

Induction of IgE antibodies in mice and rhesus monkeys with recombinant birch pollen allergens: Different allergenicity of Bet v 1 and Bet v 2

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Background: Serologic measurements with recombinant birch pollen allergens, rBet v 1 and rBet v 2 (birch profilin), have shown that more than 95% of patients allergic to tree pollen mount high levels of IgE against rBet v 1, whereas only approximately 10% of the patients display rather low levels of IgE against rBet v 2.

Objective: In this study an attempt was made to determine whether the different allergenicity of the major birch pollen allergen, rBet v 1, and a minor birch pollen allergen, rBet v 2, might be related to a different immunogenicity of the proteins as evaluated in experimental animal systems (mice and rhesus monkeys).

Methods: Purified recombinant allergens were injected into mice and rhesus monkeys with aluminum hydroxide as adjuvant for elicitation of specific IgE responses. Antibody responses to the allergens were detected by immunoblotting, and time courses of immune responses were measured by ELISA.

Results: In both animal models more than the 10-fold dose of rBet v 2 was required to induce IgE antibodies, and even then, the amount of specific IgE antibodies elicited with rBet v 1 was substantially higher than that induced by rBet v 2. It was noted that rBet v 2 formed stable polymers through disulfide bonds.

Conclusion: In two different animal models (mice and rhesus monkeys) the major birch pollen allergen, rBet v 1, induced substantially higher levels of IgE than rBet v 2. A reduced allergenicity of Bet v 2 caused by polymer formation would be in agreement with previous studies indicating reduced allergenicity of proteins on chemical polymerization. (*J Allergy Clin Immunol* 1996;98:913-21.)

Key words: Allergens, recombinant Bet v 1 and Bet v 2, allergenicity, immunization, mice, rhesus monkeys

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Despite increasing knowledge of the biologic functions of allergens, there is still little understanding of why certain proteins are more allergenic than others. Those allergens against which a high percentage ($\geq 60\%$) of patients are sensitized and which mostly induce high titers of IgE have been designated as major allergens. In tree pollen allergy, Bet v 1 represents a major allergen of birch pollen inducing specific IgE antibodies in more than 95% of patients allergic to birch pollen,¹ whereas birch pollen profilin (Bet v 2) induces IgE antibodies in about only 10% to 20% of patients allergic to pollen,¹⁻⁴ showing, however, extensive cross-reactivities with profilins from pollen and food.^{2,3}

Abbreviations used

PBS:	Phosphate-buffered saline
SDS-PAGE:	Sodium dodecylsulfate–polyacrylamide gel electrophoresis

In this study we investigated the possibility that the difference in allergenicity of Bet v 1 and Bet v 2 observed in patients with allergy¹ may be attributable to different immunogenicity and intrinsic molecular differences of these two allergens and might be reproduced in experimental animal models. The two allergens were recently expressed as recombinant proteins in *Escherichia coli*, and in vitro studies demonstrated that their immunologic and physicochemical (e.g., molecular weight, isoelectric point) properties were similar to those of their natural counterparts.^{3,5,6}

It has been generally accepted that induction of specific IgE responses to diverse protein antigens in mice and rats may serve as a useful model for investigating the pathogenetic mechanism underlying IgE-mediated allergies, which in part can be transferred to human beings.⁷⁻¹¹ Moreover, immunization of mice with tiny doses of antigens absorbed onto Al(OH)₃ was shown to elicit specific IgE responses in mice¹²⁻¹⁴ with features typical of T_{H2} immune responses¹⁵ and comparable to those observed in patients with allergy.¹⁶ In contrast, a T_{H1} response can be elicited in mice by injection of antigens with Freund's complete adjuvant.^{17,18}

Pure and soluble rBet v 1 and rBet v 2 were used for induction of specific IgE antibodies in mice and rhesus monkeys. For induction of anti-Bet v 2 IgE antibodies in mice and rhesus monkeys, the immunizing dose of profilin had to be 20-fold higher than that of Bet v 1; even then, the amounts of the Bet v 2-specific IgE antibodies were lower than those of the Bet v 1-specific IgE antibodies. It could be shown that rBet v 2 formed polymers through disulfide bonds, and it is hence suggested that the decreased allergenicity of rBet v 2 might be related to its tendency to polymerize. A similar capacity to form polymers was also noted for natural profilins from birch and other sources (unpublished data). Our findings fit the observation that chemical polymerization of allergens can switch T_{H2} to T_{H1} immune responses.¹⁹⁻²²

