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(54) Title: METHOD FOR EVALUATING THE ALLERGEN SENSITIVITY OF AN INDIVIDUAL

(57) Abstract: The present invention discloses a method for evaluating the allergen sensitivity of an individual and/or the clinical efficacy of an allergen immunotherapy comprising the steps: providing at least two samples selected from the group consisting of blood or fractions thereof, connective tissue, nasal, bronchial, skin or gut biopsy material from an individual subjected or intended to be subjected to an immunotherapy with at least one pure allergen or derivative thereof, wherein the samples contain cells capable of releasing mediators in response to said allergen, contacting said sample with said allergen or derivative thereof, and determining the amounts of mediators released from said sample and evaluating the allergen sensitivity of the individual prior to therapy and/or the clinical efficacy of the immunotherapy by comparing said amounts.

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Method for evaluating the allergen sensitivity of an individual

The present invention relates to methods for monitoring the efficacy of an allergen immunotherapy.

An allergy is an immune malfunction wherein an individual is hypersensitised to react immunologically to typically per se harmless substances called allergens. The principal antibody which is involved in allergic reactions is IgE. Every individual has different IgE antibodies and each allergic substance stimulates production of its own specific IgE. An IgE antibody binding a defined allergen will therefore react only against said allergen. The constant region (Fc region) of IgE is able to bind to specific receptors of cells, which are able to release histamine or other inflammatory mediators, cytokines and/or proteases into the surrounding tissue. Histamine releasing cells are mainly mast and basophilic cells. The release of histamine is initiated when cell-bound IgE is contacted and cross-linked by the allergen.

Especially histamine causes the main allergic reactions. Histamine released in the nose, eyes, and sinuses, for example, stimulates sneezing, a runny nose, and itchy eyes; released in the lungs it causes narrowing and swelling of the lining of the airways and the secretion of thick mucus; in the skin, rashes and hives; and in the digestive system, stomach cramps and diarrhea.

Typical allergens are derived from plant pollens, like rye grass, ragweed, timothy grass and birch trees pollens, mold spores, drugs, like penicillins, sulfanomides, salicylates and local anaesthetics, foods, like nuts, seafood, egg, peas, beans, peanuts and other legumes, milk, insect products, like bee-sting venom, wasp sting venom, cockroach calyx and dust mites, and animal hair and dander.

There exists a number of medical treatments for allergies. Mainly three methods are regularly used in medical practice: chemotherapy, immunotherapy and alternative medical methods.

In chemotherapy antagonistic drugs are used to block the action of allergic mediators, preventing activation of cells and degranulation processes. They include antihistamines, cortisone, adrenalin (epinephrine), theophylline and Cromolyn sodium. These drugs help alleviate the symptoms of allergy but play little role in chronic alleviation of the disorder. They can play an

imperative role in the acute recovery of someone suffering from anaphylaxis.

In alternative medicine, a number of treatments are considered effective by practitioners in the treatment of allergies, particularly traditional Chinese medicine. However, none of these have been backed up by good quality evidence.

The most promising therapy form is probably immunotherapy. In the course of an immunotherapy where an individual is gradually vaccinated against progressively larger doses of the allergen in question. This can either reduce the severity or eliminate hypersensitivity altogether. Alternatively, monoclonal anti-IgE antibodies may be injected. These antibodies bind to free IgE signalling such sources for destruction. They do not bind to IgE already bound to the Fc receptor on basophils and mast cells as this would stimulate the allergic inflammatory response.

The proteins and glycoproteins used in allergen immunotherapy are usually extracted from materials such as pollens, molds, pelt and insect venoms. Based on the clinical evaluation, repeated subcutaneous injections of a solution of the disease-causing allergen or a derivative thereof are done once or twice a week in increasing doses until a maintenance dose is reached. This maintenance dose is then injected every 2 to 4 weeks.

In order to accomplish an immunotherapy in a successful manner monitoring of the progress of said therapy has to be performed.

For instance, in Wantke et al. (Clin Exp Allergy 23 (1993) 992-995) a method for monitoring an immunotherapy for allergic rhinoconjunctivitis is disclosed. Therein the authors analysed the spontaneous histamine release, i.e., the release without addition of allergen, in patients prior and after the immunotherapy and showed that the histamine release into the blood after exposure to the allergen was significantly reduced after four months of treatment. However, this method cannot be used to assess changes in sensitivity towards a particular allergen and specific efficacy of the treatment.

Stephan et al. (Allergy 44 (1989) 453-459) investigated the effect of bee venom immunotherapy over a period of more than five years by analysing the allergen induced histamine release in whole blood. However, the authors of this study did not correlate the results of histamine release with a clinical paramet-

er, e.g., skin sensitivity and hence no data were shown which would justify to use the assay to measure and reflect clinical sensitivity to a given allergen. Furthermore, no samples obtained before and after treatment were compared among each other.

Yuta et al. (Arerugi 51 (2002) 634-648) studied the histamine release from basophilic cells to evaluate an immunotherapy of allergic rhinitis. The authors analysed samples at the beginning of the treatment and at six months after starting immunotherapy and could show the positive effect of the therapy. In this article samples obtained before and after treatment were analysed and the authors could only show that the rush protocol leads to an exhaustion of the cells but does not show a reduction of histamine release. In this context it should be noted that rush immunotherapy works already before „blocking antibodies“ are induced by immunotherapy, i.e., sometimes after hours and few days. This may be interpreted as an exhaustion of cells. However, the assessment of the effect of blocking antibodies which appear after several weeks of treatment is important. Hence an assay where the IgG antibodies are still present, e.g. whole blood, has to be used. In contrast thereto, in Yuta et al. the cells were washed and hence the interference of blocking IgG could not be measured.

In addition to histamine release also other methods for the assessment of basophil and mast cell activation are known, which include measuring the release of leutrienenes (Van Rooyen & Anderson, R. J. Immunol. Methods 2004, 288, 1-7), tryptase (Taira M et al., J. Asthma 2002, 39, 315-322) and other mast cell or basophil products which are released upon allergen-specific activation of the mast cells and basophils. Furthermore also the upregulation of activation markers such as CD63 and CD203c resulting from the exposure of an individual to an allergen can be measured by flow cytometry (Hauswirth A.W., et al. J. Allergy Clin. Immunol. 2002, 110, 102-109).

Therefore it is an object of the present invention to provide in vitro means and methods to monitor as close as possible clinical efficacy and the progress of an allergen immunotherapy and allergen sensitivity of an individual.

Therefore the present invention provides a method for evaluating the allergen sensitivity of an individual and/or the clin-

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ical efficacy of an allergen immunotherapy comprising the steps:

- providing at least two samples selected from the group consisting of blood or fractions thereof, connective tissue, nasal, bronchial, skin or gut biopsy material from an individual subjected or intended to be subjected to an immunotherapy with at least one pure allergen or derivative thereof, wherein the samples contain cells capable of releasing mediators in response to said allergen

- contacting said sample with said allergen or derivative thereof, and

- determining the amounts of mediators released from said sample and evaluating the allergen sensitivity of the individual prior to therapy and/or the clinical efficacy of the immunotherapy by comparing said amounts.

The evaluation of the allergen sensitivity of an individual and/or the clinical efficacy as well as the progress of an allergen immunotherapy is important in order to guarantee an effective treatment, e.g. by changing the dose and/or time intervals of the administered allergen. Therefore a reliable method to monitor the immunotherapy is required which directly reflects the sensitivity of an individual for a certain type of allergen prior and in the course of an immunotherapy. The measurement of the amount of IgE binding specifically to an allergen turned out to be not suited to determine the degree of sensitisation of an individual for a certain type of allergen, since there is no direct correlation between the amount of IgE present in an individual and the mediator release from mast and basophilic cells. Therefore the release of mediator of a sample of an individual comprising mediator releasing cells is preferred. It was surprisingly found that the method according to the present invention gave comparable, if not identical, results as the traditionally used skin sensitivity test.

The samples provided by an individual are preferably contacted with the same allergen, which is used for immunotherapy. However, it is also possible to perform the immunotherapy with an allergen extract and to monitor said therapy with substantially purified ("pure") allergens.

Of course the method according to the present invention may also be used to monitor the progress of an allergen immunotherapy by determining the allergen sensitivity of an individual in

the course of the therapy.

"Allergens" according to the present invention are molecules or mixtures of molecules able to induce the production of specific antibodies (IgE) which are responsible to trigger mediator release of a mediator releasing cell and to cause consequently allergic effects in the individual. Of course, "allergens" are also capable to induce the production of antibodies other than IgE (e.g. IgG). However, the allergens used in the method according to the present invention are preferably purified, i.e. the allergens consist substantially of one single allergen molecule, whereby the degree of purity exceeds 90% (w/w), preferably 95% (w/w), most preferably 99% (w/w). Due to the use of substantially purified or isolated allergens it is possible to determine and to dose in a reproducible manner the amount of allergen used in immunotherapy as well as used in a method according to the present invention. In contrast thereto allergen extracts contain varying concentrations of the specific allergen, depending on the specific purification conditions. Furthermore allergen extracts may also contain more than one allergen, which may be present in the extract in different concentrations (the amount of the allergen of interest is not definable in an accurate manner) and may further provoke cross reactions (see for instance Marth K et al. (2004) J. Allergy Clin. Immunol. 113: 470-474; Marth K et al. (2004) XXIII EAACI congress abstract book 597: 181; Akkerdaas HJ et al. (2003) Arb. Paul Ehrlich Inst. Bundesamt Sera Impfstoffe Frankf. a. M. 94: 87-95). In addition, allergen extracts may contain contaminations or substance which may influence the stability of the extract. This problem can also be avoided by using substantially purified or "pure" allergens.

The term "derivative" allergen as used herein refers to modified (deleted, point mutated, truncated etc.) allergens which still exhibit the same antigenic and IgE binding properties as the native allergen from which they are derived from.

According to a preferred embodiment of the present invention the mediators are selected from the group consisting of histamine, tryptase, prostaglandins, leukotrienes, especially cysteinyl leukotrienes, eosinophil cationic protein, cytokines, like interleukins (IL), especially IL-2R, CD63, CD203c and combinations thereof.

The allergic response of an individual after the exposure of said individual to an allergen is primarily caused by the release of mediators by mast cells. These mediators produce the early symptoms of an allergic reaction (e.g. sneezing, itching) and stimulate the production and infiltration into local tissue of circulating leukocytes (e.g. eosinophils). The mediators can be released from the cells by degranulation (histamine and proteases) or after neosynthesis of said mediators (Quraishi S.A. et al., JAOA Supplement 5, 104:S7-S15). According to the present invention also activation markers - besides mediators - can be determined (e.g. Yoshimura C., et al., (2002) J Allergy Clin Immunol. 109:817-23).

The sample is blood or fractions thereof (e.g. plasma, serum), connective tissue, nasal, bronchial, skin or gut biopsy material.

Mediator releasing cells can be found in blood and fractions thereof, in connective and several other tissues. It was surprisingly found that the method according to the present invention closely mirrors cutaneous sensitivity when using pure allergens, especially when whole blood is used. In contrast thereto, measurements of specific IgE did not correlate with cutaneous sensitivity. Therefore the sample to be used in a method according to the present invention may be a blood sample (preferably heparinised blood) or connective tissue.

