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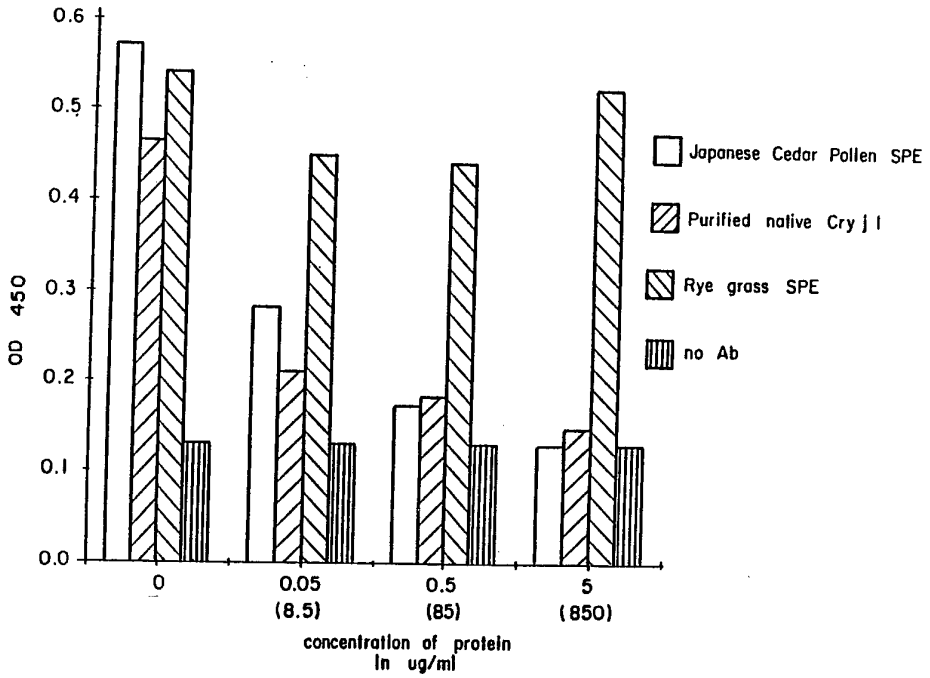

(57) Abstract

The present invention provides isolated peptides of Japanese cedar pollen protein allergen, *Cry j* I. Peptides within the scope of the invention comprise at least one T cell epitope, or preferably at least two T cell epitopes of *Cry j* I. The invention also pertains to modified peptides having similar or enhanced therapeutic properties as the corresponding, naturally-occurring allergen or portion thereof, but having reduced side effects. The invention further provides nucleic acid sequences coding for peptides of the invention. Methods of treatment or of diagnosis of sensitivity to Japanese cedar pollens in an individual and therapeutic compositions comprising one or more peptides of the invention are also provided. The present invention also provides *Jun v* I and *Jun s* I protein allergens and nucleic acid sequences coding for *Jun s* I and *Jun v* I allergens. *Jun s* I and *Jun v* I are protein allergens which are immunologically cross-reactive with *Cry j* I.

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ALLERGENIC PROTEINS AND PEPTIDES FROM JAPANESE CEDAR POLLEN

Background of the Invention

Genetically predisposed individuals, who make up about 10% of the population, become hypersensitized (allergic) to antigens from a variety of environmental sources to which they are exposed. Those antigens that can induce immediate and/or delayed types of hypersensitivity are known as allergens. (King, T.P., *Adv. Immunol.* **23**: 77-105, (1976)). Anaphylaxis or atopy, which includes the symptoms of hay fever, asthma, and hives, is one form of immediate allergy. It can be caused by a variety of atopic allergens, such as products of grasses, trees, weeds, animal dander, insects, food, drugs, and chemicals.

The antibodies involved in atopic allergy belong primarily to the IgE class of immunoglobulins. IgE binds to mast cells and basophils. Upon combination of a specific allergen with IgE bound to mast cells or basophils, the IgE may be cross-linked on the cell surface, resulting in the physiological effects of IgE-antigen interaction. These physiological effects include the release of, among other substances, histamine, serotonin, heparin, a chemotactic factor for eosinophilic leukocytes and/or the leukotrienes, C4, D4, and E4, which cause prolonged constriction of bronchial smooth muscle cells (Hood, L.E. et al. *Immunology* (2nd ed.), The Benjamin/Cumming Publishing Co., Inc. (1984)). These released substances are the mediators which result in allergic symptoms caused by a combination of IgE with a specific allergen. Through them, the effects of an allergen are manifested. Such effects may be systemic or local in nature, depending on the route by which the antigen entered the body and the pattern of deposition of IgE on mast cells or basophils. Local manifestations generally occur on epithelial surfaces at the location at which the allergen entered the body. Systemic effects can include anaphylaxis (anaphylactic shock), which is the result of an IgE-basophil response to circulating (intravascular) antigen.

Japanese cedar (Sugi; *Cryptomeria japonica*) pollinosis is one of the most important allergic diseases in Japan. The number of patients suffering from this disease is on the increase and in some areas, more than 10% of the population are affected. Treatment of Japanese cedar pollinosis by administration of Japanese cedar pollen extract to effect hyposensitization to the allergen has been attempted. Hyposensitization using Japanese cedar pollen extract, however, has drawbacks in that it can elicit anaphylaxis if high doses are used, whereas when low doses are used to avoid anaphylaxis, treatment must be continued for several years to build up a tolerance for the extract.

The major allergen from Japanese cedar pollen has been purified and designated as Sugi basic protein (SBP) or *Cry j* I. This protein is reported to be a basic protein with a molecular weight of 41-50 kDa and a pI of 8.8. There appear to be multiple isoforms of the allergen, apparently due in part to differential glycosylation (Yasueda et al. (1983) *J. Allergy Clin. Immunol.* **71**: 77-86; and Taniai et al. (1988) *FEBS Letters* **239**: 329-332. The sequence of the first twenty amino acids at the N-terminal end of *Cry j* I and a sixteen amino acid internal sequence have been determined (Taniai *supra*).

A second allergen from Japanese cedar pollen having a molecular weight of about 37 kDa known as *Cry j* II has also been reported (Sakaguchi et al. (1990) *Allergy* **45**: 309-312). This allergen was found to have no immunological cross-reactivity with *Cry j* I. Most patients with Japanese cedar pollinosis were found to have IgE antibodies to both *Cry j* I and *Cry j* II, however, sera from some patients reacted with only *Cry j* I or *Cry j* II.

In addition to hyposensitization of Japanese cedar pollinosis patients with low doses of Japanese cedar pollen extract, U.S. patent 4,939,239 issued July 3, 1990 to Matsuhashi et al. discloses a hyposensitization agent comprising a saccharide covalently linked to a Japanese cedar pollen allergen for hyposensitization of persons sensitive to Japanese cedar pollen. This hyposensitization agent is reported to enhance the production of IgG and IgM antibodies, but reduce production of IgE antibodies which are specific to the allergen and responsible for anaphylaxis and allergy. The allergens used in the hyposensitization agent preferably have an NH_2-terminal amino acid sequence of

Asp-Asn-Pro-Ile-Asp-Ser-X-Trp-Arg-Gly-Asp-Ser-Asn-Trp-Ala-Gln-Asn-Arg-Met-Lys-, wherein X is Ser, Cys, Thr, or His (SEQ ID NO: 18). Additionally, Usui et al. (1990) *Int. Arch. Allergy Appl. Immunol.* 91: 74-79 reported that the ability of a Sugi basic protein (i.e., *Cry j* I)-pullulan conjugate to elicit the Arthus reaction was markedly reduced, about 1,000 times lower than that of native Sugi basic protein and suggested that the Sugi basic protein-pullulan conjugate would be a good candidate for desensitization therapy against cedar pollinosis.

The *Cry j* I allergen found in *Cryptomeria japonica* has also been found to be cross-reactive with allergens in the pollen from other species of trees, including *Cupressus sempervirens*. Panzani et al. (*Annals of Allergy* 57: 26-30 (1986)) reported that cross reactivity was detected between allergens in the pollens of *Cupressus sempervirens* and *Cryptomeria japonica* in skin testing, RAST and RAST inhibition. A 50 kDa allergen isolated from Mountain Cedar (*Juniperus sabinoides,* also known as *Juniperus ashei*) has the NH_2-terminal sequence AspAsnProIleAsp (SEQ ID NO: 25) (Gross et al, (1978) Scand. J. Immunol. 8: 437-441) which is the same sequence as the first five amino acids of the NH_2 terminal end of the *Cry j* I allergen. The *Cry j I* allergen has also been found to be allergenically cross-reactive with the following species of trees: *Cupressus arizonica, Cupressus macrocarpa, Juniperus virginiana, Juniperus communis, Thuya orientalis* , and *Chamaecyparis obtusa.*

Despite the attention Japanese cedar pollinosis allergens have received, definition or characterization of the allergens responsible for its adverse effects on people is far from complete. Current desensitization therapy involves treatment with pollen extract with its attendant risks of anaphylaxis if high doses of pollen extract are administered, or long desensitization times when low doses of pollen extract are administered.

Summary of the Invention

The present invention provides nucleic acid sequences coding for the *Cryptomeria japonica* major pollen allergen *Cry j* I and fragments thereof. The present invention also provides isolated *Cry j* I or at least one fragment thereof produced in a host cell transformed with a nucleic acid sequence coding

for *Cry j* I or at least one fragment thereof and fragments of *Cry j* I prepared synthetically.

The present invention also provides *Jun v* I and *Jun s* I protein allergens which are immunologically cross-reactive with *Cry j* I and fragments of *Jun v* I and *Jun s* I produced in a host cell transformed with a nucleic acid sequence coding for *Jun s* I or *Jun v* I respectively and fragments of *Jun s* I and *Jun v* I prepared synthetically. The present invention further provides nucleic acid sequences coding for *Jun v* I and *Jun s* I and fragments thereof. As used herein, a fragment of the nucleic acid sequence coding for the entire amino acid sequence of *Cry j* I, *Jun s* I or *Jun v* I refers to a nucleotide sequence having fewer bases than the nucleotide sequence coding for the entire amino acid sequence of *Cry j* I, *Jun s* I or *Jun v* I and/or mature *Cry j* I, *Jun s* I or *Jun v* I. *Cry j* I, *Jun s* I or *Jun v* I and fragments thereof are useful for diagnosing, treating, and preventing Japanese cedar pollinosis as well as pollinosis caused by pollen from other species of trees wherein such pollen is immunologically cross-reactive with Japenese cedar pollen allergen.

Peptides within the scope of the invention preferably comprise at least one T cell epitope, and more preferably at least two T cell epitopes of *Cry j* I. The invention further provides peptides comprising at least two regions, each region comprising at least one T cell epitope of a Japanese cedar pollen protein allergen. The invention also provides modified peptides having similar or enhanced therapeutic properties as the corresponding, naturally-occurring allergen or portion thereof, but having reduced side effects, as well as modified peptides having improved properties such as increased solubility and stability. Peptides of the invention are capable of modifying, in a Japanese cedar pollen-sensitive individual or in an individual who is sensitive to an allergen cross-reactive with Japanese cedar pollen, to whom they are administered, the allergic response of the individual to a Japanese cedar pollen allergen or an allergen cross-reactive with Japanese cedar pollen such as *Jun s* I or *Jun v* I. Methods of treatment or diagnosis of sensitivity to Japanese cedar pollen or a cross-reactive allergen in an individual and therapeutic compositions comprising one or more peptides of the invention are also provided. This invention is more particularly

described in the appended claims and is described in its preferred embodiments in the following description.

Brief Description of the Drawings

Fig. 1a is a graphic representation of affinity purified *Cry j* I on Superdex 75 (2.6 by 60 cm) equilibrated with 10 mM sodium acetate (pH 5.0) and 0.15 M NaCl;

Fig. 1b shows an SDS-PAGE (12.5%) analysis of the fractions from the major peak shown in Fig 1a;

Fig. 2 shows a Western blot of isoforms of purified native *Cry j* I proteins separated by SDS-PAGE and probed with mAB CBF2;

Fig. 3 is a graphic representation of allergic sera titration of different purified fractions of purified native *Cry j* I using plasma from a pool of fifteen allergic patients;

Figs. 4a-b show the composite nucleic acid sequence from the two overlapping clones JC 71.6 and pUC19JC91a coding for *Cry j* I. The complete cDNA sequence for *Cry j* I is composed of 1312 nucleotides, including 66 nucleotides of 5' untranslated sequence, an open reading frame starting with the codon for an initiating methionine of 1122 nucleotides, and a 3' untranslated region. Figs. 4a-b also show the deduced amino acid sequence of *Cry j* I;

Fig. 5a is a graphic representation of the results of IgE binding reactivity wherein the coating antigen is soluble pollen extract (SPE) from Japanese cedar pollen;

Fig. 5b is a graphic representation of the results of IgE binding reactivity wherein the coating antigen is purified native *Cry j* I;

Fig. 6 is a graphic representation of the results of a competition ELISA with pooled human plasma (PHP) from 15 patients wherein the coating antigen is soluble pollen extract (SPE) from Japanese cedar pollen;

Fig. 7 is a graphic representation of the results of a competition ELISA using plasma from individual patients (indicated by patient numbers) wherein the coating antigen is soluble pollen extract (SPE) from Japanese cedar pollen and the competing antigen is purified native *Cry j* I;

Fig. 8a is a graphic representation of the results from a direct binding ELISA using plasma from seven individual patients (indicated by patient numbers) wherein the coating antigen is soluble pollen extract (SPE) from Japanese cedar pollen;

Fig. 8b is a graphic representation of the results from a direct binding ELISA using plasma from seven individual patients (indicated by patient numbers) wherein the coating antigen is denatured soluble pollen extract which has been denatured by boiling in the presence of a reducing agent, DTT;

Fig. 9 is a graphic representation of a direct ELISA where the wells were coated with recombinant *Cry j* I (r*Cry j* I) and IgE binding was assayed on individual patients;

Fig. 10a is a graphic representation of the results of a capture ELISA using pooled human plasma from fifteen patients wherein the wells were coated with CBF2 (IgG) mAb, PBS was used as a negative antigen control, and the antigen was purified recombinant *Cry j* I;

Fig. 10b is a graphic representation of the results of a capture ELISA using rabbit anti-*Amb a*I and II, wherein the wells were coated with 20 μg/ml CBF2 (IgG), PBS was used as a negative antigen control and the antigen was purified recombinant *Cry j* I;

Fig. 11 is a graphic representation of a histamine release assay performed on one Japanese cedar pollen allergic patient using SPE from Japanese cedar pollen, purified native *Cry j* I and recombinant *Cry j* I as the added antigens; and

Fig. 12 is a graphic representation of the results of a T cell proliferation assay using blood from patient #999 wherein the antigen is recombinant *Cry j* I protein, purified native *Cry j* I protein, or selected *Cry j* I peptides recombinant *Amb a* 1.1.

Fig. 13 shows various peptides of desired lengths derived from *Cry j* I.

Fig. 14 is a graphic representation depicting responses of T cell lines from twenty-five patients primed in vitro with purified native *Cry j* I and analyzed for response to various *Cry j* I peptides by percent of responses

(positive) with an S.I of at least two (shown over each bar), the mean stimulation index of positive response for the peptide (shown over each bar in parenthesis) and the positivity index (Y axis).

Fig. 15 is a graphic representation of the results of a direct binding assay of IgE to certain *Cry j* I peptides, purified native *Cry j* I and r*Cry j* I.

Figs. 16 shows the nucleotide sequence of *Jun s* I; this sequence is a composite from the two overlapping cDNA clones pUC19JS42e and pUC19JS45a as well as the full-length clone JS53iib coding for *Jun s* I; the complete cDNA sequence for *Jun s* I is composed of 1170 nucleotides, including 25 nucleotides of 5' untranslated sequence, an open reading frame of 1,101 nucleotides, and a 3' untranslated region; Fig. 16 also shows the deduced amino acid sequence of *Jun s* I.

Fig. 17 shows the nucleotide sequence of *Jun v* I; this sequence is a composite from the two overlapping cDNA clones pUC19JV46a and pUC19JV49iia coding for *Jun v* I; the complete cDNA sequence for *Jun v* I is composed of 1278 nucleotides, including 35 nucleotides of 5' untranslated sequence, an open reading frame of 1,110 nucleotides, and a 3' untranslated region; Fig. 17 also show the deduced amino acid sequence of *Jun v* I.

Fig. 18 shows various peptides of desired lengths derived from *Cry j* I.

Figs. 19a and 19b show Northern blots of pollen-derived RNA probed with *Cry j* cDNA for identification of mRNA capable of encoding *Cry j* I or a *Cry j* I homologue; Fig. 19a shows RNA from *C. japonica* (U.S. and Japanese sources), *J. sabinoides* and *J. virginiana* probed with *Cry j* I cDNA; Fig. 19b shows RNA from *J. sabinoides* and *C. arizonica* probed with the same cDNA; the position of molecular weight standards are shown in each part of the Figure.

Detailed Description of the Invention

The present invention provides nucleic acid sequences coding for *Cry j* I, the major allergen found in Japanese cedar pollen as well as nucleic acid sequences coding for *Jun v* I and *Jun s* I. The nucleic acid sequence coding for *Cry j* I preferably has the sequence shown in Figs. 4a and 4b (SEQ ID NO: 1).

The nucleic acid sequence coding for *Cry j* I shown in Figs. 4a and 4b (SEQ ID NO: 1) contains a 21 amino acid leader sequence from base 66 through base 128. This leader sequence is cleaved from the mature protein which is encoded by bases 129 through 1187. The deduced amino acid sequence of *Cry j* I is also shown in Figs. 4a and 4b (SEQ ID NO: 2). The nucleic acid sequence of the invention codes for a protein having a predicted molecular weight of 38.5 kDa, with a pI of 7.8, and five potential N-linked glycosylation sites. Utilization of these glycosylation sites will increase the molecular weight and affect the pI of the mature protein. The deduced amino acid sequence for the mature protein encoded by the nucleic acid sequence of the invention is identical with the known NH_2-terminal and internal amino acid sequences reported by Taniai et al., *supra*. The NH_2-terminal end of *Cry j* I reported by Taniai et al., *supra* has the sequence shown in SEQ ID NO: 18. The internal sequence reported by Taniai et al., *supra* has the sequence GluAlaPheAsnValGluAsnGlyAsnAlaThrProGlnLeuThrLys (SEQ ID NO: 19). There are sequence polymorphisms observed in the nucleic acid sequence of the invention. For example, single independent nucleotide substitutions at the codons encoding amino acids 38, 51 and 74 (GGA vs. GAA, GTG vs. GCG, and GGG vs. GAG, respectively) of SEQ ID #1 may result in amino acid polymorphisms (G vs. E, V vs. A, and G vs. E, respectively) at these sites. In addition, a single nucleotide substitution has been detected in one cDNA clone derived from *Cryptomeria japonica* pollen collected in Japan. This substitution in the codon for amino acid 60 (TAT vs. CAT) of SEQ ID #1 may result in an amino acid polymorphism (Y vs. H) at this site. Additional silent nucleotide substitutions have been detected. It is expected that there are additional sequence polymorphisms, and it will be appreciated by one skilled in the art that one or more nucleotides (up to about 1% of the nucleotides) in the nucleic acid sequence coding for *Cry j* I may vary among individual *Cryptomeria japonica* plants due to natural allelic variation. Any and all such nucleotide variations and resulting amino acid polymorphisms are within the scope of the invention. Furthermore, there may be one or more family members of *Cry j* I. Such family members are defined as proteins related in function and amino acid sequence to

Cry j I but encoded by genes at separate genetic loci.

Fragments of the nucleic acid sequence coding for fragments of *Cry j* I or a cross-reactive allergen are also within the scope of the invention. Fragments within the scope of the invention include those coding for parts of *Cry j* I or a cross-reactive allergen such as *Jun v* I or *Jun s* I which induce an immune response in mammals, preferably humans, such as stimulation of minimal amounts of IgE; binding of IgE; eliciting the production of IgG and IgM antibodies; or the eliciting of a T cell response such as proliferation and/or lymphokine secretion and/or the induction of T cell anergy. The foregoing fragments of *Cry j* I are referred to herein as antigenic fragments. Fragments within the scope of the invention also include those capable of hybridizing with nucleic acid from other plant species for use in screening protocols to detect allergens that are cross-reactive with *Cry j* I. As used herein, a fragment of the nucleic acid sequence coding for *Cry j* I refers to a nucleotide sequence having fewer bases than the nucleotide sequence coding for the entire amino acid sequence of *Cry j* I and/or mature *Cry j* I. Generally, the nucleic acid sequence coding for the fragment or fragments of *Cry j* I will be selected from the bases coding for the mature protein, however, in some instances it may be desirable to select all or a part of a fragment or fragments from the leader sequence portion of the nucleic acid sequence of the invention. The nucleic acid sequence of the invention may also contain linker sequences, modified restriction endonuclease sites and other sequences useful for cloning, expression or purification of *Cry j* I or fragments thereof.

A nucleic acid sequence coding for *Cry j* I may be obtained from *Cryptomeria japonica* plants. However, Applicants have found that mRNA coding for *Cry j* I could not be obtained from commercially available *Cryptomeria japonica* pollen. This inability to obtain mRNA from the pollen may be due to problems with storage or transportation of commercially available pollen. Applicants have found that fresh pollen and staminate cones are a good source of *Cry j* I mRNA. It may also be possible to obtain the nucleic acid sequence coding for *Cry j* I from genomic DNA. *Cryptomeria japonica* is a well-known species of cedar, and plant material may be obtained from wild.

cultivated, or ornamental plants. The nucleic acid sequence coding for *Cry j* I may be obtained using the method disclosed herein or any other suitable techniques for isolation and cloning of genes. The nucleic acid sequence of the invention may be DNA or RNA.

The present invention provides expression vectors and host cells transformed to express the nucleic acid sequences of the invention. A nucleic acid sequence coding for *Cry j* I, *Jun v* I or *Jun s* I or at least one fragment thereof may be expressed in bacterial cells such as *E. coli*, insect cells (baculovirus), yeast, or mammalian cells such as Chinese hamster ovary cells (CHO). Suitable expression vectors, promoters, enhancers, and other expression control elements may be found in Sambrook et al. *Molecular Cloning: A Laboratory Manual*, second edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989). Other suitable expression vectors, promoters, enhancers, and other expression elements are known to those skilled in the art. Expression in mammalian, yeast or insect cells leads to partial or complete glycosylation of the recombinant material and formation of any inter- or intra-chain disulfide bonds. Suitable vectors for expression in yeast include YepSec1 (Baldari et al. (1987) *Embo J.* 6: 229-234); pMFa (Kurjan and Herskowitz (1982) *Cell* 30: 933-943); JRY88 (Schultz et al. (1987) *Gene* 54: 113-123) and pYES2 (Invitrogen Corporation, San Diego, CA). These vectors are freely available. Baculovirus and mammalian expression systems are also available. For example, a baculovirus system is commercially available (PharMingen, San Diego, CA) for expression in insect cells while the pMSG vector is commercially available (Pharmacia, Piscataway, NJ) for expression in mammalian cells.

For expression in *E. coli*, suitable expression vectors include, among others, pTRC (Amann et al. (1988) *Gene* 69: 301-315); pGEX (Amrad Corp., Melbourne, Australia); pMAL (N.E. Biolabs, Beverly, MA); pRIT5 (Pharmacia, Piscataway, NJ); pET-11d (Novagen, Madison, WI) Jameel et al., (1990) *J. Virol.* 64:3963-3966; and pSEM (Knapp et al. (1990) *BioTechniques* 8: 280-281). The use of pTRC, and pET-11d, for example, will lead to the expression of unfused protein. The use of pMAL, pRIT5 pSEM and pGEX will lead to the expression of allergen fused to maltose E binding protein (pMAL), protein A

(pRIT5), truncated ß-galactosidase (PSEM), or glutathione S-transferase (pGEX). When *Cry j* I, fragment, or fragments thereof is expressed as a fusion protein, it is particularly advantageous to introduce an enzymatic cleavage site at the fusion junction between the carrier protein and *Cry j* I or fragment thereof. *Cry j* I or fragment thereof may then be recovered from the fusion protein through enzymatic cleavage at the enzymatic site and biochemical purification using conventional techniques for purification of proteins and peptides. Suitable enzymatic cleavage sites include those for blood clotting Factor Xa or thrombin for which the appropriate enzymes and protocols for cleavage are commercially available from, for example, Sigma Chemical Company, St. Louis, MO and N.E. Biolabs, Beverly, MA. The different vectors also have different promoter regions allowing constitutive or inducible expression with, for example, IPTG induction (PRTC, Amann et al., (1988) *supra*; pET-11d, Novagen, Madison, WI) or temperature induction (pRIT5, Pharmacia, Piscataway, NJ) . It may also be appropriate to express recombinant *Cry j* I in different *E. coli* hosts that have an altered capacity to degrade recombinantly expressed proteins (e.g. U.S. patent 4,758,512). Alternatively, it may be advantageous to alter the nucleic acid sequence to use codons preferentially utilized by *E. coli*, where such nucleic acid alteration would not affect the amino acid sequence of the expressed protein.

Host cells can be transformed to express the nucleic acid sequences of the invention using conventional techniques such as calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, or electroporation. Suitable methods for transforming the host cells may be found in Sambrook et al. *supra*, and other laboratory textbooks.

The nucleic acid sequences of the invention may also be synthesized using standard techniques.

The present invention also provides a method of producing isolated Japanese cedar pollen allergen *Cry j* I or at least one fragment thereof comprising the steps of culturing a host cell transformed with a nucleic acid sequence encoding Japanese cedar pollen allergen *Cry j* I or at least one fragment thereof in an appropriate medium to produce a mixture of cells and medium containing said Japanese cedar pollen allergen *Cry j* I or at least one fragment

thereof; and purifying the mixture to produce substantially pure Japanese cedar pollen allergen *Cry j* I or at least one fragment thereof. Host cells transformed with an expression vector containing DNA coding for *Cry j* I or at least one fragment thereof are cultured in a suitable medium for the host cell. *Cry j* I protein and peptides can be purified from cell culture medium, host cells, or both using techniques known in the art for purifying peptides and proteins including ion-exchange chromatography, gel filtration chromatography, ultrafiltration, electrophoresis and immunopurification with antibodies specific for *Cry j* I or fragments thereof. The terms isolated and purified are used interchangeably herein and refer to peptides, protein, protein fragments, and nucleic acid sequences substantially free of cellular material or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when synthesized chemically.

Another aspect of the invention provides preparations comprising Japanese cedar pollen allergen *Cry j* I or a cross-reactive allergen such as *Jun v* I or *Jun s* I or at least one fragment thereof synthesized in a host cell transformed with a nucleic acid sequence encoding all or a portion of Japanese cedar pollen allergen *Cry j* I or such cross-reactive allergen, or chemically synthesized, and isolated Japanese cedar pollen allergen *Cry j* I protein or a cross-reactive allergen such as *Jun v* I or *Jun s* I, or at least one antigenic fragment thereof produced in a host cell transformed with a nucleic acid sequence of the invention, or chemically synthesized. In preferred embodiments of the invention the *Cry j* I protein is produced in a host cell transformed with the nucleic acid sequence coding for at least the mature *Cry j* I protein.

Antigenic fragments as defined herein refer to any protein fragment or peptide which induces an immune response. Unique antigenic fragments as defined herein refer to any antigenic fragment derived from Cry j I, with the exception of the fragments consisting of amino acids 1-20 or 325-340 as shown in Figs. 4a-4b. Antigenic fragments of an allergen from Japanese cedar pollen, or a cross-reactive allergen such as *Jun v* I or *Jun s* I may be obtained, for example, by screening peptides recombinantly produced from the corresponding fragment of the nucleic acid sequence of the invention coding for

such peptides or synthesized chemically using techniques known in the art. The allergen may be arbitrarily divided into fragments of a desired length with no overlap of the peptides, or preferably divided into overlapping fragments of a desired length. The fragments are tested to determine their antigenicity (e.g. the ability of the fragment to induce an immune response). If fragments of *Cry j* I are to be used for therapeutic purposes, then the fragments of *Cry j* I allergen which are capable of eliciting a T cell response such as stimulation (i.e., proliferation or lymphokine secretion) and/or are capable of inducing T cell anergy are particularly desirable and fragments of Japanese cedar pollen which have minimal IgE stimulating activity are also desirable. Minimal IgE stimulating activity refers to IgE stimulating activity that is less than the amount of IgE production stimulated by the native *Cry j* I protein. Additionally, for therapeutic purposes, it is preferable to use isolated Japanese cedar pollen allergens, e.g. *Cry j* I, or fragments thereof which are capable of eliciting T cell responses and which do not bind IgE specific for Japanese cedar pollen or bind such IgE to a substantially lesser extent than the purified native Japanese cedar pollen allergen binds such IgE. If the isolated Japanese cedar pollen allergen or fragment or fragments thereof bind IgE, it is preferable that such binding does not result in the release of mediators (e.g. histamines) from mast cells or basophils. Furthermore, if *Jun v* I or *Jun s* I are to be used for therapeutic purposes, it is preferable to use *Juniperus* pollen allergens, e.g. *Jun v* I or *Jun s* I or a fragment thereof which are capable of eliciting T cell responses and which do not bind IgE specific for pollen from the species *Juniperus* or bind such IgE to a substantially lesser extent than the purified native *Juniperus* pollen allergen binds such IgE. If the isolated *Jun v* I or *Jun s* I or fragment or fragments thereof bind IgE, it is preferable that such binding does not result in the release of mediators (e.g. histamines) from mast cells or basophils.

Isolated protein allergens from Japanese cedar pollen or preferred antigenic fragments thereof, when administered to a Japanese cedar pollen-sensitive individual, or an individual allergic to an allergen cross-reactive with Japanese cedar pollen allergen, such as allergen from the pollen of *Juniperus virginiana* or *Juniperus sabinoides* etc. (discussed previously) are capable of

modifying the allergic response of the individual to Japanese cedar pollen or such cross-reactive allergen of the individual, and preferably are capable of modifying the B-cell response, T-cell response or both the B-cell and the T-cell response of the individual to the allergen. As used herein, modification of the allergic response of an individual sensitive to a Japanese cedar pollen allergen or cross-reactive allergen can be defined as non-responsiveness or diminution in symptoms to the allergen, as determined by standard clinical procedures (See e.g. Varney et al, *British Medical Journal,* 302:265-269 (1990)) including dimunition in Japanese cedar pollen induced asthmatic symptoms. As referred to herein, a dimunition in symptoms includes any reduction in allergic response of an individual to the allergen after the individual has completed a treatment regimen with a peptide or protein of the invention. This dimunition may be subjective (i.e. the patient feels more comfortable in the presence of the allergen). Dimunition in symptoms can be determined clinically as well, using standard skin tests as is known in the art.

The isolated *Cry j* I protein or fragments thereof are preferably tested in mammalian models of Japanese cedar pollinosis such as the mouse model disclosed in Tamura et al. (1986) *Microbiol. Immunol.* 30: 883-896, or U.S. patent 4,939,239; or the primate model disclosed in Chiba et al. (1990) *Int. Arch. Allergy Immunol.* 93: 83-88. Initial screening for IgE binding to the protein or fragments thereof may be performed by scratch tests or intradermal skin tests on laboratory animals or human volunteers, or in *in vitro* systems such as RAST (radioallergosorbent test), RAST inhibition, ELISA assay, radioimmunoassay (RIA), or histamine release (see Examples 7 and 8).

Antigenic fragments of the present invention which have T cell stimulating activity, and thus comprise at least one T cell epitope are particularly desirable. T cell epitopes are believed to be involved in initiation and perpetuation of the immune response to a protein allergen which is responsible for the clinical symptoms of allergy. These T cell epitopes are thought to trigger early events at the level of the T helper cell by binding to an appropriate HLA molecule on the surface of an antigen presenting cell and stimulating the relevant T cell subpopulation. These events lead to T cell proliferation, lymphokine

secretion, local inflammatory reactions, recruitment of additional immune cells to the site, and activation of the B cell cascade leading to production of antibodies. One isotype of these antibodies, IgE, is fundamentally important to the development of allergic symptoms and its production is influenced early in the cascade of events, at the level of the T helper cell, by the nature of the lymphokines secreted. A T cell epitope is the basic element or smallest unit of recognition by a T cell receptor, where the epitope comprises amino acids essential to receptor recognition. Amino acid sequences which mimic those of the T cell epitopes and which modify the allergic response to protein allergens are within the scope of this invention.

Exposure of cedar pollen patients to isolated protein allergens of the present invention or to the antigenic fragments of the present invention which comprise at least one T cell epitope and are derived from protein allergens may tolerize or anergize appropriate T cell subpopulations such that they become unresponsive to the protein allergen and do not participate in stimulating an immune response upon such exposure. In addition, administration of a protein allergen of the invention or an antigenic fragment of the present invention which comprises at least one T cell epitope may modify the lymphokine secretion profile as compared with exposure to the naturally-occurring protein allergen or portion thereof (e.g. result in a decrease of IL-4 and/or an increase in IL-2). Furthermore, exposure to such protein allergen or antigenic fragment of such protein allergen may influence T cell subpopulations which normally participate in the response to the allergen such that these T cells are drawn away from the site(s) of normal exposure to the allergen (e.g., nasal mucosa, skin, and lung) towards the site(s) of therapeutic administration of the fragment or protein allergen. This redistribution of T cell subpopulations may ameliorate or reduce the ability of an individual's immune system to stimulate the usual immune response at the site of normal exposure to the allergen, resulting in a dimunution in allergic symptoms.

The isolated *Cry j* I, *Jun v* I or *Jun s* I protein, and fragments or portions derived therefrom (peptides) can be used in methods of diagnosing, treating and preventing allergic reactions to Japanese cedar pollen allergen or a

cross reactive protein allergen. Thus the present invention provides therapeutic compositions comprising isolated Japanese cedar pollen allergen *Cry j* I, *Jun v* I or *Jun s* I or at least one fragment thereof produced in a host cell transformed to express *Cry j* I, *Jun v* I or *Jun s* I or at least one fragment thereof, and a pharmaceutically acceptable carrier or diluent. The therapeutic compositions of the invention may also comprise synthetically prepared *Cry j* I, *Jun v* I or *Jun s* I or at least one fragment thereof and a pharmaceutically acceptable carrier or diluent. Administration of the therapeutic compositions of the present invention to an individual to be desensitized can be carried out using known techniques. *Cry j* I, *Jun v* I or *Jun s* I protein or at least one fragment thereof may be administered to an individual in combination with, for example, an appropriate diluent, a carrier and/or an adjuvant. Pharmaceutically acceptable diluents include saline and aqueous buffer solutions. Pharmaceutically acceptable carriers include polyethylene glycol (Wie et al. (1981) *Int. Arch. Allergy Appl. Immunol.* 64:84-99) and liposomes (Strejan et al. (1984) *J. Neuroimmunol* 7: 27). For purposes of inducing T cell anergy, the therapeutic composition is preferably administered in nonimmunogenic form, e.g. it does not contain adjuvant. The therapeutic compositions of the invention are administered to Japanese cedar pollen-sensitive individuals or individuals sensitive to an allergen which is immunologically cross-reactive with Japanese cedar pollen allergen (i.e. *Juniperus virginiana*, or *Juniperus sabinoides*, etc.).

