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

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(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.

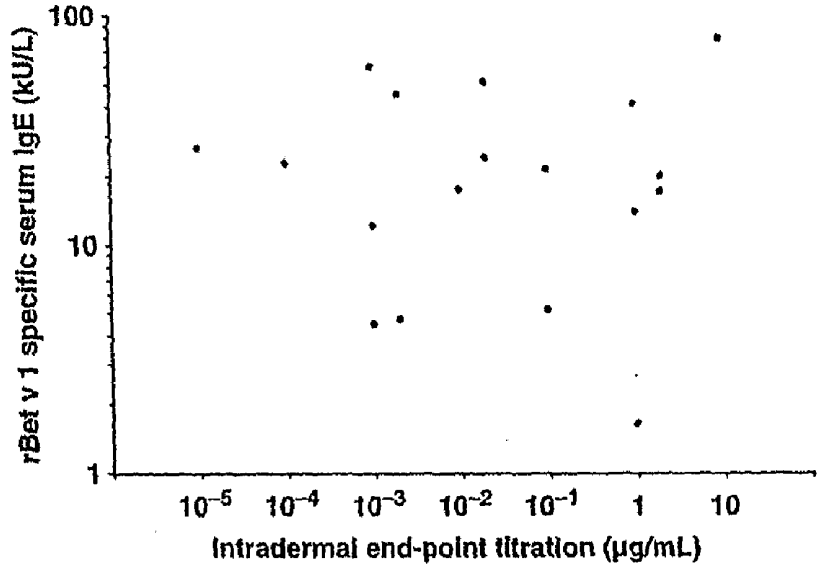
100
10
1
rBet v 1 specific serum IgE (kU/L)
10⁻⁵ 10⁻⁴ 10⁻³ 10⁻² 10⁻¹ 1 10
Intradermal end-point titration (µg/mL)

Fig. 1.

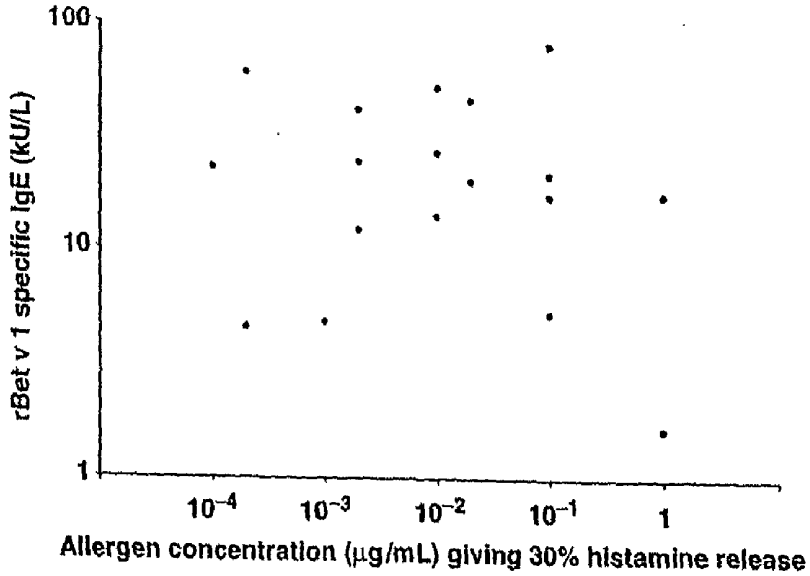
100
10
1
rBet v 1 specific IgE (kU/L)
10⁻⁴ 10⁻³ 10⁻² 10⁻¹ 1
Allergen concentration (µg/mL) giving 30% histamine release

Fig. 2.

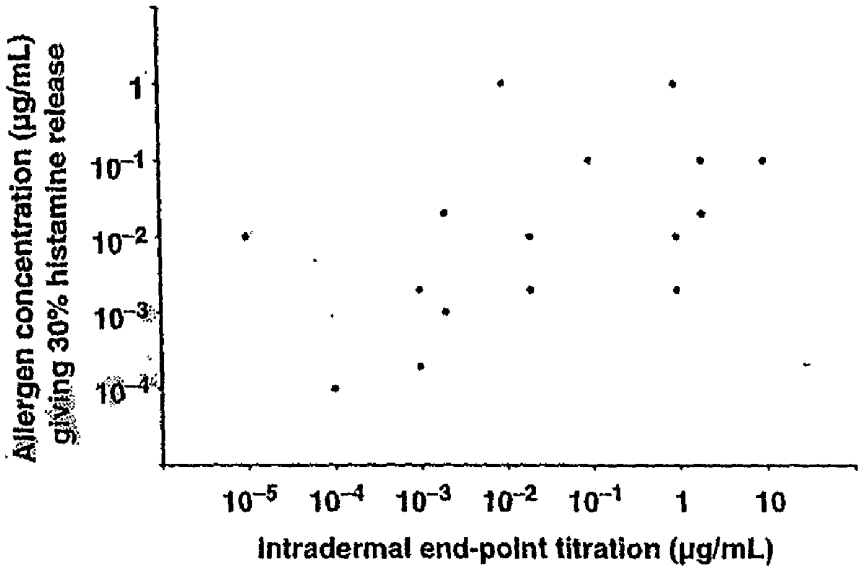
Allergen concentration (µg/mL) giving 30% histamine release
1
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5} 10^{-4} 10^{-3} 10^{-2} 10^{-1} 1 10
Intradermal end-point titration (µg/mL)

Fig. 3.