The mediator releasing cells used in the method according to the present invention may be isolated from the sample. Due to this isolation other possibly disturbing substances present in the sample may be removed. Especially considering that blood, for instance, may contain released mediator providing a high background during the determination of the amount of mediator released into the sample upon contact with an allergen. This problem may be avoided by measuring the amount of mediator present in the sample prior its exposure to the allergen. On the other hand experimental data revealed that substantially no correlation between histamine release and skin sensitivity, for instance, exists. Therefore, the samples to be used according to the present invention are not isolated or washed prior contacting the sample with the allergen or derivative thereof. This may be reasoned by the fact that when mediator releasing cells are washed all antibodies including those IgG antibodies which

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should be induced in the course of an allergen therapy and which would act as blocking antibodies in order to reduce the amount of IgE-allergen complexes (due to competition with IgE molecules) in the sample are removed (see e.g. Stahl-Skov et al. (1977) Clin. Exp. Immunol. 27: 432-439)

Preferably said cells are mast and/or basophilic and/or eosinophilic cells.

Mast and basophilic cells are those cells which release most of the mediators, especially histamine, when exposed to an allergen. Mast cells are found in connective tissues of the skin, lung and gastrointestinal tract, whereas basophilic cells are found in blood. These cells can be isolated by known methods and be used in a method according to the present invention. Isolation protocols for mast cells can be found in Jamur MC et al. (J Histochem Cytochem. 1997 45:1715-1722), Massey WA (J Immunol. 1991 147:1621-7), isolation protocols for basophilic cells in Valent P. (Proc. Natl. Acad. Sci USA 1989, 86, 5542-5546).

According to a preferred embodiment of the present invention the sample further comprises immunoglobulins (Ig), especially immunoglobulin G (IgG).

The procedure should preferably be carried out with samples containing IgG, e.g. whole blood samples. The presence of IgG in such samples is preferred since it allows the measurement of the interference of blocking IgG during the exposure of said cells to the allergen. In the course of an allergen immunotherapy IgGs directed to said allergen are produced. These IgGs bind to the allergen when an individual is contacted with said allergen and prevent that the allergen binds to IgE. Since the production of allergen binding IgGs is therefore directly involved in the response of an individual to an allergen and thus influencing the allergen sensitivity of an individual, the sample should preferably contain IgGs.

In order to evaluate the allergen sensitivity of an individual or the clinical efficacy of an allergen immunotherapy the samples are preferably provided before and after subjecting said individual to an immunotherapy.

To monitor and to evaluate the efficacy of an immunotherapy it is necessary to determine the sensitivity of an individual to an allergen prior and in the course of the therapy. Therefore the mediator release is determined at various stages of the

therapy. In the course of the therapy the sensitivity to an allergen ideally decreases. Furthermore, the determination of the mediator release at one or more time points before the immunotherapy may be useful for dosing the allergen in the course of the therapy.

According to another preferred embodiment of the present invention the samples are provided after subjecting said individual to an immunotherapy.

Of course an immunotherapy may also be evaluated solely by analysing samples after the first administration of a medicament comprising an allergen.

Preferably the at least one sample is provided after a maximum of 1 hour, 2 hours, 6 hours, 12 hours, 24 hours, 5 days, 10 days, 4 weeks, 6 months, 12 months, 24 months and 36 months, after subjecting said individual to an immunotherapy.

The sample to be analysed may be provided after a defined time period after the first administration of the allergen. Also the time intervals in between the single determinations of the mediator release may be preferably varied within the range of 1 hour, 2 hours, 6 hours, 12 hours, 2 days, 5 days, 1 week, 2 weeks, 4 weeks, 2 months, 4 months, 6 months, 12 months and 24 months.

According to a preferred embodiment said allergen is recombinantly produced.

An efficient allergen immunotherapy and an accurate method to determine the release of mediator is preferably conducted with an allergen, which is recombinantly produced. Due to genetic engineering it is possible to produce a specific allergen in a high amount and to isolate said allergen. Allergens are usually isolated directly from the source which contains the allergen (e.g. pollen) and since the allergen is contained in an extract, said allergen is always part of a mixture of different allergenic and potential allergenic substances. Even purified "natural allergens" consist of several isoforms, some of them which may be even hypo or non-allergenic and hence give false test results (Ferreira F., et al., J. Exp. Med. 1996, 183, 599-609). This problem can be avoided by the recombinant production of allergens. The allergen used for the administration to an individual may also be used in a method according to the present invention.

Said allergen comprises preferably at least one deletion, at least one substitution or at least one insertion.

Also hypoallergenic allergen or derivatives thereof can be used when it comes to the question whether the patient may become sensitised to these derivatives during treatment.

According to a preferred embodiment of the present invention said allergen is modified by reshuffling the fragments of said allergen by genetic engineering.

The sample is preferably contacted with varying concentrations of said allergen.

The amount of mediator released from a mediator releasing cell depends on the concentration of the allergen employed in the method according to the present invention. The higher the concentration of the allergen used to induce the release a distinct amount of mediator is, the lower is the sensitivity of the cells provided from an individual and vice versa. Therefore the determination of the amount of mediator released requires the use of varying concentrations of allergen.

Preferably the concentration of said allergen is selected within the range of 1ng/ml to 100µg/ml, preferably within the range of 1pg/ml to 10µg/ml.

According to a preferred embodiment the total amount of mediator of the cells contained in the sample provided by an individual is determined.

In order to determine the amount of total mediator present in the cells, these cells are lysed e.g. by several thawing and freezing cycles. The determined amount of mediator indicates the mediator potentially releasable by said cells, which value may be employed to determining the degree of cellular sensitisation of the cells to a certain allergen.

A degree of cellular sensitisation is preferably defined by determining the concentration of said allergen inducing the release of 10%, preferably 30%, of the total amount of mediator of said cells.

The degree of cellular sensitisation is an indicator of the progress of the immunotherapy because it reveals the concentration, at which a cell releases 10%, preferably 20%, 25%, 30%, of the total amount of mediator present in the mediator releasing cell. In the course of a successful allergen immunotherapy the concentration of the allergen employed should increase because a

high concentration of allergen releasing a certain amount of mediator from said cells indicates that the cells are less sensitive than in a previous measurement. Also the dose inducing maximum release of the mediator may be evaluated. This allows to create a dose response curve and to measure the shifting of said curve in the course of an allergen immunotherapy.

Therefore, the allergen sensitivity of an individual and/or the clinical efficacy of the allergen immunotherapy is preferably evaluated by observing the degree of cellular sensitisation in the course of said immunotherapy.

According to a preferred embodiment of the present invention the mediator in the sample is determined by an immunological and/or a chromatographical method, preferably the method is selected from the group consisting of radioimmunoassay (RIA), enzyme linked immunosorbent assay (ELISA), high performance liquid chromatography (HPLC), reverse transcriptase polymerase chain reaction, immunofluorescence flow cytometry and combinations thereof.

All of these methods have been established to come closer to clinical sensitivity. However, none of these methods has been used to look at a pure allergen in serology, basophil activation and skin sensitivity (e.g. Pierkes M. et al., J Allergy Clin Immunol. (1999) 103:326-32; Di Lorenzo G. et al., J Allergy Clin Immunol. (1997) 100:832-7).

Preferred allergens to be used by the present invention include all major protein allergens available e.g. under www.allergen.org/List.htm. Specifically preferred groups of allergens according to the present invention include major allergens such as major birch pollen allergens, e.g. Bet v 1, major timothy grass pollen allergens, e.g. Phl p 1, Phl p 2, Phl p 5 and Phl p 6, major house dust mite allergens, e.g. Der p 1, Der p 2, major cat allergen, e.g. Fel d 1, major bee and wasp allergens (see list), other profilins, especially Phl p 12, other birch allergens, especially Bet v 4, storage mite allergens, especially Lep d 2, and the allergens listed in table 1.

Table 1: preferred allergen to be used by the present invention (including reference examples)

ALLERGENS

Species Name

Allergen Name	Biochem.ID or Obsolete name	MW	cDNA or protein	Reference, Acc.No.
<i>Ambrosia artemisiifolia</i>				
short ragweed				
Amb a 1	antigen E	8	C	8, 20
Amb a 2	antigen K	38	C	8, 21
Amb a 3	Ra3	11	C	22
Amb a 5	Ra5	5	C	11, 23
Amb a 6	Ra6	10	C	24, 25
Amb a 7	Ra7	12	P	26
<i>Ambrosia trifida</i>				
giant ragweed				
Amb t 5	Ra5G	4.4	C	9, 10, 27
<i>Artemisia vulgaris</i>				
mugwort				
Art v 1		27-29	C	28
Art v 2		35	P	28A
Art v 3	lipid transfer protein	12	P	53
Art v 4	profilin	14	C	29
<i>Helianthus annuus</i>				
sunflower				
Hel a 1		34		29A
Hel a 2	profilin	15.7	C	Y15210
<i>Mercurialis annua</i>				
Mer a 1	profilin	14-15	C	Y13271
<i>Caryophyllales</i>				
<i>Chenopodium album</i>				
lamb's-quarters, pigweed,				
Che a 1		17	C	AY049012, 29B
white goosefoot				
Che a 2	profilin	14	C	AY082337
Che a 3	polcalcin	10	C	AY082338
<i>Salsola kali</i>				
Russian-thistle				
Sal k 1		43	P	29C

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Rosales

Humulus japonicus

Japanese hop

Hum j 4w	C	AY335187
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Parietaria judaica

Par j 1 lipid transfer protein 1	15	C	see list of isoaller-
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gens

Par j 2 lipid transfer protein 2		C	see list of isoaller-
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gens

Par j 3	profilin	C	see list of isoaller-
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gens

Parietaria officinalis

Par o 1 lipid transfer protein	15		29D
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B. Grasses

Poales

Cynodon dactylon

Bermuda grass

Cyn d 1	32	C	30, S83343
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Cyn d 7		C	31, X91256
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Cyn d 12	profilin	14	C	31a, Y08390
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Cyn d 15		9	C	AF517686
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Cyn d 22w	enolase	data		pending
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Cyn d 23	Cyn d 14	9	C	AF517685
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Cyn d 24 Pathogenesis- related p.	21	P	pending
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Dactylis glomerata

orchard grass

Dac g 1	AgDgl	32	P	32
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Dac g 2		11	C	33, S45354
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Dac g 3			C	33A, U25343
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Dac g 5		31	P	34
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Festuca pratensis

meadow fescue

Fes p 4w		60	-
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Holcus lanatus

velvet grass

Hol l 1			C	Z27084
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Lolium perenne

rye grass

Lol p 1	group I	27	C	35, 36
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Lol p 2	group II	11	P	37, 37A, X73363
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Lol p 3	group III	11	P	38
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	Lol p 5	Lol p IX, Lol p Ib	31/35	C	34, 39
	Lol p 11 hom: trypsin inhibitor		16		39A
Phalaris aquatica					
canary grass					
	Pha a 1			C	40, S80654
Phleum pratense					
timothy	Phl p 1	27	C		X78813
	Phl p 2			C	X75925, 41
	Phl p 4			P	41A
	Phl p 5	Ag25	32	C	42
	Phl p 6			C	Z27082, 43
	Phl p 11 trypsin inhibitor hom.		20	C	AF521563, 43A
	Phl p 12 profilin			C	X77583, 44
	Phl p 13 polygalacturonase		55-60	C	AJ238848
Poa pratensis					
Kentucky blue grass					
	Poa p 1	group I	33	P	46
	Poa p 5		31/34	C	34, 47
Sorghum halepense					
Johnson grass					
	Sor h 1			C	48
C. Trees					
Arecales					
Phoenix dactylifera					
date palm					
	Pho d 2	profilin	14.3	C	Asturias p.c.
Fagales					
Alnus glutinosa					
alder					
	Aln g 1		17	C	S50892
Betula verrucosa					
birch	Bet v 1		17	C	see list of isoaller-
gens					
	Bet v 2	profilin	15	C	M65179
	Bet v 3			C	X79267
	Bet v 4		8	C	X87153, S54819
	Bet v 6 h: isoflavone reductase		33.5	C	see list of isoaller-
gens					
	Bet v 7	cyclophilin	18	P	P81531

