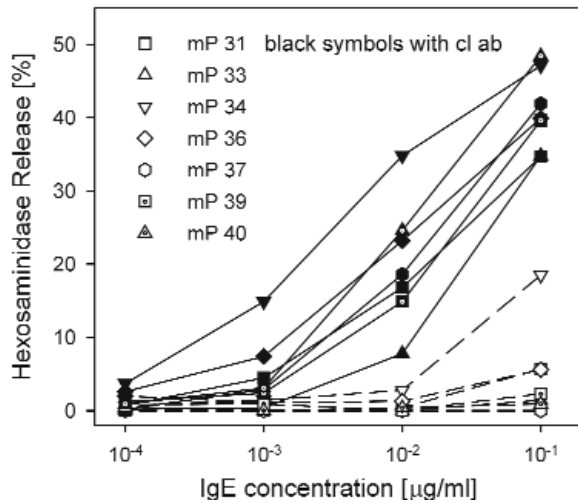


FIG 1. IgE mAb-sensitized cells required co-cross-linking antibodies to release mediators. RBL cells were sensitized with IgE mAb in different concentrations and stimulated with BPE without co-cross-linking antibodies (white symbols) or with birch pollen-specific IgG containing mouse serum (diluted 1:800) as cl ab (black symbols). Means of double determinations are shown; coefficients of variation were less than 15%.

concentration of IgE mAb (mP 34). However, an IgE concentration-dependent mediator release was induced by co-cross-linking of allergen with polyclonal allergen-specific IgG antibodies (murine serum pool) (Fig 1).

Characterization of heavy and light chains

RBL cells sensitized with the obtained mAbs and stimulated with a rat anti-mouse IgE antibody released 35% to 40% of the intracellular β -hexosaminidase, whereas stimulation with anti-mouse IgG antibodies failed to induce any mediator release (Table I, columns 1 and 2). For additional isotype confirmation, immunoblotting with BPE was performed. Detection of bound mAbs with anti-mouse IgE conjugate revealed bands in the expected molecular weight range of birch pollen allergens, whereas anti-mouse IgG conjugate did not show any reaction (data not shown). The IgE isotype was finally confirmed by sequencing the heavy chain of obtained mAbs. In an RT-PCR with a primer specific for the mouse ϵ -heavy chain amplicons of all mAbs as well as of the IGEL b4 (positive control) were obtained, revealing after cloning in the constant region the sequence of mouse ϵ -heavy chain. In contrast, the CDR 1-3 in the variable region of the heavy chain differed among all IgE mAbs, indicating that we obtained 7 different IgE mAbs (data not shown). The partial nucleotide sequences of all mAbs were submitted to the GenBank database under the accession numbers AF518231-AF518237. As expected, no amplicons were obtained from IgG-producing hybridoma cells.

The light chains were characterized by the RBL-MRA, showing that 5 antibodies had a κ -light chain (mP 33, mP 34, mP 36, mP 39, and mP 40) and that 2 IgE mAbs contained a λ -light chain (mP 31 and mP 37) (Table I, columns 3 and 4).

