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The present invention relates to a hypoallergenic protein consisting of at least one hypoallergenic molecule derived from an allergen, which is fused or conjugated to at least one second non-allergenic protein or fragment thereof.



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Vaccine carrier

The present invention relates to novel hypoallergenic molecules and uses thereof.

Type I allergy is an IgE-mediated hypersensitivity disease affecting almost 25% of the population. It is based on the recognition of harmless airborne, insect, venom, food allergen and contact allergen antigens derived from *per se* harmless antigen sources such as pollen, insects, mold and animal proteins by specific immunoglobulin E. The crosslinking of effector cell-bound IgE antibodies leads to a release of inflammatory mediators (e.g., histamine, leucotrienes) and thus to the immediate symptoms of allergy (e.g., rhinoconjunctivitis, asthma, dermatitis, anaphylaxis). T-cell activation via IgE-dependent as well as IgE-independent mechanisms contributes to chronic allergic inflammation.

The probably only causative forms of allergy treatment is allergen-specific immunotherapy, which is based on the repeated administration of increasing amounts of allergen extracts for most sources. Numerous clinical studies have documented the clinical efficacy of injection immunotherapy and there is evidence for several immunological mechanisms underlying this treatment. Due to the difficulty to prepare high quality allergen extracts for certain allergen sources and the fact that the administration of allergens to patients can cause severe side effects, allergen-specific immunotherapy can only be recommended for certain patients groups and disease manifestations. It is especially difficult to treat patients with co-sensitizations to several different allergen sources and patients suffering from severe disease manifestations such as allergic asthma. Allergic asthma is one of the most vigorous manifestations of allergy, because it severely affects the quality of daily life, causes a high rate of hospitality admissions and can manifest itself in serious, life-threatening forms requiring intensive care of the patient.

Allergen extracts prepared from natural allergen-sources are crude in nature, and it is impossible to influence the quality and amounts of individual allergens in such preparations by technical means. They also contain numerous undefined non-allergenic components, and several recent studies indicate the poor

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quality of such extracts and document their great heterogeneity.

In the last decade great progress has been made in the field of molecular allergen characterization using recombinant DNA technology. A large number of the most important disease-eliciting allergens has been characterized down to the molecular level, and recombinant allergens mimicking the epitope complexity of natural allergen extracts have been produced. Moreover, several research groups have used the knowledge regarding allergen structures to develop defined new allergy vaccines. Genetic engineering, synthetic peptide chemistry and conjugation of allergens with immunostimulatory DNA sequences have been used to reduce the allergenic activity of the new vaccines and thus the rate of therapy-induced side effects. First promising clinical studies were conducted with such allergen derivatives. Interestingly, it turned out that although IgE-reactivity of genetically engineered recombinant allergens and allergen-derived synthetic T-cell epitope-containing peptides could be strongly reduced or even abolished, these derivatives still could induce systemic side effects appearing several hours after injection. For example, it was reported that T-cell epitope peptides of the major cat allergen, Fel d 1, induced asthma and bronchial hyper reactivity several hours after intracutaneous injection, and there is strong evidence that this effect is T-cell mediated and MHC-restricted.

These results indicate that the removal of IgE-reactivity diminishes IgE-mediated side effects since no immediate reactions were recorded in the course of these immunotherapy studies. However, the allergen-specific T-cell epitopes which have been preserved in the recombinant allergen derivatives as well as in the peptide mixtures are responsible for the late side effects (e.g. very problematic or atopic dermatitis, chronic T cell-mediated allergic skin manifestation). The side effects caused in the case of recombinant allergen-derivatives were relatively mild and in the case of the T-cell peptide vaccines may be overcome by adequate dosing. Both of the two new approaches therefore seem very promising for immunotherapy of allergic rhinoconjunctivitis but may have limitations when it comes to the treatment of severe forms of allergic asthma, where the induction of late side effects in the lung may be very problematic.

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In order to administer and consequently to provoke an efficient immune response against peptides, polypeptides and proteins, adjuvants and/or carriers are regularly used. Complete Freund's adjuvant, for instance, is one of the most potent adjuvants available. However, because of its side effects, its use is not approved for humans. Therefore, there exists a need for vaccine compositions able to induce strong immune responses against peptides and polypeptides derived from allergens and of course of other antigens avoiding the use of complete Freund's adjuvant. Further, while BSA has been used successfully as a carrier in animal models it may not be appropriate for use in human vaccine compositions because of the risk of adverse reactions such as the risk of transmitting prion disease (variant Creutzfeldt-Jakob disease). A further challenge to the development of an effective vaccine against allergens is the need for an immune response able to rapidly decrease allergens in an individual or animal. Therefore, high concentrations of allergen-specific antibodies in the blood, which are mainly of the IgG subtype, are needed. In mucosal surfaces IgA antibodies are the primary subtype.

Cholera toxin, a known carrier protein in the art, is also used regularly as an adjuvant, eliminating the need for complete Freund's adjuvant in a vaccine composition. However, cholera toxin increases total and specific IgE antibody levels and leads to IgE-associated inflammatory reactions.

Due to the side effects provoked by most carrier proteins used for vaccination, there exists a need for carrier systems which are able to stimulate immune responses against allergens or other antigens, without using toxic adjuvants, without using poorly tolerated carrier proteins and, in certain situations, without stimulation of potentially pathologic immune responses. Novel carrier systems meeting these specifications can be used towards the formation of novel conjugates and compositions suitable for the treatment or prevention of diseases like allergic diseases.

In Bohle B. et al. (J. Immunol. 172 (11) (2004): 6642-6648) a recombinant fusion protein comprising an S-layer protein moiety and Bet v 1 moiety is described. This molecule comprises the native hyperallergenic Bet v 1 protein.

WO 2004/004761 relates to virus like particles which are

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fused to an immunogen and which may be used for immunisation.

In WO 2004/003143 the use of fusion proteins comprising a virus like particle and a hyperallergenic molecule as immunogen for vaccination is disclosed.

The present invention may provide medicaments and carriers which overcome the aforementioned drawbacks and allow an allergen vaccination with reduced side effects.

SUMMARY OF INVENTION

In one aspect, the present invention provides a hypoallergenic protein for use in the treatment or prevention of an allergy in a human or animal, the protein consisting of at least one hypoallergenic molecule derived from an allergen, which is fused to at least one viral capsid protein, wherein the at least one hypoallergenic molecule derived from an allergen exhibits an at least 50% reduced IgE-binding capacity and an at least 30% reduced T-cell reactivity compared to the wild-type allergen.

In another aspect, the present invention provides use of a hypoallergenic protein for the treatment or prevention of an allergy in a human or animal, the protein consisting of at least one hypoallergenic molecule derived from an allergen, which is fused to at least one viral capsid protein, wherein the at least one hypoallergenic molecule derived from an allergen exhibits an at least 50% reduced IgE-binding capacity and an at least 30% reduced T-cell reactivity compared to the wild-type allergen.

In another aspect, the present invention provides use of a hypoallergenic protein in the manufacture of a medicament for the treatment or prevention of an allergy in a human or animal, the protein consisting of at least one hypoallergenic molecule derived from an allergen, which is fused to at least one viral capsid protein, wherein the at least one hypoallergenic molecule derived

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from an allergen exhibits an at least 50% reduced IgE-binding capacity and an at least 30% reduced T-cell reactivity compared to the wild-type allergen.

In another aspect, the present invention provides a nucleic acid molecule encoding a fused hypoallergenic protein of the invention.

In another aspect, the present invention provides a vector comprising a nucleic acid molecule of the invention.

In another aspect, the present invention provides a host cell comprising a nucleic acid molecule of the invention or a vector of the invention.

In another aspect, the present invention provides a vaccine formulation comprising a hypoallergenic protein of the invention and at least one adjuvant, pharmaceutical acceptable excipient or preservative.

DESCRIPTION OF INVENTION

Therefore, the present invention relates to a hypoallergenic protein consisting of at least one hypoallergenic molecule derived from an allergen, which is fused or conjugated to at least one second non-allergenic protein or fragment thereof.

In order to provoke an enhanced immune response against a molecule, in particular of a hypoallergenic molecule according to the present invention, said molecule is fused (by genetic engineering) or conjugated (by chemical reactions) to a carrier. A conventional and regularly employed carrier is, for instance, KLH (Keyhole limpet hemocyanin). KLH, which is isolated from the giant sea mollusc *Megathura crenulata*, is one of the most popular carrier proteins used to create an immunogen for injection. KLH induces a strong antibody response because of its large mass and because it is a non-mammalian protein.

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The second protein (the "carrier" or "carrier protein") to be fused or conjugated to a hypoallergenic molecule of the invention is not derived from an allergen ("non-allergenic"). However, the carrier protein used in the present invention may exhibit T cell reactivity and/or provoke an immune response against itself and the hypoallergenic molecule fused or conjugated to it when administered to an animal or human body. Consequently, if the carrier protein is derived from a pathogen (e.g. virus, bacteria etc.), (protecting) antibodies directed to said carrier and pathogens are produced.

As used herein, "hypoallergenic protein" means a fusion protein/polypeptide of a carrier of a non-allergenic source with a hypoallergenic molecule. Furthermore, a "hypoallergenic protein" is also intended to be a conjugation product (e.g. chemical coupling, adsorption) of a carrier with a hypoallergenic molecule.

"Hypoallergenic" as used herein, refers to molecules with reduced allergenic potential. Such molecules have a decreased capacity to provoke allergic reactions in an individual compared

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to the wild-type protein from which these molecules are derived.

The at least one hypoallergenic molecule derived from an allergen and fused/conjugated to a second protein is preferably C- and/or N-terminally truncated. "C- and/or N-terminal truncation", as used herein, means that amino acid residues either from the N-terminus or from the C-terminus or both from the N- and C-terminus of the wild-type allergen are removed by deletion of at least 1, 2, 3, 4, 5, 7, 10, 15, 20, 30 amino acid residues.

The hypoallergenic molecules, i.e. peptides/polypeptides, comprise preferably 10 to 50 amino acids, more preferably 15 to 40 amino acids, in particular 20-30 amino acids and exhibit reduced IgE reactivity. These molecules are designed to exclude T-cell epitopes which may cause T-cell-mediated side effects. T-cell epitopes and molecules exhibiting reduced T-cell response may be determined and identified by methods known by the person skilled in the art (e.g., Bercovici N. et al. Clin Diagn Lab Immunol. (2000) 7:859-864).

It was found that it is possible to design peptide vaccines derived from allergens like the major grass pollen allergens, e.g., Phl p 1, and for the major birch pollen allergen, Bet v 1, using surface exposed peptides. The data obtained show that such peptide vaccines can be produced for any allergen whose primary structure is known according to IgE epitope mapping, three-dimensional structure data or computer-aided prediction of surface-exposed domains. However, the selection of suitable peptides which may be used for vaccination remains crucial, because not all peptides identified with these methods can be employed in vaccination. The peptides suitably used for vaccination purposes should exhibit reduced IgE-binding capacity and - in order to reduce or avoid late side effects - exhibit reduced T-cell reactivity.

The term "derived from an allergen", as used herein, means that the hypoallergenic molecules according to the present invention are obtained directly from an allergen by fragmentation or truncation. The amino acid sequence of the hypoallergenic molecules of the present invention are preferably at least 80% identical, more preferably at least 90% identical, most preferably at least 95% identical, in particular 100% identical, to the amino sequence stretch of the wild-type allergen, from which

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the hypoallergenic molecule is derived. However, the molecules which are not 100% identical to the wild-type allergen fragments should be able to bind with at least 60%, preferably at least 70%, more preferably at least 80%, most preferably at least 90%, strength to an antibody or to antibodies, preferably to IgG antibodies, which are directed to said wild-type allergen fragments.

The degree of identity of a first amino acid sequence to a second amino acid can be determined by a direct comparison between both amino acid sequences using certain algorithms. Such algorithms are, for instance, incorporated in various computer programs (e.g. "BLAST 2 SEQUENCES (blastp)" (Tatusova et al. (1999) FEMS Microbiol. Lett. 174:247-25; Corpet F, Nucl. Acids Res. (1988) 16:10881-10890).

The truncated molecules according to the present invention can be defined as being parts of the complete allergen that induce less activation of allergen-specific T cells than the complete wild-type allergen (preferably at least a 30%, more preferably at least a 50%, most preferably at least a 70%, reduction), exhibit a more than 50% reduced (preferably more than 70%) allergenic activity as evaluated by IgE binding assays and ability to induce IgE-mediated cell activation and when coupled to a carrier as described induce IgG antibodies which inhibit the binding of polyclonal IgE from allergic patients to the complete wild-type allergen.

The peptides should contain sequences from the allergens to avoid overlaps with the mimotopes. Mimotopes, however, which are small peptide mimics (less than 15 amino acids) of antigen pieces and are obtained from random peptide libraries do not represent original, allergen-derived molecules as defined herein. They can not be used according to the invention because they are too small to induce a robust blocking IgG response.

The hypoallergenic molecules according to the present invention may be obtained by recombinant methods or chemical synthesis. Alternatively, it is, of course, also possible to obtain the molecules by enzymatic or chemical cleavage of the wild-type allergen or a polypeptide/protein harbouring the molecule of interest.

The hypoallergenic molecule may comprise preferably at least two truncated allergen molecules derived from at least one

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allergen, wherein the order of the truncated allergen fragments differs from the order of the fragments in the wild-type allergen if the at least two molecules are derived from the same allergen.

The hypoallergenic molecule according to the present invention may comprise one or more (preferably at least 2, more preferably at least 3) hypoallergenic molecules as defined herein, thus, resulting in a fusion protein. The single hypoallergenic molecules of the fusion protein, which, of course, also lacks IgE-binding capacity and lacks T-cell epitopes, may be derived from allergens of the same and/or of different origin. If the molecules are derived from the same allergen, the order in the hypoallergenic fusion protein should not be identical to the order in the wild-type allergen (this prevents the reconstitution and formation of IgE-binding sites) (see, e.g., WO2004/065414, Linhart B and Valenta R (Int Arch Allergy Immunol. (2004) 134:324-31)).

According to a preferred embodiment of the present invention the at least one hypoallergenic molecule is fused to the N-terminus and/or C-terminus of said at least one second protein or fragment thereof.

The allergen or fragments thereof may be conjugated chemically, e.g., or by recombinant methods to each other. If the allergen or fragment thereof is conjugated chemically to a carrier, said allergen or fragment should be provided with a terminal cysteine residue (resulting in a free sulfhydryl group). To said terminal (N- or C-terminal) cysteine residue any maleimide-activated carrier protein may be conjugated, thus creating an immunogen/carrier complex. If the allergen or fragment thereof does not have a sulfhydryl group at a terminus, EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride) chemistry in order to couple amines (lysine) or carboxylic acids (glutamic, aspartic acid or 5'-phosphate) to the carrier protein may be employed.

If the hypoallergenic molecule fused to the N- or C-terminus of the carrier, recombinant methods are employed.

According to a preferred embodiment of the present invention the at least one second protein is a viral, in particular RNA or DNA viral, bacterial fungal or protozoal protein.

The at least one second protein ("carrier") may be of any of

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the above-mentioned origin. It is, however, in particular preferred to use proteins which provoke an immune response against the protein itself and the hypoallergenic molecule fused or conjugated thereto. Due to the induction of formation of (protective) antibodies directed also to the at least one second protein, the hypoallergenic protein according to the present invention may also be employed as vaccine for said second protein and its originating source (e.g. virus, bacteria, fungus). Of course it is also possible to use carrier proteins well known in the art (e.g. KLH) as the at least second protein.

The viral protein according to the present invention is preferably a capsid protein.

Viral capsid proteins are especially suited because they induce antiviral activity, provoke the formation of antibodies which block adhesion of viruses, e.g. rhinoviruses, to epithelial cells, exhibit an immuno-modulatory activity towards a Th1 response, increase the immunogenicity of the peptide (i.e., higher anti-peptide and hence higher levels of protective IgG antibodies), are suited and proved for prophylactic vaccination (virus vaccination) and are safe, when capsid proteins are used to whose humans are continuously exposed (e.g. rhinoviruses).

According to another preferred embodiment of the present invention the at least one viral capsid protein is derived from a human pathogenic virus, preferably a virus of the family of picornaviridae.

The virus of the family of picornaviridae is preferably of the genus of rhinoviruses, preferably of the species of human rhinoviruses, in particular human rhinovirus 89 and 14. The capsid protein may be VP1, VP2, VP3 and/or VP4.

The allergen to be fused to a viral capsid protein is preferably 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.

Other suited allergens to be used according to the present invention can be derived from the following table.

ALLERGENS

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

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Rosales

Humulus japonicus

Japanese hop

Hum j 4w	C	AY335187
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Parietaria judaica

Par j 1 lipid transfer protein 1	15	C	see list of
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isoallergens

Par j 2 lipid transfer protein 2		C	see list of
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isoallergens

Par j 3	profilin	C	see list of isoaller-
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gens

Parietaria officinalis

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

Poales

Cynodon dactylon

Bermuda grass

Cyn d 1		32	C	30, S83343
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

orchard grass

Dac g 1	AgDg1	32	P	32
Dac g 2		11	C	33, S45354
Dac g 3			C	33A, U25343
Dac g 5		31	P	34

Festuca pratensis

meadow fescue

Fes p 4w		60	-
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Holcus lanatus

velvet grass

Hol l 1			C	227084
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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

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

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Carpinus betulus

hornbeam

Car b 1	17	C	see list of isoaller-
gens			

Castanea sativa

chestnut

Cas s 1	22	P	52
Cas s 5 chitinase			
Cas s 8 lipid transfer protein	9.7	P	53

Corylus avellana

hazel

Cor a 1	17	C	see list of isoaller-
gens			

Cor a 2 profilin	14	C	
Cor a 8 lipid transfer protein	9	C	
Cor a 9 11S globulin-like protein	40/?	C	Beyer p.c.
Cor a 10 luminal binding prot.	70	C	AJ295617
Cor a 11 7S vicilin-like prot.	48	C	AF441864

Quercus alba

White oak

Que a 1	17	P	54
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Lamiales

Oleaceae

Fraxinus excelsior

ash

Fra e 1	20	P	58A, AF526295
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Ligustrum vulgare

privet

Lig v 1	20	P	58A
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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 Ca ²⁺ -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
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Plantaginaceae

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Plantago lanceolata

English plantain

Pla l 1

18

P

P842242

Pinales

Cryptomeria japonica

sugi

Cry j 1

41-45

C

55, 56

Cry j 2

C

57, D29772

Cupressus arisonica

cypress

Cup a 1

43

C

A1243570

Cupressus sempervirens

common cypress

Cup s 1

43

C

see list of isoaller-

gens

Cup s 3w

34

C

ref pending

Juniperus ashei

mountain cedar

Jun a 1

43

P

P81294

Jun a 2

C

57A, AJ404653

Jun a 3

30

P

57B, P81295

Juniperus oxycedrus

prickly juniper

Jun o 4 hom: calmodulin

29

C

57C, AF031471

Juniperus sabinoides

mountain cedar

Jun s 1

50

P

58

Juniperus virginiana

eastern red cedar

Jun v 1

43

P

P81825, 58B

Platanaceae

Platanus acerifolia

London plane tree

Pla a 1

18

P

P82817

Pla a 2

43

P

P82967

Pla a 3 lipid transfer protein

10

P

Iris p.c.

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D. Mites

Acarus siro arthropod

mite Aca s 13 fatty acid binding prot. 14* C AJ006774

Blomia tropicalis

mite Blo t 1 cysteine protease 39 C AF277840
 Blo t 3 trypsin 24* C Cheong p.c.
 Blo t 4 alpha amylase 56 C Cheong p.c.
 Blo t 5 C U59102
 Blo t 6 chymotrypsin 25 C Cheong p.c.
 Blo t 10 tropomyosin 33 C 61
 Blo t 11 paramyosin 110 C AF525465, 61A
 Blo t 12 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

American house dust mite

Der f 1 cysteine protease 25 C 69
 Der f 2 14 C 70, 70A, see list of

isoallergens

Der f 3 trypsin 30 C 63
 Der f 7 24-31 C SW:Q26456, 71
 Der f 10 tropomyosin C 72
 Der f 11 paramyosin 98 C 72A
 Der f 14 mag3, apolipophorin C D17686
 Der f 15 98k chitinase 98 C AF178772
 Der f 16 gelsolin/villin 53 C 71A
 Der f 17 Ca binding EF protein 53 C 71A
 Der f 18w 60k chitinase 60 C Weber p.c.

Dermatophagoides microceras

house dust mite

Der m 1 cysteine protease 25 P 68

Dermatophagoides pteronyssinus

European house dust mite

Der p 1 antigen P1, cysteine protease 25 C 62, see list of

isoallergens

Der p 2 14 C 62A-C, see list of

isoallergens

Der p 3 trypsin 28/30 C 63
 Der p 4 amylase 60 P 64
 Der p 5 14 C 65

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

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

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

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Aspergillus niger

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

Aspergillus oryzae

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

Penicillium brevicompactum

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

Pen ch 13 alkaline serine protease	34		87
Pen ch 18 vacuolar serine protease	32		87
Pen ch 20 N-acetyl 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
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1.3 Hypocreales

Fusarium culmorum

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

1.4 Onygenales

Trichophyton rubrum

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

Trichophyton tonsurans

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

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1.5 Saccharomycetales

Candida albicans

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

Candida boidinii

Cand b 2	20	C	J04984, J04985
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2. Basidiomycotina

2.1 Hymenomycetes

Psilocybe cubensis

Psi c 1			
Psi c 2	cyclophilin	16	89A

Coprinus comatus

shaggy cap

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

2.2 Urediniomycetes

Rhodotorula mucilaginosa

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

2.3 Ustilaginomycetes

Malassezia furfur

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

Malassezia sympodialis

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

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

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midge	Chi k 10	tropomyosin	32.5*	C	AJ012184
Chironomus thummi thummi					
midge	Chi t 1-9	hemoglobin	16	C	99
	Chi t 1.01	component III	16	C	P02229
	Chi t 1.02	component IV	16	C	P02230
	Chi t 2.0101	component I	16	C	P02221
	Chi t 2.0102	component IA	16	C	P02221
	Chi t 3	component II-beta	16	C	P02222
	Chi t 4	component IIIA	16	C	P02231
	Chi t 5	component VI	16	C	P02224
	Chi t 6.01	component VIIA	16	C	P02226
	Chi t 6.02	component IX	16	C	P02223
	Chi t 7	component VIIB	16	C	P02225
	Chi t 8	component VIII	16	C	P02227
	Chi t 9	component X	16	C	P02228
Ctenocephalides felis felis					
cat flea					
	Cte f 1				
	Cte f 2	M1b	27	C	AF231352
	Cte f 3		25	C	
Thaumetopoea pityocampa					
pine processionary moth					
	Tha p 1		15	P	PIR:A59396, 99A
Lepisma saccharina					
silverfish					
	Lep s 1	tropomyosin	36	C	AJ309202
Dolichovespula maculata					
white face hornet					
	Dol m 1	phospholipase A1	35	C	100
	Dol m 2	hyaluronidase	44	C	101
	Dol m 5	antigen 5	23	C	102, 103
Dolichovespula arenaria					
yellow hornet					
	Dol a 5	antigen 5	23	C	104
Polistes annularies					
wasp					
	Pol a 1	phospholipase A1	35	P	105
	Pol a 2	hyaluronidase	44	P	105
	Pol a 5	antigen 5	23	C	104
Polistes dominulus					
Mediterranean paper wasp					

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

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Ves v 2	hyaluronidase	44	P	105A	
Ves v 5	antigen 5	23	C	104	
Myrmecia pilosula					
Australian jumper ant					
Myr p 1			C	X70256	
Myr p 2			C	S81785	
Solenopsis geminata					
tropical fire ant					
Sol g 2				Hoffman p.c.	
Sol g 4				Hoffman p.c.	
Solenopsis invicta					
fire ant	Sol i 2	13	C	110, 111	
	Sol i 3	24	C	110	
	Sol i 4	13	C	110	
Solenopsis saevissima					
Brazilian fire ant					
Sol s 2				Hoffman p.c.	
Triatoma protracta					
California kissing bug					
Tria p 1	Procalin	20	C	AF179004, 111A.	
H. Foods					
Gadus callarias					
cod					
Gad c 1	allergen M	12	C	112, 113	
Salmo salar					
Atlantic salmon					
Sal s 1	parvalbumin	12	C	X97824	
Bos domesticus					
domestic cattle					
Bos d 4	alpha-lactalbumin	14.2	C	M18780	
(milk)	Bos d 5	beta-lactoglobulin	18.3	C	X14712
see also animals					
Bos d 6	serum albumin	67	C	M73993	
Bos d 7	immunoglobulin	160		77	
Bos d 8	caseins	20-30		77	
Cyprinus carpio					
(Common carp)					
Cyp c 1	parvalbumin	12	C	129	
Gallus domesticus					
chicken					
Gal d 1	ovomucoid	28	C	114, 115	

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

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SW:P80198

Secale cereale

rye Sec c 20 secalin see isoall. list

Triticum aestivum

wheat Tri a 18 agglutinin

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

Zea mays

maise, corn

Zea m 14 lipid transfer prot. 9 P P19656

Oryza sativa

rice Ory s 1 C 119B, U31771

Apium graveolens

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

Api g 4 profilin AF129423

Api g 5 55/58 P P81943

Daucus carota

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

list

Dau c 4 profilin C AF456482

Corylus avellana

hazelnut

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

gens

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 isoaller-

gens

Mal d 2 hom: thaumatin C AJ243427

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

Mal d 4 profilin 14.4* C see list of isoaller-

gens

Pyrus communis

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

Pyr c 4 profilin 14 C AF129424

Pyr c 5 hom: isoflavone reductas 33.5 C AF071477

Persea americana

avocado Pers a 1 endochitinase 32 C Z78202

Prunus armeniaca

apricot

Pru ar 1 hom: Bet v 1 C U93165

Pru ar 3 lipid transfer protein 9 P

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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

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

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Brazil nut

Ber e 1 2S albumin	9	C	P04403, M17146
Ber e 2 11S globulin seed storage protein 29 C			AY221641

Juglans nigra

black walnut

Jug n 1 2S albumin	19*	C	AY102930
Jug n 2 vicilin-like prot.	56*	C	AY102931

Juglans regia

English walnut

Jug r 1 2S albumin		C	U66866
Jug r 2 vicilin	44	C	AF066055
Jug r 3 lipid transfer protein	9	P	Pastorello

Anacardium occidentale

Cashew	Ana o 1 vicilin-like protein	50	C	see isoallergen list
	Ana o 2 legumin-like protein	55	C	AF453947
	Ana o 3 2S albumin	14	C	AY081853

Ricinus communis

Castor bean

Ric c 1 2S albumin		C	P01089
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Sesamum indicum

sesame	Ses i 1 2S albumin	9	C	121A, AF240005
	Ses i 2 2S albumin	7	C	AF091841
	Ses i 3 7S vicilin-like globulin	45	C	AF240006
	Ses i 4 oleosin	17	C	AAG23840
	Ses i 5 oleosin	15	C	AAD42942

Cucumis melo

muskmelon

Cuc m 1 serine protease	66	C	D32206
Cuc m 2 profilin	14	C	AY271295
Cuc m 3 pathogenesis-rel p. PR-1	16*	P	P83834

I. Others

Anisakis simplex

nematode

Ani s 1		24	P	121B, A59069
Ani s 2	paramyosin	97	C	AF173004
Ani s 3	tropomyosin	41	C	121C, Y19221
Ani s 4		9	P	P83885

Argas reflexus

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

References

- 1 Marsh, D.G., and L.R. Freidhoff. 1992. ALBE, an allergen database. IUIS, Baltimore, MD, Edition 1.0.
- 2 Marsh, D. G. et al. 1986. Allergen nomenclature. Bull WHO 64:767-770.
- 3 King, T.P. et al. 1964. Biochemistry 3:458-468.
- 4 Lowenstein, H. 1980. Allergy 35:188-191.
- 5 Aukrust, L. 1980. Allergy 35:206-207.
- 6 Demerec, M. et al. 1966. Genetics 54:61-75.
- 7 Bodmer, J. G. et al. 1991. Immunogenetics 33:301-309.
- 8 Griffith, I.J. et al. 1991. Int. Arch. Allergy Appl. Immunol. 96:296-304.
- 9 Roebber, M. et al. 1985. J. Immunol. 134:3062-3069.
- 10 Metzler, W. J. et al. 1992. Biochemistry 31:5117-5127.
- 11 Metzler, W. J. et al. 1992. Biochemistry 31:8697-8705.
- 12 Goodfriend, L. et al. 1979. Fed. Proc. 38:1415.
- 13 Ekramoddoullah, A. K. M. et al. 1982. Mol. Immunol. 19:1527-1534.
- 14 Ansari, A. A. et al. 1987. J. Allergy Clin. Immunol. 80:229-235.
- 15 Morgenstern, J.P. et al. 1991. Proc. Natl. Acad. Sci. USA 88:9690-9694.
- 16 Griffith, I.J. et al. 1992. Gene 113:263-268.
- 17 Weber, A. et al. 1986. Biochem. Physiol. 83B:321-324.
- 18 Weber, A. et al. 1987. Allergy 42:464-470.
- 19 Stanworth, D. R. et al. 1990. Bulletin WHO 68:109-111.
- 20 Rafnar, T. et al. 1991. J. Biol. Chem. 266: 1229-1236.
- 21 Rogers, B.L. et al. 1991. J. Immunol. 147:2547-2552.
- 22 Klapper, D.G. et al. 1980. Biochemistry 19:5729-5734.
- 23 Ghosh, B. et al. 1993. J. Immunol. 150:5391-5399.
- 24 Roebber, M. et al. 1983. J. Immunol. 131:706-711.
- 25 Lubahn, B., and D.G. Klapper. 1993. J. Allergy Clin. Immunol. 91:338.
- 26 Roebber, M., and D.G. Marsh. 1991. J. Allergy Clin. Immunol. 87:324.
- 27 Goodfriend L. et al. Mol Immunol 22: 899-906, 1985.
- 28 Himly M. et al. FASEB J 17: 106-108, 2003.
- 28A Nilsen, B. M. et al. 1991. J. Biol. Chem. 266:2660-2668.

- 31 -

- 29 Wopfner N. et al. Biol Chem 383: 1779-1789, 2002.
- 29A Jimenez A. et al. 1994. Int Arch Allergy Immunol 105:297-307.
- 29B Barderas R. et al. Int Arch Allergy Immunol 127: 47-54, 2002.
- 29C Carnés J. et al. Allergy 56, Supplement 68: 274, 2001.
- 29D Giuliani A. et al. Allergy 42: 434-440, 1987.
- 30 Smith, P.M. et al. 1996. J. Allergy Clin. Immunol. 98:331-343.
- 31 Suphioglu, C. et al. 1997. FEBS Lett. 402:167-172.
- 31a Asturias J.A. et al. 1997. Clin Exp Allergy 27:1307-1313.
- 32 Mecheri, S. et al. 1985. Allergy Appl. Immunol. 78:283-289.
- 33 Roberts, A.M. et al. 1993. Allergy 48:615-623.
- 33a Guerin-Marchand, C. et al. 1996. Mol. Immunol. 33:797-806.
- 34 Klysner, S. et al. 1992. Clin. Exp. Allergy 22: 491-497.
- 35 Perez, M. et al. 1990. J. Biol. Chem. 265:16210-16215.
- 36 Griffith, I. J. et al. 1991. FEBS Letters 279:210-215.
- 37 Ansari, A. A. et al. 1989. J. Biol. Chem. 264:11181-11185.
- 37a Sidoli, A. et al. 1993. J. Biol. Chem. 268:21819-21825.
- 38 Ansari, A. A. et al. 1989. Biochemistry 28:8665-8670.
- 39 Singh, M. B. et al. 1991. Proc. Natl. Acad. Sci. 88:1384-1388.
- 39a van Ree R. et al. 1995. J Allergy Clin Immunol 95:970-978.
- 40 Suphioglu, C. and Singh, M.B. 1995. Clin. Exp. Allergy 25:853-865.
- 41 Dolecek, C. et al. 1993. FEBS Lett. 335:299-304.
- 41A Fischer S. et al. 1996. J Allergy Clin Immunol 98:189-198.
- 42 Matthiesen, F., and H. Lowenstein. 1991. Clin. Exp. Allergy 21:297-307.
- 43 Petersen, A. et al. 1995. Int. Arch. Allergy Immunol. 108:55-59.
- 43A Marknell DeWitt A. et al. Clin Exp Allergy 32: 1329-1340, 2002.
- 44 Valenta, R. et al. 1994. Biochem. Biophys. Res. Commun. 199:106-118.
- 46 Esch, R. E., and D. G. Klapper. 1989. Mol. Immunol. 26:557-561.
- 47 Olsen, E. et al. 1991. J. Immunol. 147:205-211.
- 48 Avjioglu, A. et al. 1993. J. Allergy Clin. Immunol. 91:340.
- 52 Kos T. et al. 1993. Biochem Biophys Res Commun 196:1086-92.

- 53 Díaz-Perales A. et al. 2000. Clin Exp Allergy 30:1403-1410.
- 54 Ipsen, H., and O.C. Hansen. 1991. Mol. Immunol. 28: 1279-1288.
- 55 Taniai, M. et al. 1988. FEBS Lett. 239:329-332.
- 56 Griffith, I.J. et al. 1993. J. Allergy Clin. Immunol. 91:339.
- 57 Sakaguchi, M. et al. Allergy 45: 309-312, 1990.
- 57A Yokoyama M. et al. Biochem Biophys Res Commun 275: 195-202, 2000.
- 57B Midoro-Horiuti T. et al. J Immunol 164: 2188-2192, 2000.
- 57C Tinghino R. et al. J. Allergy Clin. Immunol. 101: 772-777, 1998.
- 58 Gross GN et al. Scand J Immunol 8: 437-441, 1978.
- 58A Obispo TM et al. Clin Exp Allergy 23: 311-316, 1993.
- 58B Midoro-Horiuti T. et al. Clin Exp Allergy 31: 771-778, 2001.
- 59 Lombardero M. et al. Clin. Exp. Allergy 24: 765-770, 1994.
- 60 Villalba, M. et al. Eur. J. Biochem. 216: 863-869, 1993.
- 60A Asturias JA et al. J Allergy Clin Immunol 100: 365-372, 1997.
- 60B Batanero E. et al. Eur J Biochem 241: 772-778, 1996.
- 60C Batanero E. et al. FEBS Lett. 410: 293-296, 1997.
- 60D Tejera ML et al. J Allergy Clin Immunol 104: 797-802, 1999.
- 60E Ledesma A. et al. FEBS Lett 466: 192-196, 2000.
- 60F Barral P. et al. J Immunol 172: 3644-3651, 2004.
- 61 Yi FC et al. Clin Exp Allergy 32: 1203-1210, 2002.
- 61A Ramos JD et al. Int Arch Allergy Immunol 126: 286-293, 2001.
- 62 Chua, K. Y. et al. J. Exp. Med. 167: 175-182, 1988.
- 62A Chua, K. Y. et al. Int. Arch. Allergy Appl. Immunol. 91: 118-123, 1990.
- 62B Smith AM et al. Int Arch Allergy Immunol 124: 61-63, 2001.
- 62C Smith AM et al. J Allergy Clin Immunol 107: 977-984, 2001.
- 63 Smith WA, Thomas WR. Int Arch Allergy Immunol 109: 133-140, 1996.
- 64 Lake, F.R. et al. J. Allergy Clin. Immunol. 87: 1035-1042, 1991.
- 65 Tovey, E. R. et al. J. Exp. Med. 170: 1457-1462, 1989.
- 66 Yasueda, H., T. Shida, T. Ando, S. Sugiyama, and H. Yamakawa. 1991. Allergenic and proteolytic properties of fourth allergens

- 33 -

- from Dermatophagoides mites. In: "Dust Mite Allergens and Asthma. Report of the 2nd international workshop" A. Todt, Ed., UCB Institute of Allergy, Brussels, Belgium, pp. 63-64.
- 67** Shen, H.-D. et al. Clin. Exp. Allergy 23: 934-940, 1993.
- 67A** O'Neil GM et al. Biochim Biophys Acta, 1219: 521-528, 1994.
- 67B** King C. et al. J Allergy Clin Immunol 98: 739-747, 1996.
- 68** Lind P. et al. J. Immunol. 140: 4256-4262, 1988.
- 69** Dilworth, R. J. et al. Clin. Exp. Allergy 21: 25-32, 1991.
- 70** Nishiyama, C. et al. Int. Arch. Allergy Immunol. 101: 159-166, 1993.
- 70A** Trudinger, M. et al. Clin. Exp. Allergy 21: 33-38, 1991.
- 71** Shen HD et al. Clin Exp Allergy 25: 1000-1006, 1995.
- 71A** Tategaki A. et al. ACI International suppl. 1: 74-76, 2000.
- 72** Aki T. et al. J Allergy Clin Immunol 96: 74-83, 1995.
- 72A** Tsai L. et al. Clin Exp Allergy 29: 1606-1613, 1999.
- 72B** Gafvelin G. et al. J Allergy Clin Immunol 107: 511-518, 2001.
- 73** van Hage-Hamsten. et al. J. Allergy Clin. Immunol. 91:353, 1993.
- 74** Varela J. et al. Eur J Biochem 225: 93-98, 1994.
- 74A** Schmidt M. et al. FEBS Lett 370: 11-14, 1995.
- 75** Eriksson TLJ et al. Eur. J. Biochem. 268: 287-294, 2001.
- 75A** Saarne T. et al. Int Arch Allergy Immunol 130: 258-265, 2003.
- 75B** Eriksson TL et al. Eur. J. Biochem. 251 (1-2), 443-447, 1998.
- 76** Rautiainen J, Rytkonen M, Pelkonen J, Pentikainen J, Perola O, Virtanen T, Zeiler T, Mantyjarvi R. BDA20, a major bovine dander allergen characterised at the sequence level is Bos d 2. Submitted.
- 77** Gjesing B, Lowenstein H. Ann Allergy 53:602, 1984.
- 78** de Groot, H. et al. J. Allergy Clin. Immunol. 87:1056-1065, 1991.
- 79** Konieczny, A. Personal communication; Immunologic Pharmaceutical Corp.
- 79A** Bulone, V. Eur J Biochem 253: 202-211, 1998.
- 79B** Swiss-Prot acc. P81216, P81217.
- 79C** Dandeu J. P. et al. (1993). J. Chromatogr. 621:23-31.
- 79D** Goubbran Botros H. et al. 1998. J. Chromatogr. B 710:57-65.
- 79E** Hilger C. et al. Allergy 52: 179-187; and Hilger C. et al.

Gene 169:295-296, 1996.

79F Ichikawa K. et al. Clin Exp Allergy, In Press 2001.

80 Fahlbusch B. et al. Allergy 57: 417-422, 2002.

81 McDonald, B. et al. 1988. J. Allergy Clin. Immunol. 83:251.

81A Clarke, A. J. et al. 1984. EMBO J 3:1045-1052.

82 Longbottom, J. L. 1983. Characterisation of allergens from the urines of experimental animals. McMillan Press, London, pp. 525-529.

83 Laperche, Y. et al. 1983. Cell 32:453-460.

83A Bush RK et al. 1999. J Allergy Clin Immunol 104:665-671.

83B Aukrust L, Borch SM. 1979. Int Arch Allergy Appl Immunol 60:68-79.

83C Sward-Nordmo M. et al. 1988. Int Arch Allergy Appl Immunol 85:288-294.

84 Shen, et al. J. Allergy Clin. Immunol. 103:S157, 1999.

84A Cramer R. Epidemiology and molecular basis of the involvement of *Aspergillus fumigatus* in allergic diseases. Contrib. Microbiol. Vol. 2, Karger, Basel (in press).

84B Shen, et al. (manuscript submitted), 1999

84C Shen HD et al. Vacuolar serine proteinase: A major allergen of *Aspergillus fumigatus*. 10th International Congress of Immunology, Abstract, 1998.

85 Kumar A. et al. 1993. J. Allergy Clin. Immunol. 91:1024-1030.

85A Saxena S. et al. 2003. Clin Exp Immunol 134:86-91.

85B Baur X. et al. Allergy 57: 943-945, 2002.

86A Shen HD et al. 1996. Clin Exp Allergy 26:444-451.

86B Shen, et al. Abstract; The XVIII Congress of the European Academy of Allergology and Clinical Immunology, Brussels, Belgium, 3-7 July 1999.

87 Shen HD et al. Clin Exp Allergy 29: 642-651, 1999.

87A Shen HD et al. Clin Exp Allergy 25: 350-356, 1995.

87B Shen HD et al. J Lab Clin Med 137: 115-124, 2001.

88 Woodfolk JA et al. 1998. J Biol Chem 273:29489-96.

88A Deuell, B. et al. 1991. J. Immunol. 147:96-101.

89 Shen, H.D. et al. 1991. Clin. Exp. Allergy 21:675-681.

89A Horner WE et al. 1995. Int Arch Allergy Immunol 107:298-300.

89B Chang CY et al. J Biomed Sci 9: 645-655, 2002.

90 Yasueda H. et al. Biochem Biophys Res Commun 248: 240-244,

- 35 -

1998. NB:strain TIMM2782 (Teikyo University Institute for Medical Mycology) equal to strain CBS1878 (Central Bureau von Schimmelkulturen).

90A Onishi Y. et al. Eur J Biochem 261: 148-154, 1999. NB: strain TIMM2782 (Teikyo University Institute for Medical Mycology) equal to strain CBS1878 (Central Bureau von Schimmelkulturen).

91 Schmidt M. et al. Eur J Biochem 246:181-185, 1997. NB: strain ATCC no. 42132 (American Type Culture Collection).

91A Rasool O. et al. Eur J Biochem 267: 4355-4361, 2000. NB: strain ATCC no. 42132 (American Type Culture Collection).

91B NB: ; strain 4625 (Indian Agricultural Research Institute, PUSA; New Delhi , India).

92 Kuchler, K. et al. 1989. Eur. J. Biochem. 184:249-254.

93 Gmachl, M., and G. Kreil. 1993. Proc. Natl. Acad. Sci. USA 90:3569-3573.

93A Hoffman DR. 1977. J Allergy Clin. Immunol. 59:364-366.

