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(54) **Title**: JAPANESE POLLEN ALLERGEN PEPTIDES FOR PREVENTING OR TREATING ALLERGY

(57) **Abstract**: A polypeptide or a pharmaceutically acceptable salt thereof and pharmaceutical formulations of at least three polypeptidesor salts thereof are provided which may be used for preventing or treating allergy to Japanese Cedar and/or Japanese Cypress pollen.

JAPANESE POLLEN ALLERGEN PEPTIDES FOR PREVENTING OR TREATING ALLERGY

Field of the Invention

The present invention relates to polypeptides and pharmaceutical formulations thereof which may be used for preventing or treating allergy to Japanese Cedar and/or Japanese Cypress pollen.

Background to the Invention

Tree pollen allergens are recognised as a major cause of allergic diseases in humans. In particular, allergy to pollen (also known as pollinosis) of the Japanese Cedar tree (Cryptomeria japonica; 'sugi' in Japanese) is one of the most common allergic respiratory diseases in Japan. The Japanese Cypress tree (Chamaecyparis obtusa, 'hinoki' in Japanese) also causes pollinosis in Japan. The Japanese Cedar pollen season runs from February to March while the Japanese Cypress pollen season runs from March to April; symptoms can also persist beyond the pollen season. A significant proportion of the Japanese population suffers from symptoms of pollinosis and allergy. Pollinosis and allergy also occur in other countries where the Japanese Cedar or Japanese Cypress trees is a native species or where the trees are cultivated, including Korea and China.

Summary of the Invention

The invention provides a pharmaceutical formulation comprising:

(a) a polypeptide comprising the amino acid sequence of RPTAIKIDYSKSVT (SEQ ID NO: 5; CRY19A); or a T cell epitope-containing variant sequence derived from said amino acid sequence,

(b) a polypeptide comprising the amino acid sequence of GAYFVSSGKYEGG (SEQ ID NO: 163, CRY11D) or a T cell epitope-containing variant sequence derived from said amino acid sequence, and

(c) a polypeptide comprising the amino acid sequence of TFKVDGIIAAYQNPASWK (SEQ ID NO: 19; CRY15) or a T cell epitope-containing variant sequence derived from said amino acid sequence,

wherein a T cell epitope-containing variant sequence of a said amino acid sequence is said amino acid sequence having up to five amino acid modifications, each of which is independently a deletion, substitution or insertion;

wherein each polypeptide is up to 30 amino acids in length;

wherein each polypeptide may be present as a pharmaceutically acceptable salt thereof;

wherein said pharmaceutical formulation further comprises a pharmaceutically acceptable carrier or diluent.

The invention also provides a polypeptide consisting of the amino acid sequence RPTAIKIDYSKSVT (SEQ ID NO: 5; CRY19A), KGAYFVSSGKYEGGK (SEQ ID NO: 165; CRY11F), TFKVEGIIAAYQNPASWK (SEQ ID NO: 54; CRY15A), KRIWLQFAKLTGFTLK (SEQ ID NO: 159; CRY17A), KSMKVTVAFNQFGP (SEQ ID NO:13, CRY08), SKNFHLQKNTIGTG (SEQ ID NO: 166; CRY21C), TYKNIRGTSAT (SEQ ID NO: 23; CRY22), KDIKLSDISLKLTSGKIAS (SEQ ID NO: 24; CRY23), or a pharmaceutically acceptable salt thereof.

The invention further provides a polypeptide, salt thereof or pharmaceutical formulation of the invention for use in a method of treating or preventing allergy to Japanese Cedar pollen and/or Japanese Cypress pollen.

The invention further provides a method of treating an individual for allergy to Japanese Cedar pollen and/or Japanese Cypress pollen or of preventing in an individual allergy to Japanese Cedar pollen and/or Japanese Cypress pollen, which method comprises administering to said individual a therapeutically or prophylactically effective amount of a polypeptide, salt thereof or pharmaceutical formulation of the invention.

The invention also provides use of a polypeptide or salt thereof of the invention for the manufacture of a medicament for the prevention or treatment of allergy to Japanese Cedar pollen and/or Japanese Cypress pollen.

The invention additionally provides an *in vitro* method of determining whether T cells recognize a polypeptide or salt thereof of the invention or a polypeptide or salt thereof of said pharmaceutical formulation of the invention comprising at least three polypeptides, which method comprises contacting said T cells with said polypeptide or salt thereof or said pharmaceutical formulation and detecting whether said T cells are stimulated by said polypeptide or salt thereof.

Description of the sequences

SEQ ID NOs 1 to 156 provide amino acid sequences as set out in Examples 1 to 9 and the Detailed Description of the Invention.

SEQ ID NOs 1, 114 to 116, 134, 135, 137, 138 correspond to amino acid sequences derived from Cry j IFR.

.SEQ ID NOs 2, 6 to 15, 26, 29 to 32, 34 to 35, 37-38, 42, 48, 67 to 73, 140-141, 148 ,150-151, 157-158 and 163-165 correspond to amino acid sequences derived from Cry j1.

SEQ ID NOs 3 to 5, 16 to 22, 51, 54, 58, 60-62, 74-88, 142-145,149, 159 to 162 and 166 correspond to amino acid sequences derived from Cry j2.

SEQ ID NOs 25 and 89-106 correspond to amino acid sequences derived from Cry j3.

SEQ ID NOs 107-113 correspond to amino acid sequences derived from Cry j chitinase.

SEQ ID NOs 27-28, 33, 36, 39-41, 43, 44, 46, 47, 49, 117-121 and 136 correspond to amino acid sequences derived from Cha o1.

SEQ ID NOs 50, 52, 53, 55-57, 59, 63-66, 122-133 , 146-147 and 167 correspond to amino acid sequences derived from Cha o2.

SEQ ID NOS: 154-156 correspond to amino acid sequences of control polypeptides used in Example 9.

NCBI accession numbers for the proteins referred to above are provided in Examples 1 to 7.

Brief Description of the Drawings

Figure 1 shows the results of a cell proliferation assay using peptides described herein, and media (negative control), Candida Albicans and Japanese Cedar whole allergen extract (positive controls). Proliferation is measured as incorporation of 3H-thymidine (CPM – Y axis).

Figures 2 and 3 show the results of cell proliferation and cytokine release assays with peptides described herein. The percentage of subjects in the study population showing positive responses is shown, together with a ranking for peptides on each

parameter. The designation CHA19 used in Figures 2 and 3 (and also Figures 4-8) is an alternative reference for peptide CRY9A.

Figure 4 shows analyses of population coverage provided by preferred peptides based on cytokine release data shown in Figures 2 and 3. Figure 4A shows the percentage of the study population responding to one, two or three peptides taken from the CRY11, CRY15 and CRY17 series. 98% of the study population responded to at least one of these peptides and 64% to all three peptides. Figure 4B shows the percentage of the study population with a positive response to various preferred peptides for one, two or three of the cytokines (CK) analysed.

Figure 5A shows a heat map for responses across the subjects in the study population, with a result for a subject showing positive responses to a peptide for at least one cytokine shaded grey. Negative responses are shown as white. The response multiplicity score and cumulative cytokine response scores are also shown together with the total scores for the entire population for all eight peptides. Figure 5B is identical to Figure 5A save that positive responses are also shown alongside the number of cytokines for which a positive response was present (1, 2 or 3). The response multiplicity score **is** the cumulative number of positive responses to each peptide for at least one cytokine.. The cumulative cytokine response score is the cumulative number of positive responses to each peptide for all three cytokines. Surrogate peptides from the CRY11, CRY17 and CRY21 series were used to represent responses for CRY11F, CRY17A and CRY21C.

Figures 6A and 6B show HLA binding analysis for various preferred peptides based on selected alleles present in the Japanese population. Figure 6A shows potential impact in the Japanese population based on REVEAL® score for each peptide (as the percentage of the Japanese population which is covered). Figure 6B shows potential impact in the Japanese Population based on stability index (as the percentage of the Japanese population which is covered). Calculation of the REVEAL® score and stability index and percentage impact in the Japanese population is described in Example 14.

Figures 7A to C provide detailed results for percentage impact in the Japanese population based on REVEAL® score for each peptide and each allele.

Figures 8A to 8C provide detailed results for percentage impact in the Japanese population based on stability index for each peptide and each allele.

Figure 9 shows individual and cumulative genotype frequencies for selected HLA alleles in the Japanese population.

Detailed Description of the Invention

The present invention is concerned with preventing or treating allergy to Japanese Cedar pollen and/or Japanese Cypress pollen and provides polypeptides, and pharmaceutically acceptable salts thereof, suitable for this use. Said polypeptides or salts thereof may be provided in pharmaceutical formulations.

Amino acid sequences and variant amino acid sequences

A polypeptide of the invention may for example comprise, consist or consist essentially of an amino acid sequence as shown in SEQ ID NO:5. Alternatively, a polypeptide of the invention may comprise, consist or consist essentially of a T cell epitope-containing variant sequence which is an amino acid sequence as shown in SEQ ID NO: 5 having up to five amino acid modifications, each of which is independently a deletion, substitution or insertion.

For the avoidance of doubt, the discussion herein of the characteristics of polypeptides and variant amino acid sequences by reference to SEQ ID NO 5 also applies to the amino acid sequences of SEQ ID NOs 1, 3, 4, 6 to 25 and 163. Thus, for example, a pharmaceutical composition of the invention may comprise a polypeptide that comprises, consists or consists essentially of an amino acid sequence as shown in SEQ ID NO: 163. Alternatively, the polypeptide may comprise, consist or consist essentially of a T cell epitope-containing variant sequence which is an amino acid sequence as shown in SEQ ID NO: 163 having up to five amino acid modifications, each of which is independently a deletion, substitution or insertion.

It is preferred that the modifications in a variant sequence do not alter the functional properties of a T cell epitope present in the corresponding original amino acid sequence. The functional properties of T cell epitopes are discussed further below.

In preferred variant sequences, sufficient contiguous amino acids of the corresponding original amino acid sequence are retained to contain a T cell epitope. Typically, such a variant sequence retains at least 8, preferably at least 9, contiguous amino acids of the original amino acid sequence. The variant sequence may retain from 8 to 12 amino acids or from 9 to 12 amino acids of the original amino acid sequence.

Where applicable, the variant sequence may retain from 8 to 15 amino acids, or from 9 to 15 amino acids of the original amino acid sequence, such as at least 12 or at least 15 amino acids of the original amino acid sequence.

A variant sequence may have fewer than five amino acid modifications. For example, said variant sequence may have up to four amino acid modifications, more preferably up to three amino modifications, and most preferably only one or two amino acid modifications. All said modifications are independently a deletion, substitution or insertion.

In a particularly preferred embodiment, the variant sequence has one or two amino acid modifications, the modification or each modification independently being a deletion or substitution.

Deletions

Where a T cell epitope-containing variant sequence has an amino acid modification that is a deletion, the deleted amino acid is preferably removed from the N- or C-terminus of the corresponding original amino acid sequence. That is, the variant sequence is a truncation of the original amino acid sequence formed by removing one or more contiguous amino acids from the N- and/or C-terminus of the original sequence. Such a variant sequence may optionally have no other deletions or no other modifications.

A deleted amino acid may less preferably be removed from an internal position in the corresponding original amino acid sequence. By removal from an internal position it is meant that a deleted amino acid is not itself at the N- or C-terminus of the original amino acid sequence and nor is it removed as part of a sequence of contiguous amino acids including the N- or C-terminus of the original amino acid sequence. That is, to be considered to be deletion from an internal position, said deletion must occur independently of deletion from the N- or C-terminus of the original amino acid sequence.

For example, given an original sequence ABCDEFGH, an example variant sequence having an internal deletion of two amino acids could be ADEFGH. Thus, B and C are removed from internal positions and the original terminal residues A and H are retained. By contrast, a deletion of two contiguous amino acids from the N-terminus of the same original sequence would result in the variant sequence CDEFGH,

in which A and B are removed and C is now at the N-terminus. The deletion of B in this case is not a removal from an internal position, because it is removed as one of the two contiguous amino acids including the N-terminus of the original sequence.

Where more than one deletion occurs in a variant sequence, the deleted amino acids may be removed from any combination of the N-terminus and/or the C-terminus and/or an internal position. Preferred variant sequences have no more than one deletion from an internal position. In particularly preferred variant sequences there is no deletion from an internal position, and the deleted amino acids are removed from any combination of the N- and/or C-terminus of the original sequence. That is, the deleted amino acids may all be removed from the N-terminus of the original sequence, or they may all be removed from the C-terminus of the original sequence, or some amino acids may be removed from each end of the original sequence.

Thus, in one embodiment, a variant sequence is an amino acid sequence of SEQ ID NO: 5 having one, two, three, four or five amino acids removed from the N-terminus of said sequence of SEQ ID NO: 5.

In another embodiment, a variant sequence is an amino acid sequence of SEQ ID NO: 5 having one, two, three, four or five amino acids removed from the C-terminus of said sequence of SEQ ID NO: 5.

In another embodiment, a variant amino acid sequence is an amino acid sequence of SEQ ID NO: 5 having a number of amino acids removed from both the N- and C-terminus of said sequence, provided that said sequence has no more than five modifications in total. A preferred embodiment of such a variant sequence is an amino acid sequence of SEQ ID NO: 5 having one, two or three amino acids removed from the N- and/or C-terminus of said sequence of SEQ ID NO: 5, and optionally no other modifications.

Specific examples of variant amino acid sequences which have at least one deletion include:

- the variant sequence KLTGFTLMGKGVIDGQ (SEQ ID NO: 151), which is the amino acid sequence of KLTGFTLMGKGVIDGQG (SEQ ID NO: 4) having one amino acid removed from the C-terminus.

Substitutions

Where a T cell epitope-containing variant sequence has an amino acid modification that is a substitution, the substitution may occur at any position in the original amino acid sequence. It is preferred that said substitution does not introduce a proline or a cysteine. It is also preferred that said substitution is a conservative substitution.

By conservative substitution, it is meant that an amino acid may be substituted with any alternative amino acid having similar properties. The following is a non-exhaustive list of examples:

The amino acids with basic side chains, such as lysine, arginine or histidine, may each be independently substituted for each other.

The amino acids with acidic side chains, such as aspartate and glutamate, may each be independently substituted for each other, or for their amide derivatives, asparagine and glutamine. A glutamate or glutamine may also preferably be replaced with pyroglutamate. The amino acids with aliphatic side chains, such as glycine, alanine, valine, leucine and isoleucine, may each be independently substituted for each other. Particularly preferred substitutions in this category are limited to the amino acids with smaller aliphatic side chains, that is glycine, alanine, valine, which may preferably each be independently substituted for each other.

Other preferred substitutions include the substitution of methionine with norleucine (Nle).

Additionally, in more general terms, a neutral amino acid may be substituted with another neutral amino acid, a charged amino acid may be substituted with another charged amino acid, a hydrophilic amino acid may be substituted with another hydrophilic amino acid, a hydrophobic amino acid may be substituted with another hydrophobic amino acid, a polar amino acid may be substituted with another polar amino acid, and an aromatic amino acid may be substituted with another aromatic amino acid. Some properties of the 20 main amino acids which can be used to select suitable substituents are as follows:

Ala	aliphatic, hydrophobic, neutral	Met	hydrophobic, neutral
Cys	polar, hydrophobic, neutral	Asn	polar, hydrophilic, neutral
Asp	polar, hydrophilic, charged (-)	Pro	hydrophobic, neutral
Glu	polar, hydrophilic, charged (-)	Gln	polar, hydrophilic, neutral
Phe	aromatic, hydrophobic, neutral	Arg	polar, hydrophilic, charged (+)
Gly	aliphatic, neutral	Ser	polar, hydrophilic, neutral
His	aromatic, polar, hydrophilic, charged (+)	Thr	polar, hydrophilic, neutral
Ile	aliphatic, hydrophobic, neutral	Val	aliphatic, hydrophobic, neutral
Lys	polar, hydrophilic, charged(+)	Trp	aromatic, hydrophobic, neutral
Leu	aliphatic, hydrophobic, neutral	Tyr	aromatic, polar, hydrophobic

Specific examples of variant amino acid sequences which have at least one substitution include:

- the variant sequence KIWLQFAQLTDFNL (SEQ ID NO: 57; Cha17), which is the amino acid sequence of RIWLQFAKLTGFTL (SEQ ID NO: 3; CRY17) having four substitutions. The amino acid R at position 1 of SEQ ID NO: 3 is substituted with lysine, the amino acid K at position 8 of SEQ ID NO: 3 is substituted with glutamine, the amino acid G at position 11 of SEQ ID NO: 3 is substituted with aspartate, and the amino acid T at position 13 of SEQ ID NO: 3 is substituted with asparagine;

- the variant sequence KLTGFTLMGKGVIEGQK (SEQ ID NO: 58; Cry18), which is the amino acid sequence of KLTGFTLMGKGVIDGQG (SEQ ID NO: 4) having two substitutions. A polypeptide having the variant sequence of SEQ ID NO: 58 is particularly preferred for use according to the invention. The amino acid D at position 14 of SEQ ID NO: 4 is substituted with glutamate, and the amino acid G at position 17 of SEQ ID NO: 4 is substituted with lysine;

- the variant sequence QLTDFNLMGTGVIDGQG (SEQ ID NO: 59; Cha18), which is the amino acid sequence of KLTGFTLMGKGVIDGQG (SEQ ID NO: 4) having four substitutions. The amino acid K at position 1 of SEQ ID NO: 4 is substituted with glutamine, the amino acid G at position 4 of SEQ ID NO: 4 is substituted with aspartate, the amino acid T at position 6 of SEQ ID NO: 4 is substituted

with asparagine, and the amino acid K at position 10 of SEQ ID NO: 4 is substituted with threonine.

In some variant sequences there may be substitutions and deletions. For example, in one embodiment, a variant sequence is the amino acid sequence of SEQ ID NO: 5 having a number of amino acids deleted from the N- and/or C-terminus of said sequence of SEQ ID NO: 5, and a number of substitutions provided that said sequence has no more than five modifications in total. A preferred embodiment of such a variant sequence is the amino acid sequence of SEQ ID NO: 5 having one or two amino acids deleted at the N-and/or C-terminus of said sequence of SEQ ID NO: 5 and one or two substitutions made within the sequence of SEQ ID NO: 5.

Insertions

Where a variant sequence has an amino acid modification that is an insertion, the added amino acid may be inserted at any position in the original amino acid sequence. It is preferred that the insertion does not introduce a proline or a cysteine.

Preferably, an amino acid may be inserted at the N-terminus and/or C-terminus of the original sequence. That is, the variant sequence is an extension of the original amino acid sequence formed by adding amino acids to the N- and/or C-terminus of the original sequence. Such a variant sequence may optionally have no other insertions or no other modifications.

Less preferably, an amino acid may be inserted at an internal position. By insertion at an internal position it is meant that an amino acid is inserted at any position which is C-terminal to the amino acid at the N-terminus of the original sequence, or that an amino acid is inserted at any position which is N-terminal to the amino acid at the C-terminus of the original sequence.

Where more than one insertion occurs in a variant sequence, the added amino acids may be inserted at any combination of the N-terminus and/or the C-terminus and/or an internal position. Preferred variant sequences have no more than one insertion at an internal position. In particularly preferred variant sequences there is no insertion at an internal position, and the added amino acids are inserted at any combination of the N- and/or C-terminus of the original sequence. That is, the added amino acids may all be inserted at the N-terminus of the original sequence, or they may all be inserted at the C-terminus of the original sequence, or some amino acids may be

inserted at each end of the original sequence. That is, the added amino acids may be considered to extend the original sequence at the N- and/or C-terminus.

Thus, in one embodiment, a variant sequence is the amino acid sequence of SEQ ID NO: 5 having one, two, three, four or five amino acids inserted at the N-terminus of said sequence of SEQ ID NO: 5.

In another embodiment, a variant sequence is the amino acid sequence of SEQ ID NO: 5 having one, two, three, four or five amino acids inserted at the C-terminus of said sequence of SEQ ID NO: 5.

In another embodiment, a variant sequence is the amino acid sequence of SEQ ID NO: 5 having a number of amino acids inserted at both the N- and C-terminus of said sequence of SEQ ID NO: 5, provided that said sequence has no more than five modifications in total. A preferred embodiment of such a variant sequence is the amino acid sequence of SEQ ID NO: 5 having one, two or three amino acids inserted at the N- and/or C-terminus of said sequence of SEQ ID NO: 5, and optionally no other modifications.

A variant sequence having a charged amino acid inserted at the N- and/or C-terminus is particularly preferred where said charged amino acid will correspond to the N- and/or C-terminus of the polypeptide of the invention which comprises, consists or consists essentially of the variant sequence. Charged residues at the N- and/or C-terminus of a polypeptide can improve the solubility of a polypeptide. Preferred charged amino acids include lysine, arginine and histidine. One, two or more amino acids selected from lysine, arginine and histidine may be added at the N- and/or C-terminus of the polypeptide. Lysine is particularly preferred. Thus, a particularly preferred variant sequence is an amino acid sequence of SEQ ID NO: 5 having one or more charged amino acids, preferably one or more lysine residues, inserted at the N- and/or C-terminus of said sequence of SEQ ID NO: 5.

Specific examples of variant amino acid sequences which include addition of charged residues are:

- the variant sequences KVFYNGAYFVSSGKYEGG (SEQ ID NO: 157; CRY11B) and KVFYNGAYFVSSGKYEGGK (SEQ ID NO:158; CRY11C) which differ from the amino acid sequence of VFYNGAYFVSSGKYEGG (SEQ ID NO: 2; CRY11) by the addition of an N-terminal lysine residue or of an N- and an C-terminal lysine residue

- the variant sequences KRIWLQFAKLTGFTLK (SEQ ID NO: 159; CRY17A) and RIWLQFAKLTGFTLK (SEQ ID NO: 160; CRY17B) which differ from the amino acid sequence of RIWLQFAKLTGFTL (SEQ ID NO: 3; CRY17) by the addition of N- and C-terminal lysine residues or of a C-terminal lysine residue;
- the variant sequence KDIFASKNFHLQKNTIGTK (SEQ ID NO: 161; CRY21A) which differs from the amino acid sequence of DIFASKNFHLQKNTIGT (SEQ ID NO: 22; CRY21) by the addition of N- and C-terminal lysine residues.

In some variant sequences there may be substitutions and insertions. For example, in one embodiment, a variant sequence is the amino acid sequence of SEQ ID NO: 5 having a number of amino acids inserted at both the N- and C-terminus of said sequence of SEQ ID NO: 5, and a number of substitutions provided that said sequence has no more than five modifications in total. A preferred embodiment of such a variant sequence is the amino acid sequence of SEQ ID NO: 5 having one or two amino acids inserted at the N-and/or C-terminus of said sequence of SEQ ID NO: 5 and one or two substitutions made within the sequence of SEQ ID NO: 5.

In some variant sequences there may be deletions and insertions. For example, the variant sequence SKNFHLQKNTIGTGK (SEQ ID NO: 162; CRY21B) differs from the amino acid sequence of DIFASKNFHLQKNTIGT (SEQ ID NO: 22; CRY21) by the deletion of four N-terminal residues and the addition of C-terminal glycine and lysine residues. The variant sequence SKNFHLQKNTIGTG (SEQ ID NO: 166; CRY21C) differs from the amino acid sequence of DIFASKNFHLQKNTIGT (SEQ ID NO:22; CRY21) by having four amino acids deleted from the N-terminus, and one amino acid deleted from the C-terminus, and the addition of a C-terminal glycine residue.

Polypeptides

A polypeptide of the invention or a polypeptide provided in a pharmaceutical composition of the invention is up to 30 amino acids in length and comprises, consists or consists essentially of an amino acid sequence or variant sequence as defined above.

Said polypeptide may preferably be up to 25 amino acids in length, more preferably up to 20 amino acids in length or up to 17 amino acids in length, and most preferably up to 15 amino acids in length. Put another way, the polypeptide may have a maximum length of 30, 25, 20, 17 or 15 amino acids.

Said polypeptide is preferably at least 8 amino acids in length, more preferably at least 9 amino acids in length, most preferably at least 12 amino acids in length. Put another way, the polypeptide may have a minimum length of 8, 9, or 12 amino acids.

Said polypeptide may be of a length defined by any combination of a said minimum and a said maximum length. For example, the polypeptide may be 8 to 30, 8 to 25, 8 to 20, 8 to 17 or 8 to 15 amino acids in length. The polypeptide may be 9 to 30, 9 to 25, 9 to 20, 9 to 17 or 9 to 15 amino acids in length. The polypeptide may be 12 to 30, 12 to 25, 12 to 20, 12 to 17 or 12 to 15 amino acids in length. A preferred polypeptide is of 9 to 30 amino acids in length, more preferably 9 to 20 amino acids in length. A particularly preferred polypeptide is of 12 to 17 amino acids in length.

Said polypeptide may comprise an amino acid sequence or variant sequence as defined above. Therefore, said polypeptide may include additional amino acids which are not defined by said amino acid sequence or variant sequence. The additional amino acids may be described as flanking said amino acid sequence or variant sequence. That is, the additional amino acids are included at the N-terminus and/or C-terminus of said amino acid sequence or variant sequence.

Put another way, said polypeptide may have a sequence consisting of said amino acid sequence or variant sequence having an N-terminal and/or C-terminal extension of a number of amino acids. The maximum number of amino acids in the N-terminal and/or C-terminal extension is determined by the maximum length of the polypeptide, as defined above.

The amino acids in an N-terminal extension of a said amino acid sequence or variant sequence preferably correspond to the amino acids immediately N-terminal to the said amino acid sequence in the native sequence of the protein from which it derives.

The amino acids in a C-terminal extension of a said amino acid sequence or variant sequence preferably correspond to the amino acids immediately C-terminal to the said amino acid sequence in the native sequence of the protein from which it derives.