Carpinus betulus					
hornbeam					
	Car b 1	17	C	see list of isoaller-	
gens					
Castanea sativa					
chestnut					
	Cas s 1	22	P	52	
	Cas s 5 chitinase				
	Cas s 8 lipid transfer protein	9.7	P	53	
Corylus avellana					
hazel	Cor a 1	17	C	see list of isoaller-	
gens					
	Cor a 2 profilin	14	C		
	Cor a 8 lipid transfer protein	9	C		
	Cor a 9 11S globulin-like protein	40/?	C	Beyer p.c.	
	Cor a 10 luminal binding prot.	70	C	AJ295617	
	Cor a 11 7S vicilin-like prot.	48	C	AF441864	
Quercus alba					
White oak					
	Que a 1	17	P	54	
Lamiales					
Oleaceae					
Fraxinus excelsior					
ash	Fra e 1	20	P	58A, AF526295	
Ligustrum vulgare					
privet	Lig v 1	20	P	58A	
Olea europea					
olive	Ole e 1	16	C	59, 60	
	Ole e 2 profilin	15-18	C	60A	
	Ole e 3	9.2		60B	
	Ole e 4	32	P	P80741	
	Ole e 5 superoxide dismutase	16	P	P80740	
	Ole e 6	10	C	60C, U86342	
	Ole e 7	?	P	60D, P81430	
	Ole e 8 Ca2+-binding protein	21	C	60E, AF078679	
	Ole e 9 beta-1,3-glucanase	46	C	AF249675	
	Ole e 10 glycosyl hydrolase hom.	11	C	60F, AY082335	
Syringa vulgaris					
lilac	Syr v 1	20	P	58A	
Plantaginaceae					

Plantago lanceolata

English plantain

Pla 1 1

18

P

P842242

Pinales

Cryptomeria japonica

sugi Cry j 1

41-45

C

55, 56

Cry j 2

C

57, D29772

Cupressus arisonica

cypress

Cup a 1

43

C

A1243570

Cupressus sempervirens

common cypress

Cup s 1

43

C

see list of isoaller-

gens

Cup s 3w

34

C

ref pending

Juniperus ashei

mountain cedar

Jun a 1

43

P

P81294

Jun a 2

C

57A, AJ404653

Jun a 3

30

P

57B, P81295

Juniperus oxycedrus

prickly juniper

Jun o 4 hom: calmodulin

29

C

57C, AF031471

Juniperus sabinoides

mountain cedar

Jun s 1

50

P

58

Juniperus virginiana

eastern red cedar

Jun v 1

43

P

P81825, 58B

Platanaceae

Platanus acerifolia

London plane tree

Pla a 1

18

P

P82817

Pla a 2

43

P

P82967

Pla a 3 lipid transfer protein

10

P

Iris p.c.

D. Mites

Acarus siro arthropod					
mite	Aca s 13 fatty acid binding prot.	14*	C		AJ006774
Blomia tropicalis					
mite	Blo t 1 cysteine protease	39	C		AF277840
	Blo t 3 trypsin	24*	C		Cheong p.c.
	Blo t 4 alpha amylase	56	C		Cheong p.c.
	Blo t 5		C		U59102
	Blo t 6 chymotrypsin	25	C		Cheong p.c.
	Blo t 10 tropomyosin	33	C		61
	Blo t 11 paramyosin	110	C		AF525465, 61A
	Blo t 12 Bt1a		C		U27479
	Blo t 13 Bt6, fatty acid bind prot.		C		U58106
	Blo t 19 anti-microbial pep. hom.	7.2	C		Cheong p.c.
Dermatophagoides farinae					
American house dust mite					
	Der f 1 cysteine protease	25	C		69
	Der f 2	14	C		70, 70A, see list of
isoallergens					
	Der f 3 trypsin	30	C		63
	Der f 7	24-31	C		SW:Q26456, 71
	Der f 10 tropomyosin		C		72
	Der f 11 paramyosin	98	C		72A
	Der f 14 mag3, apolipophorin		C		D17686
	Der f 15 98k chitinase	98	C		AF178772
	Der f 16 gelsolin/villin	53	C		71A
	Der f 17 Ca binding EF protein	53	C		71A.
	Der f 18w 60k chitinase	60	C		Weber p.c.
Dermatophagoides microceras					
house dust mite					
	Der m 1 cysteine protease	25	P		68
Dermatophagoides pteronyssinus					
European house dust mite					
	Der p 1 antigen P1, cysteine protease	25	C		62, see list of
isoallergens					
	Der p 2	14	C		62A-C, see list of
isoallergens					
	Der p 3 trypsin	28/30	C		63
	Der p 4 amylase	60	P		64
	Der p 5	14	C		65

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	Der p 6	chymotrypsin	25	P	66
	Der p 7		22/28	C	67
	Der p 8	glutathione transferase		C	67A
	Der p 9	collagenolytic serine pro.		P	67B
	Der p 10	tropomyosin	36	C	Y14906
	Der p 14	apolipophorin like prot.		C	Epton p.c.
Euroglyphus maynei					
mite	Eur m 2			C	see list of isoaller-
gens					
	Eur m 14	apolipophorin	177	C	AF149827
Glycyphagus domesticus					
storage mite					
	Gly d 2			C	72B, see isoallergen
list					
Lepidoglyphus destructor					
storage mite					
	Lep d 2	Lep d 1	15	C	73, 74, 74A, see
isoallergen list					
	Lep d 5			C	75, AJ250278
	Lep d 7			C	75, AJ271058
	Lep d 10	tropomyosin		C	75A, AJ250096
	Lep d 13			C	75, AJ250279
Tyrophagus putrescentiae					
storage mite					
	Tyr p 2			C	75B, Y12690
E. Animals					
Bos domesticus					
domestic cattle					
	Bos d 2	Ag3, lipocalin	20	C	76, see isoallergen
list					
(see also foods)					
	Bos d 3	Ca-binding S100 hom.	11	C	L39834
	Bos d 4	alpha-lactalbumin	14.2	C	M18780
	Bos d 5	beta-lactoglobulin	18.3	C	X14712
	Bos d 6	serum albumin	67	C	M73993
	Bos d 7	immunoglobulin	160		77
	Bos d 8	caseins	20-30		77
Canis familiaris					
(Canis domesticus)					
	Can f 1		25	C	78, 79

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dog	Can f 2		27	C	78, 79
	Can f 3 albumin			C	S72946
	Can f 4		18	P	A59491
Equus caballus					
domestic horse					
	Equ c 1	lipocalin	25	C	U70823
	Equ c 2	lipocalin	18.5	P	79A, 79B
	Equ c 3	Ag3 - albumin	67	C	79C, X74045
	Equ c 4		17	P	79D
	Equ c 5	AgX	17	P	Goubran Botros p.c.
Felis domesticus					
cat (saliva)					
	Fel d 1	cat-1	38	C	15
	Fel d 2	albumin		C	79E, X84842
	Fel d 3	cystatin	11	C	79F, AF238996
	Fel d 4	lipocalin	22	C	AY497902
	Fel d 5w immunoglobulin A		400		Adedoyin p.c.
	Fel d 6w immunoglobulin M				
800-					
1000					
	Adedoyin p.c.				
	Fel d 7w	immunoglobulin G	150		Adedoyin p.c.
Cavia porcellus					
guinea pig					
	Cav p 1	lipocalin homologue	20	P	SW:P83507, 80
	Cav p 2		17	P	SW:P83508
Mus musculus					
mouse (urine)					
	Mus m 1	MUP	19	C	81, 81A
Rattus norvegicus					
rat (urine)					
	Rat n 1		17	C	82, 83
F. Fungi (moulds)					
1. Ascomycota					
1.1 Dothideales					
Alternaria alternata					

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Alt a 1	28	C	U82633
Alt a 2	25	C	83A, U62442
Alt a 3 heat shock prot.	70	C	U87807, U87808
Alt a 4 prot. disulfideisomerase	57	C	X84217
Alt a 6 acid ribosomal prot. P2	11	C	X78222, U87806
Alt a 7 YCP4 protein	22	C	X78225
Alt a 10 aldehyde dehydrogenase	53	C	X78227, P42041
Alt a 11 enolase	45	C	U82437
Alt a 12 acid ribosomal prot. P1	11	C	X84216
Cladosporium herbarum			
Cla h 1	13		83B, 83C
Cla h 2	23		83B, 83C
Cla h 3 aldehyde dehydrogenase	53	C	X78228
Cla h 4 acid ribosomal prot. P2	11	C	X78223
Cla h 5 YCP4 protein	22	C	X78224
Cla h 6 enolase	46	C	X78226
Cla h 12 acid ribosomal prot. P1	11	C	X85180
1.2 Eurotiales			
Aspergillus flavus			
Asp fl 13 alkaline serine protease	34		84
Aspergillus fumigatus			
Asp f 1	18	C	M83781, S39330
Asp f 2	37	C	U56938
Asp f 3 peroxisomal protein	19	C	U20722
Asp f 4	30	C	AJ001732
Asp f 5 metalloprotease	40	C	Z30424
Asp f 6 Mn superoxide dismut.	26.5	C	U53561
Asp f 7	12	C	AJ223315
Asp f 8 ribosomal prot. P2	11	C	AJ224333
Asp f 9	34	C	AJ223327
Asp f 10 aspartic protease	34	C	X85092
Asp f 11 peptidyl-prolyl isomeras	24		84A
Asp f 12 heat shock prot. P90	90	C	85
Asp f 13 alkaline serine protease	34		84B
Asp f 15	16	C	AJ002026
Asp f 16	43	C	g3643813
Asp f 17		C	AJ224865
Asp f 18 vacuolar serine protease	34		84C
Asp f 22w enolase	46	C	AF284645
Asp f 23 L3 ribosomal protein	44	C	85A, AF464911

Aspergillus niger

Asp n 14 beta-xylosidase	105	C	AF108944
Asp n 18 vacuolar serine protease	34	C	84B
Asp n 25 3-phytase B	66-100	C	85B, P34754
Asp n ?	85	C	Z84377

Aspergillus oryzae

Asp o 13 alkaline serine protease	34	C	X17561
Asp o 21 TAKA-amylase A	53	C	D00434, M33218

Penicillium brevicompactum

Pen b 13 alkaline serine protease	33		86A
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Penicillium chrysogenum(formerly *P. notatum*)

Pen ch 13 alkaline serine protease	34		87
Pen ch 18 vacuolar serine protease	32		87
Pen ch 20 N-acetyl glucosaminidase	68		87A

Penicillium citrinum

Pen c 3 peroxisomal mem. prot.	18		86B
Pen c 13 alkaline serine protease	33		86A
Pen c 19 heat shock prot. P70	70	C	U64207
Pen c 22w enolase	46	C	AF254643
Pen c 24 elongation factor 1 beta		C	AY363911

Penicillium oxalicum

Pen o 18 vacuolar serine protease	34		87B
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1.3 Hypocreales

Fusarium culmorum

Fus c 1 ribosomal prot. P2	11*	C	AY077706
Fus c 2 thioredoxin-like prot.	13*	C	AY077707

