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(54) **CLONING OF HONEY BEE ALLERGEN**

KLONIERUNG EINES ALLERGENS AUS BIENE

CLONAGE D'UN ALLERGENE DE L'ABEILLE

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(73) Proprietor: **PLS-Design GmbH**
20255 Hamburg (DE)

(72) Inventor: **GRUNWALD, Thomas**
22459 Hamburg (DE)

(74) Representative: **Jungblut & Seuss Patentanwälte**
Max-Dohrn-Strasse 10
10589 Berlin (DE)

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Description

[0001] The present invention relates to a nucleic acid encoding a polypeptide capable of binding to IgE from subjects allergic to venom of an insect from the order *Hymenoptera* having the amino acid sequence of SEQ ID NO: 2, which is the honey bee allergen Api m3 (acid phosphatase). The invention further relates to expression vectors, host cells and polypeptides encoded by the nucleic acid, as well as diagnostic and pharmaceutical uses thereof.

[0002] It has long been recognised that allergies against insect venoms are relatively common. 4-5% of the German population react allergic to insect venoms. In Europe the relevant stinging insects are honey bees (*Apis mellifera*), wasps (*Vespula spp*.), bumble bees (*Bombus spp*.), hornets (*Vespa crabo*), midges, and horse flies (Ref.1,2). Bees, bumble bees, wasps, and hornets belong to the order *Hymenoptera*.

[0003] These social insects do not normally attack people, but will sting them in self defence if disturbed. Once stung, if the stinger remains in the skin, a honey bee is responsible, while, if no stinger is present, a wasp is likely to be the culprit. The female worker honey bee carries the stinger and dies soon after discharging a sting.

[0004] If a bee stings a vertebrate, the stinger will be detached from the insect, but the venom sack will still be attached to the stinger and if not removed, the whole venom volume (up to 50 μl) will be injected into the victim. Wasps can retract the stinger, and only inject about 20 μl venom.

[0005] The differences in stinging behaviour are based on natural evolution. Bees collect nectar, whereas wasps and hornets are insect hunters. Therefore, bees need to protect the hive, even against vertebrates like mice or larger animals. The insect dies upon the sting, but will inject the maximum volume of venom, if the stinger is not removed. Wasps and hornets do not have such natural enemies.

[0006] Since it is easy to obtain sufficient quantities of material, honey bee venom has been well studied. Honey bee venom contains at least 18 active substances. Melittin, the most prevalent substance, is one of the most potent anti-inflammatory agents known (100 times more potent than hydrocortisone). Adolapin is another strong anti-inflammatory substance, and inhibits cyclooxygenase; it thus has analgesic activity as well. Apamin inhibits complement C3 activity, and blocks calcium-dependent potassium channels, thus enhancing nerve transmission. Other substances, such as Compound X, Hyaluronidase, Phospholipase A2, Histamine, and Mast Cell Degranulating Protein (MSDP), are involved in the inflammatory response to venom, with the softening of tissue and the facilitation of flow of the other substances. Finally, there are measurable amounts of the neurotransmitters Dopamine, Norepinephrine and Serotonin. The water content varies between 55-70%. The pH range is between 4.5-5.5. A summary of the components of bee venom is given in table 1 (Ref. 3,4).

Table 1) Bee venom components

Component type	name	% weight of dry mass
Proteins	Phospholipase A2 (Api m 1)	10-12
	Hyaluronidase (Api m 2)	1-3
	Phosphatase, Glucosidase	1-2
Peptides	Melittin (Api m 4)	50-55
	Secapin, MCD-peptide	1.5-4
	Tertiapamin, Apamin, Procamin	2-5
	Other small peptides	13-15
Biogene amines	Histamine	0.5-2
	Dopamine	0.2-1
	Norepinephrine	0.1-0.5
	Sugars (Glucose, Fructose)	2
Phospholipids		5
Amino acids		-
Volatile substances	Pheromones	4-8
Minerals		3-4

[0007] The LD50 dose, i.e., the amount of bee venom which causes 50% of the tested individuals to die, is 6 mg

venom/kg body weight for mice and rats. This equals 40 stings/kg body weight. For hornets, this factor is around 154-180 stings/kg body weight. Bee venom is 1.7-1.5 more effective than those of hornets (Ref. 5,6).

[0008] Honey bees and wasps of the *Hymenoptera* order are by far the most frequent cause of serious allergic reactions. Normally, at least more than 50 stings of a bee per children or 100 per adult are necessary to induce life threatening conditions (see above). In case of allergic persons, one sting can be enough to cause death by adverse immunological reactions.

[0009] This type of allergy is mediated by IgE antibodies which react to venom components. The possibility, therefore, exists that desensitisation therapy by repeated and progressively increased doses of bee venom components would be successful. Several polypeptides from bee venom have been cloned and expressed as recombinant molecules (Ref. 7-16). One component of bee venom, acid phosphatase, is one of the more potent allergic proteins (Ref. 17). Until now, no information about the complete gene sequence has been published and only initial studies on protein level have been made (Ref. 18-20, 27).

[0010] Barboni et al. (Ref. 19) describe two different proteins with acid phosphatase activity from honey bee venom, having a molecular weight of 45 and 96 kDa. Enzymatic activity is partly lost during purification in the gel filtration step. Other publications (Ref. 18, 20, 27) report contrasting data, teaching different fragments of the protein and the corresponding nucleic acid, and coming to different conclusions about the family of phosphatases that honey bee venom acid phosphatase might belong to, either prostatic phosphatases or lysosomal phosphatases. Soldatova et al. (Ref. 18) describe the incomplete cloning of a partial cDNA possibly encoding an acid phosphatase from honey bee venom. They report difficulties in cloning and obtaining the full length sequence and do not teach the sequence they seem to have cloned.

[0011] In light of the prior art, the person skilled in the art is therefore faced with the problem of providing a nucleic acid suitable for recombinant production of acid phosphatase (api m3) from the venom of an insect from the order *Hymenoptera,* in particular the honey bee, which can be used in such desensitisation therapy as well as in diagnostic tests for the detection of allergy.

[0012] This problem is solved by the subject matter of the claims.

[0013] In particular, the present invention provides a nucleic acid encoding a polypeptide capable of binding to IgE from subjects allergic to venom of an insect from the order *Hymenoptera* wherein the polypeptide has the amino acid sequence of SEQ ID NO: 2 (note: "SEQ ID NO" relates to code <400> in the attached sequence listing under WIPO standard ST.25).

[0014] This polypeptide is designated Api m 3. In particular, the nucleic acid comprises or has the nucleotide sequence of SEQ ID NO: 1.

[0015] In the context of the present invention, the terms "polypeptide" and "protein" are used interchangeably, without any limitation as to the number of amino acids linked. The polypeptides may also comprise non-naturally occuring amino acids.

[0016] Throughout this specification, the polypeptides encoded by the nucleic acid of the invention have to be capable of binding to IgE from subjects allergic to venom of an insect from the order *Hymenoptera.*

[0017] Following unsuccessfull attempts to clone the full length sequence of Api m 3 following the common deduced primer strategy (cf. Soldatova et al., 2000) based on the peptide fragments found by Hoffmann, 1996, and their postulated sequence (Hoffmann, 1996). A completely different approach was chosen in the present invention. Using nucleic acid sequences derived from the peptide sequences published by Hoffman, small virtual probes of partial deduced sequences were constructed, which were used to scan the published bee genome for targets. Two regions on different truncated chromosome 16 sequences matched the probes. Scanning of these regions with bioinformatic tools revealed possible open reading frames and gene sequences. Scanning for a potential sequence cleavage site led to the putative N-terminus of the Api m 3 gene. Primers were then designed according to the proposed protein N- and C-terminus. These primers were used to amplify the gene from bee venom gland cDNA synthesized from total RNA with oligodT(20) primers. The amplification was successful and resulted in a DNA fragment of the expected size. The identity of the DNA was verified by sequencing, molecular weight calculation and alignment to the homologous acid phosphatases of human as well as rat sequences and the proposed peptide fragments. The protein identity was also verified.

[0018] From the resulting full length cDNA sequence, it is clear the classical approach had to fail, as Hoffman erred in several points. For example, he chose an incorrect alignment of the peptide fragments (cf. Fig. 5), erroneously thought that several peptides that are in fact separated by other peptides were contigous, and postulated existence of a fragment that does not belong to the acid phosphatase. In fact, using primers based on peptides 1 and 7, as proposed by Hoffman, one would expect to amplify a short fragment covering only the peptide sequence around amino acids 200 and 230 of the consensus sequence.

[0019] The social insects from the order, *Hymenoptera* that commonly interact with man are members of the superfamilies *Apoidea* and *Vespoidea,* bees and wasps (Ref. 20). The Vespoidea include the social wasps and hornets, *Vespidae,* as well as ants, *Formici*dae. Important wasps comprise yellowjackets of the genus *Vespula,* hornets of the genera *Dolichovespula* and *Vespa* and paper wasps of the genus *Polistes.* Bees comprise, e.g., honey bees, *Apis*

mellifera, and bumble bees of the species *Bombus terrestris.* In the context of the present invention, an insect from the order *Hymenoptera* can be from any of these species, but according to a particular embodiment, the insect is a bee from the genus *Apis.* Most preferably, the bee is the honey bee, *Apis mellifera.*

[0020] Other species from the order *Hymenoptera* produce similar allergens with antigenic cross reactivity and a high degree of amino acid homology (Ref. 24-26). Thus the present invention not only extends to the Api m3 allergen from *Apis mellifera* but also to homologous *Hymenoptera* allergens.

[0021] In particular, the polypeptides encoded by the nucleic acids of the invention have to be capable of binding to IgE from subjects allergic to venom of *Apis mellifera.* The subjects are commonly reactive to the Api m3 antigen, acid phosphatase from bee venom. For the purpose of testing, serum or purified IgE from such allergic subjects are contacted with the polypeptide, and specific binding of the polypeptide to the antibodies is detected. Such a test can, e.g., be an ELISA. For verifying the reactivity of the polypeptides with IgE antibodies, serum or IgE from several subjects are pooled, preferentially, from 5 to 20 subjects.

[0022] The nucleic acids of the invention can be either DNA or RNA.

[0023] In one embodiment, the invention also provides a nucleic acid, which is a fragment having a length of more than 255 nucleotides of a nucleic acid encoding a polypeptide of the amino acid sequence of SEQ ID NO: 2, wherein the fragment encodes a polypeptide capable of binding to IgE from subjects allergic to venom of an insect from the order *Hymenoptera.* Preferably, the nucleic acid is a fragment having a length of more than 255, more preferably of more than 600, more than 700 or more than 800 nucleotides of a nucleic acid encoding a polypeptide having the amino acid sequence of SEQ ID NO: 2.

[0024] In another embodiment, a nucleic acid fragment (polynucleotide) is provided that comprises at least 15 contiguous nucleotides of the nucleic acid encoding a polypeptide having the amino acid sequence of SEQ ID NO: 2, wherein the polynucleotide is selected from the group consisting of nucleotides 78 to 299, 348 to 437, 459 to 476, 555 to 671, 696 to 830 or 1086 to 1121 of said nucleic acid, wherein the numbering corresponds to the region encoding said polypeptide. Specifically, said nucleic acid has the nucleotide sequence shown in SEQ ID NO: 1. Preferentially, the nucleotides are from the region of nucleotides 555 to 671 or 696 to 830.

[0025] Additionally, such nucleic acids consisting of nucleotides 1 to 77, 438 to 458, 477 to 494, 504 to 554, 672 to 695, and 831 to 1085 of the nucleic acid shown in SEQ ID NO: 1 are provided.

[0026] Preferentially, the nucleic acid comprises 15 to 240, 15 to 90, 18 to 60, 21 to 30, more preferably at least 18, 21, 24, 27, 30, 60, 90 or more contiguous nucleotides from the above regions.

[0027] The invention also provides further polypeptide variants and nucleic acid sequences as indicated in the claims.

[0028] In one embodiment, the invention also provides a polypeptide encoded by a nucleic acid of the invention. Preferentially, the polypeptide is a full length acid phosphatase from the venom of an insect from the order Hymenoptera. In particular, the polypeptide has the amino acid sequence of SEQ ID NO: 2. Although not essential, it is preferred that the polypeptide has acid phosphatase activity. This activity can be tested, e.g., according to the method described in Ref. 19.