METHODS**Purification of recombinant forms of Bet v 1 and birch profilin (Bet v 2)**

Recombinant Bet v 1 was expressed in *E. coli* JM105¹ and purified to homogeneity according to the previously described protocol.⁶ The recombinant protein produced a single peak in the chromatogram obtained by high-pressure liquid chromatography and was completely soluble. Recombinant birch profilin, rBet v 2, was expressed at high levels in plasmid pMW175 *E. coli* BL21 (DE3). The purification protocol described by Valenta et al.³ was modified so that a more than 95% soluble protein was yielded.²³ Both proteins were tested for IgE- and antibody-binding capacity in ELISA and immunoblot assays and further tested for their capacity to release histamine from basophils of patients with allergy, as described previously.^{3,5,6}

Reducing and nonreducing sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS-PAGE), immunoblots, and blot overlays

The tendency of recombinant Bet v 2 to form polymers through disulfide bonds under nonreducing conditions was demonstrated by SDS-PAGE, immunoblotting, and blot overlays. SDS-PAGE was done as described by Laemmli.²⁴ Five micrograms of rBet v 2 and rBet v 1, respectively, were dissolved in 10 µl sample buffer with or without β-mercaptoethanol and subjected to SDS-PAGE. Gels were then stained with Coomassie Brilliant Blue (Bio-Rad, Richmond, Calif.) as previously described.²⁵ Higher molecular weight bands, which were assumed to represent Bet v 2 polymers, were probed with two different rabbit anti-profilin antibodies (RP1 and RP3).²⁶ The binding of rBet v 2 to itself was further investigated by blot overlays. Recombinant Bet v 2 was separated by SDS-PAGE and blotted onto nitrocellulose.²⁷ Nitrocellulose strips were then incubated with iodine 125-labeled rBet v 1 and rBet v 2 under reducing and nonreducing conditions, respectively. Each strip was incubated with radiolabeled protein (500,000 cpm) with or without the addition of β-mercaptoethanol (5 mmol/L) as described by Valenta et al.²⁶ Binding of ¹²⁵I-labeled proteins was visualized by autoradiography with Kodak x-OMAT films and intensifying screens (Kodak, Heidelberg, Germany).

Immunization of mice and rhesus monkeys with rBet v 1 and rBet v 2

Eight-week-old female BALB/c and B6D2F₁ mice were obtained from a local experimental animal care unit (Himberg, Austria). Animals were maintained in the animal care unit of the Institute of General and Experimental Pathology of the University of Vienna according to the local guidelines for animal care. Groups of mice were immunized by subcutaneous injection in the neck with 0.1 µg or 1 µg of rBet v 1 and 0.1 µg, 1 µg, or 20 µg of rBet v 2 adsorbed to 100 µl of AluGel-S