94 Habermann, E. 1972. Science 177:314-322.

95 Hoffman DR, Jacobson RS. 1996. J. Allergy Clin. Immunol. 97:812-821.

95A Hoffman DR, El-Choufani AE, Smith MM, de Groot H. 2001. Occupational allergy to bumblebee venom: Allergens of *Bombus terrestris*. J Allergy Clin Immunol In press.

95B Helm R. et al. 1996. J Allerg Clin Immunol 98:172-180.

95C Pomes A. et al. 1998. J Biol Chem 273:30801-30807.

96 Arruda LK et al. J Biol Chem 270:19563-19568, 1995.

97 Arruda LK et al. J Biol Chem 270:31196-31201, 1995.

98 Arruda LK et al. Int Arch Allergy Immunol 107:295-297, 1995.

98A Wu CH et al. 1998. J Allergy Clin Immunol 101:832-840.

98B Melen E. et al. 1999. J Allergy Clin Immunol 103:859-64.

98C Wu CH et al. J Biol Chem 271:17937-17943, 1996.

98D Wu CH et al. Molecular Immunol 34:1-8, 1997.

98E Santos ABR et al. 1999. J Allergy Clin Immunol 104:329-337.

98F Asturias JA et al. 1999. J Immunol 162:4342-4348.

99 Mazur, G. et al. 1990. Monog. Allergy 28:121-137.

99A Moneo I. et al. Allergy 58: 34-37, 2003.

100 Soldatova, L. et al. 1993. FEBS Letters 320:145-149.

101 Lu, G. et al. 1994. J. Allergy Clin. Immunol. 93:224.

102 Fang, K. S. F. et al. 1988. Proc. Natl. Acad. Sci., USA

- 36 -

85:895-899.

103 King, T. P. et al. 1990. Prot. Seq. Data Anal. 3:263-266.

104 Lu, G. et al. 1993. J. Immunol. 150: 2823-2830.

105 King, T. P. and Lu, G. 1997. Unpublished data.

105A King TP et al. 1996. J. Allergy Clin. Immunol. 98:588-600.

106 Hoffman, D.R. 1993. J. Allergy Clin. Immunol. 92:707-716.

107 Hoffman DR. 1992. Unpublished data.

108 Hoffman DR. J. Allergy Clin. Immunol. 91:187, 1993.

109 Jacobson RS et al. J. Allergy Clin. Immunol. 89:292, 1992.

110 Hoffman DR. J. Allergy Clin. Immunol 91: 71-78, 1993.

111 Schmidt M. et al. FEBS Letters 319: 138-140, 1993.

111A Paddock CD et al. J Immunol 167: 2694-2699, 2001.

112 Elsayed S, Bennich H. Scand J Immunol 3: 683-686, 1974.

113 Elsayed S. et al. Immunochemistry 9: 647-661, 1972.

114 Hoffman, D. R. 1983. J. Allergy Clin. Immunol. 71: 481-486.

115 Langeland, T. 1983. Allergy 38:493-500.

116 Daul CB, Slattery M, Morgan JE, Lehrer SB. 1993. Common crustacea allergens: identification of B cell epitopes with the shrimp specific monoclonal antibodies. In: "Molecular Biology and Immunology of Allergens" (D. Kraft and A. Sehon, eds.). CRC Press, Boca Raton. pp. 291-293.

116A Shanti KN et al. J. Immunol. 151: 5354-5363, 1993.

117 Yu CJ et al. J Immunol 170: 445-453, 2003.

117A Miyazawa M et al. J. Allergy Clin. Immunol. 98: 948-953, 1996.

117B Asturias JA et al. Int Arch Allergy Immunol 128: 90-96, 2002.

117C Lopata AL et al. J. Allergy Clin. Immunol. 100: 642-648, 1997.

117D Hoffmann-Sommergruber K. et al. Clin. Exp. Allergy 29: 840-847, 1999.

118 Monsalve RI et al. Biochem. J. 293: 625-632 1993.

118A. Monsalve RI et al. 1997. Clin Exp Allergy 27:833-841.

119 Mena, M. et al. Plant Molec. Biol. 20: 451-458, 1992.

119A Palosuo K. et al. J. Allergy Clin. Immunol. 108: 634-638, 2001.

119B Xu H. et al. Gene 164: 255-259, 1995.

119C Pastorello EA et al. J. Allergy Clin. Immunol. 94: 699-707,

- 1994.
- 119D** Diaz-Perales A. et al. J Allergy Clin Immunol 110: 790-796, 2002.
- 119E** Galleguillos F, Rodriguez JC. Clin Allergy 8: 21-24, 1978.
- 119F** Baur X. Clin Allergy 9: 451-457, 1979.
- 119G** Gailhofer G. et al. Clin Allergy 18: 445-450, 1988.
- 120** Menendez-Arias, L. et al. 1988. Eur. J. Biochem. 177:159-166.
- 120A** Gonzalez R. et al. Lancet 346:48-49, 1995.
- 120B** Kleine-Tebbe J. et al. J Allergy Clin Immunol 110: 797-804, 2002.
- 120C** Sanchez-Monge R. et al. J. Allergy Clin. Immunol. 106: 955-961, 2000.
- 121** Gavrovic-Jankulovic M. et al. J Allergy Clin Immunol 110: 805-810, 2002.
- 121A** Pastorello EA et al. J. Chromatogr. B Biomed. Sci. Appl. 756: 85-93, 2001.
- 121B** Moneo I. et al. J. Allergy Clin. Immunol. 106: 177-182, 2000.
- 121C** Asturias JA et al. 2000. Allergy 55:898-890.
- 122** Christie, J. F. et al. 1990. Immunology 69:596-602.
- 122A** Baur X. et al. Clin Allergy 12: 9-17, 1982.
- 122B** Onisuka R. et al. Int Arch Allergy Immunol 125: 135-143, 2001.
- 123** Czuppon AB et al. J Allergy Clin Immunol 92:690-697, 1993.
- 124** Attanayaka DPSTG et al. 1991. Plant Mol Biol 16:1079-1081.
- 125** Chye ML, Cheung KY. 1995. Plant Mol Biol 26:397-402.
- 126** Alenius H. et al. 1993. Int Arch Allergy Immunol 102:61-66.
- 127** Yeang HY, Cheong KF, Sunderasan E, Hamzah S, Chew NP, Hamid S, Hamilton RG, Cardoso MJ. 1996. The 14.6 kD (REF, Hev b 1) and 24 kD (Hev b 3) rubber particle proteins are recognised by IgE from Spina Bifida patients with Latex allergy. J Allerg Clin Immunol in press.
- 128** Sunderasan E. et al. 1995. J nat Rubb Res 10:82-99.
- 129** Swoboda I. et al. 2002. J Immunol. 168:4576-84.

According to a preferred embodiment of the present invention the hypoallergenic molecule exhibits reduced IgE-binding capacity.

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According to another preferred embodiment of the present invention the hypoallergenic molecule exhibits reduced T-cell reactivity.

However, also allergen fragments comprising at least one T-cell epitope may be used in the hypoallergenic protein according to the present invention.

"Exhibiting reduced IgE-binding capacity", as used herein, means that the molecules according to the present invention show significantly reduced IgE-binding capacity or activity (at least 50% less, preferably at least 70% less, more preferably at least 80% less, even more preferably at least 90% less, most preferably at least 95% less, binding capacity compared to the wild-type allergen) or even lack them at all.

IgE-binding activity/capacity of molecules like peptides and proteins can be determined by, for example, an enzyme linked immunosorbent assay (ELISA) using, for example, sera obtained from a subject, (i.e., an allergic subject) that has been previously exposed to the wild-type allergen. Briefly, a peptide to be tested is coated onto wells of a microtiter plate. After washing and blocking the wells, an antibody solution consisting of the plasma of an allergic subject, who has been exposed to the peptide being tested or the protein from which it was derived, is incubated in the wells. A labelled secondary antibody is added to the wells and incubated. The amount of IgE-binding is then quantified and compared to the amount of IgE bound by a purified wild-type allergen.

Alternatively, the binding activity of a peptide can be determined by Western blot analysis. For example, a peptide to be tested is run on a polyacrylamide gel using SDS-PAGE. The peptide is then transferred to nitrocellulose and subsequently incubated with serum from an allergic subject. After incubation with the labelled secondary antibody, the amount of IgE bound is determined and quantified.

Another assay which can be used to determine IgE-binding activity of a peptide is a competition ELISA assay. Briefly, an IgE-antibody pool is generated by combining plasma from allergic subjects who have been shown by direct ELISA to be IgE-reactive with wild-type allergen. This pool is used in ELISA competition assays to compare IgE-binding to wild-type allergen to the peptide tested. IgE-binding for the wild-type allergen and the pep-

tide being tested is determined and quantified.

A "T-cell epitope" means a protein (e.g., allergen) or fragment thereof, for which a T-cell has an antigen specific binding site, the result of binding to said binding site activates the T-cell. The term "exhibiting reduced T-cell reactivity", as used herein, refers to molecules which exhibit a T-cell reactivity which is significantly reduced compared to the stimulation induced by the wild-type allergen from which the hypoallergenic molecule is derived using equimolar amounts in standard assays known in the art (reduced T-cell reactivity means at least 30%, preferably at least 50%, more preferably at least 70%, most preferably at least 90%, less stimulation of hypoallergenic molecules compared to the wildtype allergen at equimolar amounts). In a particular preferred embodiment of this invention, the molecules may "lack" T-cell epitopes and thus molecule shows reduced T-cell reactivity in the individual(s) to be treated (i.e., who is to receive an epitope-presenting valency platform molecule). It is likely that, for example, an allergen-derived molecule may lack a T-cell epitope(s) with respect to an individual, or a group of individuals, while possessing a T-cell epitope(s) with respect to other individual(s). Methods for detecting the presence of a T-cell epitope are known in the art and include assays which detect T-cell proliferation (such as thymidine incorporation). Immunogens that fail to induce statistically significant incorporation of thymidine above background (i.e., generally p less than 0.05 using standard statistically methods) are generally considered to lack T-cell epitopes, although it will be appreciated that the quantitative amount of thymidine incorporation may vary, depending on the immunogen being tested (see, e.g., Zhen L. et al. (Infect Immun. (2003) 71:3920-3926)). Generally, a stimulation index below about 2-3, more preferably less than about 1, indicates lack of T-cell reactivity and epitopes. The presence of T-cell epitopes can also be determined by measuring secretion of T-cell-derived lymphokines according to standard methods. The stimulation index (SI) may be calculated by dividing the proliferation rate (Thymidine uptake) of stimulated cells through the proliferation rate of unstimulated cells in medium alone. $SI=1$ means no stimulation, $SI<1$ indicates toxic effects and $SI>1$ indicates stimulation of cells. Location and content of T-cell epitopes, if

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present, can be determined empirically.

The cytokine secretion may be determined in addition to the stimulation of T cells. For example, IFN-gamma has been recognized as a harmful cytokine. Other examples may be TNF-alpha, IL-5, IL-4, IL-8 etc.

The allergen fragment is preferably composed of amino acids 151 to 177, 87 to 117, 1 to 30, 43 to 70 or 212 to 241 of Phl p 1, amino acids 93 to 128, 98 to 128, 26 to 53, 26 to 58, 132 to 162, 217 to 246, 252 to 283 or 176 to 212 of Phl p 5, amino acids 1 to 34 or 35 to 70 of chain 1 of Fel d 1, amino acids 1 to 34, 35 to 63 or 64 to 92 of chain 2 of Fel d 1, amino acids 30 to 59, 50 to 79 or 75 to 104 of Bet v 1, amino acids 1 to 33, 21 to 51, 42 to 73, 62 to 103 or 98 to 129 of Der p 2, amino acids 1 to 30, 20 to 50, 50 to 80, 90 to 125, 125 to 155 or 165 to 198 of Der p 7, amino acids 1-35, 36-70, 71-110, 111-145, 140-170, 175-205, 210-250 or 250-284 of Der p 10, amino acids 1 to 35, 35 to 72, 70 to 100 or 90 to 122 of Der p 21, amino acids 1 to 32, 15 to 48 or 32 to 70 of Clone 30, amino acids 19 to 58, 59 to 95, 91 to 120 or 121 to 157 of Alt a 1, amino acids 31 to 60, 45 to 80, 60 to 96 or 97 to 133 of Par j 2, amino acids 1 to 40, 36 to 66, 63 to 99, 86 to 120 or 107 to 145 of Ole e 1, amino acids 25 to 58, 99 to 133, 154 to 183, 277 to 307, 334 to 363, 373 to 402, 544 to 573, 579 to 608, 58 to 99, 125 to 165, 183 to 224, 224 to 261, 252 to 289, 303 to 340, 416 to 457, 460 to 500 or 501 to 542 of Fel d 2, amino acids 19 to 58, 52 to 91, 82 to 119, 106 to 144 or 139 to 180 of Can f 2, amino acids 19 to 56, 51 to 90, 78 to 118, 106 to 145 or 135-174 of Can f 1, amino acids 27 to 70, 70 to 100 or 92 to 132 of Art v 1, amino acids 31 to 70, 80 to 120, 125 to 155, 160 to 200, 225 to 263, 264 to 300 305 to 350 or 356 to 396 of Amb a 1, amino acids 1 to 34, 35 to 74, 74 to 115, 125 to 165, 174 to 213, 241 to 280, 294 to 333, 361 to 400 or 401 to 438 of Alt a 6, amino acids 1 to 40, 41 to 80, 81 to 120, 121 to 160 of Alt a 2 or fragments or sequence variations thereof.

The specific amino acid sequences of the above identified allergen-derived molecules are:

Peptide	Position	Sequence	SEQ ID No.
Pep Alt a 1.1	19-58	APLESRQDTASCPVTTEGDYVWKISEFYGRKPEG-TYYNSL	23

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Peptide	Position	Sequence	SEQ ID No.
Pep Alt a 1.2	59-95	GFNIKATNGGTLDFTCSAQADKLEDHKWYSCGENSFM	24
Pep Alt a 1.3	91-120	ENSFMDFSFDSDRSGLLLKQKVSDDITYVA	25
Pep Alt a 1.4	121-157	TATLPNYCRAGGNGPKDFVCQGVADAYITLVTLPKSS	26
Pep Alt a 2.1	1-40	MHSSNNFFKDNIFRSLSKEDPDYSRNIEGQVIRLH- WDWAQ	27
Pep Alt a 2.2	41-80	LLMLSAKRMKVAFKLDIEKDQRVWDRCTADDLK- GRNGEKR	28
Pep Alt a 2.3	81-120	CLQFTLYRPRDLLSLLNEAFFSAFRENRETIINTD- LEYAA	29
Pep Alt a 2.4	121-160	KSISMARLEDLWKEYQKIFPSIQVITSAFRSIE- PELTVYT	30
Pep Alt a 2.5	161-190	CLKKIEASFELIEENGDPKITSEIQLLKAS	31
Pep Alt a 6.1	1-34	MTITKIHARSVYDSRGNPTVEVDIVTETGLHRAI	32
Pep Alt a 6.2	35-74	VTETGLHRAIVPSGASTGSHEACELRDGDKSKWG- GKGVTK	33
Pep Alt a 6.3	74-115	APALIKEKLDVKDQSAVDAFLNKLDGTTNKTNL- GANAILGVS	34
Pep Alt a 6.4	125-165	EKGVPLYAHISDLAGT KKPYPVLPVPF QNVLNG- GSHAGGRLA	35
Pep Alt a 6.5	174-213	CEAPTFSEAMRQGAEVYQKLKALAKKTYGQSAGN- VGDEGG	36
Pep Alt a 6.6	241-280	IKIAMDVASSEFYKADEKKYDLDFKNPDSKSKWL- TYEQL	37
Pep Alt a 6.7	294-333	VSIEDPFAEDDWEAWSYFFKTYDGQIVGDDLTVT- NPEFIK	38
Pep Alt a 6.8	361-400	AKDAFGAGWGMVSHRSGETEDVTIADIVVGLRS- GQIKTG	39
Pep Alt a 6.9	401-438	APARSERLAKLNQILRIEEELGDNAVYAGNNFR- TAVNL	40
Pep Amb a 1.1	31-70	EILPVNETRRLTTS GAYNIIDGCWRGKADWAEN- RKALADC	41
Pep Amb a 1.2	80-120	GGKDGDIYTVTSELDDDVANPKEGTLRFGAAQNR- PLWIIIFE	42
Pep Amb a 1.3	125-155	IRLDKEMVVNSDKTIDGRGAKVEIINAGFTL	43
Pep Amb a 1.4	160-200	NVIIHNINMHDVKVNPGLIKSNDGPAAPRAGSDG- DAISIS	44
Pep Amb a 1.5	225-263	GTTRLTVSNSLFTQHQFVLLFGAGDENIEDRGMLAT- VAF	45
Pep Amb a 1.6	264-300	NTFTDNVDQRMPCRHGFFQVVNNNYDKWGSYAIGGS	46

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Peptide	Position	Sequence	SEQ ID No.
Pep Amb a 1.7	305-350	ILSQGNRFCAPDERSKKNVLGRHGEAAAESMKWN- WRTNKDVLENGA	47
Pep Amb a 1.8	356-396	GVDPVLTPEQSAGMIPAEPGESALSLTSSAGVLSC- QPGAPC	48
Pep Art v 1.1	27-70	SKLCEKTSKTYSGKCDNKKCDKKCIEWEKAQHGACH- KREAGKES	49
Pep Art v 1.2	70-100	SCFCYFDCSKSPPGATPAPPGAAPPPAAGGS	50
Pep Art v 1.3	92-132	APPPAAGGSPSPPADGGSPPPPADGGSPVDDGG- SPPPPSTH	51
Can f 1 Pep 1	19-56	QDTPALGKDTVAVSGKWYLKAMTADQEVPEKPDSVT- PM	52
Can f 1 Pep 2	51-90	DSVTPMILKAQKGGNLEAKITMLTNGQCQNITVVL- HKTSE	53
Can f 1 Pep 3	78-118	CQNITVVLHKTSEPGKYTAYEGQRVVFIQPSVPRD- HYILYC	54
Can f 1 Pep 4	106-145	QPSPVRDHYILYCEGELHGRQIRMAKLLGRD- PEQSQEALE	55
Can f 1 Pep 5	135-174	RDPEQSQEALDFREFSRAKGLNQEILELAQSETC- SPGGQ	56
Can f 2 Pep 1	19-58	QEGNHEEPQGGLEELSGRWHSVALASNKSDLIK- WGHFRV	57
Can f 2 Pep 2	52-91	PWGHFRVFIHMSAKDGNLHGDILIPQDGQCEK- VSLTAFK	58
Can f 2 Pep 3	82-119	CEKVSLTAFKTATSNNKFDLEY- WGHNDLYLAEVDPKSYL	59
Can f 2 Pep 4	106-144	NDLYLAEVDPKSYLILYMINQYN- DDTSLVAHLMVRDLR	60
Can f 2 Pep 5	139-180	VRDLRSRQQDFLPAFESVCEDIGLHKDQIVVLS- DDDRCCQSRD	61
Fel d 2 Pep 1	25-58	EAHQSEIAHRENDLGEHFRGLVLVAFSQYLQQC	62
Fel d 2 Pep 2	99-133	CTVASLRDKYGEMADCCEKKEPERNECFQHKDDN	63
Fel d 2 Pep 3	154-183	NEQRFLGKYLYEIAARRHPYFYAPELLYYAE	64
Fel d 2 Pep 4	277-307	CADDRADLAKYICENQDSISTKLKECCGKPV	65
Fel d 2 Pep 5	334-363	VEDKEVCKNYQEAKDVFLGTFLYEYSRRHP	66
Fel d 2 Pep 6	373-402	LAKEYEATLEKCCATDDPPACYAHVFDEFK	67
Fel d 2 Pep 7	544-573	EKQIKKQSALVELLKHKPKATEEQLKTVMG	68
Fel d 2 Pep 8	579-608	VDKCCAAEDKEACFAEEGPKLVAAAQAALA	69
Fel d 2 Pep 9	58-99	CPFEDHVKLVNEVTEFAKGCVADQSAANCEK- SLHELLGDKLC	70

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Peptide	Position	Sequence	SEQ ID No.
Fel d 2 Pep 10	125-165	CFLQHKDDNPGFGQLVTPEADAMCTAFHENEQRFLG-- KLYLE	71
Fel d 2 Pep 11	183-224	EEYKGVFTECCEAADKAACLTPKVDALREKVLAS-- SAKERLKC	72
Fel d 2 Pep 12	224-261	CASLQKFGERAFAKWSVARLSQKFPKAE-- FAEISKLVTD	73
Fel d 2 Pep 13	252-289	FAEISKLVTDLAKIHKECCHGDLLECADDRADLAKY-- IC	74
Fel d 2 Pep 14	303-340	CGKPVLEKSHCISEVERDELPADLPPLAVD-- FVEDKEVC	75
Fel d 2 Pep 15	416-457	CELFEKLGEYGFQNALLVRYTKKVPQVST-- PTLVEVSRSLGKV	76
Fel d 2 Pep 16	460-500	CTHPEAERLSCAEDYLSVVLNRLCVLHEKTPVSER-- VTKC	77
Fel d 2 Pep 17	501-542	CTESLVNRRPCFSALQVDETYVPKEFSAETFTF-- HADLCTLPE	78
Pep Ole e 1.1	1-40	EDIPQPPVSQFHIOGQVYCDTCRAGFITEELSEFIP-- GASLR	79
Pep Ole e 1.2	36-66	GASLRLQCKDKENGDVTFTEVGYTRAEGLYS	80
Pep Ole e 1.3	63-99	GLYSMLVERDHKNEFCEITLISSGRKDCNEIPTEGWA	81
Pep Ole e 1.4	86-120	GRKDCNEIPTEGWAKPSLKFKLNTVNGTTTRTVNPL	82
Pep Ole e 1.5	107-145	LNTVNGTTTRTVNPLGFFKKEALPKCAQVYNKL-- GMYPPNM	83
Pep Par j 2.1	31-60	GEEACGKVVQDIMPCLHFKVKGEEKEPSKEC	84
Pep Par j 2.2	45-80	CLHFKVKGEEKEPSKECCSGTKKLSEEVKTTEQKREA	85
Pep Par j 2.3	60-96	CCSGTKKLSEEVKTTEQKREACKCIVRATKGISGIKN	86
Pep Par j 2.4	97-133	ELVAEVPKKCDIKTTLPITADFDCSKIQSTIFRGYY	87
Der p 1 Pep 1	1-30	TNACSINGNAPAEIDLRQMRTVTPIRMQGG	88
Der p 1 Pep 2	52-84	NQSLDLAEQELVDCASQHGCHGDTIPRGIEYIQ	89
Der p 1 Pep 3	85-115	HNGVVQESYRYVAREQSCRRPNAQRFGISN	90
Der p 1 Pep 4	99-135	REQSCRRPNAQRFGISNYCQIYPPNVNKiREALAQTH	91
Der p 1 Pep 5	145-175	KDLDAFRHYDGRTIIQRDNGYQPNYHAVNIV	92
Der p 1 Pep 6	155-187	GRTIIQRDNGYQPNYHAVNIVGYSNAQGVDYWI	93
Der p 1 Pep 7	175-208	VGYSNAQGVDYWIVRNSWDTNWGDNGYGYFAANI	94
Der p 1 Pep 8	188-222	VRNSWDTNWGDNGYGYFAANIDLMMIEEYPYVVIL	95
Der p 2 Pep 1	1-33	DQVDVKDCANHEIKKVLVPGCHGSEPCIIHRGK	96
Der p 2 Pep 2	21-51	CHGSEPCIIHRGKPFQLEAVFEANQNSKTAK	97
Der p 2 Pep 3	42-73	EANQNSKTAKIEIKASIEGLEVDVPGIDPNAC	98
Der p 2 Pep 4	62-103	EVDVPGIDPNACHYMKCPLVKGOQYDIKYTWIVP--	99

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Peptide	Position	Sequence	SEQ ID No.
		KIAPKSEN	
Der p 2 Pep 5	98-129	APKSENVVVTVKVMDNGVLACAIATHAKIRD	100
Der p 5 Pep 1	1-35	MEDKKHDYQNEFDLLMERIHEQIKKGELALFYLO	101
Der p 5 Pep 2	25-60	KKGELALFYLOEQINHFEEKPTKEMKDKIVAEMDTI	102
Der p 5 Pep 3	65-95	DGVRGVLDRLMQRKDLDFEQYNLEMAKKSG	103
Der p 5 Pep 4	78-114	DLDFEQYNLEMAKKSGDILERDLKKEEARVKKIEV	104
Der p 7 Pep 1	1-30	DPIHYDKITEEINKAVDEAVAAIEKSETFD	105
Der p 7 Pep 2	20-50	VAAIEKSETFDPMKVPDHSDKFERHIGIIDL	106
Der p 7 Pep 3	50-80	LKGELDMRNIQVRGLKQMKRVGDANVKSEDG	107
Der p 7 Pep 4	90-125	VHDDVVSMEYDLAYKLGLDHPNTHVISDIQDFVVEL	108
Der p 7 Pep 5	125-155	LSLEVSEEGNMTLTSFEVRQFANVVNHIGGL	109
Der p 7 Pep 6	165-198	LSDVLTALFQDTVRAEMTKVLAPAFKKELERNNO	110
Der p 10 Pep 1	1-35	MEAIKKKMQAMKLEKDNAIDRAEIAEQKARDANLR	111
Der p 10 Pep 2	36-70	AEKSEEEVRALQKKIQQIENELDQVQEQLSAANTK	112
Der p 10 Pep 3	71-110	LEEKEKALQTAEGDVAALNRRIQLIEEDLERSEER- LKIAT	113
Der p 10 Pep 4	111-145	AKLEEASQSADESERMKMLEHRSITDEERMEGLE	114
Der p 10 Pep 5	140-170	RMEGLENQLKEARMMAEDADRKYDEVARKLA	115
Der p 10 Pep 6	175-205	DLERAEEAETGESKIVELEEELRVVGNNLK	116
Der p 10 Pep 7	210-250	SEEKAQQREEAHEQQIRIMTTKLKEAEARAFAERS- VQKLQ	117
Der p 10 Pep 8	250-284	QKEVDRLEDELVHEKEKYKSISDELDTFAELTGY	118
Der p 21 Pep 1	1-35	MFIVGDKKEDWRMAFDRLMMEELETKIDQVEKGL	119
Der p 21 Pep 2	35-72	LHLSEQYKELEKTKSKELKEQILRELTIGENFMKGAL	120
Der p 21 Pep 3	70-100	GALKFFEMEAKRTDLNMFERYNYEFALESIK	121
Der p 21 Pep 4	90-122	YNYEFALESIKLLIKKLDELAKKVKAVNPDEYY	122
Clone 30 Pep 1	1-32	MANDNDDPTTTVHPTTTEQPDDKFECPSRFG	123
Clone 30 Pep 2	15-48	PTTTEQPDDKFECPSRFGYFADPKDPHKFYICSN	124
Clone 30 Pep 3	32-70	GYFADPKDPHKFYICSNWEAVHKDCPGNTRWNEDEE TCT	125
Bet v 1 Pep 1	30-59	LFPKVAPQAISSVENIEGNGGPGTIKKISF	126
Bet v 1 Pep 2	50-79	GPGTIKKISFPEGFPFKYVKDRVDEV DHTN	127
Bet v 1 Pep 3	75-104	VDHTNFKYNYSVIEGGPIGDTLEKISNEIK	128
Fel d 1 chain 1 Pep 1	1-34	EICPAVKRDVDLFLTGTPEYVEQVAQYKALPVVC	129
Fel d 1 chain 1 Pep 2	35-70	LENARILKNCVDAKMTEEDKENALSLLDKIYTSPLC	130
Fel d 1 chain 2 Pep 1	1-34	VKMAITCPIFYDVFFAVANGNELLLDLSLTKVNAC	131

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Peptide	Position	Sequence	SEQ ID No.
Fel d 1 chain 2 Pep 2	35-63	TEPERTAMKKIQDCYVENGLISRVL DGLVC	132
Fel d 1 chain 2 Pep 3	64-92	CMTTISSSKDCMGEAVQNTVEDLKLNTLGR	133
Phl p 5 Pep 1	98-128	CGAASNKAFAEGLSGEPKGAAESSSKAALTSK	134
Phl p 5 Pep 2	26-58	ADLGYPATPAAPAAGYTPATPAAPAEAPAGKC	135
Phl p 5 Pep 3	132-162	AYKLAYKTAEGATPEAKYDAYVATLSEALRIC	136
Phl p 5 Pep 4	217-246	CEAAFNDAIKASTGGAYESYKFIPALEAAVK	137
Phl p 5 Pep 5	252-283	TVATAPEVKYTVFETALKKAITAMSEAQKAAC	138
Phl p 5 Pep 6	176-212	CAEEVKVIPAGELQVIEKVDAAFK- VAATAANAAPANDK	139
Phl p 5 Pep 1a	93-128	CFVATFGAASNKAFAEGLSGEPKGAAESSSKAALTSK	141
Phl p 5 Pep 2b	26-53	ADLGYPATPAAPAAGYTPATPAAPAEAC	142

The terms „fragments thereof“ and „sequence variations thereof“ refer to peptides which are deduced from the allergen-derived molecules disclosed herein and show biochemical properties (e.g. the capacity to prevent IgE binding to the allergen from which those molecules are derived from) which are comparable or identical to said allergen-derived molecules. The fragments of the present invention comprise at least 5, preferably at least 7, more preferably at least 10, successive and/or a maximum of 95%, preferably a maximum of 90%, more preferably a maximum of 80% amino acid residues of the allergen-derived molecule. The term „sequence variation“ includes modifications of the peptides such as fragmentation (see above), amino acid substitutions (e.g. with other natural or non-natural amino acids or amino acid derivatives), deletions or additions. „Sequence variation“ refers also to said allergen-derived molecules of the above table, wherein at least 1, preferably at least 2, more preferably at least 3, even more preferably at least 4 (5, 6, 7, 8, 9, 10, 15, 20) amino acid residues are added to the C- and/or N-terminus.

It is noted that the clone 30 allergen is an allergen derived from the house dust mite *Dermatophagoides pteronyssinus* and consists of the following sequence: MANDNDDPTTTVHPTTTEQP-DDKFECPSRFGYFADPKDPHKFYICSNWEAVHKDCPGNTRWNEDEETCT (SEQ ID No. 140; see also AT A 733/2006).

According to the present invention also peptides are encom-

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passed which are at least 80% identical, preferably 90% identical, to the amino sequences disclosed above.

Another aspect of the present invention relates to a nucleic acid molecule encoding a fused hypoallergenic protein according to the present invention.

Another aspect of the present invention relates to a vector comprising a nucleic acid molecule according to the present invention.

Said vector is preferably an expression vector.

The vector harbouring the nucleic acid molecule of the present invention may be used for cloning purposes or for the production of expression vectors. Said vector can be a plasmid, cosmid, virus, bacteriophage or any other vector commonly used in genetic engineering, and can include, in addition to the nucleic acid molecule of the invention, eukaryotic or prokaryotic elements for the control of the expression, such as regulatory sequences for the initiation and the termination of the transcription and/or translation, enhancers, promoters, signal sequences and the like.

According to a preferred embodiment of the present invention the vector is a bacterial, fungal, insect, viral or mammalian vector.

The vector of the present invention may preferably be employed for cloning and expression purposes in various hosts. Therefore, said vector comprises besides a nucleic acid encoding for a hypoallergenic molecule or fusion protein according to the present invention host specific regulatory sequences.

Another aspect of the present invention relates to a host comprising a nucleic acid molecule or a vector according to the present invention.

The nucleic acid molecule and the vector according to the present invention may be introduced into a suitable host. Said molecule may be incorporated into the genome of the host. The vector may exist extrachromosomally in the cytoplasm or incorporated into the chromosome of the host.

Yet another aspect of the present invention relates to an antibody directed against a hypoallergenic molecule, hypoallergenic fusion protein or a fusion protein according to the present invention.

According to a preferred embodiment of the present invention

the antibody is a monoclonal or polyclonal antibody.

Antibodies according to the present invention include, but are not limited to, polyclonal, monoclonal, multispecific, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments and epitope-binding fragments of any of the above. Furthermore, antibodies are considered to be immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. The immunoglobulin molecules of the invention are preferably of the types IgG, IgM, IgD, IgA and IgY, class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule.

Polyclonal antibodies can be prepared by administering a polypeptide of the invention, preferably using an adjuvant, to a non-human mammal and collecting the resultant antiserum. Improved titers can be obtained by repeated injections over a period of time. There is no particular limitation to the species of mammals which may be used for eliciting antibodies; it is generally preferred to use rabbits or guinea pigs, but horses, cats, dogs, goats, pigs, rats, cows, sheep, camels etc., can also be used. In the production of antibodies, a definite amount of immunogen of the invention is, e.g., diluted with physiological saline solution to a suitable concentration, and the resulting diluted solution is mixed with, e.g., complete Freund's adjuvant to prepare a suspension or with mineral gels such as aluminum hydroxide, surface active substances such as lysol-ecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. The suspensions and mixtures are administered to mammals, e.g., intraperitoneally, e.g., to a rabbit, using from about 50 µg to about 2,500 µg polypeptide of the invention per administration. The suspension is preferably administered about every two weeks over a period of up to about 2-3 months, preferably about 1 month, to effect immunization. The antibody is recovered by collecting blood from the immunized animal after the passage of 1 to 2 weeks after the last administration, centrifuging the blood and isolating serum from the blood.

Monoclonal antibodies may, e.g., be of human or murine ori-

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gin. Murine monoclonal antibodies may be prepared by the method of Köhler and Milstein (Köhler, G. and Milstein, C., Nature 256 (1975) 495), e.g., by fusion of spleen cells of hyperimmunized mice with an appropriate mouse myeloma cell line.

A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See, e.g., Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Gillies et al., (1989) J. Immunol. Methods 125:191-202; US 5,807,715; US 4,816,567 and US 4,816,397.

Humanized antibodies are antibody molecules from a non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and framework regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modelling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions (see, e.g., Queen et al., US 5,585,089; Riechmann et al., Nature 332:323 (1988)). Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; WO 91/09967; US 5,225,539; US 5,530,101; and US 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, Molecular Immunology 28(4/5):489-498 (1991); Studnicka et al., Protein Engineering 7(6):805-814 (1994); Roguska. et al., PNAS 91:969-913 (1994)), and chain shuffling (US 5,565,332).

The antibodies according to the present invention may advantageously be used for desensitization of an individual suffering from an allergy, in particular from house dust mite allergy. For passive immunisation the antibody is preferably an IgG or a derivative thereof (e.g., chimeric or humanized antibody). Furthermore, this antibody may also be used for desensib-

ilisation of an individual.

Another aspect of the present invention relates to a vaccine formulation comprising a hypoallergenic protein or an antibody according to the present invention.

The vaccine formulation according to the present invention may be formulated as known in the art and necessarily adapted to the way of administration of said vaccine formulation.

Preferred ways of administration of the vaccine formulation (of the present invention) include all standard administration regimes described and suggested for vaccination in general and allergy immunotherapy specifically (orally, transdermally, intravenously, intranasally, via mucosa, rectally, etc). However, it is particularly preferred to administer the molecules and proteins according to the present invention subcutaneously or intramuscularly.

The vaccine formulation according to the present invention may only comprise a viral capsid protein or fragments thereof of a member of the genus of rhinovirus

Said formulation preferably further comprises at least one adjuvant, pharmaceutical acceptable excipient and/or preservative.

In order to increase the immunogenicity of the hypoallergenic molecules according to the present invention, adjuvants, for instance, may be used in a medicament according to the present invention. An adjuvant according to the present invention is an auxiliary agent which, when administered together or in parallel with an antigen, increases its immunogenicity and/or influences the quality of the immune response. Hence, the adjuvant can, e.g., considerably influence the extent of the humoral or cellular immune response. Customary adjuvants are, e.g., aluminum compounds, lipid-containing compounds or inactivated mycobacteria.

Generally, adjuvants can be of different forms, provided that they are suitable for administration to human beings. Further examples of such adjuvants are oil emulsions of mineral or vegetal origin, mineral compounds such as aluminium phosphate or hydroxide, or calcium phosphate, bacterial products and derivatives, such as P40 (derived from the cell wall of *Corynebacterium granulosum*), monophosphoryl lipid A (MPL, derivative of LPS) and muramyl peptide derivatives and conjugates thereof (derivatives

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from mycobacterium components), alum, incomplete Freund's adjuvant, liposyn, saponin, squalene, etc. (see, e.g., Gupta R. K. et al. (Vaccine 11:293-306 (1993)) and Johnson A. G. (Clin. Microbiol. Rev. 7:277-289)).

According to another preferred embodiment of the present invention said formulation comprises 10 ng to 1 g, preferably 100 ng to 10 mg, especially 0.5 µg to 200 µg of said hypoallergenic molecule or antibody.

Another aspect of the present invention relates to the use of a hypoallergenic protein or an antibody according to the present invention for manufacturing a medicament for the treatment or prevention of a viral infection and/or an allergy in a human or animal.

Said medicament preferably further comprises at least one adjuvant, pharmaceutical acceptable excipient and/or preservative.

The medicament according to the present invention may be used for active (administration of the hypoallergenic protein and/or molecules of the invention) as well as for passive immunization (antibodies directed to the hypoallergenic protein and/or molecules of the invention).

According to a preferred embodiment of the present invention said medicament comprises 10 ng to 1 g, preferably 100 ng to 10 mg, especially 0.5 µg to 200 µg of said hypoallergenic molecule, nucleic acid molecule, vector, host or antibody.

The medicament is preferably administered to an individual in amount of 0.01 mg/kg body weight to 5 mg/kg body weight, preferably 0.1 mg/kg body weight to 2 mg/kg body weight.

The particular dosage regimen, i.e., dose, timing and repetition, will depend on the particular individual and that individual's medical history. Empirical considerations, such as the half life, will generally contribute to determination of the dosage. Frequency of administration may be determined and adjusted over the course of therapy.

The individual to whom the medicament according to the present invention is administered is preferably an individual or animal which is at risk to become an allergy.

Subjects having or at risk of having an allergic condition, disorder or disease include subjects with an existing allergic condition or a known or a suspected predisposition towards de-

veloping a symptom associated with or caused by an allergic condition. Thus, the subject can have an active chronic allergic condition, disorder or disease, an acute allergic episode, or a latent allergic condition, disorder or disease. Certain allergic conditions are associated with seasonal or geographical environmental factors. Thus, at risk subjects include those at risk from suffering from a condition based upon a prior personal or family history, and the season or physical location, but which the condition or a symptom associated with the condition may not presently manifest itself in the subject.

The administration of the medicament according to the present invention, which comprises at least one hypoallergenic molecule as described herein, to an individual may prevent sensitization of said individual or may induce an appropriate immune response to allergens. If the medicament of the present invention is used to prevent sensitization, it should be administered to an individual prior to the first contact with said allergen. Therefore, it is preferred to administer the medicament according to the present invention to neonates and children. It turned out that also the administration of the medicament according to the present invention to pregnant individuals will induce the formation of antibodies directed against allergens in the unborn child. It is especially beneficiary to use hypoallergenic molecules according to the present invention for such therapies, because due to the lack of T-cell epitopes side effects occurring in the course of allergen immunotherapy can significantly be reduced or even be completely avoided.

Yet another aspect of the present invention relates to the use of a hypoallergenic protein or an antibody according to the present invention for the diagnosis of an allergy and/or a viral infection in an individual.

Another aspect of the present invention relates to the use a viral capsid protein from a virus of the family of picornaviridae as a carrier in medicaments or vaccines or for diagnosing a viral infection, in particular common cold.

As a valuable alternative to the widely spread KLH carrier protein viral capsid proteins of viruses of the family of picornaviridae may be used. The carrier may be conjugated chemically or fused with recombinant techniques to peptides, proteins and polypeptides or other antigens. Furthermore, the viral capsid

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protein may be used to detect, e.g., antibodies directed to said capsid protein in the serum of an individual.

One of the advantages of such a carrier is that not only the antigen fused or conjugated thereon may be exposed to the immune system, but also an immune response against the capsid protein of a rhinovirus is induced. Consequently, such a vaccination leads to the prevention and/or treatment of diseases caused by rhinoviruses. The virus is preferably of the species of human rhinoviruses, in particular human rhinovirus 89 and 14.

Another aspect of the present invention relates to a hypoallergenic molecule derived from Phl p 5 (Genbank Nr. X7435) having a C- and/or N-terminal truncation and lacking substantially IgE-binding capacity.

Grass pollen is one of most potent outdoor seasonal sources of airborne allergens responsible for hay fever and allergic asthma.

More than 40% of allergic individuals display IgE-reactivity with grass pollen allergens, which are divided into more than 11 groups. More than 80% of the grass pollen allergic patients react with group 5 allergens.

Group 5 allergens are non-glycosylated, highly homologous proteins with a molecular mass range from 25-33kD. Several group 5 allergens have been cloned and/or immunologically characterized.

The trial to reduce the allergenic activity by introducing pointmutations, mutations of several amino acids in row or deletions showed no effect (Schramm G, et al. J Immunol 1999; 162: 2406-1435). IgE-binding regions of Phl p 5 (Flicker S, et al. J Immunol 2000; 165: 3849-3859) have already been described and the three-dimensional structure has been solved (Maglio O, et al. 2002. Protein Eng. 15:635-642).

It turned out that in particular the Phl p 5 peptides according to the present invention, which are C- and/or N-terminally truncated and lack IgE-binding capacity, may be employed for the active vaccination of individuals.

According to a preferred embodiment of the present invention the truncated molecule lacks T-cell epitopes.

As already outlined above, late side effects of allergen immunotherapy can be significantly reduced or even be avoided if the hypoallergenic molecules substantially lack T-cell epitopes.

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Truncated Phl p 5 molecules lacking T-cell epitopes are composed of amino acids 93 to 128, 98 to 128, 26 to 53, 26 to 58 or 252 to 283 of Phl p 5 or fragments or sequence variations thereof.

In particular these truncated molecules substantially show no T-cell epitopes and are, nevertheless, able to provoke an appropriate immune response directed against the wild-type allergen.

According to another preferred embodiment of the present invention the hypoallergenic truncated Phl p 5 is composed of amino acids 132 to 162, 217 to 246 or 176 to 212 of Phl p 5 or sequence variations thereof.

