(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 528 102 B9

(12) # CORRECTED EUROPEAN PATENT SPECIFICATION

Note: Bibliography reflects the latest situation

(15) Correction information:
Corrected version no 1 (W1 B1)
Corrections, see
Description Paragraph(s) 152
Remarks

(48) Corrigendum issued on:
12.12.2007 Bulletin 2007/50

(45) Date of publication and mention of the grant of the patent:
22.11.2006 Bulletin 2006/47

(21) Application number: **05001983.5**

(22) Date of filing: **01.10.1999**

(51) Int Cl.:
C12N 9/18 (2006.01) *C12N 9/26* (2006.01)
C12N 15/10 (2006.01) *C12N 15/54* (2006.01)
A61K 39/35 (2006.01)

(54) **Cloning and recombinant production of Polistinae venom enzymes such as hyaluronidase, and immunological therapies based thereon**

Klonierung und rekombinante Herstellung von Polistinae Insektengift- Enzymen, beispielsweise Hyaluronidase, und davon abgeleitete immunologische Therapien

Clonage et production par recombinaison des enzymes du venin de Polystinae, telle que l'hyaluronidase, et therapies immunologiques basées dessus.

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE

(30) Priority: **01.10.1998 US 166205**

(43) Date of publication of application:
04.05.2005 Bulletin 2005/18

(62) Document number(s) of the earlier application(s) in accordance with Art. 76 EPC:
99950198.4 / 1 117 769

(73) Proprietor: **ROCKEFELLER UNIVERSITY**
New York, NY 10021-6399 (US)

(72) Inventor: **King, Te, Paio**
New York, NY 10021 (US)

(74) Representative: **Olsen, Lars Pallisgaard et al**
Zacco Denmark A/S
Hans Bekkevolds Allé 7
2900 Hellerup (DK)

(56) References cited:
WO-A-94/20623

- **FROHMAN M A ET AL: "RAPID PRODUCTION OF FULL-LENGTH CDNAS FROM RARE TRANSCRIPTS: AMPLIFICATION USING A SINGLE GENE-SPECIFIC OLIGONUCLEOTIDE PRIMER" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA,US,NATIONAL ACADEMY OF SCIENCE. WASHINGTON, vol. 85, 1988, page 8998-9002, XP000604678 ISSN: 0027-8424**
- **KING T.P. ET AL.,: "Yellow jacket venom allergens, hyaluronidase and phospholipase: sequence similarity and antigenic cross-reactivity with their hornet and wasp homologs and possible implications for clinical allergy." J. ALLERGY CLIN. IMMUNOL., vol. 98, no. 3, 1996, page 588-600, XP000874949**
- **LU G ET AL.: "Sequence identity and antigenic cross-reactivity of white face hornet venom allergen, also a hyaluronidase, with other proteins" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 9, 1995, pages 4457-4465, XP000876778**

Note: Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

EP 1 528 102 B9

- **DATABASE EMBL NUCLEOTIDE AND PROTEIN SEQUENCES, 7 September 1999 (1999-09-07), XP002130425 HINXTON, GB**

Description

FIELD OF THE INVENTION

[0001] The present invention is directed to nucleic acid molecules encoding *Polistinae* venom allergens, in particular hyaluronidase, or immunomodulatory fragments thereof, recombinant vectors comprising such nucleic acid molecules, and host cells containing the recombinant vectors. The invention is further directed to expression of such nucleic acid molecule to produce the recombinant *Polistinae* venom enzyme, hyaluronidase, or recombinant immunomodulatory fragments thereof. Such an allergen and immunomodulatory fragments thereof are useful for diagnosis of allergy, for therapeutic treatment of allergy, for the treatment of immune system related diseases or disorders, or symptoms related thereto, and for the modulation of immune response towards an immunogen.

BACKGROUND OF THE INVENTION

[0002] Insect sting allergy to bees and vespids is of common occurrence. The vespids include hornets, yellow jackets and wasps (Golden, et al., 1989, Am. Med. Assoc. 262:240). Susceptible people can be sensitized on exposure to minute amounts of venom proteins; as little as 2-10 μg of protein is injected into the skin on a single sting by a vespid (Hoffman and Jacobson, 1984, Ann. Allergy. 52:276).

[0003] There are many species of hornets (genus *Dolichovespula),* yellow jackets (genus *Vespula)* and wasp (genus *Polistes*) in North America (Akre, et al., 1980, "Yellowjackets of America North of Mexico," Agriculture Handbook No. 552, US Department of Agriculture). The vespids have similar venom compositions (King, et al., 1978, Biochemistry 17: 5165; King, et al., 1983, Mol. Immunol. 20:297; King, et al., 1984, Arch. Biochem. Biophys. 230:1; King, et al., 1985, J. Allergy and Clin. Immunol. 75:621; King, 1987, J. Allergy Clin. Immunol. 79:113; Hoffman, 1985, J. Allergy and Clin. Immunol. 75:611). Their venom each contains three major venom allergens, phospholipase (37 kD), hyaluronidase (43 kD) and antigen 5 (23 kD) of as yet unknown biologic function. U.S. Patent No. 5,593,877 describes cloning and expression of the vespid venom allergens phospholipase and hyaluronidase. As described in this patent, the recombinant allergens permit expression of a protein or fragments thereof for use in immunotherapy, dignostics, and to investigate T and B cell allergens, it sets forth in greater detail the rationale for cloning vespid venom enzymes. However, unique vespid venom cDNAs were not described.

[0004] In addition to the insect venom allergens described above, the complete amino acid sequence of several major allergens from different grass (Perez, et al., 1990, J. Biol. Chem. 265:16210; Ansari, et al., 1989, Biochemistry 26:8665; Silvanovich, et al., 1991, J. Biol. Chem. 266:1204), tree pollen (Breiteneder, 1989, EMBO J. 8:1935; Valenta, et al., 1991, Science, 253:557), weed pollen (Rafnar, et al., 1991, J. Biol. Chem. 266:1229; Griffith, et al., 1991, Int. Arch. Allergy Appl. Immunol. 96:296), mites (Chua, et al., 1988, J. Exp. Med. 167:175), cat dander (Griffith, et al., 1992, Gene. 113:263), and mold (Aruda, et al., 1990, J. Exp. Med. 172:1529; Han, et al., 1991, J. Allergy Clin. Immunol. 87:327) have been reported in the past few years. These major allergens are proteins of 10-40 kD and they have widely different biological functions. Nearly all allergens of known sequences have a varying extent of sequence similarity with other proteins in our environment.

[0005] Although U.S. Patent No. 5,593,877 provides for cloning and expression of vespid venom enzymes, particularly hyaluronidase and phospholipase, there remains a need to identify unusual and unexpected sequences for such enzymes, and to design effective expression systems for them. There is a particular need to delineate the B and helper T cell epitopes of the paper wasp *(e.g., Polistes annularis).* In particular, the major *Polistinae* venom allergens phospholipase and hyaluronidase are appropriate targets for determining the important B and T cell epitopes. In order to fully address the basis for allergic response to vespid allergens, and to develop allergen-based immunotherapies, the cDNA and protein sequences of several homologous allergens need to be investigated. Moreover, vectors suitable for high level expression in bacteria and eukaryotic cells of vespid allergens or their fragments should be developed. Recombinant vespid allergens and their fragments may then be used to map their B and T cell epitopes in the murine and, more importantly, human systems by antibody binding and T cell proliferation tests, respectively.

[0006] There is also a need in the art to use peptides having T or B cell epitopes of vespid venom allergens to study induction of tolerance in mice and induction of tolerance in humans.

[0007] There is a further need to test whether a modified peptide inhibits allergen T cell epitope binding to MHC class II molecule, or induces T cell anergy, or both.

[0008] Thus, there is a need in the art for unique sequence information about vespid venom allergens, and a plentiful source of such allergens for immunological investigations and for immunological therapy of the allergy.

[0009] Furthermore, due to the overuse of antibiotics throughout the world, and to the spread of numerous viruses, such as HIV, Ebolla, etc., efforts have been made to produce new "super" antibiotic medication, and compounds which have activity against viruses. For example, AZT has been developed, along with protease inhibitors to treat subjects suffering from HIV. However, the costs of developing new "super" antibiotics and anti-viral medications are enormous.

[0010] Hence, what is needed are agents and pharmaceutical compositions for treating immune system related diseases or disorders whose activity is not dependent necessarily on combating the particular virus or pathogen, but rather modulate or potentiate the immune system is ability to combat the disease or disorder, thereby ameliorating the disease or disorder, or a symptom related thereto. Hymenoptera venoms, particularly vespid venoms, provide one possible source for such agents and pharmaceutical compositions, as described in U.S. Patent Nos. 4,822,608 and 5,827,829.
[0011] The citation of references herein shall not be construed as an admission that such is prior art to the present invention.

SUMMARY OF THE INVENTION

[0012] The present invention provides a nucleic acid molecule encoding *Polistinae* venom enzyme encoding a hyaluronidase having the activity of the hyaluronidase of SEQ ID N.4, or, immunomodulatory fragments thereof. In particular, the invention is directed to such nucleic acid molecules encoding a *Polistinae* venom hyaluronidase. In specific embodiments, a nucleic acid molecule of the invention encodes an immunomodulatory portion of a T cell epitope of a *Polistinae* venom enzyme. In another embodiment, a nucleic acid molecule of the invention encodes an antigenic portion of a B cell epitope of a *Polistinae* venom enzyme.
[0013] The nucleic acid of the invention, which is not genomic, surprisingly is found in one embodiment, to contain a non-coding, e.g., an intronic sequence. In a specific embodiment, cDNA molecules for *Polistinae* venom enzyme contain what appears to be an intron. Thus, it has unexpectedly proved necessary to delete the "intronic" sequence in order to obtain a nucleic acid coding for a mature *Polistinae* venom enzyme, e.g., hyaluronidase.
[0014] Hence broadly, the present invention extends to an isolated nucleic acid molecule encoding a venom enzyme, variants thereof encoding a hyaluronidase having the activity of the hyaluronidase of SEQ ID N.4 ,or, immunomodulatory fragment thereof. As noted above, the nucleic acid molecule contains internal non-coding sequences, i.e., in addition to 5⁻ and 3⁻ untranslated (UTR) sequences., but is not a genomic sequence. Examples of *Polistinae* venom enzymes which can be encoded by an isolated nucleic acid molecule of the invention include, but are not limited to phospholipase and hyaluronidase, variants thereof having the activity of the hyaluronidase of SEQ ID N.4, immunomodulatory fragments thereof, from the venom of numerous *Polistinae* venoms can b encoded by an isolated nucleic acid molecule of the invention. A particular example comprises *Polistinae* of the genus *Polistes,* and particularly the species *annularis.*
[0015] In another particular embodiment, the present invention extends to an isolated nucleic acid molecule, that encodes hyaluronidase from *Polistes annularis* comprising an amino acid sequence of SEQ ID NO: 4, more particularly wherein the isolated nucleic acid has a nucleotide sequence of SEQ ID NO:3 or fragment thereof, or variants thereof, encoding a hyaluronidase having the activity of the hyaluronidase of SEQ ID N.4, and, immunomodulatory fragments thereof.
[0016] Moreover, the present invention further extends to an isolated nucleic acid molecule encoding a *Polistinae* venom enzyme variants thereof, encoding a hyaluronidase having the activity of the hyaluronidase of SEQ ID N.4, or an immunomodulatory fragment, wherein the isolated nucleic acid molecule encodes an immunomodulatory portion of a T cell epitope or an antigenic portion of a B cell epitope of the *Polistinae* venom enzyme. Moreover the present invention extends to an isolated nucleic acid molecule encoding a *Polistinae* venom hyaluronidase comprising an amino acid sequence of SEQ ID NO: 4, or an immunomodulatory fragment thereof; or variants of said molecule encoding a hyaluronidase having the activity of the hyaluronidase of SEQ ID NO: 4, wherein said molecule is hybridizable to the isolated nucleic acid molecule consisting of the DNA sequence of SEQ ID NO:3 under high stringency hybridisation conditions corresponding to a T_m greater than or equal to about 65°C. Likewise, the present invention extends to an isolated polypeptide comprising an immunomodulatory portion of a T cell epitope of a *Polistinae* venom enzyme, wherein the polypeptide is encoded by an isolated nucleic acid molecule of the invention. Examples of waso venom enzymes for which isolated nucleic acid molecules of the present invention encode an immunomodulatory portion of a T cell epitope include, but certainly are not limited to, hyaluronidase. In a specific embodiment, the hyaluronidase originate from a genus *Polistes,* and particularly from the species *annularis.*
[0017] The invention further provides cloning vectors and expression vectors, which permit expression of the nucleic acids. Such vectors contain nucleic acids of the invention as set forth above. In the case of expression vectors, such nucleic acids are operatively associated with an expression control sequence.
[0018] The invention advantageously provides a method of producing a *Polistinae* venom hyaluronidase, variants thereof, encoding a hyaluronidase having the activity of the hyaluronidase of SEQ. ID N.4 or immunomodulatory fragments thereof, which, comprises the steps of:

(a) culturing a host cell transformed with an expression vector comprising an isolated nucleic acid molecule hybridizable to an isolated nucleic acid molecule comprising a DNA sequence of SEQ ID NO:3, or preferably having a sequence of SEQ ID NO:3, or variants thereof, encoding a hyaluronidase having the activity of the hyaluronidase of SEQ. ID N.4, wherein the isolated nucleic acid molecule is operationally associated with a promoter, so that the

Polistinae venom hyaluronidase, conserved variant thereof, immunomodulatory fragment thereof, or analog or derivative thereof is produced by the host cell; and

(b) recovering the *Polistinae* venom hyaluronidase, or variant thereof, encoding a hyaluronidase having the activity of the hyaluronidase of SEQ. ID N.4 immunomodulatory fragment thereof, so produced, from the culture, the host cell, or both.

[0019] In a particular example, the methods set forth above yield hyaluronidase of the genus *Polistes,* and particularly from the species *annularis,* wherein the hyaluronidase comprises an amino acid sequence of SEQ ID NO:4, variants thereof having the activity of the hyaluronidase of SEQ. ID N'4 or immunomodulatory fragments thereof.

[0020] The present invention further extends to pharmaceutical compositions effective for the treatment of a venom allergen-specific allergic condition. In particular, the present invention extends to a pharmaceutical composition comprising a polypeptide encoded by an isolated nucleic acid molecule which encodes an immunomodulatory portion of a T cell or an antigenic portion of a B cell epitope of a *Polistinae* venom enzyme, *e.g.,* a hyaluronidase, and a pharmaceutically acceptable carrier thereof. Consequently, in a preferred embodiment, a pharmaceutical composition of the invention comprises an immunomodulatory T cell epitope of *Polistes annularis* venom hyaluronidase or an antigenic portion of a B cell epitope of *Polistes annularis* hyaluronidase.

[0021] Naturally, the present invention extends to a method for treating a vespid venom allergen-specific allergic condition comprising administering a therapeutically effective amount of a pharmaceutical composition of the invention, examples of which are set forth above. Administration of a pharmaceutical composition of the invention can occur parenterally, and particularly orally, pulmonarily, nasally, topically or systemically.

[0022] Furthermore, the present invention extends to use of a recombinant *Polistinae* venom enzyme of the invention in the manufacture of a medicament for, and an associated method for modulating an immune response towards an immunogen, *e.g.*, treating a vespid allergic condition or treating an immune system related disease or disorder or a symptom of the immune system related disease or disorder. The polypeptide is encoded by an isolated nucleic acid molecule which encodes a *Polistinae* venom enzyme, wherein the polypeptide comprises an immunomodulatory fragment of a *Polistinae* venom enzyme. More particularly, an agent for treating an immune system related disease or disorder, or symptom related thereto, comprises a *Polistinae* venom enzyme or a vector that permits expression of the *Polistinae* venom or enzyme *in vivo.*

[0023] Hence, an agent for treating an immune system related disorder or disease, or a symptom thereof, comprises an isolated polypeptide encoded by an isolated nucleic acid molecule which encodes a *Polistinae* venom hyaluronidase, or variants thereof encoding a hyaluronidase having the activity of the hyaluronidase of SEQ ID N.4 or immunomodulatory fragments thereof.

[0024] Furthermore, the present invention extends to a pharmaceutical composition for modulating an immune response towards an immunogen, *e.g.*, treating a vespid allergic condition or treating an immune system related disease or disorder or a symptom related thereto, wherein the pharmaceutical composition comprises a recombinant *Polistinae* venom enzyme and a pharmaceutically acceptable carrier thereof.

[0025] Administration of a pharmaceutical composition for treating an immune system related disease or disorder to a subject can be carried out parenterally, and particularly orally, pulmonarily, nasally, topically or systemically. Furthermore, numerous diseases or disorders related to the immune system can be treated with the present invention. Examples include, but are no limited to, a pathogenic disease or disorder such as a viral disease or disorder, *e.g.*, HIV, Herpes Simplex virus, or papiloma virus; an autoimmune disease *e.g.* arthritis or Lupus; or a combination of such diseases or disorders.

[0026] It is a specific object of the invention to provide the surprising DNA sequence of isolated nucleic acid (cDNA) molecules that encode *Polistes annularis* hyaluronidase, variants thereof, encoding a hyaluronidase having the activity of the hyaluronidase of SEQ ID N.4 or immunomodulatory fragments thereof.

[0027] It is still yet another object of the invention to provide amino acid sequences of *Polistes annularis* hyaluronidase, along with variants thereof, having the activity of SEQ ID N.4, including immunomodulatory portions of T cell epitopes and antigenic portions of B cell epitopes of *Polistes annularis* hyaluronidase, either containing, or more preferably free, of "intronic" sequence. The deduced amino acid sequences of hyaluronidase from *Pol a* allow comparison of their homology to analogous enzymes from other vespids. This information provides a basis for evaluating cross-reactivity of the allergens, which can be important for allergic reactions and for therapeutic treatments. Hence, in a specific embodiment, the present invention enables one of ordinary skill in the art to determine and evaluate the degree of similarity of hyaluronidase of *Pol a* to environmental proteins and/or autologous proteins. It is believed that similarity of the vespid venom enzymes to such environmental proteins, and particularly to autologous proteins, has important implications for the allergic response.

[0028] It is yet still another object of the invention to provide expression and cloning vectors comprising an isolated nucleic acid molecule encoding *Polistes annularis* hyaluronidase, including fragments comprising an immunomodulatory portion of a T cell epitope or an antigenic portion of a B cell epitope of these *Polistinae* venom enzymes so that the isolated nucleic acid molecules can be reproduced and expressed.

[0029] Yet another object of the invention comprises production of *Polistinae* venom enzymes such as hyaluronidase, along with variants thereof encoding a hyaluronidase having the activity of hyaluronidase of SEQ ID N.4 or immunomodulatory fragments thereof, using expression vectors of the invention, despite the presence of intronic sequences in cDNA clones

[0030] Yet still another object of the invention is to provide agents and pharmaceutical compositions for treating an allergen-specific allergic condition in a subject, wherein the agents and pharmaceutical composition comprise an isolated polypeptide encoded by an isolated nucleic acid molecules which encodes a *Polistinae* venom enzyme, such as hyaluronidase, particularly from *Polistes annularis,* wherein the polypeptide comprises an antigen portion of a B cell epitope, or an immunomodulatory portion of a T cell epitope of, a *Polistinae* hyaluronidase.

[0031] Yet still another object of the invention is to provide a method for treating a vespid venom allergen-specific allergy in a subject, wherein a pharmaceutical composition for treating an allergen-specific allergic condition is administered to the subject.

[0032] Yet still another object of the invention is to provide agents and pharmaceutical compositions comprising such agents that treat an immune system related disease or disorder in mammal, such as a pathogenic disease or disorder, a viral disease or disorder, an autoimmune disease or disorder, or a combination of immune system related diseases or disorders.

[0033] Still yet another object of the invention is to provide agents and pharmaceutical composition for modulating immune response towards an immunogen in a mammal. As a result, administration of such a pharmaceutical composition modulates the immune system's ability to recognize and attack the immunogen. In a particular embodiment, the ability of the immune system of the mammal to recognize and attack the immunogen is increased upon administration of the pharmaceutical composition relative to the ability of the subject's immune system to recognize and attack the immunogen prior to administration of a pharmaceutical composition of the invention.