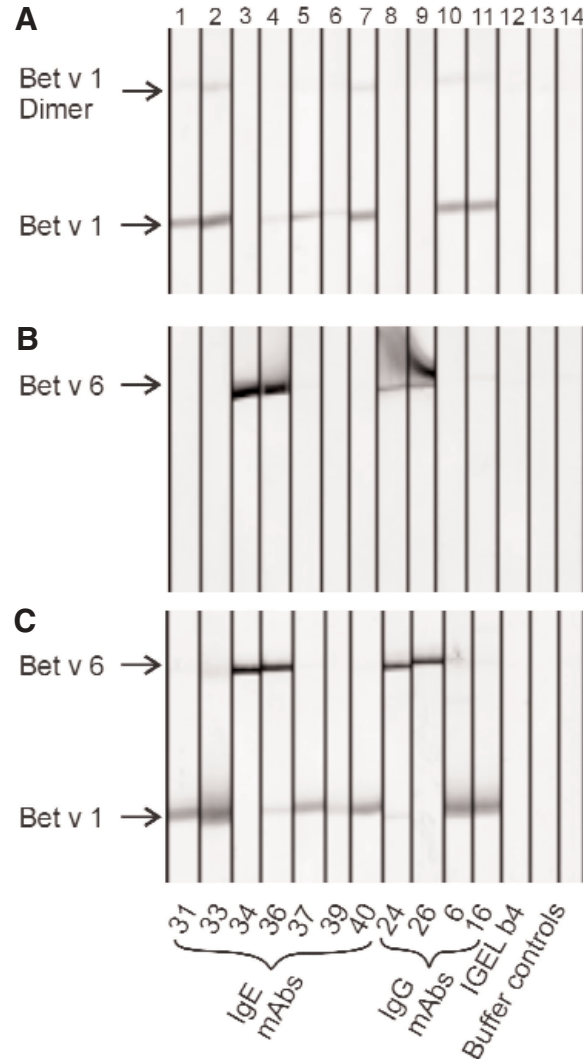


FIG 2. Detection of birch pollen allergens by IgE mAbs by immunoblotting. Blotting of rBet v 1 (A), rBet v 6 (B), or BPE (C) bands ascribed to Bet v 1, Bet v 1 dimer, or Bet v 6 are marked by an arrow. Lanes 1-7 were probed with IgE mAbs. Positive controls: lanes 8 and 9, Bet v 6-specific IgG mAbs; lanes 10 and 11, Bet v 1-specific IgG mAbs. Negative controls: lane 12, IGEL b4 (IgE mAb); lanes 13 and 14, buffer.

Bet v 1 and Bet v 6 specificity of IgE mAbs

Immunoblotting with rBet v 1 (Fig 2, A) and rBet v 6 (Fig 2, B) revealed 5 Bet v 1-specific IgE mAbs (mP 31, mP 33, mP 37, mP 39, and mP 40), 1 Bet v 6-specific IgE mAb (mP 34), and 1 Bet v 6-specific IgE mAb with slight cross-reactivity to Bet v 1 (mP 36). The same results were obtained by immunoblotting with natural BPE (Fig 2, C). Investigating the allergen specificity by ELISA with rBet v 1 or rBet v 6 as antigens, as well as by RBL-MRA using rBet v 1 or rBet v 6 as stimulant, led to the same results (data not shown). In addition, co-cross-linking with Bet v 1-specific or Bet v 6-specific IgG mAbs only stimulated mediator release from Bet v 1-specific or Bet v 6-specific IgE mAb-sensitized RBL cells, respectively (data not shown).

Combination of Bet v 1-specific IgE mAbs induces mediator release without co-cross-linking: Evidence of recognition of different epitope areas

RBL cells sensitized with a mixture of all Bet v 1-specific IgE mAbs released β -hexosaminidase after stimulation with rBet v 1 without CI ab (data not shown). To investigate the effect of mAb combinations, each possible combination of 2 IgE mAbs using the 5 Bet v 1-specific IgE mAbs and the cross-reactive mP 36 was applied for sensitization. As shown in Fig 3, there were 8 IgE mAb combinations inducing mediator release without co-cross-linking. On the basis of this functional combination, the IgE mAbs were divided into 2 groups. The first group consisted of mP 33 and mP 39, and the second consisted of mP 31, mP 36, mP 37, and mP 40. Only combinations of IgE mAbs from group I with those of group II induced mediator release, whereas combinations of IgE mAbs within 1 group did not.

IgE mAbs reveal differences in the Bet v 1 and Bet v 6 potency in PTS

PTSs are the standard tool of *in vivo* allergy diagnosis. Such allergen extracts are often characterized by inhibition assays using pooled polyclonal sera from allergic subjects. Four birch pollen PTSs from different manufacturers were compared in the RBL-MRA; BPE, rBet v 1, and rBet v 6 were used as reference allergens. After sensitizing RBL cells with the mouse polyclonal antiserum, all PTSs reacted similarly (Fig 4, A), whereas sensitizing RBL cells with mP 31 (Fig 4, B) or mP 34 (Fig 4, C) revealed different mediator release induction by the 4 PTSs. As expected, mP 31-sensitized cells were not activated by Bet v 6 (Fig 4, B), and mP 34-sensitized cells were not activated by Bet v 1 (Fig 4, C), whereas BPE induced a strong mediator release in all systems.