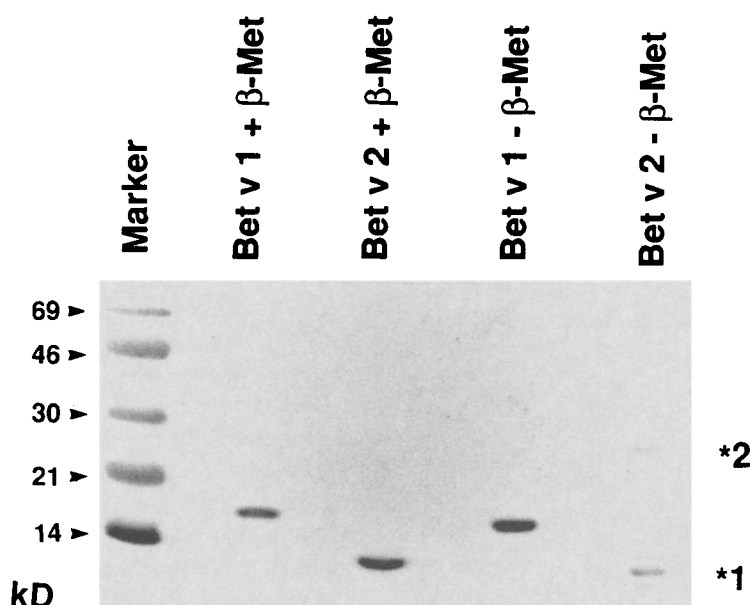


FIG. 1. Coomassie Brilliant Blue-stained SDS-PAGE showing purified recombinant Bet v 1 and recombinant birch profilin (Bet v 2) separated under reducing and nonreducing conditions. Five micrograms of purified recombinant Bet v 1 and 5 μ g of purified recombinant Bet v 2 were separated by SDS-PAGE under reducing (+ β -mercaptoethanol) and nonreducing ($-\beta$ -mercaptoethanol) conditions. Lane M is the molecular weight marker. Monomers (1) and dimers (2) in the nonreduced samples of Bet v 2 are indicated with *asterisks*. β -Met, β -Mercaptoethanol.

(Serva, Heidelberg, Germany) per mouse. A control group was immunized with 100 μ l of AluGel-S by using 0.9% sodium chloride instead of allergens. Immunizations were done with 30 mice of each of two strains (BALB/c and B6D2F1); groups of 10 mice were immunized with the same dose of antigen, and results were reproduced by using another 30 mice in a second experiment. Re-immunizations and bleedings were done at approximately 14-day intervals for a period of 9 months (in total 15 immunizations were done). Final bleedings were done after 1 year.

Groups consisting of two rhesus monkeys (one male and one female) were immunized subcutaneously with 100 μ l of AluGel-S in combination with allergen or phosphate-buffered saline (PBS) (negative control) monthly for a period of 6 months. Group I was immunized with 1 μ g of rBet v 1 per animal, group II received 10 μ g of rBet v 1 per animal, group III was injected with 1 μ g of rBet v 2 per animal, and group IV received 20 μ g of rBet v 2 per animal. The control group, consisting of one male monkey and one female monkey, was immunized with PBS without addition of allergen. In a second experiment four monkeys were immunized with 1 μ g of rBet v 1, and four monkeys received 20 μ g of rBet v 2. Monkeys were bled from the forearm 1 week before and 1 week after immunizations.

Determination of specific antibody responses by immunoblot and ELISA

Allergen-specific antibody responses were determined by immunoblotting and ELISA. For determination of

mouse antibody levels, ELISA plates (Nunc, Roskilde, Denmark) were coated with 100 μ l/well of the recombinant allergens diluted to 5 μ g/ml in PBS. The plates were washed two times with PBS–0.05% Tween and blocked for 2.5 hours with PBS–1% wt/vol bovine serum albumin–0.05% vol/vol Tween at room temperature; and 100 μ l/well of the mouse serum dilutions in PBS–0.5% bovine serum albumin–0.05% Tween was added (IgE, 1:20; IgG₁, 1:2000; IgG_{2a}, 1:200; IgG₃, 1:200). The plates were incubated with sera in duplicate overnight at 4° C and washed five times with PBS–0.05% Tween, and bound mouse immunoglobulins were detected with 100 μ l of monoclonal rat anti-mouse immunoglobulin antibodies diluted 1:1000 in PBS–0.5% wt/vol bovine serum albumin–0.05% vol/vol Tween (PharMingen, San Diego, Calif.) overnight at 4° C. After it was washed five times with PBS–0.05% vol/vol Tween, a 1:2000 diluted horseradish peroxidase–coupled sheep anti-rat antiserum (Amersham, Buckinghamshire, U.K.) was added for 30 minutes at 37° C and 30 minutes at 4° C. Plates were again washed five times with PBS–0.05% Tween, and ABTS (60 mmol/L citric acid, 77 mmol/L Na₂HPO₄ · 2H₂O, 1.7 mmol/L ABTS [Sigma, St. Louis, mo.], 3 mmol/L H₂O₂) was added. Plates were incubated in the dark for 30 minutes at room temperature, and the color reaction was stopped by addition of 100 μ l/well 0.32% NaF. Extinctions (optical density, 450 to 405 nm) were determined with an ELISA reader (Dynatech, Denkendorf, Germany). To exclude the possibility that blocking IgG antibodies can interfere with the IgE binding, sera