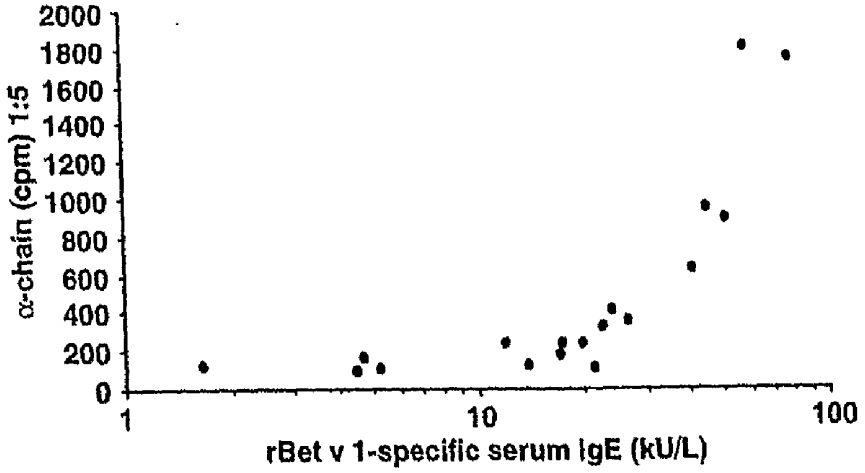
α-chain (cpm) 1:5
2000
1800
1600
1400
1200
1000
800
600
400
200
0
1 10 100
rBet v 1-specific serum IgE (kU/L)

Fig. 4.

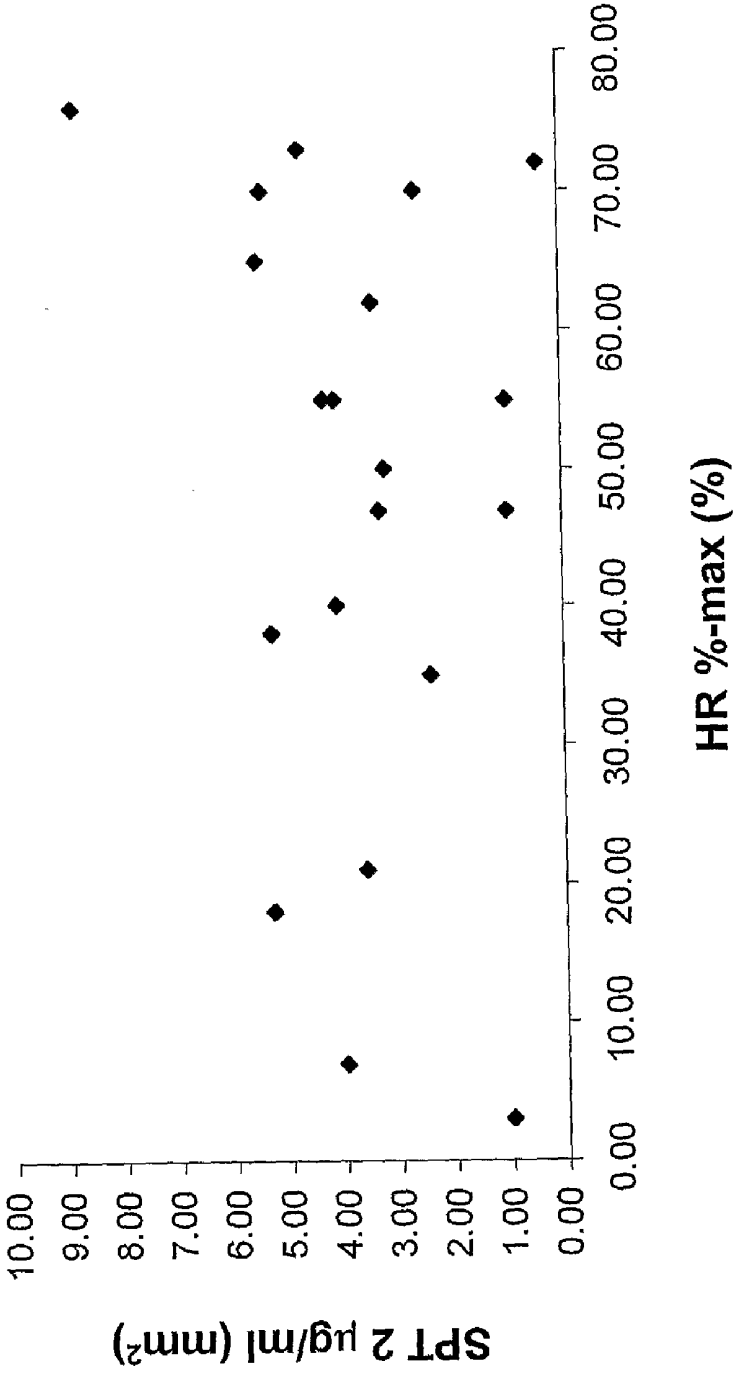
SPT 2 µg/ml (mm²)
10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
0.00
10.00
20.00
30.00
40.00
50.00
60.00
70.00
80.00
HR %-max (%)

Figure 5

METHOD FOR EVALUATING THE ALLERGEN SENSITIVITY OF AN INDIVIDUAL

[0001] The present invention relates to methods for monitoring the efficacy of an allergen immunotherapy.