ABBREVIATIONS

[0034]

Dol m Dolichovespula maculata	white face hornet
Dol a D. arenaria	yellow hornet
Pol a Polistes annularis	wasp
Pol e P. exclamans	wasp
Ves m Vespula maculifrons	yellow jacket
Ves v V. vulgaris	yellow jacket
PCR	polymerase chain reaction
RACE	rapid amplification of cDNA ends
TCR	T cell receptor for antigen

BRIEF DESCRIPTION OF THE DRAWINGS

[0035]

Figure 1. The cDNA nucleotide sequence encoding *Pol a* venom phospholipase A_1 (SEQ ID NO:1) and the amino acid sequence of *Pol a* venom phospholipase A_1 (SEQ ID NO:2). Note that the first 18 amino acid residues of SEQ ID NO:2 represent a portion of a signal sequence. Hence, amino acid residue 19 of SEQ ID NO:2 (glycine) is the N-terminus amino acid residue in mature *Pol a* phospholipase A_1.

Figures 2A and 2B. *Pol a* phospholipase cDNA contains two introns. **(A)** The nucleotide sequence of *papla intron 1* (SEQ ID NO:5), an intron in *Pol a* venom phospholipase A_1 cDNA located between nucleotides 111 and 112 of SEQ ID NO:1. **(B)** The nucleotide sequences of *papla intron 2* (SEQ ID NO:6), an intron in *Pol a* venom phospholipase A_1 cDNA located between nucleotides 720 and 721 of SEQ ID NO:1.

Figure 3. Amino acid residue sequence similarity among hornet venom phospholipase (SEQ ID NO:7), yellowjacket phospholipase (SEQ ID NO:8) and paper wasp phospholipase A_1 (SEQ ID NO:2).

Figure 4. The cDNA nucleotide sequence encoding *Pol a* venom hyaluronidase (SEQ ID NO:3) and the amino acid sequence *of Pol a* hyaluronidase (SEQ ID NO:4). Note that the first 23 amino acid residues of SEQ ID NO:4 represent a portion of a signal sequence. Hence, amino acid residue 30 of SEQ ID NO:4 (serine) is the N-terminus amino acid residue of mature *Pol a* hyaluronidase.

Figure 5. The nucleotide sequence of *Pahya* (SEQ ID NO:9), an intron in *Pol a* hyaluronidase cDNA, located between nucleotides 733 and 734 of SEQ ID NO:3.

Figure 6. Amino acid residue sequence similarity among bee venom (bv) hyaluronidase (SEQ ID NO:10), *Dol m* (wfh) hyaluronidase (SEQ ID NO:11), *Ves v* (vv) hyaluronidase (SEQ ID NO:12), and *Pol a* (pa) hyaluronidase (SEQ ID NO:4).

DETAILED DESCRIPTION OF THE INVENTION

[0036] The present invention is directed to recombinant nucleic acid molecules encoding *Polistinae* venom enzymes, such as hyaluronidase, and immunomodulatory fragments, and polypeptides encoded by such nucleic acid molecules useful in the diagnosis and therapy of vespid venom-specific allergy. In specific embodiments, the present invention is directed to a recombinant nucleic acid molecule encoding *Pol a* hyaluronidase, variants thereof, encoding a hyaluronidase having the activity of the hyaluronidase of SEQ ID N.4 and immunomodulatory fragments thereof.

[0037] The present invention is based, on the surprising and wholly unexpected discovery of internal non-coding segments of cDNA encoding *Pol a* hyaluronidase. Prior to this discovery, cDNAs for vespid venom enzymes did not contain such apparent "intronic" sequences.

[0038] This discovery has two significant implications. The first is that *Polistinae,* and more particularly, *Polistes,* and more particularly still, *Pol a,* cDNAs appear to contain "introns". Thus, *Polistinaes* of this subfamily express unique mRNAs, have unique mRNA processing capabilities, and potentially represent interesting splice variants.

[0039] The term "introns" is used to refer to nucleic acid sequences that are not expected to be present in a cDNA coding for hyaluronidase, and that are not 5' or 3' UTR sequences. The sequences may represent unexpected splice variants of the proteins, incomplete processing of mRNAs, or some regulatory feature found in this subfamily, genus, and species of vespid.

[0040] The presence of these "intron" sequences significantly impacts preparation of expression vectors. While it is possible to express the unique polypeptides encoded by these cDNAs, in another embodiment an unpredictable modification of the cDNA is required to eliminate these "introns" in order to express mature forms of the *Polistinae* venom enzymes, e.g., for use in immunotherapy. Thus, it has unexpectedly proven necessary to further engineer coding sequences for *Polistinae* hyaluronidase. Once these "intron" sequences are deleted, hyaluronidase proteins comprising the natural amino acid sequence can be obtained.

[0041] The invention is further directed to expression vectors comprising said hyaluronidase nucleic acid molecule, and to methods for producing *Polistinae* venom enzyme polypeptides of the invention by expressing such expression vectors and recovering the produced *Polistinae* venom enzyme polypeptides.

[0042] The invention also provides pharmaceutical compositions effective for the treatment of a vespid venom, and likely even a hymenoptera venom, allergen-specific allergic condition comprising a polypeptide of the invention, and methods for treating such allergic conditions comprising administering a therapeutically effective amount of the pharmaceutical compositions of the invention.

[0043] The polypeptides of the invention can also be useful for diagnosis of vespid, particularly *Polistinae ,* venom-specific allergic conditions.

[0044] In addition, it has been discovered that, unexpectedly, administration of a pharmaceutical compositions comprising *Polistinae* venom hyaluronidase can be used to treat an immune system related disease or disorder, such as a pathogenic disease or disorder, a viral disease or disorder, an autoimmune disease or disorder, or a combination of such diseases or disorders.

[0045] Accordingly, as used herein, the term *"Polistinae* venom allergen" refers to a protein found in the venom of a *Polistinae,* such as the paper wasp (*Polistes annularis*), to which susceptible people are sensitized on exposure to the sting of the insect. While most antigens are characterized by being reactive with specific IgG class antibodies, an allergen is characterized by also being reactive with IgE type antibodies. The IgE type antibodies are responsible for mediating the symptoms of an allergic condition, *i.e.*, immediate-type hypersensitivity.

[0046] As used herein, the term "vespid" is used according to the practice of those in the field of allergy, and refers to insects belonging to the worldwide family of Vespidae, *i.e.,* social wasps including hornets, yellowjackets, and paper wasps. In particular, vespids of the subfamily *Vespinae* include the subfamilies *Vespinae* and *Polistinae.* More particularly, the vespids of the subfamily include the genera *Vespa* Linnaeus, *Vespula* Thomson, *Dolichovespula* Rohwer, and *Polistes* Latreille. *Vespula* and *Dolichovespula* can be considered subgenera of the genus *Vespula* Species in the genus

Vespa include but are not limited to *V. crabro* (L.) and *V. orientalis* (Linnaeus). Species in the genus *Vespula* include but are not limited to *V. germanica* (Fab.), *V. squamosa* (Drury), *V. maculifrons* (Buysson), *V. flavopilosa* (Jacobson), *V. vulgaris* (L.), and *V. pensylvanica* (Saussure). Species in the genus *Dolichovespula* include but are not limited to *P. dominulus, D. maculata* (L.) and *D. arenaria* (Fab.).

[0047] The subfamily *Polistinae* includes the genus *Polistes.* Species in the genus *Polistes* include but are not limited to *P. dominulus, Pol a* (Linnaeus), *P. exclamans* (Viereck), P. *metricus* (Say), *P. fuscatus* (Fabricius), *P. gallicus,* pacificus, *P. canadensis, P. kaibabensis, P. comanchus, P. commanchus, P. annularis, P. exclamans, P. instabilis, P. carnifex, P. major, P. metricus, P. perplexus, P. carolinus, P. flavus, P. fuscatus, P. aurifer, P. dorsalis, P. bellicosus, P. apachus, P. sulcifer, P. semenowi, P. atrimandibularis, P. biglumis, P. bischoffi, P. dominulus, P. nimpha, P. Pgallicus, P. associus, P. gigas, P. stigma, P. adustus, P. snelleni, P. mandarinus, P. chinensis, P. sulcatus, P. formosanus, P. japonicus, P. watttii, P. macaensis, P. jadwigae, P. olivaceus, P. rothneyi, P. jokohamae, P. poeyi, P. paraguayensis, P. rossi, P. cinctus, P. cavapyta, P. buysonni, P. brevifissus, P. ferreri, P. infuscatus, P. satan, P. melanotus, P. erythrocephalus, P. lanio, P. penai, P. aterrimus, P. huacapistana, P. versicolor, P. ninabamba, P. simillimus, P. adelphus, P. biguttatus, P. binotatus, P. consobrinus, P. peruvianus, P. weyrauchorum, P. xanthogaster, P. maranonensis, P. myersi, P. veracrucis, P. eburneus, P. stabilinus, P. pseudoculatus, P. apicalis, P. oculatus, P. crinitus, P. cubensis, P. minor, P. incertus, P. franciscanus, P. goeldii, P. olivaceus, P. bicolor, P. thoracicus, P. rufiventrus, P. moraballi, P. angulinus, P. subsericeus, P. testaceicolor, P. claripennis, P. billardieri, P. davillae, P. occipitalis, P. atrox, P. deceptor, P. niger, P. candidoi, P. geminatus, P. melanosoma, P. actaeon, P. obscurus, P. bequaertianus, P. cinerascens,* and *P. apachus* (Saussure).

[0048] As used herein, the term "hyaluronidase" refers to the class of enzymes that act on the disaccharide unit of D-glucuronic acid and N-acetyl-D-glucosamine. Such enzymes mediate the hydrolysis of polymers of repeating disaccharides comprising D-glucuronic acid and N-acetyl-D-glucosamine. One example of such polymer is hyaluronic acid. Hyaluronidase catalyzes the release of reducing groups of N-acetylglucosamine from hyaluronic acid.

[0049] A "genomic" sequence contains all introns 5' and 3' untranslated sequences, and 5' and 3' untranscribed, (and often regulatory) sequences of a gene. Thus, a coding sequence is not genomic when it lacks one or more introns and 5' and 3' untranscribed sequences, particularly regulatory sequences.

[0050] As used herein, the term "immunomodulatory" refers to an ability to increase or decrease an antigen-specific immune response, either at the B cell or T cell level. Immunomodulatory activity can be detected *e.g.*, in T cell proliferation assays, by measurement of antibody production, lymphokine production or T cell responsiveness. In particular, in addition to affects on T cell responses, the immunomodulatory polypeptides of the invention may bind to immunoglobulin (*i.e.,* antibody) molecules on the surface of B cells, and affect B cell responses as well.

[0051] As used herein, the term "derivative" refers to a modified nucleic acid encoding a *Polistinae,* particularly a *Polistes,* hyaluronidase venom enzyme that contains a substitution, deletion, or insertion, and the protein encoded thereby. The term "derivative" specifically refers to a low IgE binding derivative (or analog) that contains amino acid substitutions at key amino acid residues, resulting in reduced IgE binding without disrupting the overall conformation or secondary and tertiary structure of the protein. Low IgE binding derivatives are described in PCT/DK99/00136.

[0052] As used herein, the phrase "immune system related disease or disorder" refers to a disease or disorder which evokes an immune response in a subject, or effects the ability of the immune system to respond to an immunogen. Hence, examples of immune system related diseases or disorders comprise a pathogenic disease or disorder; a viral disease or disorder, *e.g.* HIV, Herpes Simplex virus, or papiloma virus; an autoimmune disease, *e.g.* arthritis or Lupus.

[0053] A "nucleic acid molecule" refers to the phosphate ester polymeric form of ribonucleosides (adenosine, guanosine, uridine or cytidine; "RNA molecules") or deoxyribonucleosides (deoxyadenosine, deoxyguanosine, deoxythymidine, or deoxycytidine; "DNA molecules") in either single stranded form, or a double-stranded helix. Double stranded DNA-DNA, DNA-RNA and RNA-RNA helices are possible. The term nucleic acid molecule, and in particular DNA or RNA molecule, refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter alia,* in linear or circular DNA molecules (e.g., restriction fragments), viruses, plasmids, and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences may be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the nontranscribed strand of DNA (i.e., the strand having a sequence homologous to the mRNA). A "recombinant DNA molecule" is a DNA molecule that has undergone a molecular biological manipulation.

[0054] A nucleic acid molecule is "hybridizable" to another nucleic acid molecule, such as a cDNA, genomic DNA, or RNA, when a single stranded form of the nucleic acid molecule can anneal to the other nucleic acid molecule under the appropriate conditions of temperature and solution ionic strength *(see* Sambrook et al., *supra).* The conditions of temperature and ionic strength determine the "stringency" of the hybridization. For preliminary screening for homologous nucleic acid molecules, low stringency hybridization conditions, corresponding to a T_m of 55°, can be used, *e.g.*, 5x SSC, 0.1% SDS, 0.25% milk, and no formamide; or 30% formamide, 5x SSC, 0.5% SDS). Moderate stringency hybridization conditions correspond to a higher T_m (about 60°), e.g., 40% formamide, with 5x or 6x SSC. High stringency hybridization conditions correspond to the highest T_m (greater than or equal to about 65°), *e.g.*, 50% formamide, 5x or 6x SSC.

Hybridization requires that the two nucleic acid molecules contain complementary sequences, although depending on the stringency of the hybridization, mismatches between bases are possible. The appropriate stringency for hybridizing nucleic acid molecules depends on the length of the nucleic acid molecules and the degree of complementation, variables well known in the art. The greater the degree of similarity or homology between two nucleotide sequences, the greater the value of T_m for hybrids of nucleic acid molecules having those sequences. The relative stability (corresponding to higher T_m) of nucleic acid hybridizations decreases in the following order: RNA:RNA, DNA:RNA, DNA:DNA. For hybrids of greater than 100 nucleotides in length, equations for calculating T_m have been derived *(see* Sambrook et al., *supra,* 9.50-0.51). For hybridization with shorter nucleic acid molecules, *i.e.,* oligonucleotides, the position of mismatches becomes more important, and the length of the oligonucleotide determines its specificity *(see* Sambrook et al., *supra,* 11.7-11.8). Preferably a minimum length for a hybridizable nucleic acid molecule is at least about 10 nucleotides; preferably at least about 10 nucleotides; and more preferably the length is at least about 20 nucleotides; even more preferably 30 nucleotides; and most preferably 40 nucleotides.

[0055] The term "standard hybridization conditions" refers to a T_m of 55°C, and utilizes conditions as set forth above. In a preferred embodiment, the T_m is 60°C; in a more preferred embodiment, the T_m is 65°C.

[0056] A DNA "coding sequence" is a double-stranded DNA sequence which is transcribed and translated into a polypeptide *in vivo* when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxyl) terminus. A coding sequence can include, but is not limited to, prokaryotic sequences, cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic (e.g., mammalian) DNA, and even synthetic DNA sequences. If the coding sequence is intended for expression in a eukaryotic cell, a polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

[0057] Transcriptional and translational control sequences are DNA regulatory sequences, such as promoters, enhancers, terminators, and the like, that provide for the expression of a coding sequence in a host cell. In eukaryotic cells, polyadenylation signals are control sequences.

[0058] A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of defining the present invention, the promoter sequence is bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site (conveniently defined for example, by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase. Eukaryotic promoters will often, but not always, contain "TATA" boxes and "CAT" boxes.

[0059] A coding sequence is "under the control" of transcriptional and translational control sequences in a cell when RNA polymerase transcribes the coding sequence into mRNA, which is then translated into the protein encoded by the coding sequence.

[0060] A "signal sequence" can be included before the coding sequence. This sequence encodes a signal peptide, N-terminal to the polypeptide, that directs the host cell to transport the polypeptide to the cell surface or secrete the polypeptide into the media, and this signal peptide is usually selectively degraded by the cell upon exportation. Signal sequences can be found associated with a variety of proteins native to prokaryotes and eukaryotes.

[0061] In accordance with the present invention there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, *e.g.*, Sambrook, Fritsch & Maniatis, "Molecular Cloning: a Laboratory Manual," Second Edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (herein "Sambrook et al., 1989"); "DNA Cloning: a Practical Approach," Volumes I and II (D.N. Glover ed. 1985); "Oligonucleotide Synthesis" (M.J. Gait ed. 1984); "Nucleic Acid Hybridization" [B.D. Hames & S.J. Higgins eds. (1985)]; "Transcription And Translation" [B.D. Hames & S.J. Higgins, eds. (1984)]; "Animal Cell Culture" [R.I. Freshney, ed. (1986)]; "Immobilized Cells And Enzymes" [IRL Press, (1986)]; B. Perbal, "a Practical Guide To Molecular Cloning" (1984).

[0062] The present invention is based, on the cloning and sequence determination of a *Polistinae* venom hyaluronidase. The cloning and sequence determination of this *Polistinae* venom enzymes is highly significant, since the cDNA clones unexpectedly contain extra nucleotide sequences that do not appear to encode polypeptide . Vespid venom allergic conditions are common, and in some sensitive individuals an allergic reaction can proceed to anaphylaxis, which is potentially fatal. As with vespids in general, *Polistinae* venom components are likely to play an important role in production of allergy. It is therefore of great importance that the nucleotide and amino acid sequence information for the *Polistinae* venom allergens is known. so that accurate diagnostic information about the nature of the allergic condition, especially specific allergen sensitivities, can be determined and effective therapeutic treatments of the underlying allergic condition can be effected. It has unexpectedly been the case here, since *Polistinae* cDNAs were surprisingly found with non-transcribed sequences.

Isolation of a Nucleic Acid Molecule Encoding a Wasp Venom Enzyme

[0063] Isolation of nucleic acid molecules encoding vespid venom enzymes was fully described in U.S. Patent No. 5,593,877. The present invention concerns the unexpected and surprising discoveries that *Polistinae* cDNAs contain "introns". Typically, introns are spliced out of mRNA and are, therefore, not usually found in cDNAs. The sequences may represent splice variants.

[0064] Derivatives of a *Polistinae* venom enzyme, fragments, and fusion proteins (see *infra*)*,* are additionally provided, as well as nucleic acid molecules encoding the same.

[0065] The present invention provides the complete nucleic acid sequence of a *Polistinae* venom enzyme. In particular, the present invention provides the nucleic acid sequence of a *Polistinae* hyaluronidase (paper wasp) and hyaluronidase, in particular *Pol a* hyaluronidase.

[0066] In a specific embodiment, to obtain a nucleic acid molecule encoding a *Polistinae* venom enzyme, polymerase chain reaction (PCR) is combined with the rapid amplification of cDNA ends (RACE) technique described by Frohman et al. (Proc. Nat. Acad. Sci. USA, 1998, 85:8998-9002; see also Frohman, 1990, Amplifications: A Forum for PCR Users 5:11) to amplify a fragment encoding a sequence comprising the *Polistinae* venom enzyme prior to selection. Oligonucleotide primers representing a *Polistinae* venom enzyme of the invention can be used as primers in PCR. Generally, such primers are prepared synthetically. Sequences for such oligonucleotide primers can be deduced from amino acid sequence information. Such oligonucleotide sequences may be non-degenerate, but more frequently the sequences are degenerate. More preferably, the primers are based on the nucleic acid sequences for the *Polistinae* venom enzymes disclosed herein. The oligonucleotides may be utilized as primers to amplify by PCR sequences from a source (RNA or DNA), preferably a cDNA library, of potential interest. For example, PCR can be used to amplify a *Polistinae* venom enzyme coding sequence from a *Polistinae* acid gland cDNA library. PCR can be carried out, *e.g.,* by use of a Perkin-Elmer Cetus thermal cycler and Taq polymerase (Gene Amp™).

[0067] For isolating a homolog of *a Polistinae* venom enzyme from any species of *Polistinae* one can choose to synthesize several different degenerate primers for use, *e.g.*, in PCR reactions. It is also possible to vary the stringency of hybridization conditions used in priming PCR reactions, to allow for greater or lesser degrees of nucleotide sequence similarity between a homolog of a *Polistinae* venom enzyme and a specific *Polistinae* venom enzyme disclosed herein. After successful amplification of a segment of a homolog of a *Polistinae* venom enzyme, that segment may be cloned and sequenced, and utilized as a probe to isolate a complete cDNA or genomic clone. This, in turn, will permit the determination of the complete nucleotide sequence, the analysis of its expression, and the production of its protein product for functional analysis, as described *infra.* In this fashion, additional genes encoding *Polistinae* venom enzymes, in particular, hyaluronidases, may be identified and expressed.

[0068] Genes encoding a *Polistinae* venom enzyme can be isolated from a suitable library by screening with a probe. Useful probes for isolating a *Polistinae* venom enzyme gene can be generated from the sequence information provided herein.