[0029] Alternatively, the polypeptide is a fragment of the full length protein capable of binding to IgE from subjects allergic to venom of an insect from the order *Hymenoptera* having a length of more than 85, more than 200 or more than 250 amino acids. Other fragments are provided, wherein the polypeptides are capable of binding to IgE from subjects allergic to venom of an insect from the order *Hymenoptera,* and comprise at least 5, preferably at least 6, 7, 8, 9, 10, 15, 20 or more amino acids of a polypeptide selected from the group consisting of amino acid 26 to 99, 116 to 145, 153 to 158, 185 to 223, 232 to 276 or 362 to 373 of the polypeptide shown in SEQ ID NO: 2. Alternatively, the polypeptides are capable of binding to IgE from subjects allergic to venom of an insect from the order *Hymenoptera,* and comprise at least 5, preferably at least 6, 7, 8, 9, 10, 15, 20 or more amino acids of the polypeptide shown in SEQ ID NO: 2, except for the polypeptides from the group consisting of amino acids 1 to 34, 63 to 80, 100 to 115, 142 to 149, 168 to 176, 224-239 and 258 to 343 of the polypeptide shown in SEQ ID NO: 2 or except for the polypeptides shown in Fig. 5. Additionally, such polypeptides consisting of amino acids 1 to 25, 146 to 152, 159 to 164, 168 to 184, 224 to 231, and 277 to 361 are provided.

[0030] Preferably, the polypeptide of the invention is recombinantly expressed. This has the advantage, e.g., that the polypeptide can be expressed as a fusion protein linked to an additional polypeptide. For example, the polypeptide or fusion protein is attached to a signal sequence ensuring its secretion into the extracellular space or supernatant of the cultured cells, where appropriate. Due to novel techniques in molecular biology, the use of recombinant proteins in therapy and diagnostics is expected to increase the efficiency and diagnostic value in these medical applications (Ref. 21-23).

[0031] Depending on the host cell producing the recombinant protein, the protein is glycosylated (after expression in mammalian or yeast cells) or non-glycosylated (after expression in bacterial cells). The glycosylation pattern can vary depending on the host cell used, and can thus differ from the glycosylation pattern of natural acid phosphatase isolated from bee venom. In one alternative, the glycosylation pattern is identical to the glycosylation pattern of acid phosphatase isolated from bee venom. Glycosylation can have profound effects on the binding of specific antibodies.

[0032] When expressed in bacterial cells, the polypeptide of the invention lacks glycosylation. The protein thus differs from the native protein in respect to epitope presentation, and potentiality for folding and functionality. It was shown that carbohydrates can represent IgE epitopes and contribute to observed non-specific cross-reactivity of allergens, e.g., between bee and wasp proteins, due to similar features of the carbohydrate chains (Huby et al., Tox Sci 55: 235-246, 2000; Tretter et al., Int. Arch. Allergy Immunol. 102: 259-266, 1993; Hemmer et al., Cli. Exp. Allergy 34: 460-460, 2004). The cross-reactivity is one reason for false positive results in *in vitro* immunological tests (Petersen A., Mundt C., J Chromat B 756, 141-150, 2001). Expression of the non-glycosylated polypeptide eliminates these false positives, and can therefore be used to advantage in diagnostic and therapeutic applications.

[0033] The glycosylation pattern in eukaryotic cells other than insect cells, e.g., in mammalian cells, also varies from the glycosylation pattern of the native protein (Jenkins et al., Nat. Biotech. 14: 975-981, 1996). Even in insect cells, the glycosylation pattern is likely to be different due to overex-pression of the protein.

[0034] Sequence analysis of Api m 3 shows that the protein comprises three putative glycosylation sites of the sequence Asn-Xaa-Ser/Thr. In one embodiment, the polypeptides of the invention comprise mutated glycosylation sites instead of glycosylation sites. In particular, in a mutated glycosylation site, the Asparagine (Asn) in the glycosylation site(s) can be exchanged against any other amino acid, preferably against Glutamine (Gln) (Elbein A.D. et al., 1991, Trends Biotechnol. 9(10):346-352). Alternatively, in a mutated glycosylation site, the Serine (Ser) can be exchanged against another amino acid or deleted. Accordingly, the invention also provides a nucleic acid encoding a polypeptide of the invention comprising at least one, preferably 2, or 3 mutated glycosylation sites instead of glycosylation sites. Most preferably, all glycosylation sites are mutated.

[0035] The present invention also relates to an expression vector comprising a nucleic acid of the invention operationally linked to an expression control sequence. In one alternative, the nucleic acid is linked in frame to a nucleic acid encoding an additional polypeptide, so the expression vector can be used for expression of a fusion protein. The additional polypeptide can be selected from the group comprising a poly-Histidine tag (His tag), glutathione-S-transferase, β-galactosidase, a cytokine, and an IgG-Fc. In particular, tags that simplify purification of the recombinant protein, e.g., a His tag, are employed. Such a tag may be cleaved off after purification of the protein.

[0036] Alternatively, it can be beneficial for therapeutic applications to express the polypeptide of the invention linked to a therapeutic polypeptide, e.g. a cytokine. For example, a fusion protein with a cytokine enhancing T_H1 and down-regulating T_H2 responses or inducing class switch to IgG, such as IFN-y, IL-10, IL-12 or TGF-β, can improve efficiency of desensitisation. If the expression vector is used for gene therapy, it is envisaged to use sequences rich in CpG (unmethylated cytosine guanidine dinucleotides), which promote T_H1 responses. Additionally or alternatively, the polypeptide of the invention can be linked to another polypeptide or protein, such as in the form of a fusion protein or as separate proteins expressed by the same vector. Preferably, the further polypeptides or proteins are other *Hymenoptera* venom proteins or antigenic fragments thereof.

[0037] The expression vector can be suitable for expression in different cell types, such as bacterial, yeast or mammalian cells. Preferentially, the vector is suitable for expression in insect cells, e.g., HighFive insect cells (Invitrogen, Karlsruhe, Germany). Alternatively, especially for gene therapy applications, the vector is suitable for expression in human cells. In this context, the expression of the encoded polypeptide can be directed by the choice of a suitable expression control sequence, e.g., an expression control sequence mainly or specifically operational in different cell types, such as lymphoid cells, for example dendritic cells, B cells or macrophages.

[0038] In one embodiment of the invention, the expression vector is pIB/V5-His (Invitrogen, Karlsruhe, Germany, Invitrogen Manual: InsectSelect BSD System with pIB/V5-His, Version G, 30 May 2003).

[0039] In particular, the vector can be pIB/Mel opt-H10-Api m3, comprising the Api m3 cDNA sequence (SEQ ID NO: 1), which was modified to facilitate isolation and purification. A melittin signal sequence for secretion of the recombinant protein was added and the Kozak sequence was optimised for higher expression rates in insect cells (see Fig. 4 and Example 2). Alternatively, other signal sequences can be used for secretion of the protein. The expression vector can also be a different plasmid or a viral, e.g., baculoviral or adenoviral, vector. The expression vector further comprises a stop codon and a polyadenylation signal.

[0040] The present invention further relates to a host cell comprising said expression vector. This host cell can be a bacterial, yeast or mammalian cell, in particular an insect cell.

[0041] A method of producing a polypeptide encoded by a nucleic acid of the invention is provided, wherein the host cell is cultured under appropriate conditions for expression of said polypeptide and said polypeptide is purified. If the polypeptide is a fusion protein with a fusion partner facilitating purification, e.g., a His Tag or a GST-tag, a corresponding affinity column can be used for purification, e.g., a Ni^{2+} or glutathione affinity column. For purification of an IgG fusion protein, a protein A or protein G column is suitable.

[0042] The expression vector of the invention can be used for the preparation of a pharmaceutical composition for treating subjects allergic to the venom of an insect from the order *Hymenoptera.* Treatment regimens using gene therapy approaches to desensitisation are known in the state of the art (e.g., Ref. 28) .

[0043] The invention thus also provides a method of treating subjects allergic to the venom of an insect from the order

Hymenoptera comprising administering to a subject with such an allergy an expression vector of the invention. The expression vector can be administered directly, e.g., by intravenous, intramuscular or subcutaneous injection, gene gun or together with cells taken from the subject which were transfected ex *vivo.*

[0044] As used herein, "subject" encompasses human subjects (patients), grown-ups as well as children, and animals.

[0045] A pharmaceutical composition comprising an expression vector of the invention, and, optionally, comprising a suitable adjuvant or expedient, can be employed for this purpose. In particular, this expression vector is rich in CpG sequences and/or encodes a cytokine which shifts the balance between T_H1 and T_H2 immune responses.

[0046] Alternatively, the polypeptide of the invention is used for the preparation of a pharmaceutical composition for treating subjects allergic to the venom of an insect from the order *Hymenoptera.* The invention thus provides a method of treating subjects allergic to the venom of an insect from the order *Hymenoptera,* comprising administering a polypeptide of the invention to a subject having such an allergy.

[0047] Desensitisation approaches are well known in the state of the art. In principle, repeated treatments of allergic individuals with suitable, normally progressively increased doses of allergen diverts the immune response to one dominated by T cells that favour the production of IgG and IgA antibodies over production of IgE antibodies. The IgG and IgA antibodies are thought to desensitise the subject by binding to the small amounts of allergen normally encountered, and preventing the allergen from binding to IgE. Desensitisation to insect or bee venom is almost universally successful (Ref. 29). Different protocols and time schedules can be used, from traditional protocols, rush protocols to ultrarush protocols (e.g., Ref. 30), all of which are incorporated herein by reference. The efficacy of such protocols can be evaluated by testing the adjustment of IgE and IgG (different isotypes) and/or IgA levels in the subject's blood or by challenging the subject in a controlled manner and determining the allergic response.

[0048] The polypeptide of the invention can be administered alone or combination with other allergens, e.g. other *Hymenoptera* venom proteins or fragments thereof. In particular, combinations with bee or *Hymenoptera* venom phospholipase A2, hyaluronidase, glucosidase and/or mellitin are suitable, as this therapy induces generation of IgG/IgA antibodies to several venom allergens and can thus lead to full protection. The identified bee allergens are shown in Table 2.

Table 2: Identified bee allergens

Allergen	Common name	Size (processed)	Weight	SwissProt	Reference
Api m 1	Phospholipase A2	134 aa	15.2 kDa	P00630	Kuchler et al 1989
Api m 2	Hyaluronidase	349 aa	40.7 kDa	Q08169	Gmachl and Kreil 1993
Api m 3	Acid Phosphatase	nd	45 kDa	-	Barboni et al 1987
Api m 4	Melittin	26 aa	2.8 kDa	P01501	Vlasak ct al 1983
Api m 5	Allergen C	nd	105 kDa	-	Hoffman et al 1977
Api m 6	-	71 aa	7.5 kDa	P83563	Kettner et al 2001

[0049] The polypeptide of the invention can also be used for the preparation of a diagnostical composition for diagnosing or identifying subjects allergic to the venom of an insect from the order *Hymenoptera.* A method of diagnosing an allergy to venom of an insect from the order *Hymenoptera* is thus provided, comprising the steps of

a) contacting a subject with a polypeptide of the invention and

b) detecting an allergic reaction, wherein detecting an allergic reaction indicates said allergy.

[0050] In vivo tests for diagnosis of an allergy can easily be adapted to the polypeptide of the invention. Typically, a suitable amount of allergen is injected subcutaneously into a subject's limb, and, after a certain amount of time, the degree of localised inflammation in comparison to controls is determined (skin prick test). Such tests are well known in the art (Hamilton RG, Diagnosis of hymenoptera venom sensitivity, Current Opinion in Allergy Clin Immunol 2(4) (2002) 347-351; Poulsen LK, In vivo and in vitro techniques to determine the biological activity of food allergens, J Chromat B 756 (2001) 41-55;Schmid-Grendelmeier P, Crameri R, Recombinant allergens for skin testing, Int Arch Allergy Immunol 125 (2001) 96-111; Williams LW, Bock SA, Skin testing and food challenges in allergy and immunology practice, Clin Rev Allergy Immunol 17(3) (1999) 323-38; Barbee RA, Lebowitz MD, Thompson HC, Burrows B, Immediate skin-test reactivity in a general population sample, Ann Intern Med 84(2) (1976) 129-33).

[0051] An allergy to the venom of an insect from the order *Hymenop*tera can also be diagnosed by an *in vitro* method comprising the steps of

a) *in vitro* contacting a blood sample from a subject with a polypeptide of the invention and
b) detecting binding of IgE antibodies to the polypeptide, wherein detecting IgE antibodies binding to the polypeptide indicates said allergy.

[0052] Binding of IgE antibodies to the polypeptide can, e.g., be detected in an ELISA or by an *in vitro* release assay employing stripped mast cells and measuring the amount of released mediator, e.g., histamine. To determine specific binding, the results are compared with a specificity control, e.g., with an unrelated antibody. The diagnostic tests can in parallel be carried out to determine the levels of specific IgG (in particular IgG1 and/or IgG4) and/or IgA. For this, an ELISA with specific secondary antibodies recognising the different isotypes can be employed. Parallel testing is particularly useful for following and evaluating a course of specific immunotherapy.