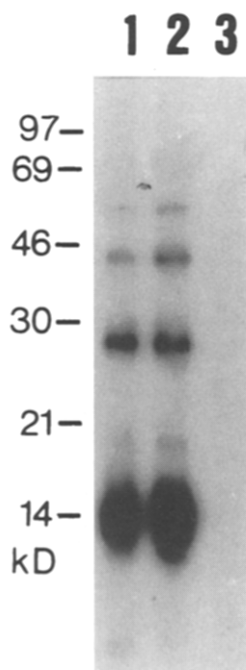


FIG. 2. Detection of Bet v 2 polymers with profilin-specific antisera. Recombinant Bet v 2 was separated by SDS-PAGE and blotted onto nitrocellulose under nonreducing conditions and detected with two different anti-profilin antisera (lane 1: RP1; lane 2: RP3). In lane 3 a normal rabbit serum was used as a negative control.

were also depleted for IgG₁ and IgG_{2a/b} by preincubation with an anti-mouse IgG₁ and IgG_{2a/b} monoclonal rat antibody (PharMingen) coupled to Sepharose 4B (Pharmacia, Uppsala, Sweden) (data not shown). IgE and IgG antibodies of immunized rhesus monkeys were determined by immunoblotting²⁸ and ELISA. Recombinant Bet v 1 and rBet v 2 (2 µg/cm gel) were separated by SDS-PAGE²⁴ and blotted onto nitrocellulose.²⁷ Nitrocellulose strips were incubated with 1:10 diluted monkey sera for IgE detection and 1:1000 diluted sera for IgG detection. Bound IgE and IgG were detected with rabbit anti-human IgE or IgG antisera (Dako, Glostrup, Denmark). Both antisera were shown to cross-react with rhesus monkey immunoglobulins and to be specific for IgE and IgG in a previous study.²⁸ To compare IgE levels of Bet v 1- and Bet v 2-immunized rhesus monkeys, ELISAs were done. The ELISA was performed as described for the mouse ELISA, except that IgE was detected with a rabbit anti-human IgE antiserum (Dako) and a horseradish peroxidase-labeled donkey anti-rabbit antiserum (Amersham).

RESULTS

Characterization of purified recombinant allergens, rBet v 1 and recombinant birch profilin (rBet v 2)

The investigation of the capacity of rBet v 1 and rBet v 2 to elicit a specific IgE response in vivo

required purification protocols delivering highly pure and soluble proteins, which contained no lipopolysaccharide or other contaminants. Recombinant Bet v 1 was purified from *E. coli* JM105 transformed with pKK 223-3 containing the Bet v 1 complementary DNA¹ according to the protocol described by Ferreira et al.⁶ The cDNA coding for Bet v 2, which was originally expressed in *E. coli* JM105 pKK 223-3,^{1,2} was transferred into plasmid pMW175 for high level expression in *E. coli* BL21 (DE3).²³ The recombinant protein was purified as previously described.³ Pure and more than 95% soluble recombinant proteins were obtained, which according to different in vitro assays (binding of patients' IgE,^{1,3} binding of specific antibodies,^{3,6} capacity to release histamine from basophils of patients with allergy^{3,5,6}) were found to behave similarly to the natural allergens. Lack of lipopolysaccharide and contaminants in the recombinant allergen preparations was determined by specific histamine release with human basophils and limulus assays (data not shown). Fig. 1 shows a Coomassie Brilliant Blue-stained SDS-polyacrylamide gel. Five micrograms of purified rBet v 1 and rBet v 2 were separated under reducing (+β-mercaptoethanol) and nonreducing conditions (−β-mercaptoethanol). Purified rBet v 1 produced one band at 17 kd under reducing and nonreducing conditions, whereas rBet v 2 migrated as a single band at 14 kd only under reducing conditions. When β-mercaptoethanol was omitted from the sample buffer, additional bands could be stained, of which one migrated at 28 kd. To further investigate whether the higher molecular bands that were observed in the Bet v 2 preparation under nonreducing conditions might be due to formation of polymers through disulfide bonds, immunoblots were done. Two different anti-profilin antisera identified the higher molecular weight bands as Bet v 2 polymers (Fig. 2). Blot overlays done with ¹²⁵I-labeled rBet v 2 showed specific binding of rBet v 2 to blot-immobilized rBet v 2, whereas no reaction with rBet v 1 (negative control) was observed. Under reducing (+β-mercaptoethanol) conditions, rBet v 2 did not bind to itself (data not shown). It is hence suggested that the binding occurs through the formation of disulfide bonds.