1.4 Onygenales

Trichophyton rubrum

Tri r 2		C	88
Tri r 4 serine protease		C	88

Trichophyton tonsurans

Tri t 1	30	P	88A
Tri t 4 serine protease	83	C	88

1.5 Saccharomycetales

Candida albicans

Cand a 1	40	C	89
Cand a 3 peroxisomal protein	29	C	AY136739

Candida boidinii

Cand b 2	20	C	J04984, J04985
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2. Basidiomycotina

2.1 Hymenomycetes

Psilocybe cubensis

Psi c 1			
Psi c 2 cyclophilin	16		89A

Coprinus comatus

shaggy cap

Cop c 1 leucine zipper protein	11	C	AJ132235
Cop c 2			AJ242791
Cop c 3			AJ242792
Cop c 5			AJ242793
Cop c 7			AJ242794

2.2 Urediniomycetes

Rhodotorula mucilaginosa

Rho m 1 enolase	47	C	89B
Rho m 2 vacuolar serine protease	31	C	AY547285

2.3 Ustilaginomycetes

Malassezia furfur

Mala f 2 MF1, peroxisomal membrane protein	21	C	AB011804, 90
Mala f 3 MF2, peroxisomal membrane protein	20	C	AB011805, 90
Mala f 4 mitochondrial malate dehydrogenase	35	C	AF084828, 90A

Malassezia sympodialis

Mala s 1		C	X96486, 91
Mala s 5	18*	C	AJ011955
Mala s 6	17*	C	AJ011956
Mala s 7		C	AJ011957, 91A

Mala s 8		19*	C	AJ011958, 91A
Mala s 9		37*	C	AJ011959, 91A
Mala s 10 heat shock prot. 70		86	C	AJ428052
Mala s 11 Mn superoxide dismut.		23	C	AJ548421
3. Deuteromycotina				
3.1 Tuberculariales				
Epicoccum purpurascens				
(formerly E. nigrum)				
Epi p 1	serine protease	30	P	SW:P83340, 91B
G. Insects				
Aedes aegyptii				
mosquito				
Aed a 1	apyrase	68	C	L12389
Aed a 2		37	C	M33157
Apis mellifera				
honey bee				
Api m 1	phospholipase A2	16	C	92
Api m 2	hyaluronidase	44	C	93
Api m 4	melittin	3	C	94
Api m 6		7-8	P	Kettner p.c.
Api m 7	CUB serine protease	39	C	AY127579
Bombus pennsylvanicus				
bumble bee				
Bom p 1	phospholipase	16	P	95
Bom p 4	protease		P	95
Blattella germanica				
German cockroach				
Bla g 1 Bd90k			C	
Bla g 2 aspartic protease		36	C	96
Bla g 4 calycin		21	C	97
Bla g 5 glutathione transferase		22	C	98
Bla g 6 troponin C		27	C	98
Periplaneta americana				
American cockroach				
Per a 1	Cr-P11		C	
Per a 3	Cr-PI	72-78	C	98A
Per a 7	tropomyosin	37	C	Y14854
Chironomus kiliensis				

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midge	Chi k 10	tropomyosin	32.5*	C	AJ012184
Chironomus thummi thummi					
midge	Chi t 1-9	hemoglobin	16	C	99
	Chi t 1.01	component III	16	C	P02229
	Chi t 1.02	component IV	16	C	P02230
	Chi t 2.0101	component I	16	C	P02221
	Chi t 2.0102	component IA	16	C	P02221
	Chi t 3	component II-beta	16	C	P02222
	Chi t 4	component IIIA	16	C	P02231
	Chi t 5	component VI	16	C	P02224
	Chi t 6.01	component VIIA	16	C	P02226
	Chi t 6.02	component IX	16	C	P02223
	Chi t 7	component VIIB	16	C	P02225
	Chi t 8	component VIII	16	C	P02227
	Chi t 9	component X	16	C	P02228
Ctenocephalides felis felis					
cat flea					
	Cte f 1				
	Cte f 2	Mlb	27	C	AF231352
	Cte f 3		25	C	
Thaumetopoea pityocampa					
pine processionary moth					
	Tha p 1		15	P	PIR:A59396, 99A
Lepisma saccharina					
silverfish					
	Lep s 1	tropomyosin	36	C	AJ309202
Dolichovespula maculata					
white face hornet					
	Dol m 1	phospholipase A1	35	C	100
	Dol m 2	hyaluronidase	44	C	101
	Dol m 5	antigen 5	23	C	102, 103
Dolichovespula arenaria					
yellow hornet					
	Dol a 5	antigen 5	23	C	104
Polistes annularies					
wasp					
	Pol a 1	phospholipase A1	35	P	105
	Pol a 2	hyaluronidase	44	P	105
	Pol a 5	antigen 5	23	C	104
Polistes dominulus					
Mediterranean paper wasp					

Pol d 1						Hoffman p.c.
Pol d 4 serine protease			32-34	C		Hoffman p.c.
Pol d 5						P81656
Polistes exclamans						
wasp	Pol e 1	phospholipase A1	34	P		107
	Pol e 5	antigen 5	23	C		104
Polistes fuscatus						
wasp	Pol f 5	antigen 5	23	C		106
Polistes gallicus						
wasp	Pol g 5	antigen 5	24	C		P83377
Polistes metricus						
wasp	Pol m 5	antigen 5	23	C		106
Vespa crabo						
European hornet						
	Vesp c 1	phospholipase	34	P		107
	Vesp c 5	antigen 5	23	C		106
Vespa mandarina						
giant asian hornet						
	Vesp m 1					Hoffman p.c.
	Vesp m 5					P81657
Vespula flavopilosa						
yellowjacket	Ves f 5	antigen 5	23	C		106
Vespula germanica						
yellowjacket	Ves g 5	antigen 5	23	C		106
Vespula maculifrons						
yellowjacket						
	Ves m 1	phospholipase A1	33.5	C		108
	Ves m 2	hyaluronidase	44	P		109
	Ves m 5	antigen 5	23	C		104
Vespula pennsylvanica						
yellowjacket						
	Ves p 5	antigen 5	23	C		106
Vespula squamosa						
yellowjacket						
	Ves s 5	antigen 5	23	C		106
Vespula vidua						
wasp	Ves vi 5	antigen 5	23	C		106
Vespula vulgaris						
yellowjacket						
	Ves v 1	phospholipase A1	35	C		105A

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Ves v 2	hyaluronidase	44	P	105A
Ves v 5	antigen 5	23	C	104
Myrmecia pilosula				
Australian jumper ant				
Myr p 1			C	X70256
Myr p 2			C	S81785
Solenopsis geminata				
tropical fire ant				
Sol g 2				Hoffman p.c.
Sol g 4				Hoffman p.c.
Solenopsis invicta				
fire ant Sol i 2		13	C	110, 111
Sol i 3		24	C	110
Sol i 4		13	C	110
Solenopsis saevissima				
Brazilian fire ant				
Sol s 2				Hoffman p.c.
Triatoma protracta				
California kissing bug				
Tria p 1	Procalin	20	C	AF179004, 111A.
H. Foods				
Gadus callarias				
cod				
Gad c 1	allergen M	12	C	112, 113
Salmo salar				
Atlantic salmon				
Sal s 1	parvalbumin	12	C	X97824
Bos domesticus				
domestic cattle				
Bos d 4	alpha-lactalbumin	14.2	C	M18780
(milk) Bos d 5	beta-lactoglobulin	18.3	C	X14712
see also animals				
Bos d 6	serum albumin	67	C	M73993
Bos d 7	immunoglobulin	160		77
Bos d 8	caseins	20-30		77
Gallus domesticus				
chicken				
Gal d 1	ovomucoid	28	C	114, 115

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	Gal d 2	ovalbumin	44	C	114, 115
	Gal d 3	Ag22, conalbumin	78	C	114, 115
	Gal d 4	lysozyme	14	C	114, 115
	Gal d 5	serum albumin	69	C	X60688
Metapenaeus ensis					
shrimp	Met e 1	tropomyosin		C	U08008
Penaeus aztecus					
shrimp	Pen a 1	tropomyosin	36	P	116
Penaeus indicus					
shrimp	Pen i 1	tropomyosin	34	C	116A
Penaeus monodon					
black tiger shrimp					
	Pen m 1	tropomyosin	38	C	
	Pen m 2	arginine kinase	40	C	AF479772, 117
Todarodes pacificus					
squid	Tod p 1	tropomyosin	38	P	117A
Helix aspersa					
brown garden snail					
	Hel as 1	tropomyosin	36	C	Y14855, 117B
Haliotis midae					
abalone					
	Hal m 1		49		117C
Rana esculenta					
edible frog					
	Ran e 1	parvalbumin alpha	11.9*	C	AJ315959
	Ran e 2	parvalbumin beta	11.7*	C	AJ414730
Brassica juncea					
oriental mustard					
	Bra j 1	2S albumin	14	C	118
Brassica napus					
rapeseed					
	Bra n 1	2S albumin	15	P	118A, P80208
Brassica rapa					
turnip	Bra r 2	hom: prohevein	25		P81729
Hordeum vulgare					
barley	Hor v 15	BMAI-1	15	C	119
	Hor v 16	alpha-amylase			
	Hor v 17	beta-amylase			
	Hor v 21	gamma-3 hordein	34	C	119A,

SW:P80198

Secale cereale

rye Sec c 20 secalin see isoall. list

Triticum aestivum

wheat Tri a 18 agglutinin

Tri a 19 omega-5 gliadin 65 P PIR:A59156

Zea mays

maise, corn

Zea m 14 lipid transfer prot. 9 P P19656

Oryza sativa

rice Ory s 1 C 119B, U31771

Apium graveolens

celery Api g 1 hom: Bet v 1 16* C Z48967

Api g 4 profilin AF129423

Api g 5 55/58 P P81943

Daucus carota

carrot Dau c 1 hom: Bet v 1 16 C 117D, see isoallergen list

Dau c 4 profilin C AF456482

Corylus avellana

hazelnut

Cor a 1.04 hom: Bet v 1 17 C see list of isoallergens

Cor a 2 profilin 14 C AF327622

Cor a 8 lipid transfer protein 9 C AF329829

Malus domestica

apple Mal d 1 hom: Bet v 1 C see list of isoallergens

Mal d 2 hom: thaumatin C AJ243427

Mal d 3 lipid transfer protein 9 C Pastorello p.c.