The N-terminal and/or C-terminal extension may be the one, two, three, four, five, six, seven, eight, nine or ten amino acids corresponding respectively to the one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acids immediately N- terminal or C-terminal to said amino acid sequence in the sequence of the protein from which it derives.

That is, the N-terminal and/or C-terminal extension is of from one to ten amino acids corresponding respectively to the one to ten contiguous amino acids immediately N-terminal or C-terminal to the said amino acid sequence in the native sequence of the protein from which it derives.

Preferably, the N-terminal and/or C-terminal extension is of from one to six amino acids corresponding respectively to the one to six contiguous amino acids immediately N-terminal or C-terminal to the said amino acid sequence.

More preferably, the N-terminal and/or C-terminal extension is of from one to four amino acids corresponding respectively to the one to four contiguous amino acids immediately N-terminal or C-terminal to the said amino acid sequence.

Most preferably, the N-terminal and/or C-terminal extension is of from one to two amino acids corresponding respectively to the one to two contiguous amino acids immediately N-terminal or C-terminal to the said amino acid sequence.

Specific examples of polypeptides which include an N-terminal and/or C-terminal extension to an amino acid sequence or variant sequence are provided below. In each instance, the N- and/or C-terminally extended polypeptides may be used in place of the corresponding original polypeptide in accordance with the invention. Examples for SEQ ID NO: 1 include the following:

- DLKIKLRRTIEAEGIP (SEQ ID NO: 1) may have a N-terminal extension of one, two, three, four, five, six, seven, eight, nine or ten amino acids corresponding to the one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acids immediately N-terminal to DLKIKLRRTIEAEGIP in the native sequence of Cry IFR, that is the amino acids H, A, V, E, P, M, K, S, M and F. For example, where all ten said contiguous amino acids are present, the polypeptide of the invention

has the amino acid sequence of HAVEPMKSMFDLKIKLRRTIEAEGIP (SEQ ID NO: 137; N-terminal extension underlined).

- DLKIKLRRTIEAEGIP (SEQ ID NO: 1) may have a C-terminal extension of one, two, three, four, five, six, seven, eight, nine or ten amino acids corresponding to the one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acids immediately C-terminal to DLKIKLRRTIEAEGIP in the native sequence of Cry IFR, that is the amino acids H, T, Y, V, V, P, H, C, F and A. For example, where all ten said contiguous amino acids are present, the polypeptide of the invention has the amino acid sequence of DLKIKLRRTIEAEGIPHTYVVPHCFA (SEQ ID NO: 138; C-terminal extension underlined).

- DLKIKLRRTIEAEGIP (SEQ ID NO: 1) may have a N-terminal extension of one, two, three, four, five, six, seven or eight amino acids corresponding to the one, two, three, four, five, six, seven or eight contiguous amino acids immediately N-terminal to DLKIKLRRTIEAEGIP in the native sequence of Cry IFR, that is the amino acids V, E, P, M, K, S, M and F. It may also have a C-terminal extension of one amino acid corresponding to the one contiguous amino acid immediately C-terminal to DLKIKLRRTIEAEGIP in the native sequence of Cry IFR, that is the amino acid H. For example, where all eight contiguous amino acids are present in the N-terminal extension and the one contiguous amino acid is present in the C-terminal extension, the polypeptide of the invention has the amino acid sequence of VEPMKSMFDLKIKLRRTIEAEGIPH (SEQ ID NO: 114; N- and C-terminal extensions are underlined).

Examples for SEQ ID NO: 163 include the following:

- GAYFVSSGKYEGG (CRY11D; SEQ ID NO: 163) may have a N-terminal extension of one, two, three, four, five, six, seven, eight, nine, ten or more amino acids corresponding to the one, two, three, four, five, six, seven, eight, nine or ten or more contiguous amino acids immediately N-terminal to one, two, three, four, five, six, seven, eight, nine, ten or more contiguous amino acids immediately N-terminal to GAYFVSSGKYEGG in the native sequence of Cry j1, that is the amino acids S, N, W, V, W, Q, S, T, Q, D, V, F, Y and N. For example, where all fourteen said contiguous amino acids are present, the polypeptide has the amino acid sequence of SNWVWQSTQDVFYNGAYFVSSGKYEGG (SEQ ID NO: 140; N-terminal extension underlined).

- GAYFVSSGKYEGG (CRY11D; SEQ ID NO: 163) may have a C-terminal extension of one, two, three, four, five, six, seven, eight, nine or ten amino acids corresponding to the one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acids immediately C-terminal to GAYFVSSGKYEGG in the native sequence of Cry j1, that is the amino acids N, I, Y, T, K, K, E, A, F and N. For example, where all ten said contiguous amino acids are present, the polypeptide has the amino acid sequence of GAYFVSSGKYEGGNIYTKKEAFN (SEQ ID NO: 141; C-terminal extension underlined).

Examples for SEQ ID NO: 3 include the following:

- RIWLQFAKLTGFTL (SEQ ID NO: 3; CRY17) may have a N-terminal extension of one, two, three, four, five, six, seven, eight, nine or ten amino acids corresponding to the one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acids immediately N-terminal to one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acids immediately N-terminal to RIWLQFAKLTGFTL in the native sequence of Cry j2, that is the amino acids Y, Q, N, P, A, S, W, K, N and N. For example, where all ten said contiguous amino acids are present, the polypeptide has the amino acid sequence of YQNPASWKNNRIWLQFAKLTGFTL (SEQ ID NO: 142; N-terminal extension underlined);

- RIWLQFAKLTGFTL (SEQ ID NO: 3; CRY17) may have a C-terminal extension of one, two, three, four, five, six, seven, eight, nine or ten amino acids corresponding to the one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acids immediately C-terminal to RIWLQFAKLTGFTL in the native sequence of Cry j2, that is the amino acids M, G, K, G, V, I, D, G, Q, and G. For example, where all ten said contiguous amino acids are present, the polypeptide has the amino acid sequence of RIWLQFAKLTGFTLMGKGVIDGQG (SEQ ID NO: 143; C-terminal extension underlined);

- RIWLQFAKLTGFTL (SEQ ID NO: 3; CRY17) may have an N-terminal extension of one amino acid corresponding to the one amino acid immediately N-terminal to RIWLQFAKLTGFTL in the native sequence of Cry j2, that is the amino acid N, and a C-terminal extension of one, two, three, four, five, six, seven, eight, or nine amino acids corresponding to the one, two, three, four, five, six, seven, eight, or nine contiguous amino acids immediately C-terminal to RIWLQFAKLTGFTL in the native sequence of Cry j2, that is the amino acids M, G, K, G, V, I, D, G, and Q. For

example, where one amino acid is present in the N-terminal extension and all eight contiguous amino acids are present in the C-terminal extension, the polypeptide has the amino acid sequence of NRIWLQFAKLTGFTLMGKGVIDGQ (SEQ ID NO: 77; N- and C-terminal extensions underlined);

Examples for SEQ ID NO: 4 include the following:

- KLTGFTLMGKGVIDGQG (SEQ ID NO: 4) may have a N-terminal extension of one, two, three, four, five, six, seven, eight, nine or ten amino acids corresponding to the one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acids immediately N-terminal to one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acids immediately N-terminal to KLTGFTLMGKGVIDGQG in the native sequence of Cry j2, that is the amino acids K, N, N, R, I, W, L, Q, F and A. For example, where all ten said contiguous amino acids are present, the polypeptide has the amino acid sequence of KNNRIWLQFAKLTGFTLMGKGVIDGQG (SEQ ID NO: 144; N-terminal extension underlined).
- KLTGFTLMGKGVIDGQG (SEQ ID NO: 4) may have a C-terminal extension of one, two, three, four, five, six, seven, eight, nine or ten amino acids corresponding to the one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acids immediately C-terminal to KLTGFTLMGKGVIDGQG in the native sequence of Cry j2, that is the amino acids. For example, where all ten said contiguous amino acids are present, the polypeptide has the amino acid sequence of KLTGFTLMGKGVIDGQGKQWWAGQCKW (SEQ ID NO: 145; C-terminal extension underlined).

Examples for SEQ ID NO: 5 include the following:

- RPTAIKIDYSKSVT (SEQ ID NO: 5; CRY19A) may have a N-terminal extension of one, two, three, four, five, six, seven, eight, nine or ten amino acids corresponding to the one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acids immediately N-terminal to one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acids immediately N-terminal to RPTAIKIDYSKSVT in the native sequence of Cha o2, that is the amino acids N, G, R, T, V, C, N, D, R, and N. For example, where all ten said contiguous amino acids are present, the polypeptide of the invention has the amino acid sequence of NGRTVCNDRNRPTAIKIDYSKSVT (SEQ ID NO: 146; N-terminal extension underlined).

- RPTAIKIDYSKSVT (SEQ ID NO: 5; CRY19A) may have a C-terminal extension of one, two, three, four, five, six, seven, eight, nine or ten amino acids corresponding to the one, two, three, four, five, six, seven, eight, nine or ten contiguous amino acids immediately C-terminal to RPTAIKIDYSKSVT in the native sequence of Cha o2, that is the amino acids V, K, E, L, T, L, M, N, S and P. For example, where all ten said contiguous amino acids are present, the polypeptide of the invention has the amino acid sequence of RPTAIKIDYSKSVTVKELTLMNSP (SEQ ID NO: 147; C-terminal extension underlined).

The amino acids in the N-terminal and/or C-terminal extension may not correspond exactly to amino acids in the native sequence of the protein from which the amino acid sequence or variant sequence derives. The N-terminal and/or C-terminal extension may include a sequence derived from said native sequence which has been modified, for example to improve stability, solubility or manufacturability of the polypeptide. For example, a methionine in the native sequence may be substituted with nor-leucine, and/or one or more charged residues may be added at the N-terminus of an N-terminal extension and/or the C-terminus of a C-terminal extension. Preferably positively charged residues such as arginine and lysine are added. Amino acids selected from histidine, glutamate and aspartate may be added. One, two or more amino acids selected from lysine, arginine and histidine may be added at the N- and/or C-terminus of the polypeptide.

Alternatively, the amino acids of an N-terminal and/or C-terminal extension may not correspond to amino acids in native sequence of the protein from which the amino acid sequence or variant sequence derives. They may instead be any suitable amino acids, preferably selected to improve stability, solubility or manufacturability of the polypeptide. For example, one or more charged residues may be added at the N- and/or C-terminus of SEQ ID NO: 5. Preferably positively charged residues such as arginine and lysine are added. Amino acids selected from histidine, glutamate and aspartate may be added.

In some embodiments, suitable polypeptides may have a sequence consisting of an N- or C-terminal extension of a said amino acid sequence of SEQ ID NOs 1, 3 to 25 and 163 which further comprises one or more deletions. In particular, the polypeptide may comprise an extension of a said amino acid sequence within the native sequence at

one terminus, such as the N-terminus, and a deletion of amino acids at the other terminus, such as the C-terminus.

The polypeptide may have a sequence consisting of an N-terminal extension of a said amino acid sequence with one, two, three, four, five, six, seven, eight, nine, ten or more amino acids corresponding respectively to the one, two, three, four, five, six, seven, eight, nine or ten or more contiguous amino acids immediately N- terminal to said amino acid sequence in the sequence of the protein from which it derives, and a deletion of one, two, three, four or five amino acids from the C-terminus of the said amino acid sequence.

The polypeptide may have a sequence consisting of a C-terminal extension of a said amino acid sequence with one, two, three, four, five, six, seven, eight, nine, ten or more amino acids corresponding respectively to the one, two, three, four, five, six, seven, eight, nine or ten or more contiguous amino acids immediately C-terminal to said amino acid sequence in the sequence of the protein from which it derives, and a deletion of one, two, three, four or five amino acids from the N-terminus of the said amino acid sequence.

Specific examples of such polypeptides include the following:

- GAYFVSSGKYEGG (CRY11D; SEQ ID NO: 163) may have a N-terminal extension of one, two, three, four, five, six, seven, eight, nine, ten, eleven or more amino acids corresponding to the one, two, three, four, five, six, seven, eight, nine, ten, eleven or more contiguous amino acids immediately N-terminal to one, two, three, four, five, six, seven, eight, nine, ten, eleven or more contiguous amino acids immediately N-terminal to GAYFVSSGKYEGG in the native sequence of Cry j1, that is the amino acids C, S, N, W, V, W, Q, S, T, Q D, F, W and N and one, two, three, four, or five deletions from the C-terminus. For example, where all eleven said N-terminal contiguous amino acids and all five said deletions are present, the polypeptide has the amino acid sequence of CSNWVWQSTQDVFYNGAYFVSSG (SEQ ID NO: 148; N-terminal extension underlined). In the polypeptide of SEQ ID NO: 148, the N-terminal Cysteine may further preferably be substituted with S, T, G, A or V.
- KLTGFTLMGKGVIDGQG (SEQ ID NO: 4) may have a C-terminal extension of one, two, three, four, five, six, seven, or eight amino acids corresponding to the one, two, three, four, five, six, seven, or eight contiguous amino acids immediately C-terminal to KLTGFTLMGKGVIDGQG in the native sequence of Cry j2, that is the

amino acids N, R, I, W, L, Q, F and A, and one deletion from the C-terminus. For example, where all eight said contiguous amino acids and said one deletion are present, the polypeptide of the invention has the amino acid sequence of NRIWLQFAKLTGFTLMGKGVIDGQ (SEQ ID NO: 149; N-terminal extension underlined).

Additional polypeptides

In a broader aspect, the invention provides a polypeptide of up to 30 amino acids in length and comprising, consisting essentially or consisting of the amino acid sequence of any one of SEQ ID NOs 3 to 25 and 163 or a corresponding variant sequence. In this aspect, the invention preferably provides the polypeptide having the amino acid sequence KGAYFVSSGKYEGGK (SEQ ID NO: 165; CRY11F), which is a variant of the amino acid sequence of SEQ ID NO: 163; CRY11D. The invention also preferably provides the polypeptide having the amino acid sequence KRIWLQFAKLTGFTLK (SEQ ID NO: 159; CRY17A), which is a variant of the amino acid sequence of SEQ ID NO: 3, CRY17. The invention additionally preferably provides the polypeptide having the amino acid sequence TFKVEGIIAAYQNPASWK (SEQ ID NO: 54; CRY15A), which is a variant of the amino acid sequence of SEQ ID NO:19; CRY15. The invention also preferably provides the polypeptide having the amino acid sequence SKNFHLQKNTIGTG (SEQ ID NO: 166; CRY21C), which is a variant of the amino acid sequence of SEQ ID NO:22, CRY21 .

The invention also provides the polypeptide having the amino acid sequence SGNLNIKLKMPLYIAGYK (SEQ ID NO: 34; CRY04), which is a variant of SEQ ID NO: 9. The invention further provides the polypeptide having the amino acid sequence SSNWVWQSTQDVF (SEQ ID NO: 45: CRY10A), which is a variant of SEQ ID NO:15; CRY10. The invention also provides the polypeptide having the amino acid sequence KLTGFTLMGKGVIEGQK (SEQ ID NO 58; CRY18), which is a variant of the amino acid sequence of SEQ ID NO: 4. Specific examples of variant sequences derived from the amino acid sequences of SEQ ID NOs 3 to 25 and 163 which are suitable for use according to the invention include:

- The amino acid sequences of SEQ ID NOs 48, 157, 158 and 164-165 which are variants of SEQ ID NO:163. SEQ ID NO 48 is an extension of the amino acid sequence of SEQ ID NO:163 within the native sequence of the protein from which it derives, and

additionally including an amino acid substitution. SEQ ID NOs 157 and 158 are extensions of the amino acid sequence of SEQ ID NO:163 within the native sequence of the protein from which it derives, and additionally including amino acid insertions. SEQ ID NOs 164 and 165 are insertional variants of SEQ ID NO:163.

- The amino acid sequences of SEQ ID NOs 159 and 160 which are insertional variants of SEQ ID NO:3

- The amino acid sequence of SEQ ID NO: 58 which is a substitutional variant of SEQ ID NO: 4.

-The amino acid sequences of SEQ ID NOs 26 to 28, which are substitutional variants of SEQ ID NO: 6.

- The amino acid sequences of SEQ ID NOs 29 and 32 which are variants of SEQ ID NO: 7. SEQ ID NOs 29 and 31 are substitutional variants of SEQ ID NO: 7. SEQ ID NO 30 is a deletional variant of SEQ ID NO: 7. SEQ ID NO: 31 has deletions and substitutions with respect to SEQ ID NO: 7.

-The amino acid sequence of SEQ ID NO: 33, which is a substitutional variant of SEQ ID NO: 8.

- The amino acid sequences of SEQ ID NOs 34 and 136, which are substitutional variants of SEQ ID NO: 9.

- The amino acid sequences of SEQ ID NOs 35 and 36, which are substitutional variants of SEQ ID NO: 10.

- The amino acid sequences of SEQ ID NOs 37 and 38, which are variants of SEQ ID NO: 11. SEQ ID NO: 37 is an insertional variant of SEQ ID NO: 11, having a Lysine residue inserted both at the N-terminus and the C-terminus of SEQ ID NO: 11. SEQ ID NO: 38 has a substitution with respect to SEQ ID NO: 37, i.e. has insertions and substitutions with respect to SEQ ID NO:11.

- The amino acid sequences of SEQ ID NOs 42 and 43, which are substitutional variants of SEQ ID NO: 14.

- The amino acid sequences of SEQ ID NOs 45 to 47, which are substitutional variants of SEQ ID NO: 15.

- The amino acid sequence of SEQ ID NO: 50, which is a substitutional variant of SEQ ID NO: 16.

- The amino acid sequences of SEQ ID NOs 51 and 52, which are substitutional variants of SEQ ID NO: 17.

- The amino acid sequence of SEQ ID NO: 53, which is a substitutional variant of SEQ ID NO: 18.

- The amino acid sequences of SEQ ID NOs 54 and 55, which are variants of SEQ ID NO: 19. SEQ ID NO: 54 is a substitutional variant of SEQ ID NO: 19.

- The amino acid sequence of SEQ ID NO: 56, which is a substitutional variant of SEQ ID NO: 20.

- The amino acid sequences of SEQ ID NOs 61 to 63, which are substitutional variants of SEQ ID NO: 21.

- The amino acid sequences of SEQ ID NOs 64,161 to 162 and 166 which are variants of SEQ ID NO: 22. SEQ ID NO: 64 is a substitutional variant of SEQ ID NO: 22. SEQ ID NO: 161 is an insertional variant of SEQ ID NO: 22. SEQ ID NOs: 162 and 166 include insertions and deletions with respect to SEQ ID NO: 22.

- The amino acid sequence of SEQ ID NO: 65, which is a substitutional variant of SEQ ID NO: 23.

The above variant sequences may be used in place of the corresponding original polypeptide in accordance with the invention, such as in a polypeptide combination of the invention described below.

T cell epitopes

A polypeptide of the invention (or a polypeptide provided in a pharmaceutical composition of the invention) is up to 30 amino acids in length and comprises, consists or consists essentially of an amino acid sequence or variant sequence as defined above. The amino acid sequence and each said variant sequence contains at least one T cell epitope. The T cell epitope is preferably an MHC class II-binding T cell epitope.

In preferred variant sequences, sufficient contiguous amino acids of the corresponding original amino acid sequence are retained to contain a T cell epitope. Typically, such a variant sequence retains at least 8, preferably at least 9, contiguous amino acids of the original amino acid sequence

The presence of a T cell epitope may preferably be confirmed by analysis performed *in silico*, for example using bioinformatic software as described in Example 1. Alternatively, the presence of a T cell epitope may be confirmed by direct evaluation of its functional properties. Particular functional properties of T cell epitopes include the ability of a polypeptide comprising the epitope to bind to an MHC molecule,

preferably an MHC class-II molecule, and/or the ability of a polypeptide comprising the epitope to activate a T cell, preferably when bound to an MHC Class II molecule.

The ability of a polypeptide to bind to an MHC molecule may be evaluated using any suitable method, such as a competition binding assay. Preferred *in vitro* binding assays are described in Examples 9 and 16.

The ability of a polypeptide to activate a T cell may also be evaluated using any suitable method. Preferred methods include the measurement of one or more parameters associated with T cell activation, such as proliferation or cytokine release. Preferred assays for these parameters are described in Examples 10 and 15. Relevant cytokines include IFN-gamma, IL-13 and IL-10. In the context of the present invention, a polypeptide is typically considered to have activated a T cell if it induces release of one, two, or all of IFN-gamma, IL-13 and IL-10, such as release of both IFN-gamma and IL-13. Typically, a positive proliferation or cytokine response is greater than the mean +/- 3* standard deviations of the background response (probability >99%). The polypeptide may induce a release of greater than 50 pg/ml of the given cytokine(s), such as a release of greater than 100 pg/ml of the given cytokine(s).

A polypeptide comprising, consisting or consisting essentially of a variant amino acid sequence may have similar MHC class-II binding properties and/or similar T cell activation properties as a polypeptide comprising, consisting or consisting essentially of the corresponding original amino acid sequence.

Typically, a polypeptide has similar MHC class-II binding characteristics as another polypeptide if both polypeptides are capable of binding specifically to one or more MHC class-II molecules belonging to the same MHC class-II allele supertype family. Examples of MHC class-II allele supertype families include HLA-DR1, HLA-DR3, HLA-DR4, HLA-DR7, HLA-DR8, HLA-DR11, HLA-DR13, HLA-DR15 and HLA-DR51. Both polypeptides may bind specifically to the same MHC class-II molecule, that is to an MHC class-II molecule encoded by the same allele.

Typically, a polypeptide has similar T cell activation properties as another polypeptide if both polypeptides specifically activate a T cell expressing the same T cell receptor. The level of activation induced by each polypeptide may be similar. The level of activation may be assessed by monitoring proliferation and/or cytokine release, as described above.

Suitable polypeptides comprising, consisting or consisting essentially a variant sequence may be derived empirically or selected according to known criteria. Within a single polypeptide there are certain residues which contribute to binding within the MHC antigen binding groove and other residues which interact with hypervariable regions of the T cell receptor (Allen et al (1987) Nature 327: 713-5). Advantageously, peptides may be designed to favour T-cell proliferation and induction of tolerance. Metzler and Wraith have demonstrated improved tolerogenic capacity of polypeptides in which substitutions increasing polypeptide-MHC affinity have been made (Metzler & Wraith (1993) Int Immunol: 1159-65). That an altered polypeptide ligand can cause long-term and profound anergy in cloned T cells was demonstrated by Sloan-Lancaster et al (1993) Nature 363: 156-9.

Sequence identity

Suitable T cell epitope-containing variant sequences according to the invention may alternatively be described in terms of their sequence identity to a corresponding original amino acid sequence. For example, a variant sequence may have at least 65% identity to the amino acid sequence of SEQ ID NO: 5. More preferably, a variant sequence may have at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% amino acid identity to an amino acid sequence of SEQ ID NO: 5. Variants of the amino acid sequences of SEQ ID NOs 3, 4, 6 to 25 and 163 may also have the above levels of identity to their corresponding original amino acid sequence.

Sequence identity is typically evaluated over a number of contiguous amino acids in the original amino acid sequence. For example, sequence identity may be measured over at least 9, 10, 11, 12, 13, 14, or 15 contiguous amino acids in the original amino acid sequence, depending on the size of the peptides of comparison. It is preferred that sequence identity be measured over at least 9 contiguous amino acids in the original amino acid sequence. It is particularly preferred that sequence identity is measured over the entire length of the corresponding original amino acid sequence.

In connection with amino acid sequences, "sequence identity" refers to sequences which have the stated value when assessed using ClustalW (Thompson et al., 1994, supra) with the following parameters:

Pairwise alignment parameters - Method: accurate, Matrix: PAM, Gap open penalty: 10.00, Gap extension penalty: 0.10; Multiple alignment parameters -Matrix:

PAM, Gap open penalty: 10.00, % identity for delay: 30, Penalize end gaps: on, Gap separation distance: 0, Negative matrix: no, Gap extension penalty: 0.20, Residue-specific gap penalties: on, Hydrophilic gap penalties: on, Hydrophilic residues: G, P, S, N, D, Q, E, K, and R. Sequence identity at a particular residue is intended to include identical residues which have simply been derivatized.

Salts

The invention encompasses any pharmaceutically acceptable salt of a polypeptide described herein. Said pharmaceutically acceptable salts include, for example, mineral acid salts such as chlorides, hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, trifluoroacetates, propionates, malonates, benzoates, and the like; and salts of monocationic metal ions such as sodium and potassium and the like; and salts of bases such as ammonia. A hydrochloride salt or an acetate salt is preferred. Where the polypeptide is polypeptide comprising the amino acid sequence of RPTAIKIDYSKSVT (SEQ ID NO: 5; CRY19A); or a T cell epitope-containing variant sequence derived from said amino acid sequence, a trifluoroacetate or hydrochloride salt thereof is preferred. As discussed below, trifluoroacetate and hydrochloride salts of such a polypeptide have enhanced stability. A hydrochloride salt is particularly preferred for pharmaceutical use and as having optimal stability.

Synthesis

The polypeptides described herein can be prepared by any suitable technique. Solid-phase peptide synthesis (SPPS) is a preferred technique. This involves formation of the peptide on small solid beads.

Using SPPS, the peptide remains covalently attached to a bead during synthesis. The peptide is synthesised using repeated cycles of coupling-washing-deprotection-washing. In particular, the free N-terminal amine of a solid-phase attached peptide is coupled to a single N-protected amino acid unit. This unit is then deprotected, revealing a new N-terminal amine to which a further protected amino acid is attached. These steps are repeated until the peptide is complete. The peptide is then cleaved from the beads using a suitable reagent.

Suitable protecting groups, reagents, solvents and reaction conditions for SPPS are well known to those skilled in the art and as such conditions can be determined by one skilled in the art by routine optimization procedures.