Administration of the therapeutic compositions of the present invention to an individual to be desensitized can be carried out using known procedures at dosages and for periods of time effective to reduce sensitivity (i.e., reduce the allergic response) of the individual to the allergen. Effective amounts of the therapeutic compositions will vary according to factors such as the degree of sensitivity of the individual to Japanese cedar pollen, the age, sex, and weight of the individual, and the ability of the protein or fragment thereof to elicit an antigenic response in the individual.

The active compound (i.e., protein or fragment thereof) may be administered in a convenient manner such as by injection (subcutaneous, intravenous, etc.), oral administration, inhalation, transdermal application, or

rectal administration. Depending on the route of administration, the active compound may be coated within a material to protect the compound from the action of enzymes, acids and other natural conditions which may inactivate the compound.

For example, preferably about 1 µg- 3 mg and more preferably from about 20-500 µg of active compound (i.e., protein or fragment thereof) per dosage unit may be administered by injection. Dosage regimen may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

To administer protein or peptide by other than parenteral administration, it may be necessary to coat the protein with, or co-administer the protein with, a material to prevent its inactivation. For example, protein or fragment thereof may be administered in an adjuvant, co-administered with enzyme inhibitors or in liposomes. Enzyme inhibitors include pancreatic trypsin inhibitor, diisopropylfluorophosphate (DEP) and trasylol. Liposomes include water-in-oil-in-water CGF emulsions as well as conventional liposomes (Strejan *et al.*, (1984) J. Neuroimmunol. 7:27).

The active compound may also be administered parenterally or intraperitoneally. Dispersions can also be prepared in glycerol, liquid polyethyline glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations may contain a preservative to prevent the growth of microorganisms.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions of dispersion. In all cases, the composition must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glyceral, propylene glycol, and liquid polyetheylene glycol, and the like),

suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as licithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thirmerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol and sorbitol or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about, including in the composition, an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating active compound (i.e., protein or peptide) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile indectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient (i.e., protein or peptide) plus any additional desired ingredient from a previously sterile-filtered solution thereof.

When protein or peptide thereof is suitably protected, as described above, the protein may be orally administered, for example, with an inert diluent or an assimilable edible carrier. The protein and other ingredients may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the individual's diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 1% by weight of active compound. The percentage of the composition and preparations may, of course, be varied and may conveniently be

between about 5 to 80% of the weight of the unit. The amount of active compound in such therapeutically useful compositions is such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit contains between from about 10 μg to about 200 mg of active compound.

The tablets, troches, pills, capsules and the like may also contain the following: a binder such as gum gragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservative, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and formulations.

As used herein "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the therapeutic compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit from as used herein refers to physically discrete units suited as unitary

dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the novel dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

The *Cry j* I cDNA (or the mRNA from which it was transcribed) or a portion thereof can be used to identify similar sequences in any variety or type of plant and thus, to identify or "pull out" sequences which have sufficient homology to hybridize to the *Cry j* I cDNA or mRNA or portion thereof, for example, DNA from allergens of *Juniperus virginiana, Juniperus sabinoides etc.*, under conditions of low stringency. Those sequences which have sufficient homology (generally greater than 40%) can be selected for further assessment using the method described herein. Alternatively, high stringency conditions can be used. In this manner, DNA of the present invention can be used to identify, in other types of plants, preferably related families, genera, or species such as *Juniperus*, or *Cupressus*, sequences encoding polypeptides having amino acid sequences similar to that of Japanese cedar pollen allergen *Cry j* I, and thus to identify allergens in other species. Thus, the present invention includes not only *Cry j* I, but also other allergens encoded by DNA which hybridizes to DNA of the present invention. The invention further includes isolated allergenic proteins or fragments thereof that are immunologically related to *Cry j* I or fragments thereof, such as by antibody cross-reactivity wherein the isolated allergenic proteins or fragments thereof are capable of binding to antibodies specific for the protein and peptides of the invention, or by T cell cross-reactivity wherein the isolated allergenic proteins or fragments thereof are capable of stimulating T cells specific for the protein and peptides of this invention.

Proteins or peptides encoded by the cDNA of the present invention can be used, for example as "purified" allergens. Such purified allergens are useful in the standardization of allergen extracts which are key

reagents for the diagnosis and treatment of Japanese cedar pollinosis. Furthermore, by using peptides based on the nucleic acid sequence of *Cry j* I, anti-peptide antisera or monoclonal antibodies can be made using standard methods. For example, an animal such as a mouse or rabbit can be immunized with an immunogenic from of the isolated *Cry j* I protein (e.g., *Cry j* I protein or antigenic fragment which is capable of eliciting an antibody response). Techniques for conferring immunogenicity on a protein or peptide subunit include conjugation to carriers or other techniques well-known in the art. The *Cry j* I protein or peptide therof can be administered in the presence of adjuvant. The progress of immunization can be monitored by detection of antibody titers in plasma or serum standard ELISA or other immunoassay can be used with the immunogen as antigen to assess the levels of antibodies.

Following immunization, anti-*Cry j* I antisera can be obtained and, if desired, polyclonal anti-*Cry j* I antibodies from the serum. To produce monoclonal antibodies, antibody producing cells (lymphocytes) can be harvested from an immunized animal and fused by standard somatic cell fusion procedures with immortalizing cells such as myeloma cells to yield hybridoma cells. Hybridoma cells can be screened immunochemically for production of antibodies reactive with the *Cry j* I protein or peptide thereof. These sera or monoclonal antibodies can be used to standardize allergen extracts.

Through use of the peptides and protein of the present invention, preparations of consistent, well-defined composition and biological activity can be made and administered for therapeutic purposes (e.g. to modify the allergic response of a Japanese cedar sensitive individual to pollen of such trees). Administration of such peptides or protein may, for example, modify B-cell response to *Cry j* I allergen, T-cell response to *Cry j* I allergen or both responses. Isolated peptides can also be used to study the mechanism of immunotherapy of *Cryptomeria japonica* allergy and to design modified derivatives or analogues useful in immunotherapy.

Work by others has shown that high doses of allergens generally produce the best results (i.e., best symptom relief). However, many people are unable to tolerate large doses of allergens because of allergic reactions to the

allergens. Modification of naturally-occurring allergens can be designed in such a manner that modified peptides or modified allergens which have the same or enhanced therapeutic properties as the corresponding naturally-occurring allergen but have reduced side effects (especially anaphylactic reactions) can be produced. These can be, for example, a protein or peptide of the present invention (e.g., one having all or a portion of the amino acid sequence of *Cry j* I), or a modified protein or peptide, or protein or peptide analogue.

It is also possible to modify the structure of a peptide of the invention for such purposes as increasing solubility, enhancing therapeutic or preventive efficacy, or stability (e.g., shelf life ex vivo, and resistance to proteolytic degradation in vivo). A modified peptide can be produced in which the amino acid sequence has been altered, such as by amino acid substitution, deletion, or addition, to modify immunogenicity and/or reduce allergenicity, or to which a component has been added for the same purpose.

For example, a peptide can be modified so that it maintains the ability to induce T cell anergy and bind MHC proteins without the ability to induce a strong proliferative response or possibly, and proliferative response when administered in immunogenic form. In this instance, critical binding residues for the T cell receptor can be determined using known techniques (e.g., substitution of each residue and determination of the presence or absence of T cell reactivity). Those residues shown to be essential to interact with the T cell receptor can be modified by replacing the essential amino acid with another, preferably similar amino acid residue (a conservative substitution) whose presence is shown to enhance, diminish but not eliminate, or not affect T cell reactivity. In addition, those amino acid residues which are not essential for T cell receptor interaction can be modified by being replaced by another amino acid whose incorporation may enhance, diminish or not affect T cell reactivity but does not eliminate binding to relevant MHC.

Additionally, peptides of the invention can be modified by replacing an amino acid shown to be essential to interact with the MHC protein complex with another, preferably similar amino acid residue (conservative substitution) whose presence is shown to enhance, diminish but not eliminate or

not affect T cell activity. In addition, amino acid residues which are not essential for interaction with the MHC protein complex but which still bind the MHC protein complex can be modified by being replaced by another amino acid whose incorporation may enhance, not affect, or diminish but not eliminate T cell reactivity. Preferred amino acid substitutions for non-essential amino acids include, but are not limited to substitutions with alanine, glutamic acid, or a methyl amino acid.

In order to enhance stability and/or reactivity, the protein or peptides of the invention can also be modified to incorporate one or more polymorphisms in the amino acid sequence of the protein allergen resulting from natural allelic variation. Additionally, D-amino acids, non-natural amino acids or non-amino acid analogues can be substituted or added to produce a modified protein or peptide within the scope of this invention. Furthermore, proteins or peptides of the present invention can be modified using the polyethylene glycol (PEG) method of A. Sehon and co-workers (Wie et al. *supra*) to produce a protein or peptide conjugated with PEG. In addition, PEG can be added during chemical synthesis of a protein or peptide of the invention. Modifications of proteins or peptides or portions thereof can also include reduction/ alyklation (Tarr in: *Methods of Protein Microcharacterization,* J.E. Silver ed. Humana Press, Clifton, NJ, pp 155-194 (1986)); acylation (Tarr, *supra*); chemical coupling to an appropriate carrier (Mishell and Shiigi, eds, *Selected Methods in Cellular Immunology,* WH Freeman, San Francisco, CA (1980); U.S. Patent 4,939,239; or mild formalin treatment (Marsh *International Archives of Allergy and Applied Immunology,* 41:199-215 (1971)).

To facilitate purification and potentially increase solubility of proteins or peptides of the invention, it is possible to add reporter group(s) to the peptide backbone. For example, poly-histidine can be added to a peptide to purify the peptide on immobilized metal ion affinity chromatography (Hochuli, E. et al., *Bio/Technology,* 6:1321-1325 (1988)). In addition, specific endoprotease cleavage sites can be introduced, if desired, between a reporter group and amino acid sequences of a peptide to facilitate isolation of peptides free of irrelevant sequences. In order to successfully desensitize an individual to

a protein antigen, it may be necessary to increase the solubility of a protein or peptide by adding functional groups to the peptide or by not including hydrophobic T cell epitopes or regions containing hydrophobic epitopes in the peptides or hydrophobic regions of the protein or peptide.

To potentially aid proper antigen processing of T cell epitopes within a peptide, canonical protease sensitive sites can be recombinantly or synthetically engineered between regions, each comprising at least one T cell epitope. For example, charged amino acid pairs, such as KK or RR, can be introduced between regions within a peptide during recombinant construction of the peptide. The resulting peptide can be rendered sensitive to cathepsin and/or other trypsin-like enzymes cleavage to generate portions of the peptide containing one or more T cell epitopes. In addition, such charged amino acid residues can result in an increase in solubility of a peptide.

Site-directed mutagenesis of DNA encoding a peptide or protein of the invention (e.g. *Cry j* I or a fragment thereof) can be used to modify the structure of the peptide or protein by methods known in the art. Such methods may, among others, include PCR with degenerate oligonucleotides (Ho et al., *Gene,* 77:51-59 (1989)) or total synthesis of mutated genes (Hostomsky, Z. et al., *Biochem. Biophys, Res. Comm.,* 161:1056-1063 (1989)). To enhance bacterial expression, the aforementioned methods can be used in conjunction with other procedures to change the eucaryotic codons in DNA constructs encoding protein or peptides of the invention to ones preferentially used in *E. coli*, yeast, mammalian cells, or other eukaryotic cells.

Using the structural information now available, it is possible to design *Cry j* I peptides which, when administered to a Japanese cedar pollen sensitive individual in sufficient quantities, will modify the individual's allergic response to Japanese cedar pollen. This can be done, for example, by examining the structure of *Cry j* I, producing peptides (via an expression system, synthetically or otherwise) to be examined for their ability to influence B-cell and/or T-cell responses in Japanese cedar pollen sensitive individuals and selecting appropriate peptides which contain epitopes recognized by the cells. In referring to an epitope, the epitope will be the basic element or smallest unit of

recognition by a receptor, particularly immunoglobulins, histocompatibility antigens and T cell receptors where the epitope comprises amino acids essential to receptor recognition. Amino acid sequences which mimic those of the epitopes and which are capable of down regulating allergic response to *Cry j* I can also be used.

It is now also possible to design an agent or a drug capable of blocking or inhibiting the ability of Japanese cedar pollen allergen to induce an allergic reaction in Japanese cedar pollen sensitive individuals. Such agents could be designed, for example, in such a manner that they would bind to relevant anti-*Cry j* I IgEs, thus preventing IgE-allergen binding and subsequent mast cell degranulation. Alternatively, such agents could bind to cellular components of the immune system, resulting in suppression or desensitization of the allergic response to *Cryptomeria japonica* pollen allergens. A non-restrictive example of this is the use of appropriate B- and T-cell epitope peptides, or modifications thereof, based on the cDNA/protein structures of the present invention to suppress the allergic response to Japanese cedar pollen. This can be carried out by defining the structures of B- and T-cell epitope peptides which affect B- and T-cell function in *in vitro* studies with blood components from Japanese cedar pollen sensitive individuals.

Protein, peptides or antibodies of the present invention can also be used for detecting and diagnosing Japanese cedar pollinosis. For example, this could be done by combining blood or blood products obtained from an individual to be assessed for sensitivity to Japanese cedar pollen with an isolated antigenic peptide or peptides of *Cry j* I, or isolated *Cry j* I protein, under conditions appropriate for binding of components in the blood (e.g., antibodies, T-cells, B- cells) with the peptide(s) or protein and determining the extent to which such binding occurs.

The present invention also provides a method of producing *Cry j* I or fragment thereof comprising culturing a host cell containing an expression vector which contains a nucleic acid sequence e.g. DNA, encoding all or at least one fragment of *Cry j* I under conditions appropriate for expression of *Cry j* I or at least one fragment. The expressed product is then recovered, using known

techniques. Alternatively, *Cry j* I or fragment thereof can be synthesized using known mechanical or chemical techniques.

The DNA used in any embodiment of this invention can be cDNA obtained as described herein, or alternatively, can be any oligodeoxynucleotide sequence having all or a portion of a sequence represented herein, or their functional equivalents. Such oligodeoxynucleotide sequences can be produced chemically or enzymatically, using known techniques. A functional equivalent of an oligonucleotide sequence is one which is 1) a sequence capable of hybridizing to a complementary oligonucleotide to which the sequence (or corresponding sequence portions) of SEQ ID NO: 1 or fragments thereof hybridizes, or 2) the sequence (or corresponding sequence portion) complementary to SEQ ID NO: 1, and/or 3) a sequence which encodes a product (e.g., a polypeptide or peptide) having the same functional characteristics of the product encoded by the sequence (or corresponding sequence portion) of SEQ ID NO: 1. Whether a functional equivalent must meet one or both criteria will depend on its use (e.g., if it is to be used only as an oligoprobe, it need meet only the first or second criteria and if it is to be used to produce a *Cry j* I allergen, it need only meet the third criterion).

The present invention also provides isolated peptides derived from Japanese cedar pollen protein. As used herein, a peptide or fragment of a protein refers to an amino acid sequence having fewer amino acid residues than the entire amino acid sequence of the protein from which it is derived. Peptides of the invention include peptides derived from *Cry j* I which comprise at least one T cell epitope of the allergen.

Peptides comprising at least two regions, each region comprising at least one T cell epitope of Japanese cedar pollen are also within the scope of the invention. Isolated peptides or regions of isolated peptides, each comprising at least two T cell epitopes of a Japanese cedar pollen protein allergen are particularly desirable for increased therapeutic effectiveness. Peptides which are immunologically related (e.g., by antibody or T cell cross-reactivity) to peptides of the present invention are also within the scope of the invention.

Isolated peptides of the invention can be produced by recombinant

DNA techniques in a host cell transformed with a nucleic acid having a sequence encoding such peptide as discussed above. The isolated peptides of the invention can also be produced by chemical synthesis. With regard to isolated *Jun v* I protein or peptides, such protein or peptides may be produced by biochemically purifying the native *Jun v* I proteins from *Juniperus virginiana* pollen as is known in the art. When a peptide is produced by recombinant techniques, host cells transformed with a nucleic acid having a sequence encoding the peptide or the functional equivalent of the nucleic acid sequence are cultured in a medium suitable for the cells and peptides can be purified from cell culture medium, host cells, or both using techniques known in the art for purifying peptides and proteins including ion-exchange chromatography, gel filtration chromatography, ultrafiltration, electrophoresis or immunopurification with antibodies specific for the peptide, the protein allergen Japanese cedar pollen from which the peptide is derived, or a portion thereof. Isolated peptides of the invention are substantially free of cellular material or culture medium when produced by recombinant DNA techniques, or substantially free of chemical precursors or other chemicals when synthesized chemically.

To obtain isolated peptides of the present invention, *Cry j* I is divided into non-overlapping peptides of desired length or overlapping peptides of desired lengths as discussed in Example 6 which can be produced recombinantly, or synthetically. Peptides comprising at least one T cell epitope are capable of eliciting a T cell response, such as T cell proliferation or lymphokine secretion, and/or are capable of inducing T cell anergy (i.e., tolerization). To determine peptides comprising at least one T cell epitope, isolated peptides are tested by, for example, T cell biology techniques, to determine whether the peptides elicit a T cell response or induce T cell anergy. Those peptides found to elicit a T cell response or induce T cell anergy are defined as having T cell stimulating activity.

As discussed in Example 6, human T cell stimulating activity can be tested by culturing T cells obtained from an individual sensitive to Japanese cedar pollen allergen, (i.e., an individual who has an IgE mediated immune response to Japanese cedar pollen allergen) with a peptide derived from the allergen and

determining whether proliferation of T cells occurs in response to the peptide as measured, e.g., by cellular uptake of tritiated thymidine. Stimulation indices for responses by T cells to peptides can be calculated as the maximum CPM in response to a peptide divided by the control CPM. A stimulation index (S.I.) equal to or greater than two times the background level is considered "positive". Positive results are used to calculate the mean stimulation index for each peptide for the group of patients tested. Preferred peptides of this invention comprise at least one T cell epitope and have a mean T cell stimulation index of greater than or equal to 2.0. A peptide having a mean T cell stimulation index of greater than or equal to 2.0 is considered useful as a therapeutic agent. Preferred peptides have a mean T cell stimulation index of at least 2.5, more preferably at least 3.5, more preferably at least 4.0, more preferably at least 5, even more preferably at least 7 and and most preferably at least about 9. For example, peptides of the invention having a mean T cell stimulation index of at least 5, as shown in Fig. 14, include CJ1-2, CJ1-7, CJ1-10, CJ1-16, CJ1-17, CJ1-20, CJ1-22, CJ1-23, CJ1-24, CJ1-27, CJ1-31, CJ1-32 and CJ1-35. For example, peptides of the invention having a mean T cell stimulation index of at least 7, as shown in Fig. 14, include CJI-16, CJ1-20, CJ1-22, and CJ1-32.

In addition, preferred peptides have a positivity index (P.I.) of at least about 100, more preferably at least about 250 and most preferably at least about 350. The positivity index for a peptide is determined by multiplying the mean T cell stimulation index by the percent of individuals, in a population of individuals sensitive to Japanese cedar pollen (e.g., preferably at least 15 individuals, more preferably at least 30 individuals or more), who have a T cell stimulation index to such peptide of at least 2.0. Thus, the positivity index represents both the strength of a T cell response to a peptide (S.I.) and the frequency of a T cell response to a peptide in a population of individuals sensitive to Japanese cedar pollen. For example, as shown in Fig. 14, peptide CJ1-22 has a mean S.I. of 14.5 and 60.0% of positive responses in the group of individuals tested resulting in a positivity index of 870.00. Peptides of *Cry j* I having a positivity index of at least about 100 and a mean T cell stimulation index of at least about 4 include: CJ1-16, CJ1-17, CJ1-20, CJ1-22, CJ1-23, CJ1-

24, CJ1-26, CJ1-27, CJ1-32 and CJ1-35.

In order to determine precise T cell epitopes by, for example, fine mapping techniques, a peptide having T cell stimulating activity and thus comprising at least one T cell epitope as determined by T cell biology techniques is modified by addition or deletion of amino acid residues at either the amino or carboxy terminus of the peptide and tested to determine a change in T cell reactivity to the modified peptide. If two or more peptides which share an area of overlap in the native protein sequence are found to have human T cell stimulating activity, as determined by T cell biology techniques, additional peptides can be produced comprising all or a portion of such peptides and these additional peptides can be tested by a similar procedure. Following this technique, peptides are selected and produced recombinantly or synthetically. Peptides are selected based on various factors, including the strength of the T cell response to the peptide (e.g., stimulation index), the frequency of the T cell response to the peptide in a population of individuals sensitive to Japanese cedar pollen, and the potential cross-reactivity of the peptide with other allergens from other species of trees as discussed earlier (e.g. *Cupressus sempervirens, Cupressus arizonica, Juniperus virginiana, Juniperus sabinoides*, etc.) or ragweed (*Amb a* I.1) . The physical and chemical properties of these selected peptides (e.g., solubility, stability) are examined to determine whether the peptides are suitable for use in therapeutic compositions or whether the peptides require modification as described herein. The ability of the selected peptides or selected modified peptides to stimulate human T cells (e.g., induce proliferation, lymphokine secretion) is determined.

Additionally, preferred T cell epitope-containing peptides of the invention do not bind immunoglobulin E (IgE) or bind IgE to a substantially lesser extent than the protein allergen from which the peptide is derived binds IgE. The major complications of standard immunotherapy are IgE-mediated responses such as anaphylaxis. Immunoglobulin E is a mediator of anaphylactic reactions which result from the binding and cross-linking of antigen to IgE on mast cells or basophils and the release of mediators (e.g., histamine, serotonin, eosinophil chemotacic factors). Thus, anaphylaxis in a substantial percentage of

a population of individuals sensitive to *Cry j* I could be avoided by the use in immunotherapy of a peptide or peptides which do not bind IgE in a substantial percentage (e.g., at least about 75%) of a population of individuals sensitive to *Cry j* I allergen, or if the peptide binds IgE, such binding does not result in the release of mediators from mast cells or basophils. The risk of anaphylaxis could be reduced by the use in immunotherapy of a peptide or peptides which have reduced IgE binding. Moreover, peptides which have minimal IgE stimulating activity are desirable for therapeutic effectiveness. Minimal IgE stimulating activity refers to IgE production that is less than the amount of IgE production and/or IL-4 production stimulated by the native *Cry j* I protein allergen.

A T cell epitope containing peptide of the invention, when administered to a Japanese cedar pollen-sensitive individual, is capable of modifying the allergic response of the individual to the allergen. Particularly, peptides of the invention comprising at least one T cell epitope of *Cry j* I or at least two regions derived from *Cry j* I, each comprising at least one T cell epitope, when administered to an individual sensitive to Japanese cedar pollen are capable of modifying T cell response of the individual to the allergen.

A preferred isolated peptide of the invention comprises at least one T cell epitope of the Japanese cedar pollen allergen, *Cry j* I and accordingly the peptide comprises at least approximately seven amino acid residues. For purposes of therapeutic effectiveness, preferred therapeutic compositions of the invention preferably comprise at least two T cell epitopes of *Cry j* I, and accordingly, the peptide comprises at least approximately eight amino acid residues and preferably at least fifteen amino acid residues. Additionally, therapeutic compositions comprising preferred isolated peptides of the invention preferably comprise a sufficient percentage of the T cell epitopes of the entire protein allergen such that a therapeutic regimen of administration of the composition to an individual sensitive to Japanese cedar pollen, results in T cells of the individual being tolerized to the protein allergen. Synthetically produced peptides of the invention comprising up to approximately forty-five amino acid residues in length, and most preferably up to approximately thirty amino acid residues in length are particularly desirable as increases in length may result in

difficulty in peptide synthesis. Peptides of the invention may also be produced recombinantly as described above, and it is preferable that peptides of 45 amino acids or longer be produced recombinantly.

Preferred peptides comprise all or a portion of the areas of major T cell reactivity within the *Cry j* I protein allergen designated herein as, Region 1, Region 2, Region 3, Region 4 and Region 5. Each major area of T cell activity is defined as follows and is shown in Fig. 4 a-b. Region 1 comprises amino acid residues 1-50 of *Cry j* I ; Region 2 comprises amino acid residues 61-120 of *Cry j* I; Region 3 comprises amino acid residues 131-180 of *Cry j* I; Region 4 comprises amino acid residues 191-280 of *Cry j* I; Region 5 comprises amino acid residues 291-353 of the *Cry j* I. Preferred areas of major T cell reactivity within each Region as shown in Fig. 4 a-b and comprise: amino acid residues 1-40; amino acid residues 81-110; amino acid residues 151-180; amino acid residues 191-260; and amino acid residues 291-330.

Peptides derived from the *Cry j* I protein allergen which can be used for therapeutic purposes comprise all or a portion of the following peptides: CJ1-1, CJ1-2, CJ1-3, CJ1-4, CJ1-7, CJ1-8, CJ1-9, CJ1-10, CJ1-11, CJ1-12, CJ1-14, CJ1-15, CJ1-16, CJ1-17, CJ1-18, CJ1-19, CJ1-20, CJ1-21, CJ1-22, CJ1-23, CJ1-24, CJ1-25, CJ1-26, CJ1-27, CJ1-28, CJ1-30, CJ1-31, CJ1-32, CJ1-33, CJ1-34 and CJ1-35 wherein the portion of the peptide preferably has a mean T cell stimulation index equivalent to, or greater than the mean T cell stimulation index of the peptide from which it is derived as shown in Fig. 14. Even more preferably peptides derived from the *Cry j* I protein allergen which can be used for therapeutic purposes comprise all or a portion of the following peptides: CJ1-2, CJ1-9, CJ1-10, CJ1-16, CJ1-17, CJ1-20, CJ1-22, CJ1-23, CJ1-24, CJ1-25, CJ1-26, CJ1-27, CJ1-30, CJ1-31, CJ1-32 and CJ1-35 as shown in Fig. 14. Additionally, other preferred peptides derived from the Cry j I protein comprise the following peptides: CJ1-41, CJ1-41.1, CJ1-41.2, CJ1-41.3, CJ1-42, CJ1-42.1, CJ1-42.2, CJ1-43, CJ1-43.1, CJ1-43.6,CJ1-43.7, CJ1-43.8, CJ1-43.9, CJ1-43.10, CJ1-43.11, CJ1-43.12, CJ1-45, CJ1-45.1, CJ1-45.2, CJ1-44, CJ1-44.1, CJ1-44.2 and CJ1-44.3, all as shown in Fig. 18. Another preferred antigenic peptide of the invention may comprise more than one Region, i.e., all or a portion of amino

acids 151-352 of the amino acid sequence of *Cry j* I, as shown in Fig. 4a-b.

One embodiment of the present invention features a peptide or portion thereof of *Cry j* I which comprises at least one T cell epitope of the protein allergen and has a formula X_n-Y-Z_m. According to the formula, Y is an amino acid sequence selected from the group consisting of CJ1-1, CJ1-2, CJ1-3, CJ1-4, CJ1-7, CJ1-8, CJ1-9, CJ1-10, CJ1-11, CJ1-12, CJ1-14, CJ1-15, CJ1-16, CJ1-17, CJ1-18, CJ1-19, CJ1-20, CJ1-21, CJ1-22, CJ1-23, CJ1-24, CJ1-25, CJ1-26, CJ1-27, CJ1-28 CJ1-30, CJ1-31, CJ1-32, CJ1-33, CJ1-34 and CJ1-35, and preferably selected from the group consisting of CJ1-2, CJ1-9, CJ1-10, CJ1-16, CJ1-17, CJ1-20, CJ1-22, CJ1-23, CJ1-24, CJ1-25, CJ1-26, CJ1-27, CJ1-30, CJ1-31, CJ1-32 and CJ1-35. In addition, X_n are amino acid residues contiguous to the amino terminus of Y in the amino acid sequence of the protein allergen and Z_m are amino acid residues contiguous to the carboxy terminus of Y in the amino acid sequence of the protein allergen. In the formula, n is 0-30 and m is 0-30. Preferably, the peptide or portion thereof has a mean T cell stimulation index equivalent to greater than the mean T cell stimulation index of Y as shown in Fig. 14.

Another embodiment of the present invention provides peptides comprising at least two regions, each region comprising at least one T cell epitope of *Cry j* I and accordingly each region comprises at least approximately seven amino acid residues. These peptides comprising at least two regions can comprise as many amino acid residues as desired and preferably comprise at least about 14, even more preferably about 30, and most preferably at least about 40 amino acid residues of a *Cry j* I allergen. If desired, the amino acid sequences of the regions can be produced and joined by a linker to increase sensitivity to processing by antigen-presenting cells. Such linker can be any non-epitope amino acid sequence or other appropriate linking or joining agent. To obtain preferred peptides comprising at least two regions, each comprising at least one T cell epitope, the regions are arranged in a configuration different from a naturally-occurring configuration of the regions in the allergen. For example, the regions containing T cell epitope(s) can be arranged in a noncontiguous configuration and can preferably be derived from the same protein allergen.

Noncontiguous is defined as an arrangement of regions containing T cell epitope(s) which is different than that of an amino acid sequence present in the protein allergen from which the regions are derived. Furthermore, the noncontiguous regions containing T cell epitopes can be arranged in a nonsequential order (e.g., in an order different from the order of the amino acids of the native protein allergen from which the region containing T cell epitope(s) are derived in which amino acids are arranged from an amino terminus to a carboxy terminus). A peptide can comprise at least 15%, at least 30%, at least 50% or up to 100% of the T cell epitopes of *Cry j* I.

The individual peptide regions can be produced and tested to determine which regions bind immunoglobulin E specific for *Cry j* I and which of such regions would cause the release of mediators (e.g., histamine) from mast cells or basophils. Those peptide regions found to bind immunoglobulin E and cause the release of mediators from mast cells or basophils in greater than approximately 10-15% of the allergic sera tested are preferably not included in the peptide regions arranged to form preferred peptides of the invention.

Additionally, regions of a peptide of the invention preferably comprise all or a portion of the above discussed preferred areas of major T cell reactivity within *Cry j* I (i.e., Regions 1-5) or the above discussed preferred areas of major T cell activity within each Region (i.e. amino acids from residues 1-40, 81-110, 151-180, 191-260 and 291-330). For example, one region can comprise all or a portion of Region 1 (amino acid residues 1-51) and one region can comprise all or a portion of Region 2 (amino acid residues 61-120). Peptides of the invention can comprise all or a portion of two or more of these Regions (i.e., Regions 1-5) and preferred resulting peptides do not bind IgE and cause the release of mediators from most cells or basophils. Preferred peptides derived from *Cry j* I comprise all or a portion of Region 3, Region 4 and Region 5, and, optionally, Region 1 or Region 2. Further, if one of these Regions is found to bind IgE and cause the release of mediators from mast cells or basophils, then it is preferred that the peptide not comprise such Region, but rather comprises various regions derived from such Region which do not bind IgE or cause release of mediators from mast cells or basophils.

Examples of preferred regions include: CJ1-1, CJ1-2, CJ1-3, CJ1-4, CJ1-7, CJ1-8, CJ1-9, CJ1-10, CJ1-11, CJ1-12, CJ1-14, CJ1-15, CJ1-16, CJ1-17, CJ1-18, CJ1-19, CJ1-20, CJ1-21, CJ1-22, CJ1-23, CJ1-24, CJ1-25, CJ1-26, CJ1-27, CJ1-28, CJ1-30, CJ1-31, CJ1-32, CJ1-33, CJ1-34, CJ1-35, CJ1-41, CJ1-41.1, CJ1-41.2, CJ1-41.3, CJ1-42, CJ1-42.1, CJ1-42.2, CJ1-43, CJ1-43.1, CJ1-43.6,CJ1-43.7, CJ1-43.8, CJ1-43.9, CJ1-43.10, CJ1-43.11, CJ1-43.12, CJ1-45, CJ1-45.1, CJ1-45.2, CJ1-44, CJ1-44.1, CJ1-44.2 and CJ1-44.3, the amino acid sequences of such regions being shown in Fig. 13 and Fig. 18, or portions of said regions comprising at least one T cell epitope.

Preferred peptides comprise various combinations of two or more regions, each region comprising all or a portion of the above-discussed preferred areas of major T cell reactivity. Preferred peptides comprise a combination of two or more regions (each region having an amino acid sequence as shown in Fig. 13), including:

CJ1-1, CJ1-2 and CJ1-3;
CJ1-1 and CJ1-2;
CJ1-9 and CJ1-10;
CJ1-14, CJ1-15, CJ1-16 and CJ1-17;
CJ1-20, CJ1-21, CJ1-22, CJ1-23;
CJ1-20, CJ1-22 and CJ1-23;
CJ1-22 and CJ1-23;
CJ1-22, CJ1-23 and CJ1-24;
CJ1-24 and CJ1-25;
CJ1-30, CJ1-31 and CJ1-32;
CJ1-31 and CJ1-32;
CJ1-22, CJ1-23, CJ1-16 and CJ1-17;
CJ1-22, CJ1-23, CJ1-31 and CJ1-32;
CJ1-16, CJ1-17, CJ1-31 and CJ1-32;
CJ1-9, CJ1-10 and CJ1-16;
CJ1-16 and CJ1-17;
CJ1-17, CJ1-22 and CJ1-23;
CJ1-16, CJ1-17 and CJ1-20;

CJ1-31, CJ1-32 and CJ1-20;
CJ1-22, CJ1-23, CJ1-1, CJ1-2 and CJ1-3;
CJ1-16, CJ1-17, CJ1-22 and CJ1-23, CJ1-31 and CJ1-32;
CJ1-9, CJ1-10, CJ1-16, CJ1-17, CJ1-22 and CJ1-23;
CJ1-9, CJ1-10, CJ1-16, CJ1-17, CJ1-31 and CJ1-32;
CJ1-9, CJ1-10, CJ1-22, CJ1-23, CJ1-31 and CJ1-32;
CJ1-9, CJ1-10, CJ1-16, CJ1-17, CJ1-22, CJ1-23, CJ1-31 and CJ1-32;
CJ1-1, CJ1-2, CJ1-16, CJ1-17, CJ1-22 and CJ1-23;
CJ1-22, CJ1-23, CJ1-24, CJ1-9, and CJ1-10;
CJ1-22, CJ1-23, CJ1-24, CJ1-9, CJ1-10, CJ1-16, and CJ1-17;
CJ1-22, CJ1-23, CJ1-24, CJ1-16, CJ1-17, CJ1-31 and CJ1-32;
CJ1-22, CJ1-23, CJ1-24, CJ1-16, and CJ1-17;
CJ1-22, CJ1-23, CJ1-24, CJ1-9, CJ1-10, CJ1-31 and CJ1-32;
CJ1-22, CJ1-23, CJ1-24, CJ1-9, CJ1-10, CJ1-16, CJ1-17, CJ1-31 and CJ1-32; and
CJ1-22, CJ1-23, CJ1-24, CJ1-31, and CJ1-32.