Mal d 4 profilin 14.4* C see list of isoallergens

gens

Pyrus communis

pear Pyr c 1 hom: Bet v 1 18 C AF05730

Pyr c 4 profilin 14 C AF129424

Pyr c 5 hom: isoflavone reductas 33.5 C AF071477

Persea americana

avocado Pers a 1 endochitinase 32 C Z78202

Prunus armeniaca

apricot

Pru ar 1 hom: Bet v 1 C U93165

Pru ar 3 lipid transfer protein 9 P

Prunus avium					
sweet cherry					
	Pru av 1 hom: Bet v 1		C		U66076
	Pru av 2 hom: thaumatin		C		U32440
	Pru av 3 lipid transfer protein	10	C		AF221501
	Pru av 4 profilin	15	C		AF129425
Prunus domestica					
European plum					
	Pru d 3 lipid transfer protein	9	P		119C
Prunus persica					
peach					
	Pru p 3 lipid transfer protein	10	P		P81402
	Pru p 4 profilin	14	C		see isoallergen list
Asparagus officinalis					
Asparagus					
	Aspa o 1 lipid transfer protein	9	P		119D
Crocus sativus					
saffron crocus					
	Cro s 1	21			Varasteh A-R p.c.
Lactuca sativa					
lettuce					
	Lac s 1 lipid transfer protein	9			Vieths p.c.
Vitis vinifera					
grape					
	Vit v 1 lipid transfer protein	9	P		P80274
Musa x paradisiaca					
banana					
	Mus xp 1 profilin	15	C		AF377948
Ananas comosus					
pineapple					
	Ana c 1 profilin	15	C		AF377949
	Ana c 2 bromelain	22.8*	C		119E-G, D14059
Citrus limon					
lemon					
	Cit l 3 lipid transfer protein	9	P		Torrejon p.c.
Citrus sinensis					
sweet orange					
	Cit s 1 germin-like protein	23	P		Torrejon p.c.
	Cit s 2 profilin	14	P		Torrejon p.c.
	Cit s 3 lipid transfer protein	9	P		Torrejon p.c.
Litchi chinensis					
litchi					
	Lit c 1 profilin	15	C		AY049013
Sinapis alba					
yellow mustard					
	Sin a 1 2S albumin	14	C		120

Glycine max						
soybean	Gly m 1	HPS	7	P	120A	
	Gly m 2			8	P	A57106
	Gly m 3	profilin		14	C	see list of isoaller-
gens						
	Gly m 4	(SAM22) PR-10 prot.	17	C		X60043, 120B
Vigna radiata						
mung bean						
	Vig r 1	PR-10 protein	15	C		AY792956
Arachis hypogaea						
peanut	Ara h 1	vicilin	63.5	C		L34402
	Ara h 2	conglutin	17	C		L77197
	Ara h 3	glycinin	60	C		AF093541
	Ara h 4	glycinin	37	C		AF086821
	Ara h 5	profilin	15	C		AF059616
	Ara h 6	hom: conglutin	15	C		AF092846
	Ara h 7	hom: conglutin	15	C		AF091737
	Ara h 8	PR-10 protein	17	C		AY328088
Lens culinaris						
lentil	Len c 1	vicilin	47	C		see list of isoaller-
gens						
	Len c 2	seed biotinylated prot.	66	P	120C	
Pisum sativum						
pea	Pis s 1	vicilin	44	C		see list of isoaller-
gens						
	Pis s 2	convicilin	63	C		pending
Actinidia chinensis						
kiwi	Act c 1	cysteine protease	30	P		P00785
	Act c 2	thaumatin-like protein	24	P		SW:P81370, 121
Capsicum annuum						
bell pepper						
	Cap a 1w	osmotin-like protein	23	C		AJ297410
	Cap a 2	profilin	14	C		AJ417552
Lycopersicon esculentum						
tomato	Lyc e 1	profilin	14	C		AJ417553
	Lyc e 2	b-fructofuranosidase	50	C		see isoallergen list
	Lyc e 3	lipid transfer prot.	6	C		U81996
Solanum tuberosum						
potato	Sola t 1	patatin	43	P		P15476
	Sola t 2	cathepsin D inhibitor	21	P		P16348
	Sola t 3	cysteine protease inhibitor	21	P		P20347
	Sola t 4	aspartic protease inhibitor	16+4	P		P30941
Bertholletia excelsa						

Brazil nut					
	Ber e 1 2S albumin	9	C		P04403, M17146
	Ber e 2 11S globulin seed storage protein 29	C			AY221641
Juglans nigra					
black walnut					
	Jug n 1 2S albumin	19*	C		AY102930
	Jug n 2 vicilin-like prot.	56*	C		AY102931
Juglans regia					
English walnut					
	Jug r 1 2S albumin		C		U66866
	Jug r 2 vicilin	44	C		AF066055
	Jug r 3 lipid transfer protein	9	P		Pastorello
Anacardium occidentale					
Cashew					
	Ana o 1 vicilin-like protein	50	C		see isoallergen list
	Ana o 2 legumin-like protein	55	C		AF453947
	Ana o 3 2S albumin	14	C		AY081853
Ricinus communis					
Castor bean					
	Ric c 1 2S albumin		C		P01089
Sesamum indicum					
sesame					
	Ses i 1 2S albumin	9	C		121A, AF240005
	Ses i 2 2S albumin	7	C		AF091841
	Ses i 3 7S vicilin-like globulin	45	C		AF240006
	Ses i 4 oleosin	17	C		AAG23840
	Ses i 5 oleosin	15	C		AAD42942
Cucumis melo					
muskmelon					
	Cuc m 1 serine protease	66	C		D32206
	Cuc m 2 profilin	14	C		AY271295
	Cuc m 3 pathogenesis-rel p. PR-1	16*	P		P83834
I. Others					
Anisakis simplex					
nematode					
	Ani s 1	24	P		121B, A59069
	Ani s 2 paramyosin	97	C		AF173004
	Ani s 3 tropomyosin	41	C		121C, Y19221
	Ani s 4	9	P		P83885
Argas reflexus					

pigeon tick					
	Arg r 1		17	C	AJ697694
Ascaris suum					
worm	Asc s 1		10	P	122
Carica papaya					
papaya	Car p 3w	papain	23.4*	C	122A, M15203
Dendronephthya nipponica					
soft coral					
	Den n 1		53	P	122B
Hevea brasiliensis					
rubber (latex)					
	Hev b 1	elongation factor	58	P	123, 124
	Hev b 2	1,3-glucanase	34/36	C	125
	Hev b 3		24	P	126, 127
	Hev b 4	component of	100-	P	128
		microhelix complex	115		
	Hev b 5		16	C	U42640
	Hev b 6.01	hevein precursor	20	C	M36986, p02877
	Hev b 6.02	hevein	5	C	M36986, p02877
	Hev b 6.03	C-terminal fragment	14	C	M36986, p02877
	Hev b 7.01	hom: patatin from B-serum	42	C	U80598
	Hev b 7.02	hom: patatin from C-serum	44	C	AJ223038
	Hev b 8	profilin	14	C	see list of isoaller-
gens					
	Hev b 9	enolase	51	C	AJ132580
	Hev b 10	Mn superoxide dismut.	26	C	see list of isoaller-
gens					
	Hev b 11	class 1 chitinase		C	see list of isoaller-
gens					
	Hev b 12	lipid transfer protein	9.3	C	AY057860
	Hev b 13	esterase	42	P	P83269
Homo sapiens					
human autoallergens					
	Hom s 1		73*	C	Y14314
	Hom s 2		10.3*	C	X80909
	Hom s 3		20.1*	C	X89985
	Hom s 4		36*	C	Y17711
	Hom s 5		42.6*	C	P02538
Triplochiton scleroxylon					
obeche	Trip s 1	class 1 chitinase	38.5	P	Kespolh p.c.

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The knowledge of the nucleic acid sequences encoding these allergens allows their recombinant production. Therefore especially these allergens are preferably used in immunotherapies and in methods according to the present invention.

Another aspect of the present invention relates to a method for evaluating the allergen sensitivity of an individual and/or the clinical efficacy of an allergen immunotherapy comprising the steps:

- providing cells capable of releasing mediators in response to an IgE-allergen complex,

- contacting said cells with serum and/or plasma of said individual spiked with at least one pure allergen or derivative thereof, and

- determining the amounts of mediators released from said sample and evaluating the allergen sensitivity of the individual prior to therapy and/or the clinical efficacy of the immunotherapy by comparing said amounts.

The cells which are capable of releasing mediators comprise normally IgE molecules bound thereto. Such cells can be isolated from samples which are obtained from the individual subjected to the method according to the present invention or from other individuals. Of course, it is also possible to use cell lines capable of binding IgE in a method according to the present invention.

The method according to the present invention is especially suited for the determination of the allergen sensitivity of an individual because it allows to determine the ratio between the allergen specific IgE and IgG molecules in the plasma and serum of said individual. Since only IgE-allergen complexes and not free IgE are able to induce the release of mediators from mediator-releasing cells like leukocytes the level of released mediator correlates with the amount of IgE-complex present in the sample. In turn the amount of IgE-complex in said sample correlates with the amount of allergen specific IgE, allergen and allergen specific antibodies other than IgE such as IgG, IgA or IgM which compete with IgE for the free allergen and consequently inhibits the formation of an IgE-allergen complex. This means that a low level of allergen specific IgE or a high level of allergen specific IgG leads to the formation of a low number of IgE complex and thus to a reduced mediator release.

The concentration of allergen in said serum and/or plasma is preferably within 1 ng/ml to 100 µg/ml, more preferably within 1 pg/ml to 10 µg/ml.

Another aspect of the present invention relates to a kit for

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evaluating the allergen sensitivity of an individual and/or the clinical efficacy of an allergen immunotherapy for at least one allergy comprising

- at least one allergen for inducing a mediator release of cells capable of releasing the mediator in response to an allergen,

- means for detecting mediator, and

- optionally at least one mediator standard

The kit provided herein comprises at least one allergen, which can be used to induce the release of a mediator from mediator releasing cells contained in a sample. The released mediator is then detected directly or preferably - after the removal of solid parts of the sample - in the supernatant of the reaction mixture. Optionally also means for the detection of IgE molecules binding said allergen are enclosed in the kit according to the present invention. IgE is able to bind a distinct allergen and to mediate, when bound to a mediator releasing cell and the allergen, the release of mediator from said cells. However, IgE specific for an allergen is not normally detected in the blood and is only produced when a person becomes sensitised to an allergen. In order to accurately determine the amount of mediator in the sample (for the provision of a standard curve) a mediator standard may be optionally part of the kit.

Preferably the cells are mast and/or basophilic and/or eosinophilic cells.

According to another preferred embodiment of the present invention the allergen is selected from the group consisting of major birch pollen allergens, in particular Bet v 1 and Bet v 4, major timothy grass pollen allergens, in particular Phl p 1, Phl p 2, Phl p 5, Phl p 6 and Phl p 7, major house dust mite allergens, in particular Der p 1 and Der p 2, major cat allergen Fel d 1, major bee allergens, major wasp allergens, profilins, especially Phl p 12, and storage mite allergens, especially Lep d 2 and the allergens listed in table 1.

The means for detecting mediators are preferably antibodies.

A mediator, as outlined above, is preferably detected by immunological methods. Therefore the kit may provide at least one antibody which is able to bind specifically mediator. Preferably enzyme linked immuno sorbent assays (ELISA), radio immuno assays (RIA) or lateral flow devices are employed.

Another aspect of the present invention relates to a kit for evaluating the allergen sensitivity of an individual or the clinical efficiency of an allergen immunotherapy for at least one allergy comprising at least two of the following components:

- at least one allergen for inducing a mediator release of cells capable of releasing mediators in response to an allergen,
- means for detecting the mediator,
- at least one mediator standard, and
- cells capable of releasing mediators in response to an IgE-allergen complex.

The present invention is further illustrated by the following figures and example, without being restricted thereto.

Fig. 1 shows the association of results from intradermal end-point titration (x-axis: Allergen concentration giving the first positive reaction) and rBet v 1- specific serum IgE (y-axis: kU/L CAP System).

Fig. 2 shows the association of results from basophil histamine release (x-axis: Allergen concentration giving 30% histamine release) and rBet v 1-specific serum IgE (y-axis: kU/L CAP System).

Fig. 3 shows the association of results from intradermal end-point titration (x-axis: Allergen concentration giving the first positive reaction) and results from basophil histamine release (y-axis: Allergen concentration giving 30% histamine release).

Fig. 4 shows the association of Bet v 1-specific IgE determined by CAP (x-axis: kU/ L) and of rBet v 1-specific IgE determined with labelled α -chain (y-axis: counts per minute (c.p.m.); 1 : 5 serum dilution).