[0002] 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.

[0003] 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.

[0004] Typical allergens are derived from plant pollens, like rye grass, ragweed, timothy grass and birch trees pollens, mold spores, drugs, like penicillins, sulfonamides, salicylates and local anesthetics, 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.

[0005] There exists a number of medical treatments for allergies. Mainly three methods are regularly used in medical practice: chemotherapy, immunotherapy and alternative medical methods.

[0006] 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.

[0007] 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.

[0008] 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.

[0009] 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.

[0010] In order to accomplish an immunotherapy in a successful manner monitoring of the progress of said therapy has to be performed.

[0011] 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.

[0012] 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 parameter, 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.

[0013] 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.

[0014] 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 leutrienes (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).

[0015] 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.

[0016] Therefore the present invention provides a method for evaluating the allergen sensitivity of an individual and/or the clinical efficacy of an allergen immunotherapy comprising the steps:

[0017] 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

[0018] contacting said sample with said allergen or derivative thereof, and

[0019] 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.

[0020] 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 prion and in the course of on 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.

[0021] 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.

[0022] 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.

[0023] "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 H J 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.

[0024] 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.

[0025] 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.

[0026] 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).

[0027] The sample is blood or fractions thereof (e.g. plasma, serum), connective tissue, nasal, bronchial, skin or gut biopsy material.

[0028] 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.

[0029] 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 anti-

bodies including those IgG antibodies which 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)

[0030] Preferably said cells are mast and/or basophilic and/or eosinophilic cells.

[0031] 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 M C et al. (J Histochem Cytochem. 1997 45:1715-1722), Massey W A (J. Immunol. 1991 147:1621-7), isolation protocols for basophilic cells in Valent P. (Proc. Natl. Acad. Sci USA 1989, 86, 5542-5546).

[0032] According to a preferred embodiment of the present invention the sample further comprises immunoglobulins (Ig), especially immunoglobulin G (IgG).

[0033] 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.

[0034] 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.

[0035] 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.

[0036] According to another preferred embodiment of the present invention the samples are provided after subjecting said individual to an immunotherapy.

[0037] Of course an immunotherapy may also be evaluated solely by analysing samples after the first administration of a medicament comprising an allergen.

[0038] 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.

[0039] 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.

[0040] According to a preferred embodiment said allergen is recombinantly produced.

[0041] 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.

[0042] Said allergen comprises preferably at least one deletion, at least one substitution or at least one insertion.

[0043] 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.

[0044] According to a preferred embodiment of the present invention said allergen is modified by reshuffling the fragments of said allergen by genetic engineering.

[0045] The sample is preferably contacted with varying concentrations of said allergen.

[0046] 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.

[0047] Preferably the concentration of said allergen is selected within the range of 1 ng/ml to 100 μg/ml, preferably within the range of 1 pg/ml to 10 μg/ml.

[0048] According to a preferred embodiment the total amount of mediator of the cells contained in the sample provided by an individual is determined.

[0049] 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.

[0050] 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.

[0051] 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 indi-

cates 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.

[0052] 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.

[0053] 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.

[0054] 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).

[0055] 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.
Ambrosia artemisiifolia	Amb a 1	antigen E	8	C	8, 20
short ragweed	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
Ambrosia trifida	Amb t 5	Ra5G	4.4	C	9, 10, 27
giant ragweed					
Artemisia vulgaris	Art v 1		27-29	C	28
mugwort	Art v 2		35	P	28A
	Art v 3	lipid transfer protein	12	P	53
	Art v 4	profilin	14	C	29
Helianthus annuus	Hel a 1		34		29A
sunflower	Hel a 2	profilin	15.7	C	Y15210
Mercurialis annua	Mer a 1	profilin	14-15	C	Y13271
Caryophyllales					
Chenopodium album	Che a 1		17	C	AY049012, 29B
lamb's-quarters, pigweed,	Che a 2	profilin	14	C	AY082337
white goosefoot	Che a 3	polcalcin	10	C	AY082338
Salsola kali	Sal k 1		43	P	29C
Russian-thistle					
Rosales					
Humulus japonicus	Hum j 4w			C	AY335187
Japanese hop					
Parietaria judaica	Par j 1	lipid transfer protein 1	15	C	see list of isoallergens
	Par j 2	lipid transfer protein 2		C	see list of isoallergens
	Par j 3	profilin		C	see list of isoallergens
Parietaria officinalis	Par o 1	lipid transfer protein	15		29D
		B. Grasses			
Poales					
Cynodon dactylon	Cyn d 1		32	C	30, S83343
Bermuda grass	Cyn d 7			C	31, X91256
	Cyn d 12	profilin	14	C	31a, Y08390
	Cyn d 15		9	C	AF517686
	Cyn d 22w	enolase	data		pending
	Cyn d 23	Cyn d 14	9	C	AF517685
	Cyn d 24	Pathogenesis-related p.	21	P	pending
Dactylis glomerata	Dac g 1	AgDg1	32	P	32
orchard grass	Dac g 2		11	C	33, S45354
	Dac g 3			C	33A, U25343
	Dac g 5		31	P	34
Festuca pratensis	Fes p 4w		60	—	
meadow fescue					