Using rBet v 1 or rBet v 6 as reference to calculate a standard curve, the concentrations of Bet v 1 or Bet v 6 in extracts can be determined on the basis of the biological activity of the allergens (Table II). In summary, the mouse polyclonal antiserum pool determined the general allergenic potency, whereas the IgE mAbs revealed the individual allergen-specific potencies. PTS no. 1 revealed the highest allergenic activity with anti-Bet v 1 and anti-Bet v 6 mAbs, indicating a high amount of both allergens. PTS no. 2 and no. 3 seemed to contain similar Betv1 concentrations, but the Bet v 6 concentration of PTS no. 2 was found to be low. Furthermore, PTS no. 4 showed a remarkable decrease in both the Bet v 1 and Bet v 6 content. These differences in the content of individual allergens were undetectable with the polyclonal antiserum.

DISCUSSION

Until now, only a few mouse IgE mAbs have been produced and scarcely allergen-specific ones. The aim of our study was to produce birch pollen-specific IgE mAbs that may serve as a novel tool for allergen characterization and epitope analysis. A highly sensitive screening system for

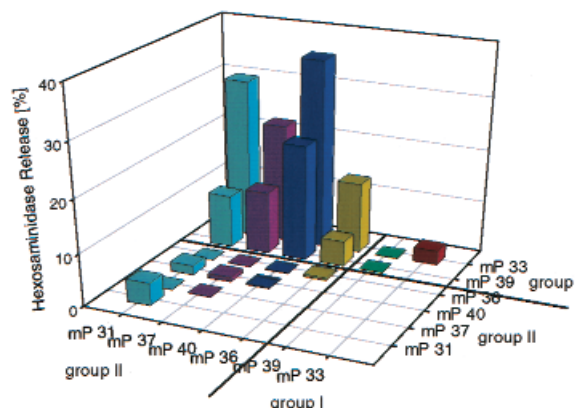


FIG 3. Combination of suitable IgE mAbs induce mediator release without co-cross-linking. RBL cells sensitized with all possible pairs of the Bet v 1-reactive IgE mAbs were tested for induction of mediator release after stimulation with rBet v 1 (0.1 μ g/mL) without co-cross-linking. Means of double determinations are shown; coefficients of variation were less than 15%.

IgE antibodies is required to detect IgE-producing hybridomas, because most B lymphocytes produce IgG antibodies even by using an immunization schedule that triggers the IgE response. In addition, it is preferable to screen directly for biologically active antibodies that are able to induce mediator release from mast cells. Thus, other authors often used PCA for detection of IgE-secreting hybridomas. Because PCA is very laborious, difficult to standardize, and many laboratory animals are required, we have established the RBL-MRA as an alternative to PCA.¹⁸ However, monoclonal IgE binds to a single epitope and fails to induce mediator release, because the cell-bound IgE molecules are not bridged. Thus, mediator release is only induced if the relevant allergen has repetitive epitopes, which was so far only shown for very few allergens, for example, the major codfish allergen,¹⁹ if the allergen forms dimers or polymers, or by additional cross-linking with allergen-specific IgG antibodies. Using the highly sensitive RBL-MRA with co-cross-linking as screening procedure, we detected 7 biologically active birch pollen-specific IgE mAbs.

The determination of partial amino acid sequences of the constant domain showed that all mAbs matched the ϵ -heavy chain, but they all had different CDR sequences. Consequently, the results suggested that the paratopes of the 7 IgE mAbs were different.

Five IgE mAbs contained a κ -light chain, and 2 mAbs contained a λ -light chain. These findings are in accordance with results of other authors, who reported that mice produce more antibodies with κ -light chains than with λ -light chains.²⁰ As for the heavy chains, we tried to sequence the light chains of the IgE mAbs. The λ -light chains could be sequenced (data not shown), but difficulties occurred for the κ -light chains because of the production of an additional aberrant mRNA transcript by MOPC-21 tumor-derived myeloma cells,^{21,22} which might exceed those generated from the productive light chain gene.^{23,24}