These hypoallergenic molecules comprise one or more T-cell epitopes but lack IgE-binding capacity.

Another aspect of the present invention relates to a hypoallergenic molecule derived from Fel d 1 (Genbank Nr. X62477 and X62478) having a C- and/or N-terminal truncation and lacking IgE-binding capacity.

Allergies to animals affect up to 40% of allergic patients. In the domestic environment, allergies to the most popular pets, cats and dogs, are particularly prevalent and connected with perennial symptoms. Animal allergens are present in dander, epithelium, saliva, serum or urine. Exposure to the allergens can occur either by direct skin contact or by inhalation of particles carrying the allergens. The major cat and dog allergens were shown to be present widespread and could even be detected in non-pet owning households and in public places, e.g., schools. This can be attributed to the high and increasing number of households keeping pets in industrialized countries (about 50%) and the high stability of the allergens, which are carried off and distributed.

Fel d 1 was identified as the major cat allergen, which is recognized by more than 90% of cat allergic patients. Fel d 1 represents a 38 kDa acidic glycoprotein of unknown biological function. It consists of two identical non-covalently linked heterodimers, which, again, are composed of two polypeptide chains antiparallely linked by three disulfide bonds. Chain 1 and chain 2 are encoded on different genes, each consisting of 3 exons. Recombinant Fel d 1 (rFel d 1), expressed as a chain 2 - to chain 1 fusion protein, has been generated in *E. coli*. This

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recombinant Fel d 1 is able to completely mimick the immunological properties of the wild-type allergen.

Peptides derived from the major cat allergen Fel d 1, and lacking IgE-binding capacity are suitable, e.g., for immunotherapy and prophylactic allergy vaccination. These peptides may be comprised in a larger polypeptide or be coupled to a suitable carrier protein such as keyhole limpet hemocyanin (KLH). The Fel d 1-derived synthetic peptides - like the Phl p 5 and allergen-derived peptides disclosed herein - are capable of inducing an IgG response, i.e., the production of so called "blocking antibodies" or "protective antibodies". These antibodies prevent IgE-binding to the allergen Fel d 1. A significant reduction in allergic symptoms may thus be achieved.

According to a preferred embodiment of the present invention the truncated molecule exhibits reduced T-cell reactivity.

In order to avoid or to significantly reduce late side effects the Fel d 1 derived hypoallergenic molecule exhibits reduced T-cell reactivity as defined in the present invention.

The truncated Fel d 1 is preferably composed of amino acids 1 to 34 or 35 to 70 of chain 1 of Fel d 1, amino acids 1 to 34, 35 to 63 or 64 to 92 of chain 2 of Fel d 1 or sequence variations thereof.

Another aspect of the present invention relates to hypoallergenic molecules being composed of or comprising amino acids 1 to 33, 21 to 51, 42 to 73, 62 to 103 or 98 to 129 of Der p 2, amino acids 1 to 30, 20 to 50, 50 to 80, 90 to 125, 125 to 155 or 165 to 198 of Der p 7, amino acids 1 to 35, 35 to 72, 70 to 100 or 90 to 122 of Der p 21, amino acids 1 to 32, 15 to 48 or 32 to 70 of Clone 30, amino acids 19 to 58, 59 to 95, 91 to 120 or 121 to 157 of Alt a 1, amino acids 31 to 60, 45 to 80, 60 to 96 or 97 to 133 of Par j 2, amino acids 1 to 40, 36 to 66, 63 to 99, 86 to 120 or 107 to 145 of Ole e 1, amino acids 25 to 58, 99 to 133, 154 to 183, 277 to 307, 334 to 363, 373 to 402, 544 to 573, 579 to 608, 58 to 99, 125 to 165, 183 to 224, 224 to 261, 252 to 289, 303 to 340, 416 to 457, 460 to 500 or 501 to 542 of Fel d 2, amino acids 19 to 58, 52 to 91, 82 to 119, 106 to 144 or 139 to 180 of Can f 2, amino acids 19 to 56, 51 to 90, 78 to 118, 106 to 145 or 135-174 of Can f 1, amino acids 27 to 70, 70 to 100 or 92 to 132 of Art v 1, amino acids 31 to 70, 80 to 120, 125 to 155, 160 to 200, 225 to 263, 264 to 300 305 to 350 or 356

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to 396 of Amb a 1, amino acids 1 to 34, 35 to 74, 74 to 115, 125 to 165, 174 to 213, 241 to 280, 294 to 333, 361 to 400 or 401 to 438 of Alt a 6, amino acids 1 to 40, 41 to 80, 81 to 120, 121 to 160 of Alt a 2 or fragments or sequence variations thereof.

Another aspect of the present invention relates to a hypoallergenic fusion protein comprising at least two hypoallergenic molecules according to the present invention exhibiting reduced IgE-binding capacity and exhibiting optionally reduced T-cell reactivity.

The hypoallergenic molecules of the present invention which are derived from an allergen and lack IgE-binding capacity may be fused recombinantly or conjugated chemically to each other. As single components (allergen fragments) of the fusion protein/polypeptide also said fusion protein/polypeptide lacks IgE-binding capacity.

The fusion protein according to the present invention may comprise at least two, preferably at least three, more preferably at least four, even more preferably at least five, hypoallergenic molecules according to the present invention. It is, of course, also possible to fuse the hypoallergenic molecules to other peptides, polypeptides and proteins not derived from allergens. These peptides, polypeptides and proteins may when administered to an individual also induce an immunologic reaction or may act as a carrier or exhibit enzymatic activities. The hypoallergenic molecules in the fusion protein according to the present invention may be coupled directly to each other or via a linker which is preferably composed of amino acid residues.

Methods for the production of fusion proteins are well known in the art and can be found in standard molecular biology references such as Sambrook et al. (Molecular Cloning, 2nd ed., Cold Spring Harbor Laboratory Press, 1989) and Ausubel et al. (Short Protocols in Molecular Biology, 3rd ed; Wiley and Sons, 1995). In general, a fusion protein is produced by first constructing a fusion gene which is inserted into a suitable expression vector, which is, in turn, used to transfect a suitable host-cell. In general, recombinant fusion constructs are produced by a series of restriction enzyme digestions and ligation reactions which result in the desired sequences being incorporated into a plasmid. If suitable restriction sites are not available, synthetic oligonucleotide adapters or linkers can be used as is known by

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those skilled in the art and described in the references cited above. The polynucleotide sequences encoding allergens and native proteins can be assembled prior to insertion into a suitable vector or the sequence encoding the allergen can be inserted adjacent to a sequence encoding a native sequence already present in a vector. Insertion of the sequence within the vector should be in frame so that the sequence can be transcribed into a protein. It will be apparent to those of ordinary skill in the art that the precise restriction enzymes, linkers and/or adaptors required as well as the precise reaction conditions will vary with the sequences and cloning vectors used. The assembly of DNA constructs, however, is routine in the art and can be readily accomplished by a person skilled in the art.

According to a preferred embodiment of the present invention the molecules are fused to each other in an order differing from the order of the fragments in the wild-type allergen if the at least two molecules are derived from the same allergen.

The fusion protein according to the present invention may comprise at least two hypoallergenic molecules which are derived from the same wild-type allergen. In such a case the single molecules (allergen fragments) are fused to each other in an order differing from the order in the wild-type allergen. Such an approach prevents the re-formation of potential IgE-binding sites/epitopes in the hypoallergenic fusion protein.

Another aspect of the present invention relates to a nucleic acid molecule coding for a hypoallergenic molecule and a fusion protein according to the present invention.

The nucleic acid molecule of the present invention may be employed, e.g., for producing said molecules recombinantly.

Said nucleic acid molecule may - according to another aspect of the present invention - be comprised in a vector.

This vector is preferably an expression vector.

Another aspect of the present invention relates to a vaccine formulation comprising a hypoallergenic molecule, a fusion protein or an antibody according to the present invention.

The formulation further comprises preferably at least one adjuvant, pharmaceutical acceptable excipient and/or preservative.

The use of particular carrier substances such as KLH (key-hole Limpet Hemocyanin) is also among the latest current methods

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to increase immune responses. The hypoallergenic molecules of the present invention may also be fused or conjugated to viral capsid proteins which may act also as a carrier (see above).

According to a preferred embodiment of the present invention said formulation comprises 10 ng to 1 g, preferably 100 ng to 10 mg, especially 0.5 μ g to 200 μ g of said hypoallergenic molecule or antibody.

The vaccine formulation may be substantially composed as the medicament of the present invention (see above).

Another aspect of the present invention relates to the use of a hypoallergenic molecule, a fusion protein, or an antibody according to the present invention for manufacturing a medicament for the treatment or prevention of an allergy in an individual.

The hypoallergenic molecule, fusion protein and antibody according to the present invention may be used for vaccination of an individual. This vaccination may reduce or prevent the allergic response caused by a wild-type allergen.

According to a preferred embodiment of the present invention said medicament further comprises at least one adjuvant, pharmaceutical acceptable excipient and/or preservative.

The medicament according to the present invention comprises preferably 10 ng to 1 g, preferably 100 ng to 10 mg, especially 0.5 μ g to 200 μ g of said immunogenic molecule, nucleic acid molecule, vector, host or antibody.

According to another preferred embodiment of the present invention the medicament is administered to an individual in the amount of 0.01 mg/kg body weight to 5 mg/kg body weight, preferably 0.1 mg/kg body weight to 2 mg/kg body weight.

According to a preferred embodiment of the present invention said individual is at risk to get an allergy.

Another aspect of the present invention relates to the use of a hypoallergenic molecule, a fusion protein or an antibody according to the present invention for diagnosing an allergy or monitoring the progress of an allergy therapy in an individual.

The hypoallergenic molecule, fusion protein or antibody according to the present invention may not only be used in medicaments but can also be suitably employed for various diagnostic purposes. For instance, these molecules and fusion proteins may be used for diagnosing an allergy by exposing, e.g., a sample of

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an individual comprising histamine releasing cells to said polypeptide (see, e.g., Purohit et al., Clin.Exp.Allergy 35 (2005): 186-192). Furthermore, these molecules, fusion proteins and antibodies may be immobilized on a surface in order to form a polypeptide array/chip. Such arrays may be used, e.g., in high throughput screening in order to diagnose an allergy in a number of samples taken from a number of individuals.

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

Fig. 1A shows a schematic overview of the vector p89VP1.

Fig. 1B shows the DNA sequence of the multiple cloning site of the pET-17b vector and the 89VP1 encoding gene.

Fig. 1C shows the schematic representation of three possibilities for creating nucleic acid fusions.

Fig. 2 shows a Coomassie blue stained 12% SDS-PAGE gel containing purified 89VP1 his-tagged protein (Lane 1: 5 µg molecular marker; Lane 2-5: 10 µl 89VP1 elution samples).

Fig. 3 shows IgG recognition of 14VP1: Immunoblotting of 14VP1 and controls. Dots are visualized by autoradiography (Lane 1-6: Incubation with 1:500-1:16000 diluted rabbit anti-14VP1 antiserum; Lane 7-12: Incubation with 1:500-1:16000 diluted preimmune serum).

Fig. 4 shows 89VP1-specific IgG1 response in mice. Groups of mice were immunized with different antigens as indicated on top of each box. 89VP1-specific IgG1 titers were measured by ELISA and are expressed as OD values on the y-axis. The results are shown as box plots, where 50% of the values are within the boxes and non-outliers between the bars. The line within the boxes indicates the median values.

Fig. 5 shows Phl p 1-specific IgG1 response in mice. Groups of mice were immunized with different antigens as indicated on top of each box. rPhl p 1-specific IgG1 titers were measured by ELISA and are expressed as optical value (OD 405nm) on the y-axis. The optical value corresponds to the level of IgG1 antibody in the mouse sera. The results are shown as box plots where 50% of the values are within the boxes and non-outliers between the bars. The line within the boxes indicates the median values.

Fig. 6 shows Timothy grass pollen extract-specific IgG1 response in immunized mice. Groups of mice were immunized with

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different antigens as indicated on top of each box. Timothy grass pollen extract-specific IgG1 titers were measured by ELISA and are expressed as optical value (OD 405nm) on the y-axis. The optical value corresponds to the level of IgG1 antibody in the mouse sera. The results are shown as box plots, where 50% of the values are within the boxes and non-outliers between the bars. The line within the boxes indicates the median values.

Fig. 7 shows the mean of %inhibition of patient's IgE-binding to rPhl p 1 by preincubation with antisera against rPhl p 1, r89P5 and KLHP5 of all 19 patients. The %inhibition is shown on the y-axis. The results are shown as bars.

Fig. 8 shows the proliferation of spleen cells of immunized mice. T-cells of immunized mice with different antigens as indicated on top of each box were stimulated with peptide 5, 89VP1(89) and KLH. Medium was used as a reference. At the y-axis the stimulation index is shown. The results are displayed in bars.

Fig. 9 shows IgG1, IgG2, IgG4 and IgA response to 14VP1, 89VP1 and rPhl p 1 detected in human sera by ELISA measurement. 10 patient's sera were tested for four antibodies specific for 89VP1, 14VP1 and rPhl p 1 as indicated on top of each box. Sera taken in autumn and winter are shown on the left hand and right hand of each "bar pair", respectively. Titers were measured by ELISA and are expressed as optical value (OD 405nm) on the y-axis. The optical value corresponds to the level of antibody in the human sera. The results are shown as box plots, where 50% of the values are within the boxes and non-outliers between the bars. The line within the boxes indicates the median values.

Fig. 10 shows the detection of anti-89VP1 and anti-rPhl p 1 antibodies in sera of allergic patients. 5 Phl p 1 allergic patient's sera were tested for seven antibodies specific for 89VP1 (left bar of each pair) and rPhl p 1 (right bar of each pair). Titers were measured by ELISA and are expressed as optical value (OD 405nm) on the y-axis. The optical value corresponds to the level of antibody in the human sera. The results are shown as box plots, where 50% of the values are within the boxes and non-outliers between the bars. The line within the boxes indicates the median values.

Fig. 11 shows anti-14VP1 IgG binding to HRV14 protein extract and purified 14VP1 (Lane 1 and 4: 5µg Marker; lane 2 and

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4: Virus extract; lane 2 and 5: 5 μ g 14VP1). Blot A und B was incubated with anti-14VP1 immune serum and preimmune serum, respectively. Bound IgG was detected by Western blotting and visualized by autoradiography.

Fig. 12 shows the neutralization of HRV14 (Lane A (cell control): Cells after preincubation of HRV14 with a dilution of anti-14VP1 immune serum 1:10²-1:10⁸ (row A1-A6); Lane B: Cells after preincubation of HRV14 with a dilution of preimmune serum 1:10²-1:10⁸ (row B1-B6); Lane C: cells after Incubation with HRV14 10 TCD50-10⁶ TCD50 (row C1- C6); D5: cells after incubation with preimmune serum; D6: cells after incubation with immune serum). Cells were plated out in all wells and colored with crystal violet after three days.

Fig. 13 shows IgG reactivity of anti-peptide antisera with the complete Phl p 5 allergen. IgG reactivities (OD values) to Phl p 5 are shown for 3 serum samples (bleeding 1: preimmune serum; bleedings 2-3: serum samples collected in monthly intervals) obtained from 6 rabbits, each of which was immunized with one of the KLH-conjugated peptides.

Fig. 14 shows allergenic activity of rPhl p 5, and the peptide mix as detected by CD203c expression. Heparinized blood samples from three patients allergic to Phl p 5 were incubated with serial dilutions from 10⁻⁴ to 10 μ g/mL of recombinant allergen, an equimolar mix of Phl p 5 derived peptides, anti-IgE or control buffer (co, x-axis). Cells were then stained with CD203c mAb and analyzed for CD203c expression on a FACScan. The stimulation index (SI) is displayed on the y-axis.

Fig. 15 shows identification of Phl p 5-derived peptides which induce low lymphoproliferative responses. PBMCs from timothy pollen allergic patients were stimulated with different concentrations of peptides and, for control purposes, with interleukin-2 (x-axis). Stimulation indices (SI) are indicated on the y-axis.

Fig. 16 shows Fel d 1-derived synthetic peptides which induce Fel d 1 specific IgG immune responses in rabbits. Six rabbits were immunized with the KLH-conjugated Fel d 1-derived synthetic peptides or unconjugated rFel d 1 and 3-4 bleedings were drawn in monthly intervals. IgG reactivities of the preimmune sera and the antisera to ELISA plate-bound rFel d 1 are shown as optical densities (O.D. values, y-axis).

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Fig. 17 shows the low allergenic activity of Fel d 1-derived synthetic peptides as determined by CD63 and CD203c expression on basophils of allergic patients. PBMCs from 5 cat allergic patients were incubated with serial dilutions of Fel d 1 (closed boxes) or a mixture of Fel d 1-derived synthetic peptides (open boxes) (x-axis). For patient RR PBMCs were also probed with serial dilutions of Fel d 1-derived synthetic peptides as single components. Induction of expression of surface markers CD203c and CD63 was measured as mean fluorescence intensities, and calculated stimulation indices are shown on the y-axis.

Fig. 18 shows that a treatment with KLH-coupled Bet v 1-derived peptides reduces lymphoproliferative responses to rBet v 1 in sensitized mice. T-cell proliferation was measured in spleen cell cultures after in vitro stimulation with the recombinant birch pollen allergen Bet v 1 (white bars), KLH (black bars), or the peptide mix (grey bars). The bars represent the mean stimulation indices (SI $\pm$ SD) for the different groups of mice.

Fig. 19 shows the prophylactic vaccination of naive mice with KLH-coupled Bet v 1-derived peptides which reduces lymphoproliferative responses to rBet v 1 after sensitization.

Fig. 20 shows that the prophylactic vaccination of naive mice with KLH-coupled Bet v 1-derived peptides induces a Bet v 1-specific IgG response and primes for the induction of allergen-specific IgG responses by the complete allergen. IgG responses (OD values: y-axis) to Bet v 1 were measured in the four treatment groups at different points of time (x-axis).

Fig. 21 shows a comparison of IgE-reactivity: IgE binding capacity of Phl p 5 derived peptides (1, 2) and variants (1a, 2b) was determined in dot blot assays applying 0.2 μ g/dot using sera from 7 grass-pollen allergic patients (p1-p7) and the serum from a non-atopic individual (NHS). rPhl p 5 was used as positive control and HSA as negative control. Bound IgE was detected with ¹²⁵I-labelled anti-human IgE.

Fig. 22 shows a lymphoproliferative responses of Phl p 5 derived peptides (1, 2) and variants (1a, 2b). PBMCs from grass pollen allergic patients were stimulated with different concentrations of peptides and, for control purposes, with equimolar concentrations of rPhl p 5. Stimulation indices (SI) are indicated on the y-axis.

Fig. 23 shows the cross protection of anti VP1 antibodies.

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EXAMPLES:*Example 1: Construction of vector p89VP1*

Virus stock samples were prepared for RT-PCR by addition of 1µl of RNase inhibitor (Boehringer GmbH, Germany) to a final concentration of 0.01 U/µl after RNA extraction from cell culture supernatants by QIAamp viral RNA kit (Qiagen, Germany).

Plasmid p89VP1 (Fig. 1A) was constructed by replacing the NdeI/EcoRI fragment of the multiple cloning site of pET-17b with the cDNA sequence encoding for the VP1 protein of human rhinovirus strain 89 (89VP1). The NdeI and EcoRI restriction sites (in pET-17b) were used for insertion of AseI/EcoRI linked 89VP1 with ATG at the 5' end, six histidin followed by the stop-codon TGA at the 3' end (Fig.1B).

The insertion of 89VP1 in pET-17b was confirmed by nucleotide sequencing.

After NdeI/AseI fusion instead of the NdeI site CATAAT was created and could not be cut with any available enzyme. Therefore, the site was mutated to CTTAAG, the restriction site of Afl II. To insert a further allergen fragment, the ACCGTT sequence at the 3' end was mutated to ACCGGT, the restriction site of AgeI. The amino acid sequences are displayed below the nucleotide sequences of 89VP1. The restriction sites are shown underlined in Fig. 1B.

Said Afl II and AgeI restriction sites were created with the Quick change site mutagenesis Kit (Stratagene).

cDNAs for gene fragments can thus be inserted for easy purification into the remaining restrictions sites either at the 5' end (using Afl II) or at the 3' end (using AgeI) of 89VP1 or into both sites as indicated in Figure 1C. Recombinant allergen fragments will thus be expressed at the N-terminus and/or the C-terminus of the 89VP1.

Table I: Cloning and mutagenesis primers (5' to 3')

89VP1 cloning		SEQ ID No.
89VP1forward	CGGAATTCATTAATATGAACCCAGTTGAAAAT- TATATAGATAGTGTATTA	1
89VP1 reverse	CGATTAAATTCAGTGGTGGTGGTGGTGGTG- GACGTTTGTAACGGTAA	2

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<i>89VP1 cloning</i>		<i>SEQ ID No.</i>
<i>Mutagenesis</i>		
Afl II for-ward	CTTTAAGAAGGAGATATACTTAAGATGAAC-CCAGTTG	3
Afl II re-verse	CAACTGGGTTTCATCTTAAGTATATCTCCTTCT-TAAAG	4
AgeI forward	CCTGATGTTTTTACCGGTACAAACGTCCACCAC	5
AgeI reverse	GTGGTGGACGTTTGTACCGGTAAAAACATCAGG	6

Example 2: Cloning of a construct expressing a 89VP1-allergen fragment fusion protein

The approach described above was exemplified for a C-terminal Phl p 1 allergen fragment, namely peptide 5 (CVRYTTEGGTK-TEAEDVIPEGWKADTAYESK; M. Focke et al. FASEB J (2001) 15:2042-4). The peptide 5 DNA sequence was amplified by PCR using the Phl p 1 cDNA (GenBank: X78813) as a template (Primers: Phl p 1 forward 5'-CGCGCTTAAGATGGTCCGCTACACCACCGAGGGC-3' (SEQ ID No. 7); Phl p 1 reverse 5'-CGCGCTTAAGCTTGGACTCGTAGGCGGTGTCGGC-3' (SEQ ID No. 8)). The PCR product was inserted into the Afl II restriction site of the p89VP1 vector, and the resulting construct is referred to as vector p89P5 and gene product r89P5.

Example 3: Expression and purification of 89VP1 peptide 5-fusion protein and 89VP1

In order to achieve expression of 89VP1 or r89P5 (recombinant 89VP1-peptide 5 fusion protein), plasmids were transformed into E. coli BL21 (DE3). Transformed E. coli cells were grown in 250 ml LB medium containing 100 mg/l ampicillin at 37°C to an optical density (600nm) of 0.4, and protein expression was induced by addition of isopropyl-beta-D-thiogalactosidase (IPTG) to a final concentration of 1 mM. E. coli cells were harvested after 4 hours by centrifugation at 3500 rpm at 4°C for 10 min. Purification was performed with the Qiagen protocol using Qiagen Ni-NTA and columns. The cell pellet was resuspended under denaturing conditions in 5 ml 6 M guanidinium hydrochloride for 1 hour. After centrifugation (20 min, 10000 x g) the supernatant was incubated with 1 ml Ni-NTA for an additional hour. The suspension was then loaded on a column, washed twice with 5 ml wash

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buffer (8 M urea pH 6.3) and then eluted with 4 ml elution buffer (8 M urea pH 3.5). Renaturation was achieved after dialysis with decreasing molarity of urea.

Purity and size of the purified protein were analyzed by SDS-PAGE as shown in Figure 2. Protein bands correlated with the calculated protein size of 33.6 kD. Integrity of the proteins was also confirmed by Western blot analysis, using anti-histidine tag antibody.

Example 4: Detection of 14VP1-specific rabbit antibodies by immunoblotting

5 µg 14VP1 (VP1 protein of the human rhinovirus strain 14) and controls (Bet v 1, Phl p 5, BSA) were dotted onto nitrocellulose membrane strips. These strips were exposed to a dilution of rabbit anti-14VP1 antiserum (lane 1-6) and preimmune serum (lane 8-12). Bound rabbit IgG antibodies were detected with ¹²⁵I-labeled donkey anti-rabbit IgG 1:1000 and visualized by autoradiography (Fig.3).

Dot blot analysis of rabbit anti-14 VP1 serum shows that 14VP1 is strongly immunogenic. IgG antibodies were still detected with a 1:16000 dilution of the antiserum in contrast to the preimmune serum and the Bet v 1, Phl p 5 and BSA controls.

Example 5: 89VP1-specific antibody response in immunized mice determined by ELISA

In order to determine the immunogenicity of 89VP1 and its ability to act as a carrier for peptide 5, groups of six week old female balb/c mice (Charles River) were immunized with the following antigens: KLH, KLH maleimide coupled peptide 5 (KLHP5) and KLH EDC coupled peptide 5 (KLHP5edc). The chemical coupling was made with the Imject maleimide activated mCKLH and Imject Immunogen EDC Kit (Pierce). The maleimide group reacts with SH groups and the EDC reacts with carboxyl and amino groups to form stable bonds. Groups of 4 mice were immunized with 89VP1, r89P5 and 89P5edc and 2 mice were immunized with peptide 5 only (5 µg each and mixed 1:2 with aluminium hydroxide). The mice were immunized subcutaneously in approximately three-week intervals and bled from the tail veins. The 89VP1-specific IgG1 antibody level was determined by ELISA.

ELISA plates (Nunc) were coated with 5 µg/ml 89VP1. The mice anti-89VP1, anti- r89P5 and peptide 5 sera were diluted 1:500. Bound IgG1 was detected with rat anti-mouse IgG1 (BD Pharmingen)

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1:1000 and then with goat anti rat IgG POX-coupled (Amersham Bioscience) 1:2000. The optical value (OD 405nm) is displayed on the y-axis and corresponds to the level of IgG1 antibody in the mouse sera (Fig.4).

KLHP5, KLHP5edc, KLH and peptide 5 were used as controls. IgG1 antibodies were detected with an increasing titer during immunization in mice injected with 89VP1 (89VP1, 89P5edc and r89P5). Rabbits immunized with 89VP1, r89P5 and KLHP5 show the same result.

Example 6: rPhl p 1-specific antibody response in immunized mice determined by ELISA

To evaluate whether immunization with r89P5 will induce IgG antibodies that react with complete Phl p 1, the same method and the same mice sera were used as described in example 5. ELISA plates were coated with 5 µg/ml rPhl p 1 and the IgG1 antibody titer was determined (Fig.5).

All Phl p 1 derived peptides either coupled to KLH or 89VP1 induced rPhl p 1 specific IgG1 antibodies with increasing responses during immunizations. Rabbits immunized with r89P5 and KLHP5 show the same result.

Example 7: ELISA detection of timothy grass pollen extract-specific IgG1 antibodies

Immunization of mice and ELISA analysis was performed as described in section 5. Whole timothy grass pollen extract was coated (100 µg/ml) on ELISA plates and the IgG1 antibody titer was determined (Fig.6).

After three immunizations extract-specific IgG1 antibodies could be detected in mice immunized with peptide 5.

Example 8: Rabbit anti-r89P5 antibodies block patient's IgE-binding to rPhl p 1

To determine the ability of peptide-induced rabbit Ig to inhibit the binding of allergic patients' IgE antibodies to rPhl p 1, ELISA plates were coated with 1µg/ml rPhl p 1, washed and blocked. The plates were preincubated with 1:100-diluted rabbit anti-peptide (89P5, KLHP5), a rabbit anti rPhl p 1 and, for control purposes, with the corresponding preimmune sera. After washing, plates were incubated with human sera from Phl p 1-allergic patients (1:3 diluted) and bound IgE was detected with mouse anti-human IgE (Pharmingen 1:1000) and then with sheep anti-mouse IgG POX-coupled (Amersham Bioscience) 1:2000.

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The percentage of inhibition of IgE-binding achieved by preincubation with the anti-peptide antisera was calculated as follows: $100 - OD_i / OD_p \times 100$.

OD_i and OD_p represent the extinctions after preincubation with the rabbit immune and preimmune serum, respectively. Table 2 shows the capacity of anti-Phl p 1 peptide antibodies to inhibit the binding of 19 allergic patients' IgE to complete rPhl p 1. Figure 7 displays the mean inhibition (in %) of all anti-rPhl p 1, anti-r89P5 and anti-KLHP5 immune sera. Anti-peptide sera blocked the IgE-binding much better than rPhl p 1. The ability for inhibition is with 89P5 and KLHP5 almost alike. Table 2 shows the inhibition (in %) of all 19 patients.

Table 2: %inhibition of 19 patients' IgE-binding to rPhl p 1 after incubation with rabbit anti-rPhl p 1, r89P5 and anti-KLHP5 antisera

<i>patient</i>	<i>%inhibition</i>		
	<i>rPhl p 1</i>	<i>r89P5</i>	<i>KLHP5</i>
1	32.343	68.291	68.213
2	29.373	64.915	61.509
3	10.367	59.469	66.270
4	28.087	73.906	71.330
5	13.808	49.358	45.372
6	22.597	66.259	67.331
7	5.375	26.667	18.902
8	22.478	42.612	47.979
9	5.019	39.822	56.837
10	13.756	53.878	63.047
11	26.444	58.430	57.944
12	25.795	67.243	62.458
13	41.330	75.694	79.517
14	35.543	85.714	87.012
15	45.796	84.255	75.185
16	32.641	76.508	77.412
17	26.483	63.171	47.735
18	19.229	85.750	86.642
19	31.142	62.428	71.086

Example 9: T-cell proliferation of mouse spleen cells after

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antigen stimulation

Groups of three mice were immunized with KLH, KLHP5 and KLHP5edc. Groups of 4 mice were immunized four times with 89VP1, r89P5 and 89P5edc, and 2 mice were immunized with peptide 5 only (5 µg each). Spleen cells were taken 10 days after the last immunization and single cell cultures were stimulated in triplicates in 96 well plates with peptide 5 (P5), 89VP1, KLH, Con A and a medium as a positive and negative control, respectively. After four days radioactive [³H]thymidine 0.5 µCi was added to each well. Cells were then harvested with a Packard Cell Harvester onto unifilter plates after 15 hours. Cell-associated radioactivity was measured with a beta-counter. Stimulation indices were calculated and are shown at the y-axis. The antigen which was used for stimulation is shown on the x-axis. Each box represents the data of the antigen which was used for immunization of the mice (Fig. 8). All values above two count as positive. The KLH and KLHP5 immunized mice are only positive when stimulated with KLH and the peptide 5 mice are completely negative. The KLHP5edc group is also negative which corresponds to the ELISA results. Cells from r89P5, 89P5edc and 89VP1 immunized mice proliferated only after stimulation with 89VP1. The naive control mouse shows no proliferation in all cases. These results show that T-cell epitopes are provided by the carrier 89VP1 and not by the peptide 5.

Example 10: Detection of 14VP1-, 89VP1- and rPhl p 1-specific antibodies in human sera obtained in autumn and winter by ELISA

Five human sera of randomly chosen persons were taken in autumn and five in winter. The IgG1, IgG2, IgG4 and IgA antibody level against 14VP1, 89VP1 and rPhl p 1 was determined by ELISA as described in example 5. Human IgG1, IgG2, IgG4 and IgA were detected (BD Pharmingen) 1:1000 with sheep anti mouse IgG POX-coupled (Amersham Bioscience) 1:2000. A high anti-14VP1 and 89VP1 IgG1 titer of sera taken in autumn and winter could be detected (Fig. 9). The anti-rPhl p 1 IgG1 antibody titer was much lower. IgG2, IgG4 and IgA antibodies could be detected in all cases at a very low level. The VP1 proteins of the different HRV strains are closely related and cross-reactivity has been shown in other studies.

Example 11: Anti- 89VP1 and anti-rPhl p 1 antibodies of

Phl p 1 allergic patients

Sera of five Phl p 1 allergic patients were taken and an ELISA experiment was performed as described in example 5. ELISA plates were coated with rPhl p 1 and 89VP1 and the specific IgM, IgG1, IgG2, IgG3, IgG4, IgA and IgE antibody titer were determined (Fig. 10). More anti-89VP1 IgG1 antibodies than anti-rPhl p 1 IgG1 antibodies could be detected.

Example 12: Detection of anti-14VP1 antibody binding to the whole virus by Western blot analysis

The IgG antibody binding of sera of the 14VP1 injected rabbit to the whole virus was confirmed by using the whole HRV14 virus (lane 2 and 5) and 5 µg purified 14VP1 (lane 3 and 6) as control. The virus extract was separated by 12% SDS-Page and blotted onto nitrocellulose membrane. Rabbit anti-14VP1 antiserum (lane 1-3) 1:500 and preimmune serum (lane 4-6) 1:500 were tested for binding to HRV14 and 14VP1. Bound IgG was detected with ¹²⁵I-labelled donkey anti-rabbit antibody and visualized by autoradiography (Fig.11).

The binding of 14VP1-antiserum could be detected at the same size (33,6 kD) as 14VP1.

Example 13: Anti-14VP1 antibodies neutralization of intact human rhinovirus 14

The tissue culture dose 50 (TCD50) of HRV14 was determined. Therefore, a virus dilution from 1:10²-1:10⁸ in MEM-Eagle 1% FCS and 40mM MgCl₂ was performed and incubated in 24 well plates at 34°C in a humidified 5% CO₂ atmosphere together with HeLa Ohio cells for three days. A control without the virus was also spread out.

The cytotoxic effect of the virus was visualized with crystal violet after three days and the TCD50 (the dilution where 50% of the cells are dead) was calculated.

Serum dilutions and virus (100TCD50) in row A and B were incubated at 34 °C. After 2 hours cells were spread out in all wells. D5 and D6 are serum controls. The experimental schema is shown in Fig. 12. The neutralization effect of the antibodies was detected after three days with crystal violet (Fig. 12).

Example 14: Characteristics of Phl p 5-derived synthetic peptides

Peptides were synthesized using Fmoc-strategy with HBTU-activation (0.1 mmol small-scale cycles) on the Applied Biosystems

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peptide synthesizer Model 433A as described. (Focke et al. Faseb J (2001) 15:2042). After in-depth analysis of the Phl p 5 allergen six Phl p 5-derived peptides ranging from 31 (P1: 3026 Dalton) to 38 (P6: 3853 Dalton) amino acids in length which are rich in solvent-exposed amino acids were prepared (Table 3).

These peptides have isoelectric points between 4.32 and 8.98 and three of them (peptide 3, 4 and 6) may contain human T-cell epitopes.

Table 3. Characteristics of non-allergenic Phl p 5-derived synthetic peptides. Position (in relation to the Phl p 5 molecule), sequence, length, molecular weight (MW), isoelectric point (pI) and presence of T-cell epitopes of the Phl p 5-derived peptides are displayed. The cysteine residue added to facilitate the coupling is marked in bold and underlined.

	<i>aa posi- tion</i>	<i>Sequence</i>	<i>aa length</i>	<i>MW</i>	<i>pI</i>	<i>T-cell epi- tope</i>
Pept. 1 (SEQ ID No. 9)	98- 128	<u>C</u> GAASNKAFAEGLSGEP- KGAAESSSKAALTSK	32	3026	8,16	-
Pept. 2 (SEQ ID No. 10)	26-58	ADLGYPATPAAPAAGYT- PATPAAPAEAAPAGK <u>C</u>	34	3068	4,37	-
Pept. 3 (SEQ ID No. 11)	132- 162	AYKLAYKTAEGATPEAKY- DAYVATLSEALRI <u>C</u>	32	3482	6,29	+
Pept. 4 (SEQ ID No. 12)	217- 246	<u>C</u> EAAFNDAIKASTG- GAYESYKFIPALEAAVK	31	3236	4,87	+
Pept. 5 (SEQ ID No. 13)	252- 283	TVATA- PEVKYTVFETALKKAITAM- SEAQKAAK <u>C</u>	33	3501	8,98	-
Pept. 6 (SEQ ID No. 14)	176- 212	<u>C</u> AEEVKVIPAGELQVIEK- VDAAFKVAATAANAAPANDK	38	3853	4,66	+

Example 15: Phl p 5-derived peptides lack IgE reactivity and allergenic activity

15.1. Lack of IgE reactivity

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To analyze the IgE reactivity of the six Phl p 5-derived peptides, the isolated as well as KLH-coupled Phl p 5-derived peptides were compared with complete rPhl p 5 regarding IgE-binding capacity by ELISA using sera from 29 grass pollen allergic patients (Table 4).

Table 4: Serological characterization of 29 grass pollen allergic patients and a non-allergenic control. Sex, age, total serum IgE levels (kU/L), timothy extract-specific IgE (kUA/L), IgE antibodies specific for rPhl p 5 and the 6 isolated (P1-P6) and KLH-coupled (KLH-P1-KLH-P6) peptides were measured by ELISA and ODs (optical densities) are shown. Dashes indicate the lack of IgE reactivity to the isolated as well as to the KLH-coupled peptides.

Patient	sex	age	total IgE (kU/L)	IgE timothy kUA/L)	IgE (OD)						
					rPhl p 5	P1	P2	P3	P4	P5	P6
1	m	29	140.0	25.90	1.437	-	-	-	-	-	-
2	m	39	76.2	10.50	0.456	-	-	-	-	-	-
3	f	29	100.0	33.50	0.699	-	-	-	-	-	-
4	f	31	261.0	28.10	0.930	-	-	-	-	-	-
5	m	33	380.0	32.00	0.545	-	-	-	-	-	-
6	f	31	278.0	37.00	1.720	-	-	-	-	-	-
7	m	43	128.0	20.70	1.118	-	-	-	-	-	-
8	f	29	200.0	18.40	0.489	-	-	-	-	-	-
9	f	34	76.6	18.70	0.571	-	-	-	-	-	-
10	m	35	144.0	39.30	0.157	-	-	-	-	-	-
11	f	33	79.2	29.60	0.574	-	-	-	-	-	-
12	f	30	30.3	10.70	0.350	-	-	-	-	-	-
13	f	34	106.0	20.80	0.395	-	-	-	-	-	-
14	f	52	448.0	43.00	1.320	-	-	-	-	-	-
15	f	25	294.0	95.50	1.638	-	-	-	-	-	-
16	m	30	471.0	82.60	0.752	-	-	-	-	-	-
17	m	44	2000.0	100.00	2.500	-	-	-	-	-	-
18	f	30	168.0	66.60	0.806	-	-	-	-	-	-
19	m	42	512.0	50.30	1.175	-	-	-	-	-	-
20	f	28	253.0	54.00	1.954	-	-	-	-	-	-
21	m	30	315.0	100.00	1.054	-	-	-	-	-	-
22	f	42	401.0	89.50	2.297	-	-	-	-	-	-
23	f	28	100.0	82.10	1.802	-	-	-	-	-	-
24	m	42	52.5	3.52	0.885	-	-	-	-	-	-
25	m	34	136.0	6.11	2.036	-	-	-	-	-	-
26	m	30	31.2	9.17	1.909	-	-	-	-	-	-
27	m	36	24.9	4.34	0.233	-	-	-	-	-	-
28	f	41	41.5	2.19	0.281	-	-	-	-	-	-
29	f	51	370.0	90.10	1.296	-	-	-	-	-	-
NHS	m	39	0.0	0.00	0.065	-	-	-	-	-	-

ELISA plates (Nunc Maxisorp, Denmark) were coated with Phl p 5-derived peptides (5 µg/ml) or rPhl p 5 as control (5 µg/ml), washed and blocked. Subsequently, plates were incubated with 1:3 diluted sera from 29 grass pollen allergic patients and from a non-atopic individual overnight at 4°C. Grass pollen allergic patients suffering from allergic rhinoconjunctivitis and/or asthma were selected according to case history indicative for seasonal grass pollinosis and characterized by skin prick testing with timothy pollen extract and serological CAP-FEIA (Pharmacia Diagnostics, Uppsala, Sweden) testing. Total IgE levels in the sera were determined by CAP measurements (Pharmacia). IgE

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antibodies specific for rPhl p 5 were determined by ELISA. Sera from 29 grass pollen allergic patients and a non-atopic individual were used for IgE competition studies. The grass pollen allergic patients group consisted of 13 males and 16 females with a mean age of 35 years (ranging from 25-51 years) (Table 4).

Bound IgE antibodies were detected with a 1:1000 diluted alkaline-phosphatase-coupled mouse monoclonal anti-human IgE antibody (Pharmingen, CA).

Total IgE levels and grass pollen extract-specific IgE ranged from 24.9-2000 kU/L (mean: 262.7) and 2.2-100 kUA/L (mean: 41.5), respectively. All patients contained rPhl p 5-specific IgE antibodies ranging between 0.157-2.530 OD units (mean: 1.082 OD units), but none of the 29 patients showed IgE reactivity to any of the peptides (P1-P6) or to an equimolar mix of the peptides ($OD \leq 0.08$). This result demonstrates that no serum contained detectable IgE antibodies with specificity for any of the six Phl p 5 derived peptides.

15.2. Reduced allergenic activity of the peptides as detected by CD 203 c expression on basophils: Basophil activation and flow cytometry:

The upregulation of CD 203 c has been described as a surrogate marker for allergen-induced basophil activation and degranulation (Hauswirth et al., J Allergy Clin Immunol 2002; 110:102). Therefore, the allergenic activity of complete rPhl p 5 allergen and an equimolar mix of peptides by measuring CD 203 c upregulation on basophils of grass pollen allergic patients was compared.

Peripheral blood cells were obtained from 3 allergic donors after informed consent had been given. Heparinized blood aliquots (100 μ l) were incubated with serial dilutions of recombinant allergens (10^{-4} to 10 μ g/ml), anti-IgE antibody (1 μ g/ml) or control buffer (phosphate-buffered saline = PBS) for 15 minutes at 37°C. After incubation, cells were washed in PBS containing 20 mM EDTA. Cells were then incubated with 10 μ l of PE-conjugated CD203c mAb 97A6 for 15 minutes at room temperature (RT). Thereafter, erythrocytes were lysed with 2 mL FACS™ Lysing Solution. Cells were then washed, resuspended in PBS, and analyzed by two-color flow cytometry on a FACScan (Becton Dickinson), using Paint-a-Gate Software. Allergen-induced upregulation of

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CD203c was calculated from mean fluorescence intensities (MFIs) obtained with stimulated (MFIstim) and unstimulated (MFIcontrol) cells, and expressed as stimulation index (MFIstim : MFIcontrol). An SI of ≥ 2.0 (≥ 2 -fold upregulation) was considered indicative of a specific response.