Fig. 5 shows the association of results from basophil histamine release (x-axis: maximal histamine release (%)) and results from skin prick testing (y-axis: weal reaction (mm²) induced by skin prick testing with 2 μ g/ml of recombinant Bet v 1).

EXAMPLES:

Example 1:

The cross-linking of effector cell (mast cells and basophils)- bound IgE antibodies by allergens is a crucial event for the induction of the immediate symptoms of type I allergy (Kawakami T, et al., Nat Rev Immunol (2002) 2:773-86). As described

in the classical experiments by Prausnitz and Küstner (Prausnitz C, et al., *Centralbe F Bact 1 Abt Orig* (1921) 86:160-8), this event depends on three major factors, i.e. allergen-specific IgE antibodies, effector cells and allergens. Because the characterisation of IgE antibodies and the development of diagnostic tests capable of measuring the precise amount of allergen-specific IgE antibodies, several studies have investigated the association of allergen-specific serum IgE levels and biological sensitivity to allergens in allergic patients (Stenius B, et al., *Clin Allergy* (1971) 1:37-55; Bryant DH, et al., *Clin Allergy* (1975) 5:145-57; Pauli G, et al., *Clin Allergy* (1977) 7:337-46; Bousquet J, et al., *Clin Allergy* (1987) 17:529-36; Witteman AM, et al., *J Allergy Clin Immunol* (1996) 97:16-25; Niederberger V, et al. *J Invest Dermatol* (2001) 117:848-51; Norman PS, et al., *J Allergy Clin Immunol* (1973) 52:210-24; Lichtenstein LM, et al. *J Allergy Clin Immunol* (1971) 47:103 (A37)). It is well established that the presence of allergen-specific serum IgE is a pre-requisite for the occurrence of an immediate type of reaction, but whether the amount of allergen-specific IgE correlates with immediate type sensitivity to the given allergen has been a matter of great debate. To address the problem almost all of the investigations carried out in the past have used allergen extracts, i.e. mixtures of allergens and non-allergenic molecules (Stenius B, et al., *Clin Allergy* (1971) 1:37-55; Bousquet J, et al., *Clin Allergy* (1987) 17:529-36; Norman PS, et al., *J Allergy Clin Immunol* (1973) 52:210-24; Lichtenstein LM, et al. *J Allergy Clin Immunol* (1971) 47:103 (A37)). This is the reason why these studies could not analyse the association between allergen-specific IgE levels and biological activities at molecular levels. Recent studies using purified natural and recombinant allergens to re-investigate the relation between skin sensitivity and allergen-specific IgE levels report considerable discrepancies between these parameters (Witteman AM, et al., *J Allergy Clin Immunol* (1996) 97:16-25; Niederberger V, et al. *J Invest Dermatol* (2001) 117:848-51). In this example, purified recombinant Bet v 1, the major birch pollen allergen, was used as a paradigmatic tool to further investigate the association between allergen-specific IgE levels, effector cell responses and in vivo sensitivity. In a population of 18 birch pollen-allergic patients, selected on well-defined clinical criteria, and out of

the pollen season, skin sensitivity and basophil degranulation in response to defined amounts of structurally folded recombinant Bet v 1 was quantified. The results of the biological and of the serological tests were compared. For the measurement of Bet v 1-specific IgE antibody levels two different assays were used: one to detect any Bet v 1-specific IgE, and the other to detect Bet v 1-specific IgE able to bind to effector cells.

Material and methods

Study population

The examination of the patients was performed between January and April before the beginning of the birch pollen season. Eighteen patients, eight women and 10 men aged between 28 and 58 years (mean age: 45.6 years), were included in the study on the basis of clinical history of birch pollinosis and positive skin prick tests to birch pollen extract. All patients had moderate to severe rhino-conjunctivitis first diagnosed at least 3 years before. Five patients had mild asthma during birch pollen season and 12 patients had oral allergy syndrome with fruits of the Rosaceae family (apple, peach, apricot and almonds) and vegetables from Solanaceae (potato, tomato) and Apiaceae family (celery, carrot). Skin prick tests with a standard panel of respiratory allergens (Stallergènes, France) consisting of house dust mites, mixtures of fungal allergens, dog and cat dander, cockroach, grass, trees (including birch, olive and ash) and weed pollens were performed to identify the sensitisation profile. Patients' characteristics are displayed in the following Table 1.

Study design

To analyse the possible association between allergen-specific IgE levels, skin sensitivity, and basophil degranulation, patients were bled and their skin was tested on the same day. The analyses were carried out strictly out of the birch pollen season to exclude effects because of seasonal allergen contact. Patients were not allowed to take anti-allergic or anti-inflammatory medication at least 1 week before the study was performed. None of the patients had received allergenspecific immunotherapy over the last 5 years. After informed consent was given, blood was collected for basophil histamine release and for serum sampling. Immediately thereafter, intradermal skin tests were performed using the end-point titration method (Grammer LC, et al., J Allergy Clin Immunol (1985) 76:123-7).

Detection and quantification of allergen-specific antibodies

Allergen-specific IgG1 to IgG4 subclass levels as well as allergen-specific IgM and IgA levels were measured by ELISA using isotype-specific monoclonal antibodies as described (Vrtala S, et al., J Allergy Clin Immunol (1996) 97:781-7). Results represent means of duplicate determinations and are shown as OD values corresponding to the amount of bound antibodies.

Basophil histamine release test

The challenge of whole blood with rBet v 1 and anti-IgE as a positive control was performed in a dose response fashion according to the method described by Tanisaki et al. (Tanisaki Y, et al., Int Arch Allergy Appl Immunol (1984) 73:141-5). Ten millilitres of venous blood was drawn into a plastic syringe containing 1ml of heparin. 250µl of different concentrations of rBet v 1 (from 10^{-4} to 10 mg/ml) or anti-IgE (from 10^{-4} to 10^{-3} ; e-specific, Dako, Glostrup, Denmark) were added to the test tubes containing 500 µl of whole blood diluted 1:4 in Tris buffer (10 mmol/l Tris, 136 mmol/l NaCl, 2.7 mmol/l KCl, 0.23 mmol/l MgCl₂, 1.8 mmol/L CaCl₂, 5.5 mmol/l glucose; pH 7.3). The mixed solution was incubated for 30 min at 37°C. The reaction was stopped, and the cells were separated by cold centrifugation (4°C) at 375 x g

Table 1. Clinical data, results of serology, basophil histamine release and skin testing for the study population

N	Initials	Age	Symptom	Positive prick test	Food allergy	+ID test	30% HR	IgE class	Specific IgE (kU/L) (S)	Total IgE (kU/L) (T)	(S/T)%	IgG1	IgG2	IgG3	IgG4
1	F-T	58	R-C	m, b, o, g	a, c, p, al, n	10^{-3}	0.3×10^{-2}	3	12.1	30.6	39.5	0.24	0.15	0.181	0.131
2	S-F	33	R-C	b		0.3×10^{-1}	0.3×10^{-2}	4	24.1	142	16.9	0.798	0.111	0.092	0.408
3	W-F	53	R-C	b, o	a, p, n	10^{-1}	10^{-1}	3	5.22	11.5	45.4	0.537	0.079	0.788	0.082
4	F-JJ	51	R-C	b		10^{-3}	0.3×10^{-3}	5	59.9	128	46.8	1.066	0.106	0.072	0.198
5	G-S	49	R-C	m, b	a, p, ap	0.3×10	10^{-1}	3	17.1	33	51.8	0.187	0.074	0.065	0.075
6	S-S	50	R-C	b, o, w, c		1	0.3×10^{-2}	4	41.1	168	24.5	0.287	0.081	0.081	0.103
7	B-A	39	R-C, A	b, a		0.3×10	0.3×10^{-1}	4	20	43	46.5	0.298	0.083	0.081	0.071
8	O-C	37	R-C	b, o, a, w	a, c, p	10	10^{-1}	5	79.9	231	34.6	0.658	0.09	0.83	0.209
9	L-N	44	R-C, A	b, a	a, n	10^{-5}	10^{-2}	4	26.6	115	23.1	1.325	0.098	0.074	0.136
10	M-C	43	R-C, A	b, o		10^{-3}	0.3×10^{-3}	3	4.51	6.9	65.4	0.5	0.173	0.083	0.06
11	H-C	58	R-C	b, o, a	a	10^{-1}	10^{-4}	4	22.7	113	20	0.505	0.1	0.088	0.208
12	P-D	49	R-C, A	m, b, a, g	a, c, ap, p, n, ce, ca	10^{-2}	1	3	17.4	94.5	18.4	1.043	0.075	0.065	0.062
13	H-M	41	R-C	m, b, o	a, c, p, al, n	0.3×10^{-1}	10^{-2}	5	51.5	82.2	62.6	0.183	0.059	0.055	0.889
14	W-S	53	R-C, A	m, b	a	0.3×10^{-2}	0.3×10^{-1}	4	45.5	72.3	62.9	0.236	0.161	0.075	0.078
15	B-E	28	R-C	b, g	n, al	1	10^{-2}	3	14	90.9	15.4	0.389	0.069	0.079	0.065
16	B-M	50	R-C	b, o, g	a, p	0.3×10^{-2}	10^{-3}	3	4.74	16.5	28.7	0.14	0.064	0.068	0.055
17	W-B	46	R-C	m, b, g, o, w, c	a, ca, k	10^{-1}	10^{-1}	4	21.4	91.3	23.4	0.344	0.086	0.075	0.111
18	S-B	40	R-C	b	a, ca, k	1	1	2	1.65	NA	NA	0.113	0.061	0.065	0.074

Symptoms: R, rhinitis; C, conjunctivitis; A, asthma.

Positive prick test: m, millet; b, birch; o, olive; g, grass; w, weeds; a, ash; c, cat.

Food allergy: a, apple; ap, apricot; c, cherry; p, peach; al, almond; n, nuts; k, kiwi; ce, celery; ca, carrot.

ID test, intradermal test; HR, histamine release (values in $\mu\text{g/mL}$); c.p.m., counts per minute.

for 5 min. 200µl of the cell-free supernatant was used for histamine quantification in a radioimmunoassay with acylated histamine monoclonal antibodies (Immunotech, Marseille, France) as described previously (Morel AM, et al. J Allergy Clin Immunol (1988) 82:646-54). Total histamine was measured after cell lysis by repeated thawing and freezing. All experiments were performed in duplicate. The parameter used to describe the degree of basophil sensitivity was the lowest allergen concentration inducing 30% of total histamine release.

Intradermal testing

Threshold intradermal skin tests were performed by injection of 0.03ml of 10-fold dilutions of rBet v 1 on the lateral part of the arm. Serial dilutions were prepared from a solution of 1000 mg/ml and the first dilution tested was 10 mg/ml. The tests were read 15 min after injection. The area of weal and erythema was recorded. The test was considered positive when the induced weal area exceeded that of the weal induced by injection and the lowest concentration of allergen inducing a positive test result was used for comparison with other parameters (Grammer LC, et al., J Allergy Clin Immunol (1985) 76:123-7).

Statistical analysis of the data

Correlation between different parameters was tested by Spearman s non-parametric tests using VisualStats Professional software (version 2003).