TABLE 1-continued

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.
Holcus lanatus velvet grass	Hol l 1			C	Z27084
Lolium perenne rye grass	Lol p 1	group I	27	C	35, 36
	Lol p 2	group II	11	P	37, 37A, X73363
	Lol p 3	group III	11	P	38
	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 isoallergens
	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 isoallergens
	Bet v 7	cyclophilin	18	P	P81531
Carpinus betulus hornbeam	Car b 1		17	C	see list of isoallergens
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 isoallergens
	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	AY295617
	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

TABLE 1-continued

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.
Plantaginaceae	Pla l 1		18	P	P842242
Plantago lanceolata					
English plantain					
Pinales					
Cryptomeria japonica	Cry j 1		41-45	C	55, 56
sugi	Cry j 2			C	57, D29772
Cupressus arisonica	Cup a 1		43	C	A1243570
cypress					
Cupressus sempervirens	Cup s 1		43	C	see list of isoallergens
common cypress	Cup s 3w		34	C	ref pending
Juniperus ashei	Jun a 1		43	P	P81294
mountain cedar	Jun a 2			C	57A, AJ404653
	Jun a 3		30	P	57B, P81295
Juniperus oxycedrus	Jun o 4	hom: calmodulin	29	C	57C, AF031471
prickly juniper					
Juniperus sabinoides	Jun s 1		50	P	58
mountain ceder					
Juniperus virginiana	Jun v 1		43	P	P81825, 58B
eastern red cedar					
Platanaceae	Pla a 1		18	P	P82817
Platanus acerifolia	Pla a 2		43	P	P82967
London plane tree	Pla a 3	lipid transfer protein	10	P	Iris p.c.
		D. Mites			
Acarus siro	Aca s 13	arthropod	14*	C	AJ006774
mite		fatty acid binding prot.			
Blomia tropicalis	Blo t 1	cysteine protease	39	C	AF277840
mite	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	Bt11a		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	Der f 1	cysteine protease	25	C	69
American house dust mite	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	Der m 1	cysteine protease	25	P	68
house dust mite					
Dermatophagoides pteronyssinus	Der p 1	antigen P1, cysteine protease	25	C	62, see list of isoallergens
European house dust mite					
	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
	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	Eur m 2			C	see list of isoallergens
mite	Eur m 14	apolipophorin	177	C	AF149827
Glycyphagus domesticus	Gly d 2			C	72B, see isoallergen list
storage mite					

TABLE 1-continued

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.
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 (see also foods)	Bos d 2	Ag3, lipocalin	20	C	76, see isoallergen list
	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*) dog	Can f 1		25	C	78, 79
	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, AF238998
	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 norvegius rat (urine)	Rat n 1		17	C	82, 83
		F. Fungi (moulds)			
1. Ascomycota					
1.1 Dothideales					
Alternaria alternata	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

TABLE 1-continued

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.
	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 sarine 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	bata-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
Penicillium chrysogenum	Pen ch 13	alkaline serine protease	34		87
(formerly *P. notatum*)	Pen ch 18	vacuolar serine protease	32		87
	Pen ch 20	N-acetyl glucosaminidas	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
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
2. Basidiomycotina					
2.1 Hymenomycetes					
Psilocybe cubensis	Psi c 1				
	Psi c 2	cyclophilin	16		89A
Coprinus comatus	Cop c 1	leucine zipper protein	11	C	AJ132235
shaggy cap	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

TABLE 1-continued

Species Name	Allergen Name	Biochem.ID or Obsolete name	Mw	cDNA or protein	Reference, Acc. No.
		preferred allergen to be used by the present invention (including reference examples) ALLERGENS			
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	Aed a 1	apyrase	68	C	L12389
mosquito	Aed a 2		37	C	M33157
Apis mellifera	Api m 1	phospholipase A2	16	C	92
honey bee	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	Bom p 1	phospholipase	16	P	95
bumble bee	Bom p 4	protease		P	95
Blattella germanica	Bla g 1	Bd90k		C	
German cockroach	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	Per a 1	Cr-PII		C	
American cockroach	Per a 3	Cr-PI	72-78	C	98A
	Per a 7	tropomyosin	37	C	Y14854
Chironomus kiiensis	Chi k 10	tropomyosin	32.5*	C	AJ012184
midge					
Chironomus thummi thummi	Chi t 1-9	hemoglobin	16	C	99
midge	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	Cte f 1				
cat flea	Cte f 2	M1b	27	C	AF231352
	Cte f 3		25	C	
Thaumetopoea pityocampa	Tha p 1		15	P	PIR: A59396, 99A
pine processionary moth					
Lepisma saccharina	Lep s 1	tropomyosin	36	C	AJ309202
silverfish					
Dolichovespula maculata	Dol m 1	phospholipase A1	35	C	100
white face hornet	Dol m 2	hyaluronidase	44	C	101
	Dol m 5	antigen 5	23	C	102, 103
Dolichovespula arenaria	Dol a 5	antigen 5	23	C	104
yellow hornet					
Polistes annularies	Pol a 1	phospholipase A1	35	P	105
wasp	Pol a 2	hyaluronidase	44	P	105
	Pol a 5	antigen 5	23	C	104
Polistes dominulus	Pol d 1				Hoffman p.c.
Mediterranean paper wasp	Pol d 4	serine protease	32-34	C	Hoffman p.c.
	Pol d 5				P81656
Polistes exclamans	Pol e 1	phospholipase A1	34	P	107
wasp	Pol e 5	antigen 5	23	C	104
Polistes fuscatus	Pol f 5	antigen 5	23	C	106
wasp					