Pharmaceutically acceptable salts of polypeptides can be prepared by any suitable technique. Typically, salification involves reaction of the polypeptide or a salt thereof with a suitable reagent, typically acid, to obtain the pharmaceutically acceptable salt selected.

For example, a hydrochloride salt of a polypeptide can be prepared by initially cleaving the polypeptide from the solid phase using trifluoroacetic acid. The polypeptide will thus initially be a trifluoroacetate salt. The trifluoroacetate salt can then be converted into a hydrochloride salt by any known technique, such as ion exchange on a suitable column using hydrochloric acid as an eluent.

The polypeptide or polypeptide salt products can be purified, where required, by any suitable technique. High pressure liquid chromatography (HPLC) can be used, for example.

The term "polypeptide" includes not only molecules in which amino acid residues are joined by peptide (-CO-NH-) linkages but also molecules in which the peptide bond is reversed. Such retro-inverso peptidomimetics may be made using methods known in the art, for example such as those described in Meziere et al (1997) J. Immunol.159, 3230-3237. This approach involves making pseudopolypeptides containing changes involving the backbone, and not the orientation of side chains. Meziere et al (1997) show that, at least for MHC class-II and T helper cell responses, these pseudopolypeptides are useful. Retro-inverse polypeptides, which contain NH-CO bonds instead of CO-NH peptide bonds, are much more resistant to proteolysis.

Similarly, the peptide bond may be dispensed with altogether provided that an appropriate linker moiety which retains the spacing between the carbon atoms of the amino acid residues is used; it is particularly preferred if the linker moiety has substantially the same charge distribution and substantially the same planarity as a peptide bond. It will also be appreciated that the peptide may conveniently be blocked at its N-or C-terminus so as to help reduce susceptibility to exoproteolytic digestion. For example, the N-terminal amino group of the polypeptides may be protected by reacting with a carboxylic acid and the C-terminal carboxyl group of the peptide may be protected by reacting with an amine. Other examples of modifications include

glycosylation and phosphorylation. Another potential modification is that hydrogens on the side chain amines of R or K may be replaced with methylene groups ($-NH_2 \rightarrow -NH(Me)$ or $-N(Me)_2$).

Analogues of polypeptides according to the invention may also include peptide variants that increase or decrease the polypeptide's half-life in vivo. Examples of analogues capable of increasing the half-life of polypeptides used according to the invention include peptoid analogues of the peptides, D-amino acid derivatives of the peptides, and peptide-peptoid hybrids. A further embodiment of the variant polypeptides used according to the invention comprises D-amino acid forms of the polypeptide. The preparation of polypeptides using D-amino acids rather than L-amino acids greatly decreases any unwanted breakdown of such an agent by normal metabolic processes, decreasing the amounts of agent which needs to be administered, along with the frequency of its administration.

The polypeptides provided by the present invention may be derived from splice variants of the parent proteins encoded by mRNA generated by alternative splicing of the primary transcripts encoding the parent protein chains. The polypeptides may also be derived from amino acid mutants, glycosylation variants and other covalent derivatives of the parent allergen proteins. Exemplary derivatives include molecules wherein the polypeptides of the invention are covalently modified by substitution, chemical, enzymatic, or other appropriate means with a moiety other than a naturally occurring amino acid. Further included are naturally occurring variant amino acid sequences of the parent proteins. Such a variant amino acid sequence may be encoded by an allelic variant or represent an alternative splicing variant.

Modifications as described above may be prepared during synthesis of the peptide or by post-production modification, or when the polypeptide is in recombinant form using the known techniques of site-directed mutagenesis, random mutagenesis, or enzymatic cleavage and/or ligation of nucleic acids.

The polypeptides described herein may also be modified to improve physicochemical characteristics. Thus, for example, original amino acid sequences may be altered to improve their solubility, and accordingly a polypeptide of the invention having a variant sequence will preferably be more soluble than a polypeptide having the corresponding original amino acid sequence under equivalent conditions. Methods for evaluating the solubility of polypeptides are well known in the art.

Improved solubility is advantageous for the tolerisation of subjects to the allergen from which the polypeptide derives, since administration of poorly soluble agents to subjects causes undesirable, non-tolerising inflammatory responses. The solubility of the polypeptides may be improved by altering the residues which flank the region containing a T cell epitope. For example, N- and C-terminal to the residues of the polypeptide which flank a T cell epitope, at least one amino acid may be added selected from arginine, lysine, histidine, glutamate and aspartate. In other examples:

i) any hydrophobic residues in the up to three amino acids at the N- or C-terminus of the native sequence of the polypeptide, which are not comprised in a T cell epitope, are deleted; and/or

ii) any two consecutive amino acids comprising the sequence Asp-Gly in the up to four amino acids at the N- or C-terminus of the native sequence of the polypeptide, which are not comprised in a T cell epitope, are deleted; and/or

iii) one or more positively charged residues are added at the N and/or C terminus of the native sequence of the polypeptide.

Optionally, any polypeptides containing cysteine residues may be engineered to prevent dimer formation such that any cysteine residues are replaced with serine or 2-aminobutyric acid.

Polypeptide combinations

The invention provides combinations of polypeptides and salts thereof including combinations of at least three polypeptides or salts thereof. Such a combination of polypeptides or salts thereof is preferably provided in a pharmaceutical formulation as described below. Each polypeptide in the combination of polypeptides is up to 30 amino acids in length. A typical combination of polypeptides of the invention comprises at least:

(a) a polypeptide comprising the amino acid sequence of RPTAIKIDYSKSVT (SEQ ID NO: 5; CRY19A); or a T cell epitope-containing variant sequence derived from said amino acid sequence,

(b) a polypeptide comprising the amino acid sequence of GAYFVSSGKYEGG (SEQ ID NO: 163, CRY11D) or a T cell epitope-containing variant sequence derived from said amino acid sequence, and

(c) a polypeptide comprising the amino acid sequence of TFKVDGIIAAYQNPASWK (SEQ ID NO: 19; CRY15) or a T cell epitope-containing variant sequence derived from said amino acid sequence,

wherein a T cell epitope-containing variant sequence of a said amino acid sequence is said amino acid sequence having up to five amino acid modifications, each of which is independently a deletion, substitution or insertion, wherein each polypeptide is up to 30 amino acids in length; and wherein each polypeptide may be present as a pharmaceutically acceptable salt thereof. Thus, the invention provides a pharmaceutical formulation comprising the above polypeptides of (a) to (c) or salts thereof, and a pharmaceutically acceptable carrier or diluent. The polypeptide of (a) may be present as a trifluoroacetate salt or hydrochloride salt thereof. The polypeptide of (a) is preferably present as a hydrochloride salt thereof.

In any combination of the invention, the polypeptide CRY11D of (b) is preferably replaced by a polypeptide having the corresponding variant sequence KGAYFVSSGKYEGGK (SEQ ID NO: 165; CRY11F) or a salt thereof. The polypeptide CRY15 of (c) is also preferably replaced by a polypeptide having the corresponding variant sequence TFKVEGIIAAYQNPASWK (SEQ ID NO: 54; CRY15A) or a salt thereof. It is thus preferred that the pharmaceutical formulation comprises at least one polypeptide or salt thereof selected from:

(a1) a polypeptide comprising, consisting essentially of or consisting of the amino acid sequence RPTAIKIDYSKSVT (SEQ ID NO: 5; CRY19A or a said salt thereof,

(b1) a polypeptide comprising, consisting essentially of or consisting of the amino acid sequence KGAYFVSSGKYEGGK (SEQ ID NO: 165; CRY11F) or a said salt thereof, and

(c1) a polypeptide comprising, consisting essentially of or consisting of the amino acid sequence TFKVEGIIAAYQNPASWK (SEQ ID NO: 54; CRY15A) or a said salt thereof.

The pharmaceutical formulation of at least three polypeptides may preferably comprise two polypeptides or salts thereof selected from (a1) and (b1), (b1) and (c1) and (a1) and (c1) above, or all three of the polypeptides or salts thereof of (a1), (b1) and (c1).

Thus, a pharmaceutical formulation of the invention may preferably comprise the polypeptides CRY19A and CRY11F or salts thereof; the polypeptides CRY19A and CRY15A or salts thereof; the polypeptides CRY11F and CRY15A; or more preferably the polypeptides CRY19A, CRY11F and CRY15A or salts thereof.

Preferred additional polypeptides or salts thereof for inclusion in a polypeptide combination of the invention, alongside the polypeptides or salts thereof of (a) to (c), are a polypeptide of (d) and/or a polypeptide of (e). These are:

(d) a polypeptide comprising the amino acid sequence of RIWLQFAKLTGFTL (SEQ ID NO: 3; CRY17) or a T cell epitope-containing variant sequence derived from said amino acid sequence; and/or

(e) a polypeptide comprising the amino acid sequence of KSMKVTVAFNQFGP (SEQ ID NO:13, CRY08) or a T cell epitope-containing variant sequence derived from said amino acid sequence

wherein a T cell epitope-containing variant sequence of a said amino acid sequence is said amino acid sequence having up to five amino acid modifications, each of which is independently a deletion, substitution or insertion;

wherein the polypeptide is up to 30 amino acids in length; and

wherein the polypeptide may be present as a pharmaceutically acceptable salt thereof.

Thus, a pharmaceutical formulation of the invention may comprise four polypeptides or salts thereof selected from (a), (b), (c) and (d); or (a), (b), (c) and (e). In any combination of the invention, the polypeptide CRY17 of (d) is preferably replaced by a polypeptide having the corresponding variant sequence KRIWLQFAKLTGFTLK (SEQ ID NO: 159; CRY17A) or a said salt thereof. It is preferred that pharmaceutical formulations comprising polypeptides or salts thereof of (d) and/or (e) comprise at least one polypeptide or salt thereof selected from:

(d1) a polypeptide comprising, consisting essentially of or consisting of the amino acid sequence KRIWLQFAKLTGFTLK (SEQ ID NO: 159; CRY17A) or a said salt thereof, and

(e1) a polypeptide comprising, consisting essentially of or consisting of the amino acid sequence KSMKVTVAFNQFGP (SEQ ID NO:13, CRY08) or a said salt thereof.

The pharmaceutical formulation may preferably comprise polypeptides or salts thereof selected from (a1), (b1), (c1), and (d1) above; or (a1), (b1), (c1) and (e1) above.

More preferably the pharmaceutical formulation comprises all five polypeptides or salts thereof of (a) to (e), such as all five polypeptides of (a1) to (e1). It is particularly preferred that the pharmaceutical formulation comprises the five polypeptides CRY19A, CRY11F, CRY15A, CRY17A, and CRY08 or salts thereof.

Other preferred additional polypeptides for inclusion in a polypeptide combination of the invention, alongside the polypeptides or salts thereof of (a) to (c), are a polypeptide of (f); and/or a polypeptide of (g). These are:

(f) a polypeptide comprising the amino acid sequence of DIFASKNFHLQKNTIGT (SEQ ID NO: 22; CRY21) or a T cell epitope-containing variant sequence derived from said amino acid sequence, and

(g) a polypeptide comprising the amino acid sequence of TYKNIRGTSAT (SEQ ID NO: 23; CRY22) or a T cell epitope-containing variant sequence derived from said amino acid sequence,

wherein a T cell epitope-containing variant sequence of a said amino acid sequence is said amino acid sequence having up to five amino acid modifications, each of which is independently a deletion, substitution or insertion;

wherein each selected polypeptide is up to 30 amino acids in length; and wherein each selected polypeptide may be present as a pharmaceutically acceptable salt thereof.

In any combination of the invention, the polypeptide CRY21 of (f) is preferably replaced by a polypeptide having the corresponding variant sequence SKNFHLQKNTIGTG (SEQ ID NO: 166; CRY21C). It is preferred that pharmaceutical formulations comprising polypeptides or salts thereof of (f) and/or (g) comprise at least one polypeptide or salt thereof selected from:

(f1) a polypeptide comprising, consisting essentially of or consisting of the amino acid sequence SKNFHLQKNTIGTG (SEQ ID NO: 166; CRY21C) or a said salt thereof, and

(g1) a polypeptide comprising, consisting essentially of or consisting of the amino acid sequence TYKNIRGTSAT (SEQ ID NO: 23; CRY22) or a said salt thereof.

A pharmaceutical formulation of the invention may comprise polypeptides or salts thereof selected from (a), (b), (c) and (f); or (a), (b), (c) and (g). The pharmaceutical formulation may comprise four or five polypeptides or salts thereof selected in one of the following combinations: (a), (b), (c), (d) and (f); (a), (b), (c), (e)

and (f); (a), (b), (c), (d) and (g); (a), (b), (c), (e) and (g); or (a), (b), (c), (f) and (g). More preferably, the pharmaceutical formulation may comprise each of the polypeptides or salts thereof of (a) to (g). In each of the above polypeptide combinations, polypeptides of (a1), (b1), (c1), (d1), (e1), (f1) and (g1) are preferably included where applicable.

A final preferred peptide for inclusion in a polypeptide combination of the invention, alongside the polypeptides or salts thereof of (a) to (c), is a polypeptide of (h); that is:

(h) a polypeptide comprising the amino acid sequence of

KDIKLSDISLKLTSGKIAS (CRY23; SEQ ID NO: 24), or a T cell epitope-containing variant sequence derived from said amino acid sequence,

wherein a T cell epitope-containing variant sequence of a said amino acid sequence is said amino acid sequence having up to five amino acid modifications, each of which is independently a deletion, substitution or insertion;

wherein the polypeptide is up to 30 amino acids in length; and

wherein the polypeptide may be present as a pharmaceutically acceptable salt thereof.

Preferably the polypeptide of (h) or salt thereof is:

(h1) a polypeptide comprising, consisting essentially of or consisting of the amino acid sequence KDIKLSDISLKLTSGKIAS (CRY23; SEQ ID NO: 24) or a said salt thereof.

A pharmaceutical formulation of the invention may thus comprise four, five or six polypeptides or salts thereof selected in one of the following combinations: (a), (b), (c), (d) and (h); (a), (b), (c), (e) and (h); (a), (b), (c), (f) and (h); (a), (b), (c), (g) and (h); (a), (b), (c), (e), (f) and (h); (a), (b), (c), (e), (g) and (h); (a), (b), (c), (d), (e), and (h); (a), (b), (c), (d), (f) and (h); (a), (b), (c), (d), (g) and (h). In each of the above polypeptide combinations, polypeptides of (a1), (b1), (c1), (d1), (e1), (f1), (g1) and (h1) are preferably included where applicable.

Particularly preferred combinations include seven polypeptides or salts thereof selected from the polypeptides of (a) to (h) or salts thereof. For example, a combination may include the polypeptides of (a), (b), (c), (d), (f), (g) and (h) or salts thereof. More preferably, a pharmaceutical formulation of the invention comprises the polypeptides of (a) to (g) or salts thereof, the polypeptides of (a) to (f) and (h) or salts thereof, or the

polypeptides of (a) to (e), (g) and (h) or salts thereof. Especially preferred seven polypeptide combinations are:

(1)

- a polypeptide having the amino acid sequence RPTAIKIDYSKSVT (CRY19A; SEQ ID NO:5) or a salt thereof;
- a polypeptide having the amino acid sequence KGAYFVSSGKYEGGK (SEQ ID NO: 165; CRY11F) or a salt thereof;
- a polypeptide having the amino acid sequence TFKVEGIIAAYQNPASWK (SEQ ID NO: 54; CRY15A) or a salt thereof;
- a polypeptide having the amino acid sequence KRIWLQFAKLTGFTLK (SEQ ID NO: 159; CRY17A) or a salt thereof;
- a polypeptide having the amino acid sequence KSMKVTVAFNQFGP (SEQ ID NO:13, CRY08) or a salt thereof;
- a polypeptide having the amino acid sequence SKNFHLQKNTIGTG (SEQ ID NO: 166; CRY21C) or a salt thereof; and
- a polypeptide having the amino acid sequence TYKNIRGTSAT (SEQ ID NO: 23; CRY22) or a salt thereof;

(2)

- a polypeptide having the amino acid sequence RPTAIKIDYSKSVT (CRY19A; SEQ ID NO:5) or a salt thereof;
- a polypeptide having the amino acid sequence KGAYFVSSGKYEGGK (SEQ ID NO: 165; CRY11F) or a salt thereof;
- a polypeptide having the amino acid sequence TFKVEGIIAAYQNPASWK (SEQ ID NO: 54; CRY15A) or a salt thereof;
- a polypeptide having the amino acid sequence KRIWLQFAKLTGFTLK (SEQ ID NO: 159; CRY17A) or a salt thereof;
- a polypeptide having the amino acid sequence KSMKVTVAFNQFGP (SEQ ID NO:13, CRY08) or a salt thereof;
- a polypeptide having the amino acid sequence SKNFHLQKNTIGTG (SEQ ID NO: 166; CRY21C) or a salt thereof; and
- a polypeptide having the amino acid sequence KDIKLSDISLKLTSGKIAS (CRY23; SEQ ID NO: 24) or a salt thereof

and

(3)

- a polypeptide having the amino acid sequence RPTAIKIDYSKSVT (CRY19A; SEQ ID NO:5) or a salt thereof;
- a polypeptide having the amino acid sequence KGAYFVSSGKYEGGK (SEQ ID NO: 165; CRY11F) or a salt thereof;
- a polypeptide having the amino acid sequence TFKVEGIIAAYQNPASWK (SEQ ID NO: 54; CRY15A) or a salt thereof;
- a polypeptide having the amino acid sequence KRIWLQFAKLTGFTLK (SEQ ID NO: 159; CRY17A) or a salt thereof;
- a polypeptide having the amino acid sequence KSMKVTVAFNQFGP (SEQ ID NO:13, CRY08) or a salt thereof;
- a polypeptide having the amino acid sequence TYKNIRGTSAT (SEQ ID NO: 23; CRY22) or a salt thereof; and
- a polypeptide having the amino acid sequence KDIKLSDISLKLTSGKIAS (CRY23; SEQ ID NO: 24) or a salt thereof.

In a most preferred embodiment, the pharmaceutical formulation comprises each of the polypeptides of (a) to (h) or salts thereof, which are preferably polypeptides of (a1) to (h1) or salts thereof. The most preferred combination of eight such polypeptides is the combination of:

- a polypeptide having the amino acid sequence RPTAIKIDYSKSVT (CRY19A; SEQ ID NO:5) or a salt thereof;
- a polypeptide having the amino acid sequence KGAYFVSSGKYEGGK (SEQ ID NO: 165; CRY11F) or a salt thereof;
- a polypeptide having the amino acid sequence TFKVEGIIAAYQNPASWK (SEQ ID NO: 54; CRY15A) or a salt thereof;
- a polypeptide having the amino acid sequence KRIWLQFAKLTGFTLK (SEQ ID NO: 159; CRY17A) or a salt thereof;
- a polypeptide having the amino acid sequence KSMKVTVAFNQFGP (SEQ ID NO:13, CRY08) or a salt thereof;
- a polypeptide having the amino acid sequence SKNFHLQKNTIGTG (SEQ ID NO: 166; CRY21C) or a salt thereof;

- a polypeptide having the amino acid sequence TYKNIRGTSAT (SEQ ID NO: 23; CRY22) or a salt thereof; and
- a polypeptide having the amino acid sequence KDIKLSDISLKLTSGKIAS (CRY23; SEQ ID NO: 24) or a salt thereof.

Additional polypeptides that may be included in polypeptide combinations of the invention are selected from one or more of:

(i) a polypeptide comprising the amino acid sequence of KLTGFTLMGKGVIDGQG (SEQ ID NO: 4) or a T cell epitope-containing variant sequence derived from said amino acid sequence,

(j) a polypeptide comprising the amino acid sequence of KKFVVNNLFFNGP (SEQ ID NO: 18; Cry14) or a T cell epitope-containing variant sequence derived from said amino acid sequence,

(k) a polypeptide comprising the amino acid sequence of CSNWVWQSTQDVF (SEQ ID NO: 15; Cry10) or a T cell epitope-containing variant sequence derived from said amino acid sequence, and

(l) a polypeptide comprising the amino acid sequence of SWKNNRIWLQFA (SEQ ID NO: 20; Cry16) or a T cell epitope-containing variant sequence derived from said amino acid sequence,

wherein a T cell epitope-containing variant sequence of a said amino acid sequence is said amino acid sequence having up to five amino acid modifications, each of which is independently a deletion, substitution or insertion;

wherein each polypeptide is up to 30 amino acids in length; and

wherein each polypeptide may be present as a pharmaceutically acceptable salt thereof.

A polypeptide combination of the invention may comprise one, two, three, or four polypeptides selected from (i) to (l) or salts thereof. One or more polypeptides of (i) to (l) or salts thereof may be included as additional polypeptides in a pharmaceutical formulation of the invention alongside any polypeptide combination comprising polypeptides (a) to (c) or salts thereof described above. Thus, for example, a polypeptide of (i) or a salt thereof may be added to a polypeptide combination comprising polypeptides of (a), (b), (c), (d), (f), (g) and (h) or salts thereof. Alternatively, one or more polypeptides of (i) to (l) or salts thereof may replace a corresponding number of polypeptides selected from (d) to (h) or salts thereof in a

polypeptide combination described above. For example, the polypeptide (h) or a salt thereof may be replaced with a polypeptide of any of (i) to (l) or a salt thereof in any polypeptide combination comprising a polypeptide of (h) or a salt thereof described above.

In any polypeptide combination comprising a polypeptide having the amino acid sequence of (i), said polypeptide or salt thereof is preferably replaced with a polypeptide having its corresponding variant sequence KLTGFTLMGKGVIEGQK (SEQ ID NO: 58; Cry18) or a salt thereof. In any polypeptide combination comprising a polypeptide having the amino acid sequence of (k) or salt thereof, said polypeptide or salt thereof is preferably replaced with a polypeptide having its corresponding variant sequence SSNWVWQSTQDVF (SEQ ID NO: 45; CRY10A) or a salt thereof.

The above polypeptide combinations may be suitable for use in preventing or treating allergy to Japanese Cedar pollen and/or Japanese Cypress pollen, for example since a polypeptide of (a) comprising the amino acid sequence RPTAIKIDYSKSVT (SEQ ID NO: 5; CRY19A) or a corresponding variant sequence represents an epitope region which is homologous between pollen allergens from both trees (Cry j 1 and Cha o 1). The invention thus provides polypeptide combinations which are suitable for tolerisation against both Japanese Cedar pollen and/or Japanese Cypress pollen allergens by virtue of representing epitope regions which are homologous between pollen allergens from both trees.

All of the above selections are subject to the combination preferably comprising twelve polypeptides in total or less, more typically, ten polypeptides or less, preferably less than nine, more preferably eight or less polypeptides. The combination may comprise four, five, six or seven polypeptides. The combination of the invention preferably comprises seven or more preferably eight polypeptides.

Any of the polypeptide combinations described above may optionally comprise no further polypeptides or no further peptides derived from Japanese Cedar and/or Japanese Cypress allergens. Any of the polypeptide combinations described above may be incorporated in a pharmaceutical formulation of the invention as described in more detail below.

Medical uses and methods

The polypeptides, salts thereof and pharmaceutical formulations of the invention are used for treating or preventing allergy to Japanese Cedar pollen and/or Japanese Cypress pollen. They preferably treat allergy to Japanese Cedar pollen. They may prevent or treat the allergy by inducing immune tolerance to subsequent exposure to allergen. The tolerisation may be to one or more protein allergens of the *Cryptomeria* and/or *Chamaecyparis* genus. The tolerisation is typically to protein allergens from *Cryptomeria japonica* and/or *Chamaecyparis obtusa*.

A method of treating an individual for allergy to Japanese Cedar pollen and/or Japanese Cypress pollen or of preventing in an individual allergy to Japanese Cedar pollen and/or Japanese Cypress pollen is thus provided. The method comprises administering to said individual a therapeutically or prophylactically effective amount of a polypeptide or salt thereof of the invention or of a pharmaceutical formulation of the invention. The method may thus reduce or ameliorate the symptoms of allergy in the individual suffering from the allergy. The method may improve the condition of the individual suffering from the allergy. The method may prevent or delay the appearance of symptoms of allergy in the individual. Symptoms of allergy to Japanese Cedar pollen and/or Japanese Cypress pollen are discussed below.

A combination of polypeptides or salts thereof can be used for treating or preventing allergy to Japanese Cedar pollen and/or Japanese Cypress pollen. The polypeptides and/or salts thereof in a combination need not be administered together, and/or need not be part of the same pharmaceutical formulation.

The invention thus provides a polypeptide or salt thereof of the invention for use in a method of preventing or treating allergy to Japanese Cedar pollen and/or Japanese Cypress pollen as described above, wherein said method further comprises administering at least one, preferably two or more additional polypeptides derived from Japanese Cedar pollen or Japanese Cypress pollen as described above. The multiple polypeptides of this method may each be administered simultaneously, sequentially or concurrently. Thus, they may be administered separately.

The polypeptides, salts thereof and pharmaceutical formulations of the invention may treat or prevent the allergy by inducing immune tolerance to Japanese Cedar pollen and/or Japanese Cypress pollen. A polypeptide or salt thereof described herein may be used to tolerise or desensitise an individual to the allergen from which it is derived. Desensitising an individual to the allergens means inhibition or dampening of allergic

tissue reactions induced by the allergens in appropriately sensitised individuals. The term "tolerisation" refers to an ability to suppress, or abolish a response to an antigen, such as an allergic response to a protein allergen. Tolerisation is also an ability to diminish or abolish an unwanted immune response to a protein allergen. Tolerisation may be determined by *in vitro* analysis of T cell responses or by observation of a reduction in the symptoms in an individual.