Results

Poor association between rBet v 1-specific immunoglobulin E levels and skin sensitivity to rBet v 1

To compare rBet v 1-specific IgE levels and skin sensitivity, rBet v 1-specific IgE levels were measured by CAP and correlated with the threshold concentration of rBet v 1 inducing a positive intradermal test weal reaction. Fig. 1 shows that there is no association between allergen-specific IgE levels and skin sensitivity ($r=-0.007$, $P=0.977$). In individual patients a strong discrepancy between allergen-specific IgE and skin sensitivity was observed. For example, patient 8 exhibited high Bet v 1-specific IgE level (79.9 kU/l) but showed a positive ID reaction only at 10 mg/ml of rBet v 1 (Table 1). On the other hand, patient 10 had low rBet v 1-specific IgE (4.5 kU/l), yet with a 1000-fold greater skin sensitivity to Bet v 1 (positive ID test reaction at 1 ng/ml of rBet v 1) than patient eight. Seven pa-

tients (2, 5, 7, 9, 11, 12 and 17) with similar rBet v 1-specific IgE levels (17.1-26.6 kU/l) exhibited an extremely broad range of skin sensitivity to rBet v 1 (from 3 to 105 mg/ml) (Table 1).

Poor association between rBet v 1-specific immunoglobulin E levels and rBet v 1-related basophil sensitivity

Fig. 2 (IgE vs. 30% histamine release) shows that there is also a lack of association between rBet v 1-specific IgE levels and Bet v 1-induced basophil sensitivity (Fig. 2: $r = -0.113$, $P = 0.656$). The concentration of rBet v 1 required to induce 30% histamine release varied from 10^{-3} to 1 mg/ml. For given levels of rBet v 1-specific IgE (RAST class 3: 4.51-17.1 kU/l), the concentration of rBet v 1 inducing 30% histamine release varied 1000-fold (1 10^{-3} mg/ml).

Association between rBet v 1-induced basophil histamine release and skin sensitivity

Fig. 3 shows that the results of intradermal testing and basophil histamine release tests are better associated than the results of serological and biological tests. There is a significant trend between the concentrations of rBet v 1 eliciting 30% histamine release and intradermal weal reactions ($r = 0.614$; $P = 0.007$). Patients with extremely bad association between rBet v 1-specific IgE levels and results of biological testing (e.g. patients 8 and 10) showed better association when intradermal testing results were compared with basophil histamine release (Table 1). Results of other tests performed in order to explain the discrepancies between serological and biological tests are given below.

Measurements of rBet v 1-specific immunoglobulin G subclasses, immunoglobulin A and immunoglobulin M

It has been described that Bet v 1-allergic patients' sera contain Bet v 1-specific IgG antibodies that may interfere with IgE binding to Bet v 1 or recognise epitopes on the Bet v 1 molecule other than IgE and hence have no effect on IgE binding to Bet v 1 (Visco V, et al. J Immunol (1996) 157:956-62; Denepoux S, et al. FEBS Lett (2000) 465:39-46). Therefore the levels of rBet v 1-specific IgG were determined (IgG1 IgG4; Table 1). The patients exhibited varying rBet v 1-specific IgG1 IgG4 subclass responses with most pronounced responses in the IgG1 and IgG4

subclasses. No significant levels of rBet v 1-specific IgA and IgM antibodies were detected in the sera, excluding the possibility that these antibody classes may influence IgE recognition of Bet v 1.

Evaluation of Bet v 1-specific immunoglobulin E as a percentage of total immunoglobulin E

If Bet v 1-specific IgE only accounts for a low percentage of total IgE, poor histamine release and skin reactivity might be explained by the fact that basophils and mast cells are primarily occupied by IgE directed against other allergens. Therefore the total IgE values were determined and the percentage of Bet v 1-specific IgE was calculated. The patients in this example had relatively low total IgE values (<168kU/L) and no association between a low percentage of Bet v 1-specific IgE and poor biological responses was found. For example, in patient 11, who showed high sensitivity, Bet v 1-specific IgE only accounted for 20% of the total IgE. On the other hand, patient 13 was less sensitive, although 62.6% of the total IgE was directed against Bet v 1 (Table 1).

Discussion

The question of whether allergen-specific IgE antibody levels, effector cell sensitivities, and clinical sensitivity correlate remains a matter of controversy. Several studies have shown a significant correlation of allergen-specific serum IgE antibodies with allergen-induced immediate type reactions even when using a complex mixture of various allergenic and non-allergenic components, which may make it difficult to compare skin tests and RAST (Stenius B, et al., Clin Allergy (1971) 1:37-55; Bousquet J, et al., Clin Allergy (1987) 17:529-36; Norman PS, et al., J Allergy Clin Immunol (1973) 52:210-24; Lichtenstein LM, et al. J Allergy Clin Immunol (1971) 47:103 (A37)). Recently, other studies using purified allergens (Wittteman AM, et al., J Allergy Clin Immunol (1996) 97:16-25) and recombinant allergens (Niederberger V, et al. J Invest Dermatol (2001) 117:848-51) have demonstrated considerable discrepancies between antibody levels and biological sensitivity.

A clinical study using a defined purified and structurally folded allergen (i.e. the major birch pollen allergen, Bet v 1) to investigate the relation between specific IgE, basophil degranulation, and skin sensitivity at a molecular level was per-

formed. Good agreement between the three methodologies and clinical relevance of birch sensitivity was found; however, strong discrepancies were noted between the levels of allergen-specific IgE, the basophil sensitivity and in vivo sensitivity (i.e. skin sensitivity as determined by end-point titration). In certain patients, very low specific IgE levels but high sensitivity in basophil degranulation and skin tests and vice versa was observed. A review of the literature reveals the scarcity of studies comparing skin tests, basophil histamine release and specific IgE levels. The few available studies showed greatly varying results and were performed with crude allergen extracts. For example, Norman et al. (Norman PS, et al., J Allergy Clin Immunol (1973) 52:210-24) found that the three tests were in good agreement with each other in the diagnosis of ragweed hayfever. Lichtenstein et al. (Lichtenstein LM, et al. J Allergy Clin Immunol (1971) 47:103 (A37)) found a quantitatively significant relationship between skin tests and histamine release. However, no measurement of specific IgE was performed in this example. The response of sensitised leucocytes and mast cells to antigen can depend on a great variety of factors.

One possibility for low sensitivity and poor release of histamine would be that only a small proportion of the total serum IgE accounts for allergen-specific IgE. Therefore the total IgE levels were determined and the percentage of allergen-specific IgE was calculated. However, an association between low percentages of allergen-specific IgE responses and poor biological activity was found. The possibility that a low percentage of specific IgE out of the total IgE may be responsible for poor biological responses towards the given allergen may be of greater importance in polysensitised subjects (Norman PS, et al., J Allergy Clin Immunol (1973) 52:210-24; Conroy MC, et al. J Immunol (1977) 118:1317-21; MacGlashan DW Jr, et al., J Immunol (1986) 136:2231-9).

There are several other factors that may be responsible for the discrepancy between allergen-specific IgE levels and biological responses but they cannot be addressed even in a system using purified allergens. They include interindividual differences in basophil and mast cell sensitivities because of variability in IgE-receptor cell surface density, a parameter that is

regulated by serum IgE levels (Conroy MC, et al. J Immunol (1977) 118:1317-21; Malveaux FJ, et al., J Clin Invest (1978) 62:176-81; Dembo M, et al., J Immunol (1978) 121:345-53; MacGlashan DW Jr, et al. J Allergy Clin Immunol (1999) 104:492-8). Different cell sensitivities have been demonstrated by variable shifts of the dose response curves (measured by 50% or 30% sensitivity) in case of similar total and antigen-specific IgE serum concentrations (Conroy MC, et al. J Immunol (1977) 118:1317-21; MacGlashan DW Jr., J Allergy Clin Immunol (1993) 91:605-15).

Furthermore, it has been shown that persons with equivalent numbers of IgE molecules on basophils may release 0-100% of their histamine content (Conroy MC, et al. J Immunol (1977) 118:1317-21). The same has been observed for cutaneous mast cells (Petersen LJ, et al., J Allergy Clin Immunol (1996) 97:672-9; Bordignon V, et al., Invest Allergol Clin Immunol (2000) 10:78-82). In addition, it has been shown that early signal events occur involving sykkinase and IP3 products, which are not linked to the level of specific IgE or basophil sensitivity (MacGlashan DW Jr., J Allergy Clin Immunol (1993) 91:605-15; Miura K, et al., J Immunol (2001) 167:7027; MacGlashan DW Jr., J Immunol (2003) 170:4914-25).

Recent evidence indicates that mast cells may also be influenced via Toll-like receptors (Marshall JS, et al., Int Arch Allergy Immunol (2003) 132:87- 97). However, the rBet v 1 preparation used for the experiments did not contain endotoxins.

Finally, it is possible that the presence of IgE antibodies with varying affinities or binding specificities for epitopes inducing varying anaphylactic activity may have influenced serological and biological test results.

In conclusion, this study demonstrates on a molecular level that allergen-specific serum IgE levels are not necessarily related to the biological sensitivity as determined by cellular and in vivo tests. A moderate association was, however, found between the cutaneous tests and the basophil histamine release tests.

Example 2:

To determine the sensitivity of a patient before therapy to allow the choice of the correct dose a whole blood basophil histamine release test is used. Patients with high sensitivity will be injected smaller doses than less sensitive patients. Be-

fore treatment a dose response curve will be established with purified allergen. In parallel, cells will be stimulated with anti-IgE to determine overall cell sensitivity which may affect sensitivity to the allergen. Success of treatment should be controlled after IgG antibodies against the allergen become detectable which is usually the case after 4-8 weeks of treatment. Since blocking of IgG antibodies may be responsible for the reduction of sensitivity it may be useful to determine in parallel IgG levels to the given allergen. Again a dose response is determined with the purified allergen and anti-IgE. Either the dose giving maximal cell activation (i.e., maximal histamine release or CD203c upregulation) is compared or the dose giving a certain degree of activation is determined and compared with the test result obtained before treatment. Materials and methods are as described in example 1.

Example 3:

When basophil histamine release experiments were performed with washed granulocyte preparations as described (Stahl-Skov et al. 1977. J Exp Immunol 27: 432-439) no correlation between histamine release data and skin sensitivity was found.

Histamine release was done using basophils from allergic patients. They were enriched by Dextran sedimentation, isolated, washed, re-suspended in histamine release buffer, and exposed to different concentrations of recombinant Bet v 1 (10^{-5} , 10^{-4} , 10^{-3} , 10^{-2} , 10^{-1} , 1 $\mu\text{g/ml}$) or anti-IgE mAb E-124-2-8 (1 $\mu\text{g/ml}$) in 96-well microtiter plates (TPP, Trasadingen, Switzerland) for 30 minutes at 37°C. After incubation, cells were centrifuged. Cell-free supernatants were recovered and analyzed for histamine content by using a commercial radioimmunoassay (Immunotech, Marseille, France). Histamine release was expressed as a percentage of total histamine measured in cell lysates (Valent et al., 1989, Proc Natl Acad Sci USA 86: 5542-5546).

Skin prick tests were performed with serial dilutions (1:2) of recombinant Bet v 1 as described (Pauli et al., 1996, J Allergy Clin Immunol 97: 1100-1109).

Maximal histamine released from basophils exposed to recombinant Bet v 1 (HR%-max) did not correlate with skin prick test reactions (mm^2) (SPT 2 $\mu\text{g/ml}$) ($r=0.224$, $P=0.342$) (Figure 5).