TABLE 1-continued

Species Name	Allergen Name	Biochem.ID or Obsolete name	Mw	cDNA or protein	Reference, Acc. No.
		preferred allergen to be used by the present invention (including reference examples) ALLERGENS			
Polistes gallicus wasp	Pol g 5	antigen 5	24	C	P83377
Polistes metricus wasp	Pol m 5	antigen 5	23	C	106
Vespa crabo	Vesp c 1	phospholipase	34	P	107
European hornet	Vesp c 5	antigen 5	23	C	106
Vespa mandarina	Vesp m 1				Hoffman p.c.
giant asian hornet	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	Ves m 1	phospholipase A1	33.5	C	108
yellowjacket	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	Ves v 1	phospholipase A1	35	C	105A
yellowjacket	Ves v 2	hyaluronidase	44	P	105A
	Ves v 5	antigen 5	23	C	104
Myrmecia pilosula	Myr p 1			C	X70256
Australian jumper ant	Myr p 2			C	S81785
Solenopsis geminata	Sol g 2				Hoffman p.c.
tropical fire ant	Sol g 4				Hoffman p.c.
Solenopsis invicta	Sol i 2		13	C	110, 111
fire ant	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	Bos d 4	alpha-lactalbumin	14.2	C	M18780
domestic cattle	Bos d 5	beta-lactoglobulin	18.3	C	X14712
(milk)	Bos d 6	serum albumin	67	C	M73993
see also animals	Bos d 7	immunoglobulin	160		77
	Bos d 8	caseins	20-30		77
Gallus domesticus	Gal d 1	ovomucoid	28	C	114, 115
chicken	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	Pen m 1	tropomyosin	38	C	
black tiger shrimp	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	Ren e 1	parvalbumin alpha	11.9*	C	AJ315959
edible frog	Ren e 2	parvalbumin beta	11.7*	C	AJ414730
Brassica juncea oriental mustard	Bra j 1	2S albumin	14	C	118

TABLE 1-continued

Species Name	Allergen Name	Biochem.ID or Obsolete name	Mw	cDNA or protein	Reference, Acc. No.
		preferred allergen to be used by the present invention (including reference examples) ALLERGENS			
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 gravaolens 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 list	Dau c 1	hom: Bet v 1	16	C	117D, see isoallergen
	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	sae list of isoallergens
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

TABLE 1-continued

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.
Glycine max	Gly m 1	HPS	7	P	120A
soybean	Gly m 2		8	P	A57106
	Gly m 3	profilin	14	C	see list of isoallergens
	Gly m 4	(SAM22) PR-10 prot.	17	C	X60043, 120B
Vigna radiata	Vig r 1	PR-10 protein	15	C	AY792956
mung bean					
Arachis hypogaea	Ara h 1	vicilin	63.5	C	L34402
peanut	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	Len c 1	vicilin	47	C	see list of isoallergens
lentil	Len c 2	seed biotinylated prot.	66	P	120C
Pisum savitum	Pis s 1	vicilin	44	C	see list of isoallergens
pea	Pis s 2	convicilin	63	C	pending
Actinidia chinensis	Act c 1	cysteine protease	30	P	P00785
kiwi	Act c 2	thaumatin-like protein	24	P	SW: P81370, 121
Capsicum annuum	Cap a 1w	osmotin-like protein	23	C	AJ297410
bell pepper	Cap a 2	profilin	14	C	AJ417552
Lycopersicon esculentum	Lyc e 1	profilin	14	C	AJ417553
tomato	Lyc e 2	b-fructofuranosidase	50	C	see isoallergen list
	Lyc e 3	lipid transfer prot.	6	C	U81996
Solanum tuberosum	Sola t 1	patatin	43	P	P15476
potato	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	Ber e 1	2S albumin	9	C	P04403, M17146
Brazil nut	Ber e 2	11S globulin seed storage protein	29	C	AY221641
Juglans nigra	Jug n 1	2S albumin	19*	C	AY102930
black walnut	Jug n 2	vicilin-like prot.	56*	C	AY102931
Juglans regia	Jug r 1	2S albumin		C	U66866
English walnut	Jug r 2	vicilin	44	C	AF066055
	Jug r 3	lipid transfer protein	9	P	Pastorello
Anacardium occidentale	Ana o 1	vicilin-like protein	50	C	see isoallergen list
Cashew	Ana o 2	legumin-like protein	55	C	AF453947
	Ana o 3	2S albumin	14	C	AY081853
Ricinus communis	Ric c 1	2S albumin		C	P01089
Castor bean					
Sesamum indicum	Ses i 1	2S albumin	9	C	121A, AF240005
sesame	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	Cuc m 1	serine protease	66	C	D32206
muskmelon	Cuc m 2	profilin	14	C	AY271295
	Cuc m 3	pathogenesis-rel p. PR-1	16*	P	P83834
		I. Others			
Anisakis simplex	Ani s 1		24	P	121B, A59069
nematode	Ani s 2	paramyosin	97	C	AF173004
	Ani s 3	tropomyosin	41	C	121C, Y19221
	Ani s 4		9	P	P83885
Argas reflexus	Arg r 1		17	C	AJ697694
pigeon tick					
Ascaris suum	Asc s 1		10	P	122
worm					
Carica papaya	Car p 3w	papain	23.4*	C	122A, M15203
papaya					
Dendronephthya nipponica	Den n 1		53	P	122B
soft coral					
Hevea brasiliensis	Hev b 1	elongation factor	58	P	123, 124
rubber (latex)	Hev b 2	1,3-glucanase	34/36	C	125
	Hev b 3		24	P	126, 127
	Hev b 4	component of microhelix complex	100-115	P	128
	Hev b 5		16	C	U42640