In more detail, T cells can be selectively activated, and then rendered unresponsive. Moreover the anergising or elimination of these T-cells leads to tolerisation of the patient for a particular allergen. The tolerisation manifests itself as a reduction in response to an allergen or allergen-derived peptide, or preferably an elimination of such a response, on second and further administrations of the allergen or allergen-derived peptide. This second administration may be made after a suitable period of time has elapsed to allow tolerisation to occur; this is preferably any period between one day and several weeks. An interval of around four weeks is preferred.

The individual to whom the polypeptide, salt thereof or pharmaceutical formulation is administered may be asymptomatic. A prophylactically effective amount of the polypeptide or pharmaceutical formulation is administered to such an individual. A prophylactically effective amount is an amount which prevents the onset of one or more symptoms of allergy.

Alternatively, the individual to whom the polypeptide, salt thereof or pharmaceutical formulation is administered may be in need thereof. That is, the individual may exhibit one or more symptoms of allergy. A therapeutically effective amount of the polypeptide or pharmaceutical formulation is administered to such an individual. A therapeutically effective amount is an amount which is effective to ameliorate one or more symptoms of allergy.

The individual to whom the polypeptide, salt thereof or pharmaceutical formulation is administered is preferably human. The individual may be known to be sensitised to Japanese Cedar pollen and/or Japanese Cypress pollen allergens, at risk of being sensitised or suspected of being sensitised. The individual can be tested for sensitisation using techniques well known in the art and as described herein. Alternatively, the individual may have a family history of allergy to Japanese Cedar pollen and/or Japanese Cypress pollen.

It may not be necessary to test an individual for sensitisation to Japanese Cedar pollen and/or Japanese Cypress pollen because the individual may display symptoms of allergy when exposed to Japanese Cedar pollen and/or Japanese Cypress pollen. By exposure is meant proximity to, for example, a tree or pollen or a substance or product derived from a tree or pollen. By proximity is meant 10 metres or less, 5 metres or less, 2 metres or less, 1 metre or less, or 0 metres from the items described above. Symptoms of allergy can include an itching nose, sneezing, ocular tearing, an itchy throat, itchy palate, itchy eyes, runny nose, breathing difficulties, bronchospasm, asthma, red itchy skin or rash.

The individual may be of any age. However, preferably, the individual may be in the age group of 1 to 90, 5 to 60, 10 to 40, or more preferably 18 to 35.

The individual may have had allergy to Japanese Cedar pollen and/or Japanese Cypress pollen for at least 2 weeks, 1 month, 6 months, 1 year, 5 years or more than 5 years. The individual may suffer from a rash, nasal congestion, nasal discharge and/or coughing caused by the allergy. The individual may suffer from an itching nose, sneezing, ocular itching, ocular tearing, itchy throat, itchy palate, bronchospasm and/or asthma caused by the allergy.

The individual may or may not have been administered with other compositions/compounds which treat Japanese Cedar pollen and/or Japanese Cypress pollen allergy. The individual may live in a geographical region which has a temperate, semi-tropical, or tropical climate. The individual typically suffers from allergy to Japanese Cedar pollen and/or Japanese Cypress pollen in a particular season but the allergy may be perennial. Seasonal allergy to Japanese Cedar pollen and/or Japanese Cypress pollen may commonly occur either in late Winter or in Spring in the Far East.

The allergic individual is allergic to tree pollen from the Japanese Cedar and/or Japanese Cypress tree, and thus to pollen from trees in the *Cryptomeria* genus, particularly *Cryptomeria japonica* and/or trees in the *Chamaecyparis* genus, in particular *Chamaecyparis obtusa.* The allergic individual is preferably allergic to tree pollen from the Japanese Cedar tree. The allergic individual may be allergic both to tree pollens of *Cryptomeria japonica* and of *Chamaecyparis obtusa.*

The polypeptides, salts thereof or pharmaceutical formulations of the invention may be screened in panels of Japanese Cedar pollen and/or Japanese Cypress pollen allergic individuals to confirm their suitability for use. The panel of Japanese Cedar

pollen and/or Japanese Cypress pollen allergic individuals may comprise individuals known or not known to be allergic to Japanese Cedar pollen and/or Japanese Cypress pollen. In particular where multiple polypeptides are provided in combination in a pharmaceutical formulation, they may be screened for their ability to cause T cell proliferation or cytokine production in at least 20 % of samples of T cells, wherein each sample is obtained from different Japanese Cedar pollen and/or Japanese Cypress pollen allergic individuals in the population. Preferably, the pharmaceutical formulation will induce T cell proliferation or cytokines in at least 30 % of samples of T cells obtained from of a panel of Japanese Cedar pollen and/or Japanese Cypress pollen allergic individuals. More preferably, the pharmaceutical formulation will induce T cell proliferation or cytokines in 35% or more, 40 % or more, 50 % or more, 60 % or more, 70% or more, 80 % or more, or 90 % or more of samples in the panel. The number of individuals in a panel of Japanese Cedar pollen and/or Japanese Cypress pollen allergic individuals may be any number greater than one, for example at least 2, 3, 5, 10, 15, 20, 30, 50, 80, or at least 100 individuals.

It is also preferred that the polypeptides, salts thereof and pharmaceutical formulations of the invention cause T cell proliferation and/or cytokine production, but do not lead to the release of histamine from samples from a sensitised individual. The histamine release profile of a polypeptide, salt thereof or pharmaceutical formulation may thus be confirmed. Suitable samples include whole blood, enriched basophils or mast cell preparations. There may be some histamine release, but preferably the amounts released are not significant.

Significant histamine release may be considered to be the release of 20% or more of the total available histamine when a sample from an individual is stimulated with a pharmaceutical formulation *in vitro*. A polypeptide, salt thereof or pharmaceutical formulation of the invention preferably causes the release of less than 15%, less than 10%, less than 5%, less than 4%, less than 3%, less than 2% or less than 1% of the total available histamine when a sample from an individual is stimulated with a composition in vitro. A normal individual typically has an approximate leukocyte histamine content of 150 ng/10^7 cells.

Pharmaceutical formulations

Each polypeptide or salt thereof described herein may be provided to an individual in an isolated, substantially isolated, purified or substantially purified form. For example, a polypeptide or salt thereof described herein may be provided to an individual substantially free from other polypeptides or salts thereof. Whilst it may be possible for the polypeptides or salts thereof to be presented in raw form, it is preferable to present them as a pharmaceutical formulation.

Thus, according to a further aspect of the invention, the invention further provides a pharmaceutical formulation which comprises a pharmaceutically acceptable carrier or diluent and the polypeptides of (a) to (c) described herein or pharmaceutically acceptable salts thereof. The invention also provides a pharmaceutical formulation comprising a combination of polypeptides or salts thereof as described above, and a pharmaceutically acceptable carrier or diluent.

The carrier(s) or diluent(s) present in the pharmaceutical formulation must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof. Typically, carriers for injection, and the final formulation, are sterile and pyrogen free. Preferably, the carrier or diluent is water. A pharmaceutically acceptable carrier or diluent may comprise as one of its components thioglycerol or thioanisole.

A composition containing one or more polypeptides or salts thereof described herein can be combined with one or more pharmaceutically acceptable excipients or vehicles to produce a pharmaceutical formulation. Auxiliary substances, such as wetting or emulsifying agents, pH buffering substances and the like, may be present in the excipient or vehicle. These excipients, vehicles and auxiliary substances are generally pharmaceutical agents that do not induce an immune response in the individual receiving the composition, and which may be administered without undue toxicity. Pharmaceutically acceptable excipients include, but are not limited to, liquids such as water, saline, polyethyleneglycol, hyaluronic acid, glycerol, thioglycerol and ethanol. Pharmaceutically acceptable salts can also be included therein, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. A thorough discussion of pharmaceutically acceptable excipients, vehicles and auxiliary substances is available in Remington's Pharmaceutical Sciences (Mack Pub. Co., N.J. 1991).

The polypeptides or salts thereof are typically present at 0.1% to 50% by weight in the pharmaceutical formulation, more preferably at 0.1% to 5% by weight. The polypeptides or salts thereof may be present at less than 0.1% by weight in the pharmaceutical formulation.

The pharmaceutically acceptable carrier or diluent is typically present at 50% to 99.9% by weight in the pharmaceutical formulation, more preferably at 95% to 99.9% by weight. The pharmaceutically acceptable carrier or diluents may be present at more than 99.9% by weight in the pharmaceutical formulation.

Pharmaceutical formulations include, but are not limited to pharmaceutically acceptable solutions, lyophilisates, suspensions, emulsions in oily or aqueous vehicles, pastes, and implantable sustained-release or biodegradable formulations. Such pharmaceutical formulations may further comprise one or more additional ingredients including, but not limited to, suspending, stabilizing, or dispersing agents. A lyophilisate may comprise one or more of trehalose, thioglycerol and thioanisole. In one embodiment of a pharmaceutical formulation for parenteral administration, the active ingredient is provided in dry form (e.g., a lyophilisate, powder or granules) for reconstitution with a suitable vehicle (e.g., sterile pyrogen-free water) prior to parenteral administration of the reconstituted pharmaceutical formulation.

The pharmaceutical formulations may be prepared, packaged, or sold in the form of a sterile injectable aqueous or oily suspension or solution. This suspension or solution may be formulated according to the known art, and may comprise, in addition to the active ingredient, additional ingredients such as the dispersing agents, wetting agents, or suspending agents described herein. Such sterile injectable formulations may be prepared using a non-toxic parenterally-acceptable diluent or solvent, such as water or 1,3-butane diol, for example. Other acceptable diluents and solvents include, but are not limited to, Ringer's solution, isotonic sodium chloride solution, and fixed oils such as synthetic mono-or di-glycerides.

Other parenterally-administrable pharmaceutical formulations which are useful include those which comprise the active ingredient in microcrystalline form, in a liposomal preparation, or as a component of a biodegradable polymer systems. Pharmaceutical formulations for sustained release or implantation may comprise pharmaceutically acceptable polymeric or hydrophobic materials such as an emulsion, an ion exchange resin, a sparingly soluble polymer, or a sparingly soluble salt.

Alternatively, the polypeptides of the present invention may be encapsulated, adsorbed to, or associated with, particulate carriers. Suitable particulate carriers include those derived from polymethyl methacrylate polymers, as well as PLG microparticles derived from poly(lactides) and poly(lactide-co-glycolides). See, e.g., Jeffery et al. (1993) Pharm. Res. 10:362-368. Other particulate systems and polymers can also be used, for example, polymers such as polylysine, polyarginine, polyornithine, spermine, spermidine, as well as conjugates of these molecules.

The formulation of any of the polypeptides mentioned herein will depend upon factors such as the nature of the polypeptide and the method of delivery. The pharmaceutical formulation may be administered in a variety of dosage forms. It may be administered orally (e.g. as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules), topically, parenterally, subcutaneously, by inhalation, intravenously, intramuscularly, intralymphatically (such as to lymph nodes in the groin), intrasternally, transdermally, intradermally, epidermally, sublingually, intranasally, buccally or by infusion techniques. The administration may be intratonsillar. The administration may be as suppositories. The administration may be made by iontophoresis. Preferably, the administration is intradermal, epidermal or transdermal. The administration may be made by a patch, such as a microtine patch.

A physician will be able to determine the required route and means of administration for each particular individual.

The pharmaceutical formulations of the invention are preferably provided sealed in a container. The pharmaceutical formulations are typically provided in unit dose form, for example single dose form. They may alternatively be provided in multi-dose form. Where the pharmaceutical formulation is a pharmaceutically acceptable solution, the solution may be provided in an ampoule, sealed vial, syringe, cartridge, flexible bag or glass bottle. Where the pharmaceutical formulation is a lyophilisate, it is preferably provided in a sealed vial.

The pharmaceutical formulations of the invention will comprise a suitable concentration of each polypeptide to be effective without causing adverse reaction. Where the pharmaceutical formulation is for example a lyophilisate, the relevant concentration will be that of each polypeptide following reconstitution. Typically, the concentration of each polypeptide in the pharmaceutical formulation when in solution will be in the range of 0.03 to 200 nmol/ml. The concentration of each polypeptide may

be more preferably in the range of 0.3 to 200 nmol/ml, 3 to 180 nmol/ml, 5 to 160 nmol/ml, 10 to 150 nmol/ml, 50 to 200 nmol/ml or 30 to 120 nmol/ml, for example about 100 nmol/ml. The pharmaceutical formulation should have a purity of greater than 95% or 98% or a purity of at least 99%.

An adjuvant or further therapeutic agent may be used in combination with one or more polypeptides of the invention. An adjuvant is preferably administered in an amount which is sufficient to augment the effect of the polypeptide(s) of the invention or vice versa. The adjuvant or further therapeutic agent may be an agent that potentiates the effects of a polypeptide of the invention. For example, the further therapeutic agent may be an immunomodulatory molecule which enhances the response to the polypeptide of the invention. Non-limiting examples of adjuvants include vitamin D, rapamycin and glucocorticoid steroids such as dexamethasone, fluticasone, budesonide, mometasone, beclomethasone, hydrocortisone, cortisone acetate, prednisone, prednisolone, methylprednisolone, betamethasone and triamcinolone. A preferred glucocorticoid is dexamethasone.

In an embodiment where one or more polypeptides of the invention are used for therapy in combination with one or more other therapeutic agents or adjuvants, the other therapeutic agents or adjuvants may be administered separately, simultaneously or sequentially. They may be administered in the same or different pharmaceutical formulations. A pharmaceutical formulation may therefore be prepared which comprises a polypeptide of the invention and also one or more other therapeutic agents or adjuvants. A pharmaceutical formulation of the invention may alternatively be used simultaneously, sequentially or separately with one or more other therapeutic compositions as part of a combined treatment. Accordingly, in a method of preventing or treating allergy according to the invention as described below, the subject may also be treated with a further therapeutic agent.

Routes of administration

Where a polypeptide or salt thereof of the invention is to be administered to an individual in a pharmaceutical formulation, it is preferred to administer the formulation to a site in the body of the individual where the polypeptide or salt thereof will have the ability to contact suitable antigen presenting cells, and where it, or they, will have the opportunity to contact T cells of the individual.

Once formulated the pharmaceutical formulations of the invention can be delivered to a subject in vivo using a variety of known routes and techniques. For example, a pharmaceutical formulation can be provided as an injectable solution, suspension or emulsion and administered via parenteral, subcutaneous, epidermal, intradermal, intramuscular, intralymphatic, intraarterial, intraperitoneal, or intravenous injection using a conventional needle and syringe, a microneedle and syringe or using a liquid jet injection system. The administration may be made using a patch, such as a microtine patch. Compositions can also be administered topically to skin or mucosal tissue, such as nasally, intratonsillarly, intratracheally, intestinal, rectally or vaginally, or provided as a finely divided spray suitable for respiratory or pulmonary administration. Other modes of administration include oral administration, suppositories, sublingual administration, and active or passive transdermal delivery techniques.

Dosages

Administration of the polypeptides, salts thereof or pharmaceutical formulations of the invention may be by any suitable method as described above. Suitable amounts of the polypeptides or salts thereof to be administered may be determined empirically, but typically are in the range given below. A single administration of each polypeptide or salt thereof may be sufficient to have a beneficial effect for the patient, but it will be appreciated that it may be beneficial if the polypeptide or salt thereof is administered more than once, in which case typical administration regimes may be, for example, once or twice a week for 2-4 weeks every 6 months, or once a day for a week every four to six months. As will be appreciated, each polypeptide or salt thereof in a combination of polypeptides or salts thereof may be administered to a patient singly or in combination.

Dosages for administration will depend upon a number of factors including the nature of the peptide, salt thereof or pharmaceutical formulation, the route of administration and the schedule and timing of the administration regime. Suitable doses of a polypeptide or salt thereof of the invention may be in the order of up to 10 μg, up to 15μg, up to 20μg, up to 25μg, up to 30μg, up to 50μg, up to 100μg, up to 500 μg or more per administration. Suitable doses may be less than 15μg, but at least 1ng, or at least 2ng, or at least 5ng, or at least 50ng, or least 100ng, or at least 500ng, or at least 1μg, or at least 10μg. Alternatively, the dose used may be higher, for example, up to 1

mg, up to 2 mg, up to 3 mg, up to 4 mg, up to 5 mg or higher. Doses may be provided in a liquid formulation, at a concentration suitable to allow an appropriate volume for administration by the selected route. It will be understood that the above doses refer to total dose in the case of a combination of peptides or salts thereof. For example, "up to 35μg" refers to a total peptide or peptide salt concentration of up to 35μg in a composition comprising a combination or more than one peptide or salt thereof.

Nucleic acids and vectors

The polypeptide of the invention may be administered directly, or may be administered indirectly by expression from an encoding sequence. For example, a polynucleotide may be provided that encodes a polypeptide of the invention. A polypeptide of the invention may thus be produced from or delivered in the form of a polynucleotide which encodes, and is capable of expressing, it. Any reference herein to the use, delivery or administration of a peptide of the invention is intended to include the indirect use, delivery or administration of such a peptide via expression from a polynucleotide that encodes it.

In this aspect, the invention provides for example a polynucleotide which encodes a polypeptide comprising, consisting or consisting essentially of the amino acid sequence of SEQ ID NO: 5 or a variant derived from either therefrom. A polynucleotide encoding a polypeptide comprising, consisting or consisting essentially of the amino acid sequence of SEQ ID NOs: 165, 54, 159, 13, 166, 23, 24 or a variant derived from any therefrom is also provided. A number of polynucleotide sequences each separately encoding for a polypeptide to be expressed may be provided together in a single nucleic acid molecule or vector to allow for expression of a combination of different polypeptides from a single nucleic acid molecule. The polynucleotide sequences may be expressed in a cell as a single polypeptide.

The terms "nucleic acid molecule" and "polynucleotide" are used interchangeably herein and refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof. Non-limiting examples of polynucleotides include a gene, a gene fragment, messenger RNA (mRNA), cDNA, recombinant polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, and primers. A polynucleotide may be provided in isolated or purified form.

Polynucleotides can be synthesised according to methods well known in the art, as described by way of example in Sambrook et al (1989, Molecular Cloning - a laboratory manual; Cold Spring Harbor Press).

The above polynucleotides may be used *in vitro*, *ex vivo* or *in vivo* in the production of a polypeptide of the invention. Such polynucleotides may be administered or used in the prevention or treatment of allergy to Japanese Cedar pollen and/or Japanese Cypress pollen.

Methods for gene delivery are known in the art. See, e.g., U.S. Patent Nos. 5,399,346, 5,580,859 and 5,589,466. The nucleic acid molecule can be introduced directly into the recipient subject, such as by standard intramuscular or intradermal injection; transdermal particle delivery; inhalation; topically, or by oral, intranasal or mucosal modes of administration. The molecule alternatively can be introduced ex vivo into cells that have been removed from a subject. For example, a polynucleotide, expression cassette or vector of the invention may be introduced into APCs of an individual ex vivo. Cells containing the nucleic acid molecule of interest are re-introduced into the subject such that an immune response can be mounted against the peptide encoded by the nucleic acid molecule. The nucleic acid molecules used in such immunization are generally referred to herein as "nucleic acid vaccines."

Antigen presenting cells (APCs)

The invention encompasses the use *in vitro* of a method of producing a population of APCs that present a polypeptide of the invention on their surface. Said population of APCs may be subsequently used in therapy. Said method of production may be carried out *ex vivo* on a sample of cells that have been obtained from a patient. The APCs produced in this way therefore form a pharmaceutical agent that can be used in the treatment or prevention of allergy to Japanese Cedar pollen and/or Japanese Cypress pollen. The cells should be accepted by the immune system of the individual because they derive from that individual. Delivery of cells that have been produced in this way to the individual from whom they were originally obtained, thus forms a therapeutic embodiment of the invention.

Where an APC is to be administered, it is preferred to administer the APC to a site in the body where it will have the ability to contact, and activate, suitable T cells of the individual.

In vitro method

The invention further provides an *in vitro* method of determining whether T cells recognize a polypeptide or salt thereof of the invention, or a polypeptide or salt thereof of a pharmaceutical formulation of the invention comprising at least three polypeptides, which method comprises contacting said T cells with said polypeptide or salt thereof or said pharmaceutical compositions and detecting whether said T cells are stimulated by said polypeptide or salt thereof. The method may comprise use of a polypeptide, or salt thereof, comprising, consisting or consisting essentially of the amino acid sequence of SEQ ID NO: 5 or a variant derived therefrom. The method may alternatively or additionally comprise use of a polypeptide or salt thereof comprising, consisting or consisting essentially of the amino acid sequence of any one of SEQ ID NOs: 165, 54, 159, 13, 166, 23, 24 or a variant derived from any therefrom.

The above method may be carried out to determine whether an individual has, or is at risk of having, an allergy to Japanese Cedar pollen and/or Japanese Cypress pollen.

The invention is illustrated by the following Examples:

Example 1

MHC Class II binding search

The aim of this study is to identify a distinct panel of polypeptides having sequences with strong affinities for the seven most common human MHC class-II HLA-DRB1* allotypes.

In order to identify said polypeptides in the allergens Cry j 1, Cry j 2 , Cry j 3, Cry j chitinase and Cry IFR from *Cryptomerium japonica* and Cha o 1 and Cha o 2 from *Chamaecyparis obtusa*, an *in silico* approach known as "peptide threading" was performed using the commercially available EpiMatrix algorithm (EpiVax Inc.). This is a bioinformatic method of analysing a polypeptide having a given sequence for the potential to be accommodated within the binding groove of MHC class-II HLA-DR molecules.

EpiMatrix is a matrix-based algorithm that ranks 10 amino acid long segments, overlapping by 9 amino acids, from any polypeptide sequence by estimated probability of binding to each of the selected MHC molecules. (De Groot et al., AIDS Research

and Human Retroviruses 13:539-41 (1997)). The procedure for developing matrix motifs was published by Schafer et al, 16 Vaccine 1998 (1998). In this Example, binding potential for HLA DR1, DR2, DR3, DR4, DR7, DR8, DR11, DR13 and DR15 is assessed. Putative MHC ligands are selected by scoring each 10-mer frame in a polypeptide sequence. This score is derived by comparing the sequence of the 10-mer to the matrix of 10 amino acid sequences known to bind to each MHC allele. Retrospective studies have demonstrated that EpiMatrix accurately predicts published MHC ligands (Jesdale et al., in Vaccines '97 (Cold Spring Harbor Press, Cold Spring Harbor, N.Y., 1997)). Successful prediction of polypeptides which bind to multiple MHC molecules has also been confirmed.

Estimated probability of binding to a selected MHC molecule is calculated by EpiMatrix as follows. The polypeptides having a given sequence are scored by estimating the relative promotion or inhibition of binding for each amino acid, compared to known MHC binders for a given MHC allele. This information is summed across the polypeptide and a summary score (EMX score) is assigned to the entire polypeptide. After comparing the EMX score to the scores of known MHC ligands, EpiMatrix arrives at an "estimated binding probability" (abbreviated as EBP, but not strictly a probability). The EBP describes the proportion of polypeptides with EpiMatrix scores as high or higher that will bind to a given MHC molecule. EBPs range from 100% (highly likely to bind) to less than 1% (very unlikely to bind).

EpiMatrix analyses were performed on the entire sequence of known isoforms of Cry j 1 (NCBI GI accession nos: 19570317, 493632, 493634). These analyses identified core polypeptides (and their flanking sequences) derived from the above sequence which are predicted to have good MHC class-II binding. The sequences are shown below in Table 1 and were identified as being present in one or more of the isoforms.

In Table 1: "Residues in sequence" gives the location of the sequence within the sequence of the polypeptide that was analysed. The core sequence (middle amino acids in bold) defines the actual binding sequence that was identified during the analysis. The stabilizing flanks (N-terminal and C-terminal, not bold) were included for use with the core sequence and are typically required to aid manufacture of a polypeptide. "Number of hits" refers to the number of high predicted binding affinities for all MHC types tested within the sequence. The "EpiMatrix Cluster Score" is derived from the number

of hits normalized for the length of the cluster. Cluster Score is thus the excess or shortfall in predicted aggregate MHC binding properties relative to a random polypeptide standard. A score above 10 is considered to indicate broad MHC binding properties.

Table 1

INPUT SEQUENCE	RESIDUES IN SEQUENCE (Incl. FLANKS)	SEQUENCE	Hydro-phobicity	EpiMatrix HITS (Excl FLANKS)	Epi Matrix CLUSTER SCORE (Excl FLANKS)	SEQ ID NO:
19570317	87 - 119	RPL**WIIFSGNMNIKLKMPMYI**AGY	0.35	17	26.55	67
19570317	103 - 122	MPM**YIAGYKTFDGRGAQ**VYI	0.03	10	14.82	68
19570317	127 - 147	PCV**FIKRVSNVIIHGLYL**YGC	0.98	11	18.36	69
19570317	171 - 193	GDA**LTLRTATNIWIDHNSFS**NSS	-0.38	11	15.26	70
19570317	231 - 251	DKS**MKVTVAFNQFGPNCG**QRM	-0.55	8	10.25	71
19570317	293 - 313	NES**YKKQVTIRIGCKTSS**SCS	-0.74	9	16.89	72
493632	127 - 149	PCV**FIKRVSNVIIHGLHLYG**CST	0.75	14	22.94	73

Based on a further analysis of Epimatrix data, the following additional sequences from Cry j1 were also identified as having suitable MHC-binding properties: SGNMNIKLKMPMYIAGYK (SEQ ID NO: 9), PCVFIKRVSNVIIHG (SEQ ID NO: 11).

Example 2

EpiMatrix analyses as above were performed on the entire sequence of the known isoforms of Cry j 2 (NCBI accession nos: 114841607, 114841617, 114841629, 114841635, 114841641, 114841653, 114841657, 114841663, 114841665, 1171004, 123299282, 506858, 5299958, 5299959, 5299960). This analysis identified core sequences (with their flanking sequences) derived from said Cry j 2 isoforms which are predicted to have good MHC class-II binding properties. These sequences are shown below in Table 2 and were identified as being present in one or more of the isoforms. Headings and notes for Table 2 are as with Table 1 above.