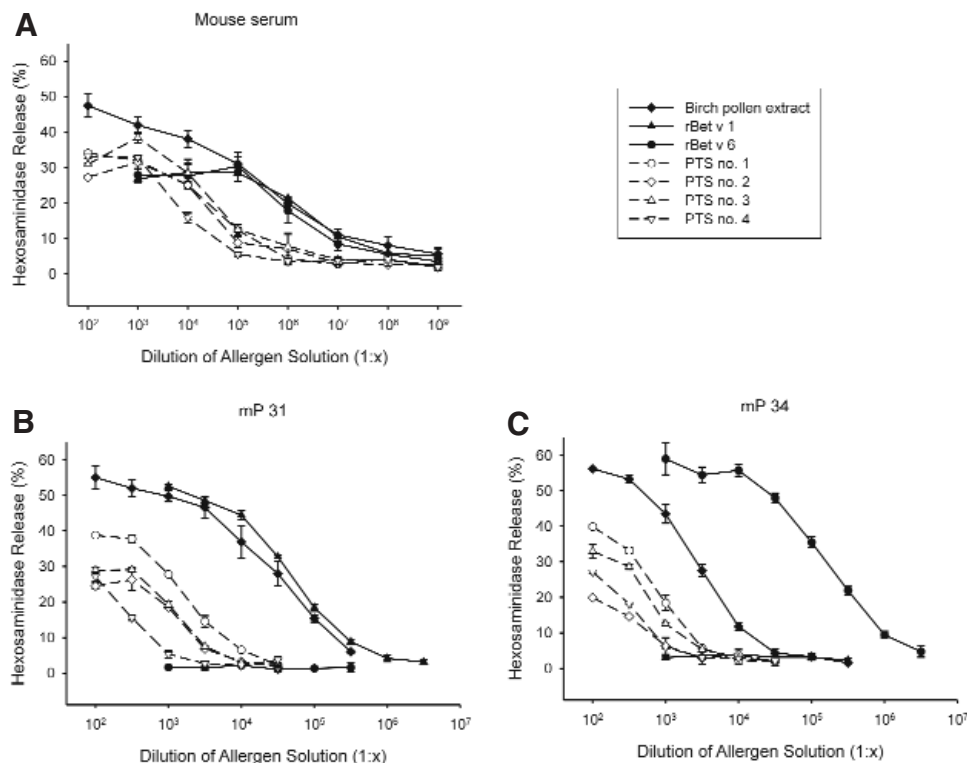


FIG 4. Determination of Bet v 1 and Bet v 6 allergenic potency in PTSs by IgE mAbs. RBL cells were sensitized with birch pollen-specific IgE containing serum (**A**), Bet v 1-specific IgE mAb mP 31 (**B**), or Bet v 6-specific IgE mAb mP 34 (**C**) and were stimulated with BPE (ranging from 10^{-10} – $10^{-2.5}$ $\mu\text{g/mL}$), rBet v 1, or rBet v 6 (both ranging from $1 \cdot 10^{-3.5}$ $\mu\text{g/mL}$) (black symbols) and with 4 PTSs (PTS nos. 1 to 4, white symbols). Co-cross-linking was applied for mP 31 and for mP 34. Means of double determinations with SDs are shown.

TABLE I. Mediator release (%) induced by H- and L-chain-specific antibodies

	Anti ϵ -chain*	Anti γ -chain*	Anti κ -chain†	Anti λ -chain†
mP 31	37.6 $\pm$ 1.5	2.1 $\pm$ 0.3	0.6 $\pm$ 0.2	21.6 $\pm$ 0.5
mP 33	34.7 $\pm$ 0.8	1.6 $\pm$ 1.1	32.0 $\pm$ 0.2	0.5 $\pm$ 0.1
mP 34	40.1 $\pm$ 2.4	3.7 $\pm$ 0.2	31.4 $\pm$ 2.5	0.0 $\pm$ 0.3
mP 36	37.1 $\pm$ 1.5	1.7 $\pm$ 0.7	35.4 $\pm$ 0.4	0.3 $\pm$ 0.4
mP 37	36.9 $\pm$ 2.3	1.5 $\pm$ 0.4	0.2 $\pm$ 0.0	28.9 $\pm$ 2.5
mP 39	40.3 $\pm$ 0.4	4.9 $\pm$ 1.4	40.2 $\pm$ 2.3	0.0 $\pm$ 0.9
mP 40	38.6 $\pm$ 0.9	0.0 $\pm$ 0.5	33.4 $\pm$ 0.2	0.0 $\pm$ 0.0

Positive releases are shown in boldface.

*IgE concentration: 0.1 $\mu\text{g/mL}$.

†IgE concentration: 0.5 $\mu\text{g/mL}$.