[0069] An expression library can be constructed by methods known in the art. Preferably, a cDNA library is prepared from cells or tissues that express a *Polistinae* venom enzyme, *i.e.*, cells from the poison gland located near the venom sac. Sometimes the poison gland is referred to as the acid gland. For example, mRNA or total RNA can be isolated, cDNA is made and ligated into an expression vector (*e.g.*, a plasmid or bacteriophage derivative) such that it is capable of being expressed by the host cell into which it is then introduced. Various screening assays can then be used to select for the positive clones. For example, PCR with appropriate primers, which can be synthesized based on the sequences provided herein, can be used. PCR is preferred as the amplified production can be directly detected, e.g., by ethydium bromide staining. Alternatively, labeled probes derived from the nucleic acid sequences of the instant application can be used to screen the colonies. Although the poison (acid) gland can be difficult to isolate, and the quantity of mRNA problematic, specific PCR based on primers of the present invention can overcome these problems by permitting specific amplification of trace amounts of mRNA or cDNA or even genomic DNA.

[0070] Alternatively, the presence of the gene may be detected by assays based on the physical, chemical, or immunological properties of its expressed product. For example, cDNA clones, or DNA clones which hybrid-select the proper mRNAs, can be selected which produce a protein that, *e.g.*, has similar or identical electrophoretic migration, isoelectric focusing behavior, proteolytic digestion maps, or antigenic properties as known for a *Polistinae* venom enzyme.

[0071] Some recombinant proteins expressed by bacteria, *e.g.,Polistinae* venom hyaluronidases, may react with antibodies specific for the native proteins. Other bacterially expressed recombinant proteins, such as venom phospholipases, may not react with antibodies specific for the native protein. Thus, in cases where the recombinant proteins are immunoreactive, it is possible to select for positive clones by immunoblot. However, bacterially expressed eukaryotic proteins may not fold in an active conformation.

[0072] Generally, according to the present invention, any method of screening for positive clones can be used.

[0073] Alternatives to isolating the *Polistinae* venom enzyme genomic DNA or cDNA include, but are not limited to, chemically synthesizing the gene sequence itself from the sequence provided herein.

[0074] The above methods are not meant to limit the methods by which clones of a *Polistinae* venom enzyme may be obtained.

[0075] A large number of vector-host systems known in the art may be used. Possible vectors include, but are not limited to, plasmids or modified viruses, but the vector system must be compatible with the host cell used. Such vectors include, but are not limited to, bacteriophages such as lambda derivatives, or plasmids such as various pBR322 derivatives, for example, pUC, CR, pGEX vectors; pmal-c, pFLAG, etc. The insertion into a cloning vector can, for example, be accomplished by ligating the DNA fragment into a cloning vector which has complementary cohesive termini. In a preferred aspect of the invention, the PCR amplified nucleic acid molecules of the invention contain 3'-overhanging A-nucleotides, and can be used directly for cloning into a pCR vector with compatible T-nucleotide overhangs (Invitrogen Corp., San Diego, CA). However, if the complementary restriction sites used to fragment the DNA are not present in the cloning vector, the ends of the DNA molecules may be enzymatically modified. Alternatively, any site desired may be produced by ligating nucleotide sequences (linkers) onto the DNA termini; these ligated linkers may comprise specific chemically synthesized oligonucleotides encoding restriction endonuclease recognition sequences. In an alternative method, the cleaved vector and a *Polistinae* venom enzyme gene may be modified by homopolymeric tailing. Recombinant molecules can be introduced into host cells via transformation, transfection, infection, electroporation, etc., so that many copies of the gene sequence are generated.

[0076] In specific embodiments, transformation of host cells with recombinant DNA molecules that incorporate the isolated *Polistinae* venom enzyme gene, cDNA, or synthesized DNA sequence enables generation of multiple copies of the gene. Thus, the gene may be obtained in large quantities by growing transformants, isolating the recombinant DNA molecules from the transformants and, when necessary, retrieving the inserted gene from the isolated recombinant DNA.

Expression of a Polistinae Venom Allergen Polypeptide or Fragment

[0077] As pointed out above, the isolated nucleic acids encoding *Polistinae* venom enzymes, particularly *Polistes* venom proteins, contain unexpected sequences that should be absent for the cDNA to encode a protein similar to other *Polistinae* venom enzymes, *e.g.*, as described in U.S. Patent No. 5,593,877. In one embodiment, the "intron"-containing nucleic acids are expressed without further modification. In another embodiment, the nucleic acids are modified using the techniques described herein and exemplified *infra,* or as described in the references cited above, such as Sambrook *et. al.,* to produce a protein having an amino acid sequence of a native *Polistinae* venom enzyme (though, as discussed below, such a protein may have a different secondary or tertiary structure, or include other polypeptide sequences fused to it).

[0078] The nucleotide sequence coding for a *Polistinae* venom enzyme, or an immunomodulatory fragment, derivative or analog thereof, can be inserted into an appropriate expression vector, *i.e.,* a vector which contains the necessary elements for the transcription and translation of the inserted protein-coding sequence. Such elements are termed herein a "promoter." Thus, the nucleic acid molecule encoding the *Polistinae* venom enzyme is operationally associated with the promoter. An expression vector also preferably includes a replication origin. The necessary transcriptional and translational signals can also be supplied by the native gene encoding a *Polistinae* venom enzyme and/or its flanking regions. Potential host-vector systems include but are not limited to mammalian cell systems infected with virus (*e.g.*, vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (*e.g.*, baculovirus); microorganisms such as yeast containing yeast vectors; or bacteria transformed with bacteriophage, DNA, plasmid DNA, or cosmid DNA. The expression elements of vectors vary in their strengths and specificities. Depending on the host-vector system utilized, any one of a number of suitable transcription and translation elements may be used.

[0079] In an alternative embodiment, a recombinant *Polistinae* venom enzyme of the invention, or an immunomodulatory fragment, or variants thereof encoding a hyaluronidase having the activity of the hyaluronidase of SEQ. ID. N.4. is expressed chromosomally, after integration of the *Polistinae* venom enzyme coding sequence by recombination. In this regard, any of a number of amplification systems may be used to achieve high levels of stable gene expression (*See,* Sambrook *et al.,* 1989, *supra,* at Section 16.28).

[0080] The cell into which the recombinant vector comprising the nucleic acid molecule encoding the *Polistinae* venom enzyme is cultured in an appropriate cell culture medium under conditions that provide for expression of the *Polistinae* venom enzyme by the cell. The expressed *Polistinae* venom enzyme can then be recovered from the culture according to methods well known in the art. Such methods are described in detail, *infra.*

[0081] In a another embodiment, a *Polistinae* venom enzyme-fusion protein can be expressed. A *Polistinae* venom enzyme-fusion protein comprises at least a functionally active portion of a non-*Polistinae* venom enzyme protein joined via a peptide bond to at least an immunomodulatory portion of a *Polistinae* venom enzyme. The non-*Polistinae* venom enzyme sequences can be amino- or carboxyl-terminal to the *Polistinae* venom enzyme sequences. A recombinant DNA molecule encoding such a fusion protein comprises a sequence encoding at least a functionally active portion of a non-*Polistinae* venom enzyme joined in-frame to the coding sequence for a *Polistinae* venom enzyme. It may encode a cleavage site for a specific protease, *e.g.*, Factor Xa, preferably at the juncture of the two proteins.

[0082] In another specific embodiment, a fragment of the *Polistinae* venom enzyme is expressed as a free (non-fusion) protein.

[0083] In another embodiment, a periplasmic form of the fusion protein (containing a signal sequence) can be produced for export of the protein to the *Escherichia coli* periplasm. Export to the periplasm can promote proper folding of the expressed protein.

[0084] Any of the methods previously described in Patent No.5,593,877 for the insertion of DNA fragments into a vector may be used to construct expression vectors containing a gene consisting of appropriate transcriptional/translational control signals and the protein coding sequences. These methods may include *in vitro* recombinant DNA and synthetic techniques and *in vivo* recombinants (genetic recombination). Expression of nucleic acid sequence encoding a *Polistinae* venom enzyme, or an immunomodulatory fragment thereof, may be regulated by a second nucleic acid sequence so that the *Polistinae* venom enzyme protein or peptide is expressed in a host transformed with the recombinant DNA molecule. For example, expression of a *Polistinae* venom enzyme protein may be controlled by any promoter/ enhancer element known in the art, but these regulatory elements must be functional in the host selected for expression. Promoters which may be used to control *Polistinae* venom enzyme gene expression include, but are not limited to, the CMV immediate early promoter, the SV40 early promoter region (Benoist and Chambon, 1981, Nature 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto, et al., 1980, Cell 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, Nature 296:39-42); prokaryotic expression vectors such as the β-lactamase promoter (Villa-Kamaroff, et al., 1978, Proc. Natl. Acad. Sci. U.S.A. 75:3727-3731), or the tac promoter (DeBoer, et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:21-25); see also "Useful proteins from recombinant bacteria" in Scientific American, 1980, 242:74-94; promoter elements from yeast or other fungi such as the Gal 4 promoter, the ADC (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) promoter, alkaline phosphatase promoter; and the animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals.

[0085] Once a particular recombinant DNA molecule is identified and isolated, several methods known in the art may be used to propagate it. Once a suitable host system and growth conditions are established, recombinant expression vectors can be propagated and prepared in quantity. As previously explained, the expression vectors which can be used include, but are not limited to, the following vectors or their derivatives: human or animal viruses such as vaccinia virus or adenovirus; insect viruses such as baculovirus; yeast vectors; bacteriophage vectors (*e.g.*, lambda), and plasmid and cosmid DNA vectors, to name but a few.

[0086] In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Different host cells have characteristic and specific mechanisms for the translational and post-translational processing and modification (*e.g.*, glycosylation, cleavage [*e.g.,* of signal sequence]) of proteins. Appropriate cell lines or host systems can be chosen to ensure the desired modification and processing of the foreign protein expressed. For example, expression in a bacterial system can be used to produce an nonglycosylated core protein product. However, the enzyme protein expressed in bacteria may not be properly folded. Expression in yeast can produce a glycosylated product. Expression in insect cells can be used to increase the likelihood of "native" glycosylation and folding of a heterologous *Polistinae* venom enzyme. Furthermore, different vector/host expression systems may affect processing reactions, such as proteolytic cleavages, to a different extent. It is interesting to note that it has been observed that glycosylation and proper refolding are not essential for immunomodulatory activity of a *Polistinae* venom allergen since bacterial-produced allergen is active in a T cell proliferation assay.

[0087] Vectors are introduced into the desired host cells by methods known in the art, *e.g.*, transfection, electroporation, electrotransfer, microinjection, transduction, cell fusion, DEAE dextran, calcium phosphate precipitation, lipofection (lysosome fusion), use of a gene gun, or a DNA vector transporter (see, e.g., Wu et al., 1992, J. Biol. Chem. 267:963-967; Wu and Wu, 1988, J. Biol. Chem. 263:14621-14624; Hartmut et al., Canadian Patent Application No. 2,012,311, filed March 15, 1990).

[0088] Preferred vectors, particularly for protein production *in vivo*, are viral vectors, such as lentiviruses, retroviruses, herpes viruses, adenoviruses, adeno-associated viruses, vaccinia viruses, baculoviruses, and other recombinant viruses with desirable cellular tropism. Thus, a vector encoding a *Polistinae* venom enzyme can be introduced *in vivo* or *ex vivo* using a viral vector or through direct introduction of DNA. Expression in targeted tissues can be effected by targeting the transgenic vector to specific cells, such as with a viral vector or a receptor ligand, or by using a tissue-specific promoter, or both. Targeted gene delivery is described in International Patent Publication WO 95/28494, published October 1995.

[0089] Viral vectors commonly used for *in vivo* or *ex vivo* targeting and expression procedures are DNA-based vectors and retroviral vectors. Methods for constructing and using viral vectors are known in the art *(see, e.g.,* Miller and Rosman, BioTechniques, 7:980-990, 1992). Preferably, the viral vectors are replication defective, that is, they are unable to replicate autonomously in the target cell. Preferably, the replication defective virus is a minimal virus, *i.e.,* it retains only the sequences of its genome which are necessary for encapsidating the genome to produce viral particles.

[0090] DNA viral vectors include an attenuated or defective DNA virus, such as but not limited to herpes simplex virus (HSV), papillomavirus, Epstein Barr virus (EBV), adenovirus, adeno-associated virus (AAV), vaccinia virus, and the like. Examples of particular vectors include, but are not limited to, a defective herpes virus 1 (HSV1) vector (Kaplitt, et al., Molec. Cell. Neurosci. 2:320-330, 1991; International Patent Publication No. WO 94/21807, published September 29, 1994; International Patent Publication No. WO 92/05263, published April 2, 1994); an attenuated adenovirus vector, such as the vector described by Stratford-Perricaudet, et al. (J. Clin. Invest. 90:626-630, 1992; see also La Salle, et al., Science 259:988-990, 1993); and a defective adeno-associated virus vector (Samulski, et al., J. Virol. 61:3096-3101, 1987; Samulski, et al., J. Virol. 63:3822-3828, 1989; Lebkowski, et al., Mol. Cell. Biol. 8:3988-3996, 1988).

[0091] Various companies produce viral vectors commercially, including but by no means limited to Avigen, Inc. (Alameda, CA; AAV vectors), Cell Genesys (Foster City, CA; retroviral, adenoviral, AAV vectors, and lentiviral vectors), Clontech (retroviral and baculoviral vectors), Genovo, Inc. (Sharon Hill, PA; adenoviral and AAV vectors), Genvec (adenoviral vectors), IntroGene (Leiden, Netherlands; adenoviral vectors), Molecular Medicine (retroviral, adenoviral, AAV, and herpes viral vectors), Norgen (adenoviral vectors), Oxford BioMedica (Oxford, United Kingdom; lentiviral vectors), and Transgene (Strasbourg, France; adenoviral, vaccinia, retroviral, and lentiviral vectors).

[0092] In another embodiment, the vector can be introduced *in vivo* by lipofection, as naked DNA, or with other transfection facilitating agents (peptides, polymers, etc.). Synthetic cationic lipids can be used to prepare liposomes for *in vivo* transfection of a gene encoding a marker (Felgner, et. al., Proc. Natl. Acad. Sci. U.S.A. 84:7413-7417, 1987; Felgner and Ringold, Science 337:387-388, 1989; see Mackey, et al., Proc. Natl. Acad. Sci. U.S.A. 85:8027-8031, 1988; Ulmer, et al., Science 259:1745-1748, 1993). Useful lipid compounds and compositions for transfer of nucleic acids are described in International Patent Publications WO95/18863 and WO96/17823, and in U.S. Patent No. 5,459,127. Lipids may be chemically coupled to other molecules for the purpose of targeting (see Mackey, *et al., supra*). Targeted peptides, *e.g.*, hormones or neurotransmitters, and proteins such as antibodies, or non-peptide molecules could be coupled to liposomes chemically.

[0093] Other molecules are also useful for facilitating transfection of a nucleic acid in vivo, such as a cationic oligopeptide (*e.g.*, International Patent Publication WO95/21931), peptides derived from DNA binding proteins (*e.g.*, International Patent Publication WO96/25508), or a cationic polymer (*e.g.*, International Patent Publication WO95/21931).

[0094] Alternatively, non-viral DNA vectors for gene therapy can be introduced into the desired host cells by methods known in the art, *e.g.*, electroporation, microinjection, cell fusion, DEAE dextran, calcium phosphate precipitation, use of a gene gun (ballistic transfection; see, *e.g.,* U.S. Pat. No. 5,204,253, U.S. Pat. No. 5,853,663, U.S. Pat. No. 5,885,795, and U.S. Pat. No. 5,702,384 and see Sanford, TIB-TECH, 6:299-302, 1988; Fynan et al., Proc. Natl. Acad. Sci. U.S.A., 90:11478-11482, 1993; and Yang et al., Proc. Natl. Acad. Sci. U.S.A., 87:1568-9572, 1990), or use of a DNA vector transporter (see, *e.g.*, Wu, et al., J. Biol. Chem. 267:963-967, 1992; Wu and Wu, J. Biol. Chem. 263:14621-14624, 1988; Hartmut, et al., Canadian Patent Application No. 2,012,311, filed March 15, 1990; Williams, et al., Proc. Natl. Acad. Sci. USA 88:2726-2730, 1991). Receptor-mediated DNA delivery approaches can also be used (Curiel, et al., Hum. Gene Ther. 3:147-154, 1992; Wu and Wu, J. Biol. Chem. 262:4429-4432, 1987). US Patent Nos. 5,580,859 and 5,589,466 disclose delivery of exogenous DNA sequences, free of transfection facilitating agents, in a mammal. Recently, a relatively low voltage, high efficiency *in vivo* DNA transfer technique, termed electrotransfer, has been described (Mir, et al., C.P. Acad. Sci., 321:893, 1998; WO 99/01157; WO 99/01158; WO 99/01175).

[0095] Both cDNA and genomic sequences can be cloned and expressed.

[0096] It is further contemplated that the *Polistinae* venom enzymes of the present invention, or variants thereof encoding a hyaluronidase having the activity hyaluronidase of SEQ ID N.4, or immunomodulatory fragments thereof can be prepared synthetically, *e.g.*, by solid phase peptide synthesis.

Isolation and Purification

[0097] Once the recombinant *Polistinae* venom enzyme protein is identified, it may be isolated and purified by standard methods including chromatography (*e.g.*, ion exchange, affinity, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins.

[0098] In a particular embodiment, a *Polistinae* venom enzyme and fragments thereof can be engineered to include about six histidyl residues, which makes possible the selective isolation of the recombinant protein on a Ni-chelation column. In a preferred aspect, the proteins are further purified by reverse phase chromatography.

[0099] In another embodiment, in which recombinant *Polistinae* venom enzyme is expressed as a fusion protein, the non-*Polistinae* venom enzyme portion of the fusion protein can be targeted for affinity purification. For example, antibody specific for the non-*Polistinae* venom enzyme portion of the fusion protein can be immobilized on a solid support, *e.g.*, cyanogen bromide-activated Sepharose, and used to purify the fusion protein. In another embodiment, a binding partner of the non-*Polistinae* venom enzyme portion of the fusion protein, such as a receptor or ligand, can be immobilized and used to affinity purify the fusion protein.

[0100] In one embodiment, a *Polistinae* venom enzyme-fusion protein, preferably purified, is used without further modification, *i.e.*, without cleaving or otherwise removing the non-*Polistinae* venom enzyme-portion of the fusion protein. In a preferred embodiment, the *Polistinae* venom enzyme-fusion protein can be used therapeutically, *e.g.*, to modulate an immune response.

[0101] In a further embodiment, the purified fusion protein is treated to cleave the non-*Polistinae* venom enzyme protein or portion thereof from the *Polistinae* venom enzyme. For example, where the fusion protein has been prepared to include a protease sensitive cleavage site, the fusion protein can be treated with the protease to cleave the protease specific site and release *Polistinae* venom enzyme.

[0102] In a particular embodiment of the present invention, such recombinant *Polistinae* venom enzymes include but certainly are not limited to those containing, as a primary amino acid sequence, all or part of the amino acid sequence substantially as depicted in Figure 4 (SEQ ID NO:4), as well as variants thereof having the activity of the hyaluronidase of SEQ ID N 4.

Derivatives and Analogs of *Polistinae* Venom Enzymes

[0103] The production and use of derivatives and analogs related to *Polistinae* venom enzymes are immunomodulatory, *i.e.,* capable of modulating an antigen-specific immune response. Moreover, analogs or derivatives *of Polistinae* venom enzymes, particularly and hyaluronidase from *Polistes annularis,* can also be used to treat immune system related diseases or disorders, or a symptom related thereto. The derivative or analog can bind to a *Polistinae* venom enzyme-specific immunoglobulin, including IgG and IgE. Derivatives or analogs of *Polistinae* venom enzyme can be tested for the desired immunomodulatory activity by procedures known in the art, including but not limited to the assays described *infra.*

[0104] In particular, *Polistinae* venom enzyme derivatives can be made by altering the nucleic acid sequences of the invention by substitutions, additions or deletions. Due to the degeneracy of nucleotide coding sequences, other DNA sequences which encode substantially the same amino acid sequence as a nucleic acid encoding a *Polistinae* venom enzyme may be used in the practice of the present invention. These include but are not limited to nucleotide sequences comprising all or portions of a gene encoding the *Polistinae* venom enzyme that are altered by the substitution of different codons that encode the same amino acid residue within the sequence, thus producing a silent change. Likewise, derivatives include, but are not limited to, those containing, as a primary amino acid sequence, all or part of the amino acid sequence of a *Polistinae* venom enzyme, including altered sequences in which functionally equivalent amino acid residues are substituted for residues within the sequence resulting in a conservative amino acid substitution. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity which acts as a functional equivalent, resulting in a silent alteration. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

[0105] Derivatives or analogs of *Polistinae* venom enzyme include but are not limited to those which are substantially homologous to a *Polistinae* venom enzyme or fragments thereof, or whose encoding nucleic acid is capable of hybridizing to a nucleic acid molecule encoding a *Polistinae* venom enzyme. Hybridization can occur under moderately stringent to highly stringent conditions, depending on the degree of sequence similarity, as is well known in the art.