Antibody responses induced with recombinant allergens in mice

Mice and monkeys that had been immunized with 1 µg rBet v 1 produced significantly higher

TABLE I. Antibody responses of BALB/c mice immunized with recombinant Bet v 1 (1 µg/injection) or Bet v 2 (20 µg/injection)

	Time of first response (week ± SD)	Time of maximal response (week ± SD)	OD of maximal response (±SD)
Bet v 1			
IgE	10.6 (±4.08)	18.4 (±8.16)	0.6 (±0.15)
IgG ₁	15.4 (±3.21)	27 (±4.36)	2.5 (±0)
IgG _{2a}	20.9 (±5.46)	35.9 (±11.63)	1.6 (±0.84)
IgG ₃	16.9 (±1.07)	27 (±8.19)	0.27 (±0.05)
Bet v 2			
IgE	20 (±3.42)	30.3 (±5.47)	0.19 (±0.05)
IgG ₁	22.6 (±6.43)	30.4 (±4.79)	2.20 (±0.57)
IgG _{2a}	24 (±2.65)	31.4 (±4.39)	0.86 (±0.91)
IgG ₃	15.7 (±3.55)	28.3 (±3.2)	0.19 (±0.04)

Time-course and antibody responses of BALB/c mice immunized with recombinant Bet v 1 (1 µg/injection) or Bet v 2 (20 µg/injection). Mean values of the times when first significant antibody responses and maximal responses were measured in a group of seven Bet v 1-immunized mice and seven Bet v 2-immunized mice are displayed in weeks together with the standard deviation (SD). Mean values of antibody responses measured at the time of maximum immune response are displayed as optical density and SD. For the measurement of specific IgE, sera were diluted 1:20; for IgG₁, 1:2000; for IgG_{2a}, 1:200; and for IgG₃, 1:200. OD, Optical density.

levels of specific IgE, whereas in the case of rBet v 2, 20 µg was necessary to induce specific IgE responses. No detectable IgE levels could be induced in mice and rhesus monkeys with 1 µg of rBet v 2. The fact that the amounts of rBet v 2, which were necessary to induce detectable IgE responses in mice and rhesus monkeys, were 20-fold (20 µg/animal) higher than the amounts of rBet v 1 (1 µg/animal) needed, prompted us to investigate Bet v 1- and Bet v 2-specific antibody responses in detail. Table I shows the antibody responses in a group of seven mice immunized with 1 µg of rBet v 1 and seven mice immunized with 20 µg of rBet v 2 determined by ELISA. IgE, IgG₁, IgG_{2a}, and IgG₃ responses were measured over a period of 57 weeks at serum dilutions in which antigen was coupled in excess to antibodies to allow quantitation of results. Mice immunized with rBet v 1 showed an earlier rise of Bet v 1-specific IgE antibodies and mounted approximately three-fold higher levels of specific IgE than the rBet v 2-immunized mice. The differences in the IgG responses were less pronounced. The rBet v 1-immunized mice displayed, for the most part, less than double the levels of specific IgG_{2a} and IgG₃ antibodies compared with the rBet v 2-immunized mice, and comparable IgG₁ levels were reached.

To exclude the possibility that blocking IgG antibodies might influence IgE binding, additional control experiments were done. Sera were depleted for IgG₁ and IgG_{2a/b} antibodies before IgE reactivity was measured, confirming that the Bet v

1-specific IgE titers were significantly higher than the Bet v 2 IgE responses. Comparable results were obtained in B6D2F₁ mice (data not shown).