TABLE 1-continued

Species Name	Allergen Name	Biochem.ID or Obsolete name	Mw	cDNA or protein	Reference, Acc. No.
preferred allergen to be used by the present invention (including reference examples) ALLERGENS					
	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 isoallergens
	Hev b 9	enolase	51	C	AJ132580
	Hev b 10	Mn superoxide dismut.	26	C	see list of isoallergens
	Hev b 11	class 1 chitinase		C	see list of isoallergens
	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	Kespohl p.c.

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[0273] 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.

[0274] 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:

[0275] providing cells capable of releasing mediators in response to an IgE-allergen complex,

[0276] contacting said cells with serum and/or plasma of said individual spiked with at least one pure allergen or derivative thereof, and

[0277] 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.

[0278] 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.

[0279] 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 leukozytes 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.

[0280] 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.

[0281] Another aspect of the present invention relates to a kit for evaluating the allergen sensitivity of an individual and/or the clinical efficacy of an allergen immunotherapy for at least one allergy comprising

[0282] at least one allergen for inducing a mediator release of cells capable of releasing the mediator in response to an allergen,

[0283] means for detecting mediator, and

[0284] optionally at least one mediator standard

[0285] 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.

[0286] Preferably the cells are mast and/or basophilic and/or eosinophilic cells.

[0287] 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.

[0288] The means for detecting mediators are preferably antibodies.

[0289] 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.

[0290] 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:

[0291] at least one allergen for inducing a mediator release of cells capable of releasing mediators in response to an allergen,

[0292] means for detecting the mediator,

[0293] at least one mediator standard, and

[0294] cells capable of releasing mediators in response to an IgE-allergen complex.

[0295] The present invention is further illustrated by the following figures and example, without being restricted thereto.

[0296] 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).

[0297] 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).

[0298] 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).

[0299] 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 a-chain (y-axis: counts per minute (c.p.m.); 1:5 serum dilution).

[0300] 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 (mm^2) induced by skin prick testing with 2 μg/ml of recombinant Bet v 1).

EXAMPLES

Example 1

[0301] 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, at 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 D H, 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 A M, et al., J Allergy Clin Immunol (1996) 97:16-25; Niederberger V, et al. J Invest Dermatol (2001) 117:848-51; Norman P S, et al., J Allergy Clin Immunol (1973) 52:210-24; Lichtenstein L M, 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 P S, et al., J Allergy Clin Immunol (1973) 52:210-24; Lichten-

stein L M, 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 A M, 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.

[0302] Material and Methods

[0303] Study Population

[0304] 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.

[0305] Study Design

[0306] 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 L C, et al., J Allergy Clin Immunol (1985) 76:123-7).

[0307] Detection and Quantification of Allergen-Specific Antibodies

[0308] 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.

[0309] Basophil Histamine Release Test

[0310] 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 1 ml of heparin. 250 µl 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 ml 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 $MgCl_2$, 1.8 mmol/L $CaCl_2$, 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×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	26.5	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	0.063	0.06
11	H-C	58	R-C	b, o, a	a	10^{-4}	10^{-4}	4	22.7	113	20	0.505	0.1	0.068	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	0.129
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	0.065	0.062
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	0.889
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	0.078

TABLE 1-continued

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
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	0.065
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		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, mites; 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 μg/mL);
c.p.m., counts per minute.

for 5 min. 200 μl 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 A M, 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.

[0311] Intradermal Testing

[0312] Threshold intradermal skin tests were performed by injection of 0.03 ml 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 L C, et al., *J Allergy Clin Immunol* (1985) 76:123-7).

[0313] Statistical Analysis of the Data

[0314] Correlation between different parameters was tested by Spearman s non-parametric tests using VisualStats Professional software (version 2003).

[0315] Results

[0316] Poor Association Between rBet v 1-Specific Immunoglobulin E Levels and Skin Sensitivity to rBet v 1

[0317] 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/1), 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 patients (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 10 5 mg/ml) (Table 1).

[0318] Poor Association Between rBet v 1-Specific Immunoglobulin E Levels and rBet v 1-Related Basophil Sensitivity

[0319] 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).

[0320] Association Between rBet v 1-Induced Basophil Histamine Release and Skin Sensitivity

[0321] 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.

[0322] Measurements of rBet v 1-Specific Immunoglobulin G Subclasses, Immunoglobulin A and Immunoglobulin M

[0323] 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.

[0324] Evaluation of Bet v 1-Specific Immunoglobulin E as a Percentage of Total Immunoglobulin E

[0325] 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 (<168 kU/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).