[0053] For the therapeutic and diagnostic uses and methods, it is preferred to employ the fusion polypeptides of the invention, non-glycosylated proteins or polypeptides that are capable of binding to IgE from subjects allergic to venom of an insect from the order *Hymenoptera,* and comprise at least 5, preferably at least 6, 7, 8, 9, 10, 15, 20 or more amino acids of a polypeptide according to the invention selected from the group consisting of amino acid 26 to 99, 116 to 145, 153 to 158, 185 to 223, 232 to 276 or 362 to 373 of the polypeptide shown in SEQ ID NO: 2. Alternatively, the employed polypeptides are capable of binding to IgE from subjects allergic to venom of an insect from the order *Hymenoptera,* and comprise at least 5, preferably at least 6, 7, 8, 9, 10, 15, 20 or more amino acids of a polypeptide shown in SEQ ID NO: 2, except for the polypeptides from the group consisiting of amino acids 1 to 34, 63 to 80, 100 to 115, 142 to 149, 168 to 176, 224-239 and 258 to 343 of the polypeptide shown in SEQ ID NO: 2 or except for the polypeptides shown in Fig. 5. Additionally, such polypeptides consisting of amino acids 1 to 25, 146 to 152, 159 to 164, 168 to 184, 224 to 231, and 277 to 361 can be used.

[0054] The invention thus also provides a pharmaceutical or diagnostical composition comprising the polypeptide of the invention. Preferentially, the composition further comprises a suitable adjuvant and/or expedient. Optionally, the composition additionally comprises other bee or *Hymenoptera* venom polypeptides, such as phospholipase A2, hyaluronidase, glucosidase and/or mellitin.

[0055] The present invention also relates to a method of diagnosing an allergy to venom of an insect from the order *Hymenoptera,* comprising the steps of

a) performing the method of producing a polypeptide encoded by the nucleic acid of the invention, wherein the host cell comprising the expression vector of the invention is cultured under appropriate conditions for expression of said polypeptide, and wherein said polypeptide is purified,
b) contacting the polypeptide obtained by the method of step a) *in vitro* with a blood sample,
c) and detecting binding of IgE antibodies to the polypeptide, wherein detecting IgE antibodies binding to the polypeptide indicates said allergy.

[0056] Furthermore, a method of diagnosing an allergy to venom of an insect from the order *Hymenoptera* is provided, comprising the steps of

a) performing the method of producing a polypeptide encoded by the nucleic acid of the invention, wherein the host cell comprising the expression vector of the invention is cultured under appropriate conditions for expression of said polypeptide, and wherein said polypeptide is purified,
b) contacting a subject with the polypeptide obtained by the method of step a) and detecting an allergic reaction, and
c) detecting an allergic reaction, which is indicative of the allergy.

[0057] The invention also provides a method of preparing a composition for diagnosing an allergy to venom of an insect from the order *Hymenoptera* comprising the step of producing a polypeptide encoded by the nucleic acid of the invention, wherein the host cell comprising the expression vector of the invention is cultured under appropriate conditions for expression of said polypeptide ane said polypeptide is purified and can be used as such for diagnosis. Optionally, the polypeptide is further formulated with stabilizers, such as a neutral protein (e.g., BSA) or detergents to give said composition.

[0058] In another embodiment, the invention teaches a method of preparing a composition for treating subjects allergic to the venom of an insect from the order *Hymenoptera,* comprising the step of performing the method of producing a polypeptide encoded by the nucleic acid of the invention, wherein the host cell comprising the expression vector of the invention is cultured under appropriate conditions for expression of said polypeptide and said polypeptide is purified and can be used as such for therapy. Optionally, the polypeptide is further formulated with appropriate excipient and/or carriers in order to provide said composition. Correspondingly, a method of treating subjects allergic to the venom of an insect from the order *Hymenoptera* is disclosed, comprising the steps of

a) performing the method of producing a polypeptide encoded by the nucleic acid of the invention, wherein the host cell comprising the expression vector of the invention is cultured under appropriate conditions for expression of said polypeptide and said polypeptide is purified, and
b) administering the polypeptide obtained by the method of step a) to a subject having such an allergy.

[0059] The present invention thus for the first time satisfies the need for a recombinantly produced *Hymenoptera* venom acid phosphatase or the cDNA encoding this polypeptide, which can be used for diagnostic and therapeutic applications.

Examples

Example 1: Cloning of cDNA

1.1 Total RNA isolation

[0060] Total RNA was isolated from the separated stinger of a honey bee with attached venom sack and additional glands. The isolation of total RNA was performed using a kit according to the manual (peqGold TriFast™, peqlab Biotechnologie GmbH, Erlangen, Germany). The organ was weighed and homogenised in a solution containing guanidiniumisothiocyanate and phenol. Phase separation was induced by addition of chloroform. The aqueous phase was separated after centrifugation, and the containing RNA precipitated with isopropyl alcohol. After washing with diluted ethanol the RNA was dissolved in RNase-free sterile water and used directly in RT-PCR experiments. To prepare RNase-free sterile water cell-culture suitable water was treated with 0.1% (v/v) diethylpyrocarbonate (DEPC) overnight, and then autoclaved for 20 minutes to destroy DEPC by causing hydrolysis of DEPC.

1.2 cDNA first strand synthesis

[0061] Reverse transcriptase was used to synthesise first strand cDNA from the isolated RNA. For this 5 μl of total bee RNA was mixed with 2 μl (20 pmol) oligonucleotide primer and 4 μl DEPC water. An universal oligo-dT of 20 base pair length was used for the purpose of transcribing the poly-adenylated portion of mRNA in the total RNA sample. The reaction mix was incubated at 70°C for 5 minutes to break secondary structures. After this, the reaction was chilled on ice. Subsequently, 1.5 μl DEPC water, 4 μl 5x reaction buffer, 2 μl dNTP mix (10 mM), and 0.5 μl RNaseOut™ recombinant ribonuclease inhibitor (Invitrogen GmbH, Karlsruhe, Germany) were added. The reaction mix was incubated at 37°C for 5 minutes. Then 1 μl (200 units) RevertAid™ M-MuLV Reverse Transcriptase (RT, Fermentas GmbH, St. Leon-Rot, Germany) was added and the reaction was incubated at 42°C for 60 minutes. After this the reaction was stopped by heating to 70°C for 10 minutes and chilled on ice.

1.3 RT-PCR

[0062] First strand cDNA from bee venom gland tissue was used as template for PCR amplification of Api m3 DNA sequences.

[0063] Known peptide fragments, public databases and bioinformatics were used to design the specific primers for Api m3. These primers have been designed to allow 5' -end blunt subcloning for native N-terminal expression and 3' -end directed Sac II restriction site subcloning. The nucleotide sequences of the oligonucleotides are:

Api m3 for, 21mer, blunt end (SEQ ID NO:3):

5' -GAA CTT AAA CAA ATA AAT GTG

Api m3 back 32mer, Sac II site (SEQ ID NO:4):

5' -AAC CGC GGT TAC TTA CTT ATT CTC AGT ACC CG.

[0064] The PCR reaction contained 41 μl DEPC water, 5 μl 10x complete Pfu PCR buffer, 1 μl Api m3 for primer (100 pmol), 1 μl Api m3 back primer (100 pmol), 1 μl dNTP mix (10 mM), 0.5 μl bee venom gland tissue cDNA, and 0.5 μl recombinant Pfu DNA polymerase (Fermentas GmbH, St. Leon-Rot, Germany), to give a total reaction volume of 50 μl.

[0065] The PCR temperature program for amplification was:

Step 1: 96°C, 1 minute
Step 2: 95°C, 30 seconds
Step 3: 55°C, 30 seconds
Step 4: 72°C, 2 minutes

Repeat steps 2-4 x29 times
Step 5: 72°C, 10 minutes
Step 6: 4°C, until end

[0066] Part of the PCR reaction was run on a 1% agarose (peqGOLD universal agarose, peqlab GmbH, Erlangen, Germany) gel in 0.5x TAE buffer and amplified DNA products visualised with ethidium bromide and UV illumination. A band at the expected size was visible.

1.4 Subcloning for sequencing

[0067] DNA from the PCR reaction was isolated using the QIAEX II gel extraction kit (Qiagen GmbH, Hilden, Germany). Subcloning for sequencing was done using the TOPO TA Cloning® Kit (Invitrogen GmbH, Karlsruhe, Germany) with pCR®2.1-TOPO® vector according to the manual. Due to use of Pfu DNA polymerase an initial TA-elongation reaction step with AGS Gold Taq DNA Polymerase (AGS Hybaid, Heidelberg) was introduced. The ligated DNA was transformed into E. coli of the strain TG1 by electroporation (2 mm cuvettes, EasyJect+, Hybaid, Heidelberg, Germany) and selected on ampicillin agar plates.

1.5 Sequencing

[0068] The sequencing reaction was done with BigDye® Terminator Cycle Sequencing Kit from ABI (Applied Biosystems Applera Deutschland GmbH, Darmstadt, Germany) according to the manual. 25 cycles were run with a 30 seconds denaturation step at 96°C, 15 seconds annealing step at 50°C, and 4 minutes elongation step at 57°C. Sequencing primer were:

M13/Uni for (SEQ ID NO:5): 5' -GTA AAA CGA CGG CCA GTG CCA A
M13/Uni rev (SEQ ID NO:6): 5' -CAG GAA ACA GCT ATG ACC ATG A

[0069] The resulting sequence is shown in Fig. 1.

Example 2: Construction of expression vector

2.1 Modification of the insect expression vector

[0070] For expression of recombinant Api m 3 with potential for native folding and posttranslational modification, the expression in insect cells was chosen. The expression vector pIB/V5-His. (Invitrogen GmbH, Karlsruhe, Germany) was modified to facilitate isolation and purification. A melittin signal sequence for secretion of the recombinant protein was added and the Kozak sequence was optimised for higher expression rates in insect cells. The melittin signal sequence was amplified from total bee RNA, synthesised as described above, using the primers:

melt leader for (SEQ ID NO:7):

5' -GGA AAG CTT TCC GCC ATG GCG AAA TTC TTA GTC

melt leader back (SEQ ID NO:8):

5' -CGG GAT CCC GCA TAG ATG TAA GAA ATG.

[0071] Underlined are the Hind III and, respectively, BamH I restriction sites in the corresponding primer. The sequence containing the 10x histidine-tag and factor Xa cleavage site has been cloned between the BamH I and EcoR V site of

the parent vector. As first template, a tag containing vector was used with the following primers:

10xHis for (SEQ ID NO:9):

5' -CTG AAT AGC GCC GGA TCC GAC CAT

10xHis back (SEQ ID NO:10):

5' -CCC TCT AGA CTC GAG CCA ATG ATG

[0072] Underlined are the bases for the introduction of the BamH I restriction site. The resulting fragment was used as second template and further modified to contain a EcoR V site at the 3' -end by use of overlapping primers and PCR extension of the sequence (splice-overlap-extension, SOE). The extension primer used was:

SOE Xa (SEQ ID NO:11):

5'-GGG ATA TCC CTT CCC TCG ATC CCT CTA GAC TC

[0073] Underlined is the newly introduced EcoR V restriction site for cloning and generation of the expression vector construct. For all PCR steps Pfu DNA polymerase (Fermentas GmbH, St. Leon-Rot, Germany) was used with standard reaction conditions. The annealing temperature was 55°C for the 10xHistidin fragment amplification and 45°C for the SOE reaction.

2.2 Re-PCR and subcloning

[0074] After sequencing of selected subcloned cDNA clones and verification of the sequence, the clone was used for secondary amplification with Pfu DNA polymerase. The PCR product was subcloned into the EcoR V / Sac II digested expression vector after restriction digest with Sac II.

2.3 Modification of the bacterial expression vector

[0075] The verified mammalian expression vector pIB/Mel opt-H10 was used as template for the construction of insert for subcloning into the prokaryontic expression vector pET26(+) (Novagen). The PCR program was done according to the temperature gradient given in 1.3. Pfu polymerase was used with the primers:

Api 3 for pro-his (SEQ ID NO: 12)	AGAATTTCATATGAAATTCTTAGTCAACG
Api 3 back pro (SEQ ID NO:13)	AAGAGCTCTTACTTACTTATTCTCAG

[0076] The amplicon was digested with Sac I and Nde I. The partly digested fragment of correct size was isolated and ligated into the pre-digested vector.

Example 3: Expression of recombinant protein

3.1 Transfection

[0077] HighFive insect cell (Invitrogen GmbH, Karlsruhe, Germany) were used as hosts for the recombinant expression of Api m 3. DNA was purified from bacterial cultures using the E.Z.N.A. Plasmid Miniprep Kit II (peqlab GmbH, Erlangen, Germany) according to the manual. For transfection of purified DNA into cells the reagent Cellfectin® (Invitrogen GmbH, Karlsruhe, Germany) was used according to the manual.

3.2 Transformation

[0078] Vectors have been transformed into prokaryontic cell by electroporation. Cells have been prepared by standard procedures. Electroporation was done with an EasyJecT+ instrument (EquiBio, Maidstone, UK) with standard settings according to the manual of the manufacturer.