Isolated *Cry j* I protein or peptides of *Cry j* I within the scope of the invention can be used in methods of treating and preventing allergic reactions to Japanese cedar pollen. Thus, one aspect of the present invention provides therapeutic compositions comprising a peptide of *Cry j* I including at least one T cell epitope, or preferably at least two T cell epitopes, and a pharmaceutically acceptable carrier or diluent. In another aspect, the therapeutic composition comprises a pharmaceutically acceptable carrier or diluent and a peptide comprising at least two regions, each region comprising at least one T cell epitope of *Cry j* I.

Preferred therapeutic compositions comprise a sufficient percentage of the T cell epitopes of *Cry j* I such that a therapeutic regimen of administration of the composition to an individual sensitive to Japanese cedar pollen allergen, results in T cells of the individual being tolerized to the protein allergen. More preferably, the composition comprises a sufficient percentage of the T cell

epitopes such that at least about 40%, and more preferably at least about 60% of the T cell reactivity of *Cry j* I is included in the composition. Such compositions can be administered to an individual to treat or prevent sensitivity to Japanese cedar pollen or to an allergen which is immunologically cross-reactive with Japanese cedar pollen allergen.

In yet another aspect of the present invention, a composition is provided comprising at least two peptides (e.g., a physical mixture of at least two peptides), each comprising at least one T cell epitope of *Cry j* I. Such compositions can be administered in the form of a therapeutic composition with a pharmaceutically acceptable carrier of diluent. A therapeutically effective amount of one or more of such compositions can be administered simultaneously or sequentially to an individual sensitive to Japanese cedar pollen.

Preferred compositions and preferred combinations of peptides which can be administered simultaneously or sequentially (comprising peptides having amino acid sequences shown in Fig. 13) include the following combinations:

CJ1-1, CJ1-2 and CJ1-3:
CJ1-1 and CJ1-2;
CJ1-9 and CJ1-10;
CJ1-14, CJ1-15, CJ1-16 and CJ1-17;
CJ1-20, CJ1-21, CJ1-22 and CJ1-23;
CJ1-20, CJ1-22 and CJ1-23;
CJ1-22 and CJ1-23;
CJ1-22, CJ1-23 and CJ1-24;
CJ1-24 and CJ1-25;
CJ1-30, CJ1-31 and CJ1-32;
CJ1-31 and CJ1-32;
CJ1-22, CJ1-23, CJ1-16 and CJ1-17;
CJ1-22, CJ1-23, CJ1-31 and CJ1-32;
CJ1-16, CJ1-17, CJ1-31 and CJ1-32;
CJ1-9, CJ1-10 and CJ1-16;
CJ1-16 and CJ1-17;
CJ1-17, CJ1-22 and CJ1-23;

CJ1-16, CJ1-17 and CJ1-20;
CJ1-31, CJ1-32 and CJ1-20;
CJ1-22, CJ1-23, CJ1-1, CJ1-2 and CJ1-3;
CJ1-16, CJ1-17, CJ1-22, CJ1-23, CJ1-31 and CJ1-32;
CJ1-9, CJ1-10, CJ1-16, CJ1-17, CJ1-22 and CJ1-23;
CJ1-9, CJ1-10, CJ1-16, CJ1-17, CJ1-31 and CJ1-32;
CJ1-9, CJ1-10, CJ1-22, CJ1-23, CJ1-31 and CJ1-32;
CJ1-9, CJ1-10, CJ1-16, CJ1-17, CJ1-22, CJ1-23, CJ1-31 and CJ1-32;
CJ1-1, CJ1-2, CJ1-16, CJ1-17, CJ1-22 and CJ1-23.
CJ1-22, CJ1-23, CJ1-24, CJ1-9, and CJ1-10;
CJ1-22, CJ1-23, CJ1-24, CJ1-9, CJ1-10, CJ1-16, and CJ1-17;
CJ1-22, CJ1-23, CJ1-24, CJ1-16, CJ1-17, CJ1-31 and CJ1-32;
CJ1-22, CJ1-23, CJ1-24, CJ1-16, and CJ1-17;
CJ1-22, CJ1-23, CJ1-24, CJ1-9, CJ1-10, CJ1-31 and CJ1-32;
CJ1-22, CJ1-23, CJ1-24, CJ1-9, CJ1-10, CJ1-16, CJ1-17, CJ1-31 and CJ1-32; and
CJ1-22, CJ1-23, CJ1-24, CJ1-31, and CJ1-32.

The invention is further illustrated by the following non-limiting examples.

Example 1

Purification of Native Japanese Cedar Pollen Allergen (*Cry j* I)

The following is a description of the work done to biochemically purify the major allergen, *Cry j* I in the native form. The purification was modified from published procedures (Yasueda et al., *J. Allergy Clin. Immunol.* 71:77, 1983).

100g of Japanese cedar pollen obtained from Japan (Hollister-Stier, Spokane, WA) was defatted in 1 L diethyl ether three times, the pollen was collected after filtration and the ether was dried off in a vacuum.

The defatted pollen was extracted at 4°C overnight in 2 L extraction buffer containing 50 mM tris-HCL, pH 7.8, 0.2 M NaCl and protease inhibitors in final concentrations: soybean trypsin inhibitor (2 µg/ml), leupeptin (1 µg/ml), pepstatin A (1 µg/ml) and phenyl methyl sulfonyl fluoride (0.17 mg/ml). The insoluble material was reextracted with 1.2 L extraction buffer at 4°C overnight and both extracts were combined together and depigmented by batch absorption with Whatman DE-52 DEAE cellulose (200 g dry weight) equilibrated with the extraction buffer.

The depigmented material was then fractionated by ammonium sulfate precipitation at 80% saturation (4°C), which removed much of the lower molecular weight material. The resultant partially purified *Cry j* I was either dialyzed in PBS buffer and used in T cell studies (see Example 6) or subjected to further purification (biochemically or by monoclonal affinity chromatography) as described below.

The enriched *Cry j* I material was then dialyzed against 50 mM Na-acetate, pH 5.0 at 4°C with 50 mM Na-acetate, pH 5.0 with protease inhibitors. The sample was next applied to a 100 ml DEAE cellulose column (Whatman DE-52) equilibrated at 4°C with 50 mM Na-acetate pH 5.0 with protease inhibitors. The unbound material (basic proteins) was then applied to a 50 ml cation exchange column (Whatman CM-52) which was equilibrated at 4°C with 10 mM Na-acetate, pH 5.0 with protease inhibitors. *Cry j* I was eluted in the early fractions of a linear gradient 0.3 M NaCl. The enriched *Cry j* I material was lyophilized and was then purified by FPLC over a 300 ml Superdex 75 column (Pharmacia) at a flow rate of 30 ml/h in 10 mM Na-acetate, pH 5.0 at 25°C.

The purified *Cry j* I was further applied to FPLC S-Sepharose 16/10 column chromatography (Pharmacia) with a linear gradient of 0 - 1 M NaCl at 25°C. *Cry j* I, eluted as the major peak, was subjected to a second gel filtration chromatography. FPLC Superdex 75 column (2.6 by 60 cm)(Pharmacia, Piscataway, NJ) was eluted with a downward flow of 10 mM Na-acetate, pH 5.0 with 0.15 M NaCl at a flow rate of 30 ml/h at 25°C. Fig. 1a shows the chromatography on gel filtration. Only *Cry j* I was detected (Fig. 1b, lane 2 to lane 8). *Cry j* I was fractionated into 3 bands as analyzed by SDS-PAGE using silver

staining (Fig. 1b) As shown in Fig. 1b, SDS PAGE (12.5%) analysis of the fractions from the major peak shown in Fig. 1a was performed under reducing conditions. The gel was silver stained using the silver staining kit from Bio-Rad. The samples in each lane were as follows: Lane 1, prestained standard proteins (Gibco BRL) including ovalbumin (43,000 kD), carbonic anhydrase (29,000 kD), and α-lactoglobulin (18,400 kD); lane 2, fraction 36 ; lane 3 fraction 37; lane 4 fraction 38; lane 5 fraction 39 ; lane 6 fraction 41, lane 7 fraction 43; and lane 8 fraction 44. All fractions are shown in Fig. 1a.

These proteins were also analyzed by Western blotting using mouse monoclonal antibody CBF2 (Fig. 2). As shown in Fig. 2, an aliquot of fraction 36 (lane 1), fraction 39, (lane 2) and fraction 43 (lane 3) purified from the Superdex 75 as shown in Fig. 1 was separated by SDS-PAGE, electroblotted onto nitrocelluslose and probed with mAB CBF2. Biotinlylated goat anti-mouse Ig was used for the second antibody and bound antibody was revealed by ^{125}I-streptavidin. The monoclonal CBF2 was raised against ragweed allergen *Amb a* I by Dr. D. Klapper (Chapel Hill, NC). Because of the homology between the *Amb a* I and *Cry j* I sequences, a number of antibodies raised against *Amb a* I were tested for reactivity with *Cry j* I. The results showed that CBF2 recognized denatured *Cry j* I as detected by ELISA and Western blotting. In addition, Western blotting also demonstrated that no other bands were detected by CBF2, other than *Cry j* I in the expected molecular weight range (Fig. 2). These results were consistent with the findings from protein sequencing. When fraction 44 and fraction 39 (Fig 1b) were subjected to N-terminal sequencing, only *Cry j* I sequence was detected.

In summary, three *Cry j* I isoforms of different molecular weight were purified from pollen extract. The molecular weights estimated by SDS-PAGE ranged from 40-35 kD under both reducing and non-reducing conditions. The isoelectric point of these isoforms is approximately 9.5-8.6, with an average pI of 9.0. The N-terminal 20 amino acid sequence was the same in these 3 bands and was identical to previously published *Cry j* I sequence (Taniai et al, *supra*). The 3 isoforms are all recognized by monoclonal antibody CBF2 as shown in the allergic sera titration of different purified subfractions of Cry j I using a pool of fifteen allergic patient plasma. They all bind allergic patient IgE (Fig. 3). The difference in

molecular weight and isoelectric point in these isoforms might in part be due to post-translational modification, e.g. glycosylation, phosphorylation or lipid content. The possibility that these different isoforms might be due to protease degradation cannot be ruled out at present even though it is unlikely due to the fact that four different protease inhibitors were used during extraction and purification. The other possibility could be due to polymorphism in the gene or alternate splicing in the mRNA though only one major form of *Cry j* I protein has been detected in cDNA cloning studies (see Example 4).

Another approach which may be used to purify native *Cry j* I or recombinant *Cry j* I is immunoaffinity chromatography. This technique provides a very selective protein purification due to the specificity of the interaction between monoclonal antibodies and antigen. For the purpose of producing *Cry j* I-reactive monoclonal antibodies, female Balbl/c mice were obtained from Jackson Labs. Each mouse was initially immunized intraperitoneally with 70-100 µg purified native *Cry j* I, (>99% purity lower band, as shown in Fig. 1b), emulsified in Freund's complete adjuvant. One further intravenous injection of 10 µg purified native *Cry j* I in PBS was given 54 days after the initial injection. The spleen was removed 3 days later and myeloma fusion was conducted as described (Current Protocols in Immunology, 1991, Coligan et al, eds.) using the myeloma line SP2.0. The cells were cultured in 10% fetal calf serum (Hybrimax), hypoxanthine and azaserine and wells containing colonies of hybridoma cells were screened for antibody production using antigen-binding ELISA.

Cells from positive wells were cloned at three-tenths cell/well in 10% fetal calf serum (Hybrimax), hypoxanthine and positive clones were subcloned one more time in hypoxanthine medium . Capture ELISA (see Example 7) was used for secondary and tertiary screening. This assay offers the advantage that a clone that recognizes the native protein may be selected and thus may be useful for immunoaffinity purification. For example, two monoclonal antibodies (4B11, 8B11) were generated. These antibodies were purified by Gammabind G. Sepharose (Pharmacia, Piscataway, NJ) according to manufacturer's procedures and were immobilized to cyanogen bromide - activated Sepharose 4B (Pharmacia, Piscataway,

NJ) according to the procedures described by Pharmacia. The ammonium sulphate preparation containing *Cry j* I was applied to the resin and unbound material was washed extensively with PBS. *Cry j* I was eluted with 2 column volumes of 0.1 M glycine, pH 2.7. Silver staining of the eluate fractions run on SDS PAGE showed that *Cry j* I was purified almost to homogeneity. These fractions did not contain detectable levels of *Cry j* II. Other methods to immobilize MAb 8B11 were also tested. Similar results were obtained using purified MAb 8B11 covalently cross-linked to Gammabind G Sepharose by dimethylpimelimidate (Schneider C., et al. *J. Biol. Chem.* (1982) volume 257:10766-10769). However, experiments using purified MAb 8B11 covalently cross-linked to Affi-gel 10 (Biorad, Richmond, CA) showed that although greater than 90% of the monoclonal antibody was covalently coupled to Affi-gel 10, the yield of *Cry j* I purified over this resin was significantly less than that purified from MAb 8B11 cross-linked to cyanogen bromide-activated Sepharose 4B (data not shown). Nevertheless, the purified *Cry j* I from these monoclonal antibodies immobilized on different resins is still intact and can be recognized by MAb 8B11 and 4B11 by capture ELISA. Thus, these MAbs will provide a useful tool in purification of *Cry j* I from pollen extracts. Similarly, monoclonal antibodies that bind to recombinant *Cry j* I can also be used for immunoaffinity chromatography. In addition, the monoclonal antibodies generated may be useful for diagnostic purposes. It may also be possible to raise different MAbs that show some specificity towards these different isoforms of *Cry j* I and thus would provide a useful tool to characterize these isoforms.

Example 2

Attempted Extraction of RNA From Japanese Cedar Pollen

Multiple attempts were made to obtain RNA from commercially-available, non-defatted, *Cryptomeria japonica* (Japanese cedar) pollen (Hollister Stier, Seattle, WA). Initially, the method of Sambrook et al., *Molecular Cloning. A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring

Harbor, New York (1989) was used in which the sample was suspended and lysed in 4 M guanidine buffer, ground under liquid nitrogen, and pelleted through 5.7 M cesium chloride by ultracentrifugation. Various amounts (3, 5 and 10 g) of pollen in varying amounts of guanidine lysis buffer (10 and 25 ml) were tried. Centrifugation through cesium resulted in viscous material in the bottom of the tube, from which it was not possible to recover an RNA pellet. Although it was possible to obtain RNA from defatted *Ambrosia artemisiifolia* (ragweed) pollen (Greer Laboratories, Lenior, NC) using this protocol, defatting the *Cryptomeria japonica* pollen with acetone before guanidine extraction also did not yield any RNA, as determined by absorbance at A_{260}.

An acid phenol extraction of RNA according to the method in *Sambrook* et al., *supra* was attempted from *Cryptomeria japonica* pollen. The pollen was ground and sheared in 4.5 M guanidine solution, acidified by addition of 2 M sodium acetate, and extracted with water-saturated phenol plus chloroform. After precipitation, the pellet was washed with 4 M lithium chloride, redissolved in 10 mM Tris/5 mM EDTA/1% SDS, chloroform extracted, and re-precipitated with NaCl and absolute ethanol. It was possible to extract *Ambrosia artemisiifolia* but not *Cryptomeria japonica* RNA with this procedure.

Next, 4 g of *Cryptomeria japonica* pollen was suspended in 10 ml extraction buffer (50 mM Tris, pH 9.0, 0.2 M NaCl, 10 mM Mg acetate and diethylpyrocarbonate (DEPC) to 0.1%), ground in a mortar and pestle on dry ice, transferred to a centrifuge tube with 1% SDS, 10 mM EDTA and 0.5% N-lauroyl sarcosine, and the mixture was extracted five times with warm phenol. The aqueous phase was recovered after the final centrifugation, 2.5 vol. absolute ethanol was added, and the mixture was incubated overnight at 4°C. The pellet was recovered by centrifugation, resuspended in 1 ml dH_20 by heating to 65°C. and reprecipitated by the addition of 0.1 vol. 3 M Na acetate and 2.0 vol. of ethanol. No detectable RNA was recovered in the pellet as judged by absorbance at A_{260} and gel electrophoresis.

Finally, 500 mg of *Cryptomeria japonica* pollen was ground by mortar and pestle on dry ice and suspended in 5 ml of 50 mM Tris pH 9.0 with

0.2 M NaCl, 1 mM EDTA, 1% SDS that had been treated overnight with 0.1% DEPC, as previously described in Frankis and Mascarhenas (1980) *Ann. Bot.* 45: 595- 599. After five extractions with phenol/chloroform/isoamyl alcohol (mixed at 25:24:1), material was precipitated from the aqueous phase with 0.1 volume 3 M sodium acetate and 2 volumes ethanol. The pellet was recovered by centrifugation, resuspended in dH_20 and heated to 65°C to solubilize the precipitated material. Further precipitations with lithium chloride were not done. There was no detectable RNA recovered, as determined by absorbance at A_{260} and gel electrophoresis.

In summary, it has not been possible to recover RNA from the commercial pollen. It is not known whether the RNA has been degraded during storage or shipment, or whether the protocols used in this example did not allow recovery of extant RNA. However, RNA was recovered from fresh *Cryptomeria japonica* pollen and staminate cone samples. (See Example 3.)

Example 3

Extraction of RNA From Japanese Cedar Pollen and Staminate Cones and Cloning of *Cry j I*

Fresh pollen and staminate cone samples, collected from a single *Cryptomeria japonica* (Japanese cedar) tree at the Arnold Arboretum (Boston, MA), were frozen immediately on dry ice. RNA was prepared from 500 mg of each sample, essentially as described by Frankis and Mascarenhas, *supra*. The samples were ground by mortar and pestle on dry ice and suspended in 5 ml of 50 mM Tris pH 9.0 with 0.2 M NaCl, 1 mM EDTA, 1% SDS that had been treated overnight with 0.1% DEPC. After five extractions with phenol/chloroform/ isoamyl alcohol (mixed at 25:24:1), the RNA was precipitated from the aqueous phase with 0.1 volume 2 M sodium acetate and 2 volumes ethanol. The pellets were recovered by centrifugation, resuspended in dH_20 and heated to 65°C for 5 min. Two ml of 4 M lithium chloride were added to the RNA preparations and they were incubated overnight at 0°C. The RNA pellets were recovered by centrifugation, resuspended in 1 ml dH_20, and again

precipitated with 3 M sodium acetate and ethanol overnight. The final pellets were resuspended in 100 µl dH_20 and stored at -80°C.

First strand cDNA was synthesized from 8 µg flowerhead and 4 µg pollen RNA using a commercially available kit (cDNA synthesis systems kit, BRL, Gaithersburg, MD) with oligo dT priming according to the method of Gubler and Hoffman (1983) *Gene* 25: 263-269. An attempt was made to amplify cDNA encoding *Cry j* I using the degenerate oligonucleotide CP-1 (which has the sequence 5'-GATAATCCGATAGATAG-3', wherein T at position 3 can also be C; T at position 6 can also be C; G at position 9 can also be A,T, or C; A at position 12 can also be T, or C; T at position 15 can also be C; A at position 16 can also be T; and G at position 17 can also be C; SEQ ID NO: 3) and primers EDT and ED. Primer EDT has the sequence 5'-GGAATTCTCTAGACTGCA-GGTTTTTTTTTTTTTT-3'(SEQ ID NO: 24). Primer ED has the sequence 5'-GGAATTCTCTAGACTGCAGGT-3' (SEQ ID NO: 23). CP-1 is the degenerate oligonucleotide sequence encoding the first six amino acids of the amino terminus (AspAsnProIleAspSer, amino acids 1-6 of SEQ ID NO: 1) of *Cry j* I. EDT will hybridize with the poly A tail of the gene. All oligonucleotides were synthesized by Research Genetics, Inc. Huntsville, AL. Polymerase chain reactions (PCR) were carried out using a commercially available kit (GeneAmp DNA Amplification kit, Perkin Elmer Cetus, Norwalk, CT) whereby 10 µl 10x buffer containing dNTPs was mixed with 1 µg of CP- 1 and 1 µg of ED/EDT primers (ED:EDT in a 3:1 M ratio), cDNA (3-5 µl of a 20 µl first strand cDNA reaction mix), 0.5 µl Amplitaq DNA polymerase, and distilled water to 100 µl.

The samples were amplified with a programmable thermal controller (MJ Research, Inc., Cambridge, MA). The first 5 rounds of amplification consisted of denaturation at 94°C for 1 minute, annealing of primers to the template at 45°C for 1.5 minutes, and chain elongation at 70°C for 2 minutes. The final 20 rounds of amplification consisted of denaturation as above, annealing at 55°C for 1.5 minutes, and elongation as above. Five percent (5 µl) of this initial amplification was then used in a secondary amplification with 1 µg each of CP-2 (which has the sequence 5'- GGGAATTCAATTGGGC-GCAGAATGG-3' wherein T at position 11 can also be C; G at position 17 can

also be A, T, or C; G at position 20 can also be A; T at position 23 can also be C; and G at position 24 can also be C) (SEQ ID NO: 4), a nested primer, and ED, as above. The sequence 5'-GGGAATTC-3' (bases 1 through 8 of SEQ ID NO: 4) in primer CP-2 represents an *Eco* R1 site added for cloning purposes; the remaining degenerate oligonucleotide sequence encodes amino acids 13-18 of *Cry j* I (AsnTrpAlaGlnAsnArg, amino acids 13 through 18 of SEQ ID NO: 1). Multiple DNA bands were resolved on a 1% GTG agarose gel (FMC, Rockport, ME), none of which hybridized with ^{32}P end- labeled probe CP-3 (SEQ ID NO: 5) in a Southern blot performed according to the method in Sambrook et al. *supra*. Therefore, it was not possible to select a specific *Cry j* I DNA band and this approach was not pursued. CP-3 has the sequence 5'- CTGCAGCCATT-TTCIACATTAAA-3' wherein A at position 9 can also be G; T at position 12 can also be C; A at position 18 can also be G; and A at position 21 can also be G) (SEQ ID NO: 5). Inosine (I) is used at position 15 in place of G or A or T or C to reduce degeneracy (Knoth et al. (1988) *Nucleic Acids Res.* 16: 10932). The sequence 5'-CTGCAG-3' (bases 1 through 6 of SEQ ID NO: 5) in primer CP-3 represent a Pst I site added for cloning purposes; the remaining degenerate oligonucleotide sequence is the non-coding strand sequence corresponding to coding strand sequence encoding amino acids PheAsnValGluAsnGly (amino acids 327 through 332 of SEQ ID NO: 1) from the internal sequence of *Cry j* I.

A primary PCR was also performed on first-strand cDNA using CP-1 (SEQ ID NO: 3) and CP-3 (SEQ ID NO: 5), as above. A secondary PCR was performed using 5% of the primary reaction using CP-2 (SEQ ID NO: 4) and CP-3 (SEQ ID NO: 5). Again, multiple bands were observed, none of which could be specifically identified in a Southern blot as *Cry j* I, and this approach was also not pursued.

Double-stranded cDNA was then synthesized from approximately 4 μg (pollen) or 8 μg (flowerhead) RNA using a commercially available kit (cDNA Synthesis System kit, BRL, Gaithersburg, MD). After a phenol extraction and ethanol precipitation, the cDNA was blunted with T4 DNA polymerase (Promega, Madison, WI), and ligated to ethanol precipitated, self-annealed, AT (SEQ ID NO: 20) and AL (SEQ ID NO: 22) oligonucleotides for

use in a modified Anchored PCR reaction, according to the method in Rafnar et al. (1991) *J. Biol. Chem.* 266: 1229-1236; Frohman et al. (1990) *Proc. Natl. Acad. Sci. USA* 85: 8998-9002; and Roux et al. (1990) *BioTech.* 8: 48-57. Oligonucleotide AT has the sequence 5'- GGGTCTAGAGGTACCGTC-CGATCGATCATT-3'(SEQ ID NO: 20) (Rafnar et al. *supra*). Oligonucleotide AL has the sequence 5'-AATGATCGATGCT-3' (SEQ ID NO: 22) (Rafnar et al. *Supra*. The amino terminus of *Cry j* I was amplified from the linkered cDNA (3 ul from a 20 µl reaction) with 1 µg each of oligonucleotides AP (SEQ ID NO: 21) and degenerate *Cry j* I primer CP-7 (which has the sequence 5'-TTCATICGATTCTGGGCCCA-3' wherein G at position 8 can also be T; A at position 9 can also be G; C at position 12 can also be T; and G at position 15 can also be A, T, or C)(SEQ ID NO: 6). Inosine (I) is used at position 6 in place of G or A or T or C to reduce degeneracy (Knoth et al. *supra*). The degenerate oligonucleotide CP-7 (SEQ ID NO: 6) is the non-coding strand sequence corresponding to coding strand sequence encoding amino acids 14-20 (TrpAlaGlnAsnArgMetLys) from the amino terminus of *Cry j* I (amino acids 14-20 of SEQ ID NO: 1). Oligonucleotide AP has the sequence 5'-GGGTCTA-GAGGTACCGTCCG-3' (SEQ ID NO: 21).

The primary PCR reaction was carried out as described herein. Five percent (5 µl) of this initial amplification was then used in a secondary amplification with 1 µg each of AP (SEQ ID NO: 21) and degenerate *Cry j* I primer CP-8 (SEQ ID NO: 7) an internally nested *Cry j* I oligonucleotide primer, as described herein. Primer CP-8 has the sequence 5'-CCTGCAGCGATTCT-GGGCCCAAATT-3' wherein G at position 9 can also be T; A at position 10 can also be G; C at position 13 can also be T; G at position 16 can also be A, T, or C; and A at position 23 can also be G)(SEQ ID NO: 7). The nucleotides 5'-CCTGCAG-3' (bases 1 through 7 of SEQ ID NO: 7) represent a *Pst* I restriction site added for cloning purposes. The remaining degenerate oligonucleotide sequence is the non-coding strand sequence corresponding to coding strand sequence encoding amino acids 13-18 of *Cry j* I (AsnTrpAlaGlnAsnArg, amino acids 13-18 of SEQ ID NO: 1) from the amino terminus of *Cry j* I. The dominant amplified product was a DNA band of approximately 193 base pairs,

as visualized on an ethidium bromide (EtBr)-stained 3% GTG agarose gel.

Amplified DNA was recovered by sequential chloroform, phenol, and chloroform extractions, followed by precipitation at -20°C with 0.5 volumes of 7.5 ammonium acetate and 1.5 volumes of isopropanol. After precipitation and washing with 70% ethanol, the DNA was simultaneously digested with *Xba* I and *Pst* I in a 15 µl reaction and electrophoresed through a preparative 3% GTG NuSieve low melt gel (FMC, Rockport, ME). The appropriate sized DNA band was visualized by EtBr staining, excised, and ligated into appropriately digested M13mp18 for sequencing by the dideoxy chain termination method (Sanger et al. (1977) *Proc. Natl Acad Sci. USA* 74: 5463-5476) using a commercially available sequencing kit (Sequenase kit, U.S. Biochemicals, Cleveland, OH). It was initially thought that ligatable material could only be derived from staminate cone-derived RNA. However, upon subsequent examination, it was shown that ligatable material could be recovered from PCR product generated from pollen-derived RNA, and from staminate cone-derived RNA.

The clone designated JC71.6 was found to contain a partial sequence of *Cry j* I. This was confirmed as an authentic clone of *Cry j* I by having complete identity to the disclosed NH_2-terminal sequence of *Cry j* I (Taniai et al. *supra*). The amino acid at position 7 was determined to be cysteine (Cys) in agreement with the sequence disclosed in U.S. patent 4, 939,239. Amino acid numbering is based on the sequence of the mature protein; amino acid 1 corresponds to the aspartic acid (Asp) disclosed as the NH_2-terminus of *Cry j* I (Taniai et al. *supra*) The initiating methionine was found to be amino acid -21 relative to the first amino acid of the mature protein. The position of the initiating methionine was supported by the presence of upstream in-frame-stop codons and by 78% homology of the surrounding nucleotide sequence with the plant consensus sequence that encompasses the initiating methionine, as reported by Lutcke et al. (1987) *EMBO J.* 6:43-48.

The cDNA encoding the remainder of *Cry j* I gene was cloned from the linkered cDNA by using oligonucleotides CP-9 (which has the sequence 5'-ATGGATTCCCCTTGCTTA-3')(SEQ ID NO: 8) and AP (SEQ ID NO: 21) in

the primary PCR reaction. Oligonucleotide CP-9 (SEQ ID NO: 8) encodes amino acids MetAspSerProCysLeu of *Cry j* I (amino acids -21 through -16 of SEQ ID NO: 1) from the leader sequence of *Cry j* I, and is based on the nucleotide sequence determined for the partial *Cry j* I clone JC76.1.

A secondary PCR reaction was performed on 5% of the initial amplification mixture, with 1 µg each of AP (SEQ ID NO: 21) and CP-10 (which has the sequence 5'-GGGAATTCGATAATCCCATAGACAGC-3')(SEQ ID NO: 9), the nested primer. The nucleotide sequence 5'-GGGAATTC-3' of primer CP- 10 (bases 1 through 8 of SEQ ID NO: 9) represent an *Eco* RI restriction site added for cloning purposes. The remaining oligonucleotide sequence encodes amino acids 1-6 of *Cry j* I (AspAsnProIleAspSer) (amino acids 1 through 6 of SEQ ID NO: 1), and is based on the nucleotide sequence determined for the partial *Cry j* I clone JC76.1. The amplified DNA product was purified and precipitated as above, followed by digestion with *Eco* RI and *Xba* I and electrophoresis through a preparative 1% low melt gel. The dominant DNA band was excised and ligated into M13mp19 and pUC19 for sequencing. Again, ligatable material was recovered from cDNA generated from pollen-derived RNA, and from staminate cone-derived RNA. Two clones, designated pUC19JC91a and pUC19JC91d, were selected for full-length sequencing. They were subsequently found to have identical sequences.

DNA was sequenced by the dideoxy chain termination method (Sanger et al. *supra*) using a commercially available kit (sequenase kit (U.S. Biochemicals, Cleveland, OH). Both strands were completely sequenced using M13 forward and reverse primers (N.E. Biolabs, Beverly, MA) and internal sequencing primers CP-13 (SEQ ID NO: 10), CP-14 (SEQ ID NO: 11), CP-15 (SEQ ID NO: 12), CP-16 (SEQ ID NO: 13), CP-18 (SEQ ID NO: 15), CP-19 (SEQ ID NO: 16), and CP-20 (SEQ ID NO: 17). CP-13 has the sequence 5'-ATGCCTATGTACATTGC-3' (SEQ ID NO: 10). CP-13 (SEQ ID NO: 10) encodes amino acids 82-87 of *Cry j* I (MetProMetTyrIleAla, amino acids 82 through 87 of SEQ ID NO: 1). CP-14 has the sequence 5'-GCAATGTACATAGGCAT-3' (SEQ ID NO: 11) and corresponds to the non-coding strand sequence of CP-13 SEQ ID NO: 10). CP-15 has the sequence 5'-

TCCAATTCTTCTGATGGT-3' ((SEQ ID NO: 12) which encodes amino acids 169-174 of *Cry j* I (SerAsnSerSerAspGly, amino acids 169 through 174 of SEQ ID NO: 1). CP-16 has the sequence 5'- TTTTGTCAATTGAGGAGT-3' (SEQ ID NO: 13) which is the non-coding strand sequence which corresponds to coding strand sequence encoding amino acids 335-340 of *Cry j* I (ThrProGlnLeuThrLys, amino acids 335 through 340 of SEQ ID NO: 1). CP-18 has the sequence 5'-TAGCAACTCCAGTCGAAGT-3' (SEQ ID NO: 15) which is the non-coding strand sequence which substantially corresponds to coding strand sequence encoding amino acids 181 through 186 of *Cry j* I (ThrSerThrGlyValThr, amino acids 181 through 186 of SEQ ID NO: 1) except that the fourth nucleotide of CP-18 (SEQ ID NO: 15) was synthesized as a C rather than the correct nucleotide, T. CP-19 which has the sequence 5'-TAGCTCTCATTTGGTGC-3' (SEQ ID NO: 16) is the non-coding strand sequence which corresponds to coding strand sequence encoding amino acids 270 through 275 of *Cry j* I (AlaProAsnGluSerTyr, amino acids 270 through 275 of SEQ ID NO: 1). CP-20 has the sequence 5'- TATGCAATTGGTGGGAGT-3' (SEQ ID NO: 17) which is the coding strand sequence for amino acids 251-256 of *Cry j* I (TyrAlaIleGlyGlySer, amino acids 251 through 256 of SEQ ID NO: 1). The sequenced DNA was found to have the sequence shown in Figs. 4a and 4b (SEQ ID NO: 1). This is a composite sequence from the two overlapping clones JC 71.6 and pUC19J91a. The complete cDNA sequence for *Cry j* I is composed of 1312 nucleotides, including 66 nucleotides of 5' untranslated sequence, an open reading frame starting with the codon for an initiating methionine, of 1122 nucleotides, and a 3' untranslated region. There is a consensus polyadenylation signal sequence in the 3' untranslated region 25 nucleotides 5' to the poly A tail (nucleotides 1279-1283 of Fig 4 and SEQ. ID NO: 1). Nucleotides 1313-1337 of Fig. 4 and SEQ. ID NO: 1 represent vector sequences. The position of the initiating methionine is confirmed by the presence of in-frame upstream stop codons and by 78% homology with the plant consensus sequence that encompasses the initiating methionine (AAAAAUGGA (bases 62 through 70 of SEQ ID NO: 1) found in *Cry j* I compared with the AACAAUGGC consensus sequence for plants, Lutcke et al. (1987) *EMBO J.* 6:

43-48). The open reading frame encodes a protein of 374 amino acids of which the first 21 amino acids comprise a leader sequence that is cleaved from the mature protein. The amino terminus of the mature protein was identified by comparison with the published NH_2-terminal sequence (Taniai et al. (1988) *supra*) and with sequence determined by direct amino acid analysis of purified native *Cry j* I (Example 1). The deduced amino acid sequence of the mature protein, comprised of 353 amino acids has complete sequence identity with the published protein sequence for *Cry j* I (Taniai et al. *supra*), including the first twenty amino acids for the NH_2-terminal and sixteen contiguous internal amino acids. The mature protein also contains five potential N-linked glycosylation sites corresponding to the consensus sequence N-X-S/T.