Table 2

INPUT SEQUENCE	RESIDUES IN SEQUENCE (Incl FLANKS)	SEQUENCE	Hydro-phobicity	Epi Matrix HITS (Excl FLANKS)	Epi Matrix CLUSTER SCORE (Excl FLANKS)	SEQ ID NO:
114841607	5 - 28	LIA**PMAFLAMQLIIMAAAEDQ**SAQ	1.09	15	29.87	74
114841607	32 - 53	DSV**VEKYLRSNRSLRKVEH**SRH	-1.39	10	14.5	75
114841607	79 - 93	STA**WQAACKKPS**AML	-0.05	6	12.21	76
114841607	136 - 159	NRI**WLQFAKLTGFTLMGKGVI**DGQ	0.15	15	27.07	77
114841607	190 - 210	FST**GLIIQGLKLMNSPEF**HLV	0.65	11	15.23	78
114841607	364 - 381	DVT**YKNIRGTSATAA**AIQ	-0.17	7	12.16	79
114841607	444 - 464	HPK**TVMVENMRAYDKGNR**TRI	-1.18	12	20.92	80
114841617	79 - 93	STA**WQAACKNPS**AML	-0.02	7	16.28	81
114841635	5 - 28	FIA**PMAFVAMQLIIMAAAEDQ**SAQ	1.06	16	23.67	82
114841635	32 - 53	DSD**IEQYLRSNRSLRKVEH**SRH	-1.70	10	14.53	83
114841635	444 - 464	HPK**TVMVENMGAYDKGNR**TRI	-0.98	8	10.83	84
114841657	183 - 210	PTA**IKFDFSTGLIIQGLRLMNSPEF**HLV	0.44	18	26.63	85
1171004	443 - 464	KHP**KTVMVKNMGAYDKGNR**TRI	-1.13	13	20.03	86
123299282	32 - 56	DSV**VEKYLRSNRSLRRVEHSRH**DAI	-1.13	16	23.96	87
75299959	5 - 28	LIA**PMAFLAMQLIIMAAVEDQ**SAQ	1.19	11	13.7	88

Example 3

EpiMatrix analyses as above were performed on the entire sequence of the known isoforms of Cry j 3 (NCBI accession nos: 75104682, 75104683, 75104684, 75153325, 75153326, 75317671). This analysis identified core sequences (with their flanking sequences) derived from said Cry j 3 isoforms which are predicted to have good MHC class-II binding properties. These sequences are shown below in Table 4 and were identified as being present in one or more of the isoforms. Headings and notes for Table 3 are as with Table 1 above.

Table 3

INPUT SEQUENCE	RESIDUES IN SEQUENCE (Incl. FLANKS)	SEQUENCE	Hydro-phobicity	EpiMatrix HITS (Excl FLANKS)	Epi Matrix CLUSTER SCORE (Excl FLANKS)	SEQ ID NO:
75104682	3 - 27	IPF**WIALIASFSVFLQGVKVKA**PTF	1.29	16	26.37	89
75104682	32 - 49	KCP**YTVWAAAFPGGG**KQL	0.01	8	11.34	90
75104682	218 - 234	GTD**YKIVFCGKSDH**IFA	0.09	8	14.49	91
75104683	1 - 25	MA**SLRLATLAMMVLFGSCRAGA**TVF	1.34	16	21.81	92
75104684	2 - 22	ARA**ILWVLLTVMAVSLLL**HAG	1.83	18	33.4	93
75104684	54 - 74	GQS**WRVNVPGGARGRFWG**RTG	-0.92	11	17.08	94
75104684	104 - 125	STL**LEYALNQYQNLDFYDI**SLV	0.07	8	10.84	95
75104684	119 - 141	FYD**ISLVDGFNLRMTVVLSN**TNC	0.51	14	22.11	96
75104684	131 - 153	RMT**VVLSNTNCKRIACNSDI**NSK	-0.36	9	10.49	97
75153324	1 - 20	RA**IGVWIALVAALSVFL**HGM	1.74	15	30.7	98
75153324	32 - 49	QCP**YTVWAAASPGGG**QQL	-0.15	8	11.26	99
75153324	52 - 70	GQT**WTIQVAAGTTQAR**IWA	0.00	10	17.35	100
75153325	1 - 13	M**ARAMHTVWI**ALV	1.28	6	11.6	101
75153325	6 - 31	HTV**WIALVPTLFVFLQGINVKAA**TFD	1.09	20	30.86	102
75153325	35 - 52	QCP**YTVWAAASPGGG**RQL	-0.21	8	11.26	103
75153326	5 - 30	IPV**WIALVATLSVFLQGINVKAA**TFD	1.35	21	34.81	104
75153326	34 - 51	QCP**YTVWAAASPGGG**RQL	-0.21	8	11.26	105
75317671	1 - 26	MA**TVSDLALLLVAGLVAISLHMQ**EAG	1.38	13	15.64	106

Example 4

EpiMatrix analyses as above were performed on the entire sequence of the known isoform of Cry J chitinase (NCBI accession no: 75285933). This analysis identified core sequences (with their flanking sequences) derived from said Cry J chitinase isoform which are predicted to have good MHC class-II binding properties. These sequences are shown below in Table 4. Headings and notes for Table 4 are as with Table 1 above.

Table 4

INPUT SEQUENCE	RESIDUES IN SEQUENCE (Incl FLANKS)	SEQUENCE	Hydro-phobicity	EpiMatrix HITS (Excl FLANKS)	Epi Matrix CLUSTER SCORE (Excl FLANKS)	SEQ ID NO:
75285933	1 - 14	**MQIMATQNSKS**NIF	-0.20	8	16.79	107
75285933	11 - 34	SNI**FWSSSASVVLVLLLLVDV**GVC	1.81	12	15.8	108
75285933	106 - 127	YDS**FISAANAFNGFGTSGS**SDV	0.06	9	11.03	109
75285933	129 - 147	KRE**IAAFFANAAHETG**GFC	0.04	10	15.24	110
75285933	177 - 199	RGP**LQLSWNYNYGAAGSYIQ**FDG	-0.57	13	20.47	111
75285933	216 - 233	TAV**WFWMVNSNCHTA**ITS	0.44	10	16.7	112
75285933	252 - 274	GNA**ATVASRVNYYQKFCQQL**NVD	-0.45	9	11.46	113

Example 5

EpiMatrix analyses as above were performed on the entire sequence of the known isoforms of Cry IFR (NCBI accession no: 75159001). This analysis identified a core sequence (with flanking sequences) derived from said Cry IFR isoform which is predicted to have good MHC class-II binding properties. These sequences are shown below in Table 5. Headings and notes for Table 5 are as with Table 1 above.

Table 5

INPUT SEQUENCE	RESIDUES IN SEQUENCE (Incl FLANKS)	SEQUENCE	Hydro-phobicity	EpiMatrix HITS (Excl FLANKS)	Epi Matrix CLUSTER SCORE (Excl FLANKS)	SEQ ID NO:
75159001	122 - 146	VEP**MKSMFDLKIKLRRTIEAEG**IPH	-0.37	19	31.54	114
75159001	155 - 170	AGY**FLTNLAQLGL**AAP	0.86	7	11.93	115
75159001	259 - 281	TFM**VSIFHTIYVKGDQTNFQ**IGP	0.20	8	10.37	116

The Epimatrix analysis further identified the peptides LKIKLRRTI (SEQ ID NO: 134) and IKLRRTIEA (SEQ ID NO: 135) as having binding potential for four or more HLA alleles.

Example 6

EpiMatrix analyses as above were performed on the entire sequence of the known isoform of Cha o 1 (NCBI accession no: 9087163). This analysis identified core sequences (with their flanking sequences) derived from said Cha o 1 isoform which are predicted to have good MHC class-II binding properties. These sequences are shown below in Table 6. Headings and notes for Table 6 are as with Table 1 above.

Table 6

INPUT SEQUENCE	RESIDUES IN SEQUENCE (Incl. FLANKS)	SEQUENCE	Hydro-phobicity	EpiMatrix HITS (Excl FLANKS)	Epi Matrix CLUSTER SCORE (Excl FLANKS)	SEQ ID NO:
9087163	86 - 110	ERS**LWIIFSKNLNIKLNMPLYI**AGN	0.16	22	37.6	117
9087163	102 - 118	NMP**LYIAGNKTIDG**RGA	-0.32	8	12.84	118
9087163	125 - 142	GGP**CLFMRTVSHVIL**HGL	0.91	11	19.33	119
9087163	231 - 251	DKS**MKVTVAFNQFGPNAG**QRM	-0.59	8	10.25	120
9087163	312 - 326	CAN**WVWRSTQDS**FNN	-0.99	6	12.86	121

Based on a further analysis of Epimatrix data and by consideration of homology with Cry j 1, the following additional sequence from Cha o 1 was also identified as having suitable MHC-binding properties: SKNLNIKLNMPLYIAGNK (SEQ ID NO: 136).

Example 7

EpiMatrix analyses as above were performed on the entire sequence of the known isoforms of Cha o 2 (NCBI accession nos: 114841683, 47606004). This analysis identified core sequences (with their flanking sequences) derived from said Cha o 2 isoforms which are predicted to have good MHC class-II binding properties. These sequences are shown below in Table 7. Headings and notes for Table 7 are as with Table 1 above.

Table 7

INPUT SEQUENCE	RESIDUES IN SEQUENCE (Incl FLANKS)	SEQUENCE	Hydro-phobicity	EpiMatrix HITS (Excl FLANKS)	Epi Matrix CLUSTER SCORE (Excl FLANKS)	SEQ ID NO:
114841683	2 - 23	DSD**IEQYLRSNRSLKKLVH**SRH	-1.35	10	14.5	122
114841683	68 - 89	NKK**FFVNNLVFRGPCQPHL**SFK	-0.37	10	11.97	123
114841683	109 - 129	WLQ**FAQLTDFNLMGTGVI**DGQ	0.23	11	16.92	124
114841683	329 - 351	AVQ**IQGVTYKNIHGTSATAA**AIQ	0.21	10	12.26	125
114841683	408 - 419	EFE**LQQQPTTVM**	-0.65	5	10.03	126
47606004	1 - 19	MG**MKFMAAVAFLALQL**IVM	1.94	15	25.98	127
47606004	13 - 28	ALQ**LIVMAAAEDQ**SAQ	0.56	8	15.62	128
47606004	32 - 53	DSD**IEQYLRSNRSLKKLVH**SRH	-1.35	10	14.5	129
47606004	98 - 119	NKK**FFVNNLVFRGPCQPHL**SFK	-0.37	10	11.97	130
47606004	139 - 159	WLQ**FAQLTDFNLMGTGVI**DGQ	0.23	11	16.92	131
47606004	359 - 381	AVQ**IQGVTYKNIHGTSATAA**AIQ	0.21	10	12.26	132
47606004	438 - 452	EFE**LQQQPTTVM**DEN	-1.22	5	10.03	133

Example 8

The sequences set out in Table 8 were selected by the inventors as having desirable characteristics. The selection was made based on the analyses performed in Examples 1 to 7, on homology with sequences identified in Examples 1 to 7 and/or a consideration of solubility and other physicochemical characteristics. Further sequences were selected based on analysis of additional epitope regions in the allergens of Examples 1 to 7. Polypeptides consisting of the sequences of Table 8 were particularly preferred for screening in subsequent assays.

Table 8

Peptide	Sequence	Residues in parent/protein of origin	SEQ ID NO.
CRY01	SCWRGDSNWAQNR	27-39 Cry j1	6
CRY01A	SSWRGDSNWAQNR	Ser variant of CRY01	26
CHA01	SCWRGDANWDQNR	Cha 01	27
CHA01A	SSWRGDANWDQNR	Ser variant of CHA01	28
CRY02	QNRMKLADCAVGFGS	37 to 51 Cry j1	7
CRY02A	PyrNRMKLADCAVGFGS	Pyr variant of CRY02	29
CRY02B	RMKLADCAVGFGS	39 to 51 Cry j1	30
CRY02C	PyrNRMKLADSAVGFGS	Pyr + Ser variant of CRY02	31
CRY02D	RMKLADSAVGFGS	Ser variant of CRY02B	32
CRY03	RPLWIIFSGNMNIKLK	87 to 102 Cry j1	8
CHA03	RSLWIIFSKNLNIKLK	87 to 102 Cha01, Lys variant	33
CRY04	SGNLNIKLKMPLYIAGYK	hybrid based on 94 to 111 Cry j1 and Cha 01	34
CRY05	YIAGYKTFDGRGAQV	106 to 120 Cry j1	10
CRY05A	YIAGYKTFEGRGAQV	Glu variant of CRY05	35
CHA05	YIAGNKTIDGRGAEV	106 to 120 Cha 01	36
CRY06	KPCVFIKRVSNVIIHGK	127 to 141 Cry j1, 2 x Lys variant	37
CRY06A	KPSVFIKRVSNVIIHGK	Ser variant of CRY06	38
CHA06	PCLFMRTVSHVILHG	127 to 141 Cha 01	39
CHA06A	KPSLFMRTVSHVILHGK	2 x Lys + Ser variant of CHA06	40
CRY07	ALTLRTATNIWIDH	173 to 186 Cry j1	12
CHA07	AITMRNVTDVWIDH	173 to 186 Cha 01	41
CRY08	KSMKVTVAFNQFGP	232 to 245 Cry j1	13
CRY09	ESYKKQVTIRIGC	294 to 306 Cry j1	14
CRY09A	PyrSYKKQVTIRIGS	Pyr + Ser variant of CRY09	42
CHA09	DSDKKEVTRRVGC	294 to 306 Cha 01	43
CHA09A	DSDKKEVTRRVGS	Ser variant of CHA09	44
CRY10	CSNWVWQSTQDVF	312 to 324 Cry j1	15
CRY10A	SSNWVWQSTQDVF	Ser variant of CRY10	45
CHA10	CANWVWRSTQDSF	312 to 324 Cha 01	46
CHA10A	SANWVWRSTQDSF	Ser variant of CHA10	47
CRY11	VFYNGAYFVSSGKYEGG	323 to 339 Cry j1	2
CRY11A	VFYQGAYFVSSGKYEGG	Gln variant of CRY11	48

CHA11	SFNNGAYFVSSGKNEGT	Cha 01	49
CRY12	VEKYLRSNRSLRKV	35 to 48 Cry j2	16
CHA12	IEQYLRSNRSLKKL	Cha 02	50
CRY13	AWQAACKKPSA	81 to 91 Cry j2	17
CRY13A	AWQAASKKPSA	Ser variant of CRY13	51
CHA13	TWNAACKKASA	Cha 02	52
CRY14	KKFVVNNLFFNGP	99 to 111 Cry j2	18
CHA14	KKFFVNNLVFRGP	99 to 111 Cha o2	53
CRY15	TFKVDGIIAAYQNPASWK	117 to 134 Cry j2	19
CRY15A	TFKVEGIIAAYQNPASWK	Glu variant of CRY15	54
CHA15	SFKVDGTIVAQPDPARWK	117 to 134 Cha 02	55
CRY16	SWKNNRIWLQFA	132 to 144 Cry j2	20
CHA16	RWKNSKIWLQFA	Cha 02	56
CRY17	RIWLQFAKLTGFTL	137 to 150 Cry j2	3
CHA17	KIWLQFAQLTDFNL	Cha 02	57
CRY18	KLTGFTLMGKGVIEGQK	Glu variant 144 to 160 Cry j2	58
CHA18	QLTDFNLMGTGVIDGQG	144 to 160 Cha 02	59
CHA18A	PyrLTDFNLMGTGVIDGQG	Pyr variant of CHA18	167
CRY19	RPTAIKFDFSTGLI	182 to 195 Cry j2	60
CRY19A	RPTAIKIDYSKSVT	Substitution variant of CRY19 (also referred to herein as CHA19)	5
CRY20	QGLKLMNSPEFH	197 to 208 Cry j2	21
CRY20A	PyrGLKLMNSPEFH	Pyr variant of CRY20	61
CRY20B	PyrGLKL-Nle-QSPEFH	Pyr/Nle variant of CRY20	62
CHA20	KELTLMNSPEFH	197 to 208 Cha 02	63
CRY21	DIFASKNFHLQKNTIGT	237 to 253 Cry j2	22
CHA21	DIFASKRFHIEKCVIGT	237 to 253 Cha 02	64
CRY22	TYKNIRGTSAT	366 to 376 Cry j2	23
CHA22	TYKNIHGTSAT	Cha 02	65
CRY23	KDIKLSDISLKLTSGKIAS	391 to 409 Cry j2	24
CHA23	TGIQLSNVSLKLTSGKPAS	Cha 02	66
CRY24	SWRVNVPGGARGR	56 to 68 Cry j3.4	25
CRY26	DLKIKLRRTIEAEGIP	130 to 144 Cry IFR	1

Example 9

In vitro binding analysis

Polypeptides having sequences identified in Examples 1 to 8 are pre-screened for solubility in an aqueous, acidic milieu and the peptides are tested in an in vitro MHC class-II binding assay.

Methods

The assay employed is a competitive MHC class II binding assay, wherein each polypeptide is analysed for its ability to displace a known control binder from each of the human MHC class II allotypes investigated. The allotypes and control polypeptides used in this study are those shown below in Table 9:

Table 9

Allotype	Control Polypeptide	Sequence
DRB1*0301	Myco. tuberculosis/leprae hsp 65 2-16	AKTIAYDEEARRGLE (SEQ ID NO: 154)
DRB1*1101	Influenza haemagglutinin 307-319	PKYVKQNTLKLAT (SEQ ID NO: 155)
DRB1*1501	Human myelin basic protein 85-99	ENPVVHFFKNIVTPR (SEQ ID NO: 156)

Each polypeptide is analysed in the competition assay and screened for relative binding compared to the control polypeptides. Due to the nature of the competitive assay the data for each polypeptide is determined as a ratio of its own IC50 to that of the control polypeptide. Thus, a polypeptide that has an IC50 value that is parity to the control polypeptide has an identical binding affinity, while peptides with a ratio less than one have a higher affinity and those with a ratio greater than one have a lower affinity.

Solubility in aqueous solution is an essential criterion for a polypeptide to be an effective therapeutic agent. Therefore, as a consequence of the solubility screen very hydrophobic polypeptides with a high frequency of large hydrophobic amino acid residues in multiple binding registers will be eliminated. This is a characteristic of promiscuous HLA-DRB1* binders. Polypeptides which bind to one or more of the MHC Class II allotypes are identified. It would be expected that such polypeptides would have the ability to bind similar allotypes that have not been tested through the homology of MHC structures.

Example 10

The following methods were used to evaluate T cell activation characteristics of polypeptides having sequences identified in Examples 1 to 8.

Cell proliferation assay

A cell proliferation assay was performed on PBMCs (0.5 x10^6 cells per test well). Proliferation was measured by the incorporation of the radiolabelled compound 3H-thymidine. In more detail, 100µl of the positive control (whole Japanese Cedar pollen allergen extract 200 µg/ml or Candida Albicans (20ug/ml) or polypeptide (200 µg/ml) was distributed into the appropriate wells of 96 well plates. Plates were prepared in advance and stored at -20°C prior to the day of assay. PBMCs isolated as described below (see description of cytokine response assay) were prepared to a concentration of 5x10^6 cells/ml in complete medium at room temperature. 100µl of cell solution was then distributed into each of the wells of the 96 well plates containing antigen/polypeptide. The plates were then incubated for 6 days into a humidified 5%CO2 incubator set at 37°C. The cultures were pulsed with tritiated thymidine solution by adding 10µl of tritiated thymidine stock solution (1.85MBq/ml in serum-free RPMI medium) to each well. The plates were then returned to the incubator for between 16 and 20 hours. Cultures were then harvested using a cell harvester. Dried filter mats were counted using an appropriate beta scintillation counter.

For all subjects, the results were expressed as Stimulation Index (SI), calculated by dividing the count from wells containing polypeptide by the average count of wells containing media alone.

A positive result was taken as a SI greater than the SI of the average of wells containing media alone +/- 3* standard deviation (probability >99%). The number of responders out of 50 Japanese Cedar pollen allergic subjects tested was calculated for each polypeptide.

Results for polypeptides tested are shown in Figure 1. Sixteen highly responding peptides are highlighted – these were CHA10A, CRY19A, CRY04, CRY08, CRY10A, CRY11, CRY11A, CRY14, CRY15A, CRY16, CRY17, CRY18, CRY19, CRY21, CRY22 and CRY23. Frequency of positive responses in the study population (and a ranking of all peptides based on proliferation data) are shown in Figure 2, again with the above peptides highlighted.

Cytokine release assay

Polypeptides for use in this assay were manufactured at small scale (approximately 10mg batch size, non-GMP). The purity of each polypeptide was at least 95% by HPLC. 96 well culture plates containing polypeptides and controls (the negative control was culture medium and the positive controls were Candida albicans extract 20 µg/ml and whole Japanese Cedar pollen allergen extract 100 µg/ml) were prepared in advance and stored at -20ºC prior to the day of assay. Polypeptides were added to wells in a volume of 100 µl containing polypeptides at a concentration of 200 µg/ml, such that subsequent addition of 100 µl of cells would create a final assay concentration of 100 µg/ml.

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinised blood by Ficoll density gradient centrifugation. A 100 µl aliquot of a $5x10^6$ cell/ml PBMC suspension was then added to each well and the plates placed in a humidified 5% CO2 incubator at 37ºC for 5 days. Following stimulation, culture supernatants (100 µl) were harvested for testing by multiplex bead assay.

Multiplex cytokine bead assays (IL-10, IL-13, Interferon gamma (IFN-g)) were performed on thawed culture supernatants according to the manufacturer's instructions. Single measurements were performed for each culture supernatant sample. After completion of the multiplex assay, individual cytokine levels were determined by interpolation from the standard curve generated in the assay. A positive result was taken as a cytokine release greater than the mean +/- 3* standard deviations of the background cytokine release (probability >99%). The number of responders out of 50 Japanese Cedar pollen allergic subjects tested was calculated for each polypeptide for each of the cytokines.

The frequency of positive IL-10, IL-13and IFN-g responses in the study population for each peptide (and a ranking of all peptides for each type of cytokine response) is shown in Figure 2. As in the proliferation analysis, peptides CHA10A, CRY19A (CHA19), CRY04, CRY08, CRY10A, CRY11, CRY11A, CRY14, CRY15A, CRY16, CRY17, CRY18, CRY19, CRY21, CRY22 and CRY23 were again highly ranked based on one or more cytokine responses.

Taking into account both the proliferation and cytokine response assays,. peptides CRY19A, CRY15A and CRY11 were in the highest ranked peptides according to all response parameters.

Example 11

The polypeptides identified in Example 10 as eliciting cytokine and proliferative responses in Japanese Cedar allergic subjects were analysed for their pharmaceutical development characteristics. Peptides determined to have particularly suitable cytokine and/or proliferative responses and pharmaceutical development characteristics included CRY04, CRY08, CRY10A, CRY11, CRY14, CRY15A, CRY16, CRY17, CRY18, CRY19, CRY19A, CRY21, CRY22, CRY23 and CRY26.

Solubility was identified as needing to be improved for peptides CRY11, CRY17 and CRY21. Variants CRY11B, CRY11C, CRY11D, CRY11E, CRY11F, CRY17A, CRY17B, CRY21A, CRY21B and CRY21C shown in Table 10 below were designed accordingly. The variants were tested for solubility in multiple buffers with variants CRY11F , CRY17A and CRY21C being the most soluble in multiple buffers (water; 2mM HCl, 10mM glycine (pH 3); 10mM phosphate (pH4); and 10 mM succinate (pH 4)). Truncation of the CRY11 N-terminus (as reflected in peptide CRY11F) was found to significantly improve solubility while maintaining activity (see results below).

Table 10

Peptide	Sequence	Residues in parent/protein of origin	SEQ ID NO.
CRY11B	KVFYNGAYFVSSGKYEGG	Lys variant of CRY11	157
CRY11C	KVFYNGAYFVSSGKYEGGK	2 x Lys variant of CRY11	158
CRY11D	GAYFVSSGKYEGG	323 to 339 Cry j1	163
CRY11E	KGAYFVSSGKYEGG	1 x Lys variant of CRY11D	164
CRY11F	KGAYFVSSGKYEGGK	2 x Lys variant of CRY11D	165
CRY17A	KRIWLQFAKLTGFTLK	2 x Lys variant of CRY17	159
CRY17B	RIWLQFAKLTGFTLK	Lys variant of CRY17	160
CRY21A	KDIFASKNFHLQKNTIGTK	2 x Lys variant of CRY21	161

CRY21B	SKNFHLQKNTIGTGK	Gly + Lys truncated variant of CRY21	162
CRY21C	SKNFHLQKNTIGTG	Gly + truncated variant of CRY21	166

Example 12 – Histamine release assay

Variant polypeptides from Example 11 were tested in a histamine release assay alongside other top performing peptides. The purpose of this assay was to identify peptides capable of activating blood basophils (as a surrogate for tissue mast cells) resulting in histamine release that may result in allergic reactions during therapy. A composition comprising peptides that induce histamine release frequently may be considered unsuitable for use as a vaccine.

Histamine release requires the crosslinking of adjacent specific IgE molecules on the surface of the basophil. The peptides being evaluated were relatively short (11 to 18 amino acids in length) and should not, therefore, possess significant tertiary structure that would enable them to retain the conformation of an IgE-binding epitope of the whole molecule. Furthermore, peptide monomers in solution, even if they are bound by IgE, should not be able to crosslink adjacent IgE molecules.