3.3 Isolation of recombinant protein

[0079] The protein was purified according to standard procedures.

[0080] In brief, cells were disrupted by sonication. Cell membranes etc. were sedimented by ultracentrifugation. Protein, including the acid phosphatase, was precipitated by ammonium sulphate at a concentration of 45%. The His-tagged protein was then purified from the extract by Ni^{2+} affinity chromatography following the manufacturer's recommendations (e.g., His Trap™ HP Kit, Amersham Biosciences). Purification was controlled by SDS-PAGE.

3.4 Test for acid phosphatase activity.

[0081] Enzymatic activity of the enzyme was confirmed according to the method described in Ref. 19.

Figur L g nds:

[0082]

Fig. 1 shows the nucleotide sequence of the isolated cDNA for Api m 3 in FASTA format (SEQ ID NO: 1).

Fig. 2 shows the predicted restriction enzyme pattern of the isolated cDNA for Api m 3.

Fig. 3 shows the predicted translated amino acid sequence of the isolated cDNA for Api m 3 (SEQ ID NO:2). The underlined peptides can be aligned to prior published fragments. See figures 5 and 6.

Fig. 4A shows a vector map of a preferred insect cell expression vector, pIB/Mel opt-H10 api m 3. The vector was modified to include a N-terminal 10xhistidine-tag, cleavable with factor Xa protease as well as the signal sequence of bee melittin for secreted expression. The gene of interest was cloned between the EcoR V and Sac II site. The gene comprises a stop codon at the 3' -end. The expressed protein should be secreted and will have a factor Xa cleavable 10x histidine-tag at the N-terminus.

Fig. 4B shows optimisation of the Kozak sequence for insect cell expression. The former sequence a) was changed into b) to be in accordance with the preferred translation initial sequence (G/A)NNATGG adding an alanine to the N-terminal sequence.

Fig. 4C shows a vector map of a preferred bacterial expression vector, pET26(+) api 3 pro. The vector was modified to contain the gene of interest between the Sac I and Nde I site. The protein sequence was taken from the verified mammalian expression vector pIB/Mel opt-H10 api m 3.

Fig. 5A shows the sequence information of potential peptide fragments of acid phosphatase publicly known prior to this invention. Peptide fragments are listed in order of alignment to human and rat prostate phosphatase as published in Ref. 20. The alignment order of fragments to derived sequence is given in the second column. Positions of aligned peptide segments can be taken from Fig. 3 and Fig 5B, as well as Fig 6. Highlighted sequence segments in the third column show amino acids present in the Api m 3 sequence. The forth column shows the length of the published peptide fragments.

Fig. 5B shows the corrected alignment of peptides originally postulated by Hoffmann et al. (Ref. 20)to Api m 3.

Fig. 6 shows a schematic alignment of peptides postulated by Hoffman et al. (Ref. 20) (A), in comparison to the corrected oder after cloning and sequencing of the Api m 3 gene (B). It is obvious that the alignment differs from the published alignment with human and rat prostate phosphatase (Ref. 20). The published peptide fragments can not be aligned to match the sequence as would be expected. Firstly, the order of alignment positions is different from the publication. Secondly, some fragments, like fragments 1 and 7 partially align at different sites in the sequence, and therefore are not continuous peptides derived from a cDNA sequence. Furthermore, some published fragment sequences, like fragment 5, cannot be aligned at all. The scheme also shows the leader peptide and is not exact regarding the number of amino acids.

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[0083]

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Claims

1. Nucleic acid encoding a polypeptide capable of binding to IgE from subjects allergic to venom of an insect from the order *Hymenoptera,* wherein the polypeptide has the amino acid sequence of SEQ ID NO: 2.

2. Nucleic acid of claim 1 having the nucleotide sequence of SEQ ID NO: 1.

3. Nucleic acid encoding a polypeptide capable of binding to IgE from subjects allergic to venom of an insect from the order Hymenoptera, wherein the encoded polypeptide has the amino acid sequence of SEQ ID No: 2 in which the glycosylation sites are mutated.

4. Nucleic acid of claims 1 to 3, wherein the insect is a bee from the genus Apis.

5. Nucleic acid of claim 4, wherein the bee is *Apis mellifera.*

6. Polypeptide comprising the polypeptide encoded by a nucleic acid of claims 1 to 3 linked to an additional polypeptide as a fusion protein.

7. Polypeptide of claim 6, wherein the protein is non-glycosylated.

8. Polypeptide encoded by a nucleic acid of claims 1 or 2, wherein the protein is expressed in insect cells.

9. Polypeptide of claims 6 to 8 having acid phosphatase activity.

10. Polypeptide of SEQ ID NO: 2, comprising mutated glycosylation sites instead of glycosylation sites.

11. Polypeptide of claim 6, wherein the additional polypeptide is selected from the group comprising a poly-Histidine tag, glutathione-Stransferase, β-galactosidase, a cytokine, an IgG-Fc or another *Hymenoptera* venom protein or

antigenic fragment thereof.

12. Expression vector comprising a nucleic acid of claims 1 to 3 operationally linked to an expression control sequence.

13. Expression vector of claim 12, wherein the nucleic acid of claims 1 to 3 is linked in frame to a nucleic acid encoding an additional polypeptide.

14. Expression vector of claim 12, wherein the additional polypeptide is selected from the group comprising a poly-Histidine tag, glutathione-Stransferase, β-galactosidase, a cytokine, an IgG-Fc or another *Hymenoptera* venom protein or antigenic fragment thereof.

15. Expression vector of claims 12 to 14, wherein the vector is suitable for expression in bacterial or insect cells.

16. Host cell comprising the expression vector-of claims 12 to 15.

17. Host cell of claim 16, wherein the cell is an insect cell or a bacterial cell.

18. Method of producing a polypeptide encoded by the nucleic acid of claims 1 to 3, wherein the host cell of claims 16 or 17 is cultured under appropriate conditions for expression of said polypeptide and said polypeptide is purified.

19. Expression vector of claims 11 to 15 for use in treating subjects allergic to the venom of an insect from the order *Hymenoptera.*

20. Pharmaceutical composition comprising an expression vector of claims 11 to 15.

21. Polypeptide of claims 6 to 11
or
polypeptide encoded by a nucleic acid, which is a fragment having a length of more than 255 nucleotides of the nucleic acid of claims 1 to 5, and which encodes a polypeptide capable of binding to IgE from subjects allergic to venom of an insect from the order *Hymenoptera,*
or
polypeptide encoded by a nucleic acid comprising at least 15 contiguous nucleotides of nucleotides 78 to 299, 348 to 437, 459 to 476, 555 to 671, 696 to 830 or 1086 to 1121 of the nucleic acid of claims 1 or 2, wherein the polypeptide encoded by the nucleic acid is capable of binding to IgE from subjects allergic to venom of an insect from the order *Hymenoptera,*
or
polypeptide encoded by a nucleic acid comprising at least 15 contiguous nucleotides of the nucleic acid of claims 1 or 2, except for the nucleotides encoding polypeptides from the group consisting of amino acids 1 to 34, 63 to 80, 100 to 115, 142 to 149, 168 to 176, 224-239 and 258 to 343 of the polypeptide shown in SEQ ID NO: 2, wherein the polypeptide encoded by the nucleic acid is capable of binding to IgE from subjects allergic to venom of an insect from the order *Hymenoptera,*
for use in treating subjects allergic to the venom of an insect from the order *Hymenoptera.*

22. Polypeptide of claims 6 to 11
or
polypeptide encoded by a nucleic acid, which is a fragment having a length of more than 255 nucleotides of the nucleic acid of claims 1 to 5, and which encodes a polypeptide capable of binding to IgE from subjects allergic to venom of an insect from the order *Hymenoptera,*
or
polypeptide encoded by a nucleic acid comprising at least 15 contiguous nucleotides of nucleotides 78 to 299, 348 to 437, 459 to 476, 555 to 671, 696 to 830 or 1086 to 1121 of the nucleic acid of claims 1 or 2, wherein the polypeptide encoded by the nucleic acid is capable of binding to IgE from subjects allergic to venom of an insect from the order *Hymenoptera,*
or
polypeptide encoded by a nucleic acid comprising at least 15 contiguous nucleotides of the nucleic acid of claims 1 or 2, except for the nucleotides encoding polypeptides from the group consisting of amino acids 1 to 34, 63 to 80, 100 to 115, 142 to 149, 168 to 176, 224-239 and 258 to 343 of the polypeptide shown in SEQ ID NO: 2, wherein the polypeptide encoded by the nucleic acid is capable of binding to IgE from subjects allergic to venom of an insect

from the order *Hymenoptera,*
for use in *in-vivo* diagnosing of subjects allergic to the venom of an insect from the order *Hymenoptera.*

23. Use of a polypeptide of claims 6 to 11
or
of a polypeptide encoded by a nucleic acid, which is a fragment having a length of more than 255 nucleotides of the nucleic acid of claims 1 to 5, and which encodes a polypeptide capable of binding to IgE from subjects allergic to venom of an insect from the order *Hymenoptera,*
or
of a polypeptide encoded by a nucleic acid comprising at least 15 contiguous nucleotides of nucleotides 78 to 299, 348 to 437, 459 to 476, 555 to 671, 696 to 830 or 1086 to 1121 of the nucleic acid of claims 1 or 2, wherein the polypeptide encoded by the nucleic acid is capable of binding to IgE from subjects allergic to venom of an insect from the order *Hymenoptera,*
or
of a polypeptide encoded by a nucleic acid comprising at least 15 contiguous nucleotides of the nucleic acid of claims 1 or 2, except for the nucleotides encoding polypeptides from the group consisting of amino acids 1 to 34, 63 to 80, 100 to 115, 142 to 149, 168 to 176, 224-239 and 258 to 343 of the polypeptide shown in SEQ ID NO: 2, wherein the polypeptide encoded by the nucleic acid is capable of binding to IgE from subjects allergic to venom of an insect from the order *Hymenoptera,*
for *ex-vivo* diagnosing of subjects allergic to the venom of an insect from the order *Hymenoptera.*

24. Method of diagnosing an allergy to the venom of an insect from the order *Hymenoptera,* comprising the steps of

a) *in vitro* contacting a blood sample from a subject with a polypeptide of claims 6 to 11, and
b) detecting binding of IgE antibodies to the polypeptide, wherein detecting IgE antibodies binding to the polypeptide indicates said allergy.

25. Pharmaceutical or diagnostical composition comprising a polypeptide of claims 6 to 11.

26. Composition of claims 20 or 25, further comprising a suitable adjuvant and/or expedient and/or further polypeptides from the venom of an insect from the order *Hymenoptera.*

27. Method of diagnosing an allergy to venom of an insect from the order *Hymenoptera,* comprising the steps of

a) contacting a polypeptide of claims 6 to 11 *in-vitro* with a blood sample from a subject, and
c) detecting binding of IgE antibodies to the polypeptide, wherein detecting IgE antibodies binding to the polypeptide indicates said allergy.

28. Method of preparing the composition of claim 25 comprising the step of producing a polypeptide encoded by the nucleic acids of claims 1 to 3, wherein the host cell of claims 16 or 17 is cultured under appropriate conditions for expression of said polypeptide and said polypeptide is purified.

Patentansprüche

1. Nukleinsäure, codierend für ein Polypeptid, das in der Lage ist, an IgE von Personen zu binden, die allergisch gegen das Gift eines Insekts der Ordnung *Hymenoptera* sind, wobei das Polypeptid die Aminosäuresequenz von SEQ ID NO: 2 aufweist.

2. Nukleinsäure nach Anspruch 1, die die Nukleotidsequenz von SEQ ID NO: 1 aufweist.

3. Nukleinsäure, codierend für ein Polypeptid, das in der Lage ist, an IgE von Personen zu binden, die allergisch gegen das Gift eines Insekts der Ordnung *Hymenoptera* sind, wobei das codierte Polypeptid die Aminosäuresequenz von SEQ ID NO: 2 aufweist, in der die Glykosylierungsstellen mutiert sind.

4. Nukleinsäure nach den Ansprüchen 1 bis 3, wobei das Insekt eine Biene der Gattung *Apis* ist.

5. Nukleinsäure nach Anspruch 4, wobei die Biene *Apis mellifera* ist.

6. Polypeptid, umfassend das durch eine Nukleinsäure nach den Ansprüchen 1 bis 3 codierte Polypeptid, verknüpft mit einem weiteren Polypeptid als Fusionsprotein.

7. Polypeptid nach Anspruch 6, wobei das Protein nichtglykosyliert ist.

8. Polypeptid, codiert durch eine Nukleinsäure nach den Ansprüchen 1 oder 2, wobei das Protein in Insektenzellen exprimiert ist.