[0106] Derivatives and analogs can be produced by various methods known in the art. The manipulations which result in their production can occur at the gene or protein level. For example, the nucleic acid sequence of the cloned *Polistinae* venom enzyme can be modified by any of numerous strategies known in the art (Maniatis, T., 1990, Molecular Cloning, A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York). The sequence can be cleaved at appropriate sites with restriction endonuclease(s), followed by further enzymatic modification if desired, isolated, and ligated *in vitro.* In the production of the gene encoding a derivative or analog of a *Polistinae* venom enzyme, care should be taken to ensure that the modified gene remains within the same translational reading frame as *Polistinae* venom enzyme, uninterrupted by translational stop signals.

[0107] Additionally, the gene encoding a *Polistinae* venom enzyme can be mutated *in vitro* or *in vivo,* to create and/or destroy translation, initiation, and/or termination sequences, or to create variations in coding regions and/or form new restriction endonuclease sites or destroy preexisting ones, to facilitate further *in vitro* modification. Any technique for mutagenesis known in the art can be used, including but not limited to, *in vitro* site-directed mutagenesis (Hutchinson, C., et al., 1978, J. Biol. Chem. 253:6551; Zoller and Smith, 1984, DNA 3:479-488; Oliphant et al., 1986, Gene 44:177; Hutchinson et al., 1986, Proc. Natl. Acad. Sci. U.S.A. 83:710), use of TAB® linkers (Phannacia), etc. PCR techniques are preferred for site directed mutagenesis (see Higuchi, 1989, "Using PCR to Engineer DNA", in PCR Technology: Principles and Applications for DNA Amplification, H. Erlich, ed., Stockton Press, Chapter 6, pp. 61-70).

[0108] Manipulations of the recombinant *Polistinae* venom enzyme may also be made at the protein level. Recombinant *Polistinae* venom enzyme fragments or other derivatives or analogs which are differentially modified during or after translation, *e.g.*, by glycosylation, acetylation, phosphorylation, amidation, reduction and carboxymethylation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. can be prepared. Any of numerous chemical modifications may be carried out by known techniques, including but not limited to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, $NaBH_4$; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

[0109] In a particular embodiment, the *Polistinae* venom enzyme or immunomodulatory fragment thereof is expressed in an insect cell expression system, *e.g.*, using a baculovirus expression vector. As pointed out above, this should yield "native" glycosylation and structure, particularly secondary and tertiary structure, of the expressed polypeptide. Native glycosylation and structure of the expressed polypeptide may be very important for diagnostic uses, since the enzyme specific antibodies detected in diagnostic assays will be specific for the native enzyme, *i.e.,* as introduced by a sting from a vespid.

Activity Assays With Peptides of the Invention

[0110] Numerous assays are known in immunology for evaluating the immunomodulatory activity of an antigen. For example, the *Polistinae* venom enzyme proteins produced by expression of the nucleic acid molecules of the invention can be used in diagnostic assays for allergic diseases, which are described in detail, *infra.* In general, such proteins can be tested for the ability to bind to antibodies specific for the enzyme. Preferably, such antibodies that are detected in the diagnostic assay are of the IgE class. However, it is important to note that natural allergen-specific antibodies have been found to bind weakly to denatured vespid venom allergens. *Polistinae* venom enzymes produced in eukaryotic expression systems, and particularly insect cell expression systems, may have the correct structure for antibody binding. *Polistinae* venom enzymes expressed in bacterial expression systems may not, and would thus require refolding prior to use in a diagnostic assay for antibody binding.

[0111] In another embodiment, the proteins of the invention can be tested in a proliferation assay for T cell responses. For such T cell response assays, the expression system used to produce the enzyme does not appear to affect the immunomodulatory activity of the protein. Generally, lymphocytes from a sensitized host are obtained. The host can be a mouse that has been immunized with a *Polistinae* venom enzyme, such as a *Polistinae* venom hyaluronidase that has been produced recombinantly according to the present invention.

[0112] In a preferred embodiment, peripheral blood leukocytes are obtained from a human who is sensitive to vespid venom. Using techniques that are well known in the art, T lymphocyte response to the protein can be measured *in vitro.* In a specific embodiment, *infra,* T cell responses are detected by measuring incorporation of ^{3}H-thymidine, which increases with DNA synthesis associated with proliferation.

[0113] Cell proliferation can also be detected using an MTT assay (Mossman, 1983, J. Immunol. Methods 65:55-63; Niks and Otto, 1990, J. Immunol. Methods 130:140-151). Any method for detecting T cell proliferation known in the art can be used with the *Polistinae* enzyme produced according to the present invention.

[0114] Similarly, lymphokine production assays can be practiced according to the present invention. In one embodiment, lymphokine production can be assayed using immunological or co-stimulation assays (see, *e.g.*, Fehlner et al., 1991, J. Immunol. 146:799) or using the ELISPOT technique (Czerkinsky, et al., 1988, J. Immunol. Methods 110:29). Alternatively, mRNA for lymphokines can be detected, *e.g.*, by amplification (see Brenner, et al., 1989, Biotechniques 7:1096) or *in situ* hybridization (see, *e.g.,* Kasaian and Biron, 1989, J. Immunol. 142:1287). Of particular interest are those individuals whose T cells produce lymphokines associated with IgE isotype switch events, *e.g.*, IL-4 and IL-5 (Purkeson and Isakson, 1992, J. Exp. Med. 175:973-982). Also of interest are the polypeptide fragments of the *Polistinae* venom enzyme that contain epitopes recognized by T cells involved in IgE switch events.

[0115] Thus, in a preferred aspect, the proteins produced according to the present invention can be used in *in vitro* assays with peripheral blood lymphocytes or, more preferably, cell lines derived from peripheral blood lymphocytes, obtained from vespid venom enzyme sensitive individuals to detect secretion of lymphokines ordinarily associated with allergic responses, *e.g.*, IL-4. Such assays may indicate which venom component or components are responsible for the allergic condition. More importantly, the fragments of the *Polistinae* venom enzyme can be tested. In this way, specific epitopes responsible for T cell responses associated with allergic response can be identified. The sequences of such epitopes can be compared to other vespid venom enzymes and to environmental or autologous proteins to determine if there are sequence similarities that suggest possible cross-reactivity. The peptides can be tested for the ability to induce T cell anergy, *e.g.*, by mega-dose administration, modification to produce an epitope antagonist, administration in the absence of the appropriate costimulatory signals, and other methods thought to result in T cell anergy. Peptides containing such epitopes are ideal candidates for therapeutics.

[0116] In a further embodiment, the polypeptide of the invention can be used directly in assays to detect the extent of cross-reactivity with other environmental proteins and/or homologous proteins, with which they share sequence similarity.

In particular, the fragments of the *Polistinae* venom enzymes that have sequence similarity with such environmental, and more particularly, homologous proteins can be evaluated for cross reactivity with antibodies or T cell specific for such proteins. In another specific embodiment, the cross reactivity of *Polistinae* venom hyaluronidase with the sperm membrane protein PH-20 is evaluated.

Diagnostic and Therapeutic Uses of the *Polistinae* Venom Enzyme Polypeptides

[0117] The present invention provides a plentiful source of a pure *Polistinae* venom enzyme, produced by recombinant techniques. Alternatively, given the sequence information provided by the present invention, polypeptide fragments, derivatives or analogs of the *Polistinae* venom enzymes can advantageously be produced by peptide synthesis.

[0118] The invention contemplates use of *Polistinae* venom enzymes, or immunomodulatory fragments, or variants having the activity of the hyaluronidase of SEQ ID N.4 for the preparation of diagnostic or therapeutic compositions, for the use in the diagnosis and therapy of vespid venom allergen-specific allergic conditions, treating vespid venom allergen-specific allergic conditions, treating immune system related conditions, and modulating immune response in a mammal against an immunogen. In particular, Polistes hyaluronidase, in particular *Pol a* hyaluronidase, or immunomodulatory fragments, variants having the activity of the hyaluronidase of SEQ ID N.4, are contemplated for use in diagnosis, therapy, treatment, and modulation of immune response according to the present invention.

Diagnostic Methods

[0119] As use herein, the term diagnostic includes *in vitro* and *in vivo* diagnostic assays. Generally, such assays are designed to measure the activity of IgE antibodies specific for a given allergen. Such diagnostic assays depend heavily on the availability of pure allergen. This is especially true for determining sensitivity to a specific allergen component of a vespid venom. *In vitro* diagnostic assays for enzyme sensitivity include radioimmunoassay (RIA), immunoradiometric immunoassay (IRMA), radio-allergosorbent tests (RAST), enzyme-linked immunosorbent assay (ELISA), ELISPOT, magnetic allergosorbent assay, immunoblots, histamine release assays, and the like.

[0120] Further one can determine the presence of epitopes that are predominantly reactive with IgE antibodies, or with other isotypes, *e.g.*, IgG. Such epitopes may overlap or be distinct. In particular, fragments of the *Polistinae* venom enzymes of the invention can be used to identify such specific B cell epitopes. Identification of specific epitopes can provide a basis for developing therapies, as described *infra.*

[0121] Furthermore, one can use *in vitro* diagnostic assays on peripheral blood lymphocytes, as described *supra.* Such diagnostic assays can give detailed information about the enzyme-specific T cell responses, the phenotype of the T cell response, and preferably the T cell epitope of the enzyme involved in T cell responses. The immunodominant epitope and the epitope involved in IgE isotype class switch events can be detected, if they are not the same. In particular, the T cell epitopes of *Polistinae* venom enzymes that stimulate proliferation and/or lymphokine secretion of T cells of a phenotype associated with IgE isotype class switching events can be identified for a specific individual, or for a class of individuals who share MHC haplotype or a predominant T cell receptor variable region expression, or both.

[0122] *In vivo* assays for allergenicity generally consist of skin prick sensitivity assays, in which serially diluted amounts of an allergen are administered either subcutaneously or intradermally into a patient's skin, and wheel and erythema reactions are detected. As with *in vitro* assays, the availability of pure venom enzyme greatly increases the value of the results of the *in vivo* diagnostic assays since cross-reactivity with impurities in extracts prepared from vespid venom sacs can be avoided.

Therapeutic Methods

[0123] Therapeutic compositions of the invention (see, *infra*) can be used in immunotherapy, also referred to as hyposensitization therapy. Immunotherapy has proven effective in allergic diseases, particular insect allergy. Allergens are administered parenterally over a long period of time in gradually increasing doses. Such therapy may be particularly effective when the allergen or allergens to which the patient is sensitive have been specifically identified and the therapy is targeted to those allergen(s). Thus, the availability of pure *Polistinae* venom enzyme in large quantities is important for immunotherapy of allergy.

[0124] In another embodiment, the present invention contemplates use of polypeptides comprising at least an immunomodulatory T cell epitope of a *Polistinae* venom enzyme to induce specific T cell allergy to a vespid venom enzyme. Identification of such peptides is described *supra.* More preferably, a peptide comprising such a T cell epitope and lacking a B cell epitope can be administered to a patient. The presence of B cell epitopes on an allergen can cause an undesirable systemic reaction when the allergen is used for immunotherapy. Thus, a particular advantage of the invention is the capability to provide allergen polypeptides that do not cause undesirable systemic effects.

[0125] In one embodiment, one or more polypeptide fragments can be injected subcutaneously to decrease the T cell response to the entire molecule, *e.g.*, as described by Brine et al. (1993, Proc. Natl. Acad. Sci. U.S.A. 90:7608-12).

[0126] In another embodiment, one or more polypeptide fragments can be administered intranasally to suppress allergen-specific responses in naive and sensitized subjects (see *e.g.*, Hoyne et al., 1993, J. Exp. Med. 178:1783-88).

[0127] Administration of a *Polistinae* venom enzyme peptide of the invention is expected to induce anergy, resulting in cessation of allergen-specific antibody production or allergen-specific T cell response, or both, and thus, have a therapeutic effect.

[0128] In a preferred aspect of the invention, peptide based therapy to induce T cell anergy is customized for each individual or a group of individuals. Using the diagnostic methods of the present invention, the specific T cell epitope or epitopes of a vespid venom enzyme involved in the allergic response can be identified. Peptides comprising these epitopes can then be used in an individualized immunotherapy regimen.

Treatment of Immune System Related Diseases or Disorders, or a Symptom Related Thereto

[0129] As explained above, the present invention relates to polypeptides for treating immune system related diseases or disorders, or for modulating immune response in a mammal towards an immunogen, wherein the polypeptides are encoded by isolated nucleic acid molecules which encode *Polistinae* venom enzymes, such hyaluronidase from *Polistes annularis.* In particular, components of vespid venom, particularly hyaluronidase, have applications in modulating a subject's immune response to various immunogens, such as pathogens and viruses, to name only a few. In a particular embodiment, components of a *Polistinae* venom, and particularly hyaluronidase from *Polistes annularis* and conserved variants thereof, fragments thereof, or analogs or derivatives thereof modulate a subject's immune system to have increased ability to combat pathogens and viruses including, but not limited to, HIV, Herpes Simplex virus, or papilloma virus. In a specific embodiment, such a method comprises administering to a subject a therapeutically effective amount of a pharmaceutical composition comprising a polypeptide encoded by an isolated nucleic acid molecule comprising a DNA sequence of SEQ ID NO 3, variants thereof, encoding a hyaluronidase having the activity of the hyaluronidase of SEQ ID N 4 immunomodulatory fragments thereof, or an isolated nucleic acid molecule hybridizable thereto, wherein the polypeptide comprises an antigenic portion of a B cell epitope or an immunomodulatory portion of a T cell epitope *of Polistes annularis* hyaluronidase.

[0130] Furthermore, it has been discovered that components of *Polistinae* venom, such hyaluronidase of *Polistes annularis,* also have applications in treating an immune system related disease or disorder, or a symptom related thereto. As used herein, the phrase "immune system related disease or disorder" refers to a disease or disorder which evokes an immune response in a subject, or effects the ability of the immune system to respond to an immunogen. Examples of immune system related diseases or disorders which can be treated with agents and pharmaceutical compositions of the invention include, but are not limited to, a pathogenic disease or disorder; a viral disease or disorder, e.g. HIV, Herpes Simplex virus, or papilloma virus; or an autoimmune disease, e.g. arthritis or Lupus. Hence, the present invention encompasses agents for treating an immune system related disease or disorder, or a symptom related thereto, in a specific embodiment comprising an isolated polypeptide encoded by an isolated nucleic acid molecule comprising a DNA sequence of SEQ ID NO 3, variants thereof, encoding a hyaluronidase having the activity of hyaluronidase of SEQ ID.N.4 immunomodulatory fragments thereof, wherein the isolated polypeptide comprises an immunomodulatory portion of a T cell epitope or an antigenic portion of a B cell epitope of *Polistes annularis* hyaluronidase.

[0131] Hence, naturally, the present invention extends to pharmaceutical compositions for treating an immune system related disease or disorder, comprising a *Polistinae* venom enzyme, or variants thereof, having the activity of the hyaluronidase of SEQ ID N4 or immunomodulatory fragments thereof. Moreover, the present invention extends to a method for treating an immune system related disease or disorder, or a symptom related thereto, comprising administering a therapeutically effective amount of a pharmaceutical composition for treating an immune system related disease or disorder to a subject. The phrase "therapeutically effective amount" is used herein to mean an amount sufficient to treat, and preferably increase by at least about 30 percent, more preferably by at least 50 percent; most preferably by at least 90 percent, the ability of the immune system of a subject to combat effectively an immunogen. As further studies are conducted, information will emerge regarding appropriate dosage levels for modulation of immune system response towards an immunogen in various patients, and the ordinary skilled worker, considering the therapeutic context, age and general health of the recipient, will be able to ascertain proper dosing. Delivery can be of the protein or a gene therapy vector. Hence, for example, should the immune system related disease or disorder involve HIV, a clinically significant change would, for example, involve an increase in white blood cell count in a subject to whom a pharmaceutical composition of the invention is administered relative to white blood cell count prior to administration. Other such examples of monitoring a clinically significant change in a subject will be readily apparent to one of ordinary skill in the art. Furthermore, as further studies are conducted, information will emerge regarding appropriate dosage levels for treating an immune system related disease or disorder, or a symptom related thereto in various patients, and the ordinary skilled worker, considering the therapeutic context, age and general health of the recipient, will be able to ascertain proper dosing. Examples of pharmaceutically acceptable compositions are described *infra.*

Pharmaceutically Acceptable Compositions

[0132] The *in vivo* diagnostic or therapeutic compositions of the invention may also contain appropriate pharmaceutically acceptable carriers, excipients, diluents and adjuvants. As used herein, the phrase "pharmaceutically acceptable" preferably means approved by a regulatory agency of a government, in particular the Federal government or a state government, or listed in the U.S. Pharmacopeia or another generally recognized pharmacopeia for use in animals, and more particularly in humans. Suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin.

[0133] Such pharmaceutically acceptable carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include mannitol, human serum albumin (HSA), starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, magnesium carbonate, magnesium stearate, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. These compositions can take the form of solutions, suspensions, tablets, pills, capsules, powders, sustained-release formulations and the like.

[0134] Such compositions will contain an effective diagnostic or therapeutic amount of the active compound together with a suitable amount of carrier so as to provide the form for proper administration to the patient. While intravenous injection is a very effective form of administration, other modes can be employed, such as by injection, or by oral, nasal or parenteral administration.

[0135] The invention will be further clarified by the following examples, example 1 not being part of this invention.

EXAMPLE 1: PAPER WASP PHOSPHOLIPASE

[0136] Based, in part, on the methods and disclosure of U.S. Patent No. 5,593,877, nucleic acids encoding *Pol a* (paper wasp) phospholipase were obtained. However, these nucleic acids surprisingly include internal sequences that do not code for an amino acid sequence found as expected on the native protein. Although the nucleic acids described in this Example are cDNAs, and are not genomic, they appear to include "introns".

MATERIALS AND METHODS

[0137] The methods used are the same as those described in U.S. Patent No. 5,593,877 using RACE.

Results

[0138] When examining paper wasp phospholipase A, cDNA produced with RACE, it was observed that its length was longer than necessary to encode paper wasp phospholipase A, protein. It was discovered that, surprisingly, this augmented length was the result introns incorporated into the paper wasp phospholipase A_1 cDNA. Such a discovery was unexpected in light of studies conducted on the cDNAs of other vespid venoms, which invariably do not contain any introns. For example, the phospholipase cDNAs of yellowjacket and hornet contain no such introns.

[0139] Because of this major unforeseen difference between paper wasp phospholipase A_1 cDNA and other vespid venom phospholipase cDNAs, special biotechniques and steps were required to isolate paper wasp phospholipase A_1 cDNA, which were not needed to obtain the venom phospholipase cDNA from other vespids, such as hornet and yellowjacket. In particular, in order to isolate the cDNA sequence encoding phospholipase A_1 for paper wasp, it was necessary to determine the size and location and number of introns.

[0140] Using the amino acid sequence derived from the cyanogen bromide degradation of paper wasp phospholipase A_1, the genetic code, and the nucleotide sequence of wasp phospholipase cDNA derived from the RACE protocol, two introns were discovered. The first intron, hereinafter referred to as *"papla intron 1"* comprises a nucleotide sequence as set forth in SEQ ID NO:5 (Figure 2A). *Papla intron 1* comprises 114 nucleotides, and is normally located between nucleotides 111 and 112 of the cDNA sequence encoding phospholipase A,, set forth In SEQ ID NO:1.

[0141] A second intron, hereinafter referred to as *"papla intron 2*" was also discovered. This intron comprises a nucleotide sequence as set forth in SEQ ID NO:6 (Figure 2B). *Papla intron 2* contains 127 nucleotides, and is normally located between nucleotides 720 and 721 of SEQ ID NO:1.