Histamine release from fresh peripheral whole blood taken from Japanese Cedar pollen allergic subjects was evaluated. Peripheral blood basophils were used as a surrogate for tissue mast cells which were not practical to assay. Blood was incubated *in vitro* with peptides identified as suitable based on the results of Examples 1 to 11 above. Histamine release in response to whole Japanese Cedar pollen allergen extract was measured in each subject to confirm basophil sensitisation. A positive control, representing total histamine release, generated by freeze/thawing the cells twice, was included in each assay. A negative control for spontaneous histamine release was generated by incubating cells in buffer only.

The assay was performed using the Immunotech Histamine Release Immunoassay kit according to the manufacturer's instructions. Following the *in vitro* challenge of blood basophils with peptide mixtures, whole allergen or buffer in microtitre plate wells, supernatants were removed and the histamine in the samples converted to acyl histamine. Acylated samples were tested by a competitive acyl histamine ELISA.

Peptide mixtures were assayed for their ability to induce histamine release over a 5 log10 range (1 to 10,000 ng/ml). The concentration range assayed was selected

based on theoretical *in vivo* doses of peptide that may be achieved during therapy. For example, a 31µg dose (approximately 3 nmol/peptide equivalent) of each peptide entering a blood volume of 5 litres, would result in a blood concentration of 6ng/ml, at the lower end of the histamine release assay dose range. The whole Japanese Cedar pollen allergen extract was used over the concentration range of 10 to 100,000 ng/mL.

Single measurements were performed for each dilution. After completion of the ELISA, individual histamine levels were determined by interpolation from the standard curve generated in the ELISA assay. Results from samples were adjusted to allow for dilution. Where two or more consecutive dilutions of a peptide/allergen preparation elicited >15% of the total histamine release seen in the freeze thawed positive control (>15% of positive control), or where a single value of >15% of positive control was achieved at the highest concentration tested (10 µg/mL for peptides), this was considered a "positive histamine release".

Table 11 below shows peptides having either no signal or a very weak signal in the histamine release assay, and thus considered very good candidates for development.

Table 11

No signal	Very weak signal (single positive, 3%)
CRY19A, CRY10A	CRY08
CRY11, 11D, 11E, 11F	CRY11B
CRY14, CRY15A	CRY17
CRY16	CRY21A
CRY17A, 17B	
CRY18	
CRY21, 21B, 21C	
CRY22, CRY23	

Example 13

Variant polypeptides from Example 11 were tested in proliferation and cytokine release assays as described in Example 10 alongside other top-performing peptides using a frozen batch of the PBMCs isolated from Japanese Cedar pollen allergic subjects previously. The cytokine release assay was optimized for use of PBMCs that had been frozen. Differently from the procedure described in Example 10, the PBMC were frozen after isolation and stored at -80C or liquid nitrogen vapors until the day of the assay. After thawing, the PBMC were cultured in the assay plates in complete media

supplemented with human IL-7 (0.5ng/ml). 5 replicates of each polypeptide in consideration, 8 replicates of negative control (media alone) and 3 replicates of positive control (whole allergen) were performed for each subject. Outliers in the negative control replicates were identified using a Grubb's test and excluded from the analysis. Results were expressed as average of replicate wells. The rest of the assay was performed as described before in Example 10.

The Results are shown in Figure 3. Highly ranked peptides based on one or more response criteria included peptides CRY19A (CHA19), CRY15A, CRY22, CRY23 and peptides from the CRY11, CRY17 and CRY21 series. As in Example 10, peptides from the CRY11, CRY17 and CRY15 series were amongst the top performers. .
The best performing variants from the CRY11, CRY17 and CRY21 series in the response assays were CRY11F, CRY17A and CRY21C, also established previously as having particularly good pharmaceutical development characteristics, and no signal in the histamine release assay. Thus, these variants were selected for inclusion in the most preferred final mixture.

Example 14 – Assessment of population coverage

The ability of various peptide combinations to provide population coverage was assessed based on the cytokine assay results from Example 10 and 11.

The use of peptides from the CRY11, CRY15 and CRY19 series in combination was found to be particularly effective. Combining peptides CRY11F, CRY15A and CRY19A (CHA19) provided for 98% coverage of the trial population of allergic individuals in terms of response to at least one peptide in the combination, as shown in Figure 4A. 64% of the population showed responses to all three of the peptides in the combination. Accordingly, a combination of three polypeptides, selected one each from CRY11D, CRY15 and CRY19A and variants thereof (most preferably CRY11F, CRY19A and CRY15A), is particularly useful for treatment or prevention of Japanese Cedar pollen and/or Japanese Cypress pollen allergy.

The ability of the further peptides to provide additional population coverage by capturing subjects not responding to other individual peptides was also assessed as a basis for further refining the final selection of peptides. Figure 4B provides an analysis of population coverage in terms of numbers of the percentage of subjects having positive responses to each peptide by production of one, two or three of the cytokines

assayed. The data show that peptides CRY22, CRY23, CRY19A, CRY17A, CRY11F, CRY15A and CRY21C are all able to provide additional population coverage over and above responses observed for other individual peptides.

Additional polypeptides from those in the CRY21 series, CRY17 series, CRY22, CRY23, CRY08 (and variants thereof) or from any of the other described T-cell reactive polypeptides may be added to the "core" combination of three polypeptides described above, to capture additional responses observed in the study population, and to further improve population coverage and redundancy.

Peptide CRY08 was found to be a particularly useful additional polypeptide alongside the "core" combination of three polypeptides described above. Peptide CRY08 produced a positive cytokine response in 10 subjects that did not respond to the other cry j 1-derived epitope and thus provides valuable redundancy by capturing subjects that do not respond to a peptide of the core combination. Additionally, adding CRY08 to the core combination increased the cumulative number of positive responses elicited by each peptide in a subject sample). Accordingly, the polypeptide CRY08 (or a variant thereof) is a preferred additional polypeptide for inclusion in polypeptide combinations described herein.

Peptide CRY17A was further selected as a useful additional polypeptide to be included alongside the core combination of three polypeptides discussed above. Peptide CRY17A had good cytokine and proliferation response data, and additionally was shown to have useful MHC binding characteristics, as discussed below. Accordingly, the polypeptide CRY17 (or a variant thereof, most preferably CRY17A) is a preferred additional polypeptide for inclusion in polypeptide combinations described herein.

Accordingly, a particularly preferred combination of polypeptides is provided by five polypeptides selected one each from CRY19A, CRY11D, CRY17, CRY15, CRY08 and variants thereof, most preferably CRY19A, CRY11F, CRY17A, CRY15A and CRY08.

Polypeptides from the CRY21 series, and CRY22 and CRY23 (and variants thereof) were further selected as additional components, as a basis for combinations of seven or eight polypeptides able to provide very high or total population coverage. Polypeptides in the CRY21 series were found to have good proliferative and cytokine responses and also to capture subjects that do not respond to other preferred peptides. The most-preferred peptide in the CRY21 series was CRY21C, which also had useful

MHC binding characteristics, as discussed below. The polypeptide CRY22 was found to have good biological activity according to both response parameters and also was a polypeptide capturing one subject (2% of the population) that did not respond to other preferred peptides. The polypeptide CRY23 was also found to have very good biological activity. Thus, polypeptides selected from CRY21, CRY22 and CRY23 (and variants thereof), particularly CRY21C, CRY22 and CRY23, were particularly preferred additional polypeptides for inclusion in polypeptide combinations described herein.

Four different polypeptide combinations designed on this basis are shown below in Table 12.

Table 12

Mixture 1	**Mixture 2**	**Mixture 3**	**Mixture 4**
CRY11F	CRY11F	CRY11F	CRY11F
CRY08	CRY08	CRY08	CRY08
CRY19A (CHA19)	CRY19A (CHA19)	CRY19A (CHA19)	CRY19A (CHA19)
CRY15A	CRY15A	CRY15A	CRY15A
CRY17A	CRY17A	CRY17A	CRY17A
CRY21C	CRY21C	CRY22	CRY21C
CRY22	CRY23	CRY23	CRY22
			CRY23

The population coverage for these four mixtures is shown below in Table 13, alongside the preferred five peptide mixture and the core three peptide combination. A detailed analysis of responses to each peptide in the combinations is also shown in Figures 5A and 5B, illustrating how the peptides each capture responses across the population. The data presented is based on the cytokine assay performed in Example 10 and thus corresponding peptides tested in Example 10 (CRY17, CRY11A, CRY21) were used as surrogates for the variants (CRY11F, CRY17A, CRY21C) later generated (as the core epitope(s) were not altered, these variants were expected to have affinity for the same T cell receptor type)s). The response multiplicity score shown is the

cumulative number of positive responses to each peptide in the combination for at least one cytokine. The cumulative cytokine response score is the cumulative number of positive responses to each peptide in the combination for all three cytokines. The mixture of the preferred five polypeptides had a high response multiplicity score and cumulative cytokine response score and provided coverage for effectively the entire population. The addition of two or three polypeptides to the preferred five polypeptides advantageously improved the response multiplicity score and cumulative cytokine response score, as shown for each of Mixtures 1-4. Additionally, population coverage was further improved. The most preferred peptide mixture (Mixture 4) was selected based on having the highest response multiplicity score and cumulative cytokine response score, and since it had a total population coverage, and only one subject responded to a single peptide in the combination, all others responding to two or more of the polypeptides.

Table 13

Peptide Combination	**Response multiplicity score**	**Frequency of subjects not responding to any peptide in the combination**	**Frequency of subjects only responding to one peptide in the combination.**	**Cumulative cytokine response score**
Core 3 peptide mixture (CRY19A, CRY11F, CRY15A)	118	1	7	192
Preferred 5 peptide mixture (CRY19A, CRY11F,	191	1	3	306

CRY17A, CRY15A and CRY08)				
Mixture 1 (7 peptides)	258	0	3	422
Mixture 2 (7 peptides)	259	1	0	415
Mixture 3 (7 peptides)	274	0	2	455
Mixture 4 (8 peptides)	300	0	1	493

Example 15 – Analysis of HLA Binding in the Japanese Population

Preferred polypeptides described above were tested in an *in vitro* MHC class-II binding assay. The assay employed was a MHC class II binding assay (REVEAL®, ProImmune), wherein each polypeptide was analysed for binding to MHC molecules compared to binding of a positive control peptide. The allotypes and control polypeptides used in this study are shown below. The panel of allotypes was selected to include 24 alleles common to supertypes presented in the Japanese population, and thus to provide further specific information on impact in the Japanese population. The individual and cumulative genotype frequencies for these HLA alleles in the Japanese population are shown in Figure 9.

Each polypeptide was analysed in the assay for binding to each of the alleles and screened for relative binding compared to the positive control polypeptide to provide a ProImmune REVEAL® binding score as a percentage of the signal generated by the positive control polypeptide. A higher REVEAL® score is thus indicative of strong binding to the MHC molecule. This binding score was calculated at 0 and 24h. The signals at these time points were used to derive an approximation to a half-life based on a one-phase dissociation equation. A stability index was then calculated by multiplying the approximated half-life by the ProImmune REVEAL® score from the binding assay. A higher stability index indicates a more stable MHC-peptide complex

The binding assay data for all peptides against each allele was then analysed in combination with the frequency of the alleles in question in the Japanese population to

provide a determination of the potential impact of each peptide in the population. The binding data was renormalized by resetting the positive binding level for each allele to the level of binding representing the 98th percentile level of all peptides where some peptides exceed the binding level of the original positive control for the allele. The normalized binding data was then multiplied by the frequency of each allele in the Japanese population to obtain the percent potential impact for each peptide/allele combination. Finally, for each peptide being studied, the percent impact for every allele was added together and plotted (with the contribution provided by each allele shown in the various sections of each bar) to provide the total percent impact in the population.

An identical analysis was also performed for the stability assay data to provide population impact analysis based on the stability index.

Results for binding at 0 and 24 hours are shown graphically in Figures 6A and B with the binding scores and stability indices for the various alleles shown in Figures 7 and 8. Seven out of the eight peptides of the most preferred polypeptide combination (Mixture 4, all except CRY08, which was not tested) were each demonstrated to have binding to Japanese MHC supertype alleles. The pattern of binding was generally pan-allele/supertype, and thus the peptides were promiscuous binders. CRY17A was identified as having particularly useful MHC II binding characteristics since it showed the greatest pan-allele binding of all of the peptides tested. Peptide CRY21C had the second greatest pan-allele binding and so also had particularly useful HLA binding characteristics. Thus peptides CRY17A and CRY21C have particular utility in a peptide combination for treating or preventing allergy to Japanese Cedar pollen in the Japanese population.

It would be expected that the polypeptides shown to have binding would have the ability to bind similar allotypes that have not been tested through the homology of MHC structures. The lower cumulative binding for certain peptides may be explained by the lack of availability of certain alleles to which the peptides could bind in the panel of alleles tested.

Example 16 - Preparation of peptides, salts thereof and pharmaceutical formulations

Peptides are prepared as follows. Synthesis is performed in a solid phase peptide synthesis (SPPS) reactor and started by suspending the substituted resin in N,N-

dimethylformamide (DMF). After washing of the resin with DMF, each coupling procedure is performed by addition of the N-α-protected amino acid derivative or the N-α-protected dipeptide to the preceding amino acid in the presence of N-[(1H-Benzotriazol-1-yl)(dimethylamino)methylene]-N-methylmethanaminium tetrafluoroborate N-oxide (TBTU) and N,N-diisopropylethylamine (DIPEA) in DMF or diisopropylcarbodiimide (DIC) and 1-hydroxybenzotriazole (HOBt) in a mixture of methylene chloride (DCM) and DMF. For each single step, the solvents and/or reagents are added and the reaction mixture is stirred and subsequently filtered to remove solvents and/or reagents from the resin.

After each successful coupling or capping procedure, an Fmoc-deprotection procedure is performed. It consists of washing of the resin with DMF, cleaving the Fmoc-group with 20% (V/V) piperidine in either DMF or 1-Methyl-2-pyrrolidone (NMP), and subsequent washings with DMF and isopropanol (IPA). For each single step, the solvents and/or reagents are added, and the reaction mixture is stirred and then filtered to remove the solvents and/or reagents from the resin.

Fmoc-deprotection and coupling procedures are repeated until the resin carries the complete peptide sequence of the required peptide. The SPPS is completed by a final Fmoc-deprotection and drying of the peptide resin under reduced pressure.

Acetate or hydrochloride salts of the specified peptides are prepared by the following methods. The peptide resin is treated with cold trifluoroacetic acid (TFA) at room temperature for 1.5 to 3 hours in the presence of 1,2-ethanedithiol (EDT), triisopropylsilane (TIS), and water. After filtering off and washing the resin with TFA, the product is precipitated in cold diisopropyl ether (IPE). It is then filtered off, washed with IPE, and dried under reduced pressure. The product is then reconstituted and purified by high-performance liquid chromatography (HPLC).

For preparation of acetate salts, the trifluoroacetate salt is reconstituted in 5% (V/V) aqueous acetic acid and loaded onto an ion exchange resin. The elution is performed with 5% (V/V) aqueous acetic acid. The acetate is filtered through a 0.2 μm membrane filter and lyophilized to yield the final product as a white to off-white powder.

For preparation of hydrochloride salts, the trifluoroacetate salt is reconstituted in 0.01 M HCl in purified water and filtered where necessary. The solution is loaded onto a preparative HPLC column for ion exchange into the hydrochloride salt. The ion

exchange is performed by washing the column with a 0.1 M ammonium chloride solution followed by 0.01 M HCl. Subsequently, the hydrochloride is filtered through a 0.2 µm membrane filter and lyophilized to yield the final product as a white to off-white powders.

Exemplary pharmaceutical formulations of the present invention contain components as set out in Table 14. The peptide salt is an acetate or hydrochloride or trifluoroacetate salt. The peptide CRY19A is preferably present as a hydrochloride salt.

Table 14

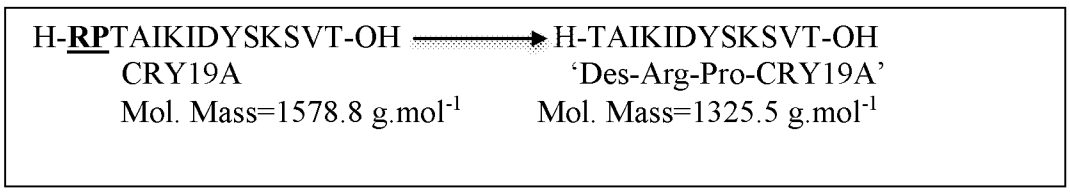
Raw material	Function	Nominal concentration for each component
CRY19A, CRY15A, CRY11F, CRY17A, CRY08, CRY21C, CRY22, CRY23	Active ingredient	40 to 220µM
L-Methionine	Antioxidant	1 to 15 mM (typically 5mM)
Phosphoric acid , Hydrochloric acid or aqueous ammonia	pH adjustment	As required
Trehalose dihydrate	Tonicity agent	260 mM
Thioglycerol (optional)	Antioxidant	2 to 50 mM (typically 15 mM)

The formulation is prepared in solution prior to being subjected to freeze-drying to produce a lyophilisate.

Example 17 – Identification of improved salt forms of peptide CRY19A

During manufacture of CRY19A as an acetate salt, formation of a degradation product was identified. In more detail, an impurity was detected by reversed phase ultra-performance liquid chromatography (UPLC), eluting at a relative retention time (RRT) of 1.034 compared to the native peptide, at a level of 1.32% area. From initial assessment of the manufacturing, it was also identified that the level of this impurity was 0.6 to 0.8 % area in sublots prior to final lyophilisation (prior to sublots of individual peptide being redissolved, combined, filtered and isolated as a single batch by lyophilisation), suggesting that the impurity had increased during the manufacturing process.

An initial investigation using liquid chromatography-mass spectrometry (LC-MS) identified that the impurity was a deletion product of CRY19A, des-Arg-Pro-CRY19A, shown below.



In order to further investigate instability of the CRY19A peptide, a study was completed during which CRY19A was kept in different solutions (acid type, solvent and concentration) and as different salt forms up to 48 hours at room temperature prior to lyophilisation. Each sample was then analysed by UPLC to monitor the level of the des-Arg-Pro-CRY19A impurity. Results are shown in Table 15 below.

Table 15: Level of des-Arg-Pro-CRY19A in CRY19A during exposure to conditions mimicking the different phases of peptide manufacturing

Test	Test Solution	Conc. (mg/mL)	% des-Arg-Pro-CRY19A impurity (Room Temperature) *vs* time (hours) / processing step					
			0 h	3 h	6 h	24 h	48 h	After lyophilisation
CRY19A trifluoroacetate (TFA) Salt								
1	TFA salt in solution in 0.1%TFA/ 18% acetonitrile (ACN)	8	1.48	1.47	1.46	1.46	1.45	1.42
2	TFA salt in solution in 0.1M NH4OAc	8	1.47	1.45	1.46	1.48	1.54	1.60
3	TFA Salt in solution in water	8	1.43	1.44	1.43	1.44	1.43	1.55
CRY19A Acetate Salt								
4	AcOH salt in solution in 0.2M HOAc/14%ACN	8	1.63	1.63	1.63	1.63	1.65	2.17
5	AcOH salt in solution in 2% AcOH	20	1.61	1.62	1.62	1.62	1.64	2.46
6	AcOH Salt in solution in water	20	1.61	1.60	1.61	1.61	1.63	1.96
CRY19A HCl Salt								
7	HCl Salt in solution in water	20	1.46	1.46	1.46	1.46	1.46	1.52
8	HCl Salt in solution in 0.1% HCl	20	1.47	1.47	1.47	1.45	1.43	1.53

On the basis of the results, it was concluded that degradation and formation of the des-Arg-Pro-CRY19A occurred mainly during the lyophilisation process for the CRY19A AcOH salt, but was much less pronounced during lyophilisation of the trifluoroacetate (TFA) and HCl salts. A lyophilisation process with isolation of

CRY19A as TFA or HCl salt was accompanied by a much lower level of degradation than for CRY19A as the AcOH salt.

Instability of CRY19A AcOH was also observed during storage of the lyophilisate over an 8 month period at -20°C with the level of the des-Arg-Pro-CRY19A observed to increase by approximately 0.2 % area. An accelerated stability study was conducted on the CRY19A AcOH salt to better quantify the instability of the peptide. In order to identify a possible remediation, this study was conducted on two other CRY19A salts, the HCl and TFA salts. Each peptide salt was held at 4 different temperature conditions for a period of 2 weeks and the sample analysed for des-Arg-Pro-CRY19A at 3 time points. The results are presented in Table_15 below (RH – relative humidity).

Table 16: Level of des-Arg-Pro-CRY19A fragment over time during storage of CRY19A at different temperatures and as different salts

Conditions	Time Point (Day)	%area (UPLC)					
		Acetate salt		TFA salt		HCl salt	
		Purity	Des-Arg-Pro-CRY19	Purity	Des-Arg-Pro-CRY19	Purity	Des-Arg-Pro-CRY19
5°C (Ambient RH)	**0**	97.50	1.62	97.69	1.47	97.68	1.47
	14	97.16	1.69	97.46	1.51	97.12	1.50
25°C (60%RH)	7	96.65	2.22	97.53	1.54	97.64	1.53
	14	95.61	2.90	97.42	1.68	97.41	1.58
40°C (Ambient RH)	7	90.31	6.89	96.79	2.26	97.14	1.93
	14	86.23	10.05	95.28	2.83	95.88	2.26
55°C (Ambient RH)	7	76.14	16.43	93.59	4.38	95.14	3.14
	14	66.09	22.15	90.43	6.00	92.79	4.07

The clear conclusion from the study was that the least stable of the 3 salts is CRY19A, AcOH and the most stable is CRY19A, HCl. Extrapolation using the Arrhenius equation calculated that during 5°C storage, the level of formation of the des-Arg-Pro-CRY19A would be in the order of 1 to 2 % in the case of the HCl and TFA salts, instead of approximately 15 % in the case of the CRY19A, AcOH over a 48 months period. Similarly the total decrease in purity would be in the order of 3 to 4 % for CRY19A TFA or HCl salt, but about 20 % for the CRY19A, AcOH salt over the same time period.

Both the TFA and HCl salts of CRY19A had significantly improved properties over the acetate salt. Based on the lyophilisation and accelerated stability studies, and

further pharmaceutical considerations, the HCl salt of CRY19A was determined to be particularly preferred for use in pharmaceutical formulations.

5

CLAIMS

1. A pharmaceutical formulation comprising:

(a) a polypeptide comprising the amino acid sequence of RPTAIKIDYSKSVT (SEQ ID NO: 5; CRY19A); or a T cell epitope-containing variant sequence derived from said amino acid sequence,

(b) a polypeptide comprising the amino acid sequence of GAYFVSSGKYEGG (SEQ ID NO: 163, CRY11D) or a T cell epitope-containing variant sequence derived from said amino acid sequence, and

(c) a polypeptide comprising the amino acid sequence of TFKVDGIIAAYQNPASWK (SEQ ID NO: 19; CRY15) or a T cell epitope-containing variant sequence derived from said amino acid sequence,

wherein a T cell epitope-containing variant sequence of a said amino acid sequence is said amino acid sequence having up to five amino acid modifications, each of which is independently a deletion, substitution or insertion;

wherein each polypeptide is up to 30 amino acids in length;

wherein each polypeptide may be present as a pharmaceutically acceptable salt thereof;

wherein said pharmaceutical formulation further comprises a pharmaceutically acceptable carrier or diluent.

2. A pharmaceutical formulation according to claim 1, comprising a said polypeptide of (a), (b) or (c) which consists respectively of the amino acid sequence or variant sequence recited in (a), (b) or (c).

3. A pharmaceutical formulation according to claim 1 or 2, wherein the variant sequence of a said amino acid sequence is said amino acid sequence having one or two amino acid modifications and the modification or each modification independently is a deletion or substitution, optionally wherein the or each substitution is a conservative substitution.

4. A pharmaceutical formulation according to claim 3, wherein the or each substitution may be:

- the substitution of lysine or arginine with each other;
- the substitution of aspartate for glutamate, or with their amide derivatives, asparagines and glutamine; and/or
- the substitution of glycine, alanine, valine, leucine and isoleucine for each other.

5 A pharmaceutical formulation according to any one of the preceding claims, wherein:

(I) the variant sequence of a said amino acid sequence is said amino acid sequence having up to two amino acids deleted from the N-terminus and/or up to two amino acids deleted from the C-terminus; and/or

(II) each polypeptide is up to 20 amino acids in length; and/or

(III) at least one polypeptide has an amino acid sequence consisting of a said amino acid sequence or variant sequence having a N-terminal and/or C-terminal extension of from one to six amino acids corresponding respectively to the one to six amino acids immediately N-terminal or C-terminal to the said amino acid sequence in the native sequence of the protein from which said amino acid sequence is derived; and/or

(IV) at least one polypeptide has an amino acid sequence consisting of a said amino acid sequence or variant sequence having an N-terminal and/or a C-terminal extension of one or two amino acids selected from the group consisting of arginine, lysine and histidine.

6. A pharmaceutical formulation according to any one of the preceding claims, which comprises:

(a) the polypeptide having the amino acid sequence RPTAIKIDYSKSVT (SEQ ID NO: 5; CRY19A or a said salt thereof, and/or

(b) the polypeptide having the amino acid sequence KGAYFVSSGKYEGGK (SEQ ID NO: 165; CRY11F) or a said salt thereof, and/or

(c) the polypeptide having the amino acid sequence TFKVEGIIAAYQNPASWK (SEQ ID NO: 54; CRY15A) or a said salt thereof.