9. Polypeptid nach den Ansprüchen 6 bis 8, das eine saure Phosphatase-Aktivität aufweist.

10. Polypeptid von SEQ ID NO: 2, umfassend mutierte Glykosylierungsstellen anstelle von Glykosylierungsstellen.

11. Polypeptid nach Anspruch 6, wobei das weitere Polypeptid ausgewählt ist aus der Gruppe umfassend ein Polyhistidin-Tag, Glutathion-S-Transferase, β-Galactosidase, ein Cytokin, ein IgG-Fc oder ein anderes Hymenoptera-Gift-Protein oder ein antigenes Fragment davon.

12. Expressionsvektor, umfassend eine Nukleinsäure nach den Ansprüchen 1 bis 3, funktionell verknüpft mit einer Expressionskontrollsequenz.

13. Expressionsvektor nach Anspruch 12, wobei die Nukleinsäure nach den Ansprüchen 1 bis 3 im Leserahmen mit einer ein weiteres Polypeptid codierenden Nukleinsäure verknüpft ist.

14. Expressionsvektor nach Anspruch 12, wobei das weitere Polypeptid ausgewählt ist aus der Gruppe umfassend ein Polyhistidin-Tag, Glutathion-S-Transferase, β-Galactosidase, ein Cytokin, ein IgG-Fc oder ein anderes *Hymenoptera-Gift-Protein* oder ein antigenes Fragment davon.

15. Expressionsvektor nach den Ansprüchen 12 bis 14, wobei der Vektor für die Expression in Bakterien-oder Insektenzellen geeignet ist.

16. Wirtszelle, die den Expressionsvektor nach den Ansprüchen 12 bis 15 umfasst.

17. Wirtszelle nach Anspruch 16, wobei die Zelle eine Insektenzelle oder eine Bakterienzelle ist.

18. Verfahren zur Herstellung eines von der Nukleinsäure nach den Ansprüchen 1 bis 3 codierten Polypeptids, wobei die Wirtszelle nach den Ansprüchen 16 oder 17 unter für die Expression des Polypeptids geeigneten Bedingungen kultiviert wird, und das Polypeptid gereinigt wird.

19. Expressionsvektor nach den Ansprüchen 11 bis 15 zur Verwendung beim Behandeln von Personen, die gegen das Gift eines Insekts der Ordnung *Hymenoptera* allergisch sind.

20. Pharmazeutische Zusammensetzung, umfassend einen Expressionsvektor nach den Ansprüchen 11 bis 15.

21. Polypeptid nach Anspruch 6 bis 11 oder
von einer Nukleinsäure codiertes Polypeptid, das ein Fragment mit einer Länge von mehr als 255 Nukleotiden der Nukleinsäure nach den Ansprüchen 1 bis 5 ist und das für ein Polypeptid codiert, das in der Lage ist, an IgE von Personen zu binden, die allergisch gegen Gift eines Insekts der Ordnung *Hymenoptera* sind, oder
von einer Nukleinsäure codiertes Polypeptid, das mindestens 15 zusammenhängende Nukleotide der Nukleotide 78 bis 299, 348 bis 437, 459 bis 476, 555 bis 671, 696 bis 830 oder 1086 bis 1121 der Nukleinsäure nach den Ansprüchen 1 oder 2 umfasst, wobei das von der Nukleinsäure codierte Polypeptid in der Lage ist, an IgE von Personen zu binden, die allergisch gegen. Gift eines Insekts der Ordnung *Hymenoptera* sind, oder
von einer Nukleinsäure codiertes Polypeptid, das mindestens 15 zusammenhängende Nukleotide der Nukleinsäure nach den Ansprüchen 1 oder 2 umfasst, außer den Nukleotiden, die für Polypeptide aus der Gruppe bestehend aus den Aminosäuren 1 bis 34, 63 bis 80, 100 bis 115, 142 bis 149, 168 bis 176, 224 bis 239 und 258 bis 343 des in SEQ ID NO: 2 gezeigten Polypeptids codieren, wobei das von der Nukleinsäure codierte Polypeptid in der Lage ist, an IgE von Personen zu binden, die allergisch gegen Gift eines Insekts der Ordnung *Hymenoptera* sind,
zur Verwendung beim Behandeln von Personen, die gegen das Gift eines Insekts der Ordnung *Hymenoptera* allergisch sind.

22. Polypeptid nach Anspruch 6 bis 11 oder
von einer Nukleinsäure codiertes Polypeptid, das ein Fragment mit einer Länge von mehr als 255 Nukleotiden der Nukleinsäure nach den Ansprüchen 1 bis 5 ist und das für ein Polypeptid codiert, das in der Lage ist, an IgE von Personen zu binden, die allergisch gegen Gift eines Insekts der Ordnung *Hymenoptera* sind, oder
von einer Nukleinsäure codiertes Polypeptid, das mindestens 15 zusammenhängende Nukleotide der Nukleotide 78 bis 299, 348 bis 437, 459 bis 476, 555 bis 671, 696 bis 830 oder 1086 bis 1121 der Nukleinsäure nach den Ansprüchen 1 oder 2 umfasst, wobei das von der Nukleinsäure codierte Polypeptid in der Lage ist, an IgE von Personen zu binden, die allergisch gegen Gift eines Insekts der Ordnung *Hymenoptera* sind, oder
von einer Nukleinsäure codiertes Polypeptid, das mindestens 15 zusammenhängende Nukleotide der Nukleinsäure nach den Ansprüchen 1 oder 2 umfasst, außer den Nukleotiden, die für Polypeptide aus der Gruppe bestehend aus den Aminosäuren 1 bis 34, 63 bis 80, 100 bis 115, 142 bis 149, 168 bis 176, 224 bis 239 und 258 bis 343 des in SEQ ID NO: 2 gezeigten Polypeptids codieren, wobei das von der Nukleinsäure codierte Polypeptid in der Lage ist, an IgE von Personen zu binden, die allergisch gegen Gift eines Insekts der Ordnung *Hymenoptera* sind,
zur Verwendung bei der *In-vivo*-Diagnose von Personen, die gegen das Gift eines Insekts der Ordnung *Hymenoptera* allergisch sind.

23. Verwendung eines Polypeptids nach Anspruch 6 bis 11 oder
eines von einer Nukleinsäure codierten Polypeptids, das ein Fragment mit einer Länge von mehr als 255 Nukleotiden der Nukleinsäure nach den Ansprüchen 1 bis 5 ist und das für ein Polypeptid codiert, das in der Lage ist, an IgE von Personen zu binden, die allergisch gegen Gift eines Insekts der Ordnung *Hymenoptera* sind, oder
eines von einer Nukleinsäure codierten Polypeptids, das mindestens 15 zusammenhängende Nukleotide der Nukleotide 78 bis 299, 348 bis 437, 459 bis 476, 555 bis 671, 696 bis 830 oder 1086 bis 1121 der Nukleinsäure nach den Ansprüchen 1 oder 2 umfasst, wobei das von der Nukleinsäure codierte Polypeptid in der Lage ist, an IgE von Personen zu binden, die allergisch gegen Gift eines Insekts der Ordnung *Hymenoptera* sind, oder
eines von einer Nukleinsäure codierten Polypeptids, das mindestens 15 zusammenhängende Nukleotide der Nukleinsäure nach den Ansprüchen 1 oder 2 umfasst, außer den Nukleotiden, die für Polypeptide aus der Gruppe bestehend aus den Aminosäuren 1 bis 34, 63 bis 80, 100 bis 115, 142 bis 149, 168 bis 176, 224 bis 239 und 258 bis 343 des in SEQ ID NO: 2 gezeigten Polypeptids codieren, wobei das von der Nukleinsäure codierte Polypeptid in der Lage ist, an IgE von Personen zu binden, die allergisch gegen Gift eines Insekts der Ordnung *Hymenoptera* sind,
für die *Ex-vivo*-Diagnose von Personen, die gegen das Gift eines Insekts der Ordnung *Hymenoptera* allergisch sind.

24. Verfahren zur Diagnose einer Allergie gegen das Gift eines Insekts der Ordnung *Hymenoptera,* das die folgenden Schritte umfasst

a) *In-vitro*-Kontaktieren einer Blutprobe einer Person mit einem Polypeptid nach den Ansprüchen 6 bis 11 und
b) Erfassen der Bindung von IgE-Antikörpern an das Polypeptid, wobei das Erfassen von an das Polypeptid bindenden IgE-Antikörpern die Allergie anzeigt.

25. Pharmazeutische oder diagnostische Zusammensetzung, umfassend ein Polypeptid nach den Ansprüchen 6 bis 11.

26. Zusammensetzung nach den Ansprüchen 20 oder 25, ferner umfassend ein geeignetes Adjuvans und/oder Hilfsmittel und/oder weitere Polypeptide aus dem Gift eines Insekts der Ordnung *Hymenoptera.*

27. Verfahren zur Diagnose einer Allergie gegen Gift eines Insekts der Ordnung *Hymenoptera,* umfassend die folgenden Schritte:

a) *In-vitro*-Kontaktieren eines Polypeptids nach den Ansprüchen 6 bis 11 mit einer Blutprobe einer Person und
c) Erfassen der Bindung von IgE-Antikörpern an das Polypeptid, wobei das Erfassen von an das Polypeptid bindenden IgE-Antikörpern die Allergie anzeigt.

28. Verfahren zum Anfertigen der Zusammensetzung nach Anspruch 25, umfassend den Schritt des Herstellens eines von der Nukleinsäure nach den Ansprüchen 1 bis 3 codierten Polypeptids, wobei die Wirtszelle nach den Ansprüchen 16 oder 17 unter für die Expression des Polypeptids geeigneten Bedingungen kultiviert wird, und das Polypeptid gereinigt wird.

Revendications

1. Acide nucléique codant pour un polypeptide capable de se lier à IgE à partir de sujets humains allergiques au venin d'un insecte de l'ordre hyménoptères, dans lequel le polypeptide a la séquence en acides aminés de SEQ ID NO: 2.

2. Acide nucléique selon la revendication 1, ayant la séquence nucléotidique de SEQ ID NO: 1.

3. Acide nucléique codant pour un polypeptide capable de se lier à IgE à partir de sujets humains allergiques au venin d'un insecte de l'ordre hyménoptères, dans lequel le polypeptide codé a la séquence en acides aminés de SEQ ID NO: 2, dans laquelle les sites de glycosylation sont mutés.

4. Acide nucléique selon les revendications 1 à 3, dans lequel l'insecte est une abeille du genre *Apis.*

5. Acide nucléique selon la revendication 4, dans lequel l'abeille est *Apis mellifera.*

6. Polypeptide comprenant le polypeptide codé par un acide nucléique selon les revendications 1 à 3 lié à un polypeptide additionnel comme protéine de fusion.

7. Polypeptide selon la revendication 6, dans lequel la protéine est non-glycosylée.

8. Polypeptide codé par un acide nucléique selon les revendications 1 ou 2, dans lequel la protéine est exprimée dans des cellules d'insecte.

9. Polypeptide selon les revendications 6 à 8 ayant une activité de phosphatase acide.

10. Polypeptide de SEQ ID NO: 2, comprenant des sites de glycosylation mutés à la place de sites de glycosylation.

11. Polypeptide selon la revendication 6, dans lequel le polypeptide additionnel est choisi à partir du groupe comprenant une étiquette poly-histidine, glutathion S-transférase, β-galactosidase, un cytokine, un IgG-Fc ou une protéine de venin d'hyménoptères ou un fragment antigénique de celle-ci.

12. Vecteur d'expression comprenant un acide nucléique selon les revendications 1 à 3 fonctionnellement lié à une séquence de contrôle d'expression.

13. Vecteur d'expression selon la revendication 12, dans lequel l'acide nucléique selon les revendications 1 à 3 est lié dans le cadre de lecture à un acide nucléique codant pour un polypeptide additionnel.

14. Vecteur d'expression selon la revendication 12, dans lequel le polypeptide additionnel est choisi à partir du groupe comprenant une étiquette poly-histidine, glutathion S-transférase, β-galactosidase, une cytokine, un IgG-Fc ou une protéine de venin d'hyménoptères ou un fragment antigénique de celle-ci.

15. Vecteur d'expression selon les revendications 12 à 14, dans lequel le vecteur est approprié pour l'expression dans des cellules de bactéries ou d'insectes.

16. Cellule d'hôte comprenant le vecteur d'expression selon les revendications 12 à 15.

17. Cellule d'hôte selon la revendication 16, dans lequel la cellule est une cellule d'insectes ou une cellule de bactéries.

18. Procédé de production d'un polypeptide codé par l'acide nucléique selon les revendications 1 à 3, dans lequel on cultive la cellule d'hôte selon les revendications 16 ou 17 sous des conditions appropriées pour l'expression de ce polypeptide, et ce polypeptide est purifié.

19. Vecteur d'expression selon les revendications 11 à 15 pour l'utilisation dans le traitement de sujets humains allergiques au venin d'un insecte de l'ordre hyménoptères.