Example 4

Extraction of RNA from Japanese Cedar Pollen Collected in Japan

Fresh pollen collected from a pool of *Cryptomeria japonica* (Japanese cedar) trees in Japan was frozen immediately on dry ice. RNA was prepared from 500 mg of the pollen, essentially as described by Frankis and Mascarenhas *Ann. Bot.* 45:595-599. The samples were ground by mortar and pestle on dry ice and suspended in 5 ml of 50 mM Tris pH 9.0 with 0.2 M NaCl, 1 mM EDTA, 1% SDS that had been treated overnight with 0.1% DEPC. After five extractions with phenol/chloroform/isoamyl alcohol (mixed at 25:24:1), the RNA was precipitated from the aqueous phase with 0.1 volume 3 M sodium acetate and 2 volumes ethanol. The pellets were recovered by centrifugation, resuspended in dH_20 and heated to 65°C for 5 minutes. Two ml of 4 M lithium chloride were added to the RNA preparations and they were incubated overnight at 9°C. The RNA pellets were recovered by centrifugation, resuspended in 1 ml dH_20, and again precipitated with 3 M sodium acetate and ethanol overnight. The final pellets were resuspended in 100 µl dH_20 and stored at -80°C.

Double stranded cDNA was synthesized from 8 µg pollen RNA using the cDNA Synthesis Systems kit (BRL) with oligo dT priming according

to the method of Gubler and Hoffman (1983) *Gene* 25:263-269. Polymerase chain reactions (PCR) were carried out using the GeneAmp DNA Amplification kit (Perkin Elmer Cetus) whereby 10 µl 10x buffer containing dNTPs was mixed with 100 pmol each of a sense oligonucleotide and an anti-sense oligonucleotide, (10 µl of a 400 µl double stranded cDNA reaction mix), 0.5 µl Amplitaq DNA polymerase, and distilled water to 100 µl.

The samples were amplified with a programmable thermal controller from MJ Research, Inc. (Cambridge, MA). The first 5 rounds of amplification consisted of denaturation at 94°C for 1 minute, annealing of primers to the template at 45°C for 1 minute, and chain elongation at 72°C for 1 minute. The final 20 rounds of amplification consisted of denaturation as above, annealing at 55°C for 1 minute, and elongation as above.

Seven different *Cry j* I primer pairs were used to amplify the double stranded cDNA as follows: CP-9 (SEQ. ID #8) and CP-17 (SEQ. ID #14), CP-10 (SEQ. ID #9) and CP-17 (SEQ. ID #14), CP-10 (SEQ. ID #9) and CP-16 (SEQ. ID #13), CP-10 (SEQ. ID #9) and CP-19 (SEQ. ID #16), CP-10 (SEQ. ID #9) and CP-18 (SEQ. ID #15), CP-13 (SEQ. ID #10) and CP-17 (SEQ. ID #14), and CP-13 (SEQ. ID #10) and CP-19 (SEQ. ID #16). CP-17 (SEQ. ID #14) has the sequence 5'- CCTGCAGAAGCTTCATCAACAACGTTTAGA-3' and corresponds to non-coding strand sequence that corresponds to coding strand sequence encoding amino acids SKRC* (amino acids 350-353 and the stop codon of SEQ. ID #1). The nucleotide sequence 5'-CCTGCAGAAGCTT-3' (bases 1 through 13 of SEQ. ID # 14) represents *Pst* I and *Hin* dIII restriction sites added for cloning purposes. The nucleotide sequence 5'-TCA-3' (bases 13 through 15 of SEQ. ID # 14) correspond to the non-coding strand sequence of a stop codon. All of the amplifications yielded products of the expected size when viewed on ethidium bromide (EtBr)-stained agarose gels. Two of these primer pairs were used in amplifications whose products were cloned into pUC19 for full-length sequencing. The PCR reaction with CP-10 (SEQ. ID #9) and CP-16 (SEQ. ID #13) on the double stranded cDNA yielded a band of approximately 1.1 kb, and was called JC130. A separate first strand cDNA reaction was done with 8 µg pollen RNA as described above and amplified with

oligonucleotide primers CP-10 (SEQ. ID #9) and CP-17 (SEQ. ID #14). This amplification yielded a full-length cDNA, named JC135, from the amino terminus of the mature protein to the stop codon.

Amplified DNA was recovered by sequential chloroform, phenol, and chloroform extractions, followed by precipitation at -20°C with 0.5 volumes of 7.5 ammonium acetate and 1.5 volumes of isopropanol. After precipitation and washing with 70% ethanol, the DNA was blunted with T4 polymerase followed by digestion with *Eco* RI, in the case of JC130 , or simultaneously digested with *Eco* RI and *Pst* I, in the case of JC135, in a 15 µl reaction and electrophoresed through a preparative 1% SeaPlaque low melt gel (FMC). Appropriate sized DNA bands were visualized by EtBr staining, excised, and ligated into appropriately digested pUC19 for dideoxy DNA sequencing by the dideoxy chain termination method (Sanger et al. (1977) *Proc. Natl. Acad. Sci. USA* 74:5463-5476) using a commercially available sequencing kit (Sequenase kit, U.S. Biochemicals, Cleveland, OH).

Both strands were sequenced using M13 forward and reverse primers (N.E. Biolabs, Beverly, MA) and internal sequencing primers CP-13 (SEQ. ID #10), CP-15 (SEQ. ID #12), CP-16 (SEQ ID #13), CP-18 (SEQ. ID #15), CP-19 (SEQ. ID #16) and CP-20 (SEQ. ID #17). Two clones from amplification JC130 (JC130a and JC130b) and one clone from amplification JC135 (JC135g) were found to be *Cry j* I clones upon sequencing. The nucleotide and deduced amino acid sequences of clones JC130a and JC135g were identical to previously known *Cry j* I sequence (SEQ. ID #1). Clone JC130b was found to contain a single nucleotide difference from the previously known *Cry j* I sequence (SEQ. ID #1). Clone JC130b had a C at nucleotide position 306 of Seq. ID #1. This nucleotide change results in a predicted amino acid change from a Tyr to a His at amino acid 60 of the mature *Cry j* I protein. This polymorphism has not yet been confirmed in an independently-derived PCR clone or by direct amino acid sequencing. However, such polymorphisms in primary nucleotide and amino acid sequences are expected.

Example 5

Expression of *Cry j* I

Expression of *Cry j* I was performed as follows. Ten µg of pUC19JC91a was digested with *Xba* I, precipitated, then blunted with T4 polymerase. *Bam*H I linkers (N.E. Biolabs, Beverly, MA) were blunt-end ligated to pUC19JC91a overnight and excess linkers were removed by filtration through a NACS ion exchange minicolumn (BRL, Gaithersburg, MD). The linkered cDNA was then digested simultaneously with *Eco*R I and *Bam*H I. The *Cry j* I insert (extending from the nucleotides encoding the amino terminus of the mature protein through the stop codon) was isolated by electrophoresis of this digest through a 1% SeaPlaque low melt agarose gel. The insert was then ligated into the appropriately digested expression vector pET-11d (Novagen, Madison, WI; Jameel et al. (1990) *J. Virol.* 64:3963-3966) modified to contain a sequence encoding 6 histidines (His 6) immediately 3' of the ATG initiation codon followed by a unique *Eco*R I endonuclease restriction site. A second *Eco*R I endonuclease restriction site in the vector, along with neighboring *Cla* I and *Hind* III endonuclease restriction sites, had previously been removed by digestion with *Eco*R I and *Hind* III, blunting and religation. The histidine (His_6) sequence was added for affinity purification of the recombinant protein (*Cry j* I) on a Ni^{2+} chelating column (Hochuli et al. (1987) *J. Chromatog.* 411:177-184; Hochuli et al. (1988) *Bio/Tech.* 6:1321-1325.). A recombinant clone was used to transform *Escherichia coli* strain BL21-DE3 which harbors a plasmid that has an isopropyl-ß-D-thiogalactopyranoside (IPTG)-inducible promoter preceding the gene encoding T7 polymerase. Induction with IPTG leads to high levels of T7 polymerase expression, which is necessary for expression of the recombinant protein in pET-11d, which has a T7 promoter. Clone pET-11dΔ HRhis_6JC91a.d was confirmed by dideoxy sequencing (Sanger et al. *Supra*) with CP-14 (SEQ. ID #11) to be a *Cry j* I clone in the correct reading frame for expression.

Expression of the recombinant protein was confirmed in an initial small culture (50 ml). An overnight culture of clone pET-11dΔHRhis_6JC91a.d was used to inoculate 50 ml of media (Brain Heart Infusion Media, Difco) containing ampicillin (200 µg/ml), grown to an A_{600} = 1.0 and then induced with IPTG (1

mM, final concentration) for 2 hrs. One ml aliquots of the bacteria were collected before and after induction, pelleted by centrifugation, and crude cell lysates prepared by boiling the pellets for 5 minutes in 50 mM Tris HCl, pH 6.8, 2 mM EDTA, 1% SDS, 1% ß-mercaptoethanol, 10% glycerol, 0.25% bromophenol blue (Studier et al., (1990) *Methods in Enzymology* 185:60-89). Recombinant protein expression was visualized as a band with the predicted molecular weight of approximately 38 kDa on a Coomassie blue-stained SDS-PAGE gel, according to the method in Sambrook et al., *supra*, on which 40 µl of the crude lysate was loaded. A negative control consisted of crude lysates from uninduced bacteria containing the plasmid with *Cry j* I and an induced lysate from bacteria carrying no plasmid.

The pET-11dΔ HRhis6JC91a.d clone was then grown on a large scale for recombinant protein expression and purification. A 2 ml culture bacteria containing the recombinant plasmid was grown for 8 hr, then streaked onto solid media (e.g. 6 petri plates (100 x 15 mm) with 1.5% agarose in LB medium (Gibco-BRL, Gaithersburg, MD) containing 200 µg/ml ampicillin), grown to confluence overnight, then scraped into 9 L of liquid media (Brain Heart Infusion media, Difco) containing ampicillin (200 µg/ml). The culture was grown until the A_{600} was 1.0, IPTG added (1 mM final concentration), and the culture grown for an additional 2 hours.

Bacteria were recovered by centrifugation (7,930 x g, 10 min), and lysed in 90 ml of 6M Guanidine-HCl, 0.1M Na_2HPO_4, pH 8.0 for 1 hour with vigorous shaking. Insoluble material was removed by centrifugation (11,000 x g, 10 min, 4° C). The pH of the lysate was adjusted to pH 8.0, and the lysate applied to an 80 ml Nickel NTA agarose column (Qiagen) that had been equilibrated with 6 M Guanidine HCl, 100 mM Na_2HPO_4, pH 8.0. The column was sequentially washed with 6 M Guanidine HCl, 100 mM Na_2HPO_4, 10 mM Tris-HCl, pH 8.0, then 8 M urea, 100 mM Na_2HPO_4, pH 8.0, and finally 8 M urea, 100 mM sodium acetate, 10 mM Tris-HCl, pH 6.3. The column was washed with each buffer until the flow through had an $A_{280} \leq 0.05$.

The recombinant protein, *Cry j* I, was eluted with 8 M urea, 100 mM sodium acetate, 10 mM Tris-HCl, pH 4.5, and collected in 10 ml aliquots. The

protein concentration of each fraction was determined by absorbance at A_{280} and the peak fractions pooled. An aliquot of the collected recombinant protein was analyzed on SDS-PAGE according to the method in Sambrook et al., *supra*.

The first 9 L prep, JCpET-1, yielded 30 mg of *Cry j* I with approximately 78% purity, as determined by densitometry (Shimadzu Flying Spot Scanner, Shimadzu Scientific Instruments, Inc., Braintree, MA) of the Coomassie-blue stained SDS-PAGE gel. A second 9 L prep prepared the same way, JCpET-2, yielded 41 mg of *Cry j* I with approximately 77% purity.

Example 6

Japanese Cedar Pollen Allergic Patient T Cell Studies with *Cry j* I - the Primary Cedar Pollen Antigen.

Synthesis of Overlapping Peptides

Japanese cedar pollen *Cry j* I overlapping peptides were synthesized using standard Fmoc/tBoc synthetic chemistry and purified by Reverse Phase HPLC. Figure 13 shows *Cry j* I peptides used in these studies. The peptide names are consistent throughout.

T Cell Responses to Cedar Pollen Antigen Peptides

Peripheral blood mononuclear cells (PBMC) were purified by lymphocyte separation medium (LSM) centrifugation of 60 ml of heparinized blood from Japanese cedar pollen-allergic patients who exhibited clinical symptoms of seasonal rhinitis and were MAST and/or skin test positive for Japanese cedar pollen. Long term T cell lines were established by stimulation of 2 X 10^6 PBL/ml in bulk cultures of complete medium (RPMI-1640, 2 mM L-glutamine, 100 U/ml penicillin/streptomycin, $5x10^{-5}$M 2-mercaptoethanol, and 10 mM HEPES supplemented with 5% heat inactivated human AB serum) with 20 μg/ml of partially purified native *Cry j* I (75% purity containing three bands similar to the three bands in Fig. 2) for 7 days at 37°C in a humidified 5% CO_2 incubator to select for *Cry j* I reactive T cells. This amount of priming antigen

was determined to be optimal for the activation of T cells from most cedar pollen allergic patients. Viable cells were purified by LSM centrifugation and cultured in complete medium supplemented with 5 units recombinant human IL-2/ml and 5 units recombinant human IL-4/ml for up to three weeks until the cells no longer responded to lymphokines and were considered "rested". The ability of the T cells to proliferate to selected peptides, recombinant *Cry j* I (r*Cry j* I), purified native *Cry j* I, or recombinant *Amb a* I.1 (r*Amb a*I.1) was then assessed. For assay, 2 X 10^4 rested cells were restimulated in the presence of 2 X 10^4 autologous Epstein-Barr virus (EBV)-transformed B cells (prepared as described below) (gamma-irradiated with 25,000 RADS) with 2-50 µg/ml of r*Cry j* I, purified native *Cry j* I or r*Amb a* I.1, in a volume of 200 µl complete medium in duplicate or triplicate wells in 96-well round bottom plates for 2-4 days. The optimal incubation was found to be 3 days. Each well then received 1 µCi tritiated thymidine for 16-20 hours. The counts incorporated were collected onto glass fiber filter mats and processed for liquid scintillation counting. Fig. 12 shows the effect of varying antigen dose in assays with recombinant *Cry j* I, purified native *Cry j* I, and recombinant *Amb a* I.1 and several antigenic peptides synthesized as described above. Some peptides were found to be inhibitory at high concentrations in these assays. The titrations were used to optimize the dose of peptides in T cell assays. The maximum response in a titration of each peptide is expressed as the stimulation index (S.I.). The S.I. is the counts per minute (CPM) incorporated by cells in response to peptide, divided by the CPM incorporated by cells in medium only. An S.I. value equal to or greater than 2 times the background level is considered "positive" and indicates that the peptide contains a T cell epitope. The positive results were used in calculating mean stimulation indices for each peptide for the group of patients tested. The results shown in Fig. 12 demonstrate that patient #999 responds well to recombinant *Cry j* I, and purified native *Cry j* I, as well as to peptides CJ1-2, 3, 20, and 22 but not to recombinant *Amb a* I.1. This indicates that *Cry j* I T cell epitopes are recognized by T cells from this particular allergic patient and that r*Cry j* I and peptides CJ1-2, 3, 20 and 22 contain such T cell epitopes. Furthermore, the epitopes were often not detected with the adjacent overlapping peptides, and

therefore probably span the non-overlapping central residues of the reactive peptides. No significant cross-reactivity was found in T cell assays using T cells primed with control antigens or with *Cry j* I primed T cells against other antigens.

The above procedure was followed with a number of other patients. Individual patient results were used in calculating the mean S.I. for each peptide if the patient responded to the *Cry j* I protein at an S.I. of 2.0 or greater and the patient responded to at least one peptide derived from *Cry j* I at an S.I. of 2.0 or greater. A summary of positive experiments from twenty-five patients is shown in Figure 14. The bars represent the positivity index. Above each bar is the percent of positive responses with an S.I. of at least two to the peptide or protein in the group of patients tested. In parenthesis above each bar are the mean stimulation indices for each peptide or protein for the group of patients tested. All twenty-five T cell lines responded to purified native *Cry j* I and 68.0% of the T cell lines responded to r*Cry j* I. These twenty-five T cell lines also responded at a significantly lower level to r*Amb a* I.1 indicating that the *Amb a* I allergens share a degree of homology with *Cry j* I and that "shared" T cell epitopes might exist between *Cry j* I and *Amb a* I. This panel of Japanese cedar allergic patients responded to peptides CJ1-1, CJ1-2, CJ1-3, CJ1-4, CJ1-7, CJ1-8, CJ1-9, CJ1-10, CJ1-11, CJ1-12, CJ1-14, CJ1-15, CJ1-16, CJ1-17, CJ1-18, CJ1-19, CJ1-20, CJ1-21, CJ1-22, CJ1-23, CJ1-24, CJ1-25, CJ1-26, CJ1-27, CJ1-28, CJ1-30, CJ1-31, CJ1-32, CJ1-33, CJ1-34 and CJ1-35 indicating that these peptides contain T cell epitopes.

Preparation of (EBV)-transformed B Cells for Use as Antigen Presenting Cells

Autologous EBV-transformed cell lines were γ-irradiated with 25,000 Rad and used as antigen presenting cells in secondary proliferation assays and secondary bulk stimulations. These cell lines were also used as a control in the immuno-fluorescence flow cytometry analysis. These EBV-transformed cell lines were made by incubating 5 X 10^6 PBL with 1 ml of B-59/8 Marmoset cell

line (ATCC CRL1612, American Type Culture Collection, Rockville, MD) conditioned medium in the presence of 1 μg/ml phorbol 12-myristate 13-acetate (PMA) at 37°C for 60 minutes in 12 X 75 mm polypropylene round-bottom Falcon snap cap tubes (Becton Dickinson Labware, Lincoln Park, NJ). These cells were then diluted to 1.25 X 10^6 cells/ml in RPMI-1640 as described above except supplemented with 10% heat-inactivated fetal bovine serum and cultured in 200 μl aliquots in flat bottom culture plates until visible colonies were detected. They were then transferred to larger wells until the cell lines were established.

Example 7

***Cry j* I as the Major Cedar Pollen Allergen**

To examine the importance of *Cry j* I , reported as the major allergen of Japanese cedar pollen, both direct and competition ELISA assays were performed. For the direct ELISA assays, wells were coated with either soluble pollen extract (SPE) of Japanese cedar pollen or purified native *Cry j* I (assayed at 90% purity by protein sequencing) and human IgE antibody binding to these antigens was analyzed. Pooled human plasma, consisting of an equal volume of plasma from 15 patients with a Japanese cedar pollen MAST score of 2.5 or greater, and two individual patient plasma samples were compared in this assay. Fig. 5 shows the results of the binding reactivity with these two antigens. The overall pattern of binding is very similar whether the coating antigen is SPE (Fig. 5a) or purified native *Cry j* I (Fig. 5b).

In the competition assay, ELISA wells were coated with Japanese cedar pollen SPE and then allergic patient IgE binding was measured in the presence of competing purified native *Cry j* I in solution. The source of allergic IgE in these assays was either the pool of plasma from 15 patients (denoted PHP) or seven individual plasma samples from patients with a Japanese cedar MAST score of 2.5 or greater. The competition assay using the pooled human plasma samples compares the competitive binding capacity of purified native *Cry j* I to Japanese

cedar pollen SPE and an irrelevant allergen source, rye grass SPE. Fig. 6 shows the graphed results of the competition ELISA with pooled human plasma. The concentration of protein present in the Japanese cedar pollen SPE is approximately 170 times greater at each competing point than is the purified native *Cry j* I . From this analysis it is clear that the purified native *Cry j* I competes very well for IgE binding to the whole range of proteins present in the Japanese cedar pollen soluble pollen extract. This implies that most of the anti-*Cry j* IgE reactivity is directed against native *Cry j* I . The negative control shows no specific competitive activity and the competing SPE in solution can completely remove binding to the coated wells. This assay was repeated with individual patients as a measure of the range of the IgE response within the allergic population. Fig. 7 shows this result where the competition of binding to SPE was performed with purified native *Cry j* I . The results demonstrate that although the patients show different dose response to Japanese cedar pollen SPE, each of the seven patients' IgE binding to Japanese cedar pollen SPE could be competed with purified native *Cry j* I. The implications of these data are that for each patient the IgE reactivity directed against *Cry j* I is predominant but that there is variation in this reactivity between patients. The overall conclusion is that these data support the previous findings (Yasueda et al., (1988) supra) that *Cry j* I is the major allergen of Japanese cedar pollen.

The reactivity of IgE from cedar pollen allergic patients to the pollen proteins is dramatically reduced when these proteins are denatured. One method of analyzing this property is through direct binding ELISA where the coating antigen is the Japanese cedar pollen SPE or denatured Japanese cedar pollen SPE which has been denatured by boiling in the presence of a reducing agent DTT. This is then examined with allergic patient plasma for IgE binding reactivity. Fig. 8a, shows the direct binding assay to the SPE with seven individual plasma samples. In Fig. 8b, the binding results with the denatured SPE demonstrates the marked decrease in reactivity following this treatment. To determine the extent of *Cry j* I binding to the ELISA wells, *Cry j* I was detected with a rabbit polyclonal antisera against the *Amb a* I & II protein family. These ragweed proteins have high sequence identity (46%) with *Cry j* I and this antisera can be

used as a cross reactive antibody detection system. In conclusion, these data demonstrate a marked loss in IgE reactivity following denaturation of the Japanese cedar pollen SPE.

Example 8

IgE Reactivity and Histamine Release Analysis

The recombinant *Cry j* I protein (r*Cry j* I), expressed in bacteria and then purified (as described in Example 5), has been examined for IgE reactivity. The first method applied to this examination was direct ELISA where wells were coated with the recombinant *Cry j* I and IgE binding was assayed on individual patients. Fig. 9 is the graphic representation of this direct ELISA. The only positive signals on this data set are from the two control antisera rabbit polyclonal anti-*Amb a* I & II prepared by conventional means (Rabbit anti-*Amb a* I & II) and CBF2, a monoclonal antibody raised against *Amb a* I that cross reacts with *Cry j* I. By this method all patients tested showed no IgE reactivity with the recombinant *Cry j* I.

Another method of analysis that was applied to the examination of IgE reactivity to the recombinant *Cry j* I was a capture ELISA. This analysis relies on the use of a defined antibody, in this case CBF2 to bind the antigen and allow for the binding of antibodies to other epitope sites. The format of this capture ELISA is 1) wells are coated with MAb CBF2, 2) antigen or PBS (as one type of negative control) is added and captured by specific interaction with the coated MAb, 3) either the control antibody anti-*Amb a* I & II (Fig. 10b) or human allergic plasma (Fig. 10a) is added as the detecting antibody, and 4) detection of antibody binding is assayed. Figs. 10a and 10b are the graphed results of these assays. For the IgE analysis, the pooled human plasma (PHP) (15 patients) was used. The conclusion from these results is that there is no indication of any specific binding of human allergic IgE to r*Cry j* I by this method of analysis. However, the capture of r*Cry j* I works as evidenced by the control antibody binding curve, shown in Fig. 10b. The lack of IgE binding to E. coli expressed r*Cry j* I may be due to absence of carbohydrate or any other post-translational

modification and/or that the majority of IgE cannot react with denatured *Cry j* I. RAST, competition ELISA and Western blotting data also demonstrates no specific IgE reactivity to the r*Cry j* I (data not shown).

A histamine release assay was performed on one Japanese cedar pollen allergic patient using Japanese cedar pollen SPE, purified native *Cry j* I and r*Cry j* I as the added antigens. This assay is a measure of IgE reactivity through human basophil mediator release. The results of this assay, shown in Fig. 11, demonstrate strong histamine release with both purified native *Cry j* I and the Japanese cedar pollen SPE over a wide concentration range. The only point where there is any measurable histamine release with the *Cry j* I is at the highest concentration, 50 µg/ml. Two possible explanations for this release by the r*Cry j* I are: 1) specific reactivity with a very low proportion of the anti-*Cry j* I IgE capable of recognizing the recombinant form of *Cry j* I , or 2) non-specific release caused by low abundance of bacterial contaminants observed only at the highest antigen concentration. Thus far, this result has only been shown in a single patient. In addition, the data shown are from single data points at each protein concentration.

It may be possible to use this recombinantly expressed *Cry j* I protein for immunotherapy as *E. coli* expressed material has T cell reactivity (Example 6), but does not appear to bind IgE from *Crytpomeria japonica* atopes nor cause histamine release from the mast cells and basophils of such atopes *in vitro*. Expression of r*Cry j* I which is capable of binding IgE could possibly be achieved in yeast, insect (baculovirus) or mammalian cells (e.g. CHO, human and mouse). A specific example of mammalian cell expression could be the use of the pcDNA I/Amp mammalian expression vector (Invitrogen, San Diego, CA) expressing recombinant *Cry j* I in COS cells. A r*Cry j* I capable of actively binding IgE may be important for the use of recombinant material for diagnostic purposes.

To analyze IgE reactivity to selected *Cry j* I peptides a direct ELISA format was used. ELSIA wells were coated with 25 peptides derived from *Cry j* I and assayed for IgE binding. Fig. 15a and 15b are graphs of these binding results using PHP (15 patients) as the cedar pollen allergic IgE source. This pool

of plasma was formulated for enrichment of IgE that could bind to denatured SPE (as determined by direct ELISA) and therefore increase the chance of reactivity toward the peptides. In this assay, the peptide IgE binding capacity was compared to that of purified native *Cry j* I and to r*Cry j* I. The only specific IgE detected in this assay was to purified native *Cry j* I which supports the finding that Japanese cedar allergic patient IgE does not bind to recombinant *Cry j* I or the recombinant *Cry j* I peptides tested (Fig. 15).

Although the invention has been described with reference to its preferred embodiments, other embodiments, can achieve the same results. Variations and modifications to the present invention will be obvious to those skilled in the art and it is intended to cover in the appended claims all such modification and equivalents and follow in the true spirit and scope of this invention.

Example 9

Extraction of RNA from *Juniperus sabinoides*, *Juniperus virginiana* and *Cupressus arizonica* pollens and the cloning of *Jun s* I and *Jun v* I, homologs of *Cry j* I.

Fresh pollen was collected from a single *Juniperus virginiana* tree at the Arnold Arboretum (Boston, MA), and was frozen immediately on dry ice; *Juniperus sabinoides* and *Cupressus arizonica* pollens were purchased from Greer Laboratories, Inc. (Lenoir, NC). Total RNA was prepared from *J. virginiana*, *J. sabinoides*, and *C. arizonica* pollens as described in Example 3. Single stranded cDNA was synthesized from 5 µg total pollen RNA from *J. virginiana* and 5 µg total pollen RNA from *J. sabinoides* using the cDNA Synthesis System kit (BRL, Gaithersburg, MD), as described in Example 3.

The initial attempt at cloning *Cry j* I homologue from the two juniper species was made using various pairs of *Cry j* I-specific oligonucleotides in PCR amplifications on both juniper cDNAs. PCRs were carried out as described in Example 3. The oligonucleotide primer pairs used were: CP-9/CP-17, CP10/CP-

17, CP-10/CP-16, CP-10/CP-19, CP-10/CP-18, CP-13/CP-17, and CP-13/CP-19. CP-10 was used in the majority of the reactions as the 5' primer since it has been reported by Gross et. al. (1978) Scand. J. Immunol. **8**: 437-441 that the first 5 amino-terminal amino acids of *J. sabinoides* are identical to those of *Cry j* I. These oligonucleotides and oligonucleotide primers pairs are described in Example 3.

None of the primer pairs cited above resulted in a PCR product for either juniper species when viewed on an EtBr-stained 1% agarose (FMC Bioproducts, Rockland, ME) minigel.

The next series of PCR amplifications attempting to clone the *Cry j* I homologues from *J. sabinoides* and *J. virginiana* from were made on double stranded linkered cDNA synthesized from RNA from each species. Double stranded cDNA was synthesized from 5 µg of *J. virginiana* and 5 µg *J. sabinoides* pollen RNA as described in Example 3. The double-stranded cDNA was ligated to ethanol precipitated, self annealed, AT and AL oligonucleotides for use in a modified Anchored PCR as described in Example 3. A number of *Cry j* I primers were then used in combination with AP in an attempt to isolate the *Cry j* I homologues from the two juniper species. The sequences of AT, AL and AP are given in Example 3. First, a primary PCR was carried out with 100 pmol each of the oligonucleotides CP-10 and AP. Three percent (3 µl) of this initial amplification was then used in a secondary PCR with 100 pmoles each of CP-10 and APA, which has the sequence 5'-GGGCTCGAGCTGCAGTTTTT-TTTTTTTTTTTTG-3', where nucleotides 1-15 represent *Pst* I and *Xho* I endonuclease restriction sites added for cloning purposes, and nucleotide 33 can also be an A or C. A broad smear, with no discreet band, was revealed upon examination of the secondary PCR reactions on an EtBr-stained agarose gel. Attempts to clone *Cry j* I homologues from these PCR products were not successful. This approach would have cloned a carboxyl portion of these genes. The degenerate *Cry j* I primers CP-1, CP-4, and CP-7 as described in Example 3 were then each used in primary PCRs with AP on the double stranded linkered *J. virginiana* and *J. sabinoides* cDNAs. Various primer pair combinations were used in secondary PCRs as follows: CP-2/AP and CP-4/AP on the CP-1/AP

primary PCR amplification mixture, CP-2/AP and CP-5/AP on the CP-4/AP primary PCR amplification mixture, and CP-8/AP on the CP-7/AP primary PCR amplification mixture. Only the last amplification, the CP-8/AP secondary PCR amplification, yielded a band upon examination on an EtBr-stained minigel; the others gave smears that could not be cloned into pUC19. Both the *J. virginiana* and *J. sabinoides* secondary PCRs with CP-8 and AP, described in Example 3, called JV21 and JS17, respectively, resulted in amplified products that were approximately 200 base pairs long. The amplified DNA was recovered as described in Example 3 and simultaneously digested with *Xba* I and *Pst* I in a 50 µl reaction, precipitated to reduce the volume to 10 µl, and electrophoresed through a preparative 2% GTG NuSeive low melt gel (FMC, Rockport, ME). The appropriate sized DNA band was visualized by EtBr staining, excised, and ligated into appropriately digested pUC19 for sequencing by the dideoxy chain termination method of Sanger et al. (supra) using a commercially available sequencing kit (Sequenase kit, U.S. Biochemicals, Cleveland, OH). Two JS17 clones (pUC19JS17d and pUC19JS17f) and one JV21 clone (pUC19JV21g) were sequenced, and found to contain sequences homologous to the *Cry j* I nucleotide and deduced amino acid sequences. The *Cry j* I homologues isolated from *J. sabinoides* and *J. virginiana* RNA were designated *Jun s* I and *Jun v* I, respectively.

The *Cry j* I primers CP-9 and CP-10 should work in primary and secondary PCRs, respectively, with AP to amplify the carboxyl portion of the *Jun s* I and *Jun v* I cDNAs. The sequence of these primers are essentially identical to the sequences of *Jun s* I and *Jun v* I, with the exception of 2 nucleotides in CP-9 (T instead of A in position 5 of CP-9, C instead of A in position 12), and 1 in CP-10 (C instead of A in position 12 for *Jun s* I only). However, primary PCRs with CP-9 and AP and secondary PCRs with CP-10 and AP did not yield identifiable *Jun s* I nor *Jun v* I product when viewed on an EtBr-stained agarose gel.