[0142] In order to isolate the cDNA sequence encoding paper wasp phospholipase A_1 (SEQ ID NO:1), these introns had to be removed from the paper wasp phospholipase A_1 cDNA derived from RACE without disturbing the reading frame of the coding nucleotides. In essence, paper wasp phospholipase A_1 cDNA had to be re-designed so that only encoding nucleotides would be included. This re-design process was technically very difficult because, should one encoding nucleotide be accidentally removed along with an intron, or should one non-coding nucleotide not be removed, a reading frame shift would be produced which would result in mutations and could cause premature termination of the expression of the cDNA.

[0143] In this re-design process, specially designed oligonucleotides were chemically synthesized, each complementary to coding nucleotides located 5' and 3' of one of the introns. The amplified paper wasp phospholipase A_1 cDNA derived from RACE was then cloned into a self-replicating plasmid. This plasmid was denatured, and, under low stringency conditions, the oligonucleotides were permitted to anneal to the paper wasp phospholipase A_1 cDNA, leaving the introns single stranded. These oligonucleotides then served as primers for DNA synthesis, which generated a double stranded plasmid wherein the introns were deleted from one of the strands. A cell was then transfected with the plasmid using methods described above, and the cell was then cloned. Since one of the two DNA strands in the original plasmid had the introns deleted, half of the transfected cells contained a double stranded plasmid in which the introns had been removed. The cloned were then screened to isolate the cells having the plasmid comprising paper wasp cDNA comprising a DNA sequence of SEQ ID NO:9 (without introns). Copies of the particular plasmid were then isolated and sequenced to confirm the deletion of the introns. The re-designed paper wasp phospholipase A_1 cDNA was then removed from the particular plasmid, sequenced, amplified, and cloned into an expression vector, using the procedures described in Example 1 and in Application Serial No. 08/474,853 and in U.S. Patent 5,593,877, which are hereby incorporated by reference in their entireties.

[0144] A comparison of the deduced amino acid sequence of paper wasp phospholipase A_1 (SEQ ID NO:2) with other vespid venom phospholipases was performed. In particular, SEQ ID NO:2 was compared with phospholipase from white face hornet (*D. maculata*) (SEQ ID NO:7) and phospholipase from yellow jacket (*V. vulgaris)* (SEQ ID NO:8). The results of this sequence comparison are shown in Figure 3.

EXAMPLE 2: PAPER WASP HYALURONIDASE

[0145] Using the procedures described in U.S. Patent No. 5,593,877, the cDNA sequence encoding paper wasp (*Pol a*) hyaluronidase (SEQ ID NO:3) and its corresponding amino acid sequence (SEQ ID NO:4) were isolated and are set forth in Figure 4. Nucleotides 449 through 536 of SEQ ID NO:3 encode a portion of a signal sequence. Hence, the amino acid residue at the N terminus of mature *Pol a* hyaluronidase is serine, which is encoded by nucleotides 536, 537, and 538.

[0146] Surprisingly, paper wasp hyaluronidase cDNA produced from the RACE protocol set forth above had greater length than necessary to encode *Pol a* hyaluronidase protein. Hence, it was concluded paper wasp hyaluronidase cDNA contained at least one intron. The presence of the at least one intron within the wasp hyaluronidase cDNA was unexpected in light of studies on hyaluronidase cDNA from other vespid venoms, such as yellowjacket and hornet, which do not contain introns. As a result, special biotechniques similar to those employed to isolate paper wasp phospholipase A_1 cDNA, and set forth in Example 1 *supra,* were required to isolate the cDNA encoding sequence of paper wasp hyaluronidase.

[0147] Initially, a determination was made as to the location and size of the introns within the paper wasp hyaluronidase cDNA. Once the introns were located, they had to be removed in such a manner as not to disturb any coding nucleotides. Hence, just as with paper wasp phospholipase A_1 cDNA, it was necessary to re-design paper wasp hyaluronidase cDNA so that only encoding nucleotides would be included. This re-design process was technically very difficult because, should one encoding nucleotide be accidentally removed along with an intron, or should one non-coding nucleotide not be removed, a missense frameshift mutation would be placed into the wasp hyaluronidase cDNA.

[0148] The cDNA encoding mature paper wasp hyaluronidase (SEQ ID NO:3) was prepared using procedure similar to that used to isolate the cDNA encoding paper wasp phospholipase A_1 *supra,* The cDNA without introns was then sequenced, amplified, and cloned into an expression vector, again using the procedures described above.

[0149] Paper wasp hyaluronidase cDNA was found to contain one intron. This intron, hereinafter referred to as *"pahya",* is 94 nucleotides long, and has a nucleotide sequence as set forth in SEQ ID NO:9 (Figure 5). Normally, this intron is located between nucleotides 733 and 734 of SEQ ID NO:3.

[0150] A comparison of the amino acid sequence of paper wasp hyaluronidase (SEQ ID NO:4) with other vespid venom phospholipases was performed. In particular, SEQ ID NO:4 was compared with hyaluronidase from bee venom (SEQ ID NO:10), hyaluronidase from white face hornet (*D. maculata*) (SEQ ID NO:11) and hyaluronidase from yellowjacket (*V. vulgaris*) (SEQ ID NO:12). The results of this sequence comparison are shown in Figure 6.

SEQUENCE LISTING

[0151]

<110> King, Te Piao

<120> CLONING AND RECOMBINANT PRODUCTION OF VESPID VENOM ENZYMES, SUCH AS PHOSPHOL-

IPASE AND HYALURONIDASE, AND IMMUNOLOGICAL THERAPIES BASED THEREON

<130> 2313/2F138

<140> Unassigned
<141> 1999-10-01

<160> 12

<170> FastSEQ for windows Version 3.0

<210> 1
<211> 1368
<212> PRT
<213> Polistes annularis

<400> 1

```
Ala Thr Thr Thr Gly Cys Thr Thr Cys Thr Thr Gly Thr Thr Ala Gly
 1               5                  10                  15
Ala Thr Gly Ala Thr Thr Cys Gly Ala Cys Gly Ala Cys Ala Thr Thr
            20                  25                  30
Thr Ala Gly Ala Ala Ala Thr Gly Gly Thr Ala Cys Cys Thr Thr Gly
        35                  40                  45
Ile Cys Phe Leu Leu Asp Asp Ser Thr Thr Phe Arg Asn Gly Thr Leu
    50                  55                  60
Ala Ala Thr Ala Gly Ala Gly Gly Cys Ala Thr Gly Thr Cys Thr Cys
65                  70                  75                  80
Cys Gly Gly Ala Thr Thr Gly Thr Ala Cys Thr Thr Thr Thr Ala Ala
                85                  90                  95
Thr Gly Ala Gly Ala Ala Ala Gly Ala Thr Ala Thr Ala Gly Thr Ala
            100                 105                 110
Asn Arg Gly Met Ser Pro Asp Cys Thr Phe Asn Glu Lys Asp Ile Val
        115                 120                 125
Thr Thr Cys Thr Ala Thr Gly Thr Thr Thr Ala Cys Thr Cys Ala Ala
    130                 135                 140
Gly Gly Gly Ala Thr Ala Ala Gly Cys Gly Ala Gly Ala Thr Gly Gly
145                 150                 155                 160
Thr Ala Thr Thr Ala Thr Thr Cys Thr Thr Ala Ala Gly Ala Ala Ala
                165                 170                 175
Phe Tyr Val Tyr Ser Arg Asp Lys Arg Asp Gly Ile Ile Leu Lys Lys
            180                 185                 190
Gly Ala Ala Ala Cys Thr Thr Thr Ala Ala Cys Gly Ala Ala Thr Thr
        195                 200                 205
Ala Cys Gly Ala Thr Cys Thr Gly Thr Thr Thr Ala Cys Ala Ala Ala
    210                 215                 220
```

Gly Thr Cys Thr Ala Cys Ala Ala Thr Ala Thr Cys Ala Ala Ala Ala
225 230 235 240
Glu Thr Leu Thr Asn Tyr Asp Leu Phe Thr Lys Ser Thr Ile Ser Lys
245 250 255
Cys Ala Ala Gly Thr Thr Gly Thr Ala Thr Thr Thr Cys Thr Thr Ala
260 265 270
Thr Ala Cys Ala Thr Gly Gly Thr Thr Thr Cys Cys Thr Thr Thr Cys
275 280 285
Ala Ala Cys Thr Gly Gly Gly Ala Ala Thr Ala Ala Thr Gly Ala Ala
290 295 300
Gln Val Val Phe Leu Ile His Gly Phe Leu Ser Thr Gly Asn Asn Glu
305 310 315 320
Ala Ala Cys Thr Thr Cys Gly Thr Thr Gly Cys Thr Ala Thr Gly Thr
325 330 335
Cys Gly Ala Ala Ala Gly Cys Thr Thr Thr Ala Ala Thr Ala Gly Ala
340 345 350
Ala Ala Ala Ala Gly Ala Thr Gly Ala Thr Thr Thr Thr Cys Thr Thr
355 360 365
Asn Phe Val Ala Met Ser Lys Ala Leu Ile Glu Lys Asp Asp Phe Leu
370 375 380
Gly Thr Ala Ala Thr Thr Thr Cys Gly Gly Thr Cys Gly Ala Cys Thr
385 390 395 400
Gly Gly Ala Ala Gly Ala Ala Gly Gly Gly Thr Gly Cys Thr Thr Gly
405 410 415
Thr Ala Ala Thr Gly Cys Thr Thr Thr Thr Gly Cys Thr Thr Cys Ala
420 425 430
Val Ile Ser Val Asp Trp Lys Lys Gly Ala Cys Asn Ala Phe Ala Ser
435 440 445
Ala Cys Ala Ala Ala Gly Gly Ala Thr Gly Cys Thr Thr Thr Gly Gly
450 455 460
Gly Thr Thr Ala Thr Thr Cys Cys Ala Ala Ala Gly Cys Cys Gly Thr
465 470 475 480
Thr Gly Gly Ala Ala Ala Cys Ala Cys Ala Cys Gly Thr Cys Ala Cys
485 490 495
Thr Lys Asp Ala Leu Gly Tyr Ser Lys Ala Val Gly Asn Thr Arg His
500 505 510
Gly Thr Thr Gly Gly Ala Ala Ala Ala Thr Thr Thr Gly Thr Ala Gly
515 520 525
Cys Thr Gly Ala Thr Thr Thr Thr Ala Cys Ala Ala Ala Ala Cys Thr
530 535 540
Ala Cys Thr Thr Gly Thr Ala Gly Ala Ala Ala Ala Ala Thr Ala Thr
545 550 555 560
Val Gly Lys Phe Val Ala Asp Phe Thr Lys Leu Leu Val Glu Lys Tyr
565 570 575
Ala Ala Ala Gly Thr Gly Cys Thr Gly Ala Thr Ala Thr Cys Ala Ala
580 585 590
Ala Thr Ala Thr Ala Cys Gly Ala Thr Thr Gly Ala Thr Cys Gly Gly
595 600 605
Gly Cys Ala Thr Ala Gly Thr Thr Thr Gly Gly Gly Cys Gly Cys Gly
610 615 620
Lys Val Leu Ile Ser Asn Ile Arg Leu Ile Gly His Ser Leu Gly Ala
625 630 635 640
Cys Ala Thr Ala Cys Thr Thr Cys Ala Gly Gly Thr Thr Thr Thr Gly

```
                645                 650                 655
Cys Gly Gly Gly Ala Ala Ala Ala Gly Ala Ala Gly Thr Thr Cys Ala
            660                 665                 670
Ala Ala Ala Gly Thr Thr Ala Ala Ala Ala Thr Thr Ala Gly Gly Ala
        675                 680                 685
His Thr Ser Gly Phe Ala Gly Lys Glu Val Gln Lys Leu Lys Leu Gly
    690                 695                 700
Ala Ala Ala Thr Ala Cys Ala Ala Gly Gly Ala Ala Ala Thr Thr Ala
705                 710                 715                 720
Thr Cys Gly Gly Gly Cys Thr Thr Gly Ala Thr Cys Cys Thr Gly Cys
                725                 730                 735
Thr Gly Gly Ala Cys Cys Gly Thr Ala Thr Thr Thr Thr Cys Ala Thr
            740                 745                 750
Lys Tyr Lys Glu Ile Ile Gly Leu Asp Pro Ala Gly Pro Tyr Phe His
        755                 760                 765
Cys Gly Gly Ala Gly Thr Gly Ala Cys Thr Gly Thr Cys Cys Gly Gly
    770                 775                 780
Ala Cys Ala Gly Ala Cys Thr Thr Thr Gly Cys Gly Thr Ala Ala Cys
785                 790                 795                 800
Ala Gly Ala Cys Gly Cys Ala Gly Ala Ala Thr Ala Thr Gly Thr Thr
                805                 810                 815
Arg Ser Asp Cys Pro Asp Arg Leu Cys Val Thr Asp Ala Glu Tyr Val
            820                 825                 830
Cys Ala Ala Gly Thr Thr Ala Thr Ala Cys Ala Thr Ala Cys Ala Thr
        835                 840                 845
Cys Ala Ala Thr Cys Ala Thr Ala Thr Thr Ala Gly Gly Ala Gly Thr
    850                 855                 860
Ala Thr Ala Thr Thr Ala Thr Ala Ala Thr Gly Thr Thr Gly Gly Thr
865                 870                 875                 880
Gln Val Ile His Thr Ser Ile Ile Leu Gly Val Tyr Tyr Asn Val Gly
                885                 890                 895
Ala Gly Cys Gly Thr Thr Gly Ala Thr Thr Thr Cys Thr Ala Cys Gly
            900                 905                 910
Thr Gly Ala Ala Thr Thr Ala Thr Gly Gly Ala Ala Ala Ala Ala Ala
        915                 920                 925
Thr Cys Ala Ala Cys Cys Thr Gly Gly Thr Thr Gly Cys Ala Ala Thr
    930                 935                 940
Ser Val Asp Phe Tyr Val Asn Tyr Gly Lys Asn Gln Pro Gly Cys Asn
945                 950                 955                 960
Gly Ala Ala Cys Cys Ala Thr Cys Cys Thr Gly Cys Thr Cys Thr Cys
                965                 970                 975
Ala Thr Ala Cys Gly Ala Ala Ala Gly Cys Cys Gly Thr Gly Ala Ala
            980                 985                 990
Ala Thr Ala Thr Cys Thr Gly Ala Cys Thr Gly Ala Gly Thr Gly Cys
        995                 1000                1005
Glu Pro Ser Cys Ser His Thr Lys Ala Val Lys Tyr Leu Thr Glu Cys
    1010                1015                1020
Ala Thr Ala Ala Ala Ala Cys Ala Thr Gly Ala Ala Thr Gly Thr Thr
1025                1030                1035                104
Gly Thr Thr Thr Ala Ala Thr Thr Gly Gly Ala Ala Cys Ala Cys Cys
                1045                1050                1055
Ala Thr Gly Gly Ala Ala Gly Ala Ala Ala Thr Ala Thr Thr Thr Cys
            1060                1065                1070
```

```
Ile Lys His Glu Cys Cys Leu Ile Gly Thr Pro Trp Lys Lys Tyr Phe
        1075                1080                1085
Ala Gly Cys Ala Cys Thr Cys Cys Ala Ala Ala Ala Cys Cys Ala Ala
    1090                1095                1100
Thr Thr Thr Cys Cys Cys Ala Gly Thr Gly Cys Ala Gly Ala Gly Gly
1105                1110                1115                112
Ala Gly Ala Cys Ala Cys Cys Thr Gly Thr Gly Thr Thr Thr Gly Cys
                1125                1130                1135
Ser Thr Pro Lys Pro Ile Ser Gln Cys Arg Gly Asp Thr Cys Val Cys
            1140                1145                1150
Gly Thr Thr Gly Gly Ala Thr Thr Gly Ala Ala Thr Gly Cys Ala Ala
        1155                1160                1165
Ala Ala Ala Gly Thr Thr Ala Thr Cys Cys Thr Gly Cys Thr Ala Gly
    1170                1175                1180
Ala Gly Gly Cys Gly Cys Ala Thr Thr Thr Thr Ala Thr Gly Cys Ala
1185                1190                1195                120
Val Gly Leu Asn Ala Lys Ser Tyr Pro Ala Arg Gly Ala Phe Tyr Ala
                1205                1210                1215
Cys Cys Gly Gly Thr Thr Gly Ala Ala Gly Cys Ala Ala Ala Thr Gly
            1220                1225                1230
Cys Ala Cys Cys Thr Thr Ala Thr Thr Gly Cys Cys Ala Thr Ala Ala
        1235                1240                1245
Cys Gly Ala Gly Gly Gly Gly Ala Thr Thr Ala Ala Ala Cys Thr Thr
    1250                1255                1260
Pro Val Glu Ala Asn Ala Pro Tyr Cys His Asn Glu Gly Ile Lys Leu
1265                1270                1275                128
Thr Ala Ala Thr Thr Ala Thr Ala Ala Ala Cys Ala Ala Ala Ala Gly
                1285                1290                1295
Thr Cys Ala Ala Thr Gly Thr Ala Cys Ala Cys Ala Ala Ala Ala Ala
            1300                1305                1310
Thr Gly Thr Ala Thr Cys Thr Ala Thr Thr Gly Ala Thr Gly Ala Ala
        1315                1320                1325
Thr Ala Thr Thr Ala Ala Ala Thr Gly Ala Ala Thr Ala Ala Ala Cys
    1330                1335                1340
Gly Ala Ala Cys Ala Gly Thr Cys Ala Ala Ala Thr Ala Ala Ala Ala
1345                1350                1355                136
Ala Ala Ala Ala Ala Ala Ala Ala
                1365
```

<210> 2
<211> 320
<212> PRT
<213> Polistes annularis

<400> 2

```
Ile Cys Phe Leu Leu Asp Asp Ser Thr Thr Phe Arg Asn Gly Thr Leu
1               5                   10                  15
Asn Arg Gly Met Ser Pro Asp Cys Thr Phe Asn Glu Lys Asp Ile Val
            20                  25                  30
Phe Tyr Val Tyr Ser Arg Asp Lys Arg Asp Gly Ile Ile Leu Lys Lys
        35                  40                  45
Glu Thr Leu Thr Asn Tyr Asp Leu Phe Thr Lys Ser Thr Ile Ser Lys
    50                  55                  60
```

```
Gln Val Val Phe Leu Ile His Gly Phe Leu Ser Thr Gly Asn Asn Glu
65                  70                  75                  80
Asn Phe Val Ala Met Ser Lys Ala Leu Ile Glu Lys Asp Asp Phe Leu
                85                  90                  95
Val Ile Ser Val Asp Trp Lys Lys Gly Ala Cys Asn Ala Phe Ala Ser
            100                 105                 110
Thr Lys Asp Ala Leu Gly Tyr Ser Lys Ala Val Gly Asn Thr Arg His
        115                 120                 125
Val Gly Lys Phe Val Ala Asp Phe Thr Lys Leu Leu Val Glu Lys Tyr
    130                 135                 140
Lys Val Leu Ile Ser Asn Ile Arg Leu Ile Gly His Ser Leu Gly Ala
145                 150                 155                 160
His Thr Ser Gly Phe Ala Gly Lys Glu Val Gln Lys Leu Lys Leu Gly
                165                 170                 175
Lys Tyr Lys Glu Ile Ile Gly Leu Asp Pro Ala Gly Pro Tyr Phe His
            180                 185                 190
Arg Ser Asp Cys Pro Asp Arg Leu Cys Val Thr Asp Ala Glu Tyr Val
        195                 200                 205
Gln Val Ile His Thr Ser Ile Ile Leu Gly Val Tyr Tyr Asn Val Gly
    210                 215                 220
Ser Val Asp Phe Tyr Val Asn Tyr Gly Lys Asn Gln Pro Gly Cys Asn
225                 230                 235                 240
Glu Pro Ser Cys Ser His Thr Lys Ala Val Lys Tyr Leu Thr Glu Cys
                245                 250                 255
Ile Lys His Glu Cys Cys Leu Ile Gly Thr Pro Trp Lys Lys Tyr Phe
            260                 265                 270
Ser Thr Pro Lys Pro Ile Ser Gln Cys Arg Gly Asp Thr Cys Val Cys
        275                 280                 285
Val Gly Leu Asn Ala Lys Ser Tyr Pro Ala Arg Gly Ala Phe Tyr Ala
    290                 295                 300
Pro Val Glu Ala Asn Ala Pro Tyr Cys His Asn Glu Gly Ile Lys Leu
305                 310                 315                 320
```