20. Composition pharmaceutique comprenant un vecteur d'expression selon les revendications 11 à 15.

21. Polypeptide selon la revendication 6 à 11 ou polypeptide codé par un acide nucléique, qui est un fragment ayant

une longueur de plus de 255 nucléotides de l'acide nucléique selon les revendications 1 à 5, et qui code pour un polypeptide capable de se lier à IgE à partir de sujets humains allergiques au venin d'un insecte de l'ordre hyménoptères, ou
polypeptide codé par un acide nucléique comprenant au moins 15 nucléotides contiguës des nucléotides 78 à 299, 348 à 437, 459 à 476, 555 à 671, 696 à 830 ou 1086 à 1121 de l'acide nucléique selon les revendications 1 ou 2, dans lequel le polypeptide codé par l'acide nucléique est capable de se lier à IgE à partir de sujets humains allergiques au venin d'un insecte de l'ordre hyménoptères, ou
polypeptide codé par un acide nucléique comprenant au moins 15 nucléotides contiguës de l'acide nucléique selon les revendications 1 ou 2, à l'exception des nucléotides codant pour des polypeptides à partir du groupe consistant en les acides aminés 1 à 34, 63 à 80, 100 à 115, 142 à 149, 168 à 176, 224 à 239 et 258 à 343 du polypeptide montré dans la SEQ ID NO: 2, dans lequel le polypeptide codé par l'acide nucléique est capable de se lier à IgE à partir de sujets humains allergiques au venin d'un insecte de l'ordre hyménoptères,
pour l'utilisation dans le traitement de sujets humains allergiques au venin d'un insecte de l'ordre hyménoptères.

22. Polypeptide selon la revendication 6 à 11 ou
polypeptide codé par un acide nucléique, qui est un fragment ayant une longueur de plus de 255 nucléotides de l'acide nucléique selon les revendications 1 à 5, et qui code pour un polypeptide capable de se lier à IgE à partir de sujets humains allergiques au venin d'un insecte de l'ordre hyménoptères, ou
polypeptide codé par un acide nucléique comprenant au moins 15 nucléotides contiguës des nucléotides 78 à 299, 348 à 437, 459 à 476, 555 à 671, 696 à 830 ou 1086 à 1121 de l'acide nucléique selon les revendications 1 ou 2, dans lequel le polypeptide codé par l'acide nucléique est capable de se lier à IgE à partir de sujets humains allergiques au venin d'un insecte de l'ordre hyménoptères, ou
polypeptide codé par un acide nucléique comprenant au moins 15 nucléotides contiguës de l'acide nucléique selon les revendications 1 ou 2, à l'exception des nucléotides codant pour des polypeptides à partir du groupe consistant en les acides aminés 1 à 34, 63 à 80, 100 à 115, 142 à 149, 168 à 176, 224 à 239 et 258 à 343 du polypeptide montré dans la SEQ ID NO: 2, dans lequel le polypeptide codé par l'acide nucléique est capable de se lier à IgE à partir de sujets humains allergiques au venin d'un insecte de l'ordre hyménoptères,
pour l'utilisation dans le diagnostic *in-vivo* de sujets humains allergiques au venin d'un insecte de l'ordre hyménoptères.

23. Utilisation d'un polypeptide selon la revendication 6 à 11 ou
d'un polypeptide codé par un acide nucléique, qui est un fragment ayant une longueur de plus de 255 nucléotides de l'acide nucléique selon les revendications 1 à 5, et qui code pour un polypeptide capable de se lier à IgE à partir de sujets humains allergiques au venin d'un insecte de l'ordre hyménoptères, ou
d'un polypeptide codé par un acide nucléique comprenant au moins 15 nucléotides contiguës des nucléotides 78 à 299, 348 à 437, 459 à 476, 555 à 671, 696 à 830 ou 1086 à 1121 de l'acide nucléique selon les revendications 1 ou 2, dans lequel le polypeptide codé par l'acide nucléique est capable de se lier à IgE à partir de sujets humains allergiques au venin d'un insecte de l'ordre hyménoptères, ou
d'un polypeptide codé par un acide nucléique comprenant au moins 15 nucléotides contiguës de l'acide nucléique selon les revendications 1 ou 2, à l'exception des nucléotides codant pour des polypeptides à partir du groupe consistant en les acides aminés 1 à 34, 63 à 80, 100 à 115, 142 à 149, 168 à 176, 224 à 239 et 258 à 343 du polypeptide montré dans la SEQ ID NO: 2, dans lequel le polypeptide codé par l'acide nucléique est capable de se lier à IgE à partir de sujets humains allergiques au venin d'un insecte de l'ordre hyménoptères,
pour le diagnostic *ex-vivo* de sujets humains allergiques au venin d'un insecte de l'ordre hyménoptères.

24. Procédé de diagnostic d'une allergie au venin d'un insecte de l'ordre hyménoptères, comprenant les étapes de

a) contacter *in-vitro* un échantillon de sang à partir d'un sujet humain avec un polypeptide selon les revendications 6 à 11 et
b) détecter le liage d'anticorps IgE au polypeptide, dans lequel la détection d'anticorps IgE se liant au polypeptide indique cette allergie.

25. Composition pharmaceutique ou diagnostique comprenant un polypeptide selon les revendications 6 à 11.

26. Composition selon les revendications 20 ou 25, en outre comprenant un adjuvant et/ou expédient approprié et/ou d'autres polypeptides à partir du venin d'un insecte de l'ordre hyménoptères.

27. Procédé de diagnostic d'une allergie au venin d'un insecte de l'ordre hyménoptères, comprenant les étapes de

a) contacter *in-vitro* un polypeptide selon les revendications 6 à 11 avec un échantillon de sang à partir d'un sujet humain et
b) détecter le liage d'anticorps IgE au polypeptide, dans lequel la détection d'anticorps IgE se liant au polypeptide indique cette allergie.

28. Procédé de préparation de la composition selon la revendication 25 comprenant l'étape de production d'un polypeptide codé par l'acide nucléique selon les revendications 1 à 3, dans lequel on cultive la cellule d'hôte selon les revendications 16 ou 17 sous des conditions appropriées pour l'expression de ce polypeptide, et ce polypeptide est purifié.

Fig. 1

```
LOCUS       Api m 3   1122 bp    DNA
SOURCE      Tissue, venom gland
ORGANISM    Apis mellifera
BASE COUNT      362 a       214 c       238 g       308 t
ORIGIN
        1 gaacttaaac aaataaatgt gatattccgg cacggcgata ggatacccga tgagaaaaac
       61 gaaatgtatc cgaaagatcc ttatttgtat tatgattttt atccactgga gcgtggcgaa
      121 ttgactaact caggtaaaat gcgagaatat caattggggc aattcttgag agagagatat
      181 ggtgactttt tgggagacat ttacacggaa gaatccgtct cggctctcag ctcgttctac
      241 gataggacga aaatgtctct gcaactcgta ctcgcggcgc tctatccgcc aaataaattg
      301 caacaatgga acgaagatct gaactggcaa ccgatcgcca cgaaatattt gcgccgctac
      361 gaggacaata tctttttgcc agaagattgt ttgttattta ccatcgaact tgatagagta
      421 ttggaatcac cgcgtggaaa gtatgaattc tcgaaatatg acaaattgaa gaaaaaattg
      481 gaagaatgga ccggaaaaaa tatcactacg ccatgggatt attattacat atatcataca
      541 ctggtggctg aacaatcgta cggtcttact ctgccatctt ggacaaataa tatattcccg
      601 agaggagaat tgttcgatgc gacggtattt acgtacaaca taaccaattc gactcctttg
      661 ttgaaaaaac tttatggagg tccgcttctt cgaatattca ccaagcatat gttagacgtg
      721 gtatcgggta cgcaaaagaa aaagcgaaag atatacttgt tcagtggaca tgaaagtaat
      781 atcgcctctg tgttgcacgc tcttcaactt tattatcctc acgttcctga atattccagt
      841 tctattataa tggagcttca caatatcgaa ggcactcact acgtaaagat cgtttactac
      901 ttgggtatcc cgtctgaagc gagagaactt caattacccg gctgcgaggt actttgccct
      961 ttgtacaaat atttacaatt gatagagaac gtgataccat cgaacgaaga gttgatctgc
     1021 gataaaagat tcgtcgacga atcggcaaac aatttgtcga tcgaagaatt agatttcgtg
     1081 aaattgaacc taataaggat agcgggtact gagaataagt aa
//
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Fig 2:

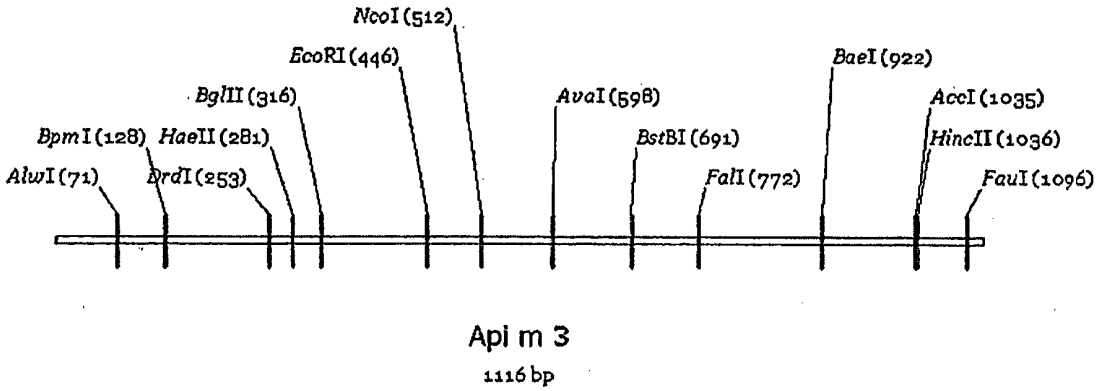

Fig. 3

```
LOCUS       Translatio    374 aa
DEFINITION  Translation of cloned Api m 3
KEYWORDS    TRANSLATED.
SOURCE      Tissue, venom gland
ORIGIN
        1 ELKQINVIFR HGDRIPDEKN EMYPKDPYLY YDFYPLERGE LTNSGKMREY QLGQFLRERY
       61 GDFLGDIYTE ESVSALSSFY DRTKMSLQLV LAALYPPNKL QQWNEDLNWQ PIATKYLRRY
      121 EDNIFLPEDC LLFTIELDRV LESPRGKYEF SKYDKLKKKL EEWTGKNITT PWDYYYIYHT
      181 LVAEQSYGLT LPSWTNNIFP RGELFDATVF TYNITNSTPL LKKLYGGPLL RIFTKHMLDV
      241 VSGTQKKKRK IYLFSGHESN IASVLHALQL YYPHVPEYSS SIIMELHNIE GTHYVKIVYY
      301 LGIPSEAREL QLPGCEVLCP LYKYLQLIEN VIPSNEELIC DKRFVDESAN NLSIEELDFV
      361 KLNLIRIAGT ENK*
```

Fig. 4A

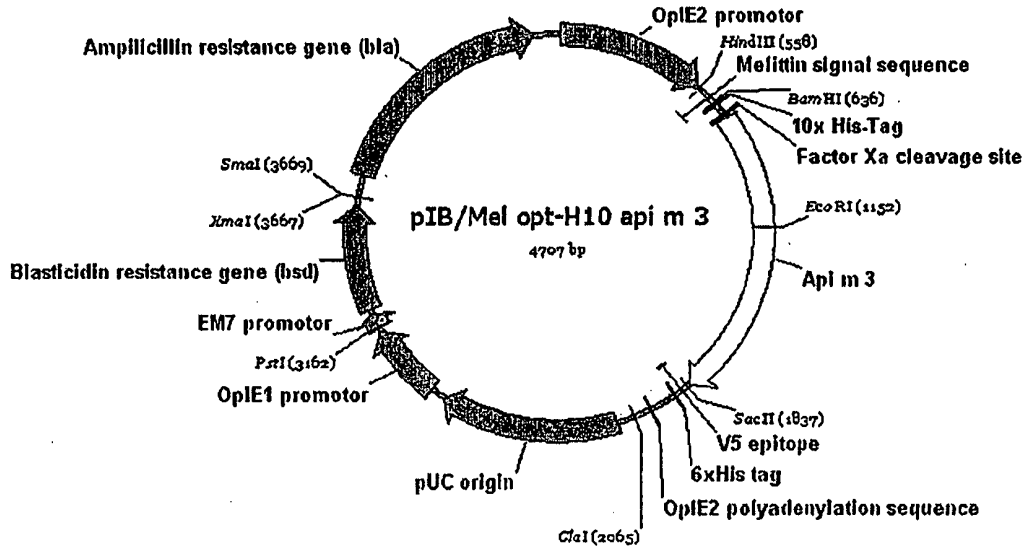

Fig. 4B

a) AAGCTTATGAAATTC
M K F

b) AAGCTTTCCGCCATGGCGAAATTC
M A K F

Fig. 4C

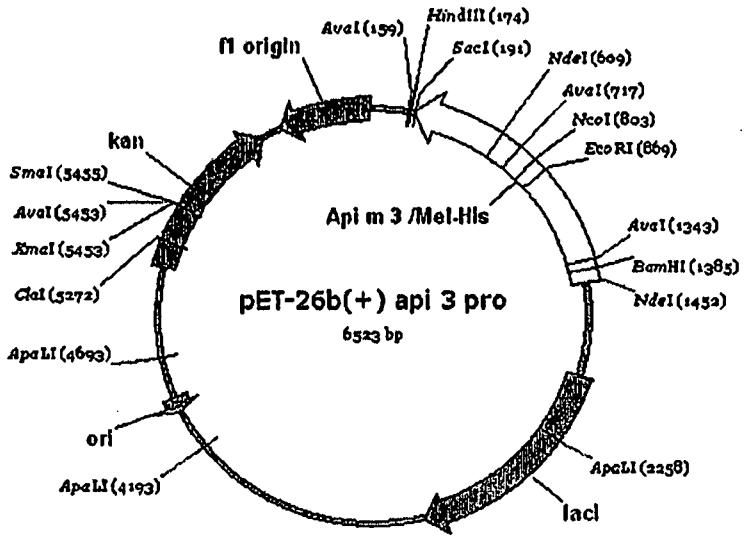
f1 origin
AvaI (159)
HindIII (174)
SacI (191)
NdeI (609)
AvaI (717)
NcoI (803)
EcoRI (869)
kan
SmaI (5455)
AvaI (5453)
XmaI (5453)
ClaI (5272)
Api m 3 /Mel-His
AvaI (1343)
BamHI (1385)
NdeI (1452)
pET-26b(+) api 3 pro
6523 bp
ApaLI (4693)
ori
ApaLI (4193)
ApaLI (2258)
lacI

Fig. 5A

Order of alignment positions (Hoffman)	Order of alignment positions (this invention)	Sequences of published peptide fragments	AA length
1	1,4	ELKQINVIFRHGDRIPDEKNEMYPKKLEEWTDK	33
2	8	FVDESANNLSIEEIDFVK	18
3	2	LQQWNEDLNWQPIATK	16
4	3	GKYEFSKR	8
5	-	YNIFAGTWK	9
6	6	LYGGPLLRDNYVGDER	16
7	5,7	DITTPKDYYYIYHTLVAENEYSSCIIMEYHNIEGTHYVKIVYY LGIPSEARELQLPGCEVLCPLEKYLQLIENVIPSNEELICDKR	86

Fig. 5B