Oligonucleotide J1 was synthesized. J1 and all subsequent oligonucleotides were synthesized on an ABI 394 DNA/RNA synthesizer (Applied Biosystems, Foster City, CA). Primary PCRs were carried out using

AP and J1 with *J. virginiana* and *J. sabinoides* cDNAs. J1 has the sequence 5'-CTAAAAATGGCTTCCCCA-3', which corresponds to nucleotides 20-37 of *Jun s* I (Fig. 16) and nucleotides 30-47 of *Jun v* I (Fig. 17). A secondary PCR amplification was performed on the primary J1/AP amplification of *J. sabinoides* cDNA using primers J2 and AP. J2 has the sequence 5'-CGGGAATTCTAGATGTGCAATTGTATCTTGTTA-3', whereby nucleotides 1-13 represent *Eco*R I and *Xba* I endonuclease restriction sites added for cloning purposes, and the remaining nucleotides correspond to nucleotides 65-84 in the *Jun s* I sequence (Fig. 16). The secondary amplification from *J. virginiana* cDNA was performed with AP and J3, which has sequence 5'-CGGGAATTCTAGATGTGCAATAGTATCTTGTTG-3' whereby nucleotides 1-13 represent *Eco*R I and *Xba* I endonuclease restriction sites added for cloning purposes and the remaining nucleotides correspond to nucleotides 75-94 in the *Jun v* I sequence (Fig. 17). No specific amplified product was observed in either secondary reaction. The primers designated ED and EDT were used at a molar ratio of 3:1 (ED:EDT) in conjunction with primers J1, J2 and J3, as described below. EDT has the sequence 5'-GGAATTCTCTAGACTGCAGG-TTTTTTTTTTTTTTT-3'. The nucleotides 1 through 20 of EDT were added to the poly-T track to create *Eco*R I, *Xba* I, and *Pst* I endonuclease restriction sites for cloning purposes. ED has the sequence 5'-GGAATTCTCTAGACTGCA-GGT-3', corresponding to nucleotides 1 to 21 of EDT. These oligonucleotides and their use have been previously described (Morgenstern et al. (1991) Proc. Natnl. Acad. Sci. USA 88:9690-9694). ED/EDT were used in primary PCRs with oligonucleotide J1 for amplifications from *J. sabinoides* and *J. virginiana* cDNAs, followed by secondary PCRs with oligonucleotides J2 and APA (for J. *sabinoides*) or J3 and APA (for *J. virginiana*). No specific product was identified from these amplifications. A final set of PCRs with J1, J2, and J3 was tried with oligonucleotide APA. APA was used in a primary PCR reaction with J1 for *J. sabinoides* and *J. virginiana*, followed by secondary amplifications with J2 (for *J. sabinoides*) or J3 (for *J. virginiana*) and APA. No specific product was identified from these amplifications. The degenerate primer CP-57 was then synthesized. CP-57 has the sequence 5'-GGCCTGCAGTTAACAGCG-

TTTGCAGAAGGTGCA-3', wherein T at position 10 can also be C, T at position 11 can also be C, A at position 13 can also be G,G at position 16 can also be A,T, or C, G at position 18 can also be T, T at position 19 can also be C, G at position 22 can also be A, T or C, C at position 23 can also be G, A at position 24 can also be C, G at position 25 can also be A, T, or C, A at position 27 can also be G, G at position 28 can also be A, T, or C, G at position 29 can also be C, T at position 30 can also be A, and G at position 31 can also be A. The nucleotides 1 through 9 of CP-57 were added to create a Pst I site for cloning purposes, the nucleotides 10 through 12 are complementary to a stop codon and nucleotides 13 through 33 are complementary to coding strand sequence essentially encoding the amino acids CysSerLeuSerLysArgCys (amino acids 347 through 353 of Figure 4b, corresponding to nucleotides 1167 through 1187 of Figure 4b). This was used in a primary PCR with J1 on both *J. sabinoides* and *J. virginiana* double stranded linkered cDNA , followed by a secondary PCRs with CP-57 and J2 for *J. sabinoides* and CP-57 and J3 for *J. virginiana*. No PCR products were recovered. Three additional degenerate *Cry j* I oligonucleotides were synthesized. CP-62 has sequence 5'-CCACTAAATATTATCCA-3', wherein A at position 3 can also be G, A at position 6 can also be G, T at position 9 can also be A or G, and T at position 12 can also be A or G; this degenerate oligonucleotide sequence is complementary to the coding strand sequence essentially encoding the amino acids TrpIleIlePheSerGly (amino acids 69 through 74 of Figure 4a, corresponding to nucleotides 333 through 349 of Figure 4a). CP-63 has sequence 5'-GCATCCCCATCTTGGGGATG-3', wherein A at position 3 can also be G, A at position 9 can also be G, T at position 12 can also be C, G at position 15 can also be A, T, or C, and A at position 18 can also be G; this degenerate oligonucleotide sequence is complementary to the sequence capable of encoding the amino acids HisProGlnAspGlyAspAla (amino acids 146-152 of Figure 4a, corresponding to nucleotides 564 to 583 of Figure 4a). CP-64 has the sequence 5'-GTCCATGGATCATAATTATT-3', wherein T at position 6 can also be C, A at position 9 can also be G, A at position 12 can also be G, A at position 15 can also be G, and A at position 18 can also be G; this degenerate oligonucleotide

sequence is complementary to the coding strand sequence capable of encoding the amino acids AsnAsnTyrAspProTrpThr (amino acids 243-249 of Figure 4b, corresponding to nucleotides 855 through 874 of Figure 4b). AP was used in a primary PCR amplification with CP-62, CP-63, CP-64 and CP-3 (described in Example 3) for both *J. sabinoides* and *J. virginiana* double-stranded linkered cDNA. A diagnostic PCR was performed on each primary reaction mixture. In this diagnostic PCR, 3% of the primary reaction was amplified as described above using AP and CP-8. For both *J. sabinoides* and *J. virginiana*, the expected bands of approximately 200 base pairs were observed in diagnostic PCRs from the primary PCR with AP and CP-63.

The degenerate primer CP-65 was then synthesized. CP-65 has the sequence 5'-GCCCTGCAGTCCCCATCTTGGGGATGGAC-3', wherein A at position 15 can also be G, T at position 18 can also be C, G at position 21 can also be G, A, T, or C, A at position 24 can also be G, and G at position 27 can also be A, T, or C. Nucleotides 1-9 of CP-65 were added to create a *Pst* I restriction site for cloning purposes, while the remaining degenerate oligonucleotide sequence is complementary to coding strand sequence essentially capable of encoding the amino acids ValHisProGlnAspGlyAsp (amino acids 145-151 of Figure 4a, corresponding to nucleotides 561 through 580 of Figure 4a). AP was used in conjunction with CP-65 in a secondary PCR of the primary AP/CP-63 amplifications of *J. sabinoides* and *J. virginiana* described above. These reactions were designated JS42 for *J. sabinoides* and JV46 for *J. virginiana*. Both secondary PCRs gave bands of approximately 600 base pairs when examined on 1% agarose minigels stained with EtBr. The DNA from the JS42 and JV46 PCRs was recovered as described in Example 3, simultaneously digested with *Xba* I and *Pst* I in 15 µl reactions then electrophoresed through a preparative 2% GTG SeaPlaque low melt gel (FMC, Rockport, ME). The appropriate sized DNA bands were visualized by EtBr staining, excised, and ligated into appropriately digested pUC19 for sequencing by the dideoxy chain termination method (Sanger et al., supra) using a commercially available sequencing kit (Sequenase kit, U.S. Biochemicals, Cleveland, OH). Clones were sequenced using M13 forward and reverse primers (N.E. Biolabs, Beverly, MA)

and internal sequencing primer J4 for both *Jun s* I and *Jun v* I. J4 has the sequence 5'-GCTCCACCATGGGAGGCA-3' (nucleotides 177-194 of Fig. 16 and nucleotides 187-204 of Fig. 17), which is the coding strand sequence that essentially encodes amino acids SerSerThrMetGlyGly (amino acids 30 through 35 of *Jun s* I and *Jun v* I as shown in Figs. 16 and 17, respectively).

The sequence of the *Jun s* I clone designated pUC19JS42e was found to be identical to that of clones pUC19JS17d and pUC19JS17f in their regions of overlap, although they had different lengths in the 5' untranslated region. Clone pUC19JS17d had the longest 5' untranslated sequence. Nucleotides 1 through 141 of Fig. 16 correspond to sequence of clone pUC19JS17d. Clone pUC19JS42e corresponds to nucleotides 1 through 538 of Fig. 16.

The sequences of the *Jun v* I clones designated pUC19JV46a and pUC19JV46b were identical to the sequence of clone pUC19JV21g in their regions of overlap, with the exception that nucleotide 83 of Figure 17 was A in clone pUC19JV21g rather than the T shown. This nucleotide difference does not result in a predicted amino acid change. Clones pUC19JV46a, pUC19JV46b and pUC19JV21g correspond to nucleotides 1 through 548, 1 through 548 and 2 through 151 of Figure 17, respectively.

The cDNAs encoding the remainder of the *Jun s* I and *Jun v* I genes were cloned from the respective linkered cDNAs by using degenerate oligonucleotide CP-66, which has the sequence 5'-CATCCGCAAGATGGGGATGC-3', wherein T at position 3 can also be C, G at position 6 can also be A, T, or C, A at position 9 can also be G, T at position 12 can also be C, and T at position 18 can also be C, and AP in a primary PCR. The sequence of CP-66 is complementary to that of CP-63. A secondary PCR was performed on 3% of the initial amplification mixture, with 100 pmoles each of AP and CP-67, which has the sequence 5'-CGGGAATTCCCTCAAGATGGGGATGCGCT-3', wherein A at position 15 can also be G, T at position 18 can also be C, T at position 24 can also be C, G at position 27 can also be A, T, or C, and C at position 28 can be T. The nucleotide sequence 5'-CGGGAATTC-3' of primer CP-67 (bases 1 through 9) were added to create an *Eco*R I restriction site for cloning purposes. The remaining oligonucleotide sequence essentially encodes amino acids

ProGlnAspGlyAspAlaLeu (amino acids 147 through 153 of Figure 4a, corresponding to nucleotides 567 through 586 of Figure 4a). The amplified DNA products, designated JS45 from the *J. sabinoides* amplification and JV49ii from the *J. virginiana* amplification, were purified as described in Example 3, digested with *Eco*R I and *Xba* I (JS45) or *Eco*R I and *Asp*718 I (JV49ii) and electrophoresed through a preparative 1% low melt gel. The dominant DNA bands, which were approximately 650 bp in length, were excised and ligated into pUC19 for sequencing. DNA was sequenced by the dideoxy chain termination method (Sanger et al. supra) using a commercially available kit (sequenase kit, U.S. Biochemicals, Cleveland, OH).

Two clones, designated pUC19JS45a and pUC19JV49iia for *Jun s* I and *Jun v* I, respectively, were sequenced using M13 forward and reverse primers (N.E. BioLabs, Beverly, MA) and internal sequencing primers J8, J9, and J12 for *Jun s* I, and J6 and J11 for *Jun v* I. J8 has the sequence 5'-TAGGACATGATGATACAT-3' (nucleotides 690-707 of Fig. 16), which is the coding strand sequence essentially encoding amino acids LeuGlyHisAspAspThr of *Jun s* I (amino acids 201-206 of Fig. 16). J9 has the sequence 5'-GAGATCTACACGAGATGC-3' (nucleotides 976-993 of Fig. 16) which is the coding strand sequence essentially encoding amino acids ArgSerThrArgAspAla of *Jun s* I (amino acids 297-302 of Fig. 16). J12 has the sequence 5'-AAAACTATTCCCTTCACT-3', wherein A at position 1 can also be G, and A at position 4 can also be T. This is the non-coding strand sequence that corresponds to coding strand sequence (nucleotides 875-892 of Fig. 16) encoding amino acids SerGluGlyAsnSerPhe of *Jun s* I (amino acids 263-268 of Fig. 16). J6 has the sequence 5'-TAGGACATAGTGATTCAT-3' (nucleotides 700-717 of Fig. 17), which is the coding strand sequence essentially encoding amino acids LeuGlyHisSerAspSer of *Jun v* I (amino acids 201-206 of Fig.17). J11 has the sequence 5'-CCGGGATCCTTACAAATAACACATTAT-3', where nucleotides 1-9 encode a *Bam*H I restriction site for cloning purposes and nucletides 10-27 correspond to noncoding strand sequence complementary to nucleotides 1165-1182 of Fig. 17 in the 3' untranslated region of *Jun v* I. The sequence of clone pUC19JS45a corresponds to nucleotides 527 through 1170 of Fig. 16. The

sequence of clone pUC29JV49iia corresponds to nucleotides 537 through 1278 of Fig. 17.

A full length clone of *Jun s* I was amplified using PCR. Oligonucleotides J7 and J10 were used in a PCR reaction as above with *J. sabinoides* double stranded, linkered cDNA. J7 has the sequence 5'-CCCGAATTCATGGCTTCC-CCATGCTTA-3', where nucleotides 1-9 encode an *Eco*R I restriction site added for cloning purposes and nucleotides 10-27 (corresponding to nucleotides 26-43 of Fig. 16) are the coding strand sequence that encode amino acids MetAlaSerProCysLeu of *Jun s* I (amino acids -21 to -16, Fig. 16). J10 has the sequence 5'-CCGGGATCCCGTTTCATAAGCAAGATT-3', where nucleotides 1-9 encode a *Bam*H I restriction site added for cloning purposes and nucleotides 10-27 are the non-coding strand sequence complementary to nucleotides 1140-1157 from the 3' untranslated region of *Jun s* I (Fig. 16). The PCR product, designated JS53ii, gave a band of approximately 1200 bp when examined on a 1% agarose minigel stained with EtBr. The DNA from the JS53ii PCR was recovered as described in Example 3. After precipitation and washing with 70% EtOH, the DNA was simultaneously digested with *Eco*R I and *Bam*H I in a 15 µl reaction and electrophoresed through a preparative 1% GTG SeaPlaque low melt gel (FMC, Rockport, ME). The appropriate sized DNA band was visualized by EtBr staining, excised, and ligated into appropriately digested pUC19 for sequencing by the dideoxy chain termination method (Sanger et al. (1977) supra) using a commercially available sequencing kit (Sequenase kit, U.S. Biochemicals, Cleveland, OH). The resultant clone, pUC19JS53iib was partially sequenced using M13 forward and reverse primers (N.E. Biolabs, Beverly, MA) and internal sequencing primer J4. The sequence of pUC19JS53iib that was determined was identical to that obtained from clones pUC19JS17d, pUC19JS42e, and pUC19JS45a. The nucleotide sequence of clone pUC19JS53iib corresponds to nucleotides 26 through 1157 of Fig. 16.

The nucleotide and predicted amino acid sequences of *Jun s* I are shown in Fig. 16. *Jun s* I has an open reading frame of 1101 nucleotides, corresponding to nucleotides 26 through 1126 of Fig. 16, that can encode a protein of 367 amino acids. Nucleotides 1-25 and 1130-1170 of Fig. 16 are untranslated 5' and

3' regions, respectively. The initiating Met, encoded by nucleotides 26-28 of Fig. 16, has been identified through the 89% identity of nucleotides 23 through 30 (AAAAATGGC) of Fig. 16 with the consensus sequence encompassing the initiating Met in plants (AACAATGGC; Lutcke, supra). There is also an in-frame stop codon just 5' of the codon encoding the initiating Met. Amino acids -21 to -1 of Fig. 16 correspond to a predicted leader sequence. The amino terminus of the mature form of *Jun s* I was identified as amino acid 1 of Fig. 16 through direct protein sequence analysis of purified *Jun s* I (Gross et al supra). The mature form of *Jun s* I, corresponding to amino acids 1 through 346 of Fig. 16, has a predicted molecular weight of 37.7 kDa. *Jun s* I has three potential N-linked glycosylation sites with the consensus sequence of Asn-Xxx-Ser/Thr.

The nucleic and predicted amino acid sequences of *Jun v* I are shown in Fig. 17. Nucleotides 1-35 and 1130-1170 are untranslated 5' and 3' regions, respectively. The initiating Met, encoded by nucleotides 36-38 of Fig. 17, was identified through the 89% identity of nucleotides 23 through 30 (AAAAATGGC) of Fig. 17 with the consensus sequence encompassing the initiating Met in plants (AACAATGGC; Lutcke, supra). The nucleic acids of *Jun s* I (Fig. 16) and *Jun v* I (Fig. 17) are identical in this region surrounding the initiating Met. There are also 2 in-frame stop codons in the 5' untranslated region of Fig. 17. *Jun v* I has an open reading frame of 1,110 nucleotides, corresponding to nucleotides 36 through 1145 of Fig. 17, that can encode a protein of 370 amino acids. Nucleotides 1146-1148 of Fig. 17 encode a stop codon. Amino acids -21 to -1 of *Jun v* I (Fig. 17) correspond to a predicted leader sequence. The amino terminus of the mature form of *Jun v* I was identified as amino acid 1 of Fig. 17 by comparison with the sequences of *Cry j* I (Fig. 4a) and *Jun s* I (Fig. 16). The mature form of *Jun v* I, corresponding to amino acids 1 through 349 of Fig. 17 has a predicted molecular weight of 38.0 kDa. *Jun v* I has four potential N-linked glycosylation sites with the consensus sequence of Asn-Xxx-Ser/Thr.

As shown in Table I, the amino acid sequences of the mature forms of *Jun s* I and *Jun v* I are 80.9% homologous (75.4% identity and 5.5% similarity) with each other. The amino acid sequences of the mature forms of *Jun s* I and

Cry j I are 87% homologous (80.1% identity, 6.9% similarity) and the sequences of the mature forms of *Jun v* I and *Cry j* I are 80.5% homologous (72.5% identity, 8% similarity). The homologies between *Cry j* I peptide sequences identified in Example 6 as containing T cell epitopes and the corresponding *Jun s* I and *Jun v* I sequences are also very high. For example, peptide CJ1-22, corresponding to amino acids 211-230 of *Cry j* I (Fig. 13), contains a major T cell epitope (Fig. 14). CJ1-22 has 95% identity (19/20 identical amino acids) and 85% homology (16/20 identical amino acids, 1/20 similar amino acid) with the corresponding regions of *Jun s* I and *Jun v* I, respectively (see Table I). This high degree of sequence homology suggests that an immunotherapy effective in treating allergic disease caused by *Cry j* I may also be effective in treating allergic diseases caused by *Cry j* I homologues. All nucleic and amino acid analyses were performed using software contained in PCGENE (Intelligenetics, Mountain View, CA).

Table I

Protein/Peptide Comparisons	Identity	Similarity	Total Homology
Jun s I vs. *Jun v* I	75.4%	5.5%	80.9%
Jun s I vs. *Cry j* I	80.1%	6.9%	87.9%
Jun v I vs. *Cry j* I	72.5%	8.0%	80.5%
CJ1-22 vs. *Jun s* $I_{211-230}$	95.0%	0.0%	95.0%
CJ1-22 vs. *Jun v* $I_{211-230}$	80.0%	5.0%	85.0%

Example 10

Northern blot analysis of *C. japonica*, *J. sabinoides*, *J. virginiana* and *C. arizonica* RNA.

A Northern blot analysis was performed on RNA isolated from *C. japonica*, *J. sabinoides* and *J. virginiana* pollens. RNA from *C. japonica* pollens collected in both the United States (Example 3) and Japan (Example 4) were examined.

Using essentially the method of Sambrook, supra, 15 µg of each RNA were run on a 1.2% agarose gel containing 38% formaldehyde and 1X MOPS (20X = 0.4M MOPS, 0.02M EDTA, 0.1M NaOAc, pH 7.0) solution. The RNA samples (first precipitated with 1/10 volume sodium acetate, 2 volumes ethanol to reduce volume and resuspended in 5.5 µl dH2O) were run with 10 µl formaldehyde/formamide buffer containing loading dyes with 15.5% formaldehyde, 42% formamide, and 1.3X MOPS solution, final concentration. The samples were transferred to Genescreen Plus (NEN Research Products, Boston, MA) by capillary transfer in 10X SSC (20X = 3M NaCl, 0.3M Sodium Citrate), after which the membrane was baked 2 hr. at 80°C and UV irradiated for 3 minutes. Prehybridization of the membrane was at 60°C for 1 hour in 4 ml 0.5M NaPO4 (pH 7.2), 1mM EDTA, 1% BSA, and 7% SDS. The antisense probe was synthesized by asymmetric PCR (McCabe, P.C., in: PCR Protocols. A Guide to Methods and Applications, Innis, M., et al., eds. Academic Press, Boston, (1990), pp 76-83) on the JC91a amplification in low melt agarose (described in Example 3), where 2 µl DNA is amplified with 2 µl dNTP mix (0.167 mM dATP, 0.167mM dTTP, 0.167mM dGTP, and 0.033mM dCTP), 2 µl 10X PCR buffer, 10 µl ^{32}P-dCTP (100 µCi; Amersham, Arlington Heights, Il), 1 µl (100 pmoles) antisense primer CP-17, 0.5 µl Taq polymerase, and dH_2O to 20 µl; the 10X PCR buffer, dNTPs and Taq polymerase were from Perkin Elmer Cetus (Norwalk, CT). Amplification consisted of 30 rounds of denaturation at 94°C for 45 sec, annealing of primer to the template at 60°C for 45 sec, and chain elongation at 72°C for 1 min. The reaction was stopped by addition of 100 µl TE, and the probe recovered over a 3cc G-50 spin column (2 ml G-50 Sephadex [Pharmacia, Uppsala, Sweden] in a 3cc syringe plugged with glass wool, equilibrated with TE) and counted on a 1500 TriCarb Liquid Scintillation Counter (Packard, Downers Grove, IL). The probe was added to the prehybridizing buffer at 10^6 cpm/ml and hybridization was carried out at 60°C for 16 hrs. The blot was washed in high stringency conditions: 3x15 min at 65°C with 0.2xSSC/1% SDS, followed by wrapping in plastic wrap and exposure to film at -80°C. A seven hour exposure of this Northern blot revealed a single thick band at approximately 1.2 kb for *C. japonica* (United States) (Fig.

19a, lane 1), *C. japonica* (Japan) (Fig. 19a, lane 2), *J. sabinoides* (Fig. 19a, lane 3) and *J. virginiana* (Fig. 19a, lane 4) RNAs. This band is the expected size for *Cry j* I, *Jun s* I and *Jun v* I as predicted by PCR analysis of the cDNA. The different band intensities in each lane may reflect differences in the amount of RNA loaded on the gel. The position of 1.6 and 1.0 kb molecular weight standards are shown on the Figs. 19a and 19b.

RNA isolated from *J. sabinoides* and *C. arizonica* were analyzed in a separate Northern blot. Five μg of total RNA from *J. sabinoides* and 5 μg of total RNA from *C. arizonica* were probed as described. The 1.2 kb band was observed in this blot for both *J. sabinoides* (Figure 19b, lane 1) and *C. arizonica* (Figure 19b, lane 2), indicating that *C. arizonica* has a *Cry j* I homologue. Other, related, trees are also expected to have a *Cry j* I homologue.

Although this invention has been described with reference to its preferred embodiments, other embodiments can achieve the same results. Variations and modifications to the present invention will be obvious to those skilled in the art and it is intended to cover in the appended claims all such modifications and equivalents that follow in the true spirit and scope of the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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Pollock, Joanne, Bond Julian

(ii) TITLE OF INVENTION: Allergenic Proteins And Peptides From Japanese Cedar Pollen

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(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1337 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Crytpomeria japonica

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 66..1187

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 129..1187

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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AGTCAATCTG CTCATAATCA TAGCATAGCC GTATAGAAAG AAATTCTACA CTCTGCTACC       60

AAAAA ATG GAT TCC CCT TGC TTA GTA GCA TTA CTG GTT TTC TCT TTT           107
      Met Asp Ser Pro Cys Leu Val Ala Leu Leu Val Phe Ser Phe
      -21 -20                 -15                 -10

GTA ATT GGA TCT TGC TTT TCT GAT AAT CCC ATA GAC AGC TGC TGG AGA         155
Val Ile Gly Ser Cys Phe Ser Asp Asn Pro Ile Asp Ser Cys Trp Arg
         -5                  1               5

GGA GAC TCA AAC TGG GCC CAA AAT AGA ATG AAG CTC GCA GAT TGT GCA         203
Gly Asp Ser Asn Trp Ala Gln Asn Arg Met Lys Leu Ala Asp Cys Ala
 10                  15                  20                  25

GTG GGC TTC GGA AGC TCC ACC ATG GGA GGC AAG GGA GGA GAT CTT TAT         251
Val Gly Phe Gly Ser Ser Thr Met Gly Gly Lys Gly Gly Asp Leu Tyr
                 30                  35                  40

ACG GTC ACG AAC TCA GAT GAC GAC CCT GTG AAT CCT GCA CCA GGA ACT         299
Thr Val Thr Asn Ser Asp Asp Asp Pro Val Asn Pro Ala Pro Gly Thr
             45                  50                  55

CTG CGC TAT GGA GCA ACC CGA GAT AGG CCC CTG TGG ATA ATT TTC AGT         347
Leu Arg Tyr Gly Ala Thr Arg Asp Arg Pro Leu Trp Ile Ile Phe Ser
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                60                  65                  70

GGG AAT ATG AAT ATA AAG CTC AAA ATG CCT ATG TAC ATT GCT GGG TAT      395
Gly Asn Met Asn Ile Lys Leu Lys Met Pro Met Tyr Ile Ala Gly Tyr
     75                  80                  85

AAG ACT TTT GAT GGC AGG GGA GCA CAA GTT TAT ATT GGC AAT GGC GGT      443
Lys Thr Phe Asp Gly Arg Gly Ala Gln Val Tyr Ile Gly Asn Gly Gly
 90                  95                 100                 105

CCC TGT GTG TTT ATC AAG AGA GTT AGC AAT GTT ATC ATA CAC GGT TTG      491
Pro Cys Val Phe Ile Lys Arg Val Ser Asn Val Ile Ile His Gly Leu
                110                 115                 120

TAT CTG TAC GGC TGT AGT ACT AGT GTT TTG GGG AAT GTT TTG ATA AAC      539
Tyr Leu Tyr Gly Cys Ser Thr Ser Val Leu Gly Asn Val Leu Ile Asn
            125                 130                 135

GAG AGT TTT GGG GTG GAG CCT GTT CAT CCT CAG GAT GGC GAT GCT CTT      587
Glu Ser Phe Gly Val Glu Pro Val His Pro Gln Asp Gly Asp Ala Leu
        140                 145                 150

ACT CTG CGC ACT GCT ACA AAT ATT TGG ATT GAT CAT AAT TCT TTC TCC      635
Thr Leu Arg Thr Ala Thr Asn Ile Trp Ile Asp His Asn Ser Phe Ser
    155                 160                 165

AAT TCT TCT GAT GGT CTG GTC GAT GTC ACT CTT ACT TCG ACT GGA GTT      683
Asn Ser Ser Asp Gly Leu Val Asp Val Thr Leu Thr Ser Thr Gly Val
170                 175                 180                 185

ACT ATT TCA AAC AAT CTT TTT TTC AAC CAT CAT AAA GTG ATG TTG TTA      731
Thr Ile Ser Asn Asn Leu Phe Phe Asn His His Lys Val Met Leu Leu
                190                 195                 200

GGG CAT GAT GAT GCA TAT AGT GAT GAC AAA TCC ATG AAG GTG ACA GTG      779
Gly His Asp Asp Ala Tyr Ser Asp Asp Lys Ser Met Lys Val Thr Val
            205                 210                 215

GCG TTC AAT CAA TTT GGA CCT AAC TGT GGA CAA AGA ATG CCC AGG GCA      827
Ala Phe Asn Gln Phe Gly Pro Asn Cys Gly Gln Arg Met Pro Arg Ala
        220                 225                 230

CGA TAT GGA CTT GTA CAT GTT GCA AAC AAT AAT TAT GAC CCA TGG ACT      875
Arg Tyr Gly Leu Val His Val Ala Asn Asn Asn Tyr Asp Pro Trp Thr
    235                 240                 245

ATA TAT GCA ATT GGT GGG AGT TCA AAT CCA ACC ATT CTA AGT GAA GGG      923
Ile Tyr Ala Ile Gly Gly Ser Ser Asn Pro Thr Ile Leu Ser Glu Gly
250                 255                 260                 265

AAT AGT TTC ACT GCA CCA AAT GAG AGC TAC AAG AAG CAA GTA ACC ATA      971
Asn Ser Phe Thr Ala Pro Asn Glu Ser Tyr Lys Lys Gln Val Thr Ile
                270                 275                 280

CGT ATT GGA TGC AAA ACA TCA TCA TCT TGT TCA AAT TGG GTG TGG CAA     1019
Arg Ile Gly Cys Lys Thr Ser Ser Ser Cys Ser Asn Trp Val Trp Gln
            285                 290                 295
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TCT ACA CAA GAT GTT TTT TAT AAT GGA GCT TAT TTT GTA TCA TCA GGG      1067
Ser Thr Gln Asp Val Phe Tyr Asn Gly Ala Tyr Phe Val Ser Ser Gly
        300                 305                 310

AAA TAT GAA GGG GGT AAT ATA TAC ACA AAG AAA GAA GCT TTC AAT GTT      1115
Lys Tyr Glu Gly Gly Asn Ile Tyr Thr Lys Lys Glu Ala Phe Asn Val
    315                 320                 325

GAG AAT GGG AAT GCA ACT CCT CAA TTG ACA AAA AAT GCT GGG GTT TTA      1163
Glu Asn Gly Asn Ala Thr Pro Gln Leu Thr Lys Asn Ala Gly Val Leu
330                 335                 340                 345

ACA TGC TCT CTC TCT AAA CGT TGT TGATGATGCA TATATTCTAG CATGTTGTAC     1217
Thr Cys Ser Leu Ser Lys Arg Cys
                350

TATCTAAATT AACATCAACA AGAAAATATA TCATGATGTA TATTGTTGTA TTGATGTCAA    1277

AATAAAAATG TATCTTTTAC TATTAAAAAA AAAAATGATC GATCGGACGG TACCTCTAGA    1337
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 374 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Asp Ser Pro Cys Leu Val Ala Leu Leu Val Phe Ser Phe Val Ile
-21 -20                 -15                 -10

Gly Ser Cys Phe Ser Asp Asn Pro Ile Asp Ser Cys Trp Arg Gly Asp
 -5                  1                   5                  10

Ser Asn Trp Ala Gln Asn Arg Met Lys Leu Ala Asp Cys Ala Val Gly
             15                  20                  25

Phe Gly Ser Ser Thr Met Gly Gly Lys Gly Gly Asp Leu Tyr Thr Val
         30                  35                  40

Thr Asn Ser Asp Asp Asp Pro Val Asn Pro Ala Pro Gly Thr Leu Arg
     45                  50                  55

Tyr Gly Ala Thr Arg Asp Arg Pro Leu Trp Ile Ile Phe Ser Gly Asn
 60                  65                  70                  75

Met Asn Ile Lys Leu Lys Met Pro Met Tyr Ile Ala Gly Tyr Lys Thr
                 80                  85                  90

Phe Asp Gly Arg Gly Ala Gln Val Tyr Ile Gly Asn Gly Gly Pro Cys
             95                  100                 105

Val Phe Ile Lys Arg Val Ser Asn Val Ile Ile His Gly Leu Tyr Leu
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                110                 115                 120

Tyr Gly Cys Ser Thr Ser Val Leu Gly Asn Val Leu Ile Asn Glu Ser
    125                 130                 135

Phe Gly Val Glu Pro Val His Pro Gln Asp Gly Asp Ala Leu Thr Leu
140                 145                 150                 155

Arg Thr Ala Thr Asn Ile Trp Ile Asp His Asn Ser Phe Ser Asn Ser
                160                 165                 170

Ser Asp Gly Leu Val Asp Val Thr Leu Thr Ser Thr Gly Val Thr Ile
            175                 180                 185

Ser Asn Asn Leu Phe Phe Asn His His Lys Val Met Leu Leu Gly His
        190                 195                 200

Asp Asp Ala Tyr Ser Asp Asp Lys Ser Met Lys Val Thr Val Ala Phe
    205                 210                 215

Asn Gln Phe Gly Pro Asn Cys Gly Gln Arg Met Pro Arg Ala Arg Tyr
220                 225                 230                 235

Gly Leu Val His Val Ala Asn Asn Asn Tyr Asp Pro Trp Thr Ile Tyr
                240                 245                 250

Ala Ile Gly Gly Ser Ser Asn Pro Thr Ile Leu Ser Glu Gly Asn Ser
            255                 260                 265

Phe Thr Ala Pro Asn Glu Ser Tyr Lys Lys Gln Val Thr Ile Arg Ile
        270                 275                 280

Gly Cys Lys Thr Ser Ser Ser Cys Ser Asn Trp Val Trp Gln Ser Thr
    285                 290                 295

Gln Asp Val Phe Tyr Asn Gly Ala Tyr Phe Val Ser Ser Gly Lys Tyr
300                 305                 310                 315

Glu Gly Gly Asn Ile Tyr Thr Lys Lys Glu Ala Phe Asn Val Glu Asn
                320                 325                 330

Gly Asn Ala Thr Pro Gln Leu Thr Lys Asn Ala Gly Val Leu Thr Cys
            335                 340                 345

Ser Leu Ser Lys Arg Cys
        350
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GAYAAYCCNA THGAYWS
17

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GGGAATTCAA YTGGGCNCAR AAYSG
25

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION: 15
(D) OTHER INFORMATION: /mod_base= i

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CTGCAGCCRT TYTCNACRTT RAA
23

2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION: 6
(D) OTHER INFORMATION: /mod_base= i

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

TTCATNCKRT TYTGNGCCCA
20

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CCTGCAGCKR TTYTGNGCCC AARTT
25

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

ATGGATTCCC CTTGCTTA
18

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GGGAATTCGA TAATCCCATA GACAGC
26

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

ATGCCTATGT ACATTGC
17

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GCAATGTACA TAGGCAT
17

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

TCCAATTCTT CTGATGGT
18

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TTTTGTCAAT TGAGGAGT
18

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CCTGCAGAAG CTTCATCAAC AACGTTTAGA
30

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TAGCAACTCC AGTCGAAGT
19

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TAGCTCTCAT TTGGTGC
17

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

TATGCAATTG GTGGGAGT
18

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Cryptomeria japonica

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 7
(D) OTHER INFORMATION: /note= "the amino acid at position

7 is Ser, Cys, Thr, or His"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Asp Asn Pro Ile Asp Ser Xaa Trp Arg Gly Asp Ser Asn Trp Ala Gln
1 5 10 15

Asn Arg Met Lys
20

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Cryptomeria japonica

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Glu Ala Phe Asn Val Glu Asn Gly Asn Ala Thr Pro Gln Leu Thr Lys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GGGTCTAGAG GTACCGTCCG ATCGATCATT

30

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GGGTCTAGAG GTACCGTCCG
20

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

AATGATCGAT GCT
13

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GGAATTCTCT AGACTGCAGG T
21

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GGAATTCTCT AGACTGCAGG TTTTTTTTTT TTTTT 35

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Juniperus sabinoides

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Asp Asn Pro Ile Asp
1 5

Claims

1. An isolated peptide of *Cry j* I or an isolated portion thereof, said peptide or portion thereof comprising at least one T cell epitope of *Cry j* I, said peptide having an amino acid sequence selected from the group consisting of CJ1-2, CJ1-3, CJ1-4, CJ1-7, CJ1-8, CJ1-9, CJ1-10, CJ1-11, CJ1-12, CJ1-14, CJ1-15, CJ1-16, CJ1-17, CJ1-18, CJ1-19, CJ1-20, CJ1-21, CJ1-22, CJ1-23, CJ1-24, CJ1-25, CJ1-26, CJ1-27, CJ1-30, CJ1-31, CJ1-32 and CJ1-35.

2. An isolated peptide or portion thereof of claim 1 wherein said portion of said peptide has a mean T cell stimulation index equivalent to or greater than the mean T cell stimulation index of said peptide as shown in Fig. 14.

3. An isolated peptide or portion thereof of claim 1 which comprises at least two T cell epitopes.

4. An isolated peptide or portion thereof of claim 1 which, when administered to an individual sensitive to Japanese cedar pollen, induces T cell anergy in the individual or modifies the lymphokine secretion profile of T cells in the individual.