As shown in Figure 14 it was found that complete rPhl p 5 shows a dose-dependent (10^{-4} to $10 \mu\text{g/mL}$) increase in expression of CD203c on peripheral blood basophils in a sensitized individual, whereas an equimolar mix of the peptides shows no effect.

Determination of CD 203c expression on basophils from grass-pollen allergic patients indicates that no allergenic activity can be observed with the Phl p 5 derived peptides.

Example 16: Immunization with Phl p 5 derived peptides induces IgG antibodies reactive with rPhl p 5 and natural allergens from different grass species

16.1. Recombinant allergens and allergen extracts

Purified recombinant Phl p 5 were expressed in *E. coli* as described (Vrtala et al. J of Immunol (1993) 151:4773-4781).

Grass pollen from *Phleum pratense*, *Lolium perenne*, *Poa pratensis*, *Dactylis glomerata*, *Secale cereale*, *Triticum aestivum*, *Avena sativa*, *Hordeum vulgare*, *Anthoxanthum odoratum* were obtained from Allergon Pharmacia (Sweden), and an aqueous pollen extract was prepared as described (Vrtala et al., Int Arch Allergy Immunol (1993) 102:160-9.).

16.2. Immunization of rabbits

HPLC-purified peptides were coupled to KLH (keyhole limpet hemocyanin, MW 4.5×10^3 - 1.3×10^7 , Pierce, USA) according to the manufacturers advice and purified using a Conjugation Kit (Sigma, USA).

Rabbits were immunized with each of the KLH conjugated-peptides ($200 \mu\text{g/injection}$) and, for control purposes, with complete rPhl p 5 using Freund's complete and incomplete adjuvant (Charles River). Serum samples were obtained in four week intervals.

16.3. Reactivity of rabbit antibodies with complete rPhl p 5 and natural allergens from different grass species

In order to investigate whether antibodies induced after immunization with KLH-coupled peptides recognize the rPhl p 5, natural Phl p 5 and Phl p 5-related grass pollen allergens from

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other grass pollen species, ELISA experiments were performed. For ELISA detection, plates (Nunc Maxisorp, Denmark) were coated with pollen allergen extracts (100 µg/ml: *Phleum pratense*, *Lolium perenne*, *Poa pratensis*, *Dactylis glomerata*, *Secale cereale*, *Triticum aestivum*, *Avena sativa*, *Hordeum vulgare*, *Anthoxanthum odoratum*) or purified recombinant allergen (5 µg/ml: rPhl p 5). ELISA plates were washed and blocked and subsequently incubated with rabbit anti-peptide antisera and corresponding pre-immun-sera diluted 1:2500. Bound rabbit IgG was detected with a HRP-coupled goat anti-rabbit Ig antiserum (Jackson Immunresearch, Pennsylvania). Results represent means of duplicate determination with an error of <5% (Figure 13, Table 5).

Table 5: Crossreactivity of anti-Phl p 5 peptide antisera with rPhl p 5 and natural group 5 allergens from *Phleum pratense*, *Lolium perenne*, *Poa pratensis*, *Dactylis glomerata*, *Secale cereale*, *Triticum aestivum*, *Avena sativa*, *Hordeum vulgare*, *Anthoxanthum odoratum*. IgG reactivities (OD values) of peptide antisera (anti-P1 to anti -P6) to Phl p 5 and pollen extracts from grass pollen are displayed for 6 rabbits which were immunized with KLH-conjugated Phl p 5-derived peptides (P1-P6).

**Crossreactivity of anti-peptide antisera with rPhl p 5
and grass pollen extracts**

	anti-P1	anti-P2	anti-P3	anti-P4	anti-P5	anti-P6
rPhl p 5a	1.115	2.418	1.336	1.600	1.540	2.142
<i>Phleum pratense</i>	0.227	1.155	0.955	0.703	1.138	1.000
<i>Lolium perenne</i>	0.056	1.320	0.834	0.238	0.163	2.500
<i>Poa pratensis</i>	0.070	1.491	1.045	1.880	2.200	2.500
<i>Dactylis glomerata</i>	0.060	0.390	0.728	0.689	0.154	0.657
<i>Secale cereale</i>	0.090	0.292	0.777	0.676	0.162	0.843
<i>Triticum aestivum</i>	0.116	1.076	0.734	0.404	0.570	0.703
<i>Avena sativa</i>	0.150	0.790	1.029	0.551	0.224	1.494
<i>Anthoxanthum</i>	0.114	1.209	1.531	0.827	1.114	1.115
<i>Hordeum vulgare</i>	0.080	1.972	1.150	1.184	0.602	1.513

16.4. Immunization with Phl p 5-derived peptides induces

cross-reactive IgG antibodies

Immunization with each of the peptides induced a robust Phl p 5-specific IgG response which became detectable four weeks after the first immunization and increased after the second immunization (Figure 13). Immunization with peptide 2 induced the highest Phl p 5 specific IgG response followed by peptides 6, 4, 5 and 1 which induced the lowest response. With the exception of anti-peptide 1 antibodies which lacked reactivity with group 5 allergens in *Lolium perenne*, *Poa pratensis*, *Dactylis glomerata*, *Secale cereale* and *Hordeum vulgare*, the other peptide antisera cross-reacted with each of the grass pollen extracts tested (Table 5).

Example 17: Immunization with Phl p 5-derived peptides induces IgG antibodies which inhibit the binding of grass pollen allergic patients IgE to Phl p 5

17.1. Inhibition of allergic patients' IgE-binding to rPhl p 5a by peptide-specific IgG

Information regarding the capacity of the peptides to induce blocking antibodies is important since blocking antibodies were shown to play a major role in immunotherapy.

In order to examine the ability of peptide-induced rabbit Ig to inhibit the binding of allergic patients' IgE to complete rPhl p 5 ELISA, competition experiments were performed using sera from 29 grass allergic patients.

ELISA plates were coated with rPhl p 5 (1 µg/ml) and preincubated either with a 1:250 dilution of each of the anti-peptide antisera (anti-P1-anti-P6), a mixture of the anti-peptide antisera, an anti-rPhl p 5 antiserum or for control purposes, with the corresponding preimmune sera or a mixture of the preimmune sera. After washing, the plates were incubated with 1:3 diluted sera from 29 grass pollen allergic patients and bound IgE antibodies were detected with the alkaline phosphatase-coupled mouse monoclonal anti-human IgE antibody (Pharmingen). The percentage inhibition of IgE-binding achieved by preincubation with the anti-peptide antisera was calculated as follows: %inhibition of IgE-binding = $100 - OD_I / OD_P \times 100$. OD_I and OD_P represent the extinctions after preincubation with the rabbit's immune and preimmune serum, respectively. Preincubation of Phl p 5 with peptide-induced rabbit IgG inhibited allergic patients IgE reactivity to a

varying degree. The average degree of inhibition of IgE binding ranged from 19.3% for anti-peptide 6 IgG to 28.5% with anti-peptide 1 IgG. Rabbit antibodies raised against complete Phl p 5 induced a mean inhibition of IgE binding of 43.6%.

Table 6: Rabbit anti-Phl p 5 peptide antisera inhibit serum IgE-binding of timothy pollen allergic patients to Phl p 5. The percentage inhibition of IgE-binding to Phl p 5 is displayed for each patient after preincubation of Phl p 5 with anti-peptide antisera (anti-P1 - anti-P6), with a mix of the six anti-peptide antisera (anti-P1-P6) or with an anti-rPhl p 5 antiserum. The mean percentage inhibition is displayed in the bottom line. N.d.: not done.

Patient	% Inhibition of IgE-binding to Phl p 5 with antisera specific for							
	P1	P2	P3	P4	P5	P6	P1-P6	Phl p 5
1	0	5	4	0	0	0	0	nd
2	1	10	4	0	0	0	0	nd
3	28	35	28	39	37	38	46	50
4	33	40	33	42	35	45	54	20
5	0	0	3	8	8	0	9	nd
6	46	34	39	47	47	34	21	56
7	41	48	46	49	50	45	49	60
8	41	8	34	18	39	0	0	8
9	6	0	0	0	0	0	0	nd
10	34	46	30	35	39	42	48	38
11	28	0	32	0	28	9	0	nd
12	33	0	27	4	33	0	0	nd
13	30	31	29	0	29	11	15	0
14	46	14	49	17	47	2	8	15
15	48	44	55	22	46	25	23	72
16	41	0	44	24	41	19	8	28
17	52	71	50	57	49	59	73	82
18	43	17	42	0	32	0	10	0
19	5	17	19	16	7	0	4	nd
20	42	54	43	38	38	41	48	65
21	39	51	46	43	43	43	40	39
22	44	49	44	46	44	40	50	70
23	38	54	40	42	48	40	50	66
24	23	0	15	0	0	0	0	nd
25	0	35	0	8	4	28	43	nd
26	51	26	31	21	24	0	19	nd
27	14	15	3	0	9	11	25	nd
28	9	0	17	0	9	0	0	nd
29	10	44	11	31	21	28	24	73
mean	28.5	25.8	28.2	20.9	27.8	19.3	23.0	43.6

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Example 18: Phl p 5-derived peptides induce low specific lymphoproliferative responses.

18.1. Lymphoproliferation assays

In order to identify peptides with the lowest possible T-cell reactivity to minimize therapy-related side effects, the T-cell reactivity was examined by lymphoproliferation assays. Peripheral blood mononuclear cells (PBMC) were isolated from 2 grass pollen allergic patients by Ficoll (Amersham Pharmacia Biotech, UK) density gradient centrifugation. PBMC (2×10^5) were cultured in triplicates in 96-well plates (Nuncclone; Nalge Nunc International, Denmark) in 200 μ l serum-free Ultra Culture medium (BioWhittaker, Rockland, ME) supplemented with 2 mM L-glutamine (SIGMA, USA), 50 μ M beta-mercaptoethanol (SIGMA) and 0.1 mg gentamicin per ml (SIGMA) at 37°C and 5% CO₂ in a humidified atmosphere. Cells were stimulated with different concentrations of synthetic peptides (1.25, 0.6 and 0.3 μ g per well) and, for comparison, with 4 U Interleukin-2 per well (Boehringer Mannheim, Germany) and with medium alone. After 6 d culture 0.5 μ Ci per well [³H]thymidine (Amersham Pharmacia Biotech) was added and 16 h thereafter incorporated radioactivity was measured by liquid scintillation counting using a microbeta scintillation counter (Wallac ADL, Germany). Mean cpm were calculated from the triplicates, and stimulation indices (SI) were calculated as the quotient of the cpm obtained by antigen or interleukin-2 stimulation and the unstimulated control.

PBMCs from timothy pollen allergic patients were stimulated with different concentrations of synthetic peptides. Stimulation indices with peptides were significantly lower than with IL2. Phl p 5-derived peptides induced low specific lymphoproliferative responses. The lowest response was seen with peptide 5 followed by peptide 4.

Example 19: Characteristics of Fel d 1-derived synthetic peptides

In order to obtain peptides suitable for cat allergy vaccination, five peptides which are 30 to 36 amino acids long and cover the whole molecule were designed according to the known amino acid sequence of Fel d 1.

Peptides were synthesized using a Fmoc (9-fluorenylmethoxycarbonyl)-strategy with HBTU (2-(1H-benzotriazol-1-yl) 1,1,3,3-tetramethyluronium hexafluorophosphate)-activation (0.1 mmol

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small-scale cycles) on the Applied Biosystems peptide synthesizer Model 433A (USA). Preloaded PEG-PS (polyethyleneglycol polystyrene) resins (0.15-0.2 mmol/g loading) (PerSeptive Biosystems, UK) were used as solid phase to build up the peptides. Chemicals were purchased from Applied Biosystems. Coupling of amino acids was confirmed by conductivity monitoring in a feedback control system. One cystein residue was added to peptides 1, 3, 4, and 5 to facilitate coupling to carriers (Table 7). Peptides were cleaved from the resins with a mixture of 250 µl distilled water, 250 µl triisopropylsilan (Fluka, Switzerland), 9.5 ml TFA for 2h and precipitated in tert-butylmethylether (Fluka, Switzerland) (Focke 2001). The identity of the peptides was checked by mass-spectrometry and peptides were purified to >90% purity by preparative HPLC (PiChem, Austria).

Table 7: Molecular characteristics of Fel d 1-derived synthetic peptides. Position within the native Fel d 1 molecule, amino acid sequence, number of amino acids, calculated molecular weight (MW) and theoretical isoelectric point (pI) of the Fel d 1-derived synthetic peptides are shown. All peptides are soluble in water.

	Position	Amino acid sequence	aa length	MW	pI
Pept. 1 SEQ ID No. 15	chain 1, aa 1-34	EICPAVKRDVDLFLTGTP- DEYVEQVAQYKALPVVC	35	3911	4.30
Pept. 2 SEQ ID No. 16	chain 1, aa 35-70	LENARILKNCVDAKMTEEDKEN- ALSLLDKIYTSPLC	36	4083	4.72
Pept. 3 SEQ ID No. 17	chain 2, aa 1-34	VKMAITCPIFYDVFFAVANG- NELLLDLSLTKVNAC	35	3835	4.56
Pept. 4 SEQ ID No. 18	chain 2, aa 35-63	TEPERTAMKKIQDCYVENG- LISRVLDGLVC	30	3382	4.93
Pept. 5 SEQ ID No. 19	chain 2, aa 64-92	CMTTISSSKD- CMGEAVQNTVEDLKLNTLGR	30	3246	4.78

The five Fel d 1-derived synthetic peptides have molecular weights in the range of 3246 to 4083 Dalton and have calculated isoelectric points of from 4.30 to 4.93. All five peptides are watersoluble and Peptides 1, 2 and 3 may contain human T-cell epitopes (Table 7).

Table 8. Reduced IgE reactivity of Fel d 1-derived synthetic peptides compared to rFel d 1

Patient	sex	age	cat							
			total	dander	rFel d	Peptide	Peptide	Peptide	Peptide	Peptide
			IgE	specific	1	1	2	3	4	5
			(kU/l)	IgE (kUA/l)	(O.D.)	(O.D.)	(O.D.)	(O.D.)	(O.D.)	(O.D.)
1	f	36	>2000	48.2	2.314	0.112	-	-	-	0.056
2	m	27	798	62.2	2.255	0.069	0.123	-	-	0.140
3	m	33	153	9.48	1.394	-	-	-	-	-
4	m	25	122	13.2	1.194	1.998	0.113	0.186	-	0.073
5	f	42	267	42.1	1.793	0.074	-	-	-	0.677
6	f	35	494	37.0	2.007	-	-	-	-	0.204
7	m	27	129	31.3	2.259	-	-	-	-	0.149
8	m	36	1150	13.5	1.384	-	-	-	-	0.130
9	f	32	580	17.3	0.569	-	-	-	-	-
10	f	22	189	4.65	0.553	0.051	-	-	-	0.057
11	f	53	>2000	>100	2.838	0.504	-	-	-	0.644
12	f	75	4567	47,3	2.519	-	0.060	-	-	0.161
13	m	34	>2000	40.0	1.244	-	-	-	-	-
14	m	n.d.	n.d.	1.99	0.178	-	-	-	-	-
NHS	f	27	<2	<0.35	-	-	-	-	-	-

Example 20: Fel d 1-derived synthetic peptides have reduced IgE reactivity compared to rFel d 1 and Fel d 1-derived synthetic peptides lack allergenic activity

Serum IgE reactivity to the Fel d 1-derived synthetic peptides in order to identify hypoallergenic peptides suited for vaccination was investigated.

The diagnosis of IgE-mediated cat allergy was based on anamnesis, skin prick testing (Allergopharma, Reinbek, Germany) and measurement of total serum IgE as well as of cat dander-specific serum IgE (CAP-FEIA, Pharmacia Diagnostics, Sweden). Non-allergic persons were included for control purposes.

20.1. IgE-binding capacity measured in ELISA assays

The IgE-binding capacity of the five Fel d 1-derived syn-

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thetic peptides was compared with that of the complete rFel d 1 allergen using sera from 14 cat allergic patients. ELISA plates (Nunc Maxisorb, Denmark) were coated with Fel d 1-derived synthetic peptides or rFel d 1 as control (0.5 µg/well), washed and blocked. Plates were then incubated overnight at 4°C with 1:5 diluted sera from cat allergic patients and from a non-atopic individual. Bound IgE antibodies were detected with a 1:2500 diluted horse-raddish-peroxidase labeled anti-human IgE antibody (KPL, USA).

Sera from 7 female and 7 male cat allergic patients at the age of 22 to 75 years were subjected to CAP-FEIA determinations. Measured total IgE levels ranged from 122 to >4000 kU/l and cat dander specific IgE levels from 1.99 to >100 kUA/l (Table 7). In ELISA assays the IgE reactivity of all 14 tested sera to the major cat allergen Fel d 1 was confirmed. Results were obtained as optical densities (OD) and ranged from 0.178 to 2.838 OD units. IgE reactivity of the 14 sera to Fel d 1-derived synthetic peptides was measured in the same ELISA assay. It was found that IgE-binding was retained for Peptides 1, 2, 3, and 5. IgE-binding was observed for 6/14 sera to Peptide 1, for 3/14 sera to Peptide 2, for 1/14 sera to Peptide 3 and for 10/14 sera to Peptide 5. Measured OD units were between 0.051 and 1.998 for Peptide 1, between 0.060 and 0.123 for Peptide 2, 0.186 for Peptide 3 and between 0.056 and 0.677 for Peptide 5. In summary, all measured OD units were considerably lower than the respective values measured for the whole Fel d 1 allergen.

This demonstrates that Fel d 1-derived synthetic peptides have a reduced IgE reactivity compared to the whole Fel d 1 allergen. Fel d 1-derived synthetic peptides can therefore be considered hypoallergenic, providing the advantage of reduced IgE-mediated side-effects, when used in SIT.

20.2. Specific induction of expression of surface markers CD203c and CD63 on human basophils (Fig. 17)

Since IgE-binding is a prerequisite but not ample for induction of type 1 allergic reactions that also require cross-link of effector cell bound specific IgE, the actual allergenic activity of Fel d 1-derived synthetic peptides was investigated in basophil activation assays. These assays detect an allergen-specific upregulation of surface markers CD203c and CD63, both recognized as markers for basophil activation (Hauswirth et al. J

Allergy Clin Immunol. (2002) 110:102-109).

Heparinized blood samples were taken from 5 cat-allergic patients after informed consent had been given. Blood aliquots (100 μ l) were incubated with serial dilutions of rFel d 1, Fel d 1-derived synthetic peptides as single components or as equimolar mixture, anti-IgE antibody or buffer (PBS) for 15 minutes at 37°C. After incubation, cells were washed in PBS containing 20 mM EDTA. Cells were then incubated with 10 μ l of PE-conjugated CD203c mAb 97A6 and 20 μ l of FITC-conjugated CD63 mAb CLB-gran12 for 15 minutes at room temperature. Thereafter, the samples were subjected to erythrocyte lysis with 2 ml FACS™ Lysing Solution. Cells were then washed, resuspended in PBS, and analyzed by two-color flow cytometry on a FACScan (Becton Dickinson, USA), using Paint-a-Gate Software. Allergen-induced upregulation of CD203c and CD63 was calculated from mean fluorescence intensities (MFIs) obtained with stimulated (MFIstim) and unstimulated (MFIcontrol) cells, and expressed as stimulation index (MFIstim : MFIcontrol). An SI of more than 2.0 (i.e. more than 2-fold upregulation) was considered indicative of a specific (diagnostic) response.

On basophils of all five studied cat-allergic patients (RR, EB, KC, MG and SM) stimulation with rFel d 1 induced an allergen-specific upregulation of surface markers CD203c and CD63. The upregulation of CD203c and CD63 was observed to be dose-dependent for 4/5 patients (RR, KC, MG and SM). For these patients CD203c stimulation indices ranged from 1.1 (SM) to 3.2 (RR) for the lowest tested concentration of 0.001 μ g rFel d 1/ml and from 3.6 (KC) to 6.2 (RR) for the highest tested concentration of 10 μ g rFel d 1/ml. CD63 stimulation indices determined in the same assay ranged from 1.1 (RR) to 2.0 (MG) for the lowest tested rFel d 1 concentration of 0.001 μ g/ml and from 3.9 (RR) to 7.3 (MG) for the highest tested rFel d 1 concentration of 10 μ g/ml. For Patient EB 0.001 μ g/ml Fel d 1 were already sufficient to induce a high-level upregulation of surface markers CD203c and CD63 preventing an observation of dose-dependency of the surface marker upregulation.

Basophils from all five cat-allergic patients were probed with five increasing concentrations (0.005, 0.05, 0.5, 5 and 50 μ g/ml) of an equimolar mix of the five Fel d 1-derived synthetic peptides. Basophils from patient RR were additionally probed

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with five increasing concentrations of the five single Fel d 1-derived synthetic peptides (0.001, 0.01, 0.1, 1 and 10 µg/ml). Peptides were found to be deficient in upregulating the basophil surface markers CD203c and CD63. Peptides were unable to induce any increased expression of CD203c and CD63 on cells of patient RR, KC and SM. A slight upregulation of CD203c (SI=2.3) and of CD63 (SI=2.5) could be detected for patient MG but only for the highest tested concentration of 50 µg peptide mixture/ml, while the lower concentrations applied had also no stimulating effect. A more pronounced upregulation of CD203c (SI=4.2) and CD63 (SI=4.3) was observed for patient EB but, again, only for the highest tested peptide mixture concentration. In both cases, patient MG and EB, the rate of upregulation after stimulation with peptides was considerably lower than the corresponding values for stimulation with the whole Fel d 1 allergen.

This demonstrates that Fel d 1-derived synthetic peptides provide the advantage of holding a lower allergenic activity than the whole Fel d 1 allergen. This is relevant for a decreased risk of IgE-mediated side-effects when Fel d 1-derived synthetic peptides are used in SIT.

Example 21: Immunization with Fel d 1-derived synthetic peptides induces IgG antibodies reactive with the whole rFel d 1 allergen

Fel d 1-derived synthetic peptides were shown to be deficient in IgE-binding. As candidate molecules for vaccination, which aims at the induction of allergen-specific IgG antibodies, peptides must retain specific allergen structures and must still be able to induce an IgG immune response specific for the whole allergen. In order to find out whether Fel d 1-derived synthetic peptides fulfill these requirements, immunization experiments in rabbits were performed.

Rabbits were immunized with uncoupled rFel d 1 and KLH-coupled Fel d 1-derived synthetic peptides. HPLC-purified peptides were coupled to KLH via their cysteine residues, using an Imject Maleimide Activated Immunogen Conjugation Kit (Pierce, USA).

Rabbits (New Zealand White rabbits) were immunized with the immunogens (200 µg/injection) using CFA (first immunization) and IFA (first booster injection after 4 weeks; a second booster injection with incomplete adjuvant was given after 7 weeks)

(Charles River Breeding Laboratories, Germany). Rabbits were bled 8 weeks after the first immunization and in four-week intervals thereafter.

The induction of peptide- and rFel d 1-specific antibodies was monitored in ELISA assays. ELISA plates (Nunc Maxisorb, Denmark) were coated with rFel d 1 (0.5 µg/well), washed and blocked. Plate-bound rFel d 1 was then probed in duplicates with 1:1000 diluted rabbit antisera and the corresponding rabbit preimmune sera, and bound IgG was detected with an 1:2000 diluted horseradish-peroxidase labelled goat anti-rabbit antiserum (Jackson ImmunoResearch Inc., USA). Means of duplicates were calculated and showed errors of less than 5%.

Immunization with Fel d 1-derived synthetic peptides induces Fel d 1 reactive IgG antibodies. Eight weeks after the first immunization with each of the five Fel d 1-derived synthetic peptides, IgG antibodies reactive to the whole Fel d 1 allergen could be detected in each of the five rabbit antisera. IgG antibody levels remained at comparable levels in the subsequent bleedings (Fig. 16).

Anti-Peptide 1, anti-Peptide 2, anti-Peptide 4 and anti-Peptide 5 rabbit antisera showed IgG reactivities to Fel d 1 at about the same magnitude than the anti-Fel d 1 rabbit antiserum. Also the anti-Peptide 3 rabbit antiserum showed a distinct but a somewhat lower IgG reactivity to Fel d 1.

This indicates that all 5 Fel d 1-derived synthetic peptides are candidate molecules to induce an Fel d 1 specific IgG antibody response.

Example 22: Fel d 1-derived synthetic peptides induce weaker lymphoproliferative responses than Fel d 1

Desired candidate molecules for improved SIT do not only offer the advantage of reduced IgE-mediated side effects but also of reduced T-cell mediated side effects. In order to investigate the T-cell activating properties of Fel d 1-derived synthetic peptides, lymphoproliferative assays were performed.

PBMCs were isolated from 7 cat-allergic patients by Ficoll (Amersham Pharmacia Biotech, UK) density gradient centrifugation. PBMC (2×10^5) were cultured in triplicates in 96-well plates (Nuncclone, Nalgene Nunc International, Denmark) in 200 µl serum-free Ultra Culture medium (Cambrex, Belgium) supplemented with 2 mM L-glutamine (Sigma, USA), 50 µM β-mercaptoethanol

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(Sigma) and 0.1 mg gentamicin per ml (Sigma) at 37°C using 5% CO₂ in a humidified atmosphere. Cells were stimulated with different concentrations (5, 2.5, 1.25 and 0.6 µg/well) of rFel d 1 and Fe d 1-derived sythetic peptides as single components or as equimolar mixture and, for control purposes, with 4U interleukin-2 or with medium alone. After 6 days of culture, 0.5 µCi per well ³H-thymidine (Amersham Pharmacia Biotech) was added and 16 h thereafter, incorporated radioactivity was measured by liquid scintillation counting using a microbeta scintillation counter (Wallac ADL, Germany), and mean cpm were calculated from the triplicates. The stimulation index (SI) was calculated as the quotient of the cpm obtained by antigen or interleukin-2 stimulation and the unstimulated medium control.

IL-2 stimulated proliferation of PBMC from all 7 tested cat-allergic patients, resulting in stimulation indices of 9.8 for RR, 5.2 for EB, 3.2 for KC, 6.7 for MG, 6.3 for SM, 15.7 for RA and of 13.9 for AR.

Fel d 1-derived sythetic peptides induced lower stimulation indices (Table 9).

Table 9: Fel d 1-derived sythetic peptides which on an equimolar basis induce weaker lymphoproliferative responses than Fel d 1 can be identified. PBMCs from 7 cat-allergic patients were stimulated with serial dilutions of rFel d 1 or Fel d 1-derived sythetic peptides as single components. Specific lymphoproliferative responses are shown as stimulation indices.

		5 µg/w	2.5 µg/w	1.25 µg/w	0.6 µg/w
Patient RR	rFel d 1	2.6	1.8	1.5	1.9
	Peptide 1	1.9	0.6	1.3	1.5
	Peptide 2	2.1	1.3	2.0	1.6
	Peptide 3	3.5	2.8	2.0	3.0
	Peptide 4	2.5	2.4	1.5	0.8
	Peptide 5	1.7	0.9	2.3	0.7
Patient EB	rFel d 1	8.2	2.9	1.6	1.5
	Peptide 1	1.3	0.9	1.0	1.2
	Peptide 2	2.4	1.7	1.8	1.6
	Peptide 3	1.1	1.2	1.4	1.7
	Peptide 4	3.6	3.6	3.2	2.3

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	Peptide 5	2.2	2.1	1.4	2.1
Patient KC	rFel d 1	0.8	1.2	1.3	5.2
	Peptide 1	0.7	1.0	1.1	1.1
	Peptide 2	1.2	1.5	1.0	1.1
	Peptide 3	0.6	0.5	0.5	0.6
	Peptide 4	1.6	1.4	1.3	1.1
	Peptide 5	1.3	1.4	0.9	1.4
Patient MG	rFel d 1	2.9	2.3	2.3	2.2
	Peptide 1	1.8	1.4	1.4	1.1
	Peptide 2	1.2	1.3	1.4	0.9
	Peptide 3	1.1	0.5	0.6	0.7
	Peptide 4	1.1	1.5	1.8	1.0
	Peptide 5	1.5	1.2	1.6	0.8
Patient SM	rFel d 1	2.3	1.6	1.8	1.1
	Peptide 1	1.1	1.0	0.8	1.0
	Peptide 2	1.8	1.1	1.3	1.2
	Peptide 3	2.6	2.1	2.1	1.5
	Peptide 4	1.9	1.6	1.7	1.1
	Peptide 5	2.3	1.3	1.4	1.0
Patient RA	rFel d 1	3.2	1.2	2.4	1.2
	Peptide 1	0.8	0.7	1.3	1.1
	Peptide 2	1.2	0.5	1.7	1.6
	Peptide 3	2.0	2.3	1.6	0.9
	Peptide 4	3.0	1.3	1.1	0.6
	Peptide 5	0.4	0.6	0.9	0.9
Patient AR	rFel d 1	1.4	0.6	0.9	1.0
	Peptide 1	1.0	0.5	1.7	0.7
	Peptide 2	0.7	0.6	0.9	0.6
	Peptide 3	1.6	1.6	2.1	1.0
	Peptide 4	1.0	0.7	0.7	0.6
	Peptide 5	0.8	0.5	0.3	0.5

Example 23: IgG antibodies induced by immunization with Fel d 1-derived synthetic peptides inhibit binding of cat-allergic patients IgE to the whole Fel d 1 allergen

The ability of peptide-induced rabbit Ig to inhibit the binding of allergic patients' IgE antibodies to complete rFel d 1 was examined in ELISA competition assays. ELISA plates (Nunc Maxisorb, Denmark) were coated with rFel d 1 (0.05 µg/well), washed and blocked. Plate-bound rFel d 1 was then pre-incubated with 1:100 diluted rabbit anti-peptide antisera (single anti-peptide antisera as well as a mixture of anti-peptide antisera were used), rabbit anti-rFel d 1 antiserum, and for control purposes also with the respective rabbit preimmune sera. After the plates had been washed, they were incubated with 1:5 diluted human sera from cat-allergic patients. Bound IgE antibodies were detected with a 1:2500 diluted horse-raddish-peroxidase labeled anti-human IgE antibody (KPL, USA). The percentage inhibition of IgE-binding achieved by preincubation with the anti-peptide antisera was calculated as follows: %inhibition of IgE-binding = $100 - \text{O.D.}_I / \text{O.D.}_P \times 100$, with O.D._I being the measured optical densitiy after preincubation with rabbit immune sera and O.D._P with rabbit preimmune sera.

Preincubation of ELISA-plate bound Fel d 1 with the 5 anti-peptide rabbit antisera resulted in inhibition patterns that varied between the 14 different tested sera from cat-allergic patients. Anti-Peptide 1 rabbit antiserum blocked patients' IgE-binding to Fel d 1 for 13/14 tested patients' sera, anti-Peptide 2 rabbit antiserum for 8/14, anti-Peptide 3 rabbit antiserum for 13/14, anti-Peptide 4 rabbit antiserum for 9/14 and anti-Peptide 5 rabbit antiserum for 5/14.

Also the range of inhibition showed variations between the different antisera. Among the single tested anti-peptide rabbit antisera, anti-Peptide 1 rabbit antiserum showed best inhibition rates with inhibitions from 0-55% (average 29%). With anti-Peptide 2 rabbit antiserum inhibition rates of 0-18% (average 5%) could be achieved, with anti-Peptide 3 rabbit antiserum of 0-29% (average 11%), with anti-Peptide 4 rabbit antiserum of 0-24% (average 8%) and with anti-Peptide 5 rabbit antiserum of 0-18% (average 4%).

A mix of all 5 anti-peptide rabbit antisera inhibited patients' IgE-binding to Fel d 1 most efficiently with inhibitions achieved for all patients' sera and inhibition rates of 25-84% (average 59%). These inhibitions were even more pronounced than that achieved by preincubation with the anti-Fel d 1 rabbit an-

tiserum (Table 10).

Table 10: Rabbit antisera raised against Fel d 1-derived synthetic peptides inhibit binding of human IgE to Fel d 1. The percentage inhibition of IgE-binding to Fel d 1 achieved by pre-incubation of Fel d 1 with rabbit antisera are shown for 14 patients and as means. Preincubations were performed with 5 rabbit antisera raised against the 5 Fel d 1-derived synthetic peptides (anti-Peptide 1-5), a mix of the 5 anti-peptide antisera (Mix) and an antiserum raised against Fel d 1 (anti-rFel d 1).

Pa- tient	anti- Peptide 1	anti- Peptide 2	anti- Peptide 3	anti- Peptide 4	anti- Peptide 5	Mix of the 5 anti- peptide antisera	anti- rFel d 1
1	48	18	29	20	18	78	64
2	24	0	8	0	0	67	43
3	55	11	5	17	8	84	74
4	38	7	11	24	8	66	49
5	10	5	5	12	0	54	48
6	33	0	12	5	0	68	46
7	6	1	10	5	0	58	45
8	44	3	17	10	0	60	53
9	26	17	12	15	16	53	43
10	0	0	10	0	0	31	26
11	38	0	0	0	0	52	56
12	47	0	22	0	7	75	51
13	27	2	8	0	0	56	41
14	16	0	6	5	0	25	25
mean	29	5	11	8	4	59	47

When the anti-Peptide 1 rabbit antiserum was combined with each of the other anti-peptide antisera, the inhibition of allergic patients IgE binding was substantially increased reaching almost values (e.g. anti-peptide 1+4:41%, anti-peptide 1+5:42%) obtained with anti-rFel d 1 antibodies (67%) (Table 11).

Table 11:

Patient	anti-Peptide 1 + anti Peptide 2	anti-Peptide 1 + anti Peptide 3	anti-Peptide 1 + anti Peptide 4	anti-Peptide 1 + anti Peptide 5	anti-rFel d 1
1	61	49	61	63	75
2	24	17	28	28	74
3	60	52	62	57	86
4	43	33	43	40	68
5	17	9	27	30	67
6	37	24	42	46	73
7	26	21	36	34	74
8	51	46	53	55	72
9	40	28	46	43	61
10	16	11	30	34	40
11	45	35	47	45	78
12	52	40	56	59	76
13	29	17	29	32	62
14	7	10	14	16	28
mean	36	28	41	42	67

Example 24: Bet v 1-derived peptides induce Bet v 1-specific IgG responses receiving T-cell help from carrier-derived T-cell epitopes and reduce Bet v 1-specific T-cell proliferation.

Bet v 1-derived, surface-exposed B cell peptides have already been shown to induce a protective Bet v 1-specific IgG response in a mouse model of therapeutic and prophylactic vaccination (Focke M et al. Clin Exp Allergy (2004) 34:1525-1533). In Focke M et al. (2004), 6 Bet v 1-derived peptides were coupled to the carrier molecule KLH before immunization of mice. In the present example it is shown that the Bet v 1-specific IgG response induced with these peptides (Table 1) is driven by the help of T-cell epitopes derived from the carrier but not from the Bet v 1 allergen. It was surprisingly found that none of the Bet v 1-derived peptides having the sequences LFPKVAPQAISS-VENIEGNGGPGTIKKISF (SEQ ID No. 20), GPGTIKKISFPEGFPFKYVKDRVDEVD-HTN (SEQ ID No. 21) and VDHTNFKYNYSVIEGGPIGDTLEKISNEIK (SEQ ID No. 22) induced relevant T-cell responses, and it could be demonstrated that the majority of T-cell responses was directed

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against the carrier molecule, KLH (Figs. 18 and 19). This finding is of great importance for the reduction of side effects during therapeutic vaccination and for reducing the risk of a potential sensitization during prophylactic vaccination. It has been demonstrated in the past that allergen-derived peptides lacking any IgE reactivity but containing allergen-derived T-cell epitopes induced side effects due to T-cell activation. Allergen-derived peptides lacking IgE and T-cell reactivity as exemplified for the Bet v 1 peptides, induce neither IgE nor T-cell mediated side-effects during therapeutic vaccination. When used for prophylactic vaccination, the peptides will induce a Bet v 1-specific protective IgG response without priming of Bet v 1-specific T-cells. This should minimize the risk of pre-priming an allergic immune response through the vaccine which might pave the road for a subsequent allergic sensitization.

In this example the allergen- and carrier-specific T-cell responses in a mouse model of therapeutic and prophylactic allergen vaccination were dissected. Bet v 1-derived peptides 2,3, and 6 (Focke M et al. (2004)) were chosen and tested on whether they contain any of the known Bet v 1-specific T-cell epitopes in BALB/c mice. The mice were immunized as follows (Table 10 shows the sensitization and treatment protocol): Groups of BALB/c mice (n=5) were immunized with 10 µg recombinant Bet v 1 (Biomay, Austria) and/or a mixture of the synthetic Bet v 1-derived peptides 2, 3, and 6 (10 µg of each). Peptides were coupled to KLH as previously described (Focke M et al. (2004)). For immunization, Bet v 1 and the peptide mix were adsorbed to aluminium hydroxide (Alu-Gel-S, Serva, Germany) in a total volume of 150 µl/mouse.

Table 12: Sensitization and treatment protocol.

groups (n=5)	Sensitization (rBet v 1)	Therapy (peptides KLH)
no Sensitization / no Therapy (S-/T-)	—	—
Sensitization / no Therapy (S+/T-)	day 0, 20, 40	—
no Sensitization / Therapy (S-/T+)	—	day 60, 80, 100
Sensitization / Therapy (S+/T+)	day 0, 20, 40	day 60, 80, 100
	Prophylaxis (peptides KLH)	Sensitization (rBet v 1)
no Prophylaxis / Sensitization (P-/S+)	—	day 60, 80, 100
Prophylaxis / no Sensitization (P+/S-)	day 0, 20, 40	—
Prophylaxis / Sensitization (P+/S+)	day 0, 20, 40	day 60, 80, 100

Allergen-, peptide-, and carrier-specific lymphoproliferation was analyzed in a T-cell proliferation assay. Spleens were removed under sterile conditions and homogenized. After lysis of erythrocytes, cells were washed and resuspended in complete medium (RPMI, 10% fetal calf serum, 0.1 mg/ml gentamycin, 2 mM glutamine). Single cell suspensions were plated into 96-well round-bottom plates at a concentration of 2×10^5 cells/well and stimulated with concavalin A (0.5 μ g/well) as a positive control, rBet v 1 (2 μ g/well), KLH (2 μ g/well), the peptide mix (0.34 μ g of each peptide/well) or the medium alone for 4 days. The cultures were pulsed with 0.5 μ Ci/well tritiated thymidine for 16 h and harvested. The proliferation responses were measured by scintillation counting. The ratio of the mean proliferation after antigen stimulation and medium control values, i.e. the stimulation index (SI), was calculated.

Interestingly, it could be shown that therapeutic vaccination with Bet v 1-derived peptides could reduce Bet v 1-specific proliferation in Bet v 1 sensitized mice (group S+/T+) compared to the sensitized but untreated group S+/T-. In the sensitized and treated group no peptide-specific proliferation could be measured, but according to the carrier effect, a KLH-specific proliferation was observed. The peptide vaccine alone (group S-/T+) induced mainly KLH-specific T-cells, but almost no Bet v 1-

specific T-cell response (Figure 18).

Prophylactic vaccination with the peptides induced almost no Bet v 1-specific proliferation (group P+/S-) compared to the Bet v 1-sensitized group P-/S+ but KLH-specific proliferation. In prophylactically vaccinated and subsequently sensitized mice (group P+/S+) Bet v 1 specific proliferation was remarkably reduced, furthermore, no peptide-specific response could be observed in any mouse group (Figure 19).

Thus, it could be shown in an allergy mouse model that prophylactic vaccination with carrier-bound allergen-derived B cell peptides did not prime peptide-specific T-cells, almost no allergen-specific but carrier-specific T-cells. Prophylactic vaccination preceding allergic sensitization but also therapeutic vaccination of sensitized mice reduces allergen-specific T-cell proliferation.

Prophylactic treatment with Bet v 1-derived peptides induced Bet v 1-specific IgG responses without help by Bet v 1-specific T-cells. Furthermore, prophylactic treatment increased Bet v 1-specific IgG responses induced by the Bet v 1 allergen already 20 and 40 days after first sensitization (Figure 20).

These results demonstrate that the peptide vaccine induces a Bet v 1-specific IgG response which can be boosted by allergen exposure.

Example 25: Der p 2-derived peptides showing reduced IgE binding capacity

The IgE binding capacity of Der p 2-derived peptides was determined as described in examples 15.1 and 20.1 employing the peptides according to table 13 and using sera of individuals suffering from house dust mite allergy.

Table 13: Der p 2-derived peptides

Peptide	Position	Sequence	SEQ ID No.
Der p 2 Pep 1	1-33	DQVDVKDCANHEIKKVLVPGCHGSEPCIIHRGK	96
Der p 2 Pep 2	21-51	CHGSEPCIIHRGKPFQLEAVFEANQNSKTAK	97
Der p 2 Pep 3	42-73	EANQNSKTAKIEIKASIEGLEVDVPGIDPNAC	98
Der p 2 Pep 4	62-103	EVDVPGIDPNACHYMKCPLVKGQQYDIKYTWIVP-KIAPKSEN	99
Der p 2 Pep 5	98-129	APKSENVVVTVKVMGDNGVLACAIATHAKIRD	100

The results clearly show that the Der p 2 derived peptides of the present invention exhibit significantly reduced IgE binding capacity.

Table 14: Results

	rDer p 2	peptide 1	peptide 2	peptide 3	peptide 4	peptide 5
means (n=50)	1,080	0,010	0,015	0,004	0,031	0,006

Example 26: Variations in the length of peptides have no effect on peptides' IgE-binding capacity, T-cell reactivity and immunogenicity.

26.1. Design of peptides

To study the effect of variation in peptides length on IgE-binding capacity, T-cell reactivity and immunogenicity variants of Phl p 5 derived peptides were designed by increasing the length of peptide 1 (P1) and decreasing the length of peptide 2 (P2) by a few amino-acids (Table 15).