Claims:

1. Method for evaluating the allergen sensitivity of an individual and/or the clinical efficacy of an allergen immunotherapy comprising the steps:

- providing at least two samples selected from the group consisting of blood or fractions thereof, connective tissue, nasal, bronchial, skin or gut biopsy material from an individual subjected or intended to be subjected to an immunotherapy with at least one pure allergen or derivative thereof, wherein the samples contain cells capable of releasing mediators in response to said allergen

- contacting said sample with said allergen or derivative thereof, and

- determining the amounts of mediators released from said sample and evaluating the allergen sensitivity of the individual prior to therapy and/or the clinical efficacy of the immunotherapy by comparing said amounts.

2. Method for evaluating the allergen sensitivity of an individual and/or the clinical efficacy of an allergen immunotherapy comprising the steps:

- providing cells capable of releasing mediators in response to an IgE-allergen complex,

- contacting said cells with serum and/or plasma of said individual spiked with at least one pure allergen or derivative thereof, and

- determining the amounts of mediators released from said sample and evaluating the allergen sensitivity of the individual prior to therapy and/or the clinical efficacy of the immunotherapy by comparing said amounts.

3. Method according to claim 1 or 2, characterised in that the mediators are selected from the group consisting of histamine, tryptase, prostaglandins, leukotrienes, especially cysteinyl leukotrienes, eosinophil cationic protein, cytokines, like interleukins (IL), especially IL-2R, CD63, CD203c and combinations thereof.

4. Method according to any one of claims 1 to 3, characterised in that said cells are mast and/or basophilic and/or eosinophil-

ic cells.

5. Method according to any one of claims 1 to 4, characterised in that the sample further comprises immunoglobulins (Ig), especially immunoglobulin G (IgG).

6. Method according to any one of claims 1 to 5, characterised in that the samples are provided before and after subjecting said individual to an immunotherapy.

7. Method according to any one of claims 1 to 6, characterised in that the samples are provided after subjecting said individual to an immunotherapy.

8. Method according to any one of claims 1 to 5, characterised in that the at least one sample is provided after a maximum of 1 hour, 12 hours, 24 hours, 10 days, 4 weeks, 6 months and 36 months, after subjecting said individual to an immunotherapy.

9. Method according to any one of claims 1 to 8, characterised in that said allergen is recombinantly produced.

10. Method according to claim 9, characterised in that said allergen comprises at least one deletion, at least one substitution or at least one insertion.

11. Method according to claim 9, characterised in that said allergen is modified by reshuffling the fragments of said allergen by genetic engineering.

12. Method according to any one of claims 1 to 11, characterised in that said sample is contacted with varying concentrations of said allergen.

13. Method according to claim 12, characterised in that the concentration of said allergen is selected within the range of 1ng/ml to 100µg/ml, preferably within the range of 1pg/ml to 10µg/ml.

14. Method according to any one of claims 1 to 13, characterised

in that further total amount of the mediator of said cells is determined.

15. Method according to claim 14, characterised in that a degree of cellular sensitisation is defined by determining the concentration of said allergen inducing the release of 10%, preferably 30%, of the total amount of the mediator of said cells.

16. Method according to claim 15, characterised in that the allergen sensitivity of an individual and/or the clinical efficiency of an allergen immunotherapy is evaluated by observing the degree of cellular sensitisation in the course of said immunotherapy.

17. Method according to any one of claims 1 to 16, characterised in that the mediator in the sample is determined by an immunological and/or a chromatographical method.

18. Method according to claim 17, characterised in that the method is selected from the group consisting of radioimmunoassay (RIA), enzyme linked immunosorbent assay (ELISA), high performance liquid chromatography (HPLC), reverse transcriptase polymerase chain reaction, immunofluorescence flow cytometry and combinations thereof.

19. Method according to any one of claims 1 to 18, characterised in that said allergen is selected from the group of the major birch pollen allergens, in particular Bet v 1 and Bet v 4, the major timothy grass pollen allergens, in particular Phl p 1, Phl p 2, Phl p 5, Phl p 6 and Phl p 7, the major house dust mite allergens, in particular Der p 1 and Der p 2, the major cat allergen Fel d 1, the major bee allergens, the major wasp allergens, profilins, especially Phl p 12, and storage mite allergens, especially Lep d 2

20. Kit for evaluating the allergen sensitivity of an individual or the clinical efficiency of an allergen immunotherapy for at least one allergy comprising

- at least one allergen for inducing a mediator release of cells capable of releasing mediators in response to an allergen,

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- means for detecting the mediator, and
- optionally at least one mediator standard.

21. Kit for evaluating the allergen sensitivity of an individual or the clinical efficiency of an allergen immunotherapy for at least one allergy comprising at least two of the following components:

- at least one allergen for inducing a mediator release of cells capable of releasing mediators in response to an allergen,
- means for detecting the mediator,
- at least one mediator standard, and
- cells capable of releasing mediators in response to an IgE-allergen complex.

22. Kit according to claim 21, characterised in that said cells are mast and/or basophilic and/or eosinophilic cells.

23. Kit according to any one of claims 20 to 22, characterised in that said allergen is selected from the group consisting of major birch pollen allergens, in particular Bet v 1 and Bet v 4, major timothy grass pollen allergens, in particular Phl p 1, Phl p 2, Phl p 5, Phl p 6 and Phl p 7, major house dust mite allergens, in particular Der p 1 and Der p 2, major cat allergen Fel d 1, major bee allergens, major wasp allergens, profilins, especially Phl p 12, and storage mite allergens, especially Lep d 2.

24. Kit according to any one of claims 20 to 23, characterised in that the means for detecting the mediator are selected from the group consisting of antibodies.

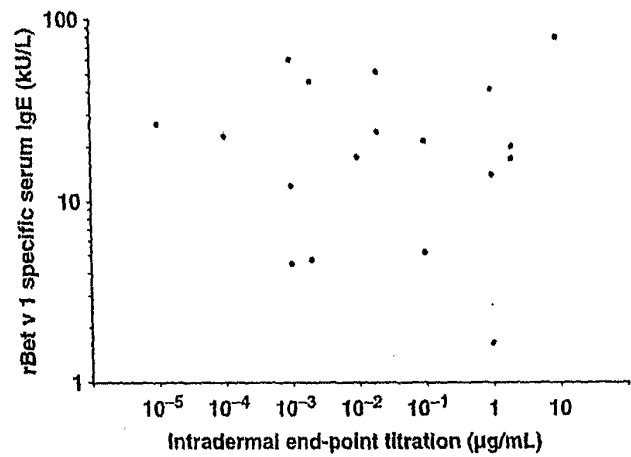


Fig. 1.

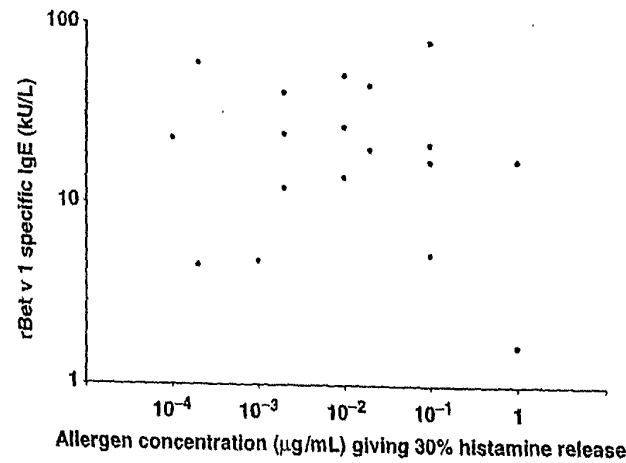


Fig. 2.

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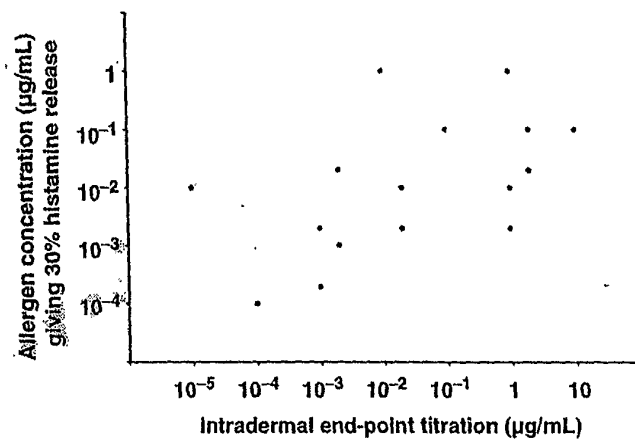


Fig. 3.

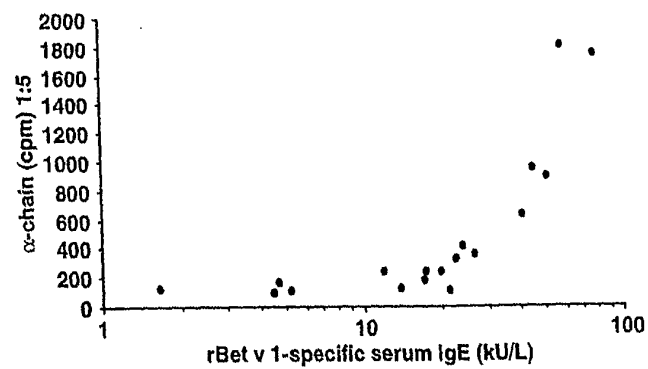


Fig. 4.

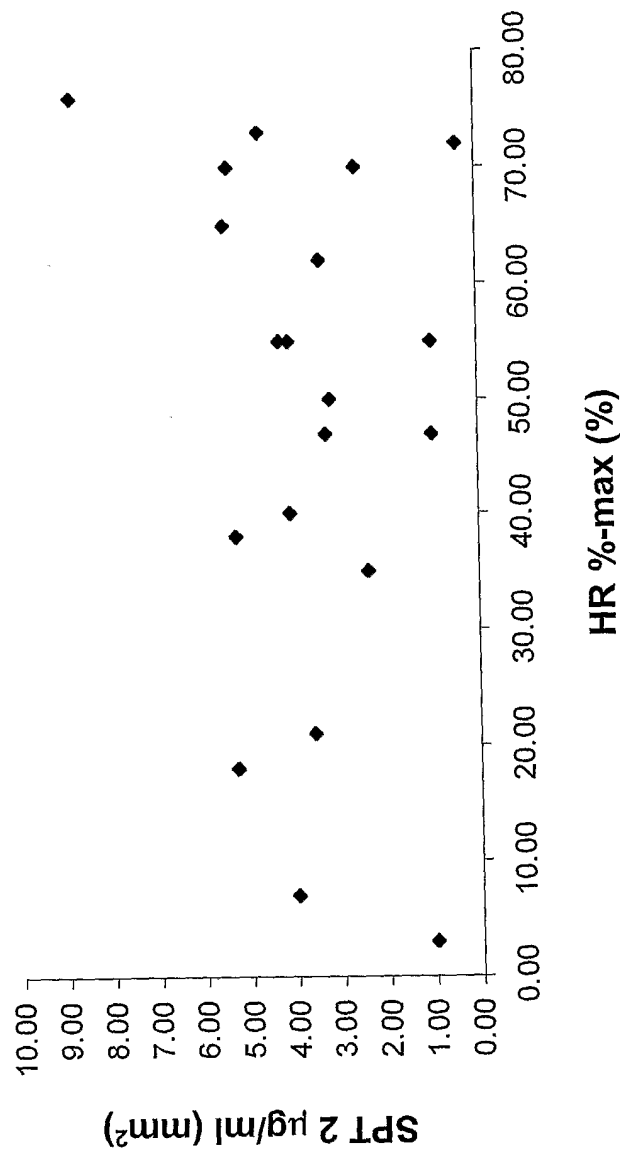


Figure 5