7. A pharmaceutical formulation according to any one of the preceding claims, which further comprises :

(d) a polypeptide comprising the amino acid sequence of RIWLQFAKLTGFTL (SEQ ID NO: 3; CRY17) or a T cell epitope-containing variant sequence derived from said amino acid sequence,

wherein a T cell epitope-containing variant sequence of a said amino acid sequence is said amino acid sequence having up to five amino acid modifications, each of which is independently a deletion, substitution or insertion; wherein the polypeptide is up to 30 amino acids in length; and

wherein the polypeptide may be present as a pharmaceutically acceptable salt thereof.

8. A pharmaceutical formulation according to claim 7, which comprises: (d) a polypeptide having the amino acid sequence KRIWLQFAKLTGFTLK (SEQ ID NO: 159; CRY17A) or a said salt thereof.

9. A pharmaceutical formulation according to any of the preceding claims, which further comprises :

(e) a polypeptide comprising the amino acid sequence of KSMKVTVAFNQFGP (SEQ ID NO:13, CRY08) or a T cell epitope-containing variant sequence derived from said amino acid sequence,

wherein a T cell epitope-containing variant sequence of a said amino acid sequence is said amino acid sequence having up to five amino acid modifications, each of which is independently a deletion, substitution or insertion;

wherein the polypeptide is up to 30 amino acids in length; and

wherein the polypeptide may be present as a pharmaceutically acceptable salt thereof.

10. A pharmaceutical formulation according to any of the preceding claims, which comprises a polypeptide (a) or a said salt thereof, a polypeptide (b) or a said salt thereof, a polypeptide (c) or a said salt thereof, a polypeptide (d) or a said salt thereof, and a polypeptide (e) or a said salt thereof.

11. A pharmaceutical formulation according to any of the preceding claims, which comprises:

(a) the polypeptide having the amino acid sequence RPTAIKIDYSKSVT (SEQ ID NO: 5; CRY19A or a said salt thereof,

(b) the polypeptide having the amino acid sequence KGAYFVSSGKYEGGK (SEQ ID NO: 165; CRY11F) or a said salt thereof,

(c) the polypeptide having the amino acid sequence TFKVEGIIAAYQNPASWK (SEQ ID NO: 54; CRY15A) or a said salt thereof,

(d) a polypeptide having the amino acid sequence KRIWLQFAKLTGFTLK (SEQ ID NO: 159; CRY17A) or a said salt thereof, and

(e) a polypeptide comprising the amino acid sequence of KSMKVTVAFNQFGP (SEQ ID NO:13, CRY08) or a said salt thereof;

12. A pharmaceutical formulation according to any one of the preceding claims, which further comprises at least one polypeptide selected from:

(f) a polypeptide comprising the amino acid sequence of DIFASKNFHLQKNTIGT (SEQ ID NO: 22; CRY21) or a T cell epitope-containing variant sequence derived from said amino acid sequence, and

(g) a polypeptide comprising the amino acid sequence of TYKNIRGTSAT (SEQ ID NO: 23; CRY22) or a T cell epitope-containing variant sequence derived from said amino acid sequence,

wherein a T cell epitope-containing variant sequence of a said amino acid sequence is said amino acid sequence having up to five amino acid modifications, each of which is independently a deletion, substitution or insertion;

wherein each selected polypeptide is up to 30 amino acids in length; and wherein each selected polypeptide may be present as a pharmaceutically acceptable salt thereof.

13. A pharmaceutical formulation according to any one of the preceding claims, which further comprises:

(h) a polypeptide comprising the amino acid sequence of KDIKLSDISLKLTSGKIAS (CRY23; SEQ ID NO: 24), or a T cell epitope-containing variant sequence derived from said amino acid sequence,

wherein a T cell epitope-containing variant sequence of a said amino acid sequence is said amino acid sequence having up to five amino acid modifications, each of which is independently a deletion, substitution or insertion;

wherein the polypeptide is up to 30 amino acids in length; and

wherein the polypeptide may be present as a pharmaceutically acceptable salt thereof.

14. A pharmaceutical formulation according to claim 12 or 13, which comprises: (f) a polypeptide having the amino acid sequence SKNFHLQKNTIGTG (SEQ ID NO: 166; CRY21C).

15. A pharmaceutical formulation according to claim 13 or 14, which comprises a polypeptide (a) or a said salt thereof, a polypeptide (b) or a said salt thereof, a polypeptide (c) or a said salt thereof, a polypeptide (d) or a said salt thereof, a polypeptide (e) or a said salt thereof, a polypeptide (g) or said salt thereof, and a polypeptide (h) or a said salt thereof.

16. A pharmaceutical formulation according to claim 15, which comprises:

(a) the polypeptide having the amino acid sequence RPTAIKIDYSKSVT (SEQ ID NO: 5; CRY19A or a said salt thereof;

(b) the polypeptide having the amino acid sequence KGAYFVSSGKYEGGK (SEQ ID NO: 165; CRY11F) or a said salt thereof;

(c) the polypeptide having the amino acid sequence TFKVEGIIAAYQNPASWK (SEQ ID NO: 54; CRY15A) or a said salt thereof;

: (d) a polypeptide having the amino acid sequence KRIWLQFAKLTGFTLK (SEQ ID NO: 159; CRY17A) or a said salt thereof.

(e) a polypeptide comprising the amino acid sequence of KSMKVTVAFNQFGP (SEQ ID NO:13, CRY08) or a said salt thereof;

(f) a polypeptide having the amino acid sequence SKNFHLQKNTIGTG (SEQ ID NO: 166; CRY21C) or a said salt thereof;

(g) a polypeptide comprising the amino acid sequence of TYKNIRGTSAT (SEQ ID NO: 23; CRY22) or a said salt thereof; and

(h) a polypeptide comprising the amino acid sequence of KDIKLSDISLKLTSGKIAS (CRY23; SEQ ID NO: 24) or a said salt thereof.

17. A pharmaceutical formulation according to any one of the preceding claims, wherein the polypeptide (a) is present as a hydrochloride salt thereof.

18. A pharmaceutical formulation according to any one of the preceding claims, which is a pharmaceutically acceptable solution or a lyophilisate.

19. A pharmaceutical formulation according to any one of the preceding claims, which is formulated for intradermal administration, subcutaneous administration, oral administration, nasal administration, topical administration, sublingual administration, buccal administration or epidermal administration.

20. A polypeptide consisting of the amino acid sequence of RPTAIKIDYSKSVT (SEQ ID NO: 5; CRY19A); KGAYFVSSGKYEGGK (SEQ ID NO: 165; CRY11F); TFKVEGIIAAYQNPASWK (SEQ ID NO: 54; CRY15A); KRIWLQFAKLTGFTLK (SEQ ID NO: 159; CRY17A); KSMKVTVAFNQFGP (SEQ ID NO:13, CRY08); SKNFHLQKNTIGTG (SEQ ID NO: 166; CRY21C); TYKNIRGTSAT (SEQ ID NO: 23; CRY22); KDIKLSDISLKLTSGKIAS or (SEQ ID NO: 24; CRY23), or a pharmaceutically acceptable salt of any thereof.

21. The polypeptide of claim 20, which is a hydrochloride salt of the polypeptide consisting of the amino acid sequence of RPTAIKIDYSKSVT (SEQ ID NO: 5; CRY19A).

22. A pharmaceutical formulation according to any one of claims 1 to 19 or a polypeptide or salt thereof according to claim 20 or 21, for use in a method of treating or preventing allergy to Japanese Cedar pollen and/or Japanese Cypress pollen.

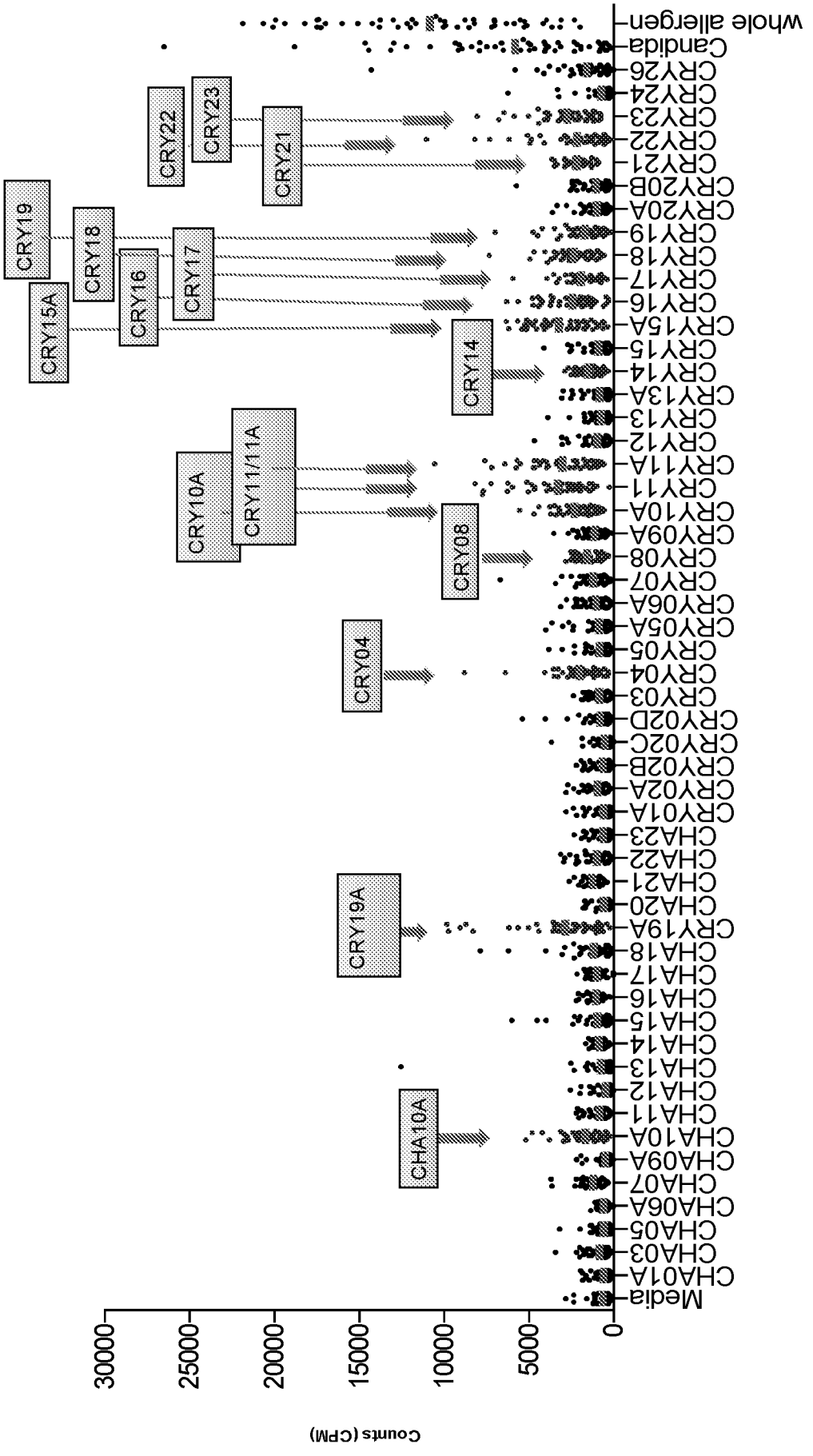
23. An *in vitro* method of determining whether T cells recognize a polypeptide or salt thereof of a pharmaceutical formulation according to any one of claims 1 to 19, or a polypeptide or salt thereof according to claim 20 or 21, which method comprises

contacting said T cells with said polypeptide or salt thereof or said pharmaceutical formulation and detecting whether said T cells are stimulated by said polypeptide or salt thereof.

24. A method according to claim 23 which is carried out to determine whether an individual has, or is at risk of having, an allergy to Japanese Cedar pollen and/or Japanese Cypress pollen.

25. Use of a polypeptide or salt thereof as defined in claim 20 or 21 for the manufacture of a medicament for the prevention or treatment of allergy to Japanese Cedar pollen and/or Japanese Cypress pollen.

26. A method of treating an individual for allergy to Japanese Cedar pollen and/or Japanese Cypress pollen or for preventing in an individual allergy to Japanese Cedar pollen and/or Japanese Cypress pollen, which method comprises administering to said individual a therapeutically or prophylactically effective amount of a pharmaceutical formulation as defined in any one of claims 1 to 19, or of a polypeptide or salt thereof as defined in claim 20 or 21.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CHA23
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CRY12
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CRY14
CRY15
CRY15A
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CRY17
CRY18
CRY19
CRY20A
CRY20B
CRY21
CRY22
CRY23
CRY24
CRY26
Candida
whole allergen
CHA10A
CRY19A
CRY04
CRY08
CRY10A
CRY11/11A
CRY14
CRY15A
CRY16
CRY17
CRY18
CRY19
CRY21
CRY22
CRY23

Figure 1

Figure 2

PROLIFERATION		
Position	Sample	% responders
1	CRY11	78.0
2	CRY18	78.0
3	CRY21	78.0
4	CRY15A	72.0
5	CRY11A	70.0
6	CRY17	70.0
7	CRY23	70.0
8	CHA19	68.0
9	CRY10A	64.0
10	CRY16	64.0
11	CRY04	58.0
12	CRY08	58.0
13	CRY14	56.0
14	CRY19	50.0
15	CHA10A	48.0
16	CHA21	44.0
17	CHA16	42.0
18	CHA17	42.0
19	CRY22	42.0
20	CHA07	40.0
21	CRY06A	38.0
22	CRY03	36.0
23	CRY09A	36.0
24	CHA14	32.0
25	CHA03	28.0
26	CHA22	28.0
27	CRY26	28.0
28	CRY07	26.0
29	CHA15	22.0
30	CRY20A	22.0
31	CRY12	20.0
32	CHA06A	18.0
33	CHA11	18.0
34	CHA18	18.0
35	CRY02A	18.0
36	CRY20B	18.0
37	CRY02B	16.0
38	CRY05	16.0
39	CHA23	14.0
40	CRY05A	14.0
41	CRY15	14.0
42	CRY02D	10.0
43	CRY13	10.0
44	CRY24	10.0
45	CHA13	8.0
46	CHA05	6.0
47	CHA01A	6.0
48	CRY01A	6.0
49	CRY02C	6.0
50	CRY13A	6.0
51	CHA20	4.0
52	CHA09A	2.0
53	CHA12	2.0
Cells w/Candida		70.0
Whole Allergen		96.0

IL10		
Position	Sample	% responders
1	CHA19	100.0
2	CRY11	100.0
3	CRY23	100.0
4	CRY16	100.0
5	CRY15A	100.0
6	CRY04	97.9
7	CRY11A	95.8
8	CHA10A	91.7
9	CRY10A	89.6
10	CRY09A	81.3
11	CRY22	81.3
12	CRY19	81.3
13	CHA23	77.1
14	CHA07	75.0
15	CRY02A	75.0
16	CHA03	70.8
17	CRY18	70.8
18	CRY17	68.8
19	CHA22	68.8
20	CHA14	66.7
21	CRY08	64.6
22	CRY03	62.5
23	CHA16	60.4
24	CHA15	60.4
25	CRY07	56.3
26	CRY02D	52.1
27	CHA17	47.9
28	CRY06A	45.8
29	CHA06A	45.8
30	CRY20A	43.8
31	CRY20B	41.7
32	CHA11	41.7
33	CRY05	39.6
34	CRY21	39.6
35	CHA05	37.5
36	CHA01A	33.3
37	CRY02B	31.3
38	CRY12	29.2
39	CRY26	25.0
40	CHA20	22.9
41	CHA18	22.9
42	CRY05A	20.8
43	CHA21	20.8
44	CRY13A	18.8
45	CHA13	16.7
46	CRY02C	16.7
47	CRY15	16.7
48	CRY14	16.7
49	CRY24	12.5
50	CRY13	10.4
51	CHA09A	8.3
52	CRY01A	4.2
53	CHA12	2.1
Cells w/Candida		93.8
Whole Allergen		100.0

IFNg		
Position	Sample	% responders
1	CHA19	81.3
2	CRY11	75.0
3	CRY23	66.7
4	CHA10A	64.6
5	CRY11A	60.4
6	CRY10A	54.2
7	CRY04	52.1
8	CRY15A	50.0
9	CRY16	37.5
10	CHA15	37.5
11	CRY09A	33.3
12	CHA18	31.3
13	CHA07	29.2
14	CHA22	29.2
15	CRY22	27.1
16	CRY15	27.1
17	CRY02B	27.1
18	CRY19	25.0
19	CRY05A	25.0
20	CHA17	22.9
21	CRY26	22.9
22	CRY05	20.8
23	CRY07	20.8
24	CHA03	18.8
25	CRY18	18.8
26	CHA01A	18.8
27	CHA11	18.8
28	CRY13	18.8
29	CRY17	18.8
30	CHA13	18.8
31	CRY20A	16.7
32	CRY03	16.7
33	CRY08	16.7
34	CRY02A	16.7
35	CRY20B	16.7
36	CRY06A	14.6
37	CHA06A	14.6
38	CRY21	14.6
39	CRY02D	14.6
40	CHA23	14.6
41	CHA05	12.5
42	CRY02C	12.5
43	CHA20	10.4
44	CRY12	8.3
45	CRY01A	8.3
46	CRY14	8.3
47	CHA09A	6.3
48	CRY13A	6.3
49	CHA12	6.3
50	CHA21	6.3
51	CHA16	4.2
52	CHA14	4.2
53	CRY24	2.1
Cells w/Candida		100.0
Whole Allergen		100.0

IL13		
Position	Sample	% responders
1	CRY11	81.3
2	CRY23	81.3
3	CHA19	77.1
4	CRY15A	77.1
5	CRY04	77.1
6	CRY16	66.7
7	CHA10A	66.7
8	CRY10A	66.7
9	CHA03	60.4
10	CRY11A	58.3
11	CRY22	54.2
12	CRY06A	50.0
13	CRY19	47.9
14	CRY09A	45.8
15	CHA07	43.8
16	CRY26	41.7
17	CRY20A	41.7
18	CRY03	39.6
19	CHA22	39.6
20	CRY08	39.6
21	CRY07	39.6
22	CRY18	35.4
23	CHA18	35.4
24	CRY02B	31.3
25	CHA06A	31.3
26	CRY15	31.3
27	CRY20B	31.3
28	CRY24	31.3
29	CRY17	29.2
30	CHA15	29.2
31	CHA16	27.1
32	CRY02D	25.0
33	CRY02A	25.0
34	CRY21	25.0
35	CRY05	25.0
36	CHA23	22.9
37	CHA01A	22.9
38	CHA20	20.8
39	CHA11	20.8
40	CRY05A	20.8
41	CRY12	18.8
42	CHA14	16.7
43	CHA05	16.7
44	CRY13A	16.7
45	CHA21	16.7
46	CRY14	16.7
47	CHA13	14.6
48	CRY02C	12.5
49	CRY01A	10.4
50	CRY13	8.3
51	CHA12	8.3
52	CHA09A	6.3
53	CHA17	4.2
Cells w/Candida		100.0
Whole Allergen		97.9

Figure 3

Proliferation		
Peptides	**Position**	**% responders**
CRY16	1	73
CRY17A	2	69
CRY17	3	60
CRY11C	4	44
CRY18	5	38
CRY21C	6	31
CRY11F	7	29
CRY21A	8	22
CRY15A	9	18
CRY21	10	13
CRY23	11	13
CHA19	12	11
CRY22	13	11
CRY11	14	11
CRY11E	15	9
Whole Allergen		100
Candida		100

IL-10		
Peptides	**Position**	**% responders**
CRY18	1	59
CRY11F	2	52
CRY15A	3	48
CRY21C	4	41
CHA19	5	39
CRY22	6	27
CRY23	7	27
CRY11E	8	25
CRY16	9	23
CRY11	10	20
CRY21A	11	11
CRY17A	12	11
CRY21	13	9
CRY11C	14	7
CRY17	15	5
Whole Allergen		100
Candida		100

IFN-g		
Peptides	**Position**	**% responders**
CRY15A	1	32
CHA19	2	27
CRY17A	3	20
CRY22	4	20
CRY23	5	16
CRY11F	6	16
CRY21A	7	14
CRY21	8	14
CRY21C	9	14
CRY17	10	11
CRY18	11	9
CRY11E	12	9
CRY11C	13	2
CRY11	14	0
CRY16	15	0
Whole Allergen		100
Candida		100

IL-13		
Peptides	**Position**	**% responders**
CRY22	1	73
CRY15A	2	68
CHA19	3	68
CRY11F	4	68
CRY23	5	66
CRY18	6	64
CRY17A	7	55
CRY21C	8	50
CRY17	9	48
CRY21A	10	48
CRY11E	11	45
CRY21	12	36
CRY11	13	32
CRY16	14	14
CRY11C	15	7
Whole Allergen		100
Candida		100

Figure 4A

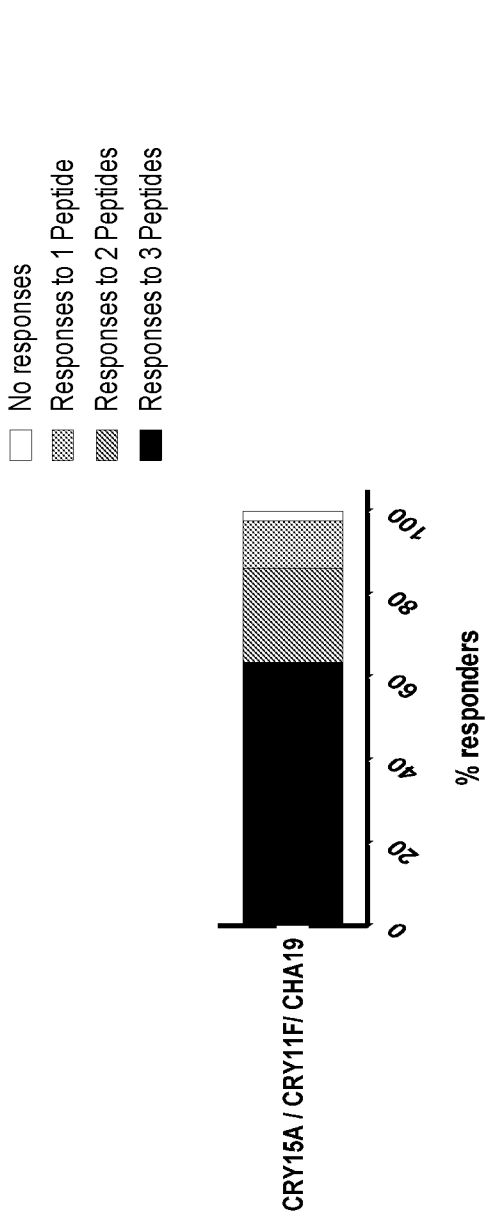


Figure 4B

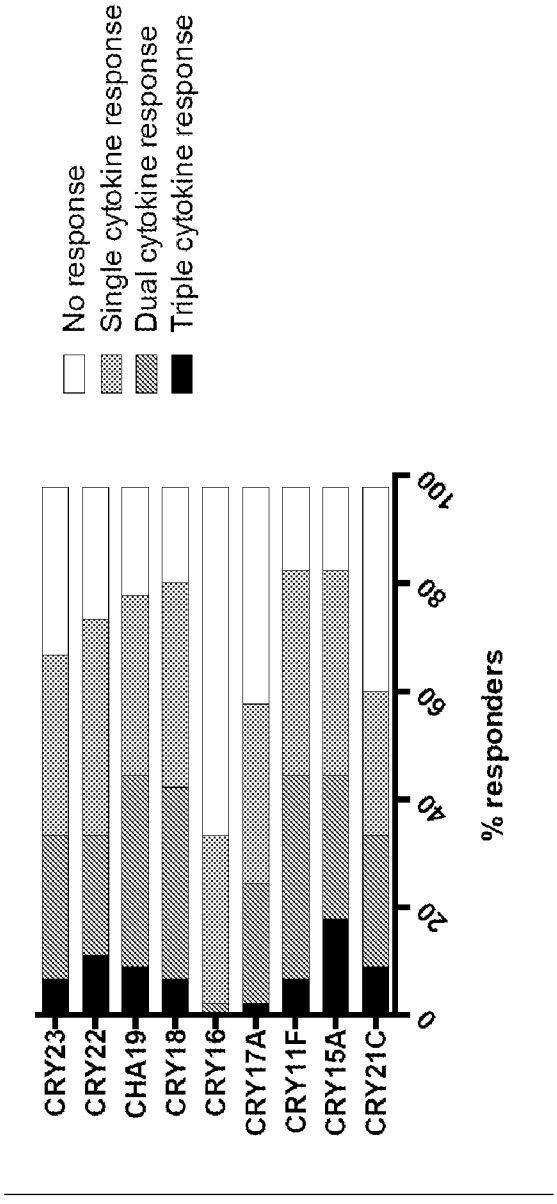
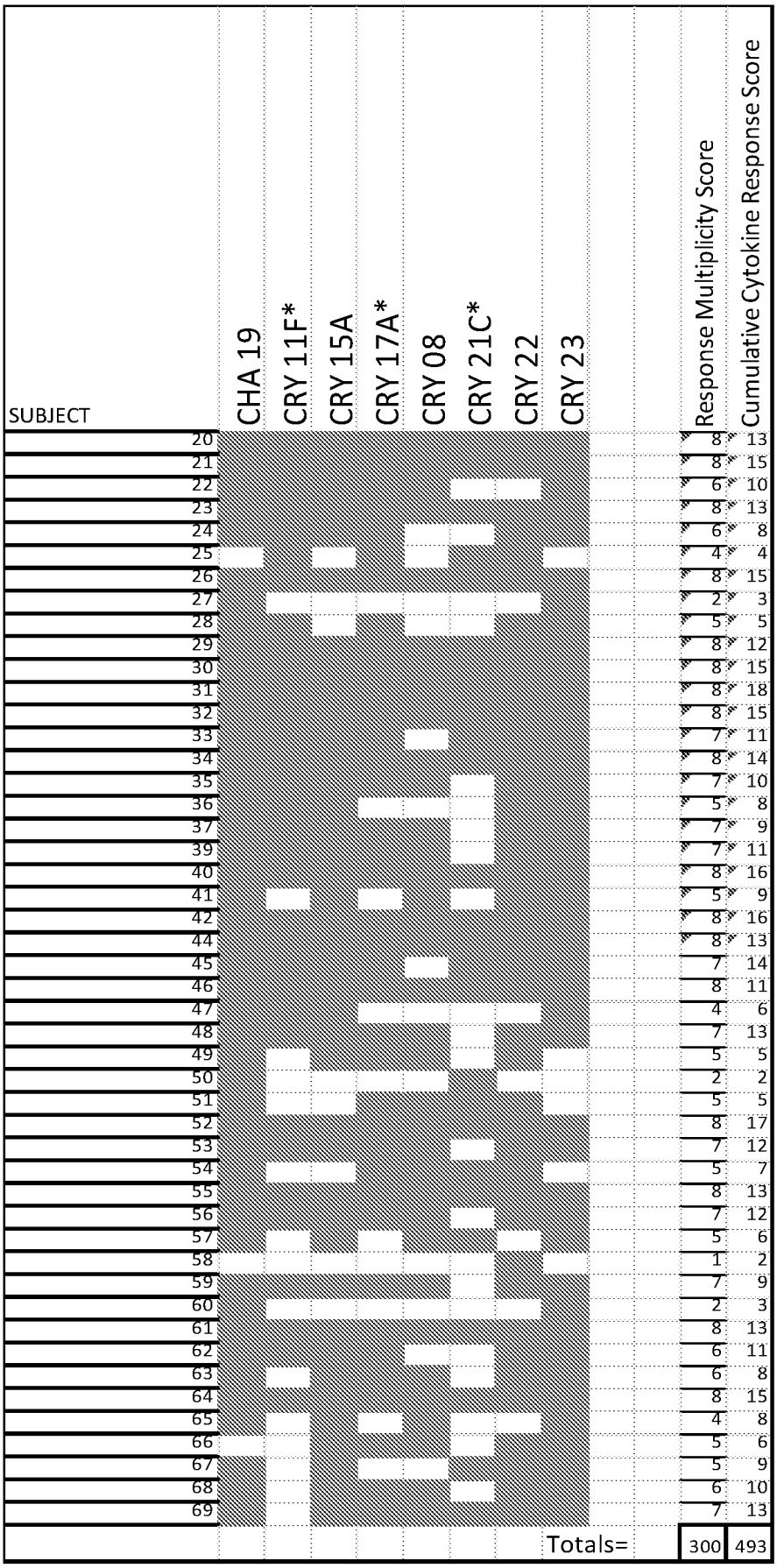