TABLE II. Calculated biologically active Bet v 1 and Bet v 6 concentrations ($\mu\text{g/mL}$) in prick test solutions

Prick test solution no.	Bet v 1	Bet v 6
1	20.8 $\pm$ 2.0	2.3 $\pm$ 0.3
2	7.6 $\pm$ 1.5	0.6 $\pm$ 0.2
3	8.6 $\pm$ 1.7	1.6 $\pm$ 0.2
4	2.2 $\pm$ 0.3	0.7 $\pm$ 0.2

As confirmed with rBet v 1 by immunoblotting, ELISA, and RBL-MRA, this major birch pollen allergen²⁵ was detected by 6 of the IgE mAbs. Bet v 1 can form dimers of 34 kDa²⁶ that were detected by mP 31, mP 33, and mP 40 (Fig 2, A). This might explain that RBL

cells sensitized with these mAbs were able to induce a weak mediator release after stimulation with rBet v 1 without co-cross-linking (Fig 3). The Bet v 6 molecule (formerly termed Bet v 5) (M_r 34 kDa)^{14,15} was detected by mP 34 and mP 36. On immunoblots and in the RBL-MRA, the mAb mP 36 presented weak cross-reactivity to Bet v 1. This cross-reactivity between Bet v 1 and Bet v 6 might be due to the so-called p-loop region, a sequence motif (-G-X-G-G-X-G-), which is highly conserved among the Bet v 1 family of Fagales allergens and other plant pathogenesis-related proteins.²⁷ Recently, the epitope character of this p-loop region was demonstrated.²⁸ Interestingly, Bet v 6 also contains such a glycine-rich motif,¹⁴ which may form a cross-reactive epitope.

Three highly conserved regions on the surface of the Bet v 1 molecule were proposed as candidates for antibody-binding epitopes.²⁹ Because the bridging of Fcε-RI-bound IgE antibodies activates the mast cells, sensitization with a mixture of 2 IgE mAbs detecting different epitopes of an allergen should be sufficient to induce mediator release. Indeed, several combinations of 2 IgE mAbs led to allergen-specific activation of RBL cells without co-cross-linking (Fig 3). On the basis of these results, we conclude that the mAbs of group I and group II detected 2 distinct epitope areas on the allergen surface. Within these areas, they may detect either the same epitope, overlapping, or adjacent epitopes. In combination with antibodies of group II, the mP 33 always led to a higher release than the mP 39. This might be due to detecting different epitopes or to recognition of the same epitope with different affinity. Recognition of only 1 epitope area on the Bet v 6 molecule may explain why a combination of the 2 Bet v 6-specific antibodies did not induce allergen-specific mediator release (results not shown). Hence, further screening of hybridomas is required to identify functional pairs of anti-Bet v 6 IgE mAbs.

To study whether allergen-specific IgE mAbs may serve as novel tools for characterization and standardization of allergen extracts, commercial standardized PTSs from different manufacturers were investigated. Standardized PTSs showed similar potency when investigated by RBL cells sensitized with polyclonal mouse IgE compared with human IgE inhibition, indicating that human and murine serum pools react similarly and present the same overall IgE pattern.³ As well, in this study all PTSs reacted similarly with the murine serum pool. However, we found that remarkable differences in the biological potency of single allergens in extracts can be detected if RBL cells are sensitized with IgE mAbs (Table II). This may help to avoid false-negative skin prick test results, for example, in patients who are monosensitized to only one of the birch pollen allergens.

Monoclonal IgE antibodies were generated from spleen cells of mice immunized with BPE. These mice did not develop a natural hypersensitization but produced IgE antibodies due to an immunization schedule that triggers the IgE response. Thus, differences between the produced IgE antibodies and such antibodies occurring in a natural sensitization could not be excluded. Moreover, little is known about whether mice produce antibodies against the same epitopes as humans. Until now, comparison between IgE antibodies of allergic patients and mouse monoclonal antibodies were performed with IgG mAbs due to the lack of allergen-specific IgE mAbs. In these studies IgG mAbs were described that inhibited the binding of human IgE to the allergen as well as those that did not.³⁰⁻³⁴ At present, we do not know whether the produced IgE mAbs detect human IgE epitopes.

In summary, to our knowledge, we created for the first time a panel of murine IgE mAbs against 2 clinically relevant birch pollen allergens that are biologically active in mast cell-based assays. These mAbs might represent a promising novel tool in allergen characterization, for epi-

tope analysis, and for component-specific standardization of allergen extracts. Further studies are in progress to analyze the epitope specificity of the mAbs compared with human IgE antibodies and to create a complete panel of IgE mAbs against all clinically relevant birch pollen allergens.

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