Difference in antibody responses of rhesus monkeys to recombinant forms of Bet v 1 and birch profilin (Bet v 2)

The IgE and IgG reactivity of a representative Bet v 1-allergic monkey and a Bet v 2-allergic monkey with nitrocellulose-blotted purified rBet v 1 and rBet v 2 are displayed in Fig. 3. Sera obtained 1 week before and after each immunization were tested for IgE and IgG reactivity with nitrocellulose-blotted purified rBet v 1 (Fig. 3, A) and rBet v 2 (Fig. 3, B). As in the mouse system, a more intense binding to rBet v 1 was observed than to rBet v 2, whereas the IgG reactivity was comparable. Because the RAST-based immunoblot assay is a semiquantitative technique, the IgE levels induced with rBet v 1 and rBet v 2 in rhesus monkeys were investigated by ELISA experiments done at conditions of antigen excess to allow quantification of responses. Fig. 4 shows the ELISA determinations of specific IgE antibodies in five rBet v 1- and five rBet v 2-immunized monkeys after the fourth month of immunization (immunizations were done monthly). Only in one rBet v 2-immunized monkey (no. 7) could specific IgE be detected by ELISA, whereas in the sera of the rBet v 1-immunized monkeys (nos. 1 to 5) high levels of specific IgE could be measured. It must be stated, however, that the five rBet v 2-immunized monkeys had