```
*         20          *          40          *
Api m 3 : ELKQINVIFRHGDRIPDEKNEMYPKDPYLYYDFYPLERGELTNSGKMREY :  50
Hoffman : ELKQINVIFRHGDRIPDEKNEMYPKKL-EEWTDK
Revised : ELKQINVIFRHGDRIPDEKNEMYPK

                   60          *          80          *          100
Api m 3 : QLGQFLRERYGDFLGDIYTEESVSALSSFYDRTKMSLQLVLAALYPPNKL : 100
Hoffman :                 IVDESANNLSIEEIDFVK                   L
Revised :                                                      L

                    *         120          *         140          *
Api m 3 : QQWNEDLNWQPIATKYLRRYEDNIFLPEDCLLFTIELDRVLESPRGKYEF : 150
Hoffman : QQWNEDLNWQPIATK                              SKYEFSKR
Revised : QQWNEDLNWQPIATK                                 GKYEF

                  160          *         180          *         200
Api m 3 : SKYDKLKKKLEEWTGKNITTPWDYYYIYHTLVAEQSYGLTLPSWTNNIFP : 200
Hoffman :                     YNIFAGTWK
Revised : SK        KLEEWT    ITTPKDYYYIYHTLVAE

                    *         220          *         240          *
Api m 3 : RGELFDATVFTYNITNSTPLLKKLYGGPLLRIFTKHMLDVVSGTQKKKRK : 250
Hoffman :                           LYGGPLLRDNYVGDER
Revised :                           LYGGPLLR

                  260          *         280          *         300
Api m 3 : IYLFSGHESNIASVLHALQLYYPHVPEYSSSIIMELHNIEGTHYVKIVYY : 300
Hoffman :         DITTPKDYYYIYHTLVAENEYSSCIIMEYHNIEGTHYVKIVYY
Revised :                             EYSSCIIMEYHNIEGTHYVKIVYY

                    *         320          *         340          *
Api m 3 : LGIPSEARELQLPGCEVLCPLYKYLQLIENVIPSNEELICDKRFVDESAN : 350
Hoffman : LGIPSEARELQLPGCEVLCPLEKYLQLIENVIPSNEELICDKR
Revised : LGIPSEARELQLPGCEVLCPLEKYLQLIENVIPSNEELICDKRFVDESAN

                  360          *
Api m 3 : NLSIEELDFVKLNLIRIAGTENK- : 373
Hoffman :
Revised : NLSIEELDFVK
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Fig. 6

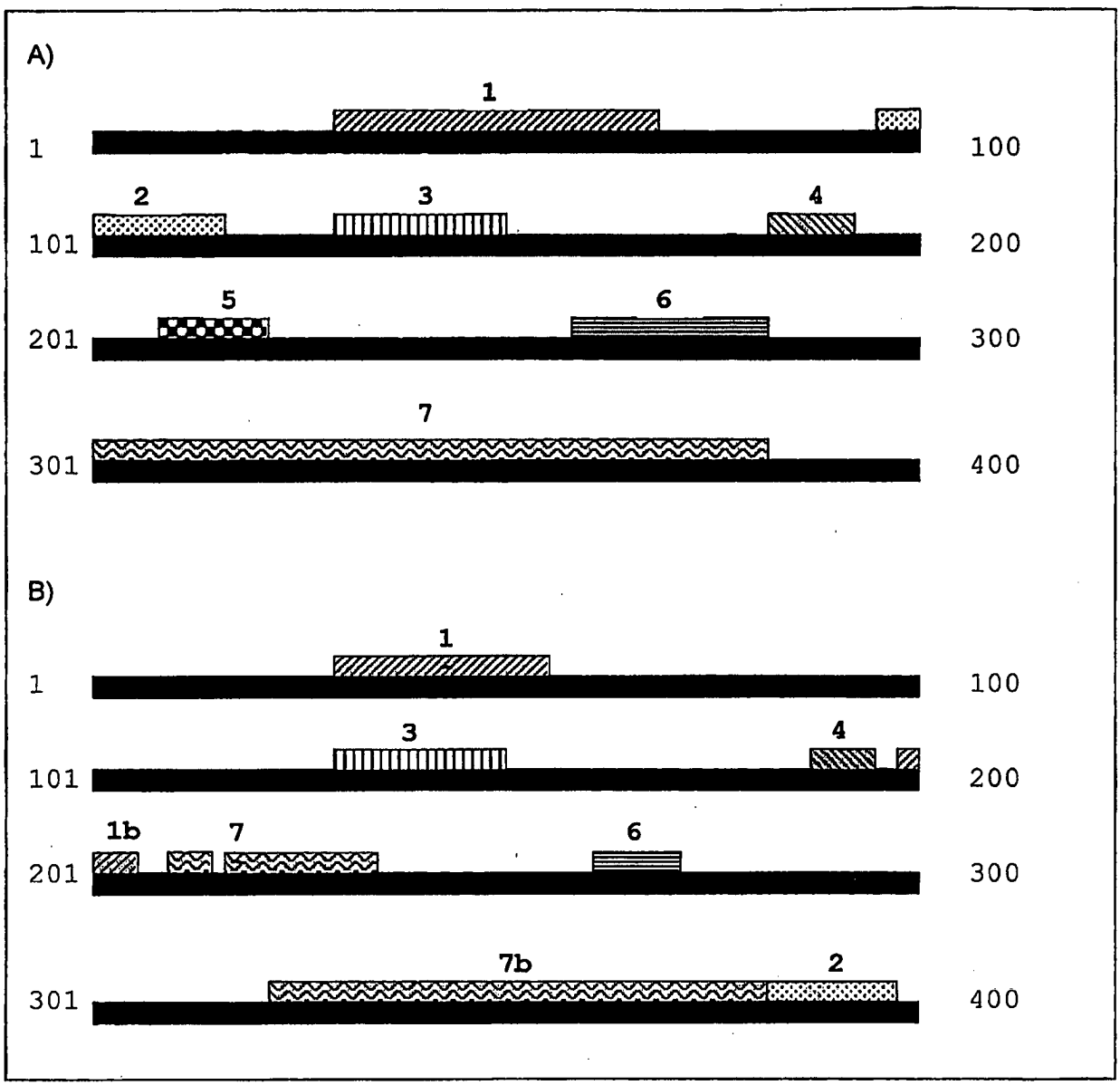

A)
1
2
3
4
5
6
7
1
101
201
301
100
200
300
400
B)
1
3
4
1b
7
6
7b
2
1
101
201
301
100
200
300
400