[0326] Discussion

[0327] 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 P S, et al., J Allergy Clin Immunol (1973) 52:210-24; Lichtenstein L M, et al. J Allergy Clin Immunol (1971) 47:103 (A37)). Recently, other studies using purified allergens (Witteman A M, 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.

[0328] 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 performed. 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 P S, 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 L M, 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.

[0329] 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 P S, et al., J Allergy Clin Immunol (1973) 52:210-24; Conroy M C, et al. J Immunol (1977) 118:1317-21; MacGlashan D W Jr, et al., J Immunol (1986) 136:2231-9).

[0330] 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 M C, et al. J Immunol (1977) 118:1317-21; Malveaux F J, et al., J Clin Invest (1978) 62:176-81; Dembo M, et al., J Immunol (1978) 121:345-53; MacGlashan D W 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 M C, et al. J Immunol (1977) 118:1317-21; MacGlashan D W Jr., J Allergy Clin Immunol (1993) 91:605-15).

[0331] Furthermore, it has been shown that persons with equivalent numbers of IgE molecules on basophils may release 0-100% of their histamine content (Conroy M C, et al. J Immunol (1977) 118:1317-21). The same has been observed for cutaneous mast cells (Petersen L J, et al., J Allergy Clin Immunol (1996) 97:672-9; Bordignon V, Pet 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 D W Jr., J Allergy Clin Immunol (1993) 91:605-15; Miura K, et al., J Immunol (2001) 167:7027; MacGlashan D W Jr., J Immunol (2003) 170:4914-25).

[0332] Recent evidence indicates that mast cells may also be influenced via Toll-like receptors (Marshall J S, et al., Int Arch Allergy Immunol (2003) 132:87-97). However, the rBet v 1 preparation used for the experiments did not contain endotoxins.

[0333] 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.

[0334] 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

[0335] 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. Before 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

[0336] 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.

[0337] 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 μg/ml) or anti-IgE mAb E-124-2-8 (1 μg/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).

[0338] 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).

[0339] 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 μg/ml) (r=0.224, P=0.342) (FIG. **5**).

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. (canceled)

3. The method according to claim **1** characterized 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), IL-2R, CD63, CD203c and combinations thereof.

4. The method according to claim **1** characterized in that said cells are mast and/or basophilic and/or eosinophilic cells.

5. The method according to claim **1** characterized in that the sample further comprises immunoglobulins (Ig).

6. The method according to claim **1** characterized in that the samples are provided before and after subjecting said individual to an immunotherapy.

7. The method according to claim **1** characterized in that the samples are provided after subjecting said individual to an immunotherapy.

8. The method according to claim **1** characterized in that the at least one sample is provided after a maximum of about 1 hour, about 12 hours, about 24 hours, about 10 days, about 4 weeks, about 6 months and about 36 months, after subjecting said individual to an immunotherapy.

9. The method according to claim **1** characterized in that said allergen is recombinantly produced.

10. The method according to claim **9**, characterized in that said allergen comprises at least one deletion, at least one substitution or at least one insertion.

11. The method according to claim **9**, characterized in that said allergen is modified by reshuffling the fragments of said allergen by genetic engineering.

12. The method according to claim **1** characterized in that said sample is contacted with varying concentrations of said allergen.

13. The method according to claim **12**, characterized in that the concentration of said allergen is selected within the range of about 1 ng/ml to about 100 μg/ml.

14. The method according to claim **1** characterized in that further total amount of the mediator of said cells is determined.

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

16. The method according to claim **15**, characterized 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. The method according to claim **1** characterized in that the mediator in the sample is determined by an immunological method, a chromatographical method, or both.

18. The 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. The method according to claim **1** characterized in that said allergen is selected from the group of the major birch pollen allergens, Bet v 1 and Bet v 4, the major timothy grass pollen allergens, Phl p 1, Phl p 2, Phl p 5, Phl p 6 and Phl p 7, the major house dust mite allergens, Der p 1 and Der p 2, the major cat allergen Fel d 1, the major bee allergens, the major wasp allergens, profilins, Phl p 12, and storage mite allergens, 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,

means for detecting the mediator, and

optionally at least one mediator standard.

21. (canceled)

22. The kit according to claim **20** characterized in that said cells are mast and/or basophilic and/or eosinophilic cells.

23. The kit according to claim **20** characterized in that said allergen is selected from the group consisting of major birch pollen allergens, Bet v 1 and Bet v 4, major timothy grass pollen allergens, Phl p 1, Phl p 2, Phl p 5, Phl p 6 and Phl p I

1 major house dust mite allergens, Der p 1 and Der p 2, major cat allergen Fel d 1, major bee allergens, major wasp allergens, profilins, Phl p 12, and storage mite allergens, Lep d 2.

24. The kit according to claim **20** characterized in that the means for detecting the mediator are selected from the group consisting of antibodies.

25. The method according to claim **5** characterized in that the sample further comprises immunoglobulin G (IgG).

26. The method according to claim **13**, characterized in that the concentration of said allergen is selected within the range of about 1 pg/ml to about 10 μg/ml.

27. 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.

28. The kit according to claim **27**, characterized in that said cells are mast and/or basophilic and/or eosinophilic cells.