<210> 3
<211> 0
<212> PRT
<213> Polistes annularis

<400> 3

```
TAT GTG TCA TTG TCC CCC GAC TCA GTA TTT AAT ATC ATC ACC GAT GAC      48
Tyr Val Ser Leu Ser Pro Asp Ser Val Phe Asn Ile Ile Thr Asp Asp
            325                 330                 335

ATC TCC CAC CAA ATT CTT TCC AGA TCG AAT TGT GAA AGA TCC AAA AGA      96
Ile Ser His Gln Ile Leu Ser Arg Ser Asn Cys Glu Arg Ser Lys Arg
        340                 345                 350

CCG AAA AGG GTC TTC AGC ATT TAT TGG AAC GTT CCT ACC TTT ATG TGC     144
Pro Lys Arg Val Phe Ser Ile Tyr Trp Asn Val Pro Thr Phe Met Cys
    355                 360                 365

CAC CAA TAT GGC ATG AAT TTC GAC GAG GTG ACA GAT TTT AAT ATC AAA     192
His Gln Tyr Gly Met Asn Phe Asp Glu Val Thr Asp Phe Asn Ile Lys
```

```
370                 375                 380                 385

CAT AAT TCT AAG GAC AAT TTT CGC GGT GAA ACT ATA TCA ATT TAT TAC      240
His Asn Ser Lys Asp Asn Phe Arg Gly Glu Thr Ile Ser Ile Tyr Tyr
                390                 395                 400

GAT CCT GGA AAA TTT CCA GCA TTG ATG CCA CTA AAA AAT GGT AAT TAT      288
Asp Pro Gly Lys Phe Pro Ala Leu Met Pro Leu Lys Asn Gly Asn Tyr
            405                 410                 415

GAG GAA AGA AAC GGA GGG GTT CCT CAG CGA GGT AAC ATC ACG ATA CAT      336
Glu Glu Arg Asn Gly Gly Val Pro Gln Arg Gly Asn Ile Thr Ile His
        420                 425                 430

TTG CAA CAA TTT AAC GAA GAT TTG GAT AAA ATG ACA CCG GAT AAA AAT      384
Leu Gln Gln Phe Asn Glu Asp Leu Asp Lys Met Thr Pro Asp Lys Asn
    435                 440                 445

TTC GGT GGT ATC GGT GTA ATC GAT TTC GAA AGA TGG AAA CCG ATT TTC      432
Phe Gly Gly Ile Gly Val Ile Asp Phe Glu Arg Trp Lys Pro Ile Phe
450                 455                 460                 465

CGA CAG AAT TGG GGT AAC ACG GAA ATA CAT AAG AAA TAT TCT ATT GAA      480
Arg Gln Asn Trp Gly Asn Thr Glu Ile His Lys Lys Tyr Ser Ile Glu
                470                 475                 480

CTC GTT CGG AAA GAA CAT CCA AAG TGG AGC GAA TCG ATG ATC GAA GCG      528
Leu Val Arg Lys Glu His Pro Lys Trp Ser Glu Ser Met Ile Glu Ala
            485                 490                 495

GAA GCT ACG AAA AAG TTC GAG AAA TAT GCG AGA TAT TTC ATG GAA GAA      576
Glu Ala Thr Lys Lys Phe Glu Lys Tyr Ala Arg Tyr Phe Met Glu Glu
        500                 505                 510

ACT TTG AAA TTG GCA AAA AAG ACT AGG AAA AGG GCT AAG TGG GGT TAT      624
Thr Leu Lys Leu Ala Lys Lys Thr Arg Lys Arg Ala Lys Trp Gly Tyr
    515                 520                 525

TAC GGA TTT CCT TAC TGC TAT AAC GTA ACA CCG AAT AAT CCT GGC CCG      672
Tyr Gly Phe Pro Tyr Cys Tyr Asn Val Thr Pro Asn Asn Pro Gly Pro
530                 535                 540                 545

GAT TGC GAT GCT AAA GCG ACA ATC GAG AAC GAT AGA CTG TCG TGG ATG      720
Asp Cys Asp Ala Lys Ala Thr Ile Glu Asn Asp Arg Leu Ser Trp Met
                550                 555                 560

TAC AAT AAT CAA GAA ATA CTT TTT CCA TCC GTC TAC GTG AGA CAT GAA      768
Tyr Asn Asn Gln Glu Ile Leu Phe Pro Ser Val Tyr Val Arg His Glu
            565                 570                 575

CAA AAA CCG GAG GAA AGG GTT TAC CTA GTG CAA GGT AGA ATT AAA GAA      816
Gln Lys Pro Glu Glu Arg Val Tyr Leu Val Gln Gly Arg Ile Lys Glu
        580                 585                 590
```

```
GCT GTT AGG ATA TCG AAT AAT TTA GAA CAT TCA CCT AGT GTG CTT GCT        864
Ala Val Arg Ile Ser Asn Asn Leu Glu His Ser Pro Ser Val Leu Ala
    595                 600                 605

TAT TGG TGG TAC GTG TAT CAG GAC AAG ATG GAC ATT TAC CTA AGC GAG        912
Tyr Trp Trp Tyr Val Tyr Gln Asp Lys Met Asp Ile Tyr Leu Ser Glu
610                 615                 620                 625

ACC GAC GTG GAA AAG ACT TTC CAA GAG ATA GTG ACT AAT GGT GGG GAT        960
Thr Asp Val Glu Lys Thr Phe Gln Glu Ile Val Thr Asn Gly Gly Asp
                630                 635                 640

GGT ATC ATA ATA TGG GGT AGC TCG TCC GAT GTT AAC AGC CTA AGT AAA       1008
Gly Ile Ile Ile Trp Gly Ser Ser Ser Asp Val Asn Ser Leu Ser Lys
            645                 650                 655

TGT AAG AGA TTG AGA GAG TAC CTG TTA AAC ACT TTA GGA CCG TTC GCG       1056
Cys Lys Arg Leu Arg Glu Tyr Leu Leu Asn Thr Leu Gly Pro Phe Ala
        660                 665                 670

GTT AAT GTA ACA GAA ACT GTC AAC GGA AGA TCA TCC CTA AAC TTC TAA       1104
Val Asn Val Thr Glu Thr Val Asn Gly Arg Ser Ser Leu Asn Phe  *
    675                 680                 685

AATAATCGAT AACGCCTAAT CACGTCGATG ATGATTATTA GGGTGTTCTT CGGTGATTGG     1164

TTTGATCTCA CTGAAAAGAC TTTTCGTTAA AAAACAAAAA GATAAATGTA ATTTATAAGT     1224

TAAAAAAACC TATACGACCA AAGAAAGAAA GAAAAAAAAA AAAAAAAAA                 1273
```

<210> 4
<211> 367
<212> PRT
<213> Polistes annularis

<400> 4

```
Tyr Val Ser Leu Ser Pro Asp Ser Val Phe Asn Ile Ile Thr Asp Asp
1               5                   10                  15
Ile Ser His Gln Ile Leu Ser Arg Ser Asn Cys Glu Arg Ser Lys Arg
            20                  25                  30
Pro Lys Arg Val Phe Ser Ile Tyr Trp Asn Val Pro Thr Phe Met Cys
        35                  40                  45
His Gln Tyr Gly Met Asn Phe Asp Glu Val Thr Asp Phe Asn Ile Lys
    50                  55                  60
His Asn Ser Lys Asp Asn Phe Arg Gly Glu Thr Ile Ser Ile Tyr Tyr
65                  70                  75                  80
Asp Pro Gly Lys Phe Pro Ala Leu Met Pro Leu Lys Asn Gly Asn Tyr
                85                  90                  95
Glu Glu Arg Asn Gly Gly Val Pro Gln Arg Gly Asn Ile Thr Ile His
            100                 105                 110
Leu Gln Gln Phe Asn Glu Asp Leu Asp Lys Met Thr Pro Asp Lys Asn
        115                 120                 125
```

```
Phe Gly Gly Ile Gly Val Ile Asp Phe Glu Arg Trp Lys Pro Ile Phe
    130                 135                 140
Arg Gln Asn Trp Gly Asn Thr Glu Ile His Lys Lys Tyr Ser Ile Glu
145                 150                 155                 160
Leu Val Arg Lys Glu His Pro Lys Trp Ser Glu Ser Met Ile Glu Ala
                165                 170                 175
Glu Ala Thr Lys Lys Phe Glu Lys Tyr Ala Arg Tyr Phe Met Glu Glu
            180                 185                 190
Thr Leu Lys Leu Ala Lys Lys Thr Arg Lys Arg Ala Lys Trp Gly Tyr
        195                 200                 205
Tyr Gly Phe Pro Tyr Cys Tyr Asn Val Thr Pro Asn Asn Pro Gly Pro
    210                 215                 220
Asp Cys Asp Ala Lys Ala Thr Ile Glu Asn Asp Arg Leu Ser Trp Met
225                 230                 235                 240
Tyr Asn Asn Gln Glu Ile Leu Phe Pro Ser Val Tyr Val Arg His Glu
                245                 250                 255
Gln Lys Pro Glu Glu Arg Val Tyr Leu Val Gln Gly Arg Ile Lys Glu
            260                 265                 270
Ala Val Arg Ile Ser Asn Asn Leu Glu His Ser Pro Ser Val Leu Ala
        275                 280                 285
Tyr Trp Trp Tyr Val Tyr Gln Asp Lys Met Asp Ile Tyr Leu Ser Glu
    290                 295                 300
Thr Asp Val Glu Lys Thr Phe Gln Glu Ile Val Thr Asn Gly Gly Asp
305                 310                 315                 320
Gly Ile Ile Ile Trp Gly Ser Ser Ser Asp Val Asn Ser Leu Ser Lys
                325                 330                 335
Cys Lys Arg Leu Arg Glu Tyr Leu Leu Asn Thr Leu Gly Pro Phe Ala
            340                 345                 350
Val Asn Val Thr Glu Thr Val Asn Gly Arg Ser Ser Leu Asn Phe
        355                 360                 365
```

<210> 5
<211> 114
<212> DNA
<213> Polistes annularis

<400> 5

```
aggtaataat ctcgattcta tgcgtacgcg attttgttga ttatttttca agaaaatgta      60
agaaaaattt ttaaaaatat attactgaag tatgaaataa aaactttata cttt           114
```

<210> 6
<211> 127
<212> DNA
<213> Polistes annularis

<400> 6

```
ggtaatattt ttatattaaa atgaacaatt ctatggaata gaaatagtac aagcatcgat      60
tatatcctat gccttgttat atgatttcgg agttagacac tattattttt aaataatttt     120
tacatta                                                               127
```

<210> 7
<211> 317
<212> PRT

<213> Dolichovespula maculata

<400> 7

Arg Leu Ile Met Phe Val Gly Asp Pro Ser Ser Ser Asn Glu Leu Asp
1   5   10   15
Arg Phe Ser Val Cys Pro Phe Ser Asn Asp Thr Val Lys Met Ile Phe
            20   25   30
Leu Thr Arg Glu Asn Arg Lys His Asp Phe Tyr Thr Leu Asp Thr Met
        35   40   45
Asn Arg His Asn Glu Phe Lys Lys Ser Ile Ile Lys Arg Pro Val Val
    50   55   60
Phe Ile Thr His Gly Phe Thr Ser Ser Ala Thr Glu Lys Asn Phe Val
65   70   75   80
Ala Met Ser Glu Ala Leu Met His Thr Gly Asp Phe Leu Ile Ile Met
                85   90   95
Val Asp Trp Arg Met Ala Ala Cys Thr Asp Glu Tyr Pro Gly Leu Lys
            100   105   110
Tyr Met Phe Tyr Lys Ala Ala Val Gly Asn Thr Arg Leu Val Gly Asn
        115   120   125
Phe Ile Ala Met Ile Ala Lys Lys Leu Val Glu Gln Tyr Lys Val Pro
    130   135   140
Met Thr Asn Ile Arg Leu Val Gly His Ser Leu Gly Ala His Ile Ser
145   150   155   160
Gly Phe Ala Gly Lys Arg Val Gln Glu Leu Lys Leu Gly Lys Phe Ser
                165   170   175
Glu Ile Ile Gly Leu Asp Pro Ala Gly Pro Ser Phe Lys Lys Asn Asp
            180   185   190
Cys Ser Glu Arg Ile Cys Glu Thr Asp Ala His Tyr Val Gln Ile Leu
        195   200   205
His Thr Ser Ser Asn Leu Gly Thr Glu Arg Thr Leu Gly Thr Val Asp
    210   215   220
Phe Tyr Ile Asn Asn Gly Ser Asn Gln Pro Gly Cys Arg Tyr Ile Ile
225   230   235   240
Gly Glu Thr Cys Ser His Thr Arg Ala Val Lys Tyr Phe Thr Glu Cys
                245   250   255
Ile Arg Arg Glu Cys Cys Leu Ile Gly Val Pro Gln Ser Lys Asn Pro
            260   265   270
Gln Pro Val Ser Lys Cys Thr Arg Asn Glu Cys Val Cys Val Gly Leu
        275   280   285
Asn Ala Lys Lys Tyr Pro Lys Arg Gly Ser Phe Tyr Val Pro Val Glu
    290   295   300
Ala Glu Ala Pro Tyr Cys Asn Asn Asn Gly Lys Ile Ile
305   310   315

<210> 8
<211> 300
<212> PRT
<213> Vespula vulgaris

<400> 8

Gly Pro Lys Cys Pro Phe Asn Ser Asp Thr Val Ser Ile Ile Ile Glu
1   5   10   15
Thr Arg Glu Asn Arg Asn Arg Asp Leu Tyr Thr Leu Gln Thr Leu Gln

```
                20                  25                  30
Asn His Pro Glu Phe Lys Lys Lys Thr Ile Thr Arg Pro Val Val Phe
        35                  40                  45
Ile Thr His Gly Phe Thr Ser Ser Ala Ser Glu Thr Asn Phe Ile Asn
    50                  55                  60
Leu Ala Lys Ala Leu Val Asp Lys Asp Asn Tyr Met Val Ile Ser Ile
65                  70                  75                  80
Asp Trp Gln Thr Ala Ala Cys Thr Asn Glu Ala Ala Gly Leu Lys Tyr
                85                  90                  95
Leu Tyr Tyr Pro Thr Ala Ala Arg Asn Thr Arg Leu Val Gly Gln Tyr
            100                 105                 110
Ile Ala Thr Ile Thr Gln Lys Leu Val Lys His Tyr Lys Ile Ser Met
        115                 120                 125
Ala Asn Ile Arg Leu Ile Gly His Ser Leu Gly Ala His Ala Ser Gly
    130                 135                 140
Phe Ala Gly Lys Lys Val Gln Glu Leu Lys Leu Gly Lys Tyr Ser Glu
145                 150                 155                 160
Ile Ile Gly Leu Asp Pro Ala Arg Pro Ser Phe Asp Ser Asn His Cys
                165                 170                 175
Ser Glu Arg Leu Cys Glu Thr Asp Ala Glu Tyr Val Gln Ile Ile His
            180                 185                 190
Thr Ser Asn Tyr Leu Gly Thr Glu Lys Thr Leu Gly Thr Val Asp Phe
        195                 200                 205
Tyr Met Asn Asn Gly Lys Asn Gln Pro Gly Cys Gly Arg Phe Phe Ser
    210                 215                 220
Glu Val Cys Ser His Ser Arg Ala Val Ile Tyr Met Ala Glu Cys Ile
225                 230                 235                 240
Lys His Glu Cys Cys Leu Ile Gly Ile Pro Lys Ser Lys Ser Ser Gln
                245                 250                 255
Pro Ile Ser Ser Cys Thr Lys Gln Glu Cys Val Cys Val Gly Leu Asn
            260                 265                 270
Ala Lys Lys Tyr Thr Ser Arg Gly Ser Phe Tyr Val Pro Val Glu Ser
        275                 280                 285
Thr Val Pro Phe Cys Asn Asn Lys Gly Lys Ile Ile
    290                 295                 300
```

<210> 9
<211> 94
<212> DNA
<213> Polistes annularis

<400> 9

```
attttctac tacagttctt tttatctctc tatcattgat gataaatcgt ttaaatcgat    60
ctattgtaaa ttatctatcg attgtttagg caaa                               94
```

<210> 10
<211> 343
<212> PRT
<213> Apis melliferis

<400> 10

```
Pro Asp Asn Asn Lys Thr Val Arg Glu Phe Asn Val Tyr Trp Asn Val
1               5                   10                  15
```

```
Pro Thr Phe Met Cys His Lys Tyr Gly Leu Arg Phe Glu Glu Val Ser
            20                  25                  30
Glu Lys Tyr Gly Ile Leu Gln Asn Trp Met Asp Lys Phe Arg Gly Glu
        35                  40                  45
Glu Ile Ala Ile Leu Tyr Asp Pro Gly Met Phe Pro Ala Leu Leu Lys
    50                  55                  60
Asp Pro Asn Gly Asn Val Val Ala Arg Asn Gly Gly Val Pro Gln Leu
65                  70                  75                  80
Gly Asn Leu Thr Lys His Leu Gln Val Phe Arg Asp His Leu Ile Asn
                85                  90                  95
Gln Ile Pro Asp Lys Ser Phe Pro Gly Val Gly Val Ile Asp Phe Glu
            100                 105                 110
Ser Trp Arg Pro Ile Phe Arg Gln Asn Trp Ala Ser Leu Gln Pro Tyr
        115                 120                 125
Lys Lys Leu Ser Val Glu Val Val Arg Arg Glu His Pro Phe Trp Asp
    130                 135                 140
Asp Gln Arg Val Glu Gln Glu Ala Lys Arg Arg Phe Glu Lys Tyr Gly
145                 150                 155                 160
Gln Leu Phe Met Glu Glu Thr Leu Lys Ala Ala Lys Arg Met Arg Pro
                165                 170                 175
Ala Ala Asn Trp Gly Tyr Tyr Ala Tyr Pro Tyr Cys Tyr Asn Leu Thr
            180                 185                 190
Pro Asn Gln Pro Ser Ala Gln Cys Glu Ala Thr Thr Met Gln Glu Asn
        195                 200                 205
Asp Lys Met Ser Trp Leu Phe Glu Ser Glu Asp Val Leu Leu Pro Ser
    210                 215                 220
Val Tyr Leu Arg Trp Asn Leu Thr Ser Gly Glu Arg Val Gly Leu Val
225                 230                 235                 240
Gly Gly Arg Val Lys Glu Ala Leu Arg Ile Ala Arg Gln Met Thr Thr
                245                 250                 255
Ser Arg Lys Lys Val Leu Pro Tyr Tyr Trp Tyr Lys Tyr Gln Asp Arg
            260                 265                 270
Arg Asp Thr Asp Leu Ser Arg Ala Asp Leu Glu Ala Thr Leu Arg Lys
        275                 280                 285
Ile Thr Asp Leu Gly Ala Asp Gly Phe Ile Ile Trp Gly Ser Ser Asp
    290                 295                 300
Asp Ile Asn Thr Lys Ala Lys Cys Leu Gln Phe Arg Glu Tyr Leu Asn
305                 310                 315                 320
Asn Glu Leu Gly Pro Ala Val Lys Arg Ile Ala Leu Asn Asn Asn Ala
                325                 330                 335
Asn Asp Arg Leu Thr Val Asp
            340
```

<210> 11
<211> 331
<212> PRT
<213> Dolichovespula maculata

<400> 11

```
Ser Glu Arg Pro Lys Arg Val Phe Asn Ile Tyr Trp Asn Val Pro Thr
1               5                   10                  15
Phe Met Cys His Gln Tyr Gly Leu Tyr Phe Asp Glu Val Thr Asn Phe
            20                  25                  30
```