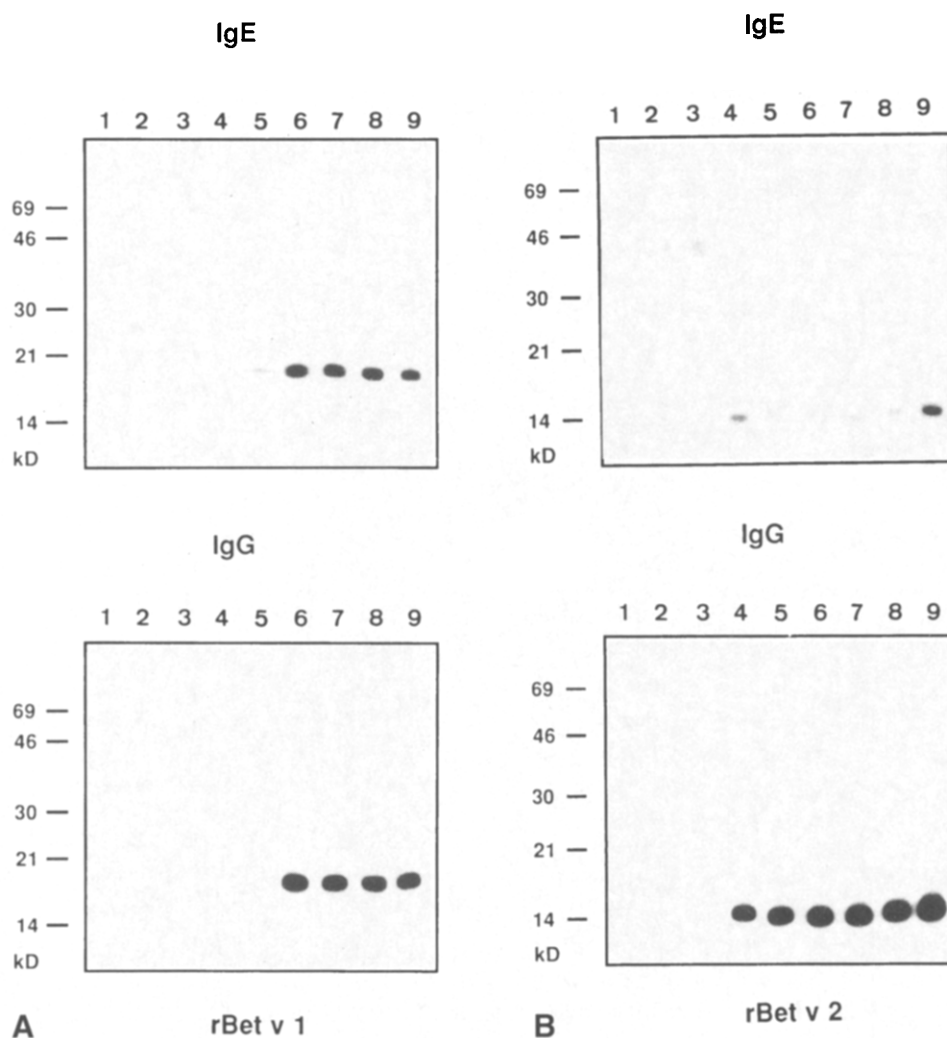


FIG. 3. IgE and IgG reactivity of a representative Bet v 1-immunized rhesus monkey (**A**) and a Bet v 2-immunized rhesus monkey (**B**) with nitrocellulose-blotted rBet v 1 and rBet v 2. For IgE detection, sera were diluted 1:10, and for IgG detection, 1:1000. To allow a comparison of signal intensity all blots were exposed for the same time (4 hours). In the first lane the monkeys' preimmune serum was used, whereas in the other lanes, strips were incubated with serum samples obtained 1 week before and after the monthly immunizations. The Bet v 1-immunized monkey had received 1 μ g of rBet v 1 per immunization, whereas the Bet v 2-immunized monkey was immunized with 20 μ g of rBet v 2 per injection.

positive rBet v 2-specific IgE when tested with the more sensitive immunoblotting technique and had mounted anti-rBet v 2 IgG titers comparable to those observed in the rBet v 1-immunized rhesus monkeys (data not shown).

DISCUSSION

Type I allergy represents a health problem of increasing importance in industrialized countries. Knowledge of the structures and sequences of allergens has increased tremendously because of the application of cDNA cloning techniques in the recent past.²⁹ Several allergens have been cloned

and sequenced and can be expressed by recombinant techniques.^{30,31} Although biologic functions of several allergens are known or suspected, there is still a lack of knowledge about why certain proteins are more effective in eliciting an IgE response.

House dust mite allergens, such as Der p 1,^{32,33} seem to have protease activity. The major birch pollen allergen Bet v 1³⁴ and the white-faced hornet venom allergen Dol m 5³⁵ are homologous to pathogenesis-related plant defense proteins, profilin (Bet v 2,² Phl p 11³⁶) represents an actin-binding protein, and Bet v 3 belongs to a novel