Table 15: Position, sequence, length in number of aminoacids and molecular weight of synthetic Phl p 5 derived peptides (1, 2) and variants thereof (1a, 2b)

Table 15: Variants of PHL p 5 - derived synthetic peptides

	Position aa	Sequence	Number of aa	Molecular Weight (MW)
Peptide 1	98-128	CGAASNKAFAEGLSGEPKGAAESSSKAALTSK	32	3026
1a	93-128	CFVATFGAASNKAFAEGLSGEPKGAAESSSKAALTSK	37	3592
Peptide 2	26-58	ADLGYPATPAAPAAGYTPATPAAPAEAPAGKC	34	3068
2b	26-53	ADLGYPATPAAPAAGYTPATPAAPAEAC	29	2644

26.2. Lack of IgE-reactivity

To analyse the IgE-reactivity of Phl p 5 derived peptides 1, 2 and their variants 1a, 2b dot-blot assays were performed applying 0.2µg peptide/dot and using sera from 7 grass-pollen allergic patients (p1-p7) and the serum from a non-atopic individual (NHS). Bound IgE was detected with 125 I- labelled anti-

human IgE (Phadia, Uppsala, Sweden). rPhl p 5 was used as positive control and HSA as negative control. Patients react with rPhl p 5 but not with the peptides and peptide variants (Fig. 21).

26.3. Lymphoproliferative responses

PBMCs from 2 grass pollen allergic patients were stimulated with different concentrations of Phl p 5 derived peptides 1, 2, their variants 1a, 2b and for control purposes with rPhl p 5. Stimulation indices obtained with the peptides were significantly lower than those obtained with rPhl p 5 (Fig. 22).

26.4. Immunogenicity of peptide variants

Rabbits were immunized with KLH-coupled Phl p 5 derived peptides and variants. ELISA experiments were used to measure IgG reactivity of the obtained rabbit antisera to peptides and their variants (Table 16). Immunization with peptides and their variants induced cross-reactive IgG antibodies recognizing the peptide and the corresponding variant.

Table 16: Cross-reactivity of anti-Phl p 5 peptide antisera raised in rabbits by immunization with KLH-conjugated peptides. IgG reactivities of peptide antisera to peptides (1, 2) and variants (1a, 2b) are displayed. No reactivity was observed with preimmune-sera (pre P1, pre P1a, pre P2, pre P2b)

- a. Anti peptide 1 antiserum (anti P1) cross-reacts with peptide 1 variant (1a) and anti peptide 1a antiserum (anti P1a) cross-reacts with peptide 1.
- b. Anti peptide 2 antiserum (anti P2) cross-reacts with peptide 2 variant (2b) and anti peptide 2b antiserum (anti P2b) cross-reacts with peptide 2.

Table 16: Cross-reactivity of rabbit-antisera

a)

	pre P1	anti P1	pre P1a	anti P1a
P1	0,041	0,880	0,052	0,947
P1a	0,038	0,705	0,048	0,859

b)

	pre P2	anti P2	pre P2b	anti P2b
P2	0,089	1,168	0,042	1,175
P2b	0,075	0,954	0,053	1,122

26.5. Peptide induced rabbit antisera inhibit grass pollen allergic patients' IgE binding to rPhl p 5

The ability of rabbit anti-peptide 2 and 2b IgGs to inhibit human IgE binding to rPhl p 5 was studied in competition ELISAs. ELISA plate bound rPhl p 5 was preincubated with anti P2, anti P2b and, for control purposes, with anti Phl p 5 antisera. Plates were then exposed to sera from 12 grass pollen allergic patients. The percentage of inhibition of IgE-binding to rPhl p 5 is displayed in Table 17. Anti peptide 2 and anti peptide 2b antisera inhibit patients' IgE binding to rPhl p 5 to the same extent.

Competition ELISAs were also performed with rabbit anti peptide 1 and 1a antisera. In example 17 (Immunization with Phl p 5 derived peptides induces IgG antibodies which inhibit the binding of grass pollen allergic patients IgE to Phl p 5) the anti peptide 1 (P1) antibodies inhibited patients IgE binding to Phl p 5 with a mean inhibition rate of 28.5%. Similar results were obtained with anti peptide 1a antiserum which gave an inhibition rate of 23.7%.

Table 17: Inhibition of patients' IgE binding to rPhl p 5 by anti peptide antisera. Anti peptide 2 and anti peptide 2b antisera inhibit patients' IgE binding to rPhl p 5 to the same extent. ELISA plate bound rPhl p 5 was preincubated with anti P2, anti P2b and, for control purposes, with anti Phl p 5 antisera. Plates were then exposed to sera from 12 grass pollen allergic patients. The percentage of inhibition of IgE-binding to rPhl p 5 is displayed.

Table 17: % Inhibition of IgE-binding

patient	anti P2	anti P2b	anti Phl p 5
1	33.38	24.40	84.77
2	52.20	57.40	87.00
3	52.70	54.85	90.81
4	51.44	59.76	78.26
5	43.19	49.15	77.93
6	47.04	52.02	83.68
7	62.67	58.00	76.62
8	52.36	50.27	74.44
9	57.63	50.91	88.13
10	35.10	37.99	75.03
11	44.44	41.24	68.39
12	47.56	45.41	77.34

Example 27: Cross-protection of anti-VP1 antibodies

Human rhinoviruses comprise over hundred different strains. In this neutralization test it is shown that the rhinovirus infection of one strain can also be inhibited by VP1 specific antibodies of another strain. HeLa cells were seeded out in the wells at equal density. 100TCD₅₀ HRV14 was preincubated with dilutions of anti-14VP1- and anti-89VP1 antibodies (undiluted; 1:2-1:32 wells 1-6) and added to the wells in lane A and D, respectively. In lanes B and C 100TCD₅₀ HRV 89 preincubated with dilutions of anti-14VP1- and anti-89VP1 antibodies, respectively, were added to the cells. After 3 days live cells were stained violet. Anti-89VP1 antibodies and anti-14VP1 antibodies block the infection of HRV14 in a comparable manner. The antibodies raised against 14VP1 and 89VP1 also inhibit the infection of HRV89 up to the same concentration.

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SEQUENCE LISTING IN ELECTRONIC FORM

In accordance with section 111(1) of the Patent Rules, this description contains a sequence listing in electronic form in ASCII text format (file: 94058-10 Seq 08-12-17 v1.txt).

A copy of the sequence listing in electronic form is available from the Canadian Intellectual Property Office.

The sequences in the sequence listing in electronic form are reproduced in the following table.

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			Val
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Thr	Gly	Ser	His	Glu	Ala	Cys	Glu	Leu	Arg	Asp	Gly	Asp	Lys	Ser	Lys
			20					25					30		

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Val	Asp	Ala	Phe	Leu	Asn	Lys	Leu	Asp	Gly	Thr	Thr	Asn	Lys	Thr	Asn
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 20 25 30

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Arg Ser Gly Gln Ile Lys Thr Gly
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		Leu	Gly
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			Asn
			Ala
			Val
			Tyr
			Ala
			Gly
			Asn
			Asn
			Phe
			30

Arg	Thr	Ala	Val	Asn	Leu
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1				5					10					15	

Tyr	Asn	Ile	Ile	Asp	Gly	Cys	Trp	Arg	Gly	Lys	Ala	Asp	Trp	Ala	Glu
				20				25					30		

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				20				25					30		

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			20					25					30		

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			20					25					30		

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<223> Amb a 1 derived hyperallergenic molecule

<400> 48

Gly	Val	Asp	Pro	Val	Leu	Thr	Pro	Glu	Gln	Ser	Ala	Gly	Met	Ile	Pro
1				5				10					15		

Ala	Glu	Pro	Gly	Glu	Ser	Ala	Leu	Ser	Leu	Thr	Ser	Ser	Ala	Gly	Val
			20					25					30		

Leu	Ser	Cys	Gln	Pro	Gly	Ala	Pro	Cys
			35				40	

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<210> 49
 <211> 44
 <212> PRT
 <213> Artificial

<220>
 <223> Art v 1 derived hyperallergenic molecule

<400> 49

Ser Lys Leu Cys Glu Lys Thr Ser Lys Thr Tyr Ser Gly Lys Cys Asp
 1 5 10 15

Asn Lys Lys Cys Asp Lys Lys Cys Ile Glu Trp Glu Lys Ala Gln His
 20 25 30

Gly Ala Cys His Lys Arg Glu Ala Gly Lys Glu Ser
 35 40

<210> 50
 <211> 31
 <212> PRT
 <213> Artificial

<220>
 <223> Art v 1 derived hyperallergenic molecule

<400> 50

Ser Cys Phe Cys Tyr Phe Asp Cys Ser Lys Ser Pro Pro Gly Ala Thr
 1 5 10 15

Pro Ala Pro Pro Gly Ala Ala Pro Pro Pro Ala Ala Gly Gly Ser
 20 25 30

<210> 51
 <211> 41
 <212> PRT
 <213> Artificial

<220>
 <223> Art v 1 derived hyperallergenic molecule

<400> 51

Ala Pro Pro Pro Ala Ala Gly Gly Ser Pro Ser Pro Pro Ala Asp Gly
 1 5 10 15

Gly Ser Pro Pro Pro Pro Ala Asp Gly Gly Ser Pro Pro Val Asp Gly
 20 25 30

Gly Ser Pro Pro Pro Pro Ser Thr His

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35

40

<210> 52
 <211> 38
 <212> PRT
 <213> Artificial

<220>
 <223> Can f 1 derived hyperallergenic molecule

<400> 52

Gln	Asp	Thr	Pro	Ala	Leu	Gly	Lys	Asp	Thr	Val	Ala	Val	Ser	Gly	Lys
1				5					10					15	

Trp	Tyr	Leu	Lys	Ala	Met	Thr	Ala	Asp	Gln	Glu	Val	Pro	Glu	Lys	Pro
			20					25					30		

Asp	Ser	Val	Thr	Pro	Met
					35

<210> 53
 <211> 40
 <212> PRT
 <213> Artificial

<220>
 <223> Can f 1 derived hyperallergenic molecule

<400> 53

Asp	Ser	Val	Thr	Pro	Met	Ile	Leu	Lys	Ala	Gln	Lys	Gly	Gly	Asn	Leu
1				5					10					15	

Glu	Ala	Lys	Ile	Thr	Met	Leu	Thr	Asn	Gly	Gln	Cys	Gln	Asn	Ile	Thr
			20					25					30		

Val	Val	Leu	His	Lys	Thr	Ser	Glu
							40

<210> 54
 <211> 41
 <212> PRT
 <213> Artificial

<220>
 <223> Can f 1 derived hyperallergenic molecule

<400> 54

Cys	Gln	Asn	Ile	Thr	Val	Val	Leu	His	Lys	Thr	Ser	Glu	Pro	Gly	Lys
1									10					15	

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Tyr Thr Ala Tyr Glu Gly Gln Arg Val Val Phe Ile Gln Pro Ser Pro
 20 25 30

Val Arg Asp His Tyr Ile Leu Tyr Cys
 35 40

<210> 55
 <211> 40
 <212> PRT
 <213> Artificial

<220>
 <223> Can f 1 derived hyperallergenic molecule

<400> 55

Gln Pro Ser Pro Val Arg Asp His Tyr Ile Leu Tyr Cys Glu Gly Glu
 1 5 10 15

Leu His Gly Arg Gln Ile Arg Met Ala Lys Leu Leu Gly Arg Asp Pro
 20 25 30

Glu Gln Ser Gln Glu Ala Leu Glu
 35 40

<210> 56
 <211> 40
 <212> PRT
 <213> Artificial

<220>
 <223> Can f 1 derived hyperallergenic molecule

<400> 56

Arg Asp Pro Glu Gln Ser Gln Glu Ala Leu Glu Asp Phe Arg Glu Phe
 1 5 10 15

Ser Arg Ala Lys Gly Leu Asn Gln Glu Ile Leu Glu Leu Ala Gln Ser
 20 25 30

Glu Thr Cys Ser Pro Gly Gly Gln
 35 40

<210> 57
 <211> 40
 <212> PRT
 <213> Artificial

<220>
 <223> Can f 2 derived hyperallergenic molecule

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<400> 57

Gln	Glu	Gly	Asn	His	Glu	Glu	Pro	Gln	Gly	Gly	Leu	Glu	Glu	Leu	Ser
1				5					10					15	

Gly	Arg	Trp	His	Ser	Val	Ala	Leu	Ala	Ser	Asn	Lys	Ser	Asp	Leu	Ile
			20					25					30		

Lys	Pro	Trp	Gly	His	Phe	Arg	Val
		35				40	

<210> 58

<211> 40

<212> PRT

<213> Artificial

<220>

<223> Can f 2 derived hyperallergenic molecule

<400> 58

Pro	Trp	Gly	His	Phe	Arg	Val	Phe	Ile	His	Ser	Met	Ser	Ala	Lys	Asp
1				5					10					15	

Gly	Asn	Leu	His	Gly	Asp	Ile	Leu	Ile	Pro	Gln	Asp	Gly	Gln	Cys	Glu
			20					25					30		

Lys	Val	Ser	Leu	Thr	Ala	Phe	Lys
		35				40	

<210> 59

<211> 38

<212> PRT

<213> Artificial

<220>

<223> Can f 2 derived hyperallergenic molecule

<400> 59

Cys	Glu	Lys	Val	Ser	Leu	Thr	Ala	Phe	Lys	Thr	Ala	Thr	Ser	Asn	Lys
1				5					10					15	

Phe	Asp	Leu	Glu	Tyr	Trp	Gly	His	Asn	Asp	Leu	Tyr	Leu	Ala	Glu	Val
			20					25					30		

Asp	Pro	Lys	Ser	Tyr	Leu
		35			

<210> 60

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<211> 39
 <212> PRT
 <213> Artificial

<220>
 <223> Can f 2 derived hyperallergenic molecule

<400> 60

Asn	Asp	Leu	Tyr	Leu	Ala	Glu	Val	Asp	Pro	Lys	Ser	Tyr	Leu	Ile	Leu
1				5					10					15	

Tyr	Met	Ile	Asn	Gln	Tyr	Asn	Asp	Asp	Thr	Ser	Leu	Val	Ala	His	Leu
			20				25						30		

Met	Val	Arg	Asp	Leu	Ser	Arg
				35		

<210> 61
 <211> 42
 <212> PRT
 <213> Artificial

<220>
 <223> Can f 2 derived hyperallergenic molecule

<400> 61

Val	Arg	Asp	Leu	Ser	Arg	Gln	Gln	Asp	Phe	Leu	Pro	Ala	Phe	Glu	Ser
1				5					10					15	

Val	Cys	Glu	Asp	Ile	Gly	Leu	His	Lys	Asp	Gln	Ile	Val	Val	Leu	Ser
			20					25					30		

Asp	Asp	Asp	Arg	Cys	Gln	Gly	Ser	Arg	Asp
				35			40		

<210> 62
 <211> 34
 <212> PRT
 <213> Artificial

<220>
 <223> Fel d 2 derived hyperallergenic molecule

<400> 62

Glu	Ala	His	Gln	Ser	Glu	Ile	Ala	His	Arg	Phe	Asn	Asp	Leu	Gly	Glu
1				5					10					15	

Glu	His	Phe	Arg	Gly	Leu	Val	Leu	Val	Ala	Phe	Ser	Gln	Tyr	Leu	Gln
			20					25					30		

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Gln Cys

<210> 63
 <211> 35
 <212> PRT
 <213> Artificial

<220>
 <223> Fel d 2 derived hyperallergenic molecule

<400> 63

Cys	Thr	Val	Ala	Ser	Leu	Arg	Asp	Lys	Tyr	Gly	Glu	Met	Ala	Asp	Cys
1				5					10					15	

Cys	Glu	Lys	Lys	Glu	Pro	Glu	Arg	Asn	Glu	Cys	Phe	Leu	Gln	His	Lys
			20					25					30		

Asp	Asp	Asn
		35

<210> 64
 <211> 30
 <212> PRT
 <213> Artificial

<220>
 <223> Fel d 2 derived hyperallergenic molecule

<400> 64

Asn	Glu	Gln	Arg	Phe	Leu	Gly	Lys	Tyr	Leu	Tyr	Glu	Ile	Ala	Arg	Arg
1				5					10					15	

His	Pro	Tyr	Phe	Tyr	Ala	Pro	Glu	Leu	Leu	Tyr	Tyr	Ala	Glu
			20					25					30

<210> 65
 <211> 31
 <212> PRT
 <213> Artificial

<220>
 <223> Fel d 2 derived hyperallergenic molecule

<400> 65

Cys	Ala	Asp	Asp	Arg	Ala	Asp	Leu	Ala	Lys	Tyr	Ile	Cys	Glu	Asn	Gln
1				5					10					15	

Asp Ser Ile Ser Thr Lys Leu Lys Glu Cys Cys Gly Lys Pro Val

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20

25

30

<210> 66
 <211> 30
 <212> PRT
 <213> Artificial

<220>
 <223> Fel d 2 derived hyperallergenic molecule

<400> 66

Val	Glu	Asp	Lys	Glu	Val	Cys	Lys	Asn	Tyr	Gln	Glu	Ala	Lys	Asp	Val
1				5					10					15	

Phe	Leu	Gly	Thr	Phe	Leu	Tyr	Glu	Tyr	Ser	Arg	Arg	His	Pro
			20					25					30

<210> 67
 <211> 30
 <212> PRT
 <213> Artificial

<220>
 <223> Fel d 2 derived hyperallergenic molecule

<400> 67

Leu	Ala	Lys	Glu	Tyr	Glu	Ala	Thr	Leu	Glu	Lys	Cys	Cys	Ala	Thr	Asp
1				5					10					15	

Asp	Pro	Pro	Ala	Cys	Tyr	Ala	His	Val	Phe	Asp	Glu	Phe	Lys
			20					25					30

<210> 68
 <211> 30
 <212> PRT
 <213> Artificial

<220>
 <223> Fel d 2 derived hyperallergenic molecule

<400> 68

Glu	Lys	Gln	Ile	Lys	Lys	Gln	Ser	Ala	Leu	Val	Glu	Leu	Leu	Lys	His
1				5					10					15	

Lys	Pro	Lys	Ala	Thr	Glu	Glu	Gln	Leu	Lys	Thr	Val	Met	Gly
			20					25					30

<210> 69
 <211> 30
 <212> PRT

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<213> Artificial

<220>

<223> Fel d 2 derived hyperallergenic molecule

<400> 69

Val	Asp	Lys	Cys	Cys	Ala	Ala	Glu	Asp	Lys	Glu	Ala	Cys	Phe	Ala	Glu
1				5					10					15	

Glu	Gly	Pro	Lys	Leu	Val	Ala	Ala	Ala	Gln	Ala	Ala	Leu	Ala
			20					25					30

<210> 70

<211> 42

<212> PRT

<213> Artificial

<220>

<223> Fel d 2 derived hyperallergenic molecule

<400> 70

Cys	Pro	Phe	Glu	Asp	His	Val	Lys	Leu	Val	Asn	Glu	Val	Thr	Glu	Phe
1				5					10					15	

Ala	Lys	Gly	Cys	Val	Ala	Asp	Gln	Ser	Ala	Ala	Asn	Cys	Glu	Lys	Ser
			20					25					30		

Leu	His	Glu	Leu	Leu	Gly	Asp	Lys	Leu	Cys
		35					40		

<210> 71

<211> 41

<212> PRT

<213> Artificial

<220>

<223> Fel d 2 derived hyperallergenic molecule

<400> 71

Cys	Phe	Leu	Gln	His	Lys	Asp	Asp	Asn	Pro	Gly	Phe	Gly	Gln	Leu	Val
1				5					10					15	

Thr	Pro	Glu	Ala	Asp	Ala	Met	Cys	Thr	Ala	Phe	His	Glu	Asn	Glu	Gln
			20					25					30		

Arg	Phe	Leu	Gly	Lys	Tyr	Leu	Tyr	Glu
		35					40	

<210> 72

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<211> 42
 <212> PRT
 <213> Artificial

<220>
 <223> Fel d 2 derived hyperallergenic molecule

<400> 72

Glu Glu Tyr Lys Gly Val Phe Thr Glu Cys Cys Glu Ala Ala Asp Lys
 1 5 10 15

Ala Ala Cys Leu Thr Pro Lys Val Asp Ala Leu Arg Glu Lys Val Leu
 20 25 30

Ala Ser Ser Ala Lys Glu Arg Leu Lys Cys
 35 40

<210> 73
 <211> 38
 <212> PRT
 <213> Artificial

<220>
 <223> Fel d 2 derived hyperallergenic molecule

<400> 73

Cys Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ser
 1 5 10 15

Val Ala Arg Leu Ser Gln Lys Phe Pro Lys Ala Glu Phe Ala Glu Ile
 20 25 30

Ser Lys Leu Val Thr Asp
 35

<210> 74
 <211> 38
 <212> PRT
 <213> Artificial

<220>
 <223> Fel d 2 derived hyperallergenic molecule

<400> 74

Phe Ala Glu Ile Ser Lys Leu Val Thr Asp Leu Ala Lys Ile His Lys
 1 5 10 15

Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp
 20 25 30

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Leu Ala Lys Tyr Ile Cys
35

<210> 75
<211> 38
<212> PRT
<213> Artificial

<220>
<223> Fel d 2 derived hyperallergenic molecule

<400> 75

Cys Gly Lys Pro Val Leu Glu Lys Ser His Cys Ile Ser Glu Val Glu
1 5 10 15

Arg Asp Glu Leu Pro Ala Asp Leu Pro Pro Leu Ala Val Asp Phe Val
20 25 30

Glu Asp Lys Glu Val Cys
35

<210> 76
<211> 42
<212> PRT
<213> Artificial

<220>
<223> Fel d 2 derived hyperallergenic molecule

<400> 76

Cys Glu Leu Phe Glu Lys Leu Gly Glu Tyr Gly Phe Gln Asn Ala Leu
1 5 10 15

Leu Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu
20 25 30

Val Glu Val Ser Arg Ser Leu Gly Lys Val
35 40

<210> 77
<211> 39
<212> PRT
<213> Artificial

<220>
<223> Fel d 2 derived hyperallergenic molecule

<400> 77

Cys Thr His Pro Glu Ala Glu Arg Leu Ser Cys Ala Glu Asp Tyr Leu

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<210> 80
<211> 31
<212> PRT
<213> Artificial
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<220>

<223> Ole e 1 derived hyperallergenic molecule

<400> 80

Gly	Ala	Ser	Leu	Arg	Leu	Gln	Cys	Lys	Asp	Lys	Glu	Asn	Gly	Asp	Val
1				5					10					15	

Thr	Phe	Thr	Glu	Val	Gly	Tyr	Thr	Arg	Ala	Glu	Gly	Leu	Tyr	Ser
			20					25					30	

<210> 81

<211> 37

<212> PRT

<213> Artificial

<220>

<223> Ole e 1 derived hyperallergenic molecule

<400> 81

Gly	Leu	Tyr	Ser	Met	Leu	Val	Glu	Arg	Asp	His	Lys	Asn	Glu	Phe	Cys
1				5					10					15	

Glu	Ile	Thr	Leu	Ile	Ser	Ser	Gly	Arg	Lys	Asp	Cys	Asn	Glu	Ile	Pro
			20					25					30		

Thr	Glu	Gly	Trp	Ala
			35	

<210> 82

<211> 35

<212> PRT

<213> Artificial

<220>

<223> Ole e 1 derived hyperallergenic molecule

<400> 82

Gly	Arg	Lys	Asp	Cys	Asn	Glu	Ile	Pro	Thr	Glu	Gly	Trp	Ala	Lys	Pro
1				5					10					15	

Ser	Leu	Lys	Phe	Lys	Leu	Asn	Thr	Val	Asn	Gly	Thr	Thr	Arg	Thr	Val
			20					25					30		

Asn	Pro	Leu
		35

<210> 83

<211> 39

<212> PRT

- 124 -

<213> Artificial

<220>

<223> Ole e 1 derived hyperallergenic molecule

<400> 83

Leu	Asn	Thr	Val	Asn	Gly	Thr	Thr	Arg	Thr	Val	Asn	Pro	Leu	Gly	Phe
1				5					10					15	

Phe	Lys	Lys	Glu	Ala	Leu	Pro	Lys	Cys	Ala	Gln	Val	Tyr	Asn	Lys	Leu
			20					25					30		

Gly	Met	Tyr	Pro	Pro	Asn	Met
						35

<210> 84

<211> 30

<212> PRT

<213> Artificial

<220>

<223> Par j 2 derived hyperallergenic molecule

<400> 84

Gly	Glu	Glu	Ala	Cys	Gly	Lys	Val	Val	Gln	Asp	Ile	Met	Pro	Cys	Leu
1				5					10					15	

His	Phe	Val	Lys	Gly	Glu	Glu	Lys	Glu	Pro	Ser	Lys	Glu	Cys
			20					25					30

<210> 85

<211> 36

<212> PRT

<213> Artificial

<220>

<223> Par j 2 derived hyperallergenic molecule

<400> 85

Cys	Leu	His	Phe	Val	Lys	Gly	Glu	Glu	Lys	Glu	Pro	Ser	Lys	Glu	Cys
1				5					10					15	

Cys	Ser	Gly	Thr	Lys	Lys	Leu	Ser	Glu	Glu	Val	Lys	Thr	Thr	Glu	Gln
			20					25					30		

Lys	Arg	Glu	Ala
			35

<210> 86

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<211> 37
 <212> PRT
 <213> Artificial

<220>
 <223> Par j 2 derived hyperallergenic molecule

<400> 86

Cys	Cys	Ser	Gly	Thr	Lys	Lys	Leu	Ser	Glu	Glu	Val	Lys	Thr	Thr	Glu
1				5					10					15	

Gln	Lys	Arg	Glu	Ala	Cys	Lys	Cys	Ile	Val	Arg	Ala	Thr	Lys	Gly	Ile
			20					25					30		

Ser	Gly	Ile	Lys	Asn
				35

<210> 87
 <211> 37
 <212> PRT
 <213> Artificial

<220>
 <223> Par j 2 derived hyperallergenic molecule

<400> 87

Glu	Leu	Val	Ala	Glu	Val	Pro	Lys	Lys	Cys	Asp	Ile	Lys	Thr	Thr	Leu
1				5					10					15	

Pro	Pro	Ile	Thr	Ala	Asp	Phe	Asp	Cys	Ser	Lys	Ile	Gln	Ser	Thr	Ile
			20					25					30		

Phe	Arg	Gly	Tyr	Tyr
				35

<210> 88
 <211> 30
 <212> PRT
 <213> Artificial

<220>
 <223> Der p 1 derived hyperallergenic molecule

<400> 88

Thr	Asn	Ala	Cys	Ser	Ile	Asn	Gly	Asn	Ala	Pro	Ala	Glu	Ile	Asp	Leu
1				5					10					15	

Arg	Gln	Met	Arg	Thr	Val	Thr	Pro	Ile	Arg	Met	Gln	Gly	Gly
			20					25					30

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<210> 89
 <211> 33
 <212> PRT
 <213> Artificial

<220>
 <223> Der p 1 derived hyperallergenic molecule

<400> 89

Asn	Gln	Ser	Leu	Asp	Leu	Ala	Glu	Gln	Glu	Leu	Val	Asp	Cys	Ala	Ser
1				5					10					15	

Gln	His	Gly	Cys	His	Gly	Asp	Thr	Ile	Pro	Arg	Gly	Ile	Glu	Tyr	Ile
			20					25					30		

Gln

<210> 90
 <211> 31
 <212> PRT
 <213> Artificial

<220>
 <223> Der p 1 derived hyperallergenic molecule

<400> 90

His	Asn	Gly	Val	Val	Gln	Glu	Ser	Tyr	Tyr	Arg	Tyr	Val	Ala	Arg	Glu
1				5					10					15	

Gln	Ser	Cys	Arg	Arg	Pro	Asn	Ala	Gln	Arg	Phe	Gly	Ile	Ser	Asn
			20					25					30	

<210> 91
 <211> 37
 <212> PRT
 <213> Artificial

<220>
 <223> Der p 1 derived hyperallergenic molecule

<400> 91

Arg	Glu	Gln	Ser	Cys	Arg	Arg	Pro	Asn	Ala	Gln	Arg	Phe	Gly	Ile	Ser
1				5					10					15	

Asn	Tyr	Cys	Gln	Ile	Tyr	Pro	Pro	Asn	Val	Asn	Lys	Ile	Arg	Glu	Ala
			20					25					30		

Leu Ala Gln Thr His

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35

<210> 92
 <211> 31
 <212> PRT
 <213> Artificial

<220>
 <223> Der p 1 derived hyperallergenic molecule

<400> 92

Lys Asp Leu Asp Ala Phe Arg His Tyr Asp Gly Arg Thr Ile Ile Gln
 1 5 10 15

Arg Asp Asn Gly Tyr Gln Pro Asn Tyr His Ala Val Asn Ile Val
 20 25 30

<210> 93
 <211> 33
 <212> PRT
 <213> Artificial

<220>
 <223> Der p 1 derived hyperallergenic molecule

<400> 93

Gly Arg Thr Ile Ile Gln Arg Asp Asn Gly Tyr Gln Pro Asn Tyr His
 1 5 10 15

Ala Val Asn Ile Val Gly Tyr Ser Asn Ala Gln Gly Val Asp Tyr Trp
 20 25 30

Ile

<210> 94
 <211> 34
 <212> PRT
 <213> Artificial

<220>
 <223> Der p 1 derived hyperallergenic molecule

<400> 94

Val Gly Tyr Ser Asn Ala Gln Gly Val Asp Tyr Trp Ile Val Arg Asn
 1 5 10 15

Ser Trp Asp Thr Asn Trp Gly Asp Asn Gly Tyr Gly Tyr Phe Ala Ala
 20 25 30

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Asn Ile

<210> 95
 <211> 35
 <212> PRT
 <213> Artificial

<220>
 <223> Der p 1 derived hyperallergenic molecule

<400> 95

Val	Arg	Asn	Ser	Trp	Asp	Thr	Asn	Trp	Gly	Asp	Asn	Gly	Tyr	Gly	Tyr
1				5					10					15	

Phe	Ala	Ala	Asn	Ile	Asp	Leu	Met	Met	Ile	Glu	Glu	Tyr	Pro	Tyr	Val
			20				25						30		

Val	Ile	Leu
		35

<210> 96
 <211> 33
 <212> PRT
 <213> Artificial

<220>
 <223> Der p 2 derived hyperallergenic molecule

<400> 96

Asp	Gln	Val	Asp	Val	Lys	Asp	Cys	Ala	Asn	His	Glu	Ile	Lys	Lys	Val
1				5					10					15	

Leu	Val	Pro	Gly	Cys	His	Gly	Ser	Glu	Pro	Cys	Ile	Ile	His	Arg	Gly
			20					25					30		

Lys

<210> 97
 <211> 31
 <212> PRT
 <213> Artificial

<220>
 <223> Der p 2 derived hyperallergenic molecule

<400> 97

Cys	His	Gly	Ser	Glu	Pro	Cys	Ile	Ile	His	Arg	Gly	Lys	Pro	Phe	Gln
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

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1	5	10	15
Leu	Glu	Ala	Val
	Phe	Glu	Ala
	Asn	Gln	Asn
	Ser	Lys	Thr
	Ala	Lys	
	20	25	30

<210> 98
 <211> 32
 <212> PRT
 <213> Artificial

<220>
 <223> Der p 2 derived hyperallergenic molecule

<400> 98

Glu	Ala	Asn	Gln	Asn	Ser	Lys	Thr	Ala	Lys	Ile	Glu	Ile	Lys	Ala	Ser
1				5					10					15	

Ile	Glu	Gly	Leu	Glu	Val	Asp	Val	Pro	Gly	Ile	Asp	Pro	Asn	Ala	Cys
			20					25					30		

<210> 99
 <211> 42
 <212> PRT
 <213> Artificial

<220>
 <223> Der p 2 derived hyperallergenic molecule

<400> 99

Glu	Val	Asp	Val	Pro	Gly	Ile	Asp	Pro	Asn	Ala	Cys	His	Tyr	Met	Lys
1				5					10					15	

Cys	Pro	Leu	Val	Lys	Gly	Gln	Gln	Tyr	Asp	Ile	Lys	Tyr	Thr	Trp	Ile
			20					25					30		

Val	Pro	Lys	Ile	Ala	Pro	Lys	Ser	Glu	Asn
		35					40		

<210> 100
 <211> 32
 <212> PRT
 <213> Artificial

<220>
 <223> Der p 2 derived hyperallergenic molecule

<400> 100

Ala	Pro	Lys	Ser	Glu	Asn	Val	Val	Val	Thr	Val	Lys	Val	Met	Gly	Asp
1				5					10					15	

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Asn Gly Val Leu Ala Cys Ala Ile Ala Thr His Ala Lys Ile Arg Asp
 20 25 30

<210> 101
 <211> 35
 <212> PRT
 <213> Artificial

<220>
 <223> Der p 5 derived hyperallergenic molecule

<400> 101

Met Glu Asp Lys Lys His Asp Tyr Gln Asn Glu Phe Asp Phe Leu Leu
 1 5 10 15

Met Glu Arg Ile His Glu Gln Ile Lys Lys Gly Glu Leu Ala Leu Phe
 20 25 30

Tyr Leu Gln
 35

<210> 102
 <211> 36
 <212> PRT
 <213> Artificial

<220>
 <223> Der p 5 derived hyperallergenic molecule

<400> 102

Lys Lys Gly Glu Leu Ala Leu Phe Tyr Leu Gln Glu Gln Ile Asn His
 1 5 10 15

Phe Glu Glu Lys Pro Thr Lys Glu Met Lys Asp Lys Ile Val Ala Glu
 20 25 30

Met Asp Thr Ile
 35

<210> 103
 <211> 31
 <212> PRT
 <213> Artificial

<220>
 <223> Der p 5 derived hyperallergenic molecule
 <400> 103

Asp Gly Val Arg Gly Val Leu Asp Arg Leu Met Gln Arg Lys Asp Leu
 1 5 10 15

Val Ala Ala Ile Glu Lys Ser Glu Thr Phe Asp Pro Met Lys Val Pro
1 5 10 15

Glu Val Arg Gln Phe Ala Asn Val Val Asn His Ile Gly Gly Leu
20 25 30

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<210> 110
 <211> 34
 <212> PRT
 <213> Artificial

<220>
 <223> Der p 7 derived hyperallergenic molecule

<400> 110

Leu	Ser	Asp	Val	Leu	Thr	Ala	Ile	Phe	Gln	Asp	Thr	Val	Arg	Ala	Glu
1				5					10					15	

Met	Thr	Lys	Val	Leu	Ala	Pro	Ala	Phe	Lys	Lys	Glu	Leu	Glu	Arg	Asn
			20					25					30		

Asn Gln

<210> 111
 <211> 35
 <212> PRT
 <213> Artificial

<220>
 <223> Der p 10 derived hyperallergenic molecule

<400> 111

Met	Glu	Ala	Ile	Lys	Lys	Lys	Met	Gln	Ala	Met	Lys	Leu	Glu	Lys	Asp
1				5				10						15	

Asn	Ala	Ile	Asp	Arg	Ala	Glu	Ile	Ala	Glu	Gln	Lys	Ala	Arg	Asp	Ala
			20					25					30		

Asn Leu Arg
 35

<210> 112
 <211> 35
 <212> PRT
 <213> Artificial

<220>
 <223> Der p 10 derived hyperallergenic molecule

<400> 112

Ala	Glu	Lys	Ser	Glu	Glu	Glu	Val	Arg	Ala	Leu	Gln	Lys	Lys	Ile	Gln
1				5					10					15	

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Gln Ile Glu Asn Glu Leu Asp Gln Val Gln Glu Gln Leu Ser Ala Ala
 20 25 30

Asn Thr Lys
 35

<210> 113
 <211> 40
 <212> PRT
 <213> Artificial

<220>
 <223> Der p 10 derived hyperallergenic molecule

<400> 113

Leu Glu Glu Lys Glu Lys Ala Leu Gln Thr Ala Glu Gly Asp Val Ala
 1 5 10 15

Ala Leu Asn Arg Arg Ile Gln Leu Ile Glu Glu Asp Leu Glu Arg Ser
 20 25 30

Glu Glu Arg Leu Lys Ile Ala Thr
 35 40

<210> 114
 <211> 35
 <212> PRT
 <213> Artificial

<220>
 <223> Der p 10 derived hyperallergenic molecule

<400> 114

Ala Lys Leu Glu Glu Ala Ser Gln Ser Ala Asp Glu Ser Glu Arg Met
 1 5 10 15

Arg Lys Met Leu Glu His Arg Ser Ile Thr Asp Glu Glu Arg Met Glu
 20 25 30

Gly Leu Glu
 35

<210> 115
 <211> 31
 <212> PRT
 <213> Artificial

<220>
 <223> Der p 10 derived hyperallergenic molecule

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<400> 115

Arg	Met	Glu	Gly	Leu	Glu	Asn	Gln	Leu	Lys	Glu	Ala	Arg	Met	Met	Ala
1				5					10					15	

Glu	Asp	Ala	Asp	Arg	Lys	Tyr	Asp	Glu	Val	Ala	Arg	Lys	Leu	Ala
			20					25					30	

<210> 116

<211> 31

<212> PRT

<213> Artificial

<220>

<223> Der p 10 derived hyperallergenic molecule

<400> 116

Asp	Leu	Glu	Arg	Ala	Glu	Glu	Arg	Ala	Glu	Thr	Gly	Glu	Ser	Lys	Ile
1				5					10					15	

Val	Glu	Leu	Glu	Glu	Glu	Leu	Arg	Val	Val	Gly	Asn	Asn	Leu	Lys
			20					25					30	

<210> 117

<211> 41

<212> PRT

<213> Artificial

<220>

<223> Der p 10 derived hyperallergenic molecule

<400> 117

Ser	Glu	Glu	Lys	Ala	Gln	Gln	Arg	Glu	Glu	Ala	His	Glu	Gln	Gln	Ile
1				5					10					15	

Arg	Ile	Met	Thr	Thr	Lys	Leu	Lys	Glu	Ala	Glu	Ala	Arg	Ala	Glu	Phe
			20					25					30		

Ala	Glu	Arg	Ser	Val	Gln	Lys	Leu	Gln
			35				40	

<210> 118

<211> 35

<212> PRT

<213> Artificial

<220>

<223> Der p 10 derived hyperallergenic molecule

<400> 118

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Gln Lys Glu Val Asp Arg Leu Glu Asp Glu Leu Val His Glu Lys Glu
 1 5 10 15

Lys Tyr Lys Ser Ile Ser Asp Glu Leu Asp Gln Thr Phe Ala Glu Leu
 20 25 30

Thr Gly Tyr
 35

<210> 119
 <211> 35
 <212> PRT
 <213> Artificial

<220>
 <223> Der p 21 derived hyperallergenic molecule

<400> 119

Met Phe Ile Val Gly Asp Lys Lys Glu Asp Glu Trp Arg Met Ala Phe
 1 5 10 15

Asp Arg Leu Met Met Glu Glu Leu Glu Thr Lys Ile Asp Gln Val Glu
 20 25 30

Lys Gly Leu
 35

<210> 120
 <211> 37
 <212> PRT
 <213> Artificial

<220>
 <223> Der p 21 derived hyperallergenic molecule

<400> 120

Leu His Leu Ser Glu Gln Tyr Lys Glu Leu Glu Lys Thr Lys Ser Lys
 1 5 10 15

Glu Leu Lys Glu Gln Ile Leu Arg Glu Leu Thr Ile Gly Glu Asn Phe
 20 25 30

Met Lys Gly Ala Leu
 35

<210> 121
 <211> 31
 <212> PRT
 <213> Artificial

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<220>

<223> Der p 21 derived hyperallergenic molecule

<400> 121

Gly	Ala	Leu	Lys	Phe	Phe	Glu	Met	Glu	Ala	Lys	Arg	Thr	Asp	Leu	Asn
1				5					10					15	

Met	Phe	Glu	Arg	Tyr	Asn	Tyr	Glu	Phe	Ala	Leu	Glu	Ser	Ile	Lys
			20				25						30	

<210> 122

<211> 33

<212> PRT

<213> Artificial

<220>

<223> Der p 21 derived hyperallergenic molecule

<400> 122

Tyr	Asn	Tyr	Glu	Phe	Ala	Leu	Glu	Ser	Ile	Lys	Leu	Leu	Ile	Lys	Lys
1				5					10					15	

Leu	Asp	Glu	Leu	Ala	Lys	Lys	Val	Lys	Ala	Val	Asn	Pro	Asp	Glu	Tyr
			20					25					30		

Tyr

<210> 123

<211> 32

<212> PRT

<213> Artificial

<220>

<223> Clone 30 derived hyperallergenic molecule

<400> 123

Met	Ala	Asn	Asp	Asn	Asp	Asp	Asp	Pro	Thr	Thr	Thr	Val	His	Pro	Thr
1				5					10					15	

Thr	Thr	Glu	Gln	Pro	Asp	Asp	Lys	Phe	Glu	Cys	Pro	Ser	Arg	Phe	Gly
			20					25					30		

<210> 124

<211> 34

<212> PRT

<213> Artificial

<220>

- 138 -

<223> Clone 30 derived hyperallergenic molecule

<400> 124

Pro	Thr	Thr	Thr	Glu	Gln	Pro	Asp	Asp	Lys	Phe	Glu	Cys	Pro	Ser	Arg
1				5					10					15	

Phe	Gly	Tyr	Phe	Ala	Asp	Pro	Lys	Asp	Pro	His	Lys	Phe	Tyr	Ile	Cys
			20					25					30		

Ser Asn

<210> 125

<211> 39

<212> PRT

<213> Artificial

<220>

<223> Clone 30 derived hyperallergenic molecule

<400> 125

Gly	Tyr	Phe	Ala	Asp	Pro	Lys	Asp	Pro	His	Lys	Phe	Tyr	Ile	Cys	Ser
1				5					10					15	

Asn	Trp	Glu	Ala	Val	His	Lys	Asp	Cys	Pro	Gly	Asn	Thr	Arg	Trp	Asn
			20					25					30		

Glu	Asp	Glu	Glu	Thr	Cys	Thr
			35			

<210> 126

<211> 30

<212> PRT

<213> Artificial

<220>

<223> Bet v 1 derived hyperallergenic molecule

<400> 126

Leu	Phe	Pro	Lys	Val	Ala	Pro	Gln	Ala	Ile	Ser	Ser	Val	Glu	Asn	Ile
1				5					10					15	