5. A portion of an isolated peptide of claim 1 which has a mean T cell stimulation index of at least 2.0.

6. All or a portion of an isolated peptide of claim 1 which does not bind immunoglobulin E specific for *Cry j* I in a substantial percentage of individuals sensitive to *Cry j* I, or if binding of the peptide or portion thereof to said immunoglobulin E occurs, such binding does not result in release of mediators from mast cells or basophils in a substantial percentage of individuals sensitive to *Cry j* I.

7. An isolated peptide of claim 1 which binds immunoglobulin E to a substantially lesser extent than *Cry j* I binds immunoglobulin E.

8. All or a portion of an isolated peptide of claim 1 which modifies in an individual sensitive to Japanese cedar pollen to whom it is administered, the allergic response of the individual to a Japanese cedar pollen.

9. A portion of an isolated peptide of claim 1 wherein the portion comprises at least 15 amino acid residues.

10. An isolated nucleic acid sequence having a sequence encoding all or a portion of a peptide of claim 1, or the functional equivalent of said nucleic acid sequence.

11. An isolated peptide which is immunologically cross-reactive with antibodies specific for all or a portion of a peptide of claim 1.

12. An isolated peptide which is immunologically cross-reactive with T cells reactive with all or a portion of a peptide of claim 1.

13. An isolated peptide or portion thereof of Japanese cedar pollen protein allergen,*Cry j* I, said peptide or portion thereof comprising at least one T cell epitope of said protein allergen, said peptide having a positivity index of at least about 100 and mean T cell stimulation index of at least about 3.5 determined in a population of individuals sensitive to said protein allergen.

14. An isolated peptide or portion thereof of claim 13 wherein said population of individuals is at least twenty-five individuals.

15. An isolated peptide or portion thereof of claim 14 wherein said population of individuals is at least thirty individuals.

16. An isolated peptide or portion thereof of claim 14 wherein said mean T cell stimulation index is at least about 5.0.

17. An isolated peptide or portion thereof of claim 14 wherein said mean T cell stimulation index is at least about 7.0.

18. A peptide or portion thereof of claim 14 wherein said peptide is selected from the group consisting of: CJ1-16, CJ1-17, CJ1-20, CJ1-22, CJ1-23, CJ1-24, CJ1-26, CJ1-27, CJ1-30, CJ1-31, CJ1-32 and CJ1-35.

19. A peptide or portion thereof of claim 17 wherein said peptide has an amino acid sequence selected from the group consisting of: CJ1-16, CJ1-20, CJ1-22, CJ1-27 and CJ1-32.

20. A modified peptide or a modified portion of a peptide of claim 1.

21. A modified peptide or a modified portion of a peptide of claim 20 which does not bind immunoglobulin E specific for *Cry j* I in a substantial percentage of individuals sensitive to *Cry j* I, or if binding of the peptide or portion thereof to said immunoglobulin E occurs, such binding does not result in release of mediators from mast cells or basophils in a substantial percentage of individuals sensitive to *Cry j* I.

22. A modified peptide or a modified portion of a peptide of claim 20 which modifies, in an individual sensitive to Japanese cedar pollen to whom it is administered, the allergic response of the individual to a Japanese cedar pollen allergen.

23. An isolated peptide of *Cry j* I or portion thereof comprising amino acids 151-352 of the amino acid sequence of *Cry j* I as shown in Fig. 4a - b.

24. A modified peptide or a modified portion of a peptide of claim 23.

25. An isolated peptide comprising at least two regions, each region comprising at least one T cell epitope of *Cry j* I, said regions each comprising all or a portion of an amino acid sequence selected from the group consisting of: CJ1-1, CJ1-2, CJ1-3, CJ1-4, CJ1-7, CJ1-8, CJ1-9, CJ1-10, CJ1-11, CJ1-12, CJ1-14, CJ1-15, CJ1-16, CJ1-17, CJ1-18, CJ1-19, CJ1-20, CJ1-21, CJ1-22, CJ1-23, CJ1-24, CJ1-25, CJ1-26, CJ1-27, CJ1-28, CJ1-30, CJ1-31, CJ1-32, CJ-33, CJ-34 and CJ1-35.

26. All or a portion of an isolated peptide of claim 25 wherein said regions comprise an amino acid sequence selected from the group consisting of: CJ1-2, CJ1-9, CJ1-10, CJ1-16, CJ1-17, CJ1-20, CJ1-22, CJ1-23, CJ1-24, CJ1-25, CJ1-26, CJ1-27, CJ1-30, CJ1-31, CJ1-32, CJ1-35.

27. An isolated peptide of claim 25, wherein said peptide comprises a combination of regions selected from the group consisting of:

CJ1-1, CJ1-2 and CJ1-3;
CJ1-1 and CJ1-2;
CJ1-9 and CJ1-10;
CJ1-14, CJ1-15, CJ1-16 and CJ1-17;
CJ1-20, CJ1-21, CJ1-22, CJ1-23;
CJ1-20, CJ1-22 and CJ1-23;
CJ1-22 and CJ1-23;
CJ1-22, CJ1-23 and CJ1-24;
CJ1-24 and CJ1-25;

CJ1-30, CJ1-31 and CJ1-32;
CJ1-31 and CJ1-32;
CJ1-22, CJ1-23, CJ1-16 and CJ1-17;
CJ1-22, CJ1-23, CJ1-31 and CJ1-32;
CJ1-16 and CJ1-17;
CJ1-16, CJ1-17, CJ1-31 and CJ1-32;
CJ1-9, CJ1-10 and CJ1-16;
CJ1-17, CJ1-22 and CJ1-23;
CJ1-16, CJ1-17 and CJ1-20;
CJ1-31, CJ1-32 and CJ1-20;
CJ1-22, CJ1-23, CJ1-1, CJ1-2 and CJ1-3;
CJ1-16, CJ1-17, CJ1-22 and CJ1-23, CJ1-31 and CJ1-32;
CJ1-9, CJ1-10, CJ1-16, CJ1-17, CJ1-22 and CJ1-23;
CJ1-9, CJ1-10, CJ1-16, CJ1-17, CJ1-31 and CJ1-32;
CJ1-9, CJ1-10, CJ1-22, CJ1-23, CJ1-31 and CJ1-32;
CJ1-9, CJ1-10, CJ1-16, CJ1-17, CJ1-22, CJ1-23, CJ1-31 and CJ1-32;
CJ1-1, CJ1-2, CJ1-16, CJ1-17, CJ1-22 and CJ1-23.

28. An isolated nucleic acid having a sequence encoding said isolated peptide or portion thereof of claim 1 or the functional equivalent of said nucleic acid sequence.

29. An isolated peptide produced in a host cell transformed with the nucleic acid of claim 28.

30. An isolated nucleic acid having a sequence encoding a peptide of claim 25, or the functional equivalent of said nucleic acid sequence.

31. An isolated peptide produced in a host cell transformed with the nucleic acid of claim 30.

32. All or a portion of an isolated peptide of *Cry j* I, said peptide or portion thereof comprising at least one T cell epitope of said protein allergen, said peptide having the formula X_n-Y-Z_m, wherein Y is an amino acid sequence selected from the group consisting of: CJ1-2, CJ1-3, CJ1-4, CJ1-7, CJ1-8, CJ1-9, CJ1-10, CJ1-11, CJ1-12, CJ1-14, CJ1-15, CJ1-16, CJ1-17, CJ1-18, CJ1-19, CJ1-20, CJ1-21, CJ1-22, CJ1-23, CJ1-24, CJ1-25, CJ1-26, CJ1-27, CJ1-28, CJ1-30, CJ1-31, CJ1-32 and CJ1-35 wherein X_n are amino acid residues contiguous to the amino terminus of Y in the amino acid sequence of said protein allergen, wherein Z_m are amino acid residues contiguous to the carboxy terminus of Y in the amino acid sequence of said protein allergen, wherein n is 0-30 and wherein m is 0-30.

33. A portion of an isolated peptide of claim 32 wherein the portion comprises at least fifteen amino acid residues.

34. All or a portion of an isolated peptide of claim 32 which does not bind immunoglobulin E specific for *Cry j* I in a substantial percentage of individuals sensitive to the protein allergen, or if binding of the peptide or portion thereof to said immunoglobulin E occurs, such binding does not result in release of mediators from mast cells or basophils in a substantial percentage of individuals sensitive to the protein allergen.

35. An isolated peptide or portion thereof of claim 32 which binds immunoglobulin E to a substantially lesser extent than *Cry j* I binds said immunoglobulin E.

36. An isolated peptide of *Cry j* I or an isolated portion thereof, said peptide or portion thereof comprising at least one T cell epitope of *Cry j* I, said peptide having an amino acid sequence comprising amino acids 20-324 or 341-353 of *Cry j* I as shown in Fig. 4a-b.

37. A therapeutic composition comprising at least one isolated peptide or a portion thereof of claim 1 and a pharmaceutically acceptable carrier or diluent.

38. A therapeutic composition comprising at least one isolated peptide or portion thereof of claim 13 and a pharmaceutically acceptable carrier or diluent.

39. A therapeutic composition comprising an isolated peptide or portion thereof of claim 23 and a pharmaceutically acceptable carrier or diluent.

40. Use of a composition of claim 37 for the manufacture of a medicament for treating sensitivity to Japanese cedar pollen allergen or an allergen which is immunologically cross-reactive with Japanese cedar pollen allergen in an individual.

41. Use of a composition of claim 39 for the manufacture of a medicament for treating sensitivity to Japanese cedar pollen allergen or an allergen which is immunologically cross-reactive with Japanese cedar pollen allergen in an individual.

42. Use of at least two different compositions of claim 37 for the manufacture of a medicament for treating sensitivity to Japanese cedar pollen allergen or an allergen which is immunologically cross-reactive with Japanese cedar pollen allergen in an individual.

43. Use of at least two different compositions of claim 38 for the manufacture of a medicament for treating sensitivity to Japanese cedar pollen allergen or an allergen which is immunologically cross-reactive with Japanese cedar pollen allergen in an individual

44. A method of detecting sensitivity to Japanese cedar pollen in an individual, comprising combining a blood sample obtained from the individual with at least one peptide of claim 1, under conditions appropriate for binding of blood components with the peptide, and determining the extent to which such binding occurs as indicative of sensitivity in the individual to Japanese cedar pollen.

45. A method of claim 44 wherein the extent to which binding occurs is determined by assessing T cell function, T cell proliferation or a combination thereof.

46. A method of detecting sensitivity to Japanese cedar pollen in an individual, comprising combining a blood sample obtained from the individual with at least one peptide of claim 13, under conditions appropriate for binding of blood components with the peptide, and determining the extent to which such binding occurs as indicative of sensitivity in the individual to Japanese cedar pollen.

47. A method of claim 46 wherein the extent to which binding occurs is determined by assessing T cell function, T cell proliferation or a combination thereof.

48. A method of detecting sensitivity to Japanese cedar pollen in an individual, comprising combining a blood sample obtained from the individual with all or a portion of at least one peptide of claim 32, under conditions appropriate for binding of blood components with the peptide or portion thereof, and determining the extent to which such binding occurs as indicative of sensitivity in the individual to Japanese cedar pollen.

49. A method of claim 48 wherein the extent to which binding occurs is determined by assessing T cell function, T cell proliferation or a combination thereof.

50. A therapeutic composition comprising a pharmaceutically acceptable carrier or diluent and at least two peptides, said peptides each comprising at least one T cell epitope of *Cry j* I.

51. A composition of claim 50 wherein said peptides are selected from the group consisting of: CJ1-1, CJ1-2, CJ1-3, CJ1-4, CJ1-7, CJ1-8, CJ1-9, CJ1-10, CJ1-11, CJ1-12, CJ1-14, CJ1-16, CJ1-17, CJ1-18, CJ1-19, CJ1-20, CJ1-21, CJ1-22, CJ1-23, CJ1-24, CJ1-25, CJ1-26, CJ1-27, CJ1-28, CJ1-30, CJ1-31, CJ1-32, CJ1-33, CJ1-34 and CJ1-35 and wherein said composition comprises a sufficient percentage of the T cell epitopes of said protein allergen such that upon administration of the composition to an individual sensitive to a Japanese cedar pollen allergen, T cells of the individual are tolerized to said at least one protein allergen.

52. A composition of claim 51 comprising a combination of peptides selected from the group consisting of:

CJ1-1, CJ1-2 and CJ1-3;
CJ1-1 and CJ1-2;
CJ1-9 and CJ1-10;
CJ1-14, CJ1-15, CJ1-16 and CJ1-17;
CJ1-20, CJ1-21, CJ1-22, CJ1-23;
CJ1-20, CJ1-22 and CJ1-23;
CJ1-22 and CJ1-23;
CJ1-22, CJ1-23 and CJ1-24;
CJ1-24 and CJ1-25;
CJ1-30, CJ1-31 and CJ1-32;
CJ1-31 and CJ1-32;
CJ1-22, CJ1-23, CJ1-16 and CJ1-17;
CJ1-22, CJ1-23, CJ1-31 and CJ1-32;
CJ1-16, CJ1-17, CJ1-31 and CJ1-32;
CJ1-9, CJ1-10 and CJ1-16;

CJ1-16 and CJ1-17;
CJ1-17, CJ1-22 and CJ1-23;
CJ1-16, CJ1-17 and CJ1-20;
CJ1-31, CJ1-32 and CJ1-20;
CJ1-22, CJ1-23, CJ1-1, CJ1-2 and CJ1-3;
CJ1-16, CJ1-17, CJ1-22 and CJ1-23, CJ1-31 and CJ1-32;
CJ1-9, CJ1-10, CJ1-16, CJ1-17, CJ1-22 and CJ1-23;
CJ1-9, CJ1-10, CJ1-16, CJ1-17, CJ1-31 and CJ1-32;
CJ1-9, CJ1-10, CJ1-22, CJ1-23, CJ1-31 and CJ1-32;
CJ1-9, CJ1-10, CJ1-16, CJ1-17, CJ1-22, CJ1-23, CJ1-31 and CJ1-32;
CJ1-1, CJ1-2, CJ1-16, CJ1-17, CJ1-22 and CJ1-23;
CJ1-22, CJ1-23, CJ1-24, CJ1-9, and CJ1-10;
CJ1-22, CJ1-23, CJ1-24, CJ1-9, CJ1-10, CJ1-16, and CJ1-17;
CJ1-22, CJ1-23, CJ1-24, CJ1-16, CJ1-17, CJ1-31 and CJ1-32;
CJ1-22, CJ1-23, CJ1-24, CJ1-16, and CJ1-17;
CJ1-22, CJ1-23, CJ1-24, CJ1-9, CJ1-10, CJ1-31 and CJ1-32;
CJ1-22, CJ1-23, CJ1-24, CJ1-9, CJ1-10, CJ1-16, CJ1-17, CJ1-31 and CJ1-32; and
CJ1-22, CJ1-23, CJ1-24, CJ1-31, and CJ1-32.

53. Use of a composition of claim 50 for the manufacture of a medicament for treating sensitivity to Japanese cedar pollen allergen or an allergen which is immunological cross-reactive with Japanese cedar pollen allergen in an individual.

54. Use of a composition of claim 52 for the manufacture of a medicament for treating sensitivity to Japanese cedar pollen allergen or an allergen which is immunologically cross-reactive with Japanese cedar pollen allergen in an individual.

55. A therapeutic composition comprising at least one peptide of *Cry j* I and a pharmaceutically acceptable carrier or diluent, said composition comprising a sufficient percentage of the T cell epitopes of *Cry j* I such that upon administration of the composition to an individual sensitive to a Japanese cedar pollen allergen, T cells of the individual are tolerized to *Cry j* I.

56. A method of treating sensitivity to Japanese cedar pollen allergen or an allergen which is immunologically cross-reactive with Japanese cedar pollen allergen in an individual, comprising administering to the individual a therapeutically effective amount of a composition of claim 55.

57. A nucleic acid sequence coding for at least one fragment of *Cry j* I thereof or the functional equivalent of said nucleic acid sequence.

58. A nucleic acid sequence of claim 57 wherein said nucleic acid sequence consists essentially of at least one fragment of the coding portion of the nucleic acid sequence of *Cry j* I as shown in Fig. 4 a-b.

59. A host cell transformed to express a peptide encoded by the nucleic acid sequence of claim 57.

60. At least one antigenic fragment of Japanese cedar pollen produced in a host cell transformed with the nucleic acid sequence of claim 57.

61. At least one antigenic fragment of claim 60 wherein said fragment does not bind immunoglobulin E specific for Japanese cedar pollen or if binding of the fragment to said immunoglobulin E occurs, such binding does not result in histamine release from mast cells or basophils.

62. The isolated antigenic fragment of claim 60 wherein said isolated

antigenic fragment is capable of modifying, in a Japanese cedar pollen-sensitive individual to which it is administered, the allergic response to Japanese cedar pollen.

63. A method of producing at least one isolated fragment of *Cry j* I comprising the steps of:

a) culturing a host cell transformed with a nucleic acid sequence encoding at least one fragment of *Cry j* I in a appropriate medium to produce a mixture of cells and medium containing at least one isolated fragment of *Cry j* I; and

b) purifying said mixture to produce at least one substantially pure fragment of *Cry j* I.

64. An isolated peptide of Cry j I or an isolated portion thereof, said peptide or portion thereof comprising at least one T cell epitope of Cry j I wherein said peptide has an amino acid sequence selected from the group consisting of CJ1-1, CJ1-2, CJ1-3, CJ1-4, CJ1-7, CJ1-8, CJ1-9, CJ1-10, CJ1-11, CJ1-12, CJ1-14, CJ1-15, CJ1-16, CJ1-17, CJ1-18, CJ1-19, CJ1-20, CJ1-21, CJ1-22, CJ1-23, CJ1-24, CJ1-25, CJ1-26, CJ1-27, CJ1-28, CJ1-30, CJ1-31, CJ1-32, CJ1-33, CJ1-34 and CJ1-35.

65. An isolated peptide or portion thereof of claim 64 wherein said peptide has an amino acid sequence selected from the group consisting of CJ1-2, CJ1-9, CJ1-10, CJ1-16, CJ1-17, CJ1-20, CJ1-22, CJ1-23, CJ1-24, CJ1-25, CJ1-26, CJ1-27, CJ1-30, CJ1-31, CJ1-32 and CJ1-35.

66. At least one modified fragment of Japanese cedar pollen allergen, which when administered to a Japanese cedar pollen-sensitive individual, reduces the allergic response of the individual to Japanese cedar pollen allergen.

67. An isolated protein allergen or antigenic fragment thereof that is

immunologically related to *Cry j* I or fragment thereof.

68. A therapeutic composition comprising at least one isolated antigenic fragment of *Cry j* I and a pharmaceutically acceptable carrier or diluent.

69. A protein preparation comprising at least one fragment of Japanese cedar pollen allergen *Cry j* I, synthesized in a host cell transformed with a nucleic acid sequence encoding a portion of Japanese cedar pollen allergen *Cry j* I.

70. A method of treating sensitivity to Japanese cedar pollen allergen or an allergen immunologically cross-reactive with Japanese cedar pollen allergen in a mammal sensitive to said allergen, comprising administering to said mammal a therapeutically effective amount of said preparation of claim 69.

71. A method of detecting sensitivity in a mammal to a Japanese cedar pollen allergen comprising combining a blood sample obtained from said mammal with a purified antigenic fragment of Japanese cedar pollen produced in a host cell transformed with the nucleic acid sequence of claim 57 or chemically synthesized under conditions appropriate for binding of blood components with the fragment and determining the extent to which such binding occurs.

72. A method of detecting sensitivity of a mammal to Japanese cedar pollen allergen comprising administering to said mammal a sufficient quantity of at least one antigenic fragment of Japanese cedar pollen allergen *Cry j* I produced in a host cell transformed with the nucleic acid sequence of claim 57 or chemically synthesized to provoke an allergic response in said mammal and determining the occurrence of an allergic response in the individual to said antigenic fragment of Japanese cedar pollen allergen.

73. A monoclonal antibody specifically reactive with at least one antigenic fragment of Japanese cedar pollen allergen, *Cry j* I.

74. All or a portion of an isolated peptide of *Cry j* I, said peptide or portion thereof comprising at least one T cell epitope of *Cry j* I, said peptide having an amino acid sequence selected from the group consisting of amino acid residues 1-40, amino acid residues 81-110, amino acid residues 151-180, amino acid residues 191-260 and amino acid residues 291-330 of *Cry j* I as shown in Fig. 4a-b.

75. A method of designing antigenic fragments of *Cry j* I, which when administered to Japanese cedar pollen sensitive individuals in sufficient quantity will modify the individual's allergic exposure to Japanese cedar pollen comprising the steps of:

(a) recombinantly or synthetically producing peptides of *Cry j* I;

(b) examining said peptides for their ability to influence B cell and/or T cell responses in Japanese cedar pollen sensitive individuals; and

(c) selecting appropriate peptides which contain epitopes recognized by the cells.

76. An isolated peptide of *Cry j* I or an isolated portion thereof, said peptide or portion thereof comprising at least one T cell epitope of *Cry j* I, said peptide having an amino acid sequence selected from the group consisting of: CJ1-41, CJ1-41.1, CJ1-41.2, CJ1-41.3, CJ1-42, CJ1-42.1, CJ1-42.2, CJ1-43, CJ1-43.1, CJ1-43.6, CJ1-43.7, CJ1-43.8, CJ1-43.9, CJ1-43.10, CJ1-43.11, CJ1-43.12, CJ1-45, CJ1-45.1, CJ1-45.2, CJ1-44, CJ1-44.1, CJ1-44.2 and CJ1-44.3.

77. A therapeutic composition comprising at least one isolated

peptide or portion thereof of claim 76 and a pharmaceutically acceptable carrier or diluent.

78. Use of a composition of claim 76 for the manufacture of a medicament for treating sensitivity to Japanese cedar pollen allergen or any allergen which is immunologically cross-reactive with Japanese cedar pollen allergen in an individual.

79. The use of claim 78 wherein said allergen which is immunologically cross-reactive with Japanese cedar pollen allergen is *Jun s* I or *Jun v* I.

80. A modified peptide or a modified portion of a peptide of claim 76.

81. A method of detecting sensitivity to Japanese cedar pollen in an individual, comprising combining a blood sample obtained from the individual with all or a portion of at least one peptide of claim 76, under conditions appropriate for binding of blood components with the peptide or portion thereof, and determining the extent to which such binding occurs as indicative of sensitivity in the individual to Japanese cedar pollen.

82. A composition of claim 76 wherein said peptides are selected from the group consisting of: CJ1-41, CJ1-41.1, CJ1-41.2, CJ1-41.3, CJ1-42, CJ1-42.1, CJ1-42.2, CJ1-43, CJ1-43.1, CJ1-43.6, CJ1-43.7, CJ1-43.8, CJ1-43.9, CJ1-43.10, CJ1-43.11, CJ1-43.12, CJ1-45, CJ1-45.1, CJ1-45.2, CJ1-44, CJ1-44.1, CJ1-44.2 and CJ1-44.3 and wherein said composition comprises a sufficient percentage of the T cell epitopes of said protein allergen such that upon administration of the composition to an individual sensitive to a Japanese cedar pollen allergen, T cells of the individual are tolerized to said at least one protein allergen.

83. Use of a composition of claim 76 in the manufacture of a medicament for treating sensitivity to Japanese cedar pollen allergen or any allergen which is immunologically cross-reactive with Japanese cedar pollen allergen in an individual.

84. The use of claim 83 wherein said allergen which is immunologically cross-reactive with Japanese cedar pollen allergen is *Jun s* I or *Jun v* I.

85. An isolated purified native protein or peptide of *Jun v* I.

86. An isolated nucleic acid having a nucleotide sequence coding for *Jun s* I, or at least one fragment thereof or the functional equivalent of said nucleotide sequence.

87. An isolated nucleic acid sequence of claim 86 wherein said nucleotide sequence consists essentially of the coding portion of the nucleotide sequence of Fig. 16.

88. An isolated nucleic acid sequence of claim 86 wherein said nucleotide sequence consists essentially of the nucleotide sequence of Fig. 16.

89. An expression vector comprising a nucleotide sequence coding for *Jun s* I, or at least one fragment thereof or the functional equivalent of said nucleotide sequence.

90. An expression vector of claim 89 wherein said nucleotide sequence consists essentially of the coding portion of the nucleotide sequence of Fig. 16.

91. A host cell transformed to express a protein or peptide encoded by the nucleic acid of claim 86.

92. Isolated *Jun s* I protein, or at least one antigenic fragment thereof, produced in a host cell transformed with the nucleic acid of claim 86.

93. An isolated nucleic acid having a nucleotide sequence coding for *Jun v* I, or at least one fragment thereof or the functional equivalent of said nucleotide sequence.

94. An isolated nucleic acid sequence of claim 93 wherein said nucleotide sequence consists essentially of the coding portion of the nucleotide sequence of Fig. 17.

95. An isolated nucleic acid sequence of claim 93 wherein said nucleotide sequence consists essentially of the nucleotide sequence of Fig. 17.

96. An expression vector comprising a nucleotide sequence coding for *Jun v* I, or at least one fragment thereof or the functional equivalent of said nucleotide sequence.

97. An expression vector of claim 96 wherein said nucleotide sequence consists essentially of the coding portion of the nucleotide sequence of Fig. 17.

98. A host cell transformed to express a protein or peptide encoded by the nucleic acid of claim 93.

99. Isolated *Jun v* I protein, or at least one antigenic fragment thereof, produced in a host cell transformed with the nucleic acid of claim 93.

100. A method of producing *Jun s* I or at least one fragment thereof comprising the steps of :

a) culturing a host cell transformed with a nucleic acid sequence encoding *Jun s* I or fragment thereof in a appropriate medium to produce a mixture of cells and medium containing said *Jun s* I or at least one fragment thereof; and

b) purifying said mixture to produce substantially pure *Jun s* I, or at lest one fragment thereof.

101. Isolated *Jun s* I or at least one antigenic fragment thereof.

102. A therapeutic composition comprising isolated *Jun s* I pollen allergen or at least one fragment thereof and a pharmaceutically acceptable carrier or diluent.

103. A protein preparation comprising *Jun s* I or at least one fragment thereof synthesized in a host cell transformed with a nucleic acid sequence encoding all or a portion of *Jun s* I.

104. A method of use of a preparation of claim 103 for the manufacture of a medicament for treating sensitivity to pollen allergen from the *Juniperus* species

in a mammal sensitive to said pollen.

105. A method of detecting sensitivity in a mammal to *Jun s* I, comprising combining a blood sample obtained from said mammal with a purified *Jun s* I allergen or antigenic fragment thereof produced in a host cell transformed with the nucleic acid sequence of claim 86 or chemically synthesized to provoke an allergic response in said mammal and determining the occurrence of an allergic response in the individual to said *Jun s* I allergen or antigenic fragment thereof.

106. A monoclonal antibody specifically reactive with *Jun s* I or at least one antigenic fragment thereof.

107. Isolated *Jun s* I or at least one antigenic fragment thereof of claim 92 wherein said allergen or fragment thereof does not bind immunoglobulin E specific for pollen from the species *Juniperus*, or if binding of the *Jun s* I allergen or fragment thereof to said immunoglobulin E occurs, such binding does not result in histamine release from mast cells or basophils.

108. The isolated allergen or antigenic fragment of claim 107 wherein said isolated allergen or said antigenic fragment is capable of modifying, in an individual sensitive to pollen from the *Juniperus* species to which it is administered, the allergic response to pollen from the Juniperus species.

109. A method of producing *Jun v* I or at least one fragment thereof comprising the steps of :

a) culturing a host cell transformed with a nucleic acid sequence encoding *Jun v* I or fragment thereof in a appropriate medium to produce a mixture of cells and medium containing said *Jun v* I or at least one fragment thereof; and

b) purifying said mixture to produce substantially pure *Jun v* I, or at least one fragment thereof.

111. Isolated *Jun v* I or at least one antigenic fragment thereof.

112. A therapeutic composition comprising isolated *Jun v* I pollen allergen or at least one fragment thereof and a pharmaceutically acceptable carrier or diluent.

113. A protein preparation comprising *Jun v* I or at least one fragment thereof synthesized in a host cell transformed with a nucleic acid sequence encoding all or a portion of *Jun v* I.

114. A method of use of a preparation of claim 113 for the manufacture of a medicament for treating sensitivity to pollen allergen from the *Juniperus* species in a mammal sensitive to said pollen.

115. A method of detecting sensitivity in a mammal to *Jun v* I, comprising combining a blood sample obtained from said mammal with a purified *Jun v* I allergen or antigenic fragment thereof produced in a host cell transformed with the nucleic acid sequence of claim 93 or chemically synthesized to provoke an allergic response in said mammal and determining the occurrence of an allergic response in the individual to said *Jun v* I allergen or antigenic fragment thereof.

116. A monoclonal antibody specifically reactive with *Jun v* I or at least one antigenic fragment thereof.

117. Isolated *Jun v* I or at least one antigenic fragment thereof of claim 99 wherein said allergen or fragment thereof does not bind immunoglobulin E specific for pollen from the species, *Juniperus*, or if binding of the *Jun v* I allergen or fragment thereof to said immunoglobulin E occurs, such binding does not result in histamine release from mast cells or basophils.

118. The isolated allergen or antigenic fragment of claim 117 wherein said isolated allergen or said antigenic fragment is capable of modifying, in an individual sensitive to pollen from the *Juniperus* species to which it is administered, the allergic response to pollen from the Juniperus species.

119. A unique antigenic fragment or portion thereof of Japanese cedar pollen protein allergen, *Cry j* I.

120. The unique antigenic fragment or portion thereof of claim 119 comprising at least one T cell epitope of *Cry j* I, said unique antigenic fragment or portion thereof having a mean T cell stimulation index of at least about 3.0 determined in a population of individuals sensitive to said protein allergen.

121. The unique antigenic fragment of claim 120 wherein said population of individuals is at least twenty-five.

122. The unique antigenic fragment of claim 120 wherein said mean T cell stimulation index is at least about 5.0.

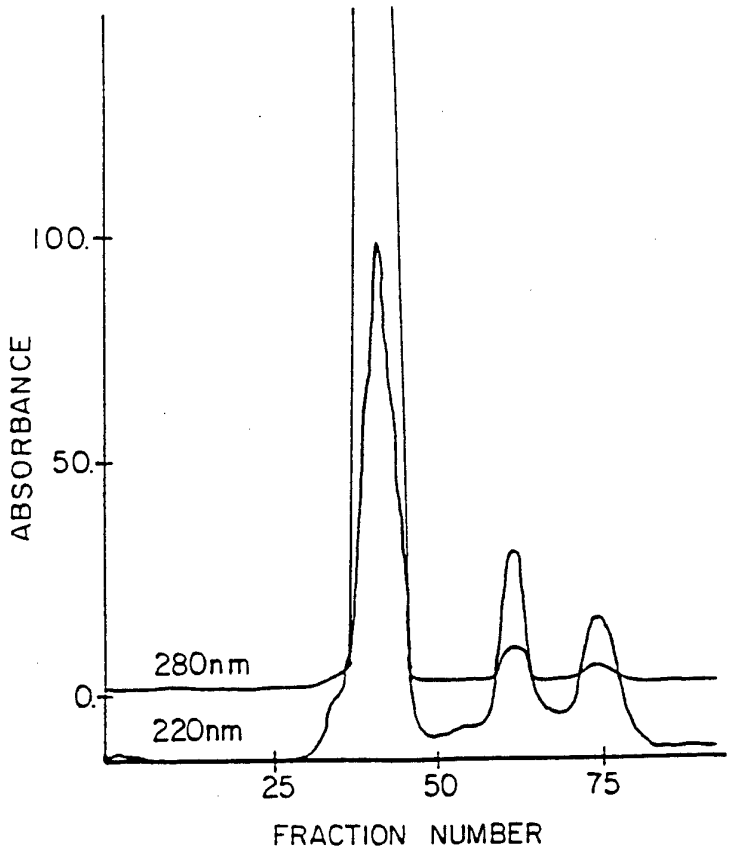

Fig. 1a

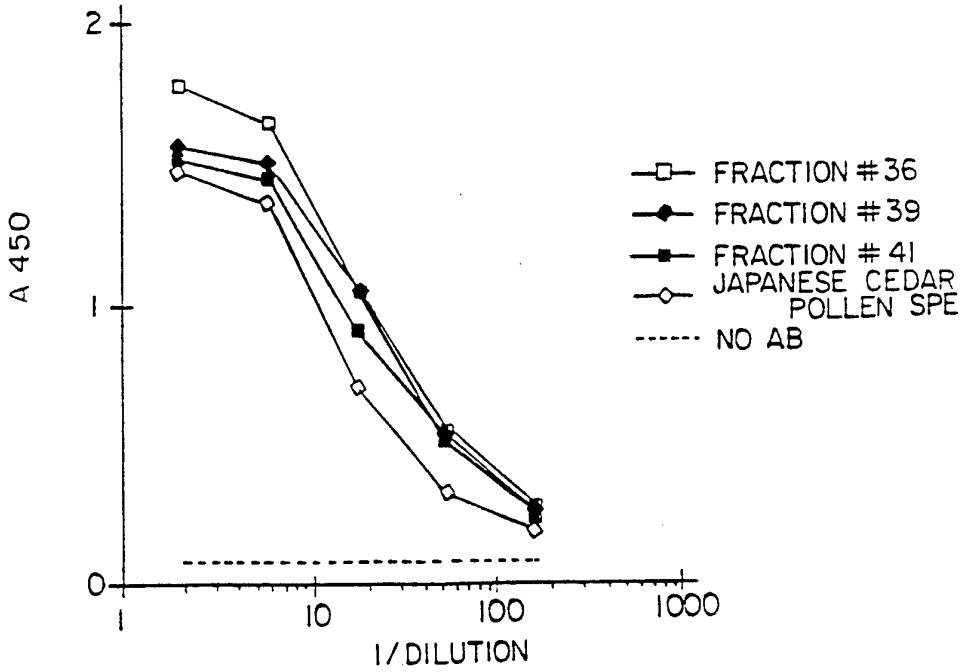

Fig. 3

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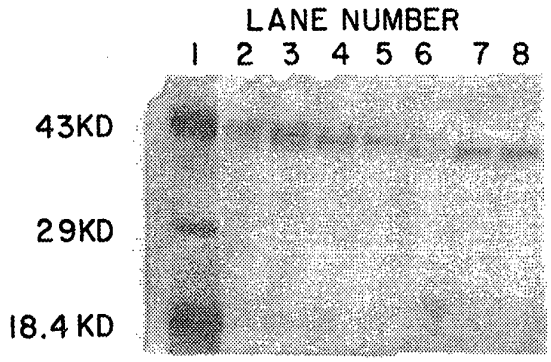

Fig. 1b

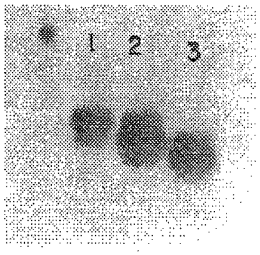

Fig. 2

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5'-AGTCAATCTG CTCATAATCA TAGCATAGCC GTATAGAAAG AAATTCTACA CTCTGCTACC    60

AAAAA ATG GAT TCC CCT TGC TTA GTA GCA TTA CTG GTT TTC TCT TTT           107
      Met Asp Ser Pro Cys Leu Val Ala Leu Leu Val Phe Ser Phe
      -21 -20                  -15                 -10

GTA ATT GGA TCT TGC TTT TCT GAT AAT CCC ATA GAC AGC TGC TGG AGA         155
Val Ile Gly Ser Cys Phe Ser Asp Asn Pro Ile Asp Ser Cys Trp Arg
         -5                   1                   5

GGA GAC TCA AAC TGG GCC CAA AAT AGA ATG AAG CTC GCA GAT TGT GCA         203
Gly Asp Ser Asn Trp Ala Gln Asn Arg Met Lys Leu Ala Asp Cys Ala
 10                  15                  20                  25

GTG GGC TTC GGA AGC TCC ACC ATG GGA GGC AAG GGA GGA GAT CTT TAT         251
Val Gly Phe Gly Ser Ser Thr Met Gly Gly Lys Gly Gly Asp Leu Tyr
                 30                  35                  40

ACG GTC ACG AAC TCA GAT GAC GAC CCT GTG AAT CCT GCA CCA GGA ACT         299
Thr Val Thr Asn Ser Asp Asp Asp Pro Val Asn Pro Ala Pro Gly Thr
             45                  50                  55

CTG CGC TAT GGA GCA ACC CGA GAT AGG CCC CTG TGG ATA ATT TTC AGT         347
Leu Arg Tyr Gly Ala Thr Arg Asp Arg Pro Leu Trp Ile Ile Phe Ser
         60                  65                  70
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Fig. 4

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GGG AAT ATG AAT ATA AAG CTC AAA ATG CCT ATG TAC ATT GCT GGG TAT          395
Gly Asn Met Asn Ile Lys Leu Lys Met Pro Met Tyr Ile Ala Gly Tyr
     75                  80                  85

AAG ACT TTT GAT GGC AGG GGA GCA CAA GTT TAT ATT GGC AAT GGC GGT          443
Lys Thr Phe Asp Gly Arg Gly Ala Gln Val Tyr Ile Gly Asn Gly Gly
 90                  95                 100                 105

CCC TGT GTG TTT ATC AAG AGA GTT AGC AAT GTT ATC ATA CAC GGT TTG          491
Pro Cys Val Phe Ile Lys Arg Val Ser Asn Val Ile Ile His Gly Leu
                110                 115                 120

TAT CTG TAC GGC TGT AGT ACT AGT GTT TTG GGG AAT GTT TTG ATA AAC          539
Tyr Leu Tyr Gly Cys Ser Thr Ser Val Leu Gly Asn Val Leu Ile Asn
            125                 130                 135

GAG AGT TTT GGG GTG GAG CCT GTT CAT CCT CAG GAT GGC GAT GCT CTT          587
Glu Ser Phe Gly Val Glu Pro Val His Pro Gln Asp Gly Asp Ala Leu
        140                 145                 150

ACT CTG CGC ACT GCT ACA AAT ATT TGG ATT GAT CAT AAT TCT TTC TCC          635
Thr Leu Arg Thr Ala Thr Asn Ile Trp Ile Asp His Asn Ser Phe Ser
    155                 160                 165
```

Fig. 4 cont.