Fig 7

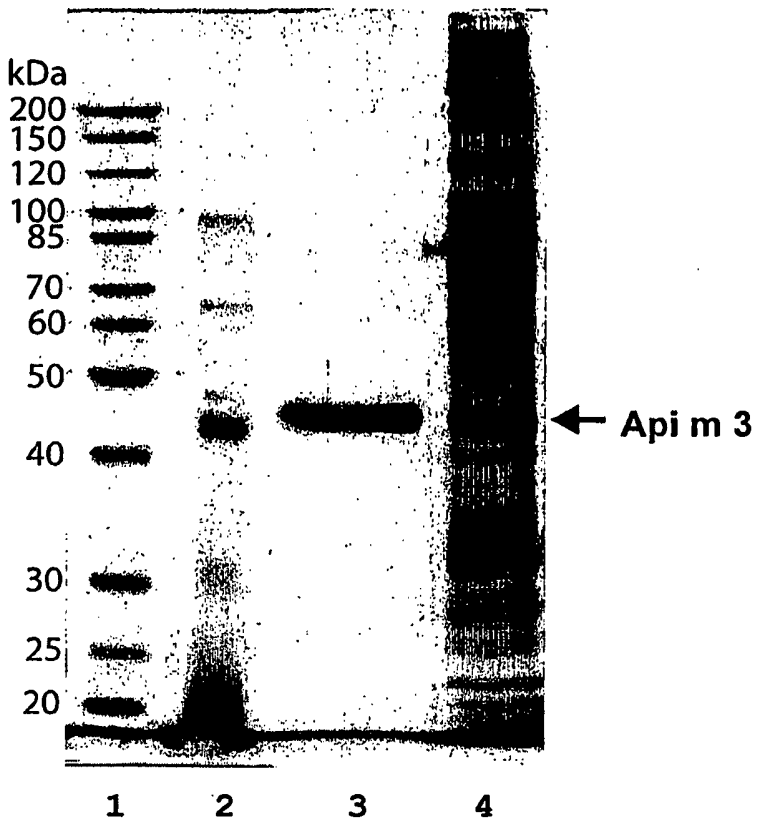


Fig 8

RHGXRXP motif

```
Api m 3    (A. mellif.)    ELKQINVIFRHCDRIPDEKNEMVPKDPYLYYDPYPLERGELTNSGKKREYQLGQFLREYGDFLGDIYTEESVSALSSFY 80
Acph 1     (D. melanog.)   QLKFVHVIYRHGDRTP---VDPYPTDPWGDRKFWPTGWGDLTNLGKQEHYDLGKNLRNRYSNLLPPIYSNEXIYVQSTDV 77
CG7899-PA  (D. melanog.)   QLKFVHVIYRHGDRTP---VDPYPTDPWGDRKFWPTGWGDLTNLGKQEHYDLGKNLRNRYSNLLPPIYSNEXIYVQSTDV 77
Acph 1     (D. suboscura.) ELKFAHVIFRHGDRTP---VDPYPTDPWNNRKFWPTGWGQLTNLGKEQHYELGKNLRNRYKSLLGSRYTNEDIFVQSTDV 77
CG9451-PA  (D. melanog.)   TLKLVHVLFRHGPRTP---VSTYPNDPYINETYEPFGWGALTNGAKVELYKIGKQLRQRYKDFLPAYYQPDAIRAQSSES 77

Api m 3    (A. mellif.)    DRTKMSLQLVLAALYPP-NKLQQWNEDLNWQPIATKYLRR-YEDNIFLPEDCLLFTIELDRVLESPRGKYEFSKYDKLKK 159
Acph 1     (D. melanog.)   DRTLMSAQSNLAGLYEP-QGEDIWNTDINWQPIPINTSPERDDPILAAKAPCPAYDYELASLESSPEFKALTEKHRNLFA 156
CG7899-PA  (D. melanog.)   DRTLMSAQSNLAGLYEP-QGEDIWNTDINWQPIPINTSPERDDPILAAKAPCPAYDYELASLESSPEFKALTEKHRNLFA 156
Acph 1     (D. suboscura.) DRTLMSAQSDLAGLYEP-QGDDIWNPRIDWQPVPVHTVPEKDDSILAAKASCPAYDYELATLEASSEFQALYVRYRELLS 156
CG9451-PA  (D. melanog.)   PRTLMSKQMVLAGLFPPENTPMEWNQLLNWQPIPIVMEPEETDVHIRMKAPCPRYDESVLEVIELPEYKKLHAESSDLLR 157

Api m 3    (A. mellif.)    KLEEMTGHNITTPWDYYYIYHTLVAESQSYGLTLPSWTNNIFFRGELFDATVFTYMITNSTPLLKKLYGGPLLRIFTKHML 236
Acph 1     (D. melanog.)   YLSEKGGRPVKTFIDAQYLNNTLFIENLYNMTLPKWTKKVYGREELTYVSNFAFAISSYTRKLARLKAGPLLKDIFQRFR 236
CG7899-PA  (D. melanog.)   YLSEKGGRPVKTFIDAQYLNNTLFIENLYNMTLPKWTRMVYGREELTYVSNFAFAISSYTRKLARLKAGPLLKDIFQRFK 236
Acph 1     (D. suboscura.) YLTQNSGRHVKSFIDAQYLNNTLFIEKLYNMTLPVWAEKVYGKEELTYVSNFAFSIATFTRSKARLRTGPLLKDIFERFD 236
CG9451-PA  (D. melanog.)   ELTTHTGLNITHAHDVTNVFITLLCEQTFGLQLPSWTNDYFP-EKMLPLAEKSYVYDAYTTEQRKMKGGFFVELLLKQMQ 236

Api m 3    (A. mellif.)    DVVSGTQK-KKRRIYLFSGHESNIASVLHALQLYYPEVPEYSSSIIMELHN--IEGTHYVKIVYYLGIPSEARELQLPGC 315
Acph 1     (D. melanog.)   EKSSGSLK-PDRSMWVYSAHDTTVASVLNALKLFELHSPFYTACIMMELRVD--ETNTPLVSIFYKNTTAEPLPLDIPGC 313
CG7899-PA  (D. melanog.)   EKSSGSLK-PDRSMWVYSAHDTTVASVLNALKLFELHSPFYTACIMMELRVD--ETNTPLVSIFYKNTTAEPLPLDIPGC 313
Acph 1     (D. suboscura.) KKLNNQLK-PDRSLWIYSAHDTTIANVLNSLKLFELHSPFYAACIMLEMRVD--DSNTPLVSVFYKNTTAEPLPLDIPGC 313
CG9451-PA  (D. melanog.)   DRISGALKPANRKMFLSCGHDWTITNVLSALNVWEAQMPRFSSLIAFELHQNPQTGEYFLEIYFQNDPHKEPQQLQIPGC 316

Api m 3    (A. mellif.)    EVLCPLYKYLQLIENVIPSNEELICDKRFVDESANNLSIEELDFVKLMEIPIAGTENK---------------------- 373
Acph 1     (D. melanog.)   GPSCPLTKLMNIYEDVLFVDWERSCKLSTMQMTYEEANLGTATGILILIVIALLFASYGLMIYYRRRNYKLYSSYSQMA 392
CG7899-PA  (D. melanog.)   GPSCPLTKLMNIYEDVLFVDWERSCKLSTMQMTYEEANLGTATGILILIVIALLFASYGLMIYYRRRNYKLYSSYSQMA 392
Acph 1     (D. suboscura.) GLSCPLKTLVKLYQDVLFGNWERSCKRSTMQMTYEEANLGAATGILIFIITVLLCASYGLMVYYRRRHYNLYTSYSQMA 392
CG9451-PA  (D. melanog.)   EKCCPIGKLLELTKDIIPDAPYAELCKAKGTQGGARISYH---------------------------------------- 356
```

Fig 9

residues	mass calculated	mass measured	sequence
28 - 34	889.52	889.55	QINVIFR
50 - 62	1753.81	1753.85	DPYLYYDFYPLER
73 - 81	1153.60	1153.64	EYQLGQFLR
248 - 255	888.53	888.54	LYGGPLLR
260 - 270	1214.62	1214.65	HMLDVVSGTQK
321 - 332	1380.75	1380.82	IVYYLGIPSEAR

Fig 10

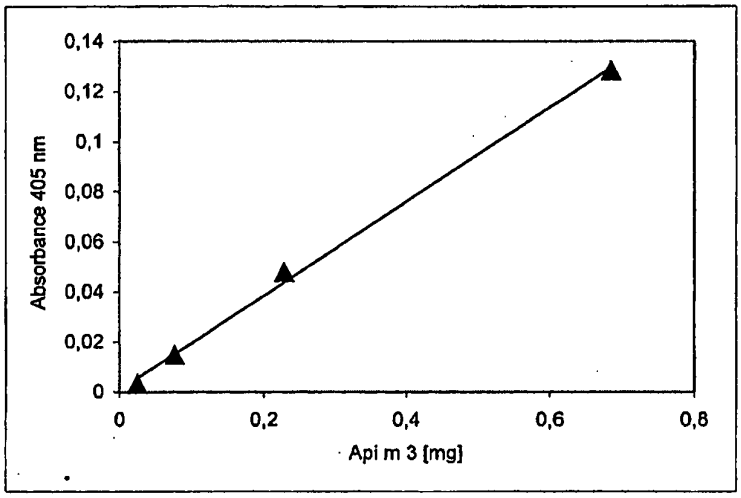


Fig 11

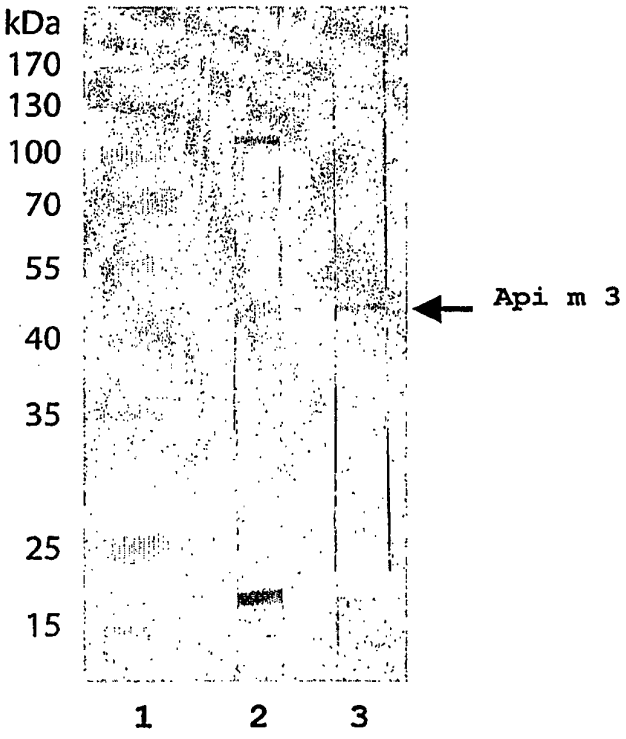
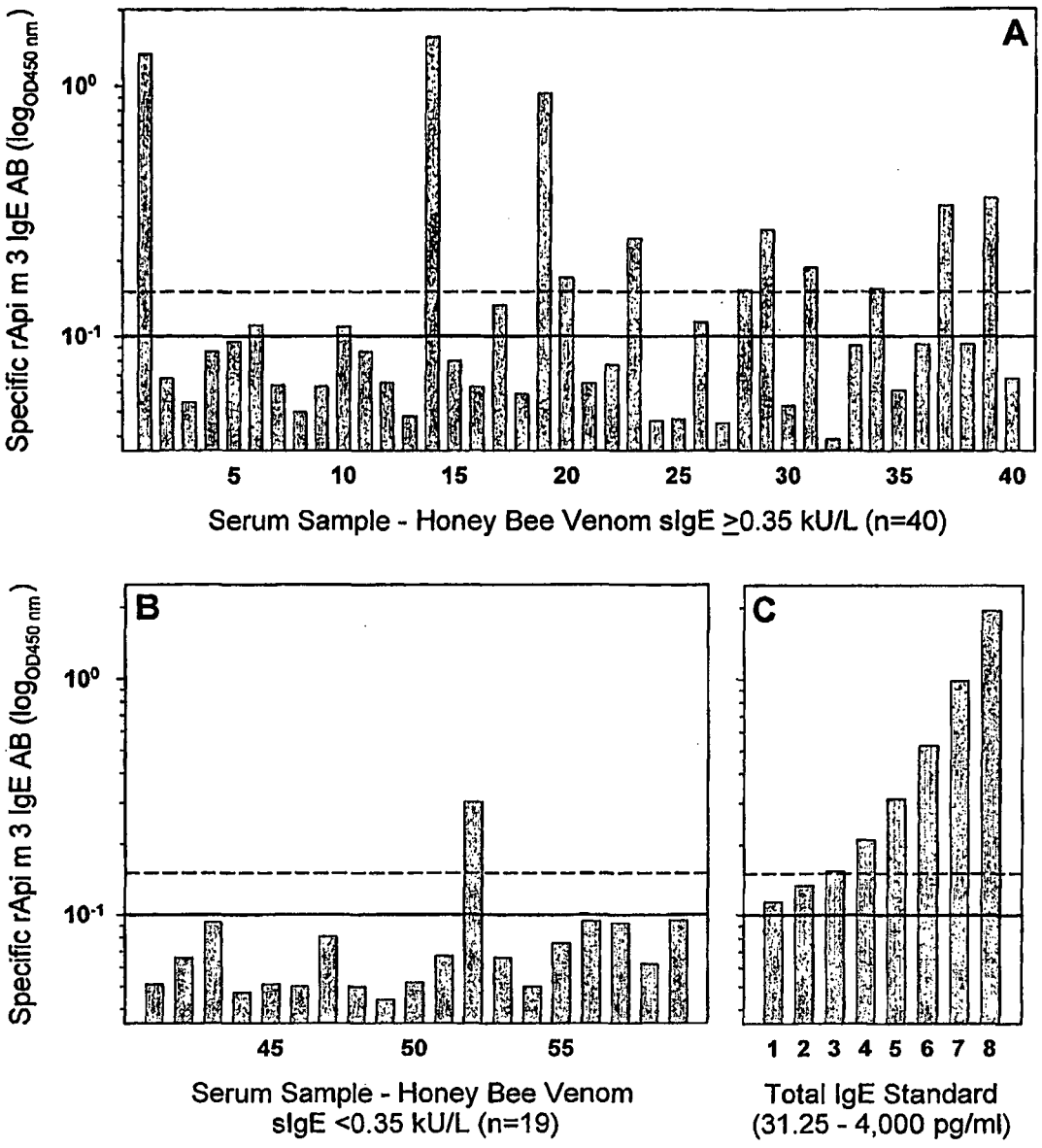


Fig 12


A
Specific rApi m 3 IgE AB ($log_{OD450\ nm}$)
10^0
10^{-1}
5
10
15
20
25
30
35
40
Serum Sample - Honey Bee Venom sIgE ≥0.35 kU/L (n=40)
B
C
Specific rApi m 3 IgE AB ($log_{OD450\ nm}$)
10^0
10^{-1}
45
50
55
1 2 3 4 5 6 7 8
Serum Sample - Honey Bee Venom
sIgE <0.35 kU/L (n=19)
Total IgE Standard
(31.25 - 4,000 pg/ml)

REFERENCES CITED IN THE DESCRIPTION

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专利名称(译)	克隆蜜蜂过敏原		
公开(公告)号	EP1846556A1	公开(公告)日	2007-10-24
申请号	EP2005815149	申请日	2005-12-13
[标]申请(专利权)人(译)	PLS DESIGN		
申请(专利权)人(译)	PLS-DESIGN GMBH		
当前申请(专利权)人(译)	PLS-DESIGN GMBH		
[标]发明人	GRUNWALD THOMAS		
发明人	GRUNWALD, THOMAS		
IPC分类号	C12N9/16 C12N15/55 C12N15/62 C12N15/11 C12N1/21 C12N5/10 C12Q1/42 A61K39/35 G01N33/53		
CPC分类号	A61K39/35 C07K14/43572		
代理机构(译)	UEXKÜLL & STOLBERG		
优先权	60/635479 2004-12-14 US		
其他公开文献	EP1846556B1		
外部链接	Espacenet		

摘要(译)

本发明涉及编码能够与来自昆虫毒液过敏的受试者的IgE结合的多肽的核酸，其与膜翅目昆虫的毒液具有与SEQ ID NO：2的氨基酸序列超过70％的同源性，其为蜜蜂过敏原Api m3（酸性磷酸酶）。本发明还涉及由核酸编码的表达载体，宿主细胞和多肽，以及其诊断和药物用途。