Glu	Gly	Asn	Gly	Gly	Pro	Gly	Thr	Ile	Lys	Lys	Ile	Ser	Phe
			20					25					30

<210> 127

<211> 30

<212> PRT

<213> Artificial

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<220>

<223> Bet v 1 derived hyperallergenic molecule

<400> 127

Gly	Pro	Gly	Thr	Ile	Lys	Lys	Ile	Ser	Phe	Pro	Glu	Gly	Phe	Pro	Phe
1				5					10					15	

Lys	Tyr	Val	Lys	Asp	Arg	Val	Asp	Glu	Val	Asp	His	Thr	Asn
			20					25					30

<210> 128

<211> 30

<212> PRT

<213> Artificial

<220>

<223> Bet v 1 derived hyperallergenic molecule

<400> 128

Val	Asp	His	Thr	Asn	Phe	Lys	Tyr	Asn	Tyr	Ser	Val	Ile	Glu	Gly	Gly
1				5					10					15	

Pro	Ile	Gly	Asp	Thr	Leu	Glu	Lys	Ile	Ser	Asn	Glu	Ile	Lys
			20					25					30

<210> 129

<211> 35

<212> PRT

<213> Artificial

<220>

<223> Fel d 1 chain 1 derived hyperallergenic molecule

<400> 129

Glu	Ile	Cys	Pro	Ala	Val	Lys	Arg	Asp	Val	Asp	Leu	Phe	Leu	Thr	Gly
1				5					10					15	

Thr	Pro	Asp	Glu	Tyr	Val	Glu	Gln	Val	Ala	Gln	Tyr	Lys	Ala	Leu	Pro
			20					25					30		

Val	Val	Cys
		35

<210> 130

<211> 36

<212> PRT

<213> Artificial

<220>

- 140 -

<223> Fel d 1 chain 1 derived hyperallergenic molecule

<400> 130

Leu	Glu	Asn	Ala	Arg	Ile	Leu	Lys	Asn	Cys	Val	Asp	Ala	Lys	Met	Thr
1				5					10					15	

Glu	Glu	Asp	Lys	Glu	Asn	Ala	Leu	Ser	Leu	Leu	Asp	Lys	Ile	Tyr	Thr
			20					25					30		

Ser	Pro	Leu	Cys
			35

<210> 131

<211> 35

<212> PRT

<213> Artificial

<220>

<223> Fel d 1 chain 2 derived hyperallergenic molecule

<400> 131

Val	Lys	Met	Ala	Ile	Thr	Cys	Pro	Ile	Phe	Tyr	Asp	Val	Phe	Phe	Ala
1				5					10					15	

Val	Ala	Asn	Gly	Asn	Glu	Leu	Leu	Leu	Asp	Leu	Ser	Leu	Thr	Lys	Val
			20					25					30		

Asn	Ala	Cys
		35

<210> 132

<211> 30

<212> PRT

<213> Artificial

<220>

<223> Fel d 1 chain 2 derived hyperallergenic molecule

<400> 132

Thr	Glu	Pro	Glu	Arg	Thr	Ala	Met	Lys	Lys	Ile	Gln	Asp	Cys	Tyr	Val
1				5					10					15	

Glu	Asn	Gly	Leu	Ile	Ser	Arg	Val	Leu	Asp	Gly	Leu	Val	Cys
			20					25					30

<210> 133

<211> 30

<212> PRT

<213> Artificial

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<220>

<223> Fel d 1 chain 2 derived hyperallergenic molecule

<400> 133

Cys	Met	Thr	Thr	Ile	Ser	Ser	Ser	Lys	Asp	Cys	Met	Gly	Glu	Ala	Val
1				5				10						15	

Gln	Asn	Thr	Val	Glu	Asp	Leu	Lys	Leu	Asn	Thr	Leu	Gly	Arg
			20				25						30

<210> 134

<211> 32

<212> PRT

<213> Artificial

<220>

<223> Phl p 5 derived hyperallergenic molecule

<400> 134

Cys	Gly	Ala	Ala	Ser	Asn	Lys	Ala	Phe	Ala	Glu	Gly	Leu	Ser	Gly	Glu
1				5				10						15	

Pro	Lys	Gly	Ala	Ala	Glu	Ser	Ser	Ser	Lys	Ala	Ala	Leu	Thr	Ser	Lys
			20					25					30		

<210> 135

<211> 34

<212> PRT

<213> Artificial

<220>

<223> Phl p 5 derived hyperallergenic molecule

<400> 135

Ala	Asp	Leu	Gly	Tyr	Gly	Pro	Ala	Thr	Pro	Ala	Ala	Pro	Ala	Ala	Gly
1				5				10						15	

Tyr	Thr	Pro	Ala	Thr	Pro	Ala	Ala	Pro	Ala	Glu	Ala	Ala	Pro	Ala	Gly
			20					25					30		

Lys Cys

<210> 136

<211> 32

<212> PRT

<213> Artificial

<220>

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<223> Phl p 5 derived hyperallergenic molecule

<400> 136

Ala	Tyr	Lys	Leu	Ala	Tyr	Lys	Thr	Ala	Glu	Gly	Ala	Thr	Pro	Glu	Ala
1				5					10					15	

Lys	Tyr	Asp	Ala	Tyr	Val	Ala	Thr	Leu	Ser	Glu	Ala	Leu	Arg	Ile	Cys
			20					25					30		

<210> 137

<211> 31

<212> PRT

<213> Artificial

<220>

<223> Phl p 5 derived hyperallergenic molecule

<400> 137

Cys	Glu	Ala	Ala	Phe	Asn	Asp	Ala	Ile	Lys	Ala	Ser	Thr	Gly	Gly	Ala
1				5					10					15	

Tyr	Glu	Ser	Tyr	Lys	Phe	Ile	Pro	Ala	Leu	Glu	Ala	Ala	Val	Lys
			20					25					30	

<210> 138

<211> 33

<212> PRT

<213> Artificial

<220>

<223> Phl p 5 derived hyperallergenic molecule

<400> 138

Thr	Val	Ala	Thr	Ala	Pro	Glu	Val	Lys	Tyr	Thr	Val	Phe	Glu	Thr	Ala
1				5					10					15	

Leu	Lys	Lys	Ala	Ile	Thr	Ala	Met	Ser	Glu	Ala	Gln	Lys	Ala	Ala	Lys
			20					25					30		

Cys

<210> 139

<211> 38

<212> PRT

<213> Artificial

<220>

<223> Phl p 5 derived hyperallergenic molecule

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<400> 139

Cys Ala Glu Glu Val Lys Val Ile Pro Ala Gly Glu Leu Gln Val Ile
 1 5 10 15

Glu Lys Val Asp Ala Ala Phe Lys Val Ala Ala Thr Ala Ala Asn Ala
 20 25 30

Ala Pro Ala Asn Asp Lys
 35

<210> 140

<211> 70

<212> PRT

<213> Artificial

<220>

<223> Clone 30 Dermatophagoides pteronyssinus allergen

<400> 140

Met Ala Asn Asp Asn Asp Asp Asp Pro Thr Thr Thr Val His Pro Thr
 1 5 10 15

Thr Thr Glu Gln Pro Asp Asp Lys Phe Glu Cys Pro Ser Arg Phe Gly
 20 25 30

Tyr Phe Ala Asp Pro Lys Asp Pro His Lys Phe Tyr Ile Cys Ser Asn
 35 40 45

Trp Glu Ala Val His Lys Asp Cys Pro Gly Asn Thr Arg Trp Asn Glu
 50 55 60

Asp Glu Glu Thr Cys Thr
 65 70

<210> 141

<211> 37

<212> PRT

<213> Artificial

<220>

<223> Phl p 5 derived peptide

<400> 141

Cys Phe Val Ala Thr Phe Gly Ala Ala Ser Asn Lys Ala Phe Ala Glu
 1 5 10 15

Gly Leu Ser Gly Glu Pro Lys Gly Ala Ala Glu Ser Ser Ser Lys Ala
 20 25 30

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Ala Leu Thr Ser Lys
35

<210> 142

<211> 29

<212> PRT

<213> Artificial

<220>

<223> Phl p 5 derived peptide

<400> 142

Ala	Asp	Leu	Gly	Tyr	Gly	Pro	Ala	Thr	Pro	Ala	Ala	Pro	Ala	Ala	Gly
1				5					10					15	

Tyr	Thr	Pro	Ala	Thr	Pro	Ala	Ala	Pro	Ala	Glu	Ala	Cys
			20					25				

Claims:

1. A hypoallergenic protein for use in the treatment or prevention of an allergy in a human or animal, the protein consisting of at least one hypoallergenic molecule derived from an allergen, which is fused to at least one viral capsid protein, wherein the at least one hypoallergenic molecule derived from an allergen exhibits an at least 50% reduced IgE-binding capacity and an at least 30% reduced T-cell reactivity compared to the wild-type allergen.
2. The hypoallergenic protein for use according to claim 1, wherein the at least one hypoallergenic molecule is fused to one or both of the N-terminus and C-terminus of said at least one viral capsid protein.
3. The hypoallergenic protein for use according to claim 1 or 2, wherein the at least one viral capsid protein is an RNA or DNA viral capsid protein.
4. The hypoallergenic protein for use according to any one of claims 1 to 3 wherein the at least one viral capsid protein is derived from a human pathogenic virus.
5. The hypoallergenic protein for use according to any one of claims 1 to 3 wherein the at least one viral capsid protein is derived from a virus of the family of picornaviridae.
6. The hypoallergenic protein for use according to claim 5, wherein the virus of the family of picornaviridae is of the genus of rhinoviruses.
7. The hypoallergenic protein for use according to claim 6, wherein the virus is of the species of human rhinoviruses.
8. The hypoallergenic protein for use according to claim 7, wherein the virus is human rhinovirus 89 or 14.
9. The hypoallergenic protein for use according to any one of claims 1 to 8, wherein the allergen is selected from the group consisting of major birch pollen allergens, major timothy grass pollen allergens, major house dust mite allergens, major cat allergens major bee allergens, major wasp allergens, profilins, olive allergens, Parietaria judaica allergens, Ragweed allergens, mugwort pollen allergens, and storage mite allergens.

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10. The hypoallergenic protein for use according to claim 9, wherein the allergen is selected from the group consisting of Bet v 1, Bet v 4, Phl p 1, Phl p 2, Phl p 5, Phl p 6, Phl p 7, Der p 1, Der p 2, Fel d 1, Fel d 2, Phl p 12, Ole e 1, Par j 2, Amb a 1, Art v 1, and Lep d 2.

11. The hypoallergenic protein for use according to any one of claims 1 to 10, wherein at least one of the at least one hypoallergenic molecule is composed of amino acids 151 to 177, 87 to 117, 1 to 30, 43 to 70 or 212 to 241 of Phl p 1, amino acids 93 to 128, 98 to 128, 26 to 53, 26 to 58, 132 to 162, 217 to 246, 252 to 283 or 176 to 212 of Phl p 5, amino acids 1 to 34 or 35 to 70 of chain 1 of Fel d 1, amino acids 1 to 34, 35 to 63 or 64 to 92 of chain 2 of Fel d 1, amino acids 30 to 59, 50 to 79 or 75 to 104 of Bet v 1, amino acids 1 to 33, 21 to 51, 42 to 73, 62 to 103 or 98 to 129 of Der p 2, amino acids 1 to 30, 20 to 50, 50 to 80, 90 to 125, 125 to 155 or 165 to 198 of Der p 1, amino acids 1 to 35, 35 to 72, 70 to 100 or 90 to 122 of Der p 21, amino acids 1 to 32, 15 to 48 or 32 to 70 of Clone 30, amino acids 19 to 58, 59 to 95, 91 to 120 or 121 to 157 of Alt a 1, amino acids 31 to 60, 45 to 80, 60 to 96 or 97 to 133 of Par j 2, amino acids 1 to 40, 36 to 66, 63 to 99, 86 to 120 or 107 to 145 of Ole e 1, amino acids 25 to 58, 99 to 133, 154 to 183, 277 to 307, 334 to 363, 373 to 402, 544 to 573, 579 to 608, 58 to 99, 125 to 165, 183 to 224, 224 to 261, 252 to 289, 303 to 340, 416 to 457, 460 to 500 or 501 to 542 of Fel d 2, amino acids 19 to 58, 52 to 91, 82 to 119, 106 to 144 or 139 to 180 of Can f 2, amino acids 19 to 56, 51 to 90, 78 to 118, 106 to 145 or 135-174 of Can f 1, amino acids 27 to 70, 70 to 100 or 92 to 132 of Art v 1, amino acids 31 to 70, 80 to 120, 125 to 155, 160 to 200, 225 to 263, 264 to 300, 305 to 350 or 356 to 396 of Amb a 1, amino acids 1 to 34, 35 to 74, 74 to 115, 125 to 165, 174 to 213, 241 to 280, 294 to 333, 361 to 400 or 401 to 438 of Alt a 6, amino acids 1 to 40, 41 to 80, 81 to 120 or 121 to 160 of Alt a 2.

12. The hypoallergenic protein for use according to claim 10, wherein at least one of the at least one hypoallergenic molecule consists of amino acids 26 to 53, 26 to 58, 93 to 128, 98 to 128, 132 to 162, 176 to 212, 217 to 246, or 252 to 283 of Phl p 5, or a fragment thereof truncated by 1 to 5 amino acids.

13. The hypoallergenic protein for use according to claim 10, wherein at least one of the at least one hypoallergenic molecule consists of amino acids 1 to 33 of SEQ ID NO: 10.

14. The hypoallergenic protein for use according to claim 10, wherein at least one of the at least one hypoallergenic molecule consists of amino acids 2 to 31 of SEQ ID NO: 12.

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15. The hypoallergenic protein for use according to claim 14, wherein at least one of the at least one hypoallergenic molecule consists of amino acids 2 to 38 of SEQ ID NO: 14.
16. The hypoallergenic protein for use according to any one of claims 1 to 15, wherein the hypoallergenic protein is formulated with at least one adjuvant, pharmaceutical acceptable excipient or preservative.
17. The hypoallergenic protein for use according to any one of claims 1 to 16, wherein the use is for active immunization.
18. The hypoallergenic protein for use according to any one of claims 1 to 16, wherein the use is for passive immunization.
19. The hypoallergenic protein for use according to any one of claims 1 to 18, wherein said use comprises use of a dose of 10 ng to 1 g of said hypoallergenic protein.
20. The hypoallergenic protein for use according to any one of claims 1 to 18, wherein said use comprises use of a dose of 100 ng to 10 mg of said hypoallergenic protein.
21. The hypoallergenic protein for use according to any one of claims 1 to 18, wherein said use comprises use of a dose of 0.5 μ g to 200 μ g of said hypoallergenic protein.
22. The hypoallergenic protein for use according to any one of claims 1 to 21, wherein the protein is for administration in the amount of 0.01 mg/kg body weight to 5 mg/kg body weight.
23. The hypoallergenic protein for use according to any one of claims 1 to 21, wherein the protein is for administration in the amount of 0.1 mg/kg body weight to 2 mg/kg body weight.
24. Use of a hypoallergenic protein for the treatment or prevention of an allergy in a human or animal, the protein consisting of at least one hypoallergenic molecule derived from an allergen, which is fused to at least one viral capsid protein, wherein the at least one hypoallergenic molecule derived from an allergen exhibits an at least 50% reduced IgE-binding capacity and an at least 30% reduced T-cell reactivity compared to the wild-type allergen.

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25. Use of a hypoallergenic protein in the manufacture of a medicament for the treatment or prevention of an allergy in a human or animal, the protein consisting of at least one hypoallergenic molecule derived from an allergen, which is fused to at least one viral capsid protein, wherein the at least one hypoallergenic molecule derived from an allergen exhibits an at least 50% reduced IgE-binding capacity and an at least 30% reduced T-cell reactivity compared to the wild-type allergen.
26. The use according to claim 25, wherein the medicament comprises the hypoallergenic protein and at least one adjuvant, pharmaceutical acceptable excipient or preservative.
27. The use according to any one of claims 24 to 26, wherein the at least one hypoallergenic molecule is fused to one or both of the N-terminus and C-terminus of said at least one viral capsid protein.
28. The use according to any one of claims 24 to 27, wherein the at least one viral capsid protein is an RNA or DNA viral capsid protein.
29. The use according to any one of claims 24 to 28 wherein the at least one viral capsid protein is derived from a human pathogenic virus.
30. The use according to any one of claims 24 to 28 wherein the at least one viral capsid protein is derived from a virus of the family of picornaviridae.
31. The use according to claim 30, wherein the virus of the family of picornaviridae is of the genus of rhinoviruses.
32. The use according to claim 31, wherein the virus is of the species of human rhinoviruses.
33. The use according to claim 32, wherein the virus is human rhinovirus 89 or 14.
34. The use according to any one of claims 24 to 33, wherein the allergen is selected from the group consisting of major birch pollen allergens, major timothy grass pollen allergens, major house dust mite allergens, major cat allergens major bee allergens, major wasp allergens, profilins, olive allergens, Parietaria judaica allergens, Ragweed allergens, mugwort pollen allergens, and storage mite allergens.

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35. The use according to claim 34, wherein the allergen is selected from the group consisting of Bet v 1, Bet v 4, Phl p 1, Phl p 2, Phl p 5, Phl p 6, Phl p 7, Der p 1, Der p 2, Fel d 1, Fel d 2, Phl p 12, Ole e 1, Par j 2, Amb a 1, Art v 1, and Lep d 2.

36. The use according to any one of claims 24 to 35, wherein at least one of the at least one hypoallergenic molecule is composed of amino acids 151 to 177, 87 to 117, 1 to 30, 43 to 70 or 212 to 241 of Phl p 1, amino acids 93 to 128, 98 to 128, 26 to 53, 26 to 58, 132 to 162, 217 to 246, 252 to 283 or 176 to 212 of Phl p 5, amino acids 1 to 34 or 35 to 70 of chain 1 of Fel d 1, amino acids 1 to 34, 35 to 63 or 64 to 92 of chain 2 of Fel d 1, amino acids 30 to 59, 50 to 79 or 75 to 104 of Bet v 1, amino acids 1 to 33, 21 to 51, 42 to 73, 62 to 103 or 98 to 129 of Der p 2, amino acids 1 to 30, 20 to 50, 50 to 80, 90 to 125, 125 to 155 or 165 to 198 of Der p 1, amino acids 1 to 35, 35 to 72, 70 to 100 or 90 to 122 of Der p 21, amino acids 1 to 32, 15 to 48 or 32 to 70 of Clone 30, amino acids 19 to 58, 59 to 95, 91 to 120 or 121 to 157 of Alt a 1, amino acids 31 to 60, 45 to 80, 60 to 96 or 97 to 133 of Par j 2, amino acids 1 to 40, 36 to 66, 63 to 99, 86 to 120 or 107 to 145 of Ole e 1, amino acids 25 to 58, 99 to 133, 154 to 183, 277 to 307, 334 to 363, 373 to 402, 544 to 573, 579 to 608, 58 to 99, 125 to 165, 183 to 224, 224 to 261, 252 to 289, 303 to 340, 416 to 457, 460 to 500 or 501 to 542 of Fel d 2, amino acids 19 to 58, 52 to 91, 82 to 119, 106 to 144 or 139 to 180 of Can f 2, amino acids 19 to 56, 51 to 90, 78 to 118, 106 to 145 or 135-174 of Can f 1, amino acids 27 to 70, 70 to 100 or 92 to 132 of Art v 1, amino acids 31 to 70, 80 to 120, 125 to 155, 160 to 200, 225 to 263, 264 to 300, 305 to 350 or 356 to 396 of Amb a 1, amino acids 1 to 34, 35 to 74, 74 to 115, 125 to 165, 174 to 213, 241 to 280, 294 to 333, 361 to 400 or 401 to 438 of Alt a 6, amino acids 1 to 40, 41 to 80, 81 to 120 or 121 to 160 of Alt a 2.

37. The hypoallergenic protein for use according to claim 35, wherein at least one of the at least one hypoallergenic molecule consists of amino acids 26 to 53, 26 to 58, 93 to 128, 98 to 128, 132 to 162, 176 to 212, 217 to 246, or 252 to 283 of Phl p 5, or a fragment thereof truncated by 1 to 5 amino acids.

38. The hypoallergenic protein for use according to claim 35, wherein at least one of the at least one hypoallergenic molecule consists of amino acids 1 to 33 of SEQ ID NO: 10.

39. The hypoallergenic protein for use according to claim 35, wherein at least one of the at least one hypoallergenic molecule consists of amino acids 2 to 31 of SEQ ID NO: 12.

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40. The hypoallergenic protein for use according to claim 35, wherein at least one of the at least one hypoallergenic molecule consists of amino acids 2 to 38 of SEQ ID NO: 14.
41. The use according to any one of claims 24 to 40, wherein the hypoallergenic protein is for active immunization.
42. The use according to any one of claims 24 to 40, wherein the hypoallergenic protein is for passive immunization.
43. The use according to any one of claims 24 to 42, wherein the use comprises use of a dose of 10 ng to 1 g of said hypoallergenic protein.
44. The use according to any one of claims 24 to 42, wherein the use comprises use of a dose of 100 ng to 10 mg of said hypoallergenic protein.
45. The use according to any one of claims 24 to 42, wherein the use comprises use of a dose of 0.5 μ g to 200 μ g of said hypoallergenic protein.
46. The use according to any one of claims 24 to 45, wherein the hypoallergenic protein is for administration in the amount of 0.01 mg/kg body weight to 5 mg/kg body weight.
47. The use according to any one of claims 24 to 45, wherein the hypoallergenic protein is for administration in the amount of 0.1 mg/kg body weight to 2 mg/kg body weight.
48. A vaccine formulation for use in the treatment or prevention of an allergy in a human or animal, the formulation comprising an hypoallergenic protein for use according to any one of claims 1 to 15 and at least one adjuvant, pharmaceutical acceptable excipient or preservative.
49. The formulation according to claim 48, wherein said formulation comprises from 10 ng to 1 g of said hypoallergenic protein.
50. The formulation for use according to claim 48, wherein said formulation comprises from 100 ng to 10 mg of said hypoallergenic protein.
51. The formulation for use according to claim 48, wherein said formulation comprises from 0.5 μ g to 200 μ g of said hypoallergenic protein.

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- 52. A nucleic acid molecule encoding a fused hypoallergenic protein for use according to any one claims 1 to 15.
- 53. A vector comprising a nucleic acid molecule according to claim 52.
- 54. The vector according to claim 53, wherein said vector is an expression vector.
- 55. The vector according to claim 53 or 54, wherein said vector is a bacterial, fungal, insect, viral or mammalian vector.
- 56. A host cell comprising a nucleic acid molecule according to claim 52 or a vector according to any one of claims 53 to 55.

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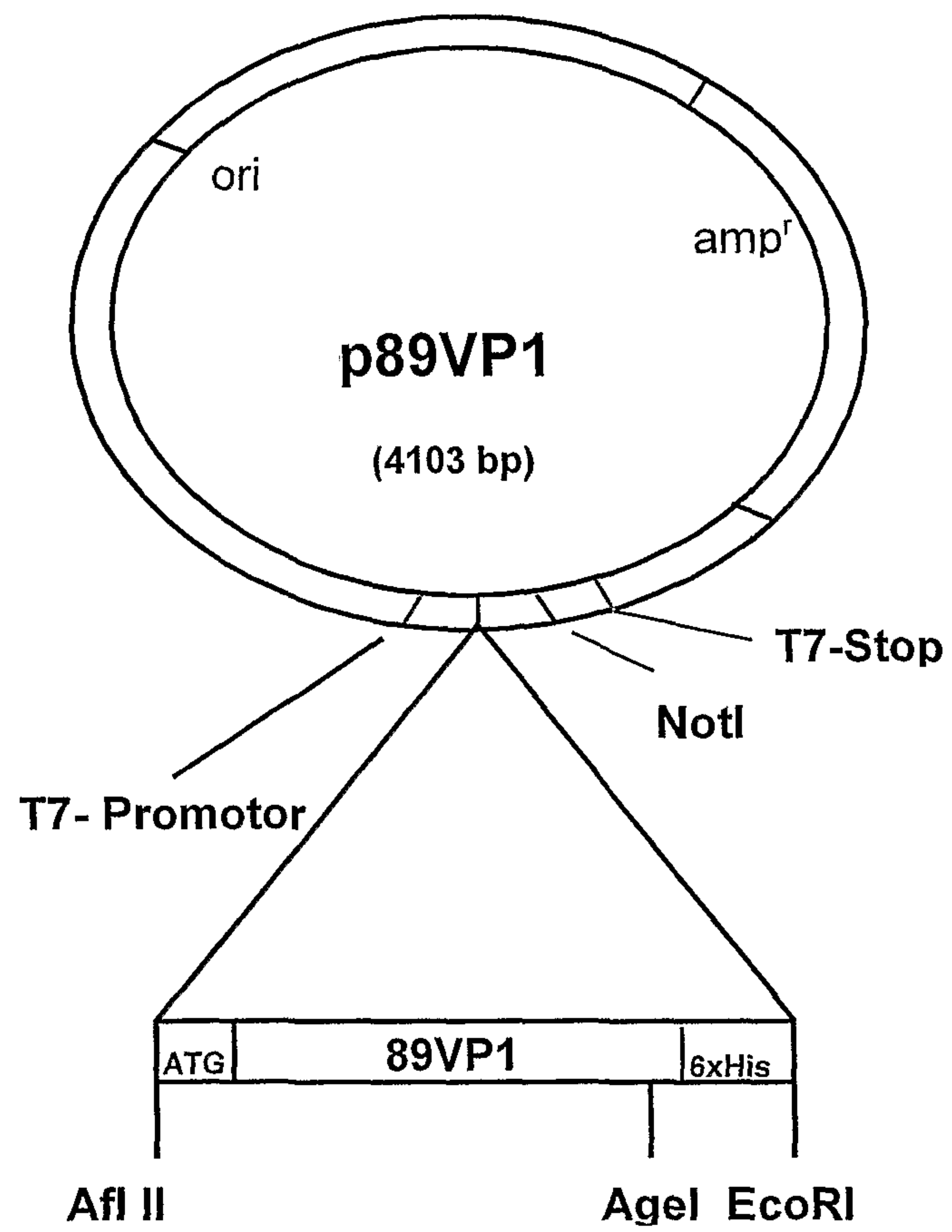


Fig. 1A

MCS p89VP1:

SD

Afl II

5'..TAAGAAGGAGATATACTTAAGATG AAC CCA GTT GAA AAT TAT ATA GAT

start N P V E N Y I D

AGT GTA TTA AAT GAA GTT CTT GTG GTG CCA AAT ATC CAA CCT AGC ACA

S V L N E V L V V P N I Q P S T

TCT GTG TCA AGT CGT GCA GCG CCT GCA TTG GAT GCT GCG GAA ACC

S V S S H A A P A L D A A E T

GGA CAC ACC AGC TCT GTT CAA CCT GAA GGT ATG ATT GAA ACT AGA

G H T S S V Q P E D M I E T R

TAT GTT ATA ACT GAT CAA ACA AGG GAT GAA ACA AGT ATT GAG AGT TTC

Y V I T D Q T R D E T S I E S F

TTA GGT AGG TCA GGG TGT ATC GCT ATG ATA GAA TTT AAT ACA AGT AGT

L G R S G C I A M I E F N T S S

GAT AAA ACT GAA CAT GAT AAA ATT GGT AAA GGA TTC AAA ACA TGG AAG

D K T E H D K I G K G F K T W K

GTT AGT CTT CAA GAA ATG GCA CAA ATC AGA AGA AAA TAT GAA TTA TTC

I S L Q E M A Q I R R K Y E L F

ACA TAT ACA AGA TTT GAT TCA GAG ATA ACA ATA GTC ACT GCA GCC GCA

T Y T R F D S E I T I V T A A A

GCT CAA GGA AAT GAT AGT GGA CAT ATA GTA TTG CAA TTT ATG TAT GTA

A Q G D D S G H I V L Q F M Y V

CCC CCA GGA GCA CCT GTC CCC GAA AAA CGT GAT GAT TAC ACA TGG

P P G A P V P E K R D D Y T W

CAA TCA GGA ACA AAT GCA TCT GTG TTC TGG CAA GAA GGA CAA CCA

Q S G T N A S V F W Q E G Q P

Fig. 1B cont.

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 Y P R F T I P F M S I A S A Y Y
 ATG TTT TAT GAT GGT TAT GAT GGT GAT AGT GCA GCA TCA AAA TAC GGT
 M F Y D G Y D G D S A A S K Y G
 TCT GTA GTC ACT AAT GAT ATG GGA ACC ATA TGT GTT AGA ATA GTG ACA
 S V V T N D M G T I C V R I V T
 TCC AAC CAA AAA CAT GAT TTA AAT ATT GTG TGC CGC ATT TAC CAC AAG
 S N Q K H D L N I V C R I Y H K
 GCC AAA CAT ATA AAA GCA TGG GT CCT CGC CCA CCA AGG GCT GTT GCC
 A K H I K A W C P R P P R A V A
 TAT CAA CAC ACA CAC TCA ACC AAT TAC ATA CCA TCC AAT GGT GAG GCC
 Y Q H T H S T N Y I P S N G E A
 ACA ACT CAG ATT AAA ACC AGA CCT GAT GTT TTT ~~ACA GGT~~ ACA AAC GTC
 T T Q I K T R P D V F T G T N V
 CAC CAC CAC CAC CAC CAC TGA GAA TTC TGC AGA TAT CCA TCA CAC
 H H H H H H stop EcoRI EcoRV
TGG CGG CCG CTC GAG CAG ATC CGG CTG CTA ACA AAG CCC GAA AGG
 NotI
 AAG CTG AGT TGG CTG CTG CCA CCG CTG AGC AAT AAC TAG .. 3'

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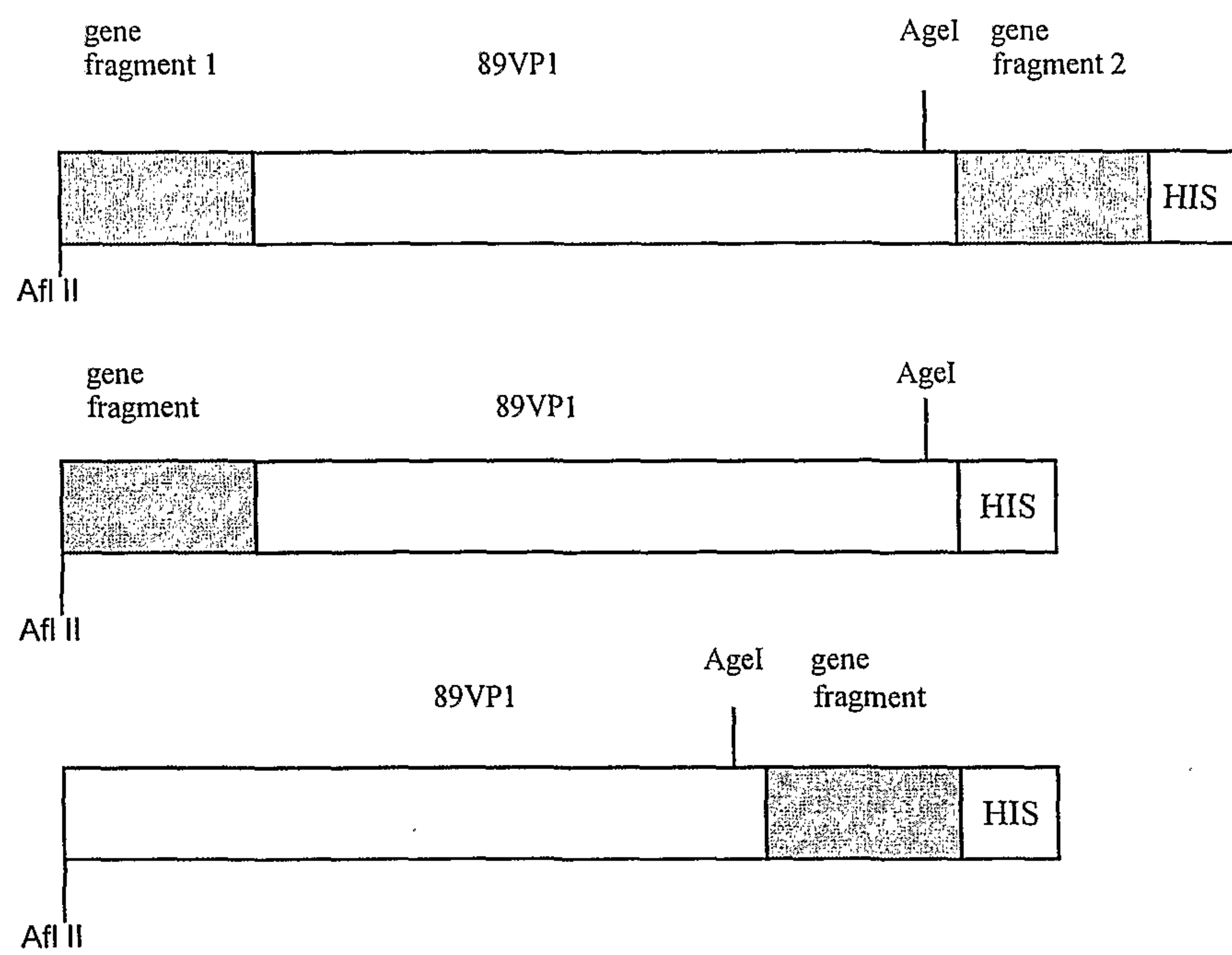


Fig. 1C

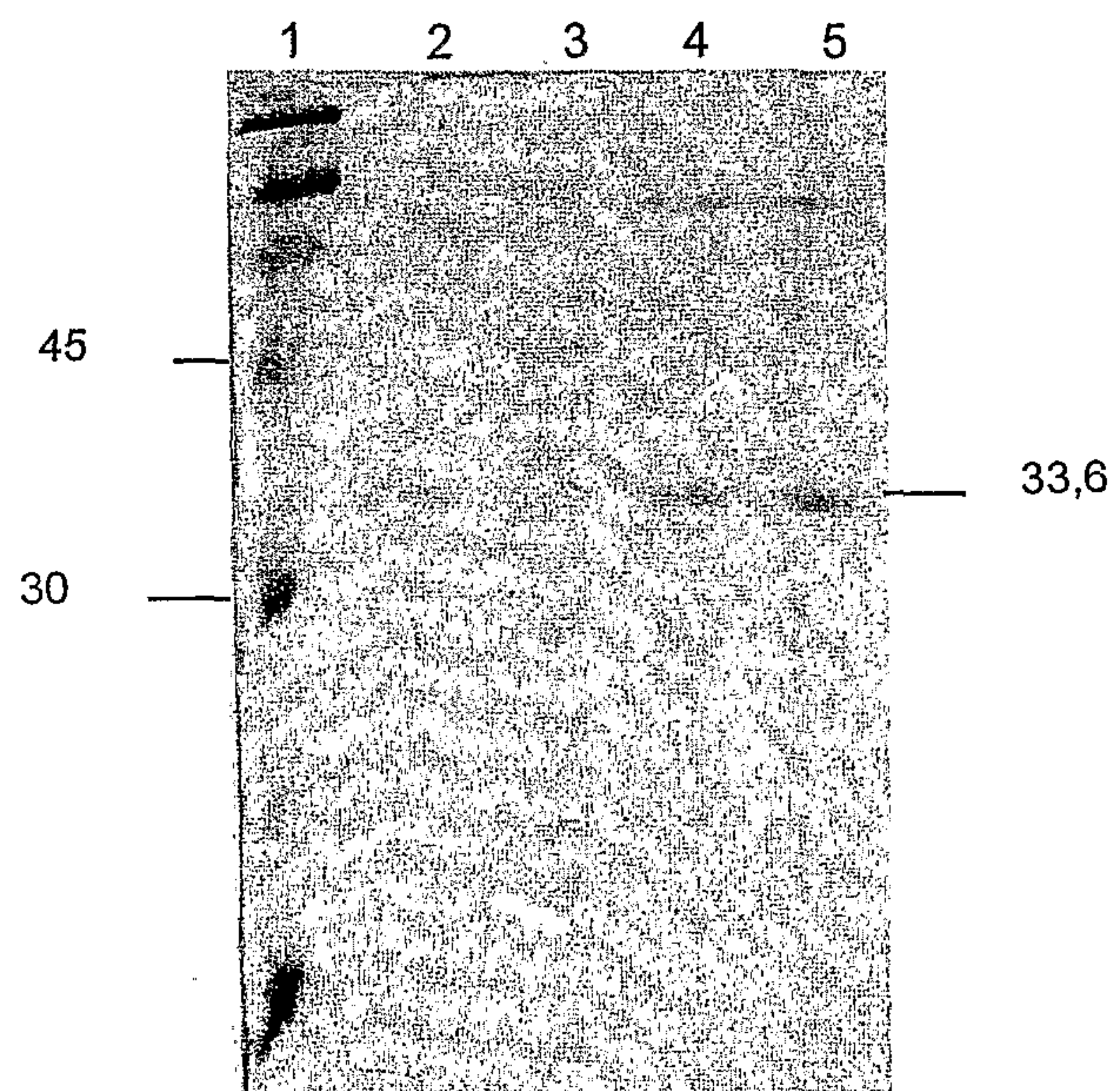


Fig. 2

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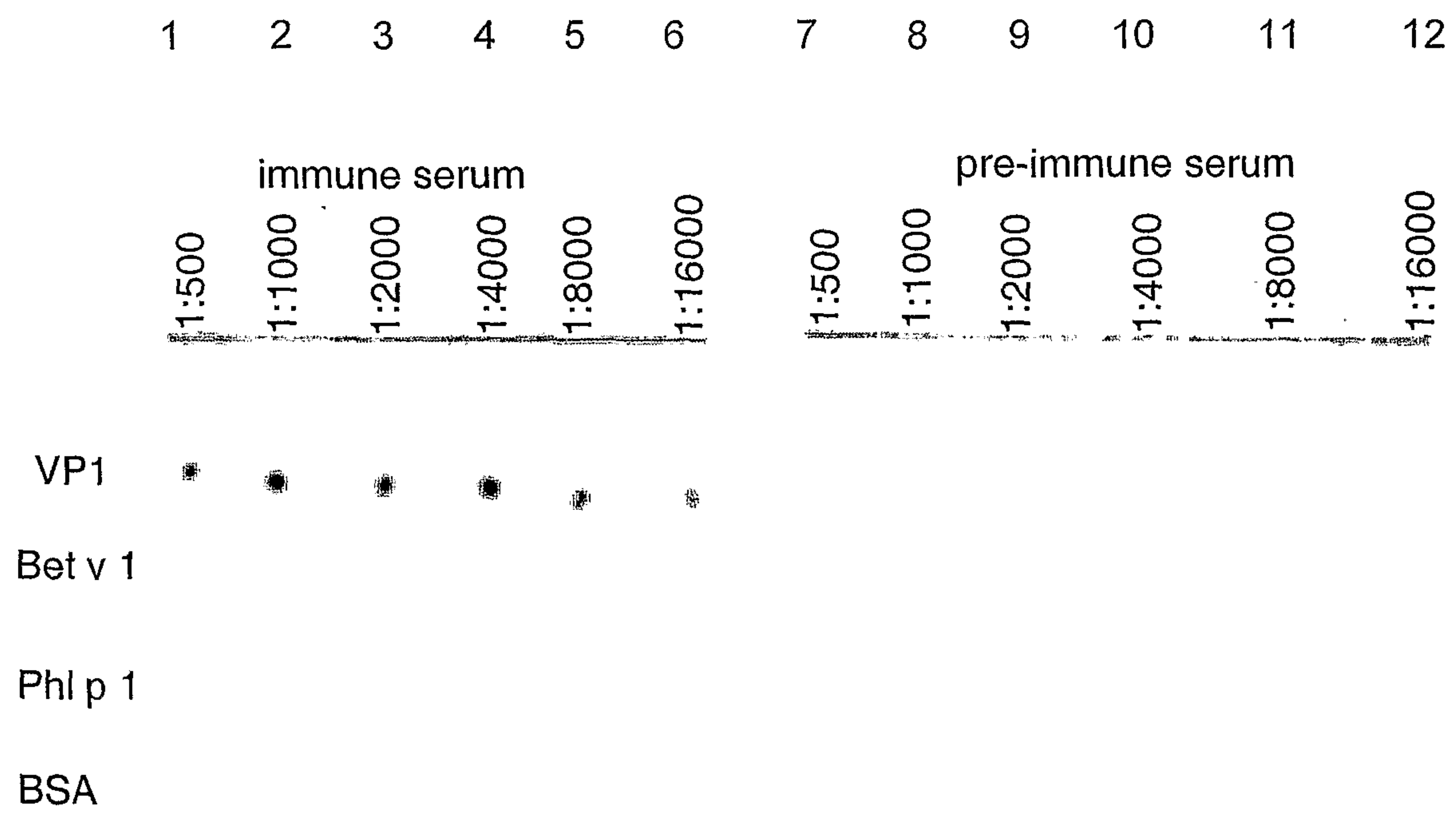