```
AAT TCT TCT GAT GGT CTG GTC GAT GTC ACT CTT ACT TCG ACT GGA GTT          683
Asn Ser Ser Asp Gly Leu Val Asp Val Thr Leu Thr Ser Thr Gly Val
170                 175                 180                 185

ACT ATT TCA AAC AAT CTT TTT TTC AAC CAT CAT AAA GTG ATG TTG TTA          731
Thr Ile Ser Asn Asn Leu Phe Phe Asn His His Lys Val Met Leu Leu
                190                 195                 200

GGG CAT GAT GAT GCA TAT AGT GAT GAC AAA TCC ATG AAG GTG ACA GTG          779
Gly His Asp Asp Ala Tyr Ser Asp Asp Lys Ser Met Lys Val Thr Val
            205                 210                 215

GCG TTC AAT CAA TTT GGA CCT AAC TGT GGA CAA AGA ATG CCC AGG GCA          827
Ala Phe Asn Gln Phe Gly Pro Asn Cys Gly Gln Arg Met Pro Arg Ala
        220                 225                 230

CGA TAT GGA CTT GTA CAT GTT GCA AAC AAT AAT TAT GAC CCA TGG ACT          875
Arg Tyr Gly Leu Val His Val Ala Asn Asn Asn Tyr Asp Pro Trp Thr
    235                 240                 245

ATA TAT GCA ATT GGT GGG AGT TCA AAT CCA ACC ATT CTA AGT GAA GGG          923
Ile Tyr Ala Ile Gly Gly Ser Ser Asn Pro Thr Ile Leu Ser Glu Gly
250                 255                 260                 265
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Fig. 4 cont.

```
AAT AGT TTC ACT GCA CCA AAT GAG AGC TAC AAG AAG CAA GTA ACC ATA          971
Asn Ser Phe Thr Ala Pro Asn Glu Ser Tyr Lys Lys Gln Val Thr Ile
                270                 275                 280

CGT ATT GGA TGC AAA ACA TCA TCA TCT TGT TCA AAT TGG GTG TGG CAA         1019
Arg Ile Gly Cys Lys Thr Ser Ser Ser Cys Ser Asn Trp Val Trp Gln
            285                 290                 295

TCT ACA CAA GAT GTT TTT TAT AAT GGA GCT TAT TTT GTA TCA TCA GGG         1067
Ser Thr Gln Asp Val Phe Tyr Asn Gly Ala Tyr Phe Val Ser Ser Gly
        300                 305                 310

AAA TAT GAA GGG GGT AAT ATA TAC ACA AAG AAA GAA GCT TTC AAT GTT         1115
Lys Tyr Glu Gly Gly Asn Ile Tyr Thr Lys Lys Glu Ala Phe Asn Val
    315 320 325

GAG AAT GGG AAT GCA ACT CCT CAA TTG ACA AAA AAT GCT GGG GTT TTA         1163
Glu Asn Gly Asn Ala Thr Pro Gln Leu Thr Lys Asn Ala Gly Val Leu
330                 335                 340                 345

ACA TGC TCT CTC TCT AAA CGT TGT TGATGATGCA TATATTCTAG CATGTTGTAC        1217
Thr Cys Ser Leu Ser Lys Arg Cys
                350

TATCTAAATT AACATCAACA AGAAAATATA TCATGATGTA TATTGTTGTA TTGATGTCAA       1277

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Fig. 4 cont.

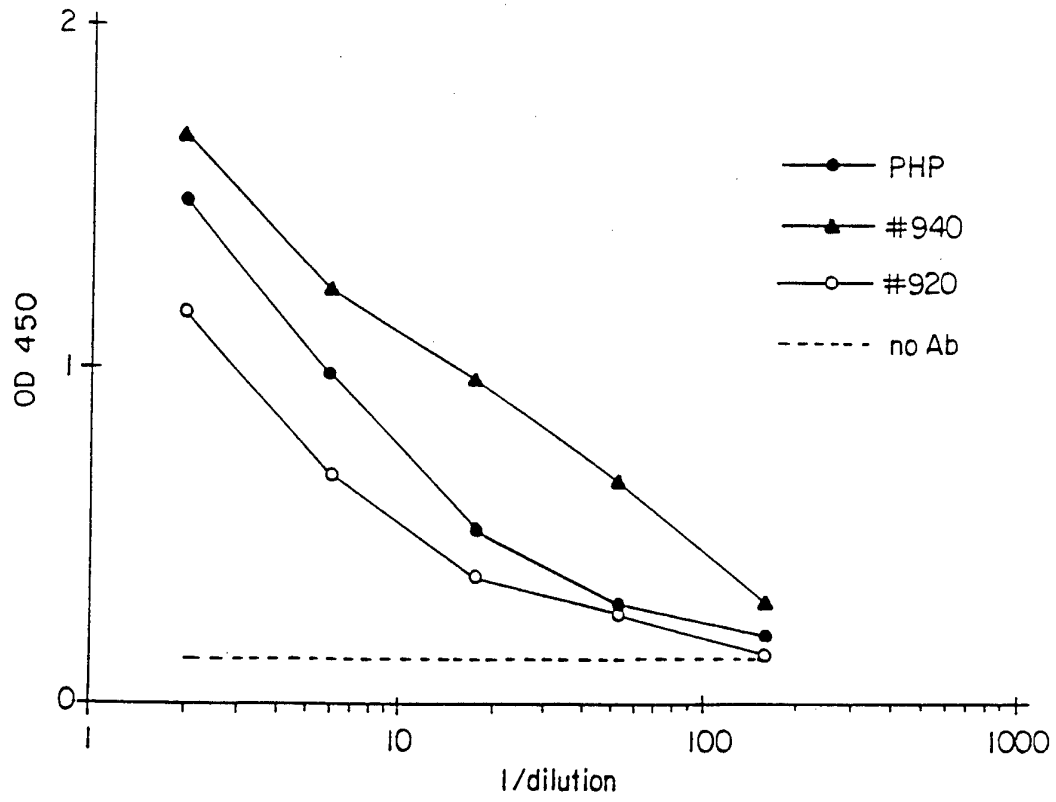
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Fig. 5a

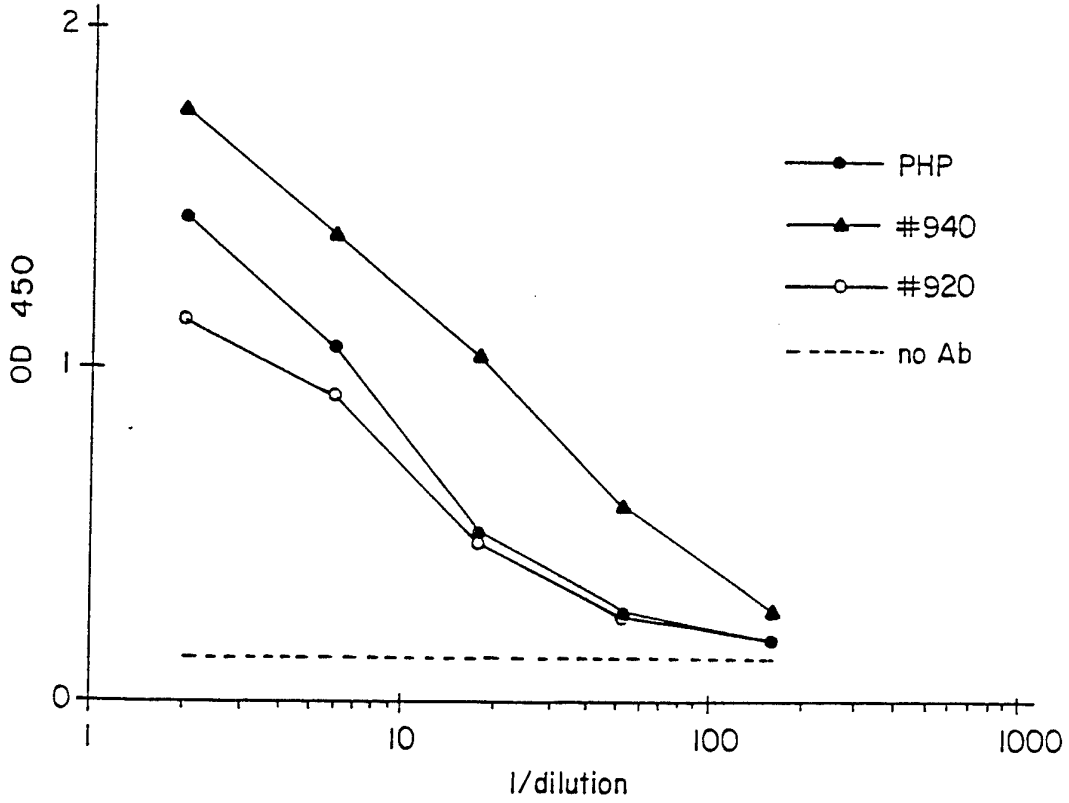
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Fig. 5b

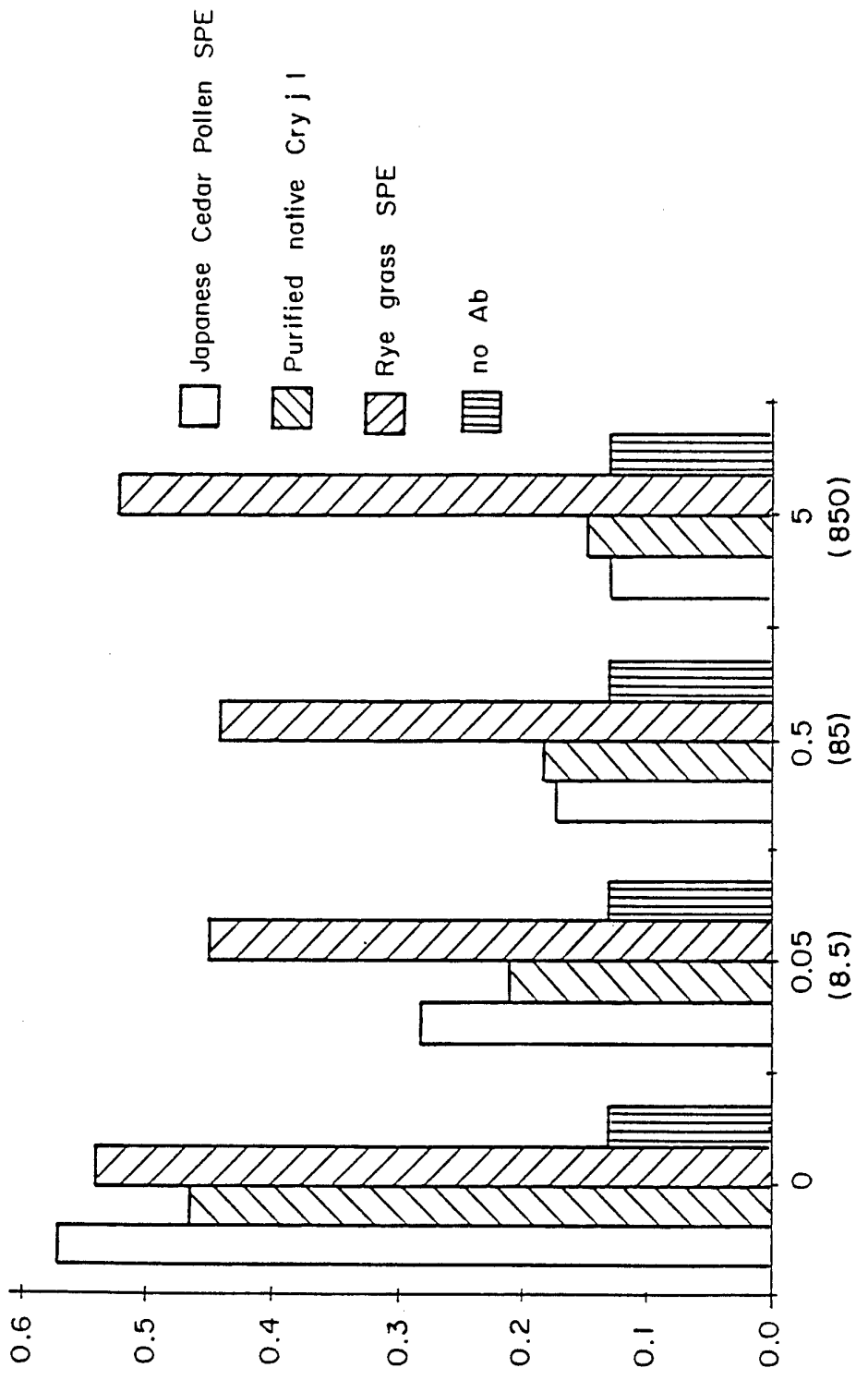

Fig. 6

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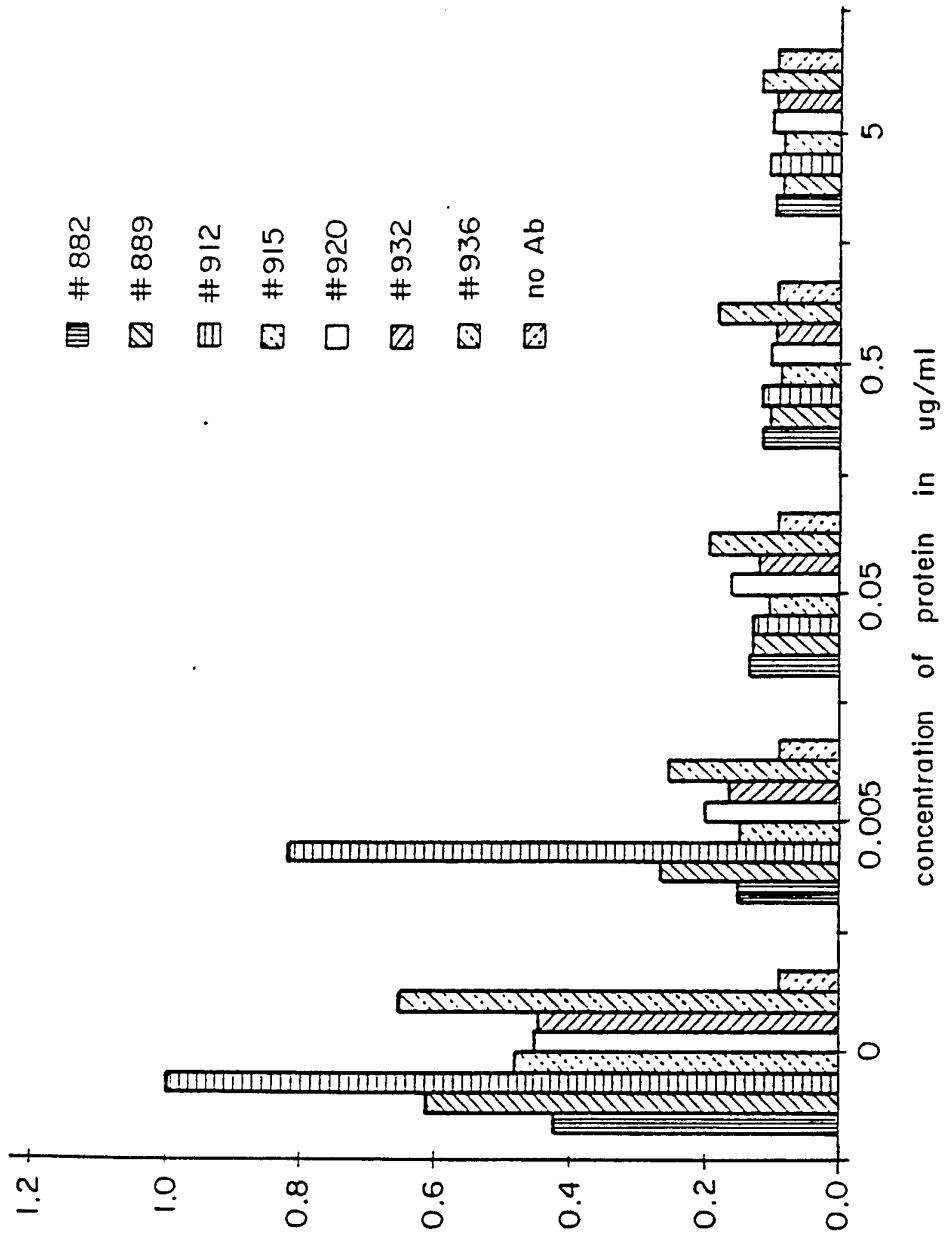

Fig. 7

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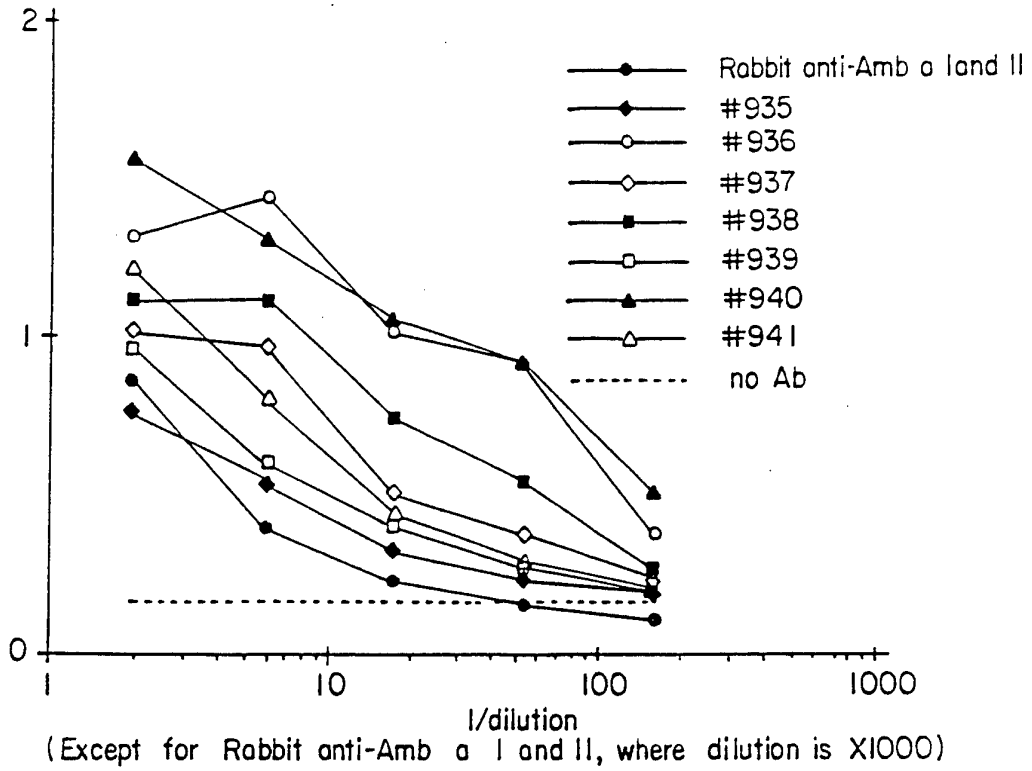
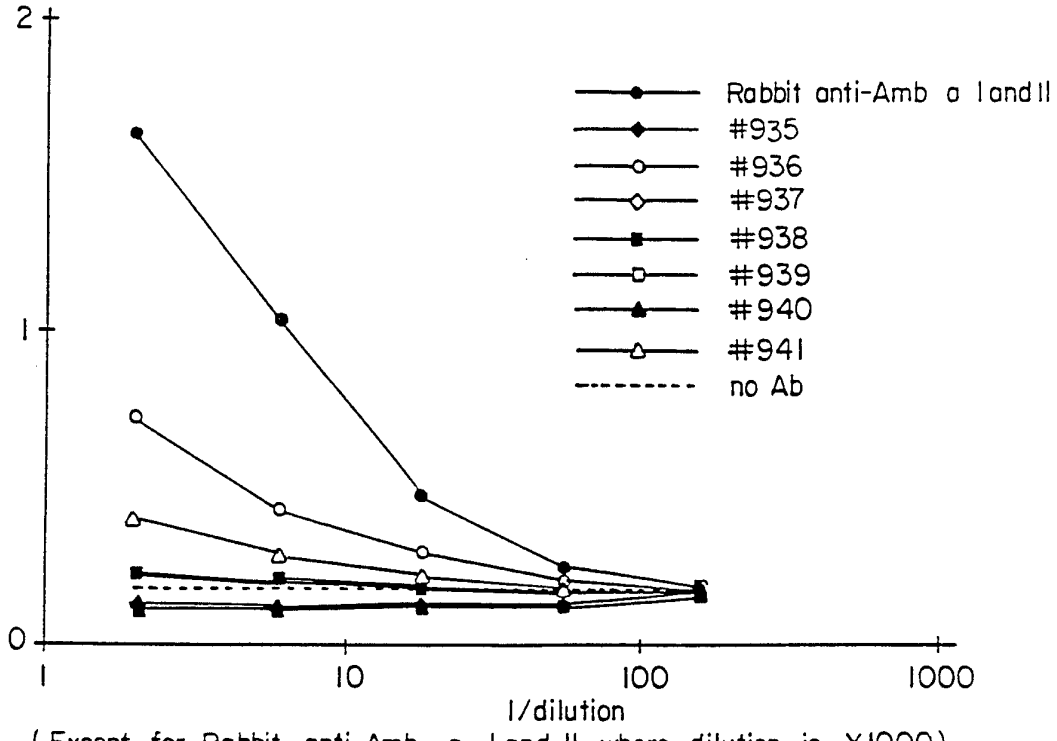


(Except for Rabbit anti-Amb a I and II, where dilution is X1000)

Fig. 8a



(Except for Rabbit anti-Amb a I and II, where dilution is X1000)

Fig. 8b

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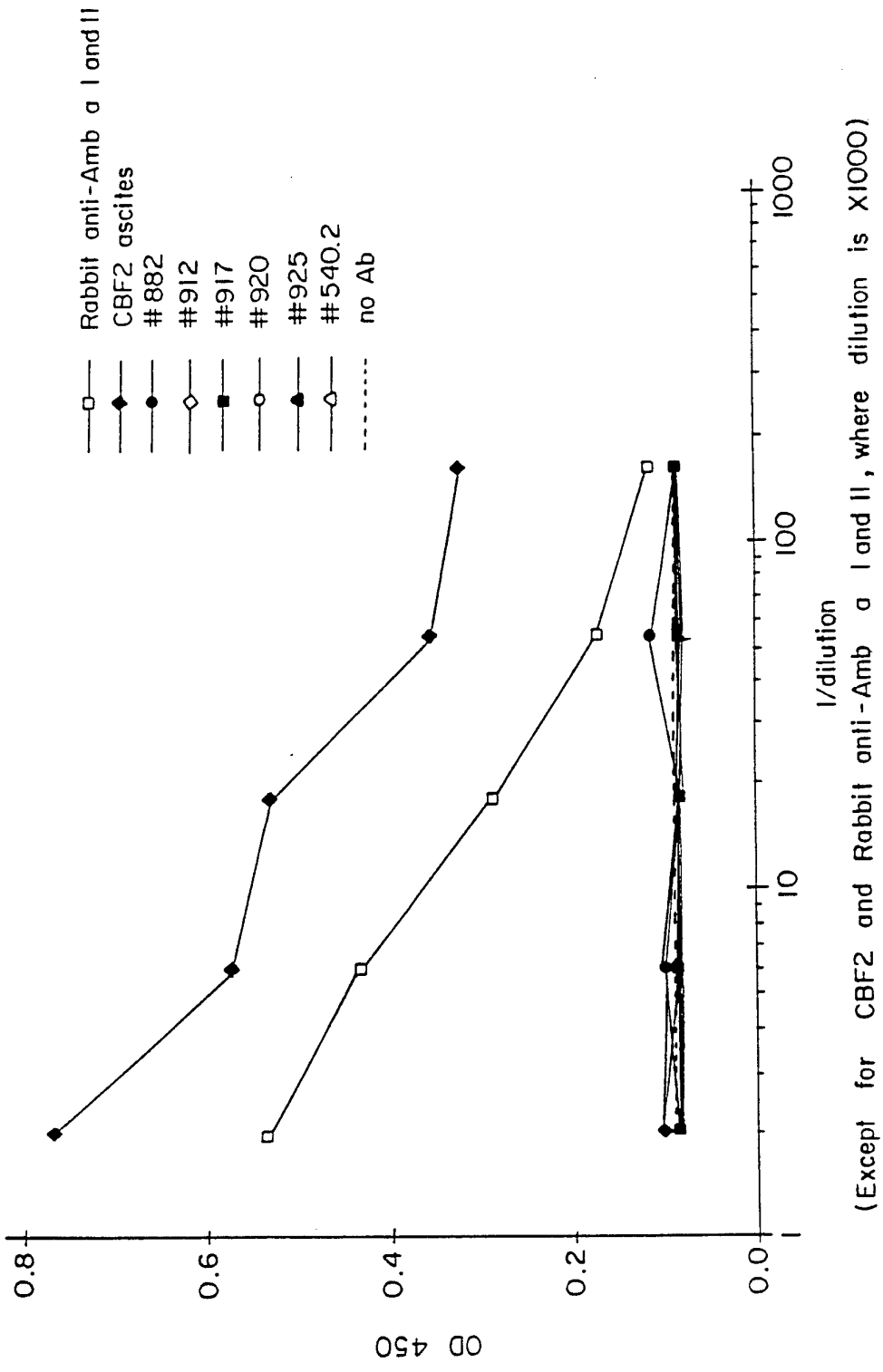

Fig. 9

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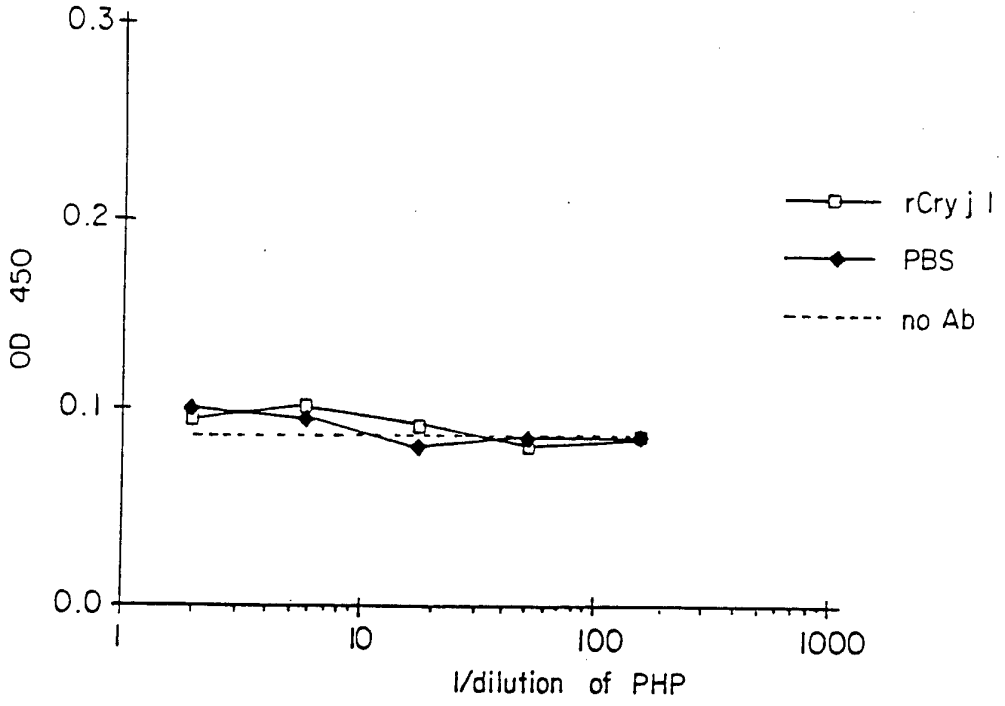

Fig. 10a

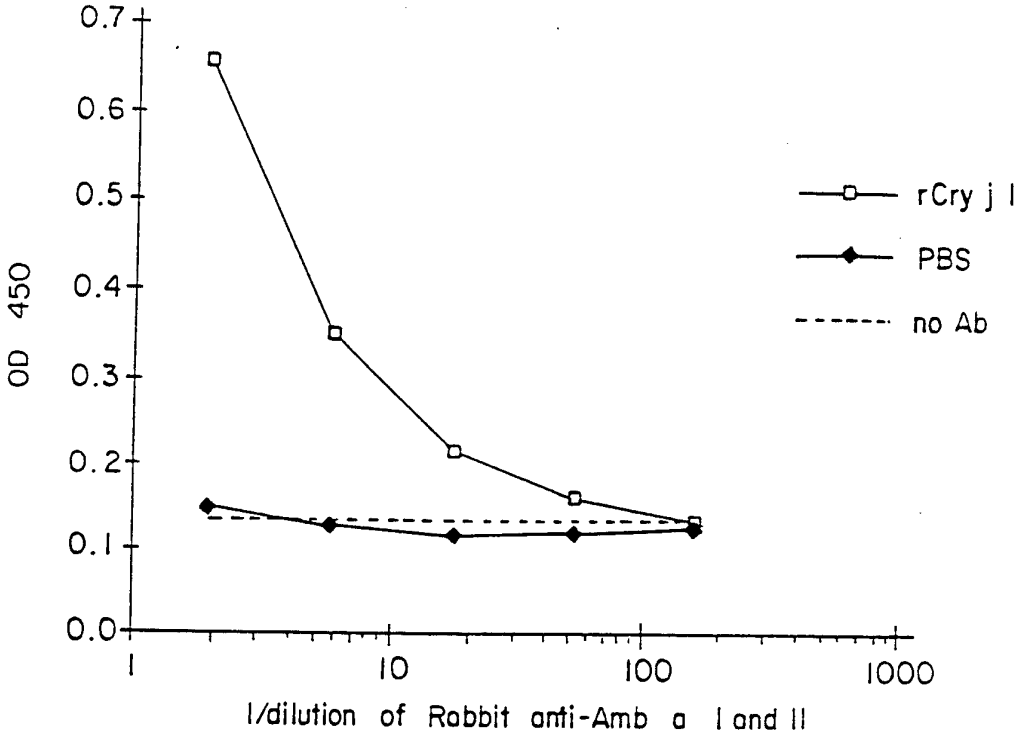

Fig. 10b

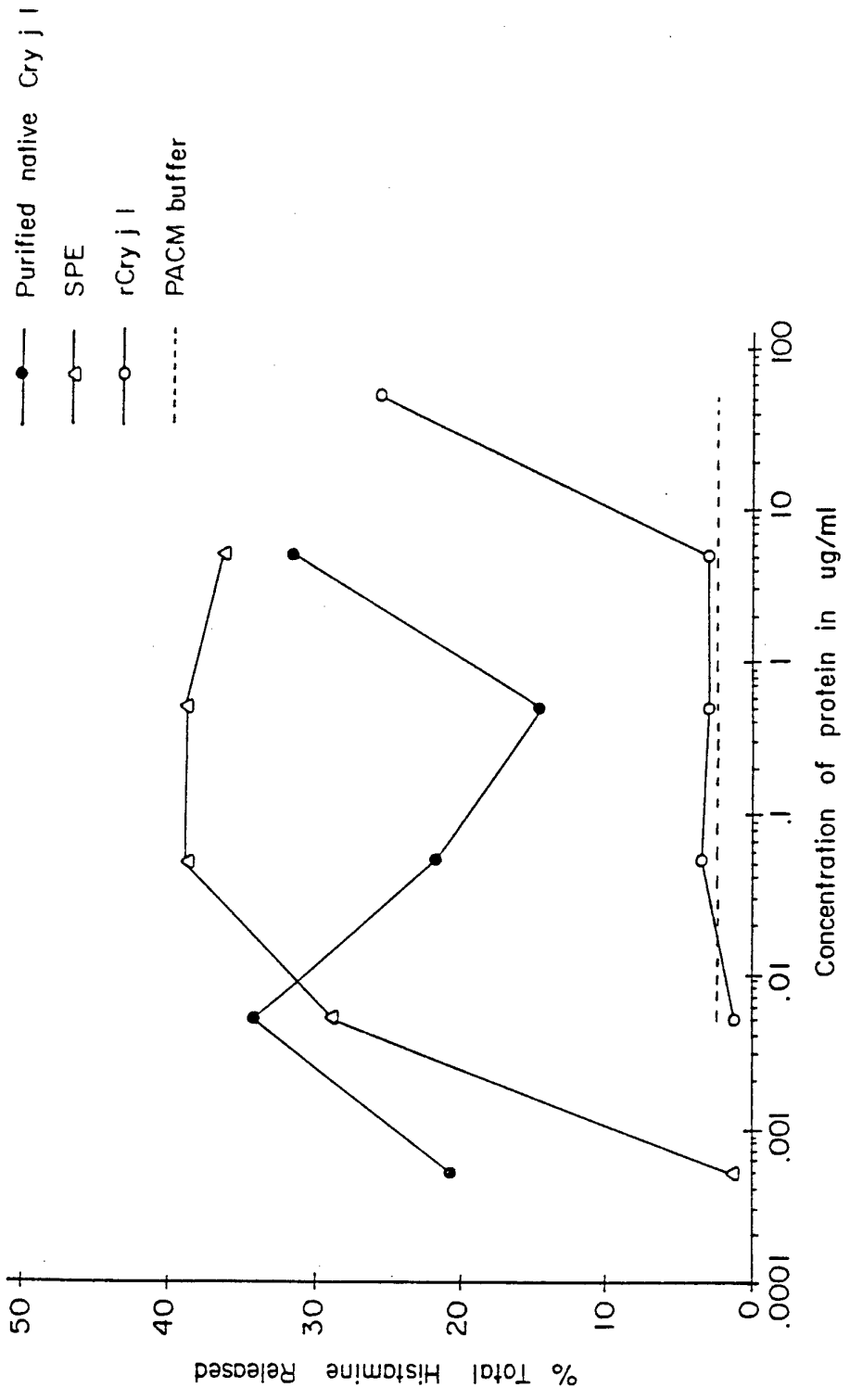

Fig. 11

SUBSTITUTE SHEET

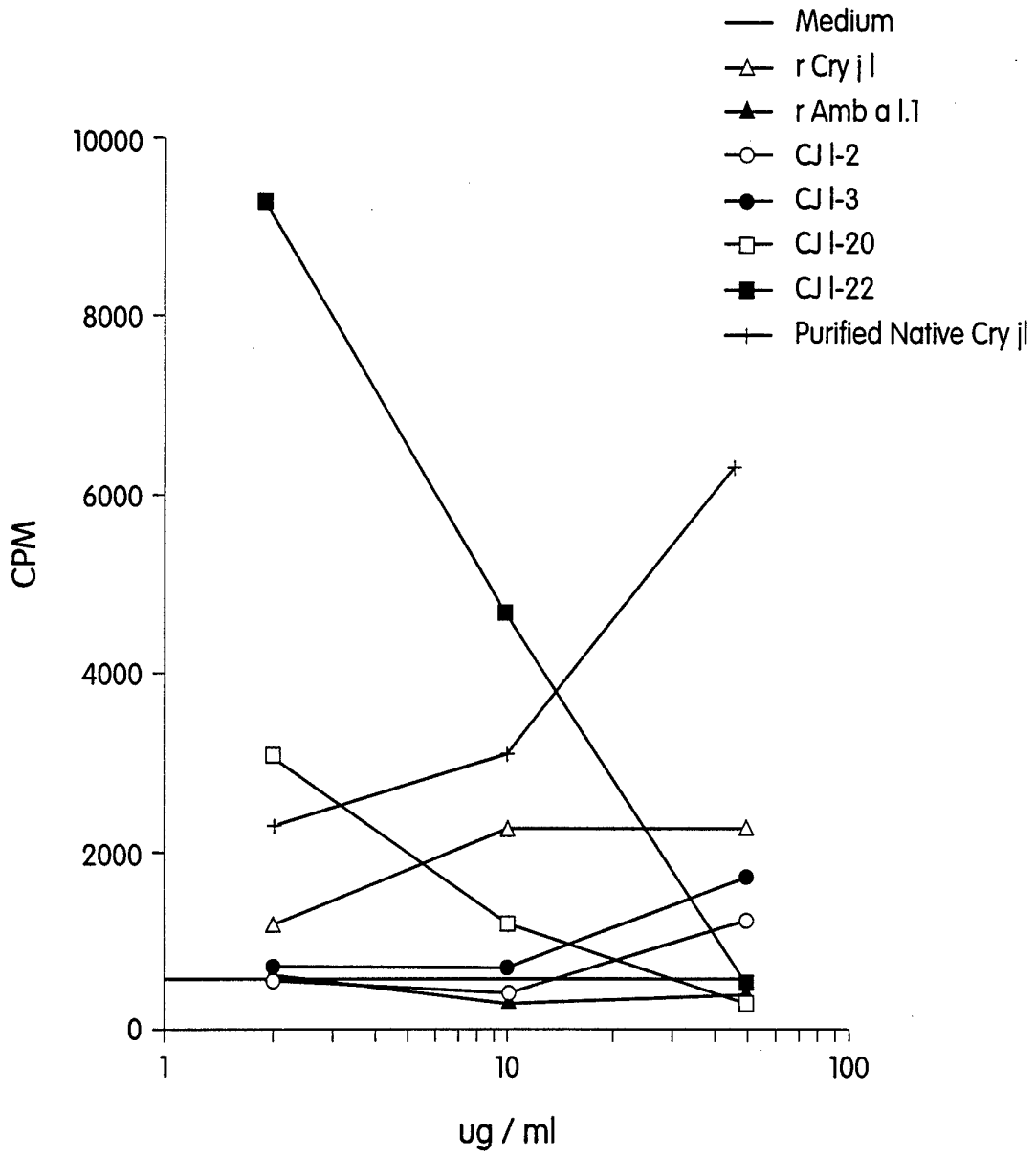

Fig. 12

PEPTIDE NAME	
CJI-1 (1-20)	DNPIDSCWRGDSNWAQNRMK
CJI-2 (11-30)	DSNWAQNRMKLADCAVGFGS
CJI-3 (21-40)	LADCAVGFGSSTMGGKGGDL
CJI-4 (31-50)	STMGGKGGDLYTVTNSDDDP
CJI-5 (41-60)	YTVTNSDDDPVNPAPGTLRY
CJI-6 (51-70)	VNPAPGTLRYGATRDRPLWI
CJI-7 (61-80)	GATRDRPLWIIFSGNMNIKL
CJI-8 (71-90)	IFSGNMNIKLKMPMYIAGYK
CJI-9 (81-100)	KMPMYIAGYKTFDGRGAQVY
CJI-10 (91-110)	TFDGRGAQVYIGNGGPCVFI
CJI-11 (101-120)	IGNGGPCVFIKRVSNVIIHG
CJI-12 (111-130)	KRVSNVIIHGLYLYGCSTSV
CJI-13 (121-140)	LYLYGCSTSVLGNVLINESF
CJI-14 (131-150)	LGNVLINESFGVEPVHPQDG
CJI-15 (141-160)	GVEPVHPQDGDALTLRTATN
CJI-16 (151-170)	DALTLRTATNIWIDHNSFSN
CJI-17 (161-180)	IWIDHNSFSNSSDGLVDVTL
CJI-18 (171-190)	SSDGLVDVTLTSTGVTISNN
CJI-19 (181-200)	TSTGVTISNNLFFNHHKVML
CJI-20 (191-210)	LFFNHHKVMLLGHDDAYSDD
CJI-21 (201-220)	LGHDDAYSDDKSMKVTVAFN
CJI-22 (211-230)	KSMKVTVAFNQFGPNCGQRM
CJI-23 (221-240)	QFGPNCGQRMPRARYGLVHV
CJI-24 (231-250)	PRARYGLVHVANNNYDPWTI
CJI-25 (241-260)	ANNNYDPWTIYAIGGSSNPT
CJI-26 (251-270)	YAIGGSSNPTILSEGNSFTA
CJI-27 (261-280)	ILSEGNSFTAPNESYKKQVT
CJI-28 (271-290)	PNESYKKQVTIRIGCKTSSS
CJI-29 (281-300)	IRIGCKTSSSCSNWVWQSTQ
CJI-30 (291-310)	CSNWVWQSTQDVFYNGAYFV
CJI-31 (301-320)	DVFYNGAYFVSSGKYEGGNI
CJI-32 (311-330)	SSGKYEGGNIYTKKEAFNVE
CJI-33 (321-340)	YTKKEAFNVENGNATPQLTK
CJI-34 (331-350)	NGNATPQLTKNAGVLTCSLS
CJI-35 (341-353)	NAGVLTCSLSKRC

Fig. 13

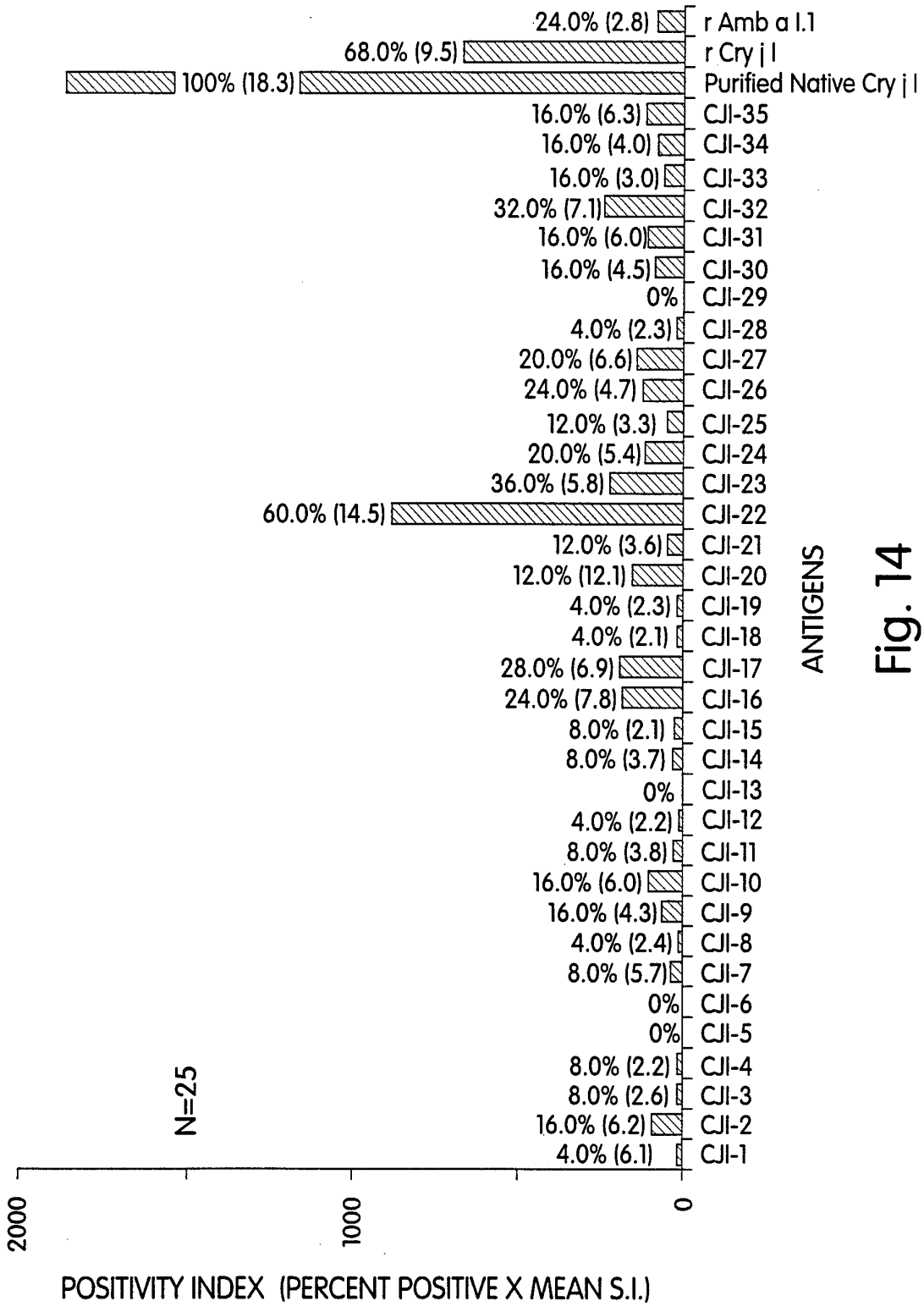

Fig. 14

SUBSTITUTE SHEET

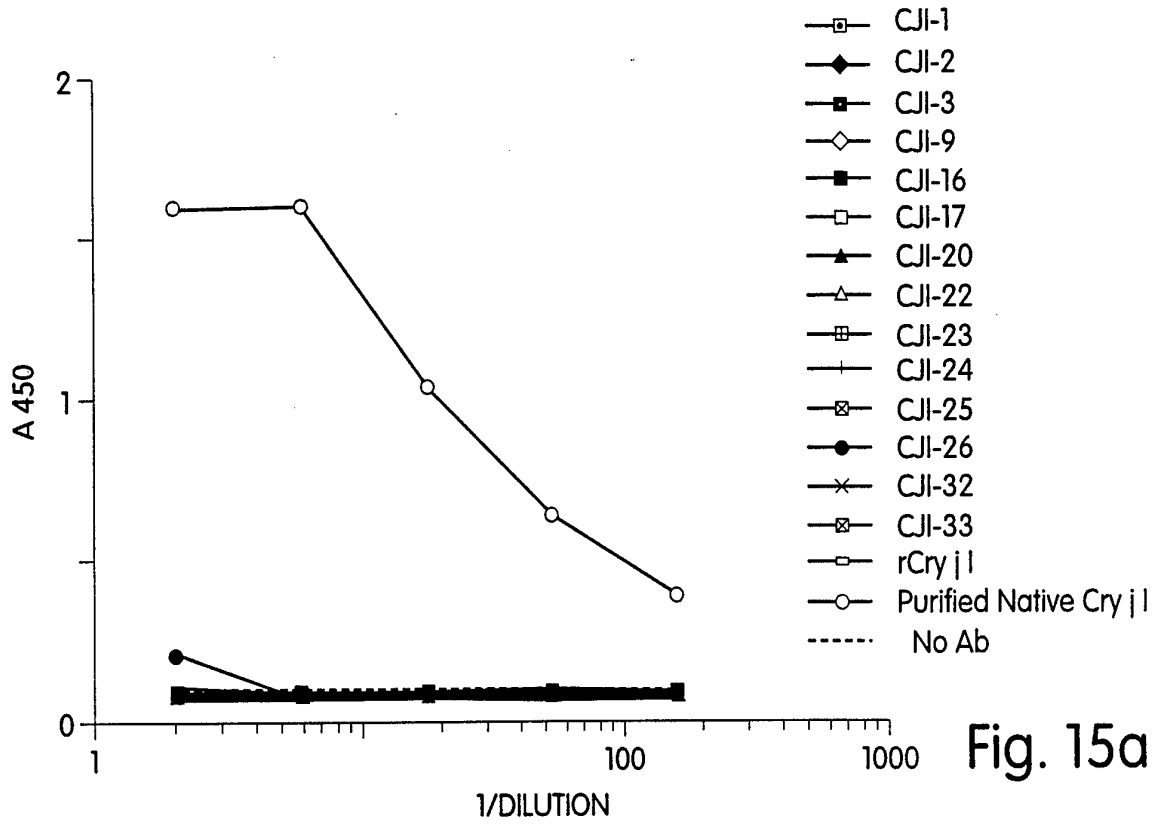

Fig. 15a

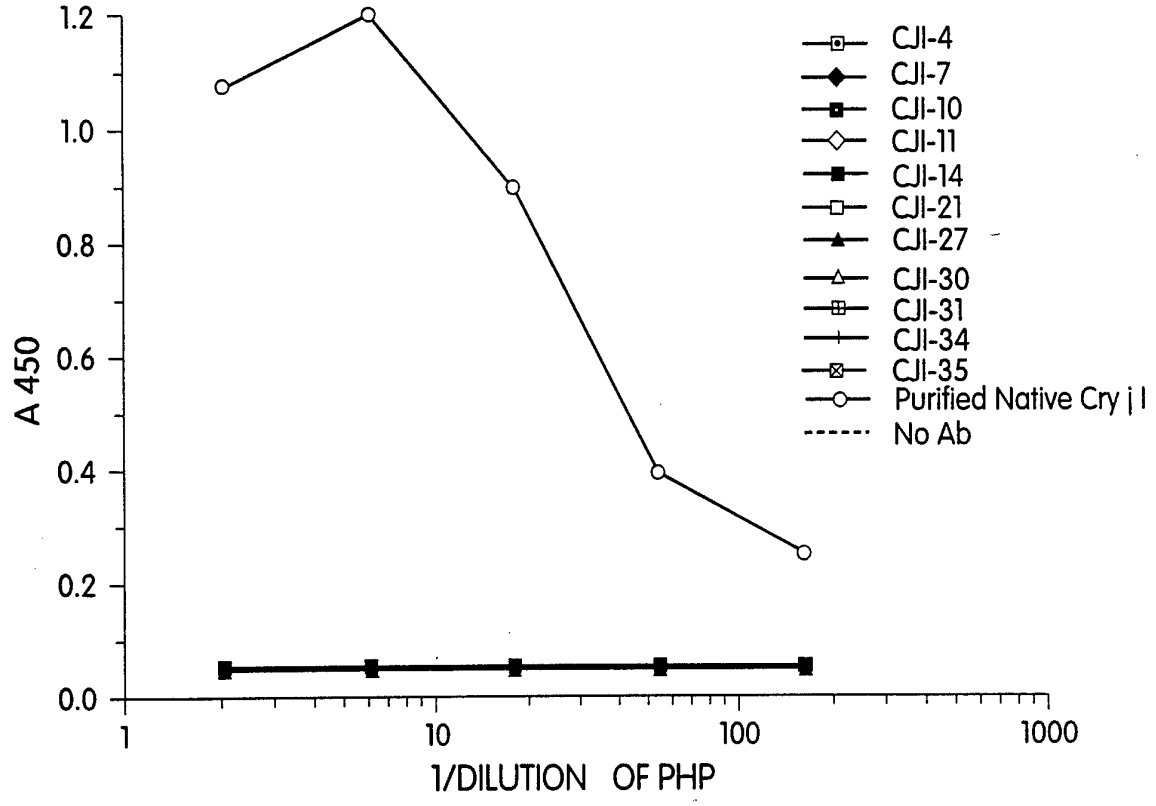

Fig. 15b