29. The kit according to claim **27** characterized in that said allergen is selected from the group consisting of major birch pollen allergens, Bet v 1, Bet v 4, major timothy grass pollen allergens, Phl p 1, Phl p 2, Phl p 5, Phl p 6, Phl p I 1 major house dust mite allergens, Der p 1, Der p 2, major cat allergen Fel d 1, major bee allergens, major wasp allergens, profilins, Phl p 12, storage mite allergens, Lep d 2 and combinations thereof.

30. The kit according to claim **27** characterized in that the means for detecting the mediator are selected from the group consisting of antibodies.

31. 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.

32. The method according to claim **31** characterized in that the mediators are selected from the group consisting of histamine, tryptase, prostaglandins, leukotrienes, cysteinyl leukotrienes, eosinophil cationic protein, cytokines, interleukins (IL), IL-2R, CD63, CD203c and combinations thereof.

33. The method according to claim **31** characterized in that said cells are mast and/or basophilic and/or eosinophilic cells.

34. The method according to claim **31** characterized in that the sample further comprises immunoglobulins (Ig).

35. The method according to claim **31** characterized in that the samples are provided before and after subjecting said individual to an immunotherapy.

36. The method according to claim **31** characterized in that the samples are provided after subjecting said individual to an immunotherapy.

37. The method according to claim **31** characterized in that the at least one sample is provided after a maximum of about 1 hour, about 12 hours, about 24 hours, about 10 days, about 4 weeks, about 6 months and about 36 months, after subjecting said individual to an immunotherapy.

38. The method according to claim **31** characterized in that said allergen is recombinantly produced.

39. The method according to claim **31**, characterized in that said allergen comprises at least one deletion, at least one substitution or at least one insertion.

40. The method according to claim **31**, characterized in that said allergen is modified by reshuffling the fragments of said allergen by genetic engineering.

41. The method according to claim **31**, characterized in that said sample is contacted with varying concentrations of said allergen.

42. The method according to claim **31**, characterized in that the concentration of said allergen is selected within the range of about 1 ng/ml to about 100 μg/ml.

43. The method according to claim **31**, characterized in that the concentration of said allergen is selected within the range of about 1 pg/ml to about 10 μg/ml.

44. The method according to claim **31**, characterized in that further total amount of the mediator of said cells is determined.

45. The method according to claim **31**, characterized in that a degree of cellular sensitisation is defined by determining the concentration of said allergen inducing the release of about 10% of the total amount of the mediator of said cells.

46. The method according to claim **31**, characterized in that a degree of cellular sensitisation is defined by determining the concentration of said allergen inducing the release of about 30% of the total amount of the mediator of said cells.

47. The method according to claim **31**, characterized 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.

48. The method according to claim **31**, characterized in that the mediator in the sample is determined by an immunological method, a chromatographical method or both methods.

49. The method according to claim **48** characterized 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.

50. The method according to claim **31**, characterized in that said allergen is selected from the group of the major birch pollen allergens, Bet v 1, Bet v 4, the major timothy grass pollen allergens, Phl p 1, Phl p 2, Phl p 5, Phl p 6 Phl p 7, the major house dust mite allergens, Der p 1 and Der p 2, the major cat allergen Fel d 1, the major bee allergens, the major wasp allergens, profilins, Phl p 12, and storage mite allergens, Lep d 2.

* * * * *

专利名称(译)	评估个体过敏原敏感性的方法		
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申请号	US11/815846	申请日	2006-02-09
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申请(专利权)人(译)	BIOMAY AG		
当前申请(专利权)人(译)	BIOMAY AG		
[标]发明人	PUROHIT ASHOK METZ FAVRE CARINE LAFFER SYLVIA VALENTA RUDOLF MOTHES LUKSCH NADINE VALENT PETER VEROT ANGELE PAULI GABRIELLE		
发明人	PUROHIT, ASHOK METZ-FAVRE, CARINE LAFFER, SYLVIA VALENTA, RUDOLF MOTHES-LUKSCH, NADINE VALENT, PETER VEROT, ANGELE PAULI, GABRIELLE		
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摘要(译)

本发明公开了评估个体的过敏原敏感性和/或过敏原免疫疗法的临床功效的方法，包括以下步骤：提供至少两种选自血液或其部分，结缔组织，鼻，支气管的样品。来自经受或意图用至少一种纯过敏原或其衍生物进行免疫疗法的个体的皮肤或肠活组织检查材料，其中所述样品含有能够响应所述过敏原释放介质的细胞，使所述样品与所述过敏原接触或其衍生物，和确定从所述样品释放的介质的量，并通过比较所述量来评估治疗前个体的过敏原敏感性和/或免疫治疗的临床功效。

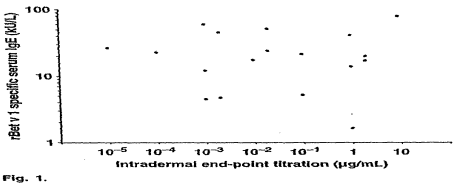

Fig. 1.

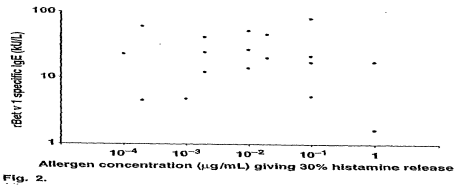

Fig. 2.