Asn Ile Lys His Asn Ser Lys Asp Asp Phe Gln Gly Asp Lys Ile Ser
35 40 45
Ile Phe Tyr Asp Pro Gly Glu Phe Pro Ala Leu Leu Pro Leu Lys Glu
50 55 60
Gly Asn Tyr Lys Ile Arg Asn Gly Gly Val Pro Gln Glu Gly Asn Ile
65 70 75 80
Thr Ile His Leu Gln Arg Phe Ile Glu Asn Leu Asp Lys Thr Tyr Pro
85 90 95
Asn Arg Asn Phe Asn Gly Ile Gly Val Ile Asp Phe Glu Arg Trp Arg
100 105 110
Pro Ile Phe Arg Gln Asn Trp Gly Asn Met Met Ile His Lys Lys Phe
115 120 125
Ser Ile Asp Leu Val Arg Asn Glu His Pro Phe Trp Asp Lys Lys Met
130 135 140
Ile Glu Leu Glu Ala Ser Lys Arg Phe Glu Lys Tyr Ala Arg Leu Phe
145 150 155 160
Met Glu Glu Thr Leu Lys Leu Ala Lys Lys Thr Arg Lys Gln Ala Asp
165 170 175
Trp Gly Tyr Tyr Gly Tyr Pro Tyr Cys Phe Asn Met Ser Pro Asn Asn
180 185 190
Leu Val Pro Asp Cys Asp Ala Thr Ala Met Leu Glu Asn Asp Lys Met
195 200 205
Ser Trp Leu Phe Asn Asn Gln Asn Val Leu Leu Pro Ser Val Tyr Ile
210 215 220
Arg His Glu Leu Thr Pro Asp Gln Arg Val Gly Leu Val Gln Gly Arg
225 230 235 240
Val Lys Glu Ala Val Arg Ile Ser Asn Asn Leu Lys His Ser Pro Lys
245 250 255
Val Leu Ser Tyr Trp Trp Tyr Val Tyr Gln Asp Asp Thr Asn Thr Phe
260 265 270
Leu Thr Glu Thr Asp Val Lys Lys Thr Phe Gln Glu Ile Ala Ile Asn
275 280 285
Gly Gly Asp Gly Ile Ile Ile Trp Gly Ser Ser Ser Asp Val Asn Ser
290 295 300
Leu Ser Lys Cys Lys Arg Leu Arg Glu Tyr Leu Leu Thr Val Leu Gly
305 310 315 320
Pro Ile Thr Val Asn Val Thr Glu Thr Val Asn
325 330

<210> 12
<211> 331
<212> PRT
<213> Vespula vulgaris

<400> 12

Ser Glu Arg Pro Lys Arg Val Phe Asn Ile Tyr Trp Asn Val Pro Thr
1 5 10 15
Phe Met Cys His Gln Tyr Asp Leu Tyr Phe Asp Glu Val Thr Asn Phe
20 25 30
Asn Ile Lys Arg Asn Ser Lys Asp Asp Phe Gln Gly Asp Lys Ile Ala
35 40 45
Ile Phe Tyr Asp Pro Gly Glu Phe Pro Ala Leu Leu Ser Leu Lys Asp
50 55 60

```
Gly Lys Tyr Lys Lys Arg Asn Gly Gly Val Pro Gln Glu Gly Asn Ile
65                  70                  75                  80
Thr Ile His Leu Gln Lys Phe Ile Glu Asn Leu Asp Lys Ile Tyr Pro
                85                  90                  95
Asn Arg Asn Phe Ser Gly Ile Gly Val Ile Asp Phe Glu Arg Trp Arg
            100                 105                 110
Pro Ile Phe Arg Gln Asn Trp Gly Asn Met Lys Ile His Lys Asn Phe
        115                 120                 125
Ser Ile Asp Leu Val Arg Asn Glu His Pro Thr Trp Asn Lys Lys Met
    130                 135                 140
Ile Glu Leu Glu Ala Ser Lys Arg Phe Glu Lys Tyr Ala Arg Phe Phe
145                 150                 155                 160
Met Glu Glu Thr Leu Lys Leu Ala Lys Lys Thr Arg Lys Gln Ala Asp
                165                 170                 175
Trp Gly Tyr Tyr Gly Tyr Pro Tyr Cys Phe Asn Met Ser Pro Asn Asn
            180                 185                 190
Leu Val Pro Glu Cys Asp Val Thr Ala Met His Glu Asn Asp Lys Met
        195                 200                 205
Ser Trp Leu Phe Asn Asn Gln Asn Val Leu Leu Pro Ser Val Tyr Val
    210                 215                 220
Arg Gln Glu Leu Thr Pro Asp Gln Arg Ile Gly Leu Val Gln Gly Arg
225                 230                 235                 240
Val Lys Glu Ala Val Arg Ile Ser Asn Asn Leu Lys His Ser Pro Lys
                245                 250                 255
Val Leu Ser Tyr Trp Trp Tyr Val Tyr Gln Asp Glu Thr Asn Thr Phe
            260                 265                 270
Leu Thr Glu Thr Asp Val Lys Lys Thr Phe Gln Glu Ile Val Ile Asn
        275                 280                 285
Gly Gly Asp Gly Ile Ile Ile Trp Gly Ser Ser Ser Asp Val Asn Ser
    290                 295                 300
Leu Ser Lys Cys Lys Arg Leu Gln Asp Tyr Leu Leu Thr Val Leu Gly
305                 310                 315                 320
Pro Ile Ala Ile Asn Val Thr Glu Ala Val Asn
                325                 330
```

Annex to the application documents - subsequently filed sequences listing

SEQUENCE LISTING

[0152]

<110> King, Te Piao

<120> CLONING AND RECOMBINANT PRODUCTION OF VESPID VENOM ENZYMES, SUCH AS PHOSPHOLIPASE AND HYALURONIDASE, AND IMMUNOLOGICAL THERAPIES BASED THEREON

<130> 2313/2F138-EP0

<140> EP 99950198.4
<141> 1999-10-01

<160> 12

<170> PatentIn version 3.0

<210> 1
<211> 1048
<212> DNA
<213> Polistes annularis

<400> 1

```
atttgcttct tgttagatga ttcgacgaca tttagaaatg gtaccttgaa tagaggcatg     60
tctccggatt gtacttttaa tgagaaagat atagtattct atgtttactc aagggataag    120
cgagatggta ttattcttaa gaaagaaact ttaacgaatt acgatctgtt tacaaagtct    180
acaatatcaa aacaagttgt atttcttata catggtttcc tttcaactgg gaataatgaa    240
aacttcgttg ctatgtcgaa agctttaata gaaaaagatg atttcttgt aatttcggtc    300
gactggaaga agggtgcttg taatgctttt gcttcaacaa aggatgcttt gggttattcc    360
aaagccgttg gaaacacacg tcacgttgga aaatttgtag ctgattttac aaaactactt    420
gtagaaaaat ataaagtgct gatatcaaat atacgattga tcgggcatag tttgggcgcg    480
catacttcag gttttgcggg aaaagaagtt caaaagttaa aattaggaaa atacaaggaa    540
attatcgggc ttgatcctgc tggaccgtat tttcatcgga gtgactgtcc ggacagactt    600
tgcgtaacag acgcagaata tgttcaagtt atacatacat caatcatatt aggagtatat    660
tataatgttg gtagcgttga tttctacgtg aattatggaa aaaatcaacc tggttgcaat    720
gaaccatcct gctctcatac gaaagccgtg aaatatctga ctgagtgcat aaaacatgaa    780
tgttgtttaa ttggaacacc atggaagaaa tatttcagca ctccaaaacc aatttcccag    840
tgcagaggag acacctgtgt ttgcgttgga ttgaatgcaa aaagttatcc tgctagaggc    900
gcattttatg caccggttga agcaaatgca ccttattgcc ataacgaggg gattaaactt    960
taattataaa caaaagtcaa tgtacacaaa aatgtatcta ttgatgaata ttaaatgaat   1020
aaacgaacag tcaaataaaa aaaaaaaa                                      1048
```

<210> 2
<211> 320
<212> PRT
<213> Polistes annularis

<400> 2

```
Ile Cys Phe Leu Leu Asp Asp Ser Thr Thr Phe Arg Asn Gly Thr Leu
1               5                   10                  15

Asn Arg Gly Met Ser Pro Asp Cys Thr Phe Asn Glu Lys Asp Ile Val
            20                  25                  30

Phe Tyr Val Tyr Ser Arg Asp Lys Arg Asp Gly Ile Ile Leu Lys Lys
        35                  40                  45

Glu Thr Leu Thr Asn Tyr Asp Leu Phe Thr Lys Ser Thr Ile Ser Lys
    50                  55                  60

Gln Val Val Phe Leu Ile His Gly Phe Leu Ser Thr Gly Asn Asn Glu
65                  70                  75                  80

Asn Phe Val Ala Met Ser Lys Ala Leu Ile Glu Lys Asp Asp Phe Leu
                85                  90                  95

Val Ile Ser Val Asp Trp Lys Lys Gly Ala Cys Asn Ala Phe Ala Ser
            100                 105                 110

Thr Lys Asp Ala Leu Gly Tyr Ser Lys Ala Val Gly Asn Thr Arg His
        115                 120                 125

Val Gly Lys Phe Val Ala Asp Phe Thr Lys Leu Leu Val Glu Lys Tyr
    130                 135                 140

Lys Val Leu Ile Ser Asn Ile Arg Leu Ile Gly His Ser Leu Gly Ala
145                 150                 155                 160

His Thr Ser Gly Phe Ala Gly Lys Glu Val Gln Lys Leu Lys Leu Gly
                165                 170                 175

Lys Tyr Lys Glu Ile Ile Gly Leu Asp Pro Ala Gly Pro Tyr Phe His
            180                 185                 190

Arg Ser Asp Cys Pro Asp Arg Leu Cys Val Thr Asp Ala Glu Tyr Val
        195                 200                 205

Gln Val Ile His Thr Ser Ile Ile Leu Gly Val Tyr Tyr Asn Val Gly
    210                 215                 220

Ser Val Asp Phe Tyr Val Asn Tyr Gly Lys Asn Gln Pro Gly Cys Asn
225                 230                 235                 240

Glu Pro Ser Cys Ser His Thr Lys Ala Val Lys Tyr Leu Thr Glu Cys
                245                 250                 255

Ile Lys His Glu Cys Cys Leu Ile Gly Thr Pro Trp Lys Lys Tyr Phe
```

260 265 270

Ser Thr Pro Lys Pro Ile Ser Gln Cys Arg Gly Asp Thr Cys Val Cys
275 280 285

Val Gly Leu Asn Ala Lys Ser Tyr Pro Ala Arg Gly Ala Phe Tyr Ala
290 295 300

Pro Val Glu Ala Asn Ala Pro Tyr Cys His Asn Glu Gly Ile Lys Leu
305 310 315 320

<210> 3
<211> 1273
<212> DNA
<213> Polistes annularis

<400> 3

```
tatgtgtcat tgtcccccga ctcagtattt aatatcatca ccgatgacat ctcccaccaa    60
attctttcca gatcgaattg tgaaagatcc aaaagaccga aaagggtctt cagcatttat   120
tggaacgttg ctacctttat gtgccaccaa tatggcatga atttcgacga ggtgacagat   180
tttaatatca aacataattc taaggacaat tttcgcggtg aaactatatc aatttattac   240
gatcctggaa aatttccagc attgatgcca ctaaaaaatg gtaattatga ggaaagaaac   300
ggaggggttc ctcagcgagg taacatcacg atacatttgc aacaatttaa cgaagatttg   360
gataaaatga caccggataa aaatttcggt ggtatcggtg taatcgattt cgaaagatgg   420
aaaccgattt tccgacagaa ttggggtaac acggaaatac ataagaaata ttctattgaa   480
ctcgttcgga aagaacatcc aaagtggagc gaatcgatga tcgaagcgga agctacgaaa   540
aagttcgaga aatatgcgag atatttcatg gaagaaactt tgaaattggc aaaaaagact   600
aggaaaaggg ctaagtgggg ttattacgga tttccttact gctataacgt aacaccgaat   660
aatcctggcc cggattgcga tgctaaagcg acaatcgaga acgatagact gtcgtggatg   720
tacaataatc aagaaatact tttccatcc gtctacgtga gacatgaaca aaaaccggag   780
gaaagggttt acctagtgca aggtagaatt aaagaagctg ttaggatatc gaataattta   840
gaacattcac ctagtgtgct tgcttattgg tggtacgtgt atcaggacaa gatggacatt   900
tacctaagcg agaccgacgt ggaaaagact ttccaagaga tagtgactaa tggtggggat   960
ggtatcataa tatggggtag ctcgtccgat gttaacagcc taagtaaatg taagagattg  1020
agagagtacc tgttaaacac tttaggaccg ttcgcggtta atgtaacaga aactgtcaac  1080
ggaagatcat ccctaaactt ctaaaataat cgataacgcc taatcacgtc gatgatgatt  1140
attagggtgt tcttcggtga ttggtttgat ctcactgaaa agacttttcg ttaaaaaaca  1200
aaaagataaa tgtaatttat aagttaaaaa aacctatacg accaaagaaa gaaagaaaaa  1260
```

aaaaaaaaaa aaa 1273

<210> 4
<211> 367
<212> PRT
<213> Polistes annularis

<400> 4

```
Tyr Val Ser Leu Ser Pro Asp Ser Val Phe Asn Ile Ile Thr Asp Asp
1               5                   10                  15

Ile Ser His Gln Ile Leu Ser Arg Ser Asn Cys Glu Arg Ser Lys Arg
            20                  25                  30

Pro Lys Arg Val Phe Ser Ile Tyr Trp Asn Val Pro Thr Phe Met Cys
        35                  40                  45

His Gln Tyr Gly Met Asn Phe Asp Glu Val Thr Asp Phe Asn Ile Lys
    50                  55                  60

His Asn Ser Lys Asp Asn Phe Arg Gly Glu Thr Ile Ser Ile Tyr Tyr
65                  70                  75                  80

Asp Pro Gly Lys Phe Pro Ala Leu Met Pro Leu Lys Asn Gly Asn Tyr
                85                  90                  95

Glu Glu Arg Asn Gly Gly Val Pro Gln Arg Gly Asn Ile Thr Ile His
            100                 105                 110

Leu Gln Gln Phe Asn Glu Asp Leu Asp Lys Met Thr Pro Asp Lys Asn
        115                 120                 125

Phe Gly Gly Ile Gly Val Ile Asp Phe Glu Arg Trp Lys Pro Ile Phe
    130                 135                 140

Arg Gln Asn Trp Gly Asn Thr Glu Ile His Lys Lys Tyr Ser Ile Glu
145                 150                 155                 160

Leu Val Arg Lys Glu His Pro Lys Trp Ser Glu Ser Met Ile Glu Ala
                165                 170                 175

Glu Ala Thr Lys Lys Phe Glu Lys Tyr Ala Arg Tyr Phe Met Glu Glu
            180                 185                 190

Thr Leu Lys Leu Ala Lys Lys Thr Arg Lys Arg Ala Lys Trp Gly Tyr
        195                 200                 205

Tyr Gly Phe Pro Tyr Cys Tyr Asn Val Thr Pro Asn Asn Pro Gly Pro
    210                 215                 220

Asp Cys Asp Ala Lys Ala Thr Ile Glu Asn Asp Arg Leu Ser Trp Met
225                 230                 235                 240

Tyr Asn Asn Gln Glu Ile Leu Phe Pro Ser Val Tyr Val Arg His Glu
                245                 250                 255
```

```
Gln Lys Pro Glu Glu Arg Val Tyr Leu Val Gln Gly Arg Ile Lys Glu
            260                 265                 270

Ala Val Arg Ile Ser Asn Asn Leu Glu His Ser Pro Ser Val Leu Ala
        275                 280                 285

Tyr Trp Trp Tyr Val Tyr Gln Asp Lys Met Asp Ile Tyr Leu Ser Glu
    290                 295                 300

Thr Asp Val Glu Lys Thr Phe Gln Glu Ile Val Thr Asn Gly Gly Asp
305                 310                 315                 320

Gly Ile Ile Ile Trp Gly Ser Ser Ser Asp Val Asn Ser Leu Ser Lys
                325                 330                 335

Cys Lys Arg Leu Arg Glu Tyr Leu Leu Asn Thr Leu Gly Pro Phe Ala
            340                 345                 350

Val Asn Val Thr Glu Thr Val Asn Gly Arg Ser Ser Leu Asn Phe
        355                 360                 365
```

<210> 5
<211> 114
<212> DNA
<213> Polistes annularis

<400> 5

```
aggtaataat ctcgattcta tgcgtacgcg attttgttga ttatttttca agaaaatgta      60

agaaaaattt ttaaaaatat attactgaag tatgaaataa aaactttata cttt           114
```

<210> 6
<211> 127
<212> DNA
<213> Polistes annularis

<400> 6

```
ggtaatattt ttatattaaa atgaacaatt ctatggaata gaaatagtac aagcatcgat      60

tatatcctat gccttgttat atgatttcgg agttagacac tattattttt aaataatttt     120

tacatta                                                               127
```

<210> 7
<211> 317
<212> PRT
<213> Dolichovespula maculata

<400> 7

```
Arg Leu Ile Met Phe Val Gly Asp Pro Ser Ser Ser Asn Glu Leu Asp
1               5                   10                  15

Arg Phe Ser Val Cys Pro Phe Ser Asn Asp Thr Val Lys Met Ile Phe
            20                  25                  30
```

```
Leu Thr Arg Glu Asn Arg Lys His Asp Phe Tyr Thr Leu Asp Thr Met
        35                  40                  45

Asn Arg His Asn Glu Phe Lys Lys Ser Ile Ile Lys Arg Pro Val Val
    50                  55                  60

Phe Ile Thr His Gly Phe Thr Ser Ser Ala Thr Glu Lys Asn Phe Val
65                  70                  75                  80

Ala Met Ser Glu Ala Leu Met His Thr Gly Asp Phe Leu Ile Ile Met
                85                  90                  95

Val Asp Trp Arg Met Ala Ala Cys Thr Asp Glu Tyr Pro Gly Leu Lys
            100                 105                 110

Tyr Met Phe Tyr Lys Ala Ala Val Gly Asn Thr Arg Leu Val Gly Asn
        115                 120                 125

Phe Ile Ala Met Ile Ala Lys Lys Leu Val Glu Gln Tyr Lys Val Pro
    130                 135                 140

Met Thr Asn Ile Arg Leu Val Gly His Ser Leu Gly Ala His Ile Ser
145                 150                 155                 160

Gly Phe Ala Gly Lys Arg Val Gln Glu Leu Lys Leu Gly Lys Phe Ser
                165                 170                 175

Glu Ile Ile Gly Leu Asp Pro Ala Gly Pro Ser Phe Lys Lys Asn Asp
            180                 185                 190

Cys Ser Glu Arg Ile Cys Glu Thr Asp Ala His Tyr Val Gln Ile Leu
        195                 200                 205

His Thr Ser Ser Asn Leu Gly Thr Glu Arg Thr Leu Gly Thr Val Asp
    210                 215                 220

Phe Tyr Ile Asn Asn Gly Ser Asn Gln Pro Gly Cys Arg Tyr Ile Ile
225                 230                 235                 240

Gly Glu Thr Cys Ser His Thr Arg Ala Val Lys Tyr Phe Thr Glu Cys
                245                 250                 255

Ile Arg Arg Glu Cys Cys Leu Ile Gly Val Pro Gln Ser Lys Asn Pro
            260                 265                 270

Gln Pro Val Ser Lys Cys Thr Arg Asn Glu Cys Val Cys Val Gly Leu
        275                 280                 285

Asn Ala Lys Lys Tyr Pro Lys Arg Gly Ser Phe Tyr Val Pro Val Glu
    290                 295                 300

Ala Glu Ala Pro Tyr Cys Asn Asn Asn Gly Lys Ile Ile
305                 310                 315
```

<210> 8
<211> 300
<212> PRT

<213> Vespula vulgaris

<400> 8

```
Gly Pro Lys Cys Pro Phe Asn Ser Asp Thr Val Ser Ile Ile Ile Glu
1               5                   10                  15

Thr Arg Glu Asn Arg Asn Arg Asp Leu Tyr Thr Leu Gln Thr Leu Gln
            20                  25                  30

Asn His Pro Glu Phe Lys Lys Lys Thr Ile Thr Arg Pro Val Val Phe
        35                  40                  45

Ile Thr His Gly Phe Thr Ser Ser Ala Ser Glu Thr Asn Phe Ile Asn
    50                  55                  60

Leu Ala Lys Ala Leu Val Asp Lys Asp Asn Tyr Met Val Ile Ser Ile
65                  70                  75                  80

Asp Trp Gln Thr Ala Ala Cys Thr Asn Glu Ala Ala Gly Leu Lys Tyr
                85                  90                  95

Leu Tyr Tyr Pro Thr Ala Ala Arg Asn Thr Arg Leu Val Gly Gln Tyr
            100                 105                 110

Ile Ala Thr Ile Thr Gln Lys Leu Val Lys His Tyr Lys Ile Ser Met
        115                 120                 125

Ala Asn Ile Arg Leu Ile Gly His Ser Leu Gly Ala His Ala Ser Gly
    130                 135                 140

Phe Ala Gly Lys Lys Val Gln Glu Leu Lys Leu Gly Lys Tyr Ser Glu
145                 150                 155                 160

Ile Ile Gly Leu Asp Pro Ala Arg Pro Ser Phe Asp Ser Asn His Cys
                165                 170                 175

Ser Glu Arg Leu Cys Glu Thr Asp Ala Glu Tyr Val Gln Ile Ile His
            180                 185                 190

Thr Ser Asn Tyr Leu Gly Thr Glu Lys Thr Leu Gly Thr Val Asp Phe
        195                 200                 205

Tyr Met Asn Asn Gly Lys Asn Gln Pro Gly Cys Gly Arg Phe Phe Ser
    210                 215                 220

Glu Val Cys Ser His Ser Arg Ala Val Ile Tyr Met Ala Glu Cys Ile
225                 230                 235                 240

Lys His Glu Cys Cys Leu Ile Gly Ile Pro Lys Ser Lys Ser Ser Gln
                245                 250                 255

Pro Ile Ser Ser Cys Thr Lys Gln Glu Cys Val Cys Val Gly Leu Asn
            260                 265                 270

Ala Lys Lys Tyr Thr Ser Arg Gly Ser Phe Tyr Val Pro Val Glu Ser
        275                 280                 285
```

```
Thr Val Pro Phe Cys Asn Asn Lys Gly Lys Ile Ile
    290                     295                 300
```