Fig. 3

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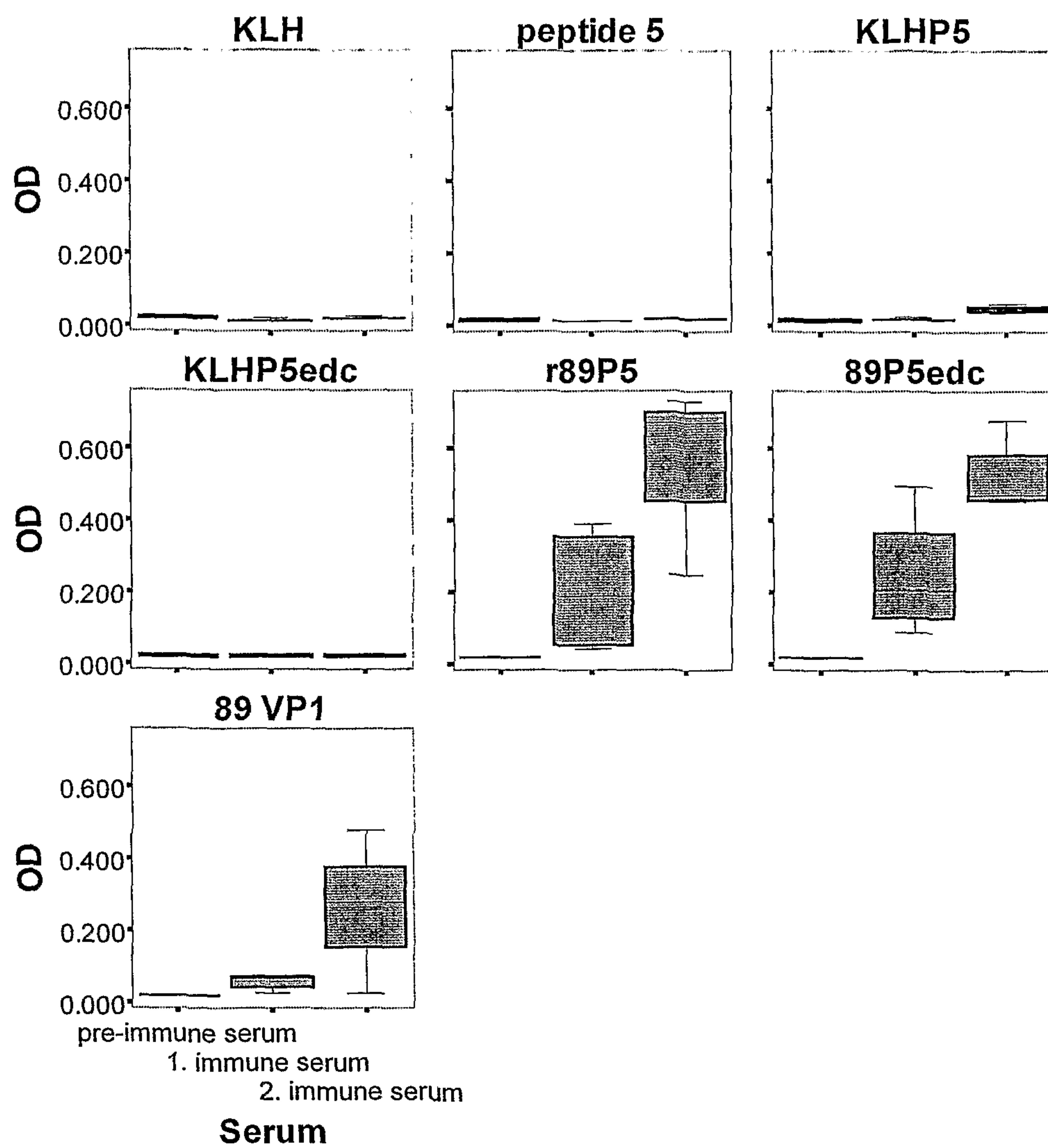


Fig. 4

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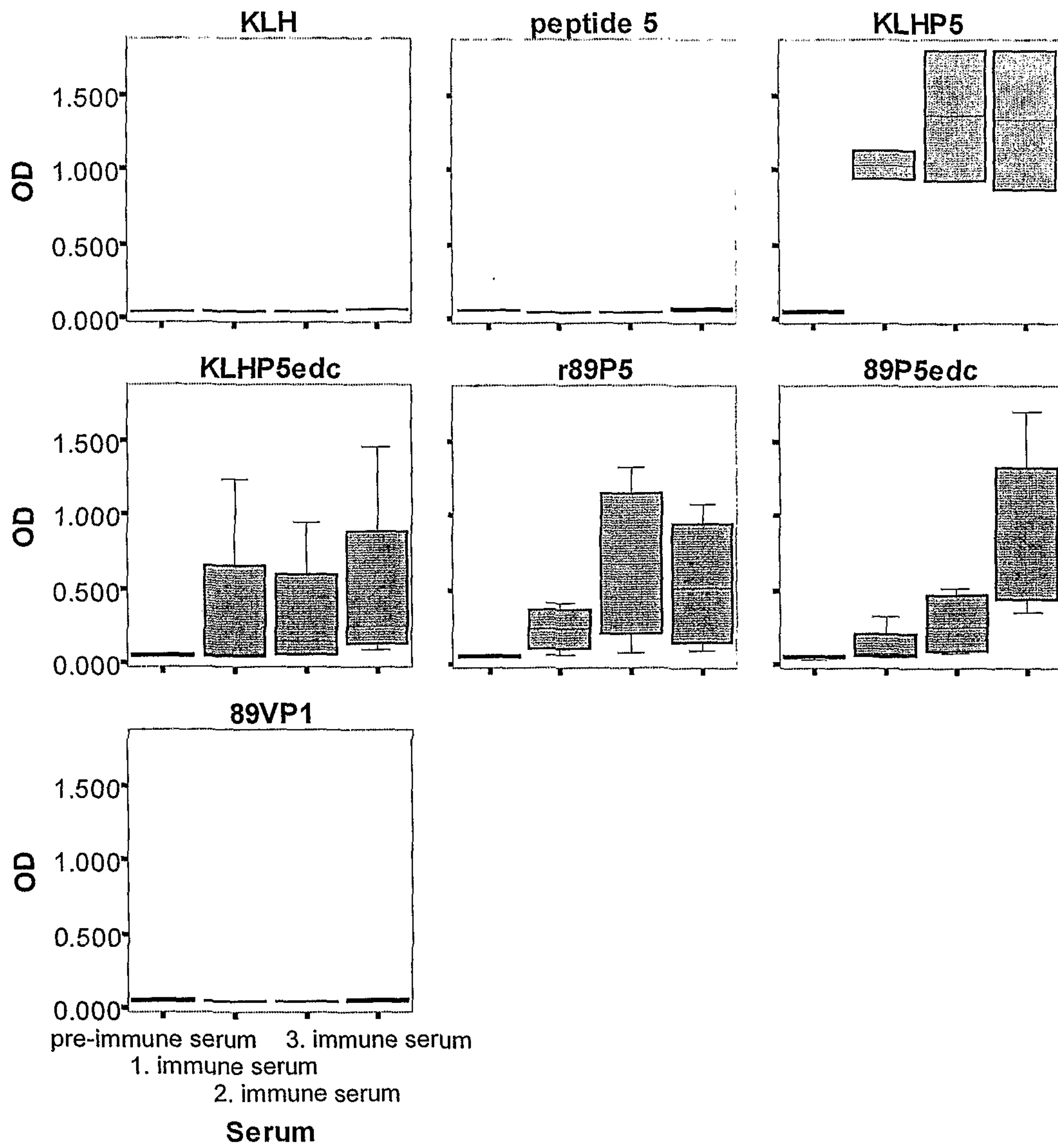


Fig. 5

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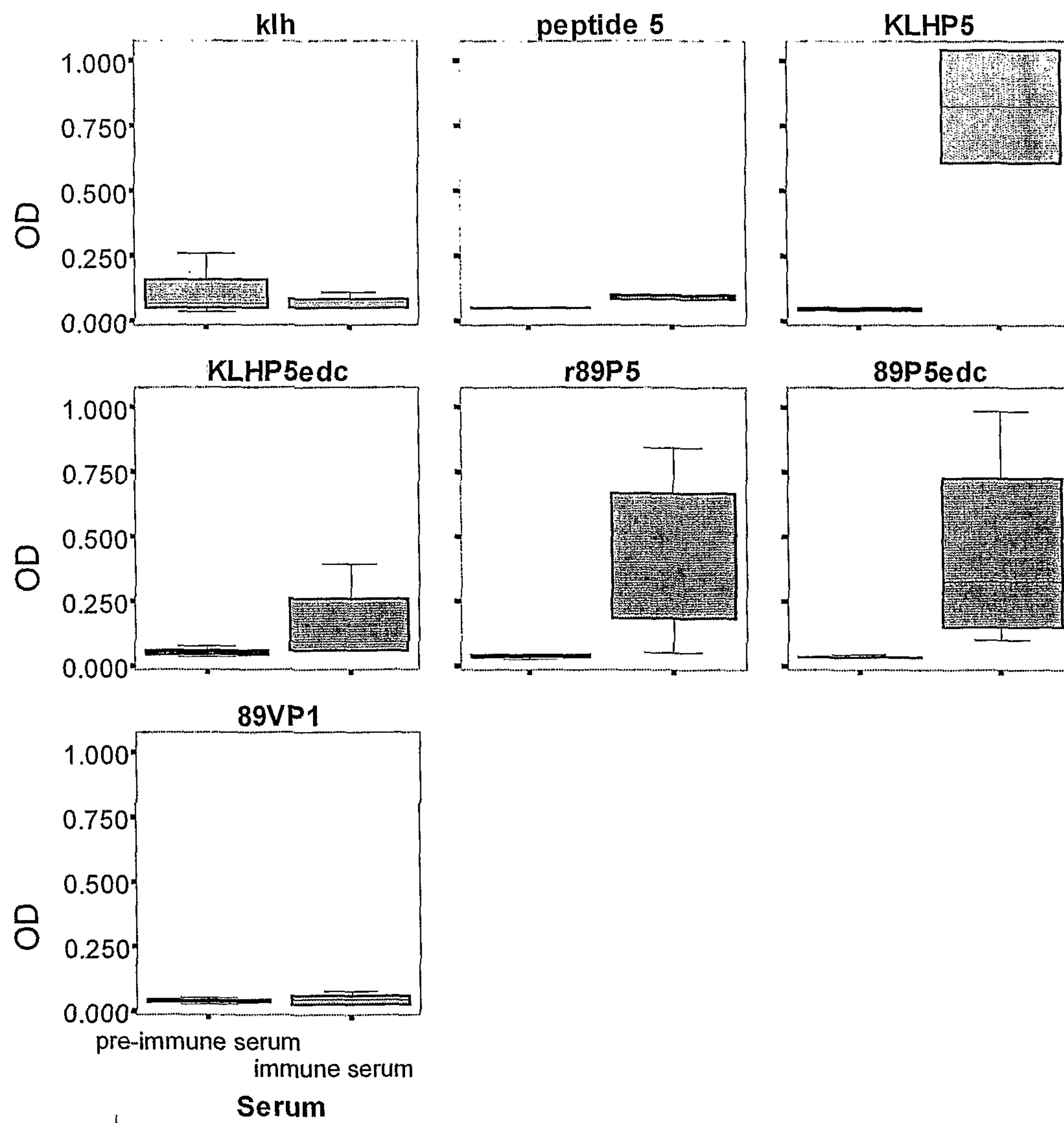


Fig. 6

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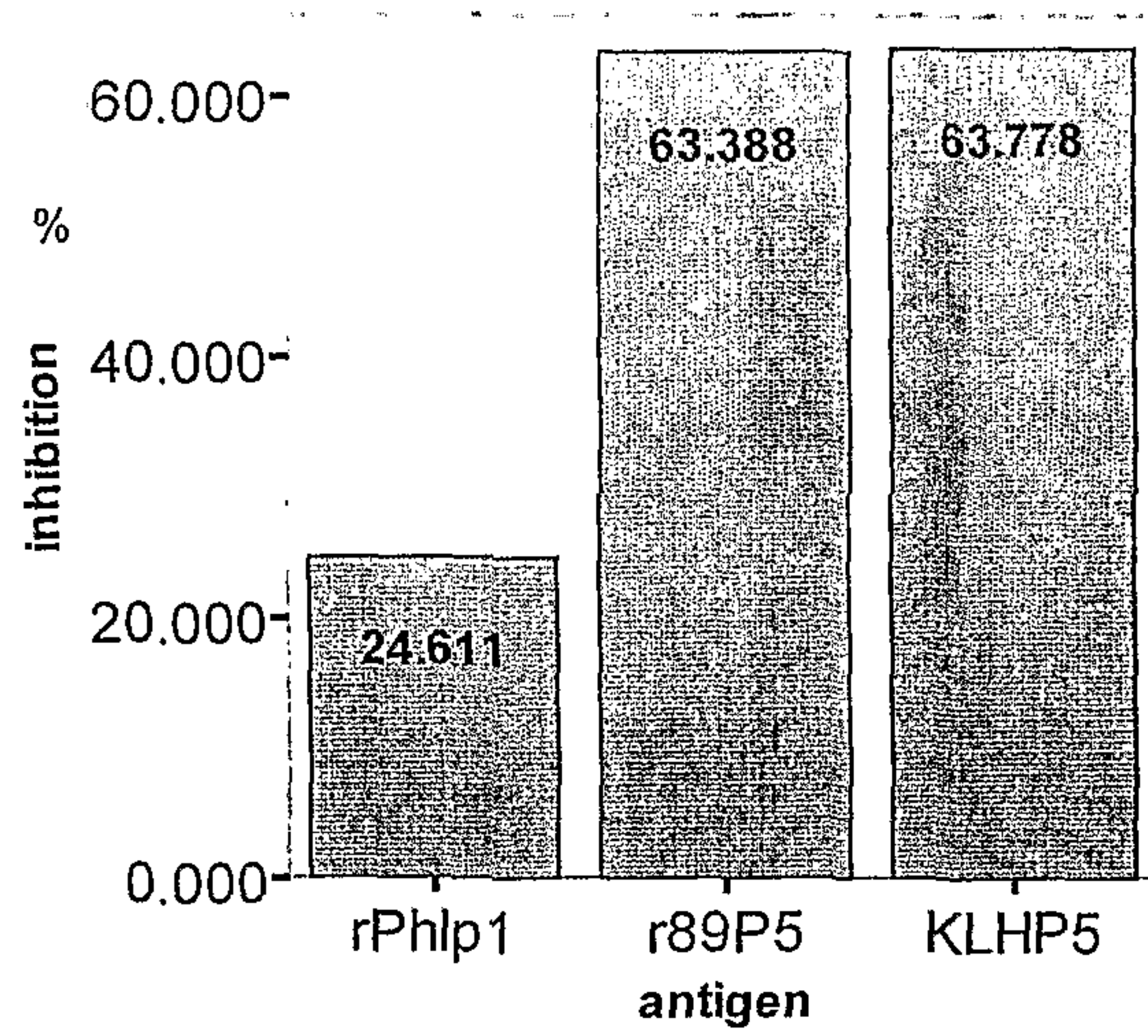


Fig. 7

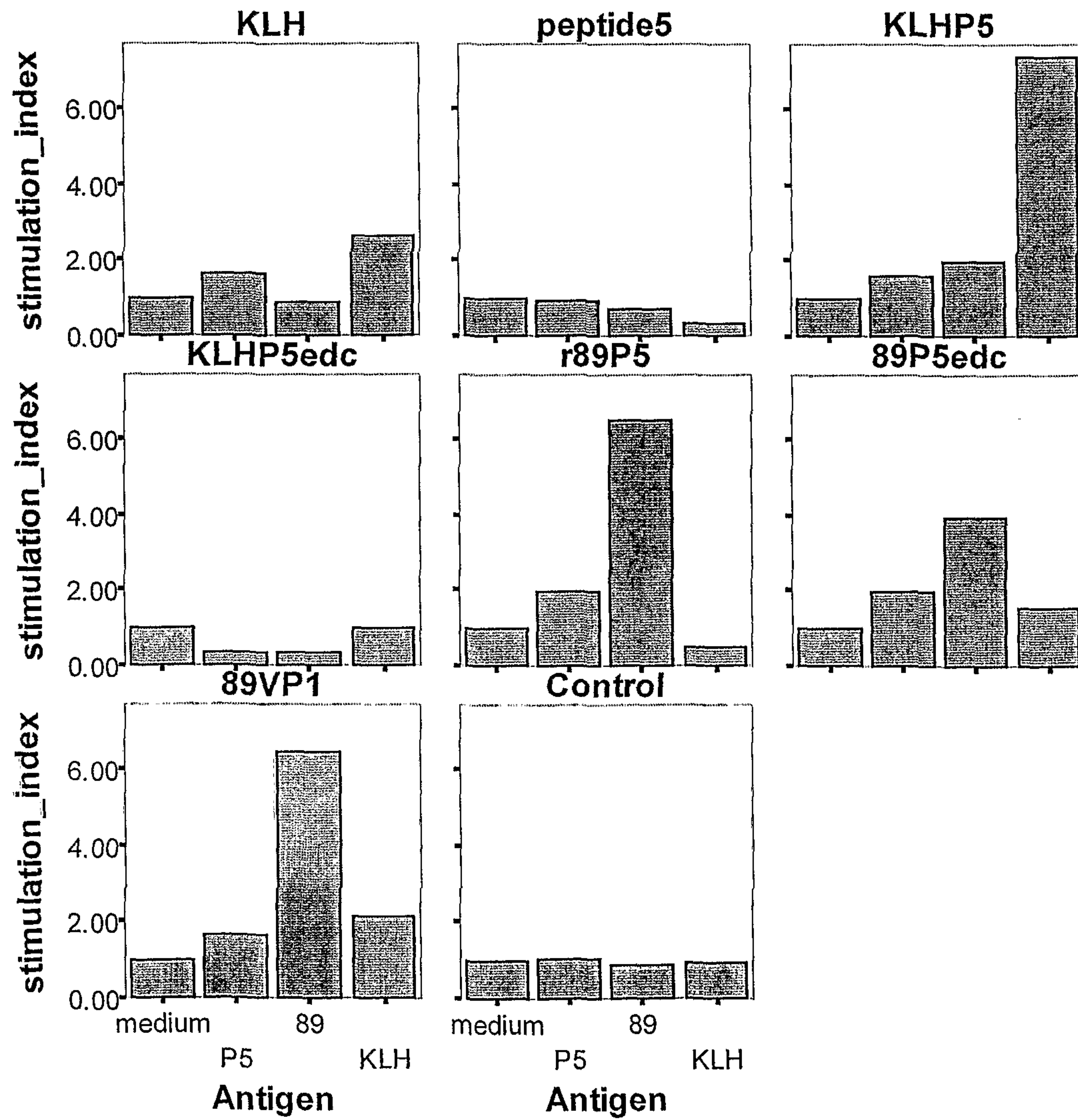


Fig. 8

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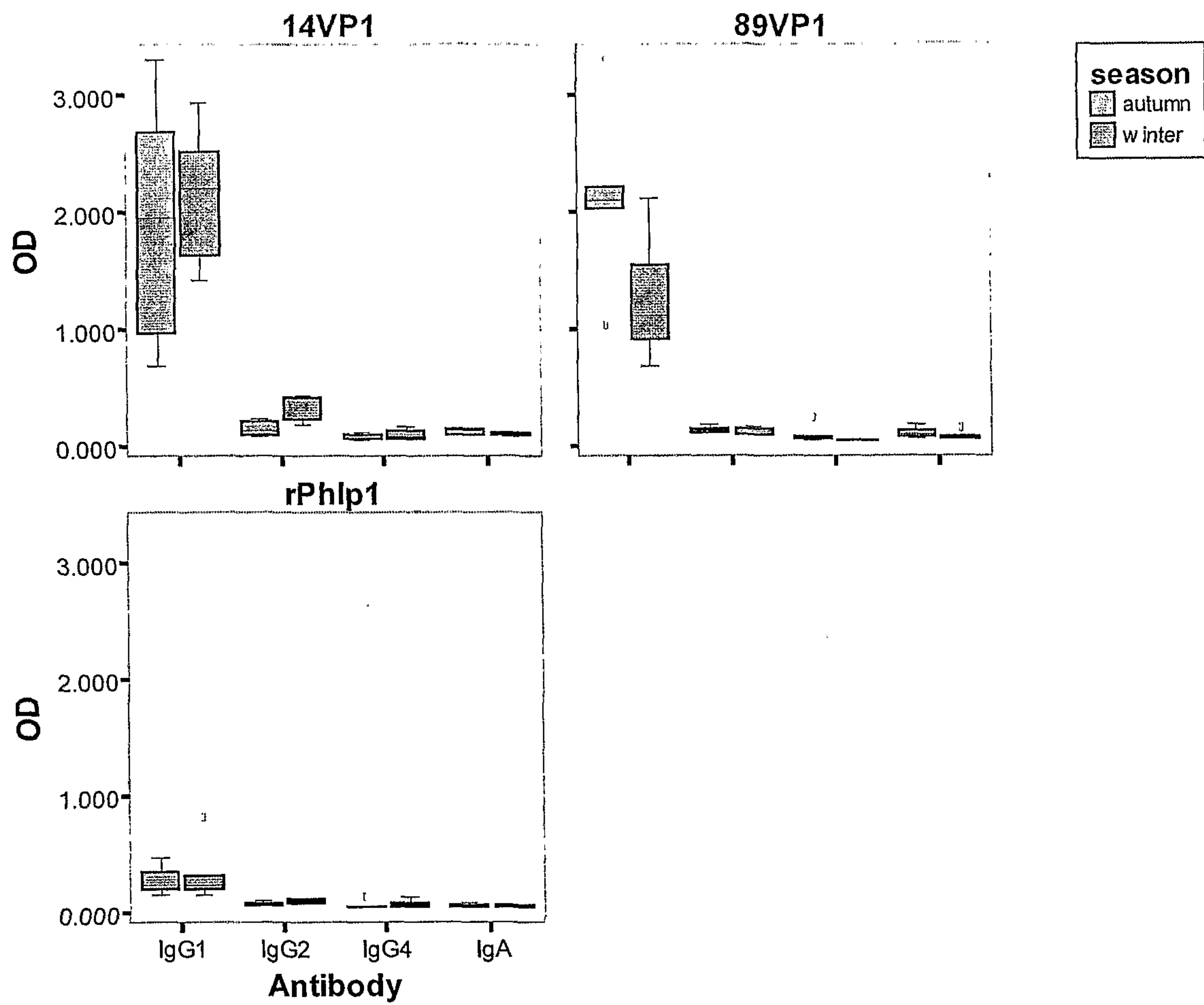


Fig. 9

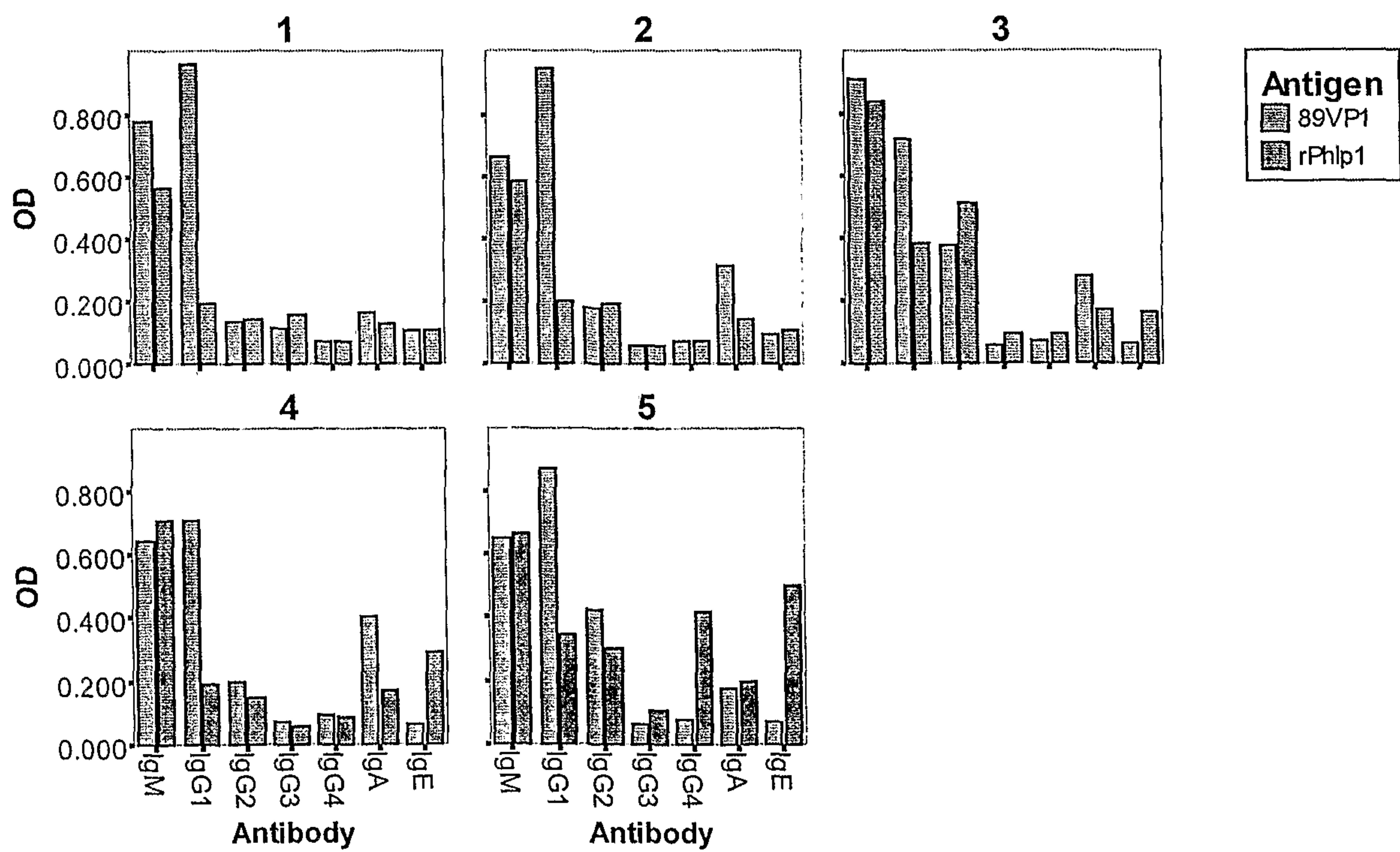


Fig. 10

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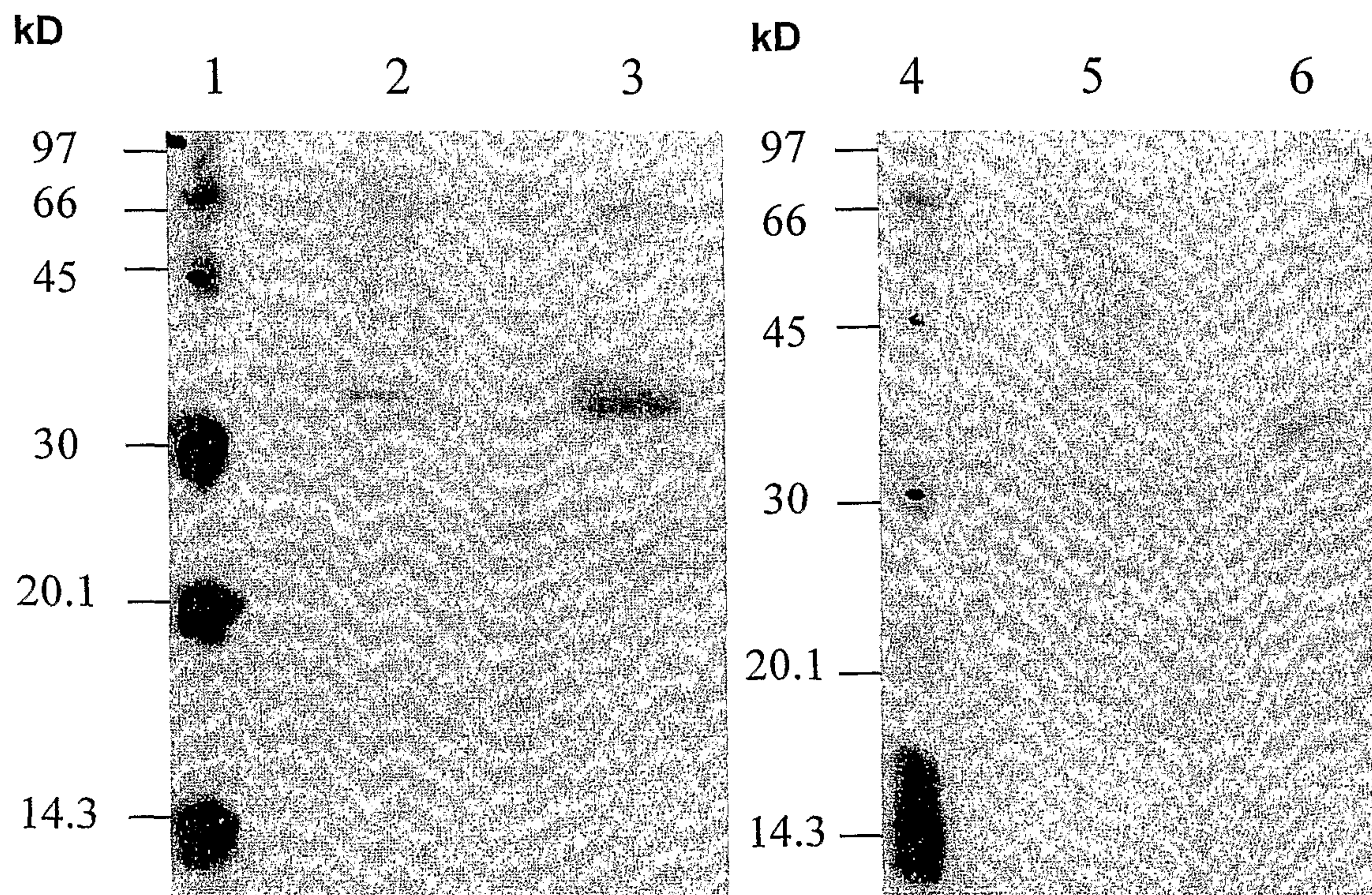


Fig. 11

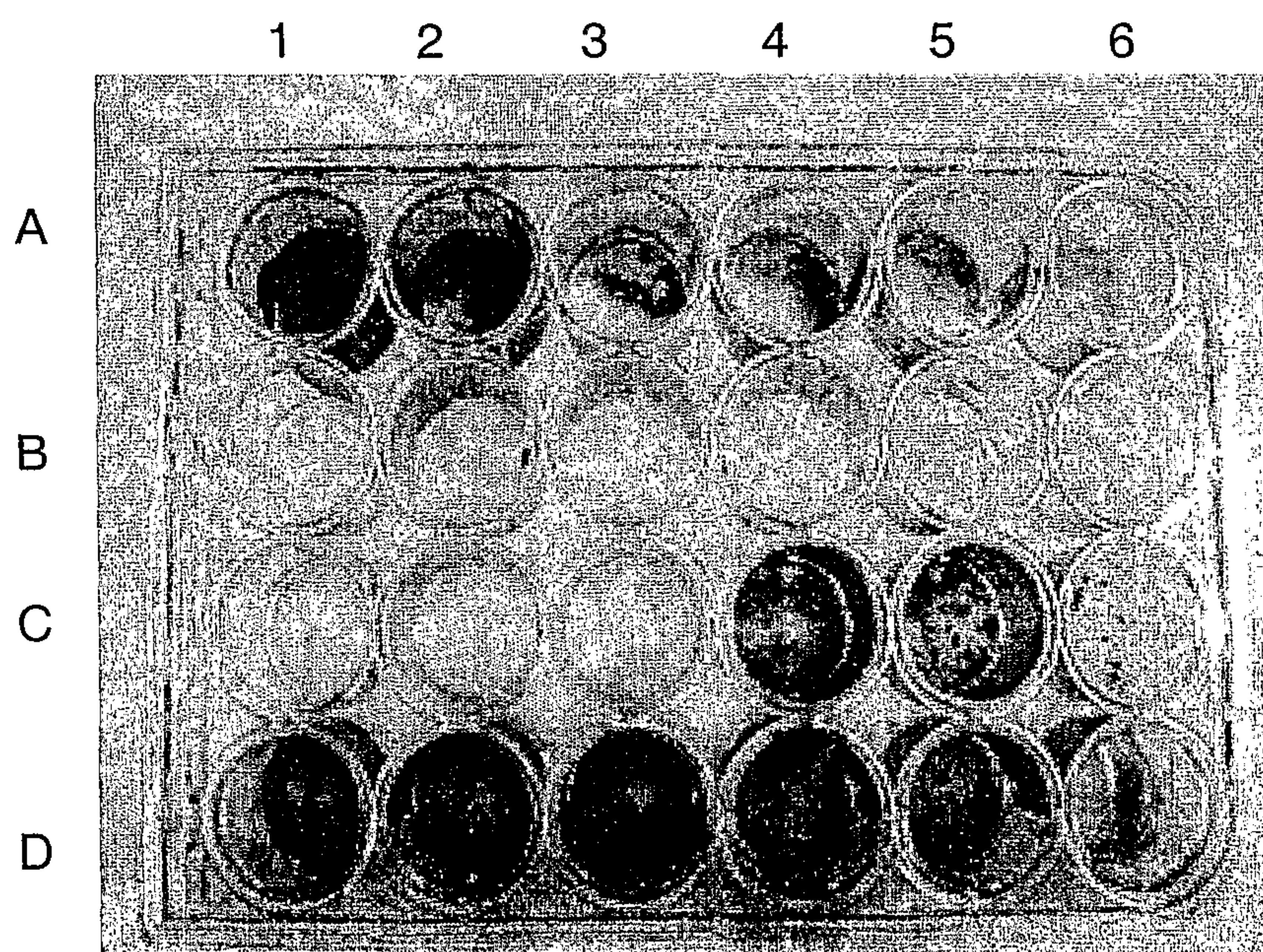


Fig. 12

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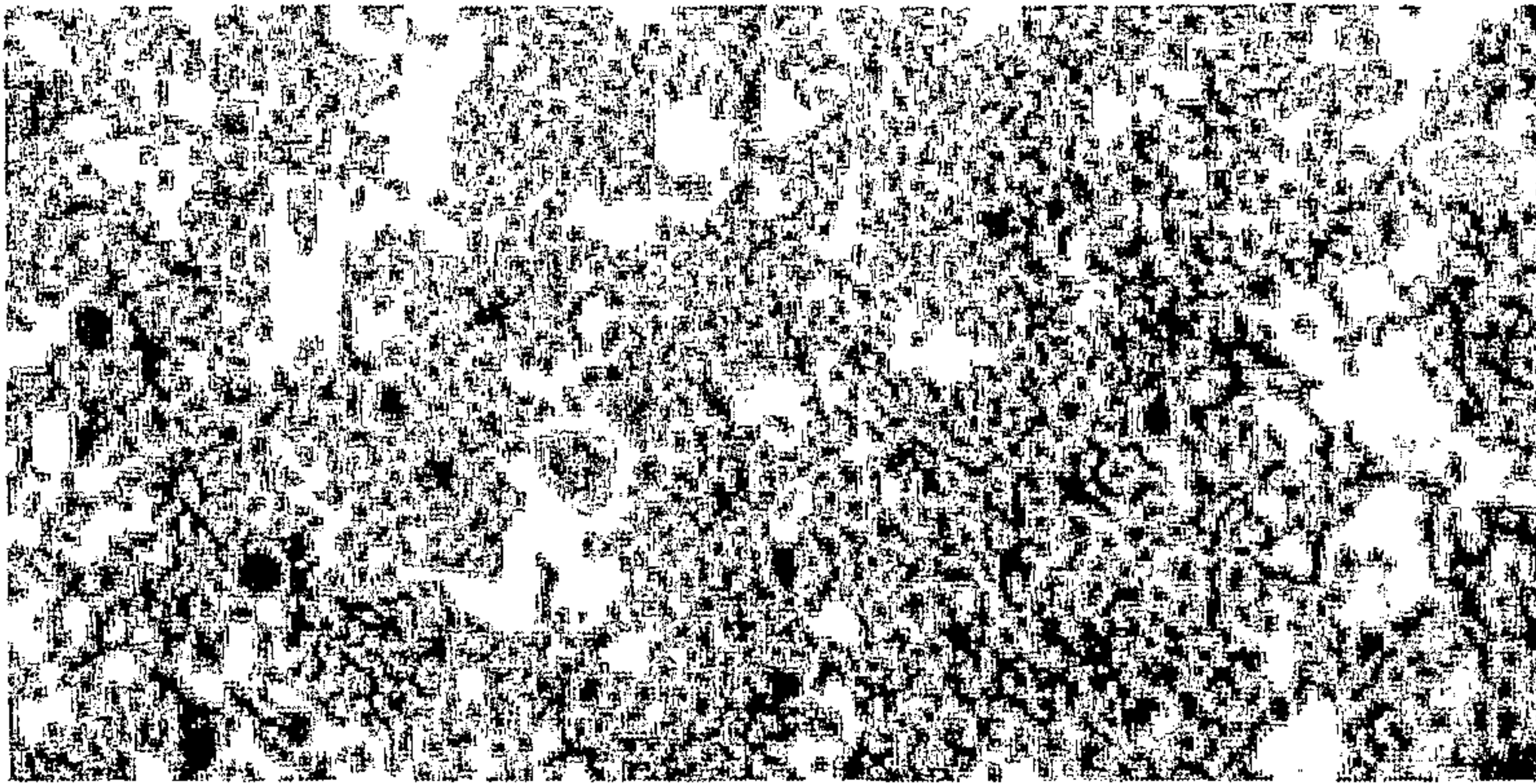


Fig. 12A

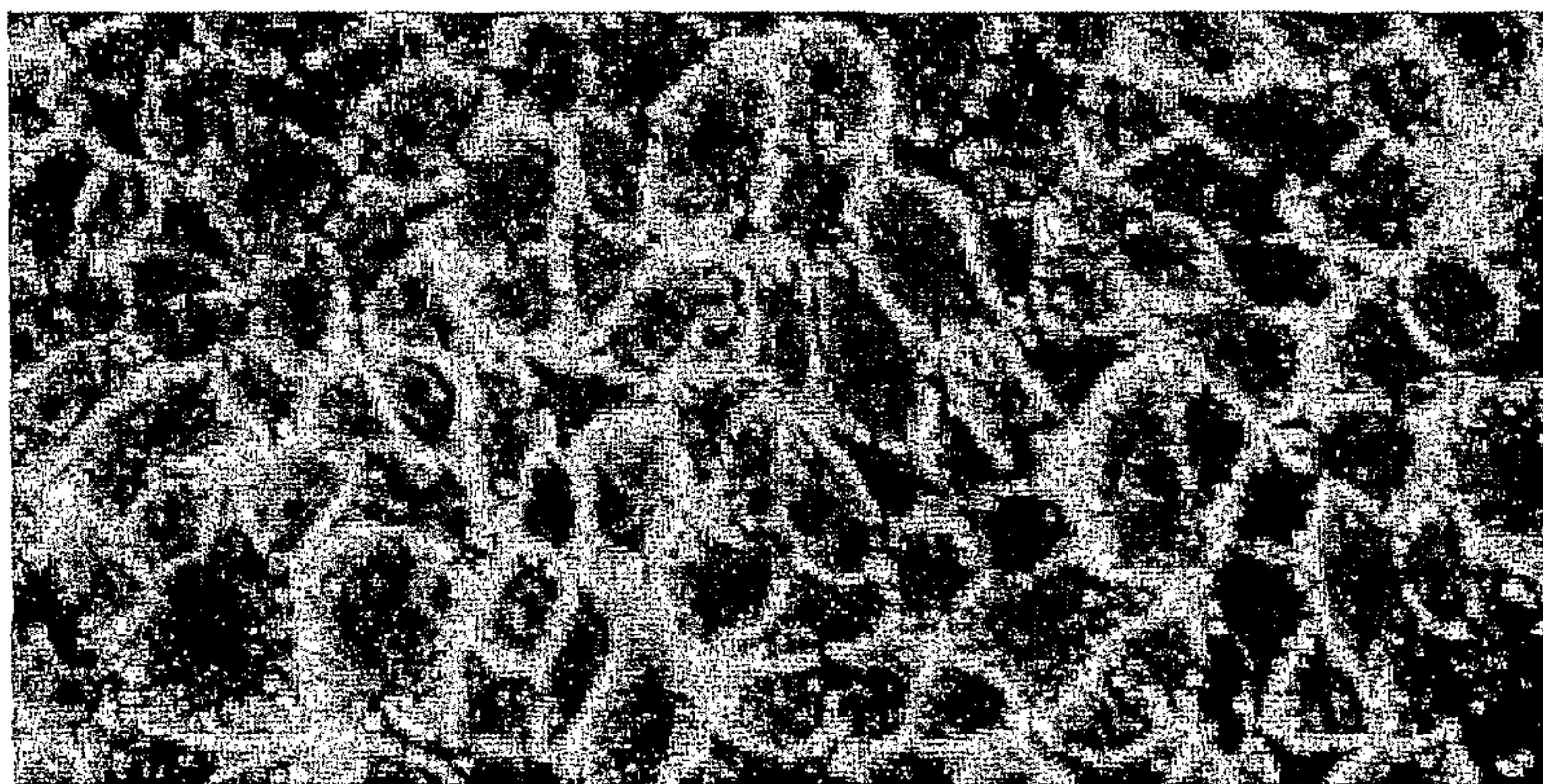


Fig. 12B

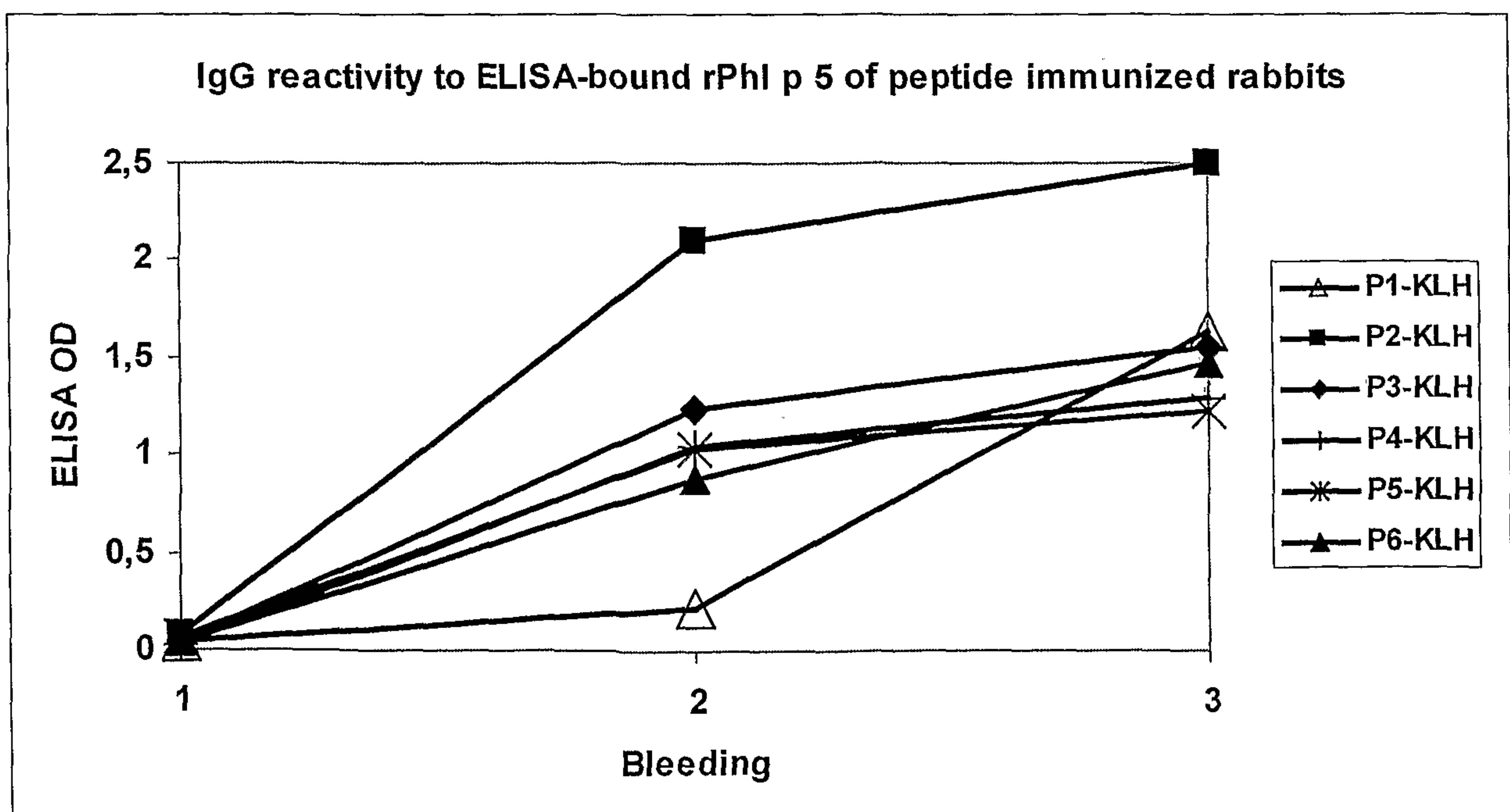


Fig. 13

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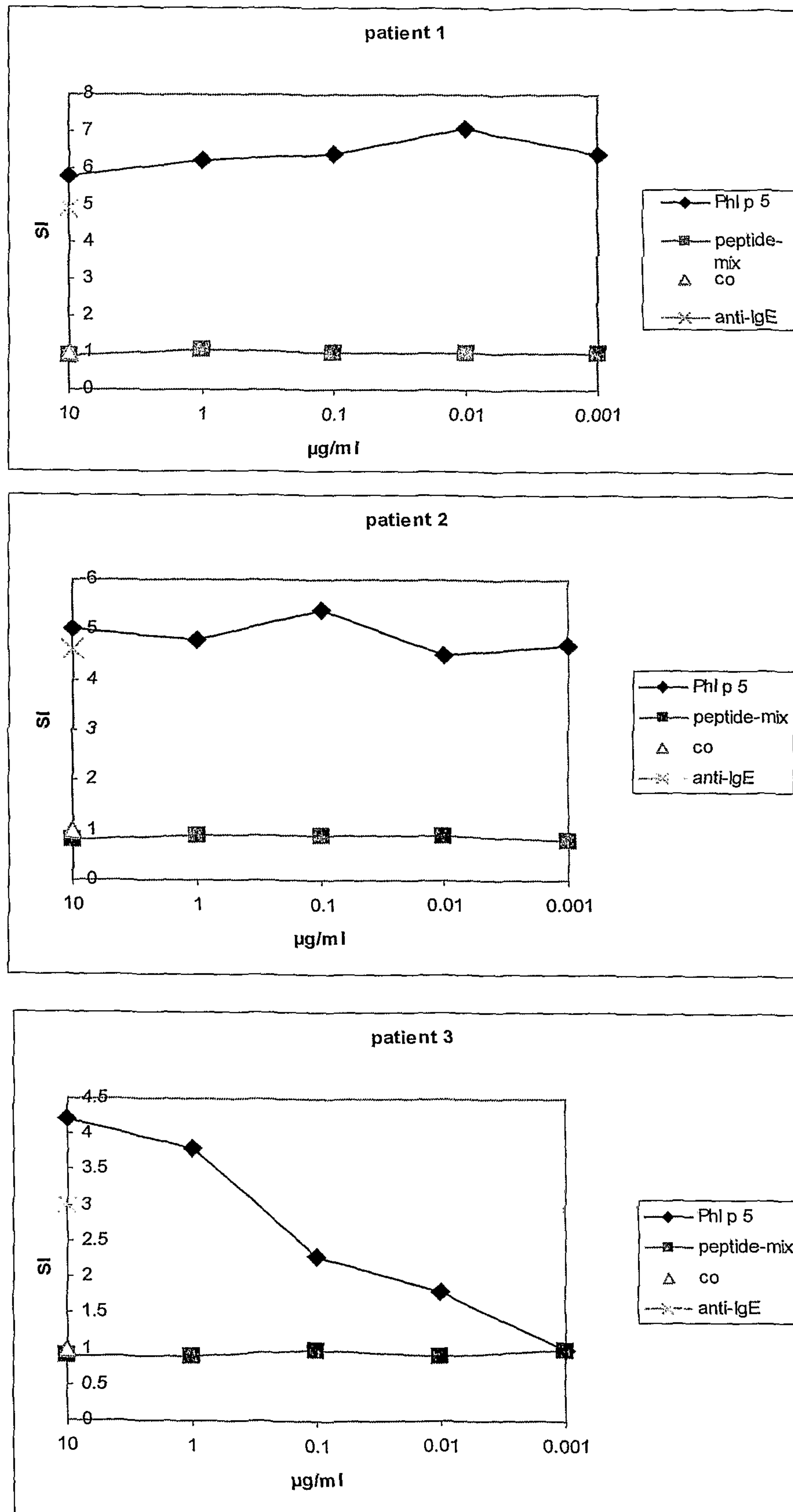