Figure 5A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CRY 15A
CRY 17A*
CRY 08
CRY 21C*
CRY 22
CRY 23
Response Multiplicity Score
Cumulative Cytokine Response Score
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21 8 15
22 6 10
23 8 13
24 6 8
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26 8 15
27 2 3
28 5 5
29 8 12
30 8 15
31 8 18
32 8 15
33 7 11
34 8 14
35 7 10
36 5 8
37 7 9
39 7 11
40 8 16
41 5 9
42 8 16
44 8 13
45 7 14
46 8 11
47 4 6
48 7 13
49 5 5
50 2 2
51 5 5
52 8 17
53 7 12
54 5 7
55 8 13
56 7 12
57 5 6
58 1 2
59 7 9
60 2 3
61 8 13
62 6 11
63 6 8
64 8 15
65 4 8
66 5 6
67 5 9
68 6 10
69 7 13
Totals= 300 493

Figure 5B

SUBJECT	CHA 19	CRY 11F*	CRY 15A	CRY 17A*	CRY 08	CRY 21C*	CRY 22	CRY 23	Response Multiplicity Score	Cumulative Cytokine Response Score
20	2	2	2	1	1	1	3	1	8	13
21	2	2	1	2	2	2	2	2	8	15
22	2	2	2	2	1	0	0	1	6	10
23	2	1	2	2	1	1	3	1	8	13
24	2	1	1	1	0	0	1	2	6	8
25	0	1	0	1	0	1	1	0	4	4
26	2	2	2	1	2	1	3	2	8	15
27	1	0	0	0	0	0	0	2	2	3
28	1	1	0	1	0	0	1	1	5	5
29	1	2	1	1	2	1	2	2	8	12
30	2	2	1	2	1	3	2	2	8	15
31	2	2	2	3	3	1	3	2	8	18
32	2	2	2	2	2	1	2	2	8	15
33	1	2	1	1	0	2	2	2	7	11
34	2	1	1	2	2	2	2	2	8	14
35	2	2	1	1	1	0	2	1	7	10
36	2	2	1	0	0	0	2	1	5	8
37	1	1	1	1	1	0	2	2	7	9
39	2	2	2	1	1	0	1	2	7	11
40	2	2	2	2	1	2	3	2	8	16
41	2	0	1	0	2	0	2	2	5	9
42	2	2	2	2	2	1	3	2	8	16
44	2	1	2	1	2	1	2	2	8	13
45	2	2	2	1	0	2	3	2	7	14
46	2	2	1	1	1	1	1	2	8	11
47	2	2	1	0	0	0	0	1	4	6
48	2	2	2	2	2	0	1	2	7	13
49	1	0	1	1	1	0	1	0	5	5
50	1	0	0	0	0	1	0	0	2	2
51	1	0	0	1	1	1	1	0	5	5
52	2	2	2	2	3	3	2	1	8	17
53	1	2	1	2	1	0	3	2	7	12
54	2	0	0	2	1	1	1	0	5	7
55	2	1	2	2	2	1	2	1	8	13
56	2	1	2	1	3	0	1	2	7	12
57	2	0	1	0	1	1	0	1	5	6
58	0	0	0	0	0	0	2	0	1	2
59	1	1	2	1	1	0	1	2	7	9
60	1	0	0	0	0	0	0	2	2	3
61	1	1	1	2	2	2	3	1	8	13
62	1	2	2	1	0	0	3	2	6	11
63	2	0	2	1	1	0	1	1	6	8
64	1	2	2	1	3	3	1	2	8	15
65	2	0	1	0	3	0	0	2	4	8
66	0	0	1	1	2	0	1	1	5	6
67	2	0	2	0	0	1	2	2	5	9
68	2	0	2	1	1	0	2	2	6	10
69	2	0	1	3	2	1	2	2	7	13
								Totals=	300	493

Figure 6A

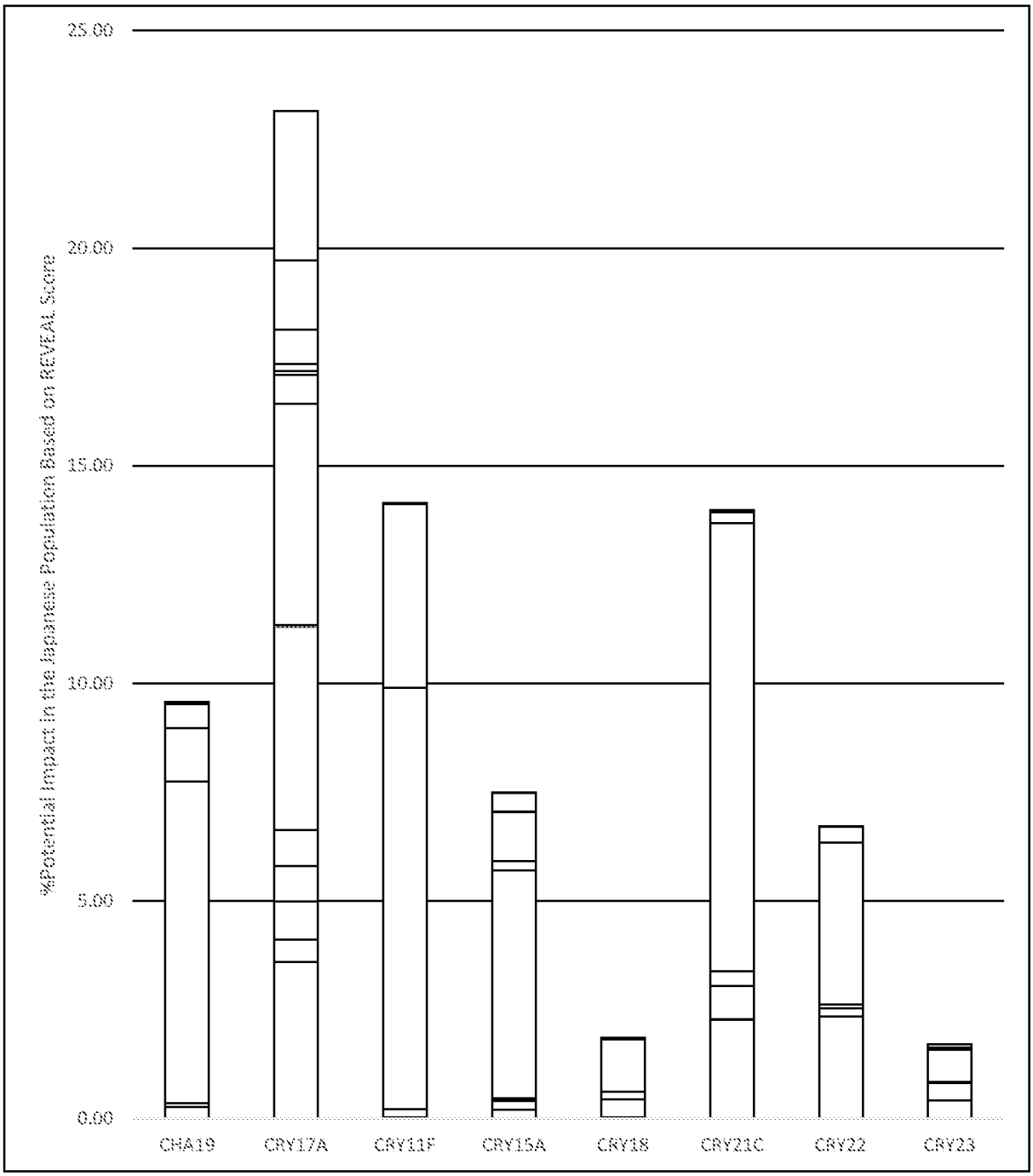
25.00
20.00
15.00
10.00
5.00
0.00
%Potential Impact in the Japanese Population Based on REVEAL Score
CHA19
CRY17A
CRY11F
CRY15A
CRY18
CRY21C
CRY22
CRY23

Figure 6B

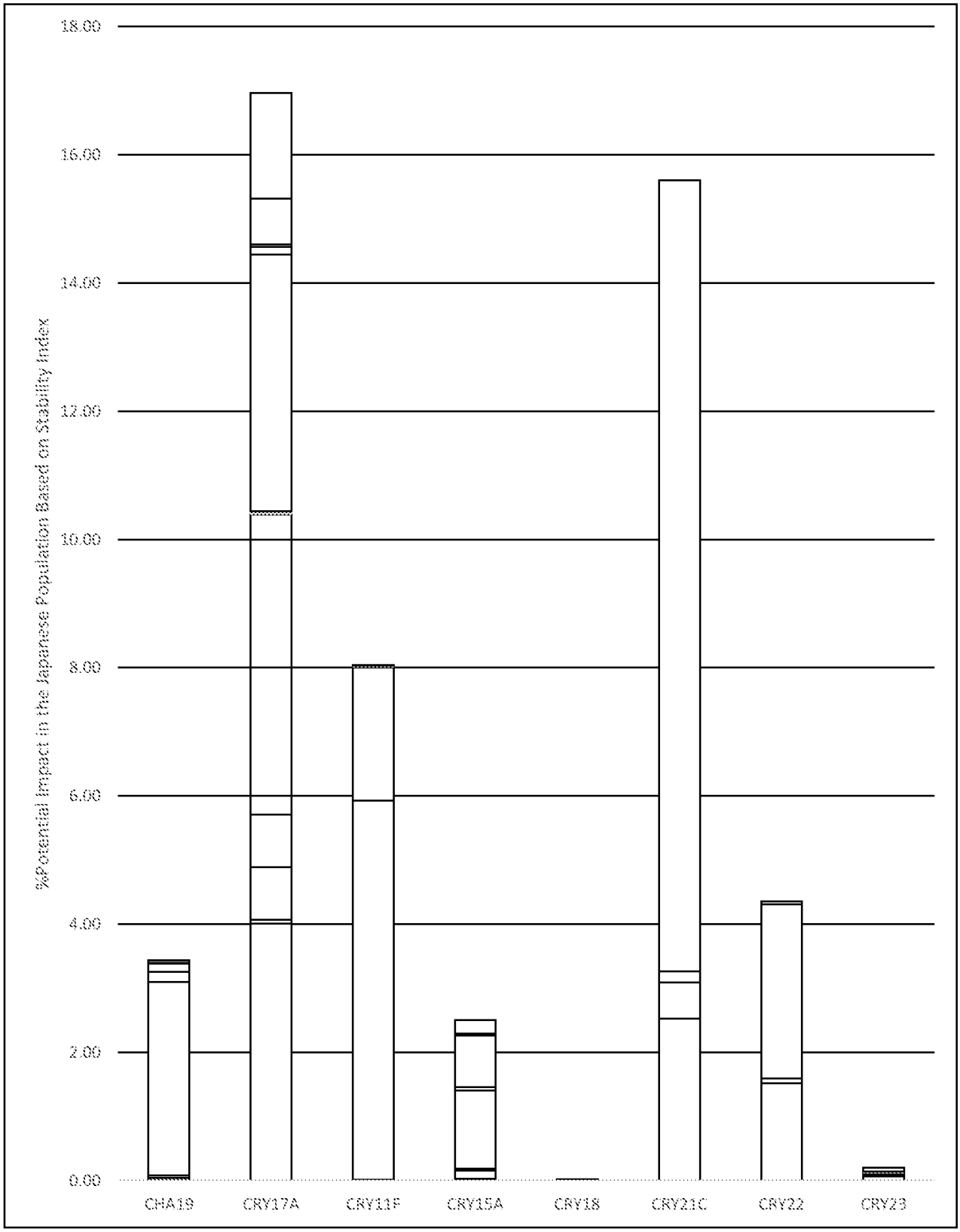
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
%Potential Impact in the Japanese Population Based on Stability Index
CHA19
CRY17A
CRY11F
CRY15A
CRY18
CRY21C
CRY22
CRY23

Figure 7A

Allele Frequency:		5.83%	6.70%	9.47%	1.90%	2.86%	0.68%	14.06%	0.04%	14.00%
Peptide ID	% Potential Impact in the Japanese Population Based on REVEAL® Score	DRA*01:01; DRB1*01:01	DRA*01:01; DRB1*15:01	DRA*01:01; DRB1*03:01	DRA*01:01; DRB1*04:01	DRA*01:01; DRB1*13:01	DRA*01:01; DRB1*13:01	DRA*01:01; DRB1*04:05	DRA*01:01; DRB1*08:04	DRA*01:01; DRB1*09:01
CHA19	9.57	0.00	0.26	0.01	0.68	0.00	0.00	7.39	0.00	1.23
CRY17A	23.15	3.59	0.51	0.00	0.01	0.87	0.00	0.00	0.00	0.00
CRY11F	14.14	0.02	0.00	0.00	0.01	0.13	0.00	9.89	0.00	4.22
CRY15A	7.48	0.13	0.21	0.00	0.04	0.02	0.00	5.24	0.00	0.21
CRY18	1.84	0.01	0.01	0.00	0.00	0.41	0.00	0.13	0.00	1.26
CRY21C	13.97	2.26	0.02	0.00	0.76	0.34	0.00	10.30	0.00	0.25
CRY22	6.70	2.33	0.00	0.00	0.13	0.09	0.00	3.71	0.00	0.37
CRY23	1.70	0.41	0.40	0.01	0.01	0.01	0.00	0.02	0.00	0.73

Figure 7B

Allele Frequency:		10.20%	0.00%	0.00%	0.82%	0.60%	0.82%	4.68%	0.05%	5.09%
Peptide ID	% Potential Impact in the Japanese Population Based on REVEAL® Score	DRA*01:01; DRB1*15:02	DRA*01:01; DRB3*02:02	DRA*01:01; DRB5*01:01	DQA1*01:01; DQB1*05:01	DQA1*05:01; DQB1*02:01	DQA1*03:02; DQB1*06:02	DQA1*03:01; DQB1*03:02	DQA1*05:01; DQB1*03:01	DQA1*03:01; DQB1*03:01
CHA19	9.57	0.56	0.00	0.00	0.00	0.00	0.01	0.02	0.00	0.00
CRY17A	23.15	0.00	0.00	0.00	0.82	0.00	0.82	4.66	0.03	5.09
CRY11F	14.14	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00
CRY15A	7.48	1.12	0.00	0.00	0.00	0.00	0.00	0.43	0.00	0.00
CRY18	1.84	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00
CRY21C	13.97	0.64	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CRY22	6.70	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00
CRY23	1.70	0.05	0.00	0.00	0.00	0.01	0.00	0.07	0.00	0.00

Figure 7C

Allele Frequency:		8.85%	1.96%	3.23%	14.43%	4.19%	6.83%
Peptide ID	% Potential Impact in the Japanese Population Based on REVEAL® Score	DPA1*01:03; DPB1*02:01	DPA1*01:03; DPB1*04:01	DPA1*01:03; DPB1*04:02	DPA1*01:03; DPB1*05:01	DPA1*02:01; DPB1*02:01	DPA1*02:01; DPB1*05:01
CHA19	9.57	0.00	0.00	0.00	0.01	0.00	0.00
CRY17A	23.15	0.66	0.10	0.17	0.79	1.58	3.44
CRY11F	14.14	0.01	0.00	0.00	0.00	0.00	0.00
CRY15A	7.48	0.00	0.00	0.00	0.00	0.00	0.00
CRY18	1.84	0.00	0.00	0.00	0.00	0.00	0.00
CRY21C	13.97	0.00	0.00	0.00	0.00	0.00	0.00
CRY22	6.70	0.00	0.00	0.00	0.00	0.00	0.00
CRY23	1.70	0.00	0.00	0.00	0.00	0.00	0.00

Figure 8A

Allele Frequency:		5.83%	6.70%	0.17%	1.10%	2.80%	0.68%	14.06%	0.04%	14.00%
Peptide ID	% Potential Impact in the Japanese Population Based on Stability Index	DRA*01:01; DRB1*01:01	DRA*01:01; DRB1*15:01	DRA*01:01; DRB1*03:01	DRA*01:01; DRB1*04:01	DRA*01:01; DRB1*11:01	DRA*01:01; DRB1*13:01	DRA*01:01; DRB1*04:05	DRA*01:01; DRB1*08:04	DRA*01:01; DRB1*09:01
CHA19	3.44	0.00	0.05	0.00	0.04	0.00	0.00	3.01	0.00	0.16
CRY17A	16.96	4.51	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00
CRY11F	8.04	0.00	0.00	0.00	0.00	0.01	0.00	5.91	0.00	2.66
CRY15A	2.50	0.03	0.13	0.00	0.02	0.00	0.00	1.22	0.00	0.05
CRY18	0.03	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00
CRY21C	15.60	2.53	0.00	0.00	0.57	0.16	0.00	12.33	0.00	0.00
CRY22	4.36	1.52	0.00	0.00	0.07	0.00	0.00	2.72	0.00	0.04
CRY23	0.21	0.07	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Figure 8B

Allele Frequency:		10.20%	0.00%	0.00%	0.82%	0.60%	0.82%	4.68%	0.05%	5.09%
Peptide ID	% Potential Impact in the Japanese Population Based on Stability Index	DRA*01:01; DRB1*15:02	DRA*01:01; DRB3*02:02	DRA*01:01; DRB5*01:01	DQA1*01:01; DQB1*05:01	DQA1*05:01; DQB1*02:01	DQA1*01:02; DQB1*06:02	DQA1*03:01; DQB1*03:02	DQA1*05:01; DQB1*03:01	DQA1*03:01; DQB1*03:01
CHA19	3.44	0.13	0.00	0.00	0.00	0.00	0.04	0.01	0.00	0.00
CRY17A	16.96	0.00	0.00	0.00	0.82	0.00	0.82	4.68	0.05	4.00
CRY11F	8.04	0.00	0.00	0.00	0.01	0.02	0.00	0.00	0.00	0.00
CRY15A	2.50	0.01	0.00	0.00	0.01	0.00	0.01	0.21	0.00	0.00
CRY18	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00
CRY21C	15.60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CRY22	4.36	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00
CRY23	0.21	0.01	0.00	0.00	0.01	0.02	0.00	0.07	0.00	0.00

Figure 8C

Allele Frequency:		8.85%	1.96%	3.23%	14.43%	4.19%	6.83%
Peptide ID	% Potential Impact in the Japanese Population Based on Stability Index	DPA1*01:03; DPB1*02:01	DPA1*01:03; DPB1*04:01	DPA1*01:03; DPB1*04:02	DPA1*01:03; DPB1*05:01	DPA1*02:01; DPB1*02:01	DPA1*02:01; DPB1*05:01
CHA19	3.44	0.00	0.00	0.00	0.00	0.00	0.00
CRY17A	16.96	0.12	0.03	0.00	0.00	0.72	1.64
CRY11F	8.04	0.00	0.00	0.00	0.00	0.00	0.00
CRY15A	2.50	0.00	0.00	0.00	0.00	0.00	0.00
CRY18	0.03	0.00	0.00	0.00	0.00	0.00	0.00
CRY21C	15.60	0.00	0.00	0.00	0.00	0.00	0.00
CRY22	4.36	0.00	0.00	0.00	0.00	0.00	0.00
CRY23	0.21	0.00	0.00	0.00	0.00	0.00	0.00

Figure 9

Allele	Global Population Frequency (%)	Japanese Population Frequency (%)
DRA*01:01;DRB1*01:01	4.39	5.83
DRA*01:01;DRB1*15:01	8.73	6.70
DRA*01:01;DRB1*03:01	9.15	0.17
DRA*01:01;DRB1*04:01	2.36	1.10
DRA*01:01;DRB1*11:01	5.69	2.80
DRA*01:01;DRB1*13:01	4.39	0.68
DRA*01:01;DRB1*04:05	2.85	14.06
DRA*01:01;DRB1*08:04	1.15	0.04
DRA*01:01;DRB1*09:01	4.87	14.00
DRA*01:01;DRB1*15:02	3.46	10.20
DRA*01:01;DRB3*02:02	0.00	0.00
DRA*01:01;DRB5*01:01	0.00	0.00
Total for HLA-DR alleles tested	**47.04**	**55.58**
DQA1*01:01;DQB1*05:01	1.16	0.82
DQA1*05:01;DQB1*02:01	2.14	0.60
DQA1*01:02;DQB1*06:02	2.20	0.82
DQA1*03:01;DQB1*03:02	1.00	4.68
DQA1*05:01;DQB1*03:01	3.00	0.05
DQA1*03:01;DQB1*03:01	2.72	5.09
Total for HLA-DQ alleles tested	**12.22**	**12.06**
DPA1*01:03;DPB1*02:01	6.92	8.85
DPA1*01:03;DPB1*04:01	14.78	1.96
DPA1*01:03;DPB1*04:02	4.97	3.23
DPA1*01:03;DPB1*05:01	5.29	14.43
DPA1*02:01;DPB1*02:01	2.31	4.19
DPA1*02:01;DPB1*05:01	1.31	6.83
Total for HLA-DP alleles tested	**35.58**	**39.49**

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2017/050246

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/36 C07K14/415 A61P37/08
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2013/119853 A1 (JOLLA INST ALLERGY IMMUNOLOG [US]; SETTE ALESSANDRO [US]; OSEROFF CARL) 15 August 2013 (2013-08-15) Abstract; sequences 642, 655, 663, 674	1-15, 17-19, 22-24,26
Y	EP 0 960 887 A1 (MEIJI MILK PROD CO LTD [JP]) 1 December 1999 (1999-12-01) Abstract; paragraphs 1, 17, 22, 23; sequence 56	1-15, 17-19, 22-24,26
Y	EP 0 958 834 A1 (MEIJI MILK PROD CO LTD [JP]) 24 November 1999 (1999-11-24) Abstract; Fig 1 211-225, 306-320; Fig 2, 66-80, 71-85, 81-95, 126-140, 131-145, 186-200, 311-325, 341-355	1-15, 17-19, 22-24,26

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 March 2017	19/05/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	López García, F

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2017/050246

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2005/152927 A1 (GRIFFITH IRWIN J [US] ET AL) 14 July 2005 (2005-07-14) Abstract; paragraphs 11,12, 159, 160, 163; Figs; Example 21; sequences 55, 56, 185, 188, 192, 231, 235 -----	1-15, 17-19, 22-24,26
Y	EP 0 700 929 A2 (HAYASHIBARA BIOCHEM LAB [JP]) 13 March 1996 (1996-03-13) Abstract; paragraphs 14; sequences 8, 9,10; examples B-1 to B-7 -----	1-15, 17-19, 22-24,26
X,P	WO 2016/209959 A2 (ALK-ABELLÓ AS [DK]; LA JOLLA INST FOR ALLERGY AND IMMUNOLOGY [US]) 29 December 2016 (2016-12-29) Abstract; claims; sequences 9, 10, 12, 29, 77, 78, 100, 105, 113, 128, 132, 170, 175, 176, 177, 185, 202, 205, 213. -----	1-15, 17-19, 22-24,26

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2017/050246

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-19(completely); 22-24, 26(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-19(completely); 22-24, 26(partially)

Pharmaceutical composition comprising the peptides of SEQ. ID. Nos.: 5, 163, 19 or variants thereof.

2-9. claims: 20, 21, 25(completely); 22-24, 26(partially)

Peptides of sequences 5, 165, 54, 159, 13, 166, 23, 24., each represent a different invention.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2017/050246

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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