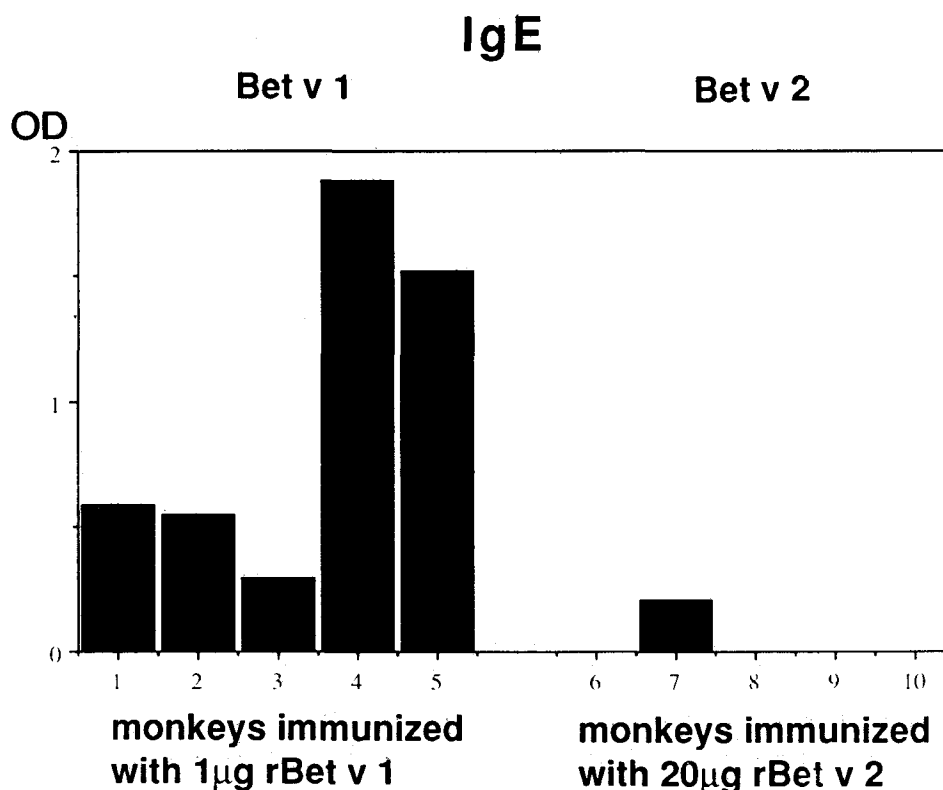


FIG. 4. ELISA measurements of Bet v 1- and Bet v 2-specific IgE antibodies in rhesus monkeys. Levels of Bet v 1- and Bet v 2-specific IgE were measured at serum dilutions of 1:5 in five rBet v 1-immunized rhesus monkeys (1 to 5) and in five rBet v 2-immunized monkeys (6 to 10). Extinctions are displayed as optical densities (OD) on the y axis. Numbers of the animals are displayed on the x axis.

class of calcium-binding proteins.³⁷ In the case of fungal allergens, homologies of Asp f 1 with mitogillin could be found.³⁸ The only obvious common properties of most allergens seem to be their low molecular weight (10 to 70 kd) and their rapid release from airborne particles on contact with aqueous solutions. Hence a relation between allergenicity and speed of release on hydration was discussed earlier.³⁹

Another way to determine whether the differences in allergenicity of allergens might be linked to intrinsic properties of proteins is to study the immunogenicity of purified proteins in experimental animal systems. We have chosen two well-characterized pollen allergens, Bet v 1 and Bet v 2, which are now available as pure recombinant proteins possessing the same immunologic and physicochemical characteristics as the natural proteins. A greatly different percentage of patients is sensitized to Bet v 1 and Bet v 2. Bet v 2 was classified as a minor allergen because only 10% to 20% of patients show IgE reactivity, whereas more than 95% of patients allergic to birch pollen are sensi-

tized against Bet v 1, which therefore represents a major allergen.¹ Both allergens have a comparable molecular weight (17 kd and 14 kd, respectively) and are not significantly glycosylated. Recombinant Bet v 1 and Bet v 2 were expressed in *E. coli* as nonfusion proteins and were purified to homogeneity as soluble proteins, which possess immunologic and physicochemical properties similar to those of their natural counterparts. Purified rBet v 1 and rBet v 2 were injected at different doses into mice and rhesus monkeys by using Al(OH)₃ as adjuvant. Two different animal models were chosen to rule out differences in the immunogenicity of the proteins caused by genetic restrictions of the hosts.

In the mouse model, as well as in the rhesus monkey model, rBet v 1 induced significantly higher titers of specific IgE already at low doses (1 µg/animal) compared with rBet v 2, which induced IgE responses only at high amounts (20 µg/animal). The comparable difference in allergenicity observed in mice and rhesus monkeys strongly suggests that this difference is not limited to the

choice of a certain animal model or a restricted genetic background of the host. In fact, when we tested patients allergic to birch pollen, we found that a high proportion of these patients displayed IgG reactivity against Bet v 2 but produced no detectable levels of IgE antibodies.

As in the animal models, the levels of Bet v 1-specific IgE were substantially higher than those of Bet v 2-specific IgE.⁴⁰ Although the experimental animal models studied certainly differ in many aspects (e.g., use of recombinant allergens, use of adjuvant) from the process of natural sensitization in human beings, it is reasonable to assume that the different allergenicity of Bet v 1 and Bet v 2 might result in part from the different immunogenicity of these allergens.

The observation that Bet v 2 induced lower levels of IgE antibodies in human beings and in experimental animal models, although still substantial IgG responses were elicited, led us to suspect that the difference in allergenicity might also be linked to intrinsic properties of the allergens. When purified rBet v 1 and rBet v 2 were analyzed by SDS-PAGE under reducing and non-reducing conditions, we found that rBet v 2 formed polymers under nonreducing conditions. Chemical polymerization of antigens was already described to favor a T_{H1} immune response in mice,¹⁹⁻²² and it is hence possible that the weaker capacity of rBet v 2 to induce IgE antibodies might be linked to the ability of Bet v 2 to form natural polymers through disulfide bonds. Although it must be stressed that there is currently no feasible experimental model to definitely prove this hypothesis, the observation is in agreement with experimental data suggesting that polymerization of antigens might be a mechanism with which to reduce the allergenicity of protein antigens in favor of a T_{H1} response.

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