```
5'-AAATTCTATATTCTGAACCCTAAAAATGGCTTCCCCATGCTTAATAGCAGTCCTTGTTTT    60
                                M  A  S  P  C  L  I  A  V  L  V  F
                              -21 -20          -15          -10

CCTTTGTGCAATTGTATCTTGTTACTCTGATAATCCCATCGACAGCTGCTGGAGAGGAGA   120
  L  C  A  I  V  S  C  Y  S  D  N  P  I  D  S  C  W  R  G  D
           -5             +1          5             10

TTCGAACTGGGATCAAAACAGAATGAAGCTCGCAGACTGTGCTGTGGGATTTGGAAGCTC   180
  S  N  W  D  Q  N  R  M  K  L  A  D  C  A  V  G  F  G  S  S
        15             20             25             30

CACCATGGGAGGCAAAGGAGGAGATTTTTACACCGTCACAAGCACAGATGATAATCCTGT   240
  T  M  G  G  K  G  G  D  F  Y  T  V  T  S  T  D  D  N  P  V
        35             40             45             50

GAATCCTACACCAGGAACTTTGCGCTATGGAGCAACAAGAGAAAAAGCACTTTGGATCAT   300
  N  P  T  P  G  T  L  R  Y  G  A  T  R  E  K  A  L  W  I  I
        55             60             65             70

TTTCTCTCAGAATATGAATATAAAGCTCAAGATGCCTTTGTATGTTGCTGGACATAAGAC   360
  F  S  Q  N  M  N  I  K  L  K  M  P  L  Y  V  A  G  H  K  T
        75             80             85             90

TATTGACGGCAGGGGAGCAGATGTTCATCTTGGCAACGGCGGTCCCTGTCTGTTTATGAG   420
  I  D  G  R  G  A  D  V  H  L  G  N  G  G  P  C  L  F  M  R
        95            100            105            110
```

Fig. 16

```
GAAAGTGAGCCATGTTATTCTCCATAGTTTGCATATACACGGTTGTAATACGAGTGTTTT  480
  K  V  S  H  V  I  L  H  S  L  H  I  H  G  C  N  T  S  V  L
        115            120            125            130

GGGGGATGTTTTGGTAAGTGAGTCTATTGGGGTCGAGCCTGTTCATGCTCAGGATGGGGA  540
  G  D  V  L  V  S  E  S  I  G  V  E  P  V  H  A  Q  D  G  D
        135            140            145            150

CGCCATTACTATGCGCCATGTTACAAATGCTTGGATTGATCATAATTCTCTCTCCGATTG  600
  A  I  T  M  R  H  V  T  N  A  W  I  D  H  N  S  L  S  D  C
        155            160            165            170

TTCTGATGGTCTTATCGATGTTACGCTTGGCTCCACTGGAATTACTATCTCCAACAATCA  660
  S  D  G  L  I  D  V  T  L  G  S  T  G  I  T  I  S  N  N  H
        175            180            185            190

CTTCTTCAACCATCATAAAGTGATGTTATTAGGACATGATGATACATATGACGATGACAA  720
  F  F  N  H  H  K  V  M  L  L  G  H  D  D  T  Y  D  D  D  K
        195            200            205            210

ATCTATGAAAGTGACAGTGGCGTTCAATCAATTTGGACCTAATGCTGGGCAAAGAATGCC  780
  S  M  K  V  T  V  A  F  N  Q  F  G  P  N  A  G  Q  R  M  P
        215            220            225            230
```

Fig. 16 cont.

```
AAGGGCACGATATGGACTTGTACATGTTGCAAACAATAATTATGATCCATGGAATATATA  840
  R  A  R  Y  G  L  V  H  V  A  N  N  N  Y  D  P  W  N  I  Y
        235            240            245            250

TGCTATTGGTGGGAGTTCAAATCCAACCATTCTGAGTGAAGGGAATAGTTTCACTGCCCC  900
  A  I  G  G  S  S  N  P  T  I  L  S  E  G  N  S  F  T  A  P
        255            260            265            270

AAGTGAGAGTTACAAGAAGCAAGTAACAAAGCGTATAGGGTGTGAATCACCATCAGCTTG  960
  S  E  S  Y  K  K  Q  V  T  K  R  I  G  C  E  S  P  S  A  C
        275            280            285            290

TGCGAACTGGGTGTGGAGATCTACACGAGATGCTTTTATTAATGGAGCTTATTTTGTATC 1020
  A  N  W  V  W  R  S  T  R  D  A  F  I  N  G  A  Y  F  V  S
        295            300            305            310

ATCGGGGAAAACTGAAGAGACCAATATATACAATAGTAATGAAGCTTTCAAAGTTGAGAA 1080
  S  G  K  T  E  E  T  N  I  Y  N  S  N  E  A  F  K  V  E  N
        315            320            325            330

TGGGAATGCAGCTCCTCAATTAACCAAAAATGCTGGAGTTGTAACCTAAGCTCTCTCTAA 1140
  G  N  A  A  P  Q  L  T  K  N  A  G  V  V  T  -
        335            340            345

ATCTTGCTTATGAAACGAAAAAATATATAG-3'                             1170
```

Fig. 16 cont.

```
5'-CGGTATAGATAGATTCTATATTCTGAGCCCTAAAAATGGCTTCCCCATGCTTAATAGCAT    60
                                          M  A  S  P  C  L  I  A
                                       -21 -20           -15

TCCTTGTTTTCCTTTGTGCAATTGTATCTTGTTGCTCTGATAATCCCATAGACAGCTGCT    120
F  L  V  F  L  C  A  I  V  S  C  C  S  D  N  P  I  D  S  C
      -10            -5             +1            5

GGAGAGGAGATTCGAACTGGGGTCAAAACAGAATGAAGCTCGCAGATTGCGCTGTGGGAT    180
W  R  G  D  S  N  W  G  Q  N  R  M  K  L  A  D  C  A  V  G
   10             15             20             25

TTGGAAGCTCCACCATGGGAGGCAAAGGAGGAGATTTTTACACCGTCACAAGCGCAGATG    240
F  G  S  S  T  M  G  G  K  G  G  D  F  Y  T  V  T  S  A  D
   30             35             40             45

ATAATCCTGTGAATCCTACACCAGGAACTTTGCGCTATGGAGCAACAAGAGAAAAAGCAC    300
D  N  P  V  N  P  T  P  G  T  L  R  Y  G  A  T  R  E  K  A
   50             55             60             65

TTTGGATCATTTTCTCTCAGAATATGAATATAAAGCTCAAGATGCCTTTGTATGTTGCTG    360
L  W  I  I  F  S  Q  N  M  N  I  K  L  K  M  P  L  Y  V  A
   70             75             80             85

GACATAAGACTATTGACGGCAGGGGAGCAGATGTTCATCTTGGCAACGGCGGTCCCTGTC    420
G  H  K  T  I  D  G  R  G  A  D  V  H  L  G  N  G  G  P  C
   90             95            100            105
```

Fig. 17

```
TGTTTATGAGGAAAGTGAGCCATGTTATTCTCCATGGTTTGCATATACACGGTTGTAATA    480
L  F  M  R  K  V  S  H  V  I  L  H  G  L  H  I  H  G  C  N
   110            115            120            125

CTAGTGTTTTGGGGGATGTTTTGGTAAGTGAGTCTATTGGGGTGGTGCCTGTACACCCCC    540
T  S  V  L  G  D  V  L  V  S  E  S  I  G  V  V  P  V  H  P
   130            135            140            145

AGGACGGAGATGCGTTTACTGTGAGGACCTCTGAACATATTTGGGTCGACCATAATACTC    600
Q  D  G  D  A  F  T  V  R  T  S  E  H  I  W  V  D  H  N  T
   150            155            160            165

TCTCCAATGGCACCGACGGCCTCGTCGACGTTACTCTTGCTTCCACTGCTGTTACTATTT    660
L  S  N  G  T  D  G  L  V  D  V  T  L  A  S  T  A  V  T  I
   170            175            180            185

CCAATAACCACTTCTTCGACCATGATGAAGTGATGTTGTTAGGACATAGTGATTCATTCT    720
S  N  N  H  F  F  D  H  D  E  V  M  L  L  G  H  S  D  S  F
   190            195            200            205

CAGATGATAAAGTGATGAAAGTCACAGTTGCATTTAACCACTTTGGACCTAATTGTGTGC    780
S  D  D  K  V  M  K  V  T  V  A  F  N  H  F  G  P  N  C  V
   210            215            220            225

AACGATTGCCAAGGGCTAGATATGGACACTTTCATGTTGTTAATAATAATTATGAGCCAT    840
Q  R  L  P  R  A  R  Y  G  H  F  H  V  V  N  N  N  Y  E  P
   230            235            240            245
```

Fig. 17 cont.

```
GGGGAAAATATGCCATTGGAGGAAGTTCTGATCCAACAATTATAAGTGAAGGGAATAGAT   900
W  G  K  Y  A  I  G  G  S  S  D  P  T  I  I  S  E  G  N  R
    250            255            260            265

TTCTTGCACCAAATGAATCTTATAAAAAGGAGGTGACAATACGTGTAGGTTGTAAATCTA   960
F  L  A  P  N  E  S  Y  K  K  E  V  T  I  R  V  G  C  K  S
    270            275            280            285

CAAGTTGTGATGCATGGGAGTGGAGATCAAAAGATGATGCCTTCCTTAATGGTGCCTATT  1020
T  S  C  D  A  W  E  W  R  S  K  D  D  A  F  L  N  G  A  Y
    290            295            300            305

TTGTACAATCAGGCAAGGGGTATAATGGTGGAGAGGCATTCAAGGTTGAAAGTGCAAATG  1080
F  V  Q  S  G  K  G  Y  N  G  G  E  A  F  K  V  E  S  A  N
    310            315            320            325

AGGTGCCAACATTGACTAAACATGCTGGAGCATTAAAATGTATACCTACCAAACAATGTG  1140
E  V  P  T  L  T  K  H  A  G  A  L  K  C  I  P  T  K  Q  C
    330            335            340            345

TGATATGAAAAGTCAATCGATATAATAATGTGTTATTTGTAATATTTCAGCTTTGAATAT  1200
V  I  -

GTATAGAAAAAGAATTTCAACAAAATGACACTATTATATAAATAAATTCTTAGTTTATTA  1260

GTTGGTATTAAAAAAAAA-3'                                         1278
```

Fig. 17 cont.

```
CJI-41     KMPMYIAGYKTFDGRGAQVYIGNGGPCVFI
CJI-41.1   PMYIAGYKTFDGRGAQVYIGNGGP
CJI-41.2   YIAGYKTFDGRGAQVYIGNGGP
CJI-41.3   KKYIAGYKTFDGRGAQVYIGNGGP


CJI-42     DALTLRTATNIWIDHNSFSNSSDGLVDVTL
CJI-42.1   RTATNIWIDHNSFSNSSDGLVD
CJI-42.2   KRTATNIWIDHNSFSNSSDGLVDK


CJI-43     KSMKVTVAFNQFGPNCGQRMPRARYGLVHVANNNYD
CJI-43.1   KSMKVTVAFNQFGPNCGQRMPRARYGLVHV
CJI-43.6   KSMKVTVAFNQFGPNSGQRMPRARYGLVHV
CJI-43.7   KSMKVTVAFNQFGPNCGQRMPRARYGLV
CJI-43.8   KSMKVTVAFNQFGPNSGQRMPRARYGLV
CJI-43.9   KSMKVTVAFNQFGPNCGQRMPRARYG
CJI-43.10  KSMKVTVAFNQFGPNSGQRMPRARYG
CJI-43.11  KSMKVTVAFNQFGPNSGQRMPRARYGKK
CJI-43.12  KSMKVTVAFNQFGPNCGQRMPRARYG


CJI-45     PRARYGLVHVANNNYDPWTIYAIGGSSNPT
CJI-45.1   RARYGLVHVANNNYDPWTIYAIGGSSNP
CJI-45.2   RARYGLVHVANNNYDPWTIYAIGGSS


CJI-44     DVFYNGAYFVSSGKYEGGNIYTKKEAFNVE
CJI-44.1   NGAYFVSSGKYEGGNIYTKKEAFNVE
CJI-44.2   NGAYFVSSGKYEGGNIYTKKEAFN
CJI-44.3   KKNGAYFVSSGKYEGGNIYTKKEAFN
```

Fig. 18

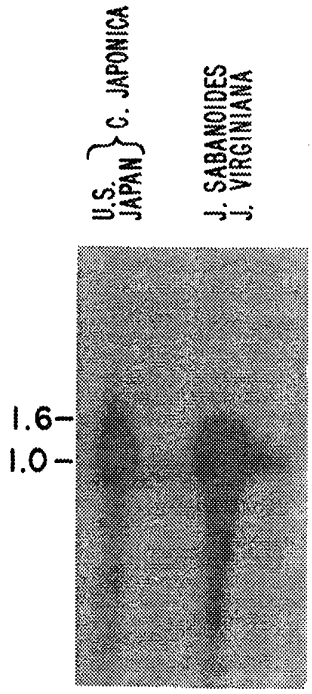

Fig. 19a

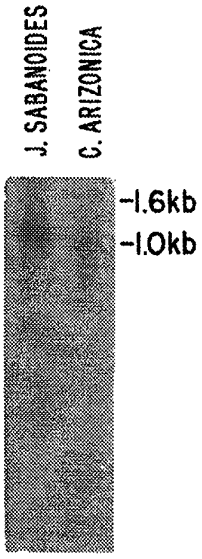

Fig. 19b

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INTERNATIONAL SEARCH REPORT

International Application No PCT/US 93/00139

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12N15/29; C07K15/10; A61K39/36; G01N33/68
C12P21/02; C12P21/08

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C12N ; C12P ; A61K ; C07K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
E	WO,A,9 301 213 (IMMULOGIC PHARMACEUTICAL CORPORATION) 21 January 1993 see the whole document	1-122
X	FEBS LETTERS. vol. 239, no. 2, November 1988, AMSTERDAM NL pages 329 - 332 TANIAI ET AL. 'N-terminal amino acid sequence of a major allergen of Japanese cedar pollen (Cry j I)' cited in the application see table 2	1-72, 74-105, 107-115, 117-122

-/--

° Special categories of cited documents : [10]

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

"E" earlier document 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

"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

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
17 JUNE 1993	28 -06- 1993
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	CUPIDO M.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	PATENT ABSTRACTS OF JAPAN vol. 13, no. 419 (C-637)(3767) 18 September 1989 & JP,A,11 56 926 (HAYASHIBARA BIOCHEM LAB INC) 20 June 1989 see abstract	1-72, 74-105, 107-115, 117-122
X	Section Ch, Week 8742, Derwent Publications Ltd., London, GB; AN 87-294838 & JP,A,62 205 794 (FUJI REBIO KK) 10 September 1987 see abstract	73,106, 116

INTERNATIONAL SEARCH REPORT

Int...ational application No.

PCT/US93/00139

Box I Observations where certain claims were found unsearchable (Continuation of item 1 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. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark : Although claims 56,70 and 72 are directed to a method of treatment of (and a diagnostic method practised on) the human body, the search has been carried out and based on the alleged efects of the composition.

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 II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

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

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 searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

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.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

US 9300139
SA 69270

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 17/06/93

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9301213	21-01-93	AU-A- 2300492	11-02-93