Fig. 14

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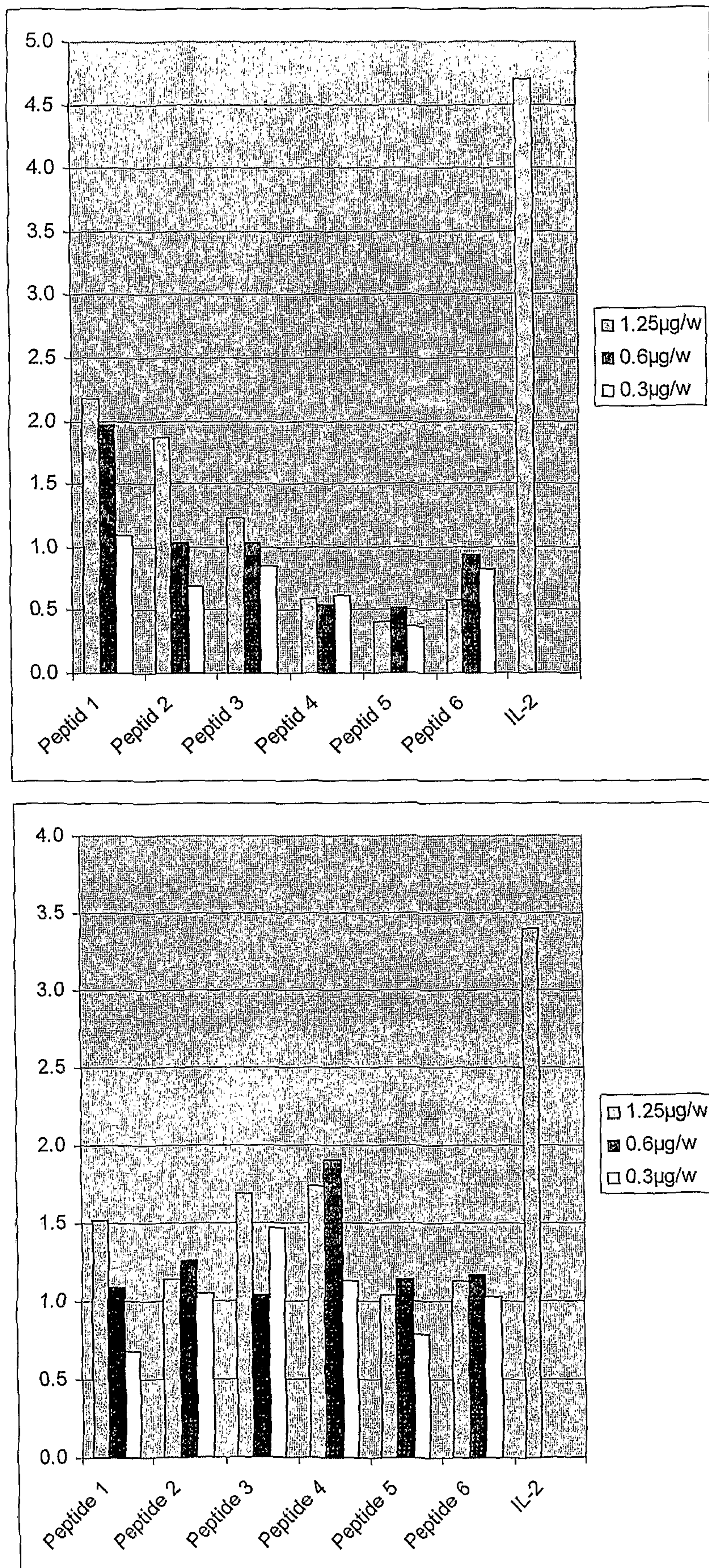


Fig. 15

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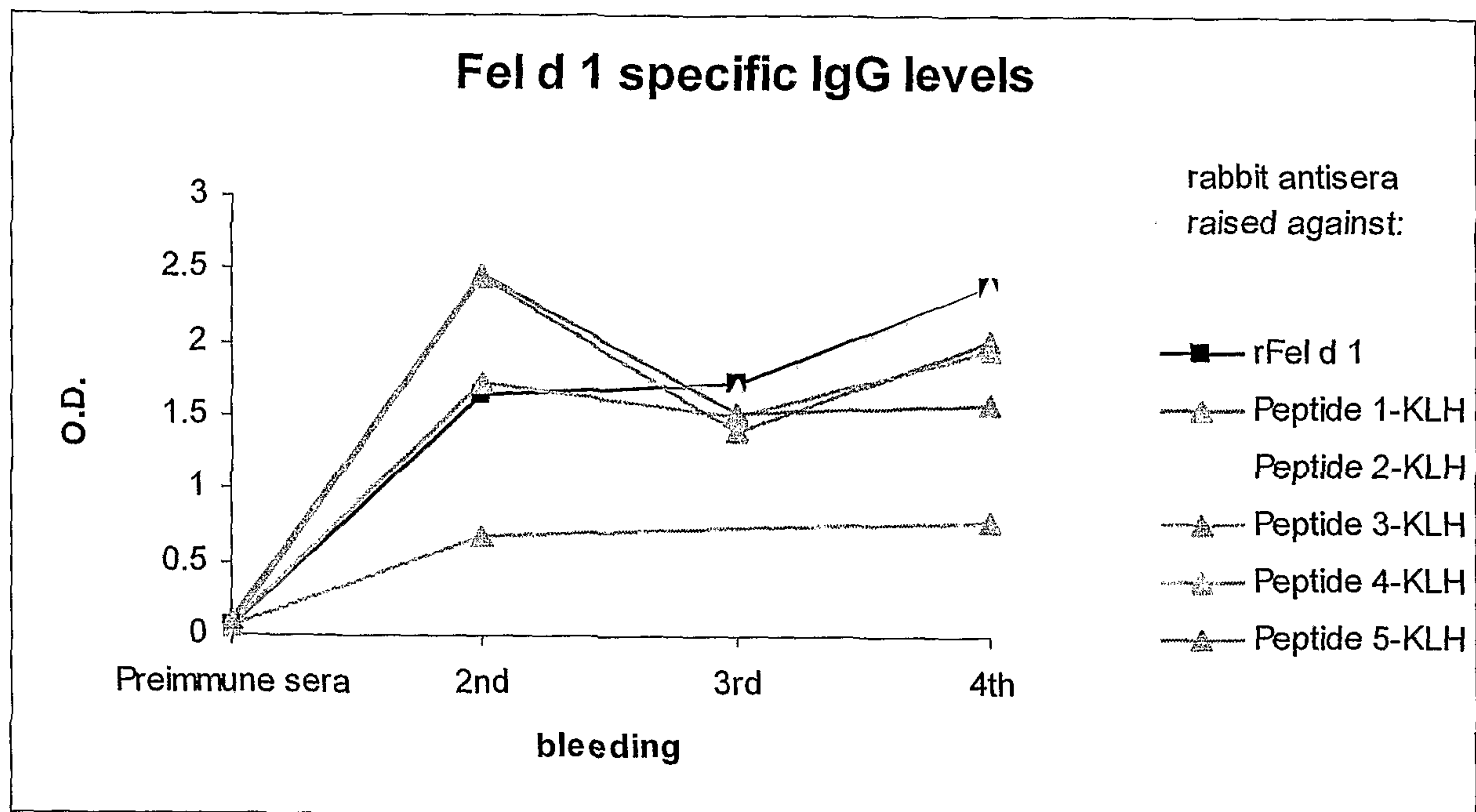


Fig. 16

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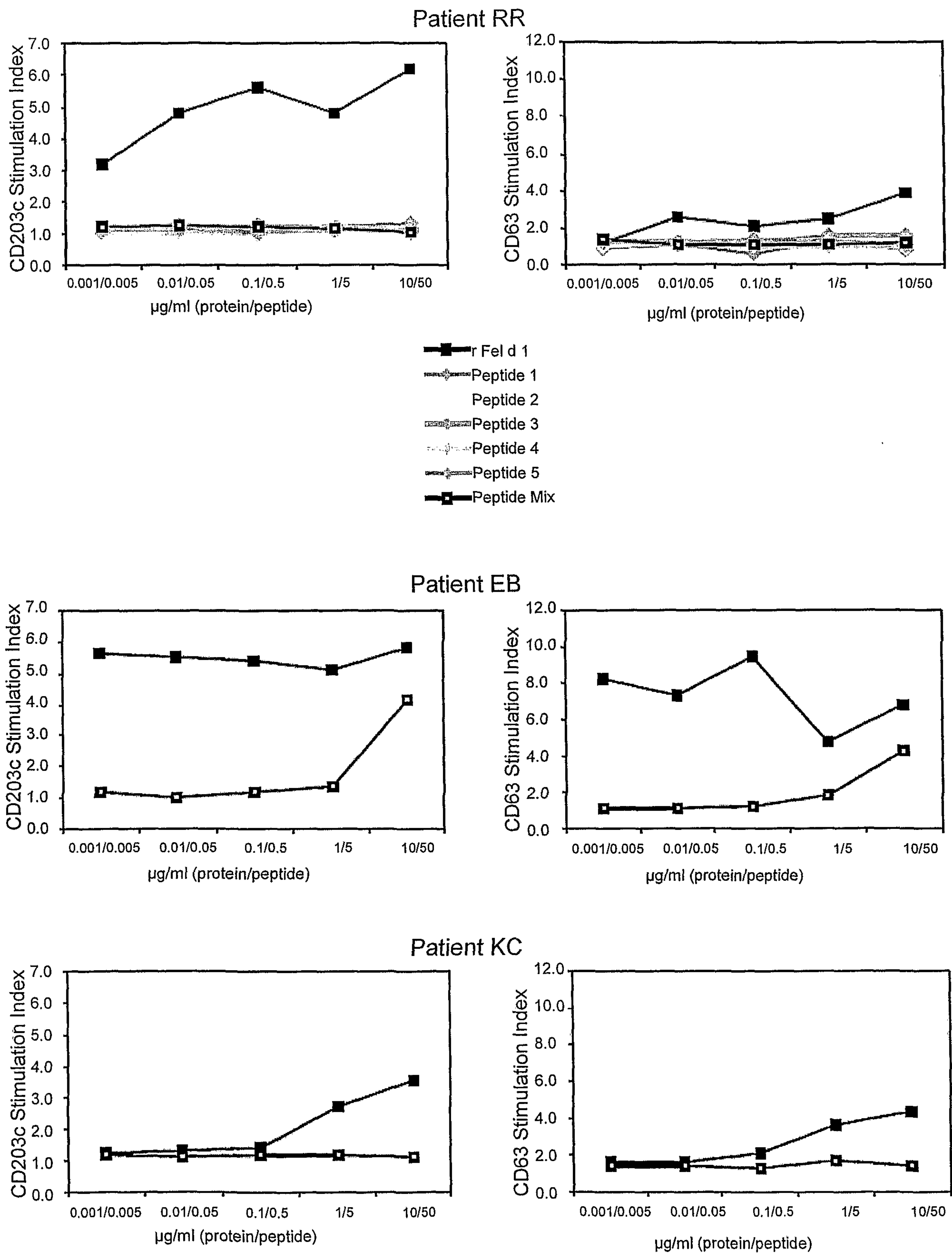
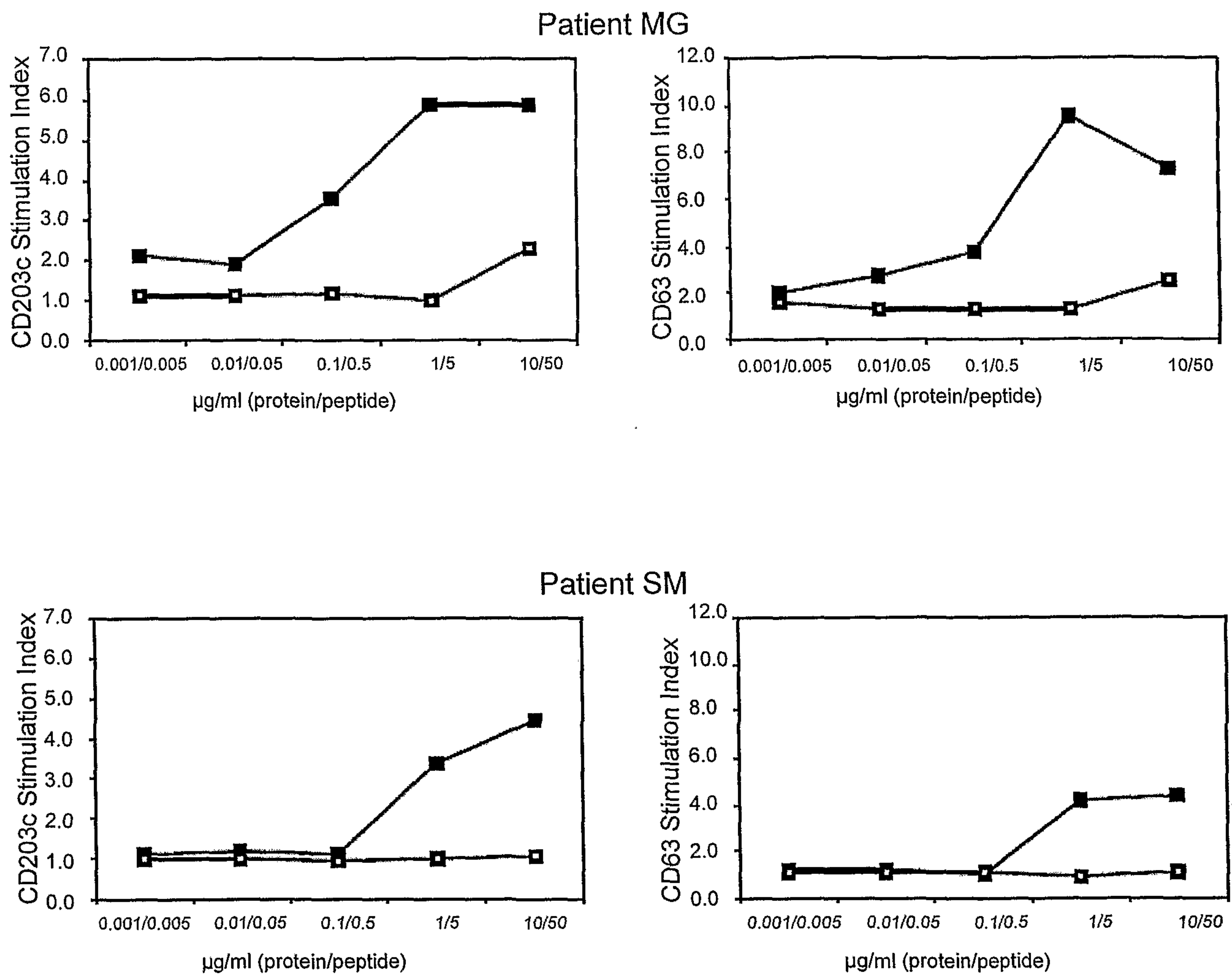


Fig. 17

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Cont. Fig. 17

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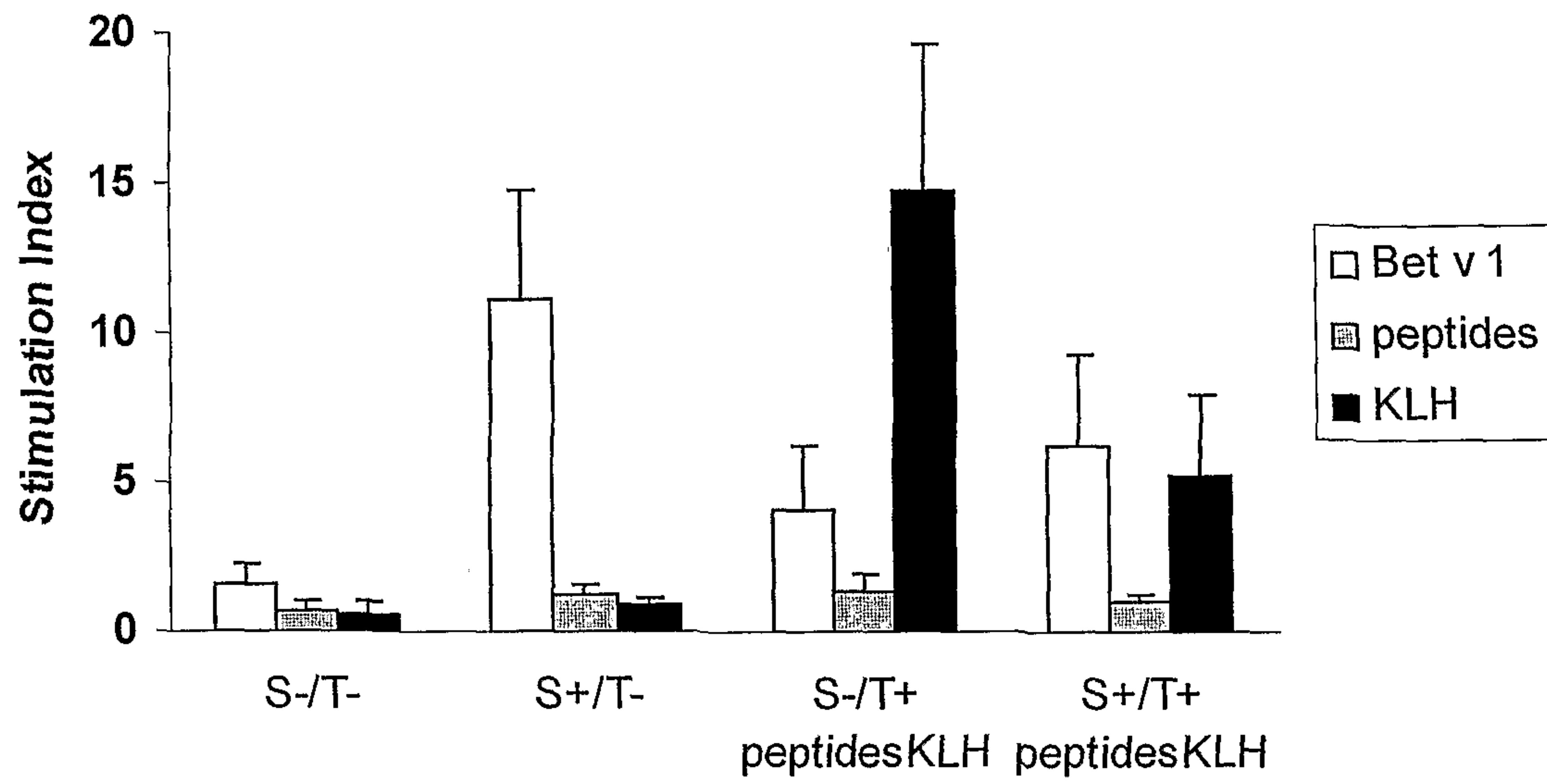


Fig. 18

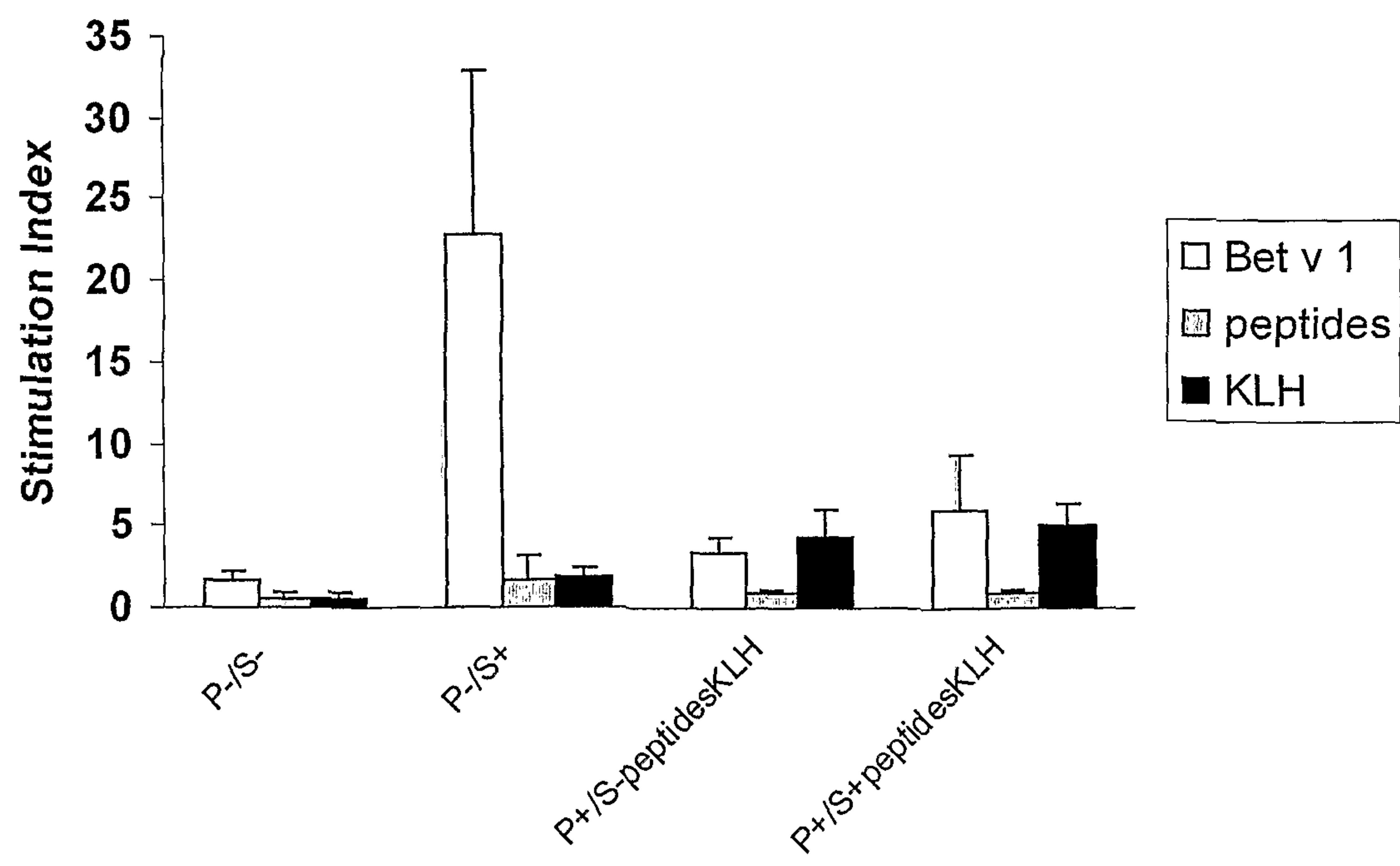


Fig. 19

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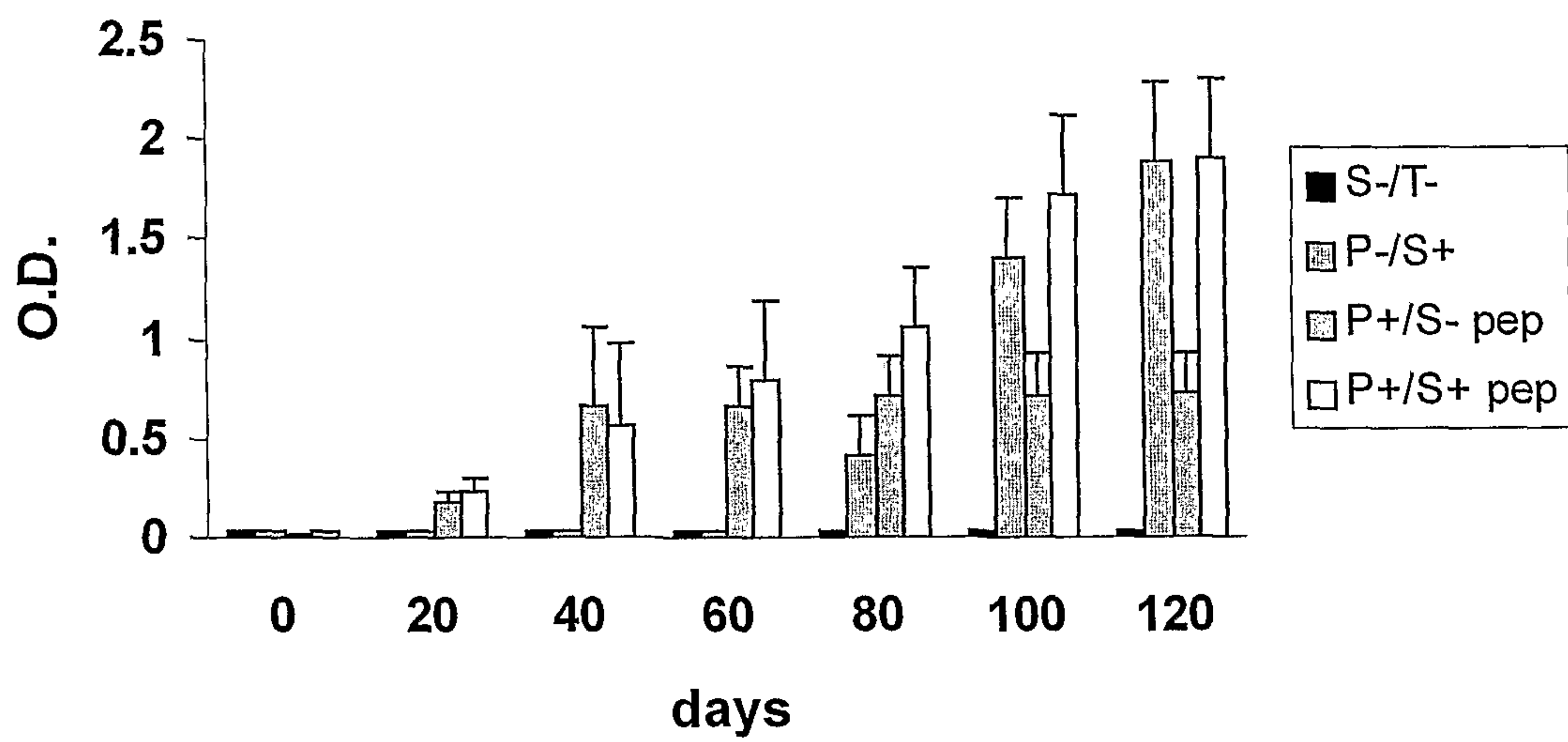


Fig. 20

IgE-reactivity

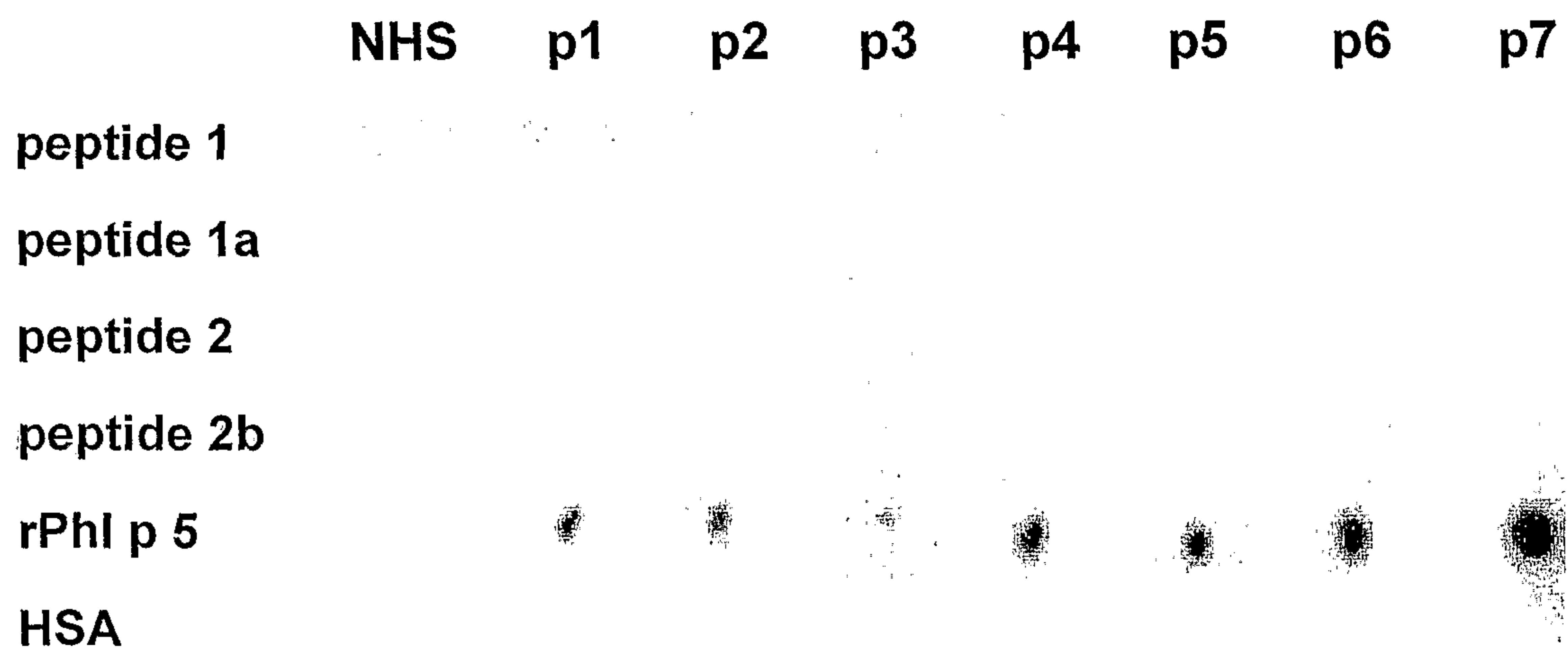


Fig. 21

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T-cell reactivity

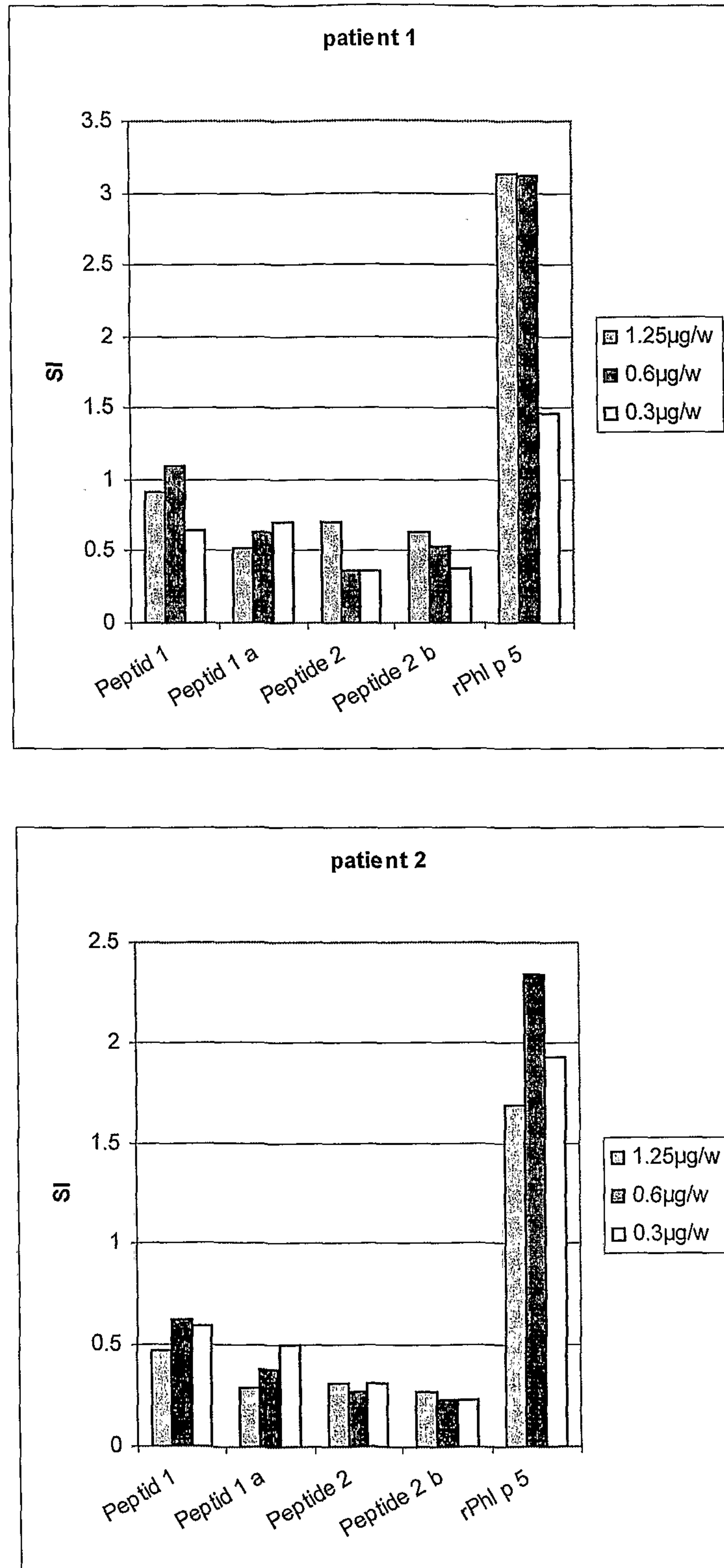


Fig. 22

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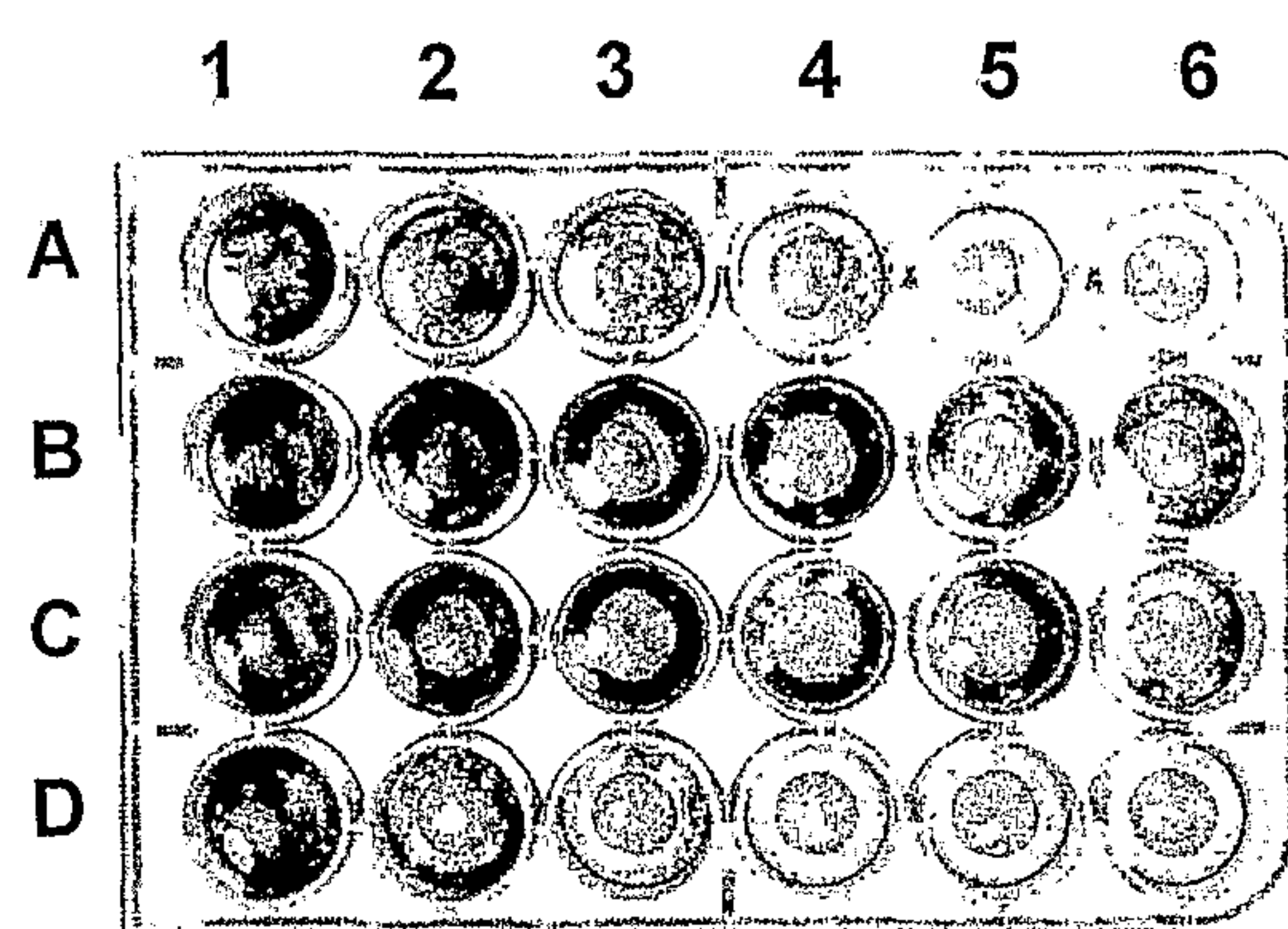


Fig. 23