<210> 9
<211> 94
<212> DNA
<213> Polistes annularis

<400> 9

```
atttttctac tacagttctt tttatctctc tatcattgat gataaatcgt ttaaatcgat    60
ctattgtaaa ttatctatcg attgtttagg caaa                                94
```

<210> 10
<211> 347
<212> PRT
<213> Apis melliferis

<400> 10

```
Asn Asn Lys Thr Val Arg Glu Phe Asn Val Tyr Trp Asn Val Pro Thr
1               5                   10                  15

Phe Met Cys His Lys Tyr Gly Leu Arg Phe Glu Glu Val Ser Glu Lys
            20                  25                  30

Tyr Gly Ile Leu Gln Asn Trp Met Asp Lys Phe Arg Gly Glu Glu Ile
        35                  40                  45

Ala Ile Leu Tyr Asp Pro Gly Met Phe Pro Ala Leu Leu Lys Asp Pro
    50                  55                  60

Asn Gly Asn Val Val Ala Arg Asn Gly Gly Val Pro Gln Leu Gly Asn
65                  70                  75                  80

Leu Thr Lys His Leu Gln Val Phe Arg Asp His Tyr Ile Asn Gln Ile
                85                  90                  95

Pro Asp Lys Ser Phe Pro Gly Val Gly Val Ile Asp Phe Glu Ser Trp
            100                 105                 110

Arg Pro Ile Phe Arg Gln Asn Trp Ala Ser Leu Gln Pro Tyr Lys Lys
        115                 120                 125

Leu Ser Val Glu Val Val Arg Arg Glu His Pro Phe Trp Asp Asp Gln
    130                 135                 140

Arg Val Glu Gln Glu Ala Lys Arg Arg Phe Glu Lys Tyr Gly Gln Leu
145                 150                 155                 160

Phe Met Glu Glu Thr Leu Lys Ala Ala Lys Arg Met Arg Pro Ala Ala
                165                 170                 175

Asn Trp Gly Tyr Tyr Ala Tyr Pro Tyr Cys Tyr Asn Leu Thr Pro Asn
            180                 185                 190

Gln Pro Ser Ala Gln Cys Glu Ala Thr Thr Met Gln Glu Asn Asp Lys
```

```
        195                     200                     205

Met Ser Trp Leu Phe Glu Ser Glu Asp Val Leu Leu Pro Ser Val Tyr
    210                     215                     220

Leu Arg Trp Asn Leu Thr Ser Gly Glu Arg Val Gly Leu Val Gly Gly
225                     230                     235                     240

Arg Val Lys Glu Ala Leu Arg Ile Ala Arg Gln Met Thr Thr Ser Arg
                245                     250                     255

Lys Lys Val Leu Pro Tyr Tyr Trp Tyr Lys Tyr Gln Asp Arg Arg Asp
            260                     265                     270

Thr Asp Leu Ser Arg Ala Asp Leu Glu Ala Thr Leu Arg Lys Ile Thr
        275                     280                     285

Asp Leu Gly Ala Asp Gly Phe Ile Ile Trp Gly Ser Ser Asp Asp Ile
    290                     295                     300

Asn Thr Lys Ala Lys Cys Leu Gln Phe Arg Glu Tyr Leu Asn Asn Glu
305                     310                     315                     320

Leu Gly Pro Ala Val Lys Arg Ile Ala Leu Asn Asn Asn Ala Asn Asp
                325                     330                     335

Arg Leu Thr Val Asp Val Ser Val Asp Gln Val
            340                     345
```

<210> 11
<211> 331
<212> PRT
<213> Dolichovespula maculata

<400> 11

```
Ser Glu Arg Pro Lys Arg Val Phe Asn Ile Tyr Trp Asn Val Pro Thr
1               5                   10                  15

Phe Met Cys His Gln Tyr Gly Leu Tyr Phe Asp Glu Val Thr Asn Phe
            20                  25                  30

Asn Ile Lys His Asn Ser Lys Asp Asp Phe Gln Gly Asp Lys Ile Ser
        35                  40                  45

Ile Phe Tyr Asp Pro Gly Glu Phe Pro Ala Leu Leu Pro Leu Lys Glu
    50                  55                  60

Gly Asn Tyr Lys Ile Arg Asn Gly Gly Val Pro Gln Glu Gly Asn Ile
65                  70                  75                  80

Thr Ile His Leu Gln Arg Phe Ile Glu Asn Leu Asp Lys Thr Tyr Pro
                85                  90                  95

Asn Arg Asn Phe Asn Gly Ile Gly Val Ile Asp Phe Glu Arg Trp Arg
            100                 105                 110

Pro Ile Phe Arg Gln Asn Trp Gly Asn Met Met Ile His Lys Lys Phe
```

```
        115                 120                 125
Ser Ile Asp Leu Val Arg Asn Glu His Pro Phe Trp Asp Lys Lys Met
    130                 135                 140
Ile Glu Leu Glu Ala Ser Lys Arg Phe Glu Lys Tyr Ala Arg Leu Phe
145                 150                 155                 160
Met Glu Glu Thr Leu Lys Leu Ala Lys Lys Thr Arg Lys Gln Ala Asp
                165                 170                 175
Trp Gly Tyr Tyr Gly Tyr Pro Tyr Cys Phe Asn Met Ser Pro Asn Asn
            180                 185                 190
Leu Val Pro Asp Cys Asp Ala Thr Ala Met Leu Glu Asn Asp Lys Met
        195                 200                 205
Ser Trp Leu Phe Asn Asn Gln Asn Val Leu Leu Pro Ser Val Tyr Ile
    210                 215                 220
Arg His Glu Leu Thr Pro Asp Gln Arg Val Gly Leu Val Gln Gly Arg
225                 230                 235                 240
Val Lys Glu Ala Val Arg Ile Ser Asn Asn Leu Lys His Ser Pro Lys
                245                 250                 255
Val Leu Ser Tyr Trp Trp Tyr Val Tyr Gln Asp Asp Thr Asn Thr Phe
            260                 265                 270
Leu Thr Glu Thr Asp Val Lys Lys Thr Phe Gln Glu Ile Ala Ile Asn
        275                 280                 285
Gly Gly Asp Gly Ile Ile Ile Trp Gly Ser Ser Ser Asp Val Asn Ser
    290                 295                 300
Leu Ser Lys Cys Lys Arg Leu Arg Glu Tyr Leu Leu Thr Val Leu Gly
305                 310                 315                 320
Pro Ile Thr Val Asn Val Thr Glu Thr Val Asn
                325                 330
```

<210> 12
<211> 331
<212> PRT
<213> Vespula vulgaris

<400> 12

```
Ser Glu Arg Pro Lys Arg Val Phe Asn Ile Tyr Trp Asn Val Pro Thr
1               5                   10                  15

Phe Met Cys His Gln Tyr Asp Leu Tyr Phe Asp Glu Val Thr Asn Phe
            20                  25                  30

Asn Ile Lys Arg Asn Ser Lys Asp Asp Phe Gln Gly Asp Lys Ile Ala
        35                  40                  45

Ile Phe Tyr Asp Pro Gly Glu Phe Pro Ala Leu Leu Ser Leu Lys Asp
```

```
 50                      55                      60

Gly Lys Tyr Lys Lys Arg Asn Gly Gly Val Pro Gln Glu Gly Asn Ile
65                  70                  75                  80

Thr Ile His Leu Gln Lys Phe Ile Glu Asn Leu Asp Lys Ile Tyr Pro
                85                  90                  95

Asn Arg Asn Phe Ser Gly Ile Gly Val Ile Asp Phe Glu Arg Trp Arg
            100                 105                 110

Pro Ile Phe Arg Gln Asn Trp Gly Asn Met Lys Ile His Lys Asn Phe
        115                 120                 125

Ser Ile Asp Leu Val Arg Asn Glu His Pro Thr Trp Asn Lys Lys Met
    130                 135                 140

Ile Glu Leu Glu Ala Ser Lys Arg Phe Glu Lys Tyr Ala Arg Phe Phe
145                 150                 155                 160

Met Glu Glu Thr Leu Lys Leu Ala Lys Lys Thr Arg Lys Gln Ala Asp
                165                 170                 175

Trp Gly Tyr Tyr Gly Tyr Pro Tyr Cys Phe Asn Met Ser Pro Asn Asn
            180                 185                 190

Leu Val Pro Glu Cys Asp Val Thr Ala Met His Glu Asn Asp Lys Met
        195                 200                 205

Ser Trp Leu Phe Asn Asn Gln Asn Val Leu Leu Pro Ser Val Tyr Val
    210                 215                 220

Arg Gln Glu Leu Thr Pro Asp Gln Arg Ile Gly Leu Val Gln Gly Arg
225                 230                 235                 240

Val Lys Glu Ala Val Arg Ile Ser Asn Asn Leu Lys His Ser Pro Lys
                245                 250                 255

Val Leu Ser Tyr Trp Trp Tyr Val Tyr Gln Asp Glu Thr Asn Thr Phe
            260                 265                 270

Leu Thr Glu Thr Asp Val Lys Lys Thr Phe Gln Glu Ile Val Ile Asn
        275                 280                 285

Gly Gly Asp Gly Ile Ile Ile Trp Gly Ser Ser Ser Asp Val Asn Ser
    290                 295                 300

Leu Ser Lys Cys Lys Arg Leu Gln Asp Tyr Leu Leu Thr Val Leu Gly
305                 310                 315                 320

Pro Ile Ala Ile Asn Val Thr Glu Ala Val Asn
                325                 330
```

Claims

1. An isolated nucleic acid molecule encoding a *Polistinae* venom hyaluronidase comprising an amino acid sequence of SEQ ID NO: 4, or an immunomodulatory fragment thereof, wherein said nucleic acid molecule is suitable for expression of a mature form of said hyaluronidase.

2. The isolated nucleic acid molecule of claim 1, comprising the DNA sequence of SEQ ID NO:3, or a fragment thereof encoding said immunomodulatory fragment.

3. An isolated nucleic acid molecule or variants thereof encoding a hyaluronidase having the activity of the hyaluronidase of SEQ ID NO: 4.

4. The isolated nucleic acid molecule of claim 1 or 3, wherein said molecule is hybridizable to the isolated nucleic acid molecule consisting of the DNA sequence of SEQ ID NO:3 under high stringency hybridisation conditions corresponding to a T_m greater than or equal to about 65° C.

5. An expression vector comprising the isolated nucleic acid molecule of any one of claims 1 to 4, operationally associated with a promoter.

6. A cell comprising the expression vector of claim 5.

7. A method for producing a recombinant *Polistinae* venom hyaluronidase or an immunomodulatory fragment thereof, which comprises:

   (a) culturing a cell of claim 6 so that the Polistinae venom hyaluronidase, or immunomodulatory fragment thereof, is produced by the cell; and
   (b) recovering the Polistinae venom hyaluronidase, or immunomodulatory fragment thereof, so produced from the culture, the cell, or both.

8. A recombinant *Polistinae* venom hyaluronidase or an immunomodulatory fragment thereof, encoded by the nucleic acid molecule of any one of claims 1 to 4.

9. A recombinant *Polistinae* venom hyaluronidase or an immunomodulatory fragment thereof, encoded by the nucleic acid molecule of claim 8 which is a fusion protein.

10. The recombinant *Polistinae* venom hyaluronidase or immunomodulatory fragment thereof, of claims 8 or 9 expressed by a bacterial or a yeast cell.

11. The fusion protein of claim 9, wherein said recombinant *Polistinae* venom hyaluronidase or immunomodulatory fragment thereof is fused via a peptide bond to at least a functionally active portion of a non-*Polistinae* venom enzyme.

12. The fusion protein of claim 11, further comprising a cleavage site for a specific protease.

13. The recombinant *Polistinae* venom hyaluronidase or immunomodulatory fragment thereof, of claim 9, which comprises a polyhistidine sequence.

14. A pharmaceutical composition for modulating an immune response towards an immunogen in a mammal comprising the recombinant *Polistinae* venom hyaluronidase or an immunomodulatory fragment thereof, of any one of claim 8 to 13, and a pharmaceutically acceptable carrier.

15. Use of the recombinant *Polistinae* venom hyaluronidase or immunomodulatory fragment thereof, of any one of claims 8 to 13, in the manufacture of a medicament for modulating an immune response towards an immunogen in a mammal.

16. The composition of claim 14 or the use of claim 15, wherein the immune response is a vespid venom allergen-specific allergic condition.

17. The composition or the use of claim 16, wherein the vespid venom allergen is a hyaluronidase.

Patentansprüche

1. Isoliertes Nukleinsäuremolekül, das eine *Polistinae*-Gift-Hyaluronidase, umfassend eine Aminosäuresequenz von SEQ ID NO:4, oder ein immunmodulatorisches Fragment davon kodiert, wobei das Nukleinsäuremolekül zur Expression einer reifen Form der Hyaluronidase geeignet ist.

2. Isoliertes Nukleinsäuremolekül nach Anspruch 1, umfassend die DNA-Sequenz von SEQ ID NO:3, oder ein Fragment davon, das das immunmodulatorische Fragment kodiert.

3. Isoliertes Nukleinsäuremolekül oder Varianten davon, die eine Hyaluronidase mit der Aktivität der Hyaluronidase von SEQ ID NO:4 kodieren.

4. Isoliertes Nukleinsäuremolekül nach Anspruch 1 oder 3, wobei das Molekül unter Hybridisierungsbedingungen hoher Stringenz, die einer T_m von größer oder gleich etwa 65 °C entsprechen, mit dem aus der DNA-Sequenz von SEQ ID NO:3 bestehenden isolierten Nukleinsäuremolekül hybridisieren kann.

5. Expressionsvektor, umfassend das isolierte Nukleinsäuremolekül nach einem der Ansprüche 1 bis 4, funktionell assoziiert mit einem Promotor.

6. Zelle, umfassend den Expressionsvektor nach Anspruch 5.

7. Verfahren zum Herstellen einer rekombinanten *Polistinae*-Gift-Hyaluronidase oder eines immunmodulatorischen Fragments davon, das umfasst:

   (a) Züchten einer Zelle nach Anspruch 6, so dass die *Polistinae*-Gift-Hyaluronidase oder das immunmodulatorische Fragment davon durch die Zelle produziert wird; und
   (b) Gewinnen der so produzierten *Polistinae*-Gift-Hyaluronidase oder des so produzierten immunmodulatorischen Fragments davon aus der Kultur, der Zelle oder beiden.

8. Rekombinante *Polistinae*-Gift-Hyaluronidase oder ein immunmodulatorisches Fragment davon, kodiert durch das Nukleinsäuremolekül nach einem der Ansprüche 1 bis 4.

9. Rekombinante *Polistinae*-Gift-Hyaluronidase oder ein immunmodulatorisches Fragment davon, kodiert durch das Nukleinsäuremolekül nach Anspruch 8, wobei es sich um ein Fusionsprotein handelt.

10. Rekombinante *Polistinae*-Gift-Hyaluronidase oder das immunmodulatorische Fragment davon nach Anspruch 8 oder 9, exprimiert durch eine Bakterien- oder Hefezelle.

11. Fusionsprotein nach Anspruch 9, wobei die rekombinante *Polistinae*-Gift-Hyaluronidase oder das immunmodulatorische Fragment davon mittels einer Peptidbindung mit mindestens einem funktionell aktiven Teilstück eines nicht aus *Polistinae*-Gift stammenden Enzyms fusioniert ist.

12. Fusionsprotein nach Anspruch 11, ferner umfassend eine Spaltungsstelle für eine spezifische Protease.

13. Rekombinante *Polistinae*-Gift-Hyaluronidase oder das immunmodulatorische Fragment davon nach Anspruch 9, die bzw. das eine Polyhistidin-Sequenz umfasst.

14. Pharmazeutische Zusammensetzung zum Modulieren einer Immunreaktion gegen ein Immunogen in einem Säuger, umfassend die rekombinante *Polistinae*-Gift-Hyaluronidase oder ein immunmodulatorisches Fragment davon nach einem der Ansprüche 8 bis 13 und einen pharmazeutisch verträglichen Träger.

15. Verwendung der rekombinanten *Polistinae*-Gift-Hyaluronidase oder des immunmodulatorischen Fragments davon nach einem der Ansprüche 8 bis 13 bei der Herstellung eines Medikaments zum Modulieren einer Immunreaktion gegen ein Immunogen in einem Säuger.

16. Zusammensetzung nach Anspruch 14 oder Verwendung nach Anspruch 15, wobei es sich bei der Immunreaktion um ein für ein Vespidengift-Attergen spezifisches allergisches Leiden handelt.

17. Zusammensetzung oder Verwendung nach Anspruch 16, wobei es sich bei dem Vespidengift-Allergen um eine Hyaluronidase handelt.

Revendications

1. Molécule d'acide nucléique isolée codant une hyaluronidase de venin de *Polistinae* comprenant une séquence en acides aminés de SEQ ID NO :4, ou un fragment immunomodulateur de celle-ci, ladite molécule d'acide nucléique étant adaptée pour l'expression d'une forme mature de ladite hyaluronidase.

2. Molécule d'acide nucléique isolée selon la revendication 1, comprenant la séquence ADN de SEQ ID NO :3 ou un fragment de celle-ci codant ledit fragment immunomodulateur.

3. Molécule d'acide nucléique isolée ou variants de celle-ci codant une hyaluronidase ayant l'activité de la hyaluronidase de SEQ ID NO :4.

4. Molécule d'acide nucléique isolée selon la revendication 1 ou 3, dans laquelle ladite molécule peut s'hybrider à la molécule d'acide nucléique isolée constituée de la séquence ADN de SEQ ID NO :3 dans des condition d'hybridation de forte stringence correspondant à une T_f supérieure ou égale à environ 65°C.

5. Vecteur d'expression comprenant la molécule d'acide nucléique isolée selon l'une quelconque des revendications 1 à 4, associée de manière opérationnelle à un promoteur.

6. Cellule comprenant le vecteur d'expression selon la revendication 5.

7. Procédé pour produire une hyaluronidase de venin de *Polistinae* recombinante ou un fragment immunomodulateur de celle-ci, qui comprend :

(a) la culture d'une cellule selon la revendication 6 de manière à ce que la hyaluronidase de venin de *Polistinae,* ou un fragment immunomodulateur de celle-ci, soit produit par la cellule ; et
(b) la récupération de la hyaluronidase de venin de *Polistinae,* ou d'un fragment immunomodulateur de celle-ci, ainsi produite à partir de la culture, la cellule ou les deux.

8. Hyaluronidase de venin de *Polistinae* recombinante, ou un fragment immunomodulateur de celle-ci, codée par la molécule d'acide nucléique selon l'une quelconque des revendications 1 à 4.

9. Hyaluronidase de venin de *Polistinae* recombinante, ou un fragment immunomodulateur de celle-ci, codée par la molécule d'acide nucléique selon la revendication 8, qui est une protéine de fusion.

10. Hyaluronidase de venin de *Polistinae* recombinante, ou un fragment immunomodulateur de celle-ci, selon la revendication 8 ou 9, exprimée dans une cellule bactérienne ou de levure.

11. Protéine de fusion selon la revendication 9, dans laquelle ladite hyaluronidase de venin de *Polistinae* recombinante, ou un fragment immunomodulateur de celle-ci, est fusionnée via une liaison peptidique à au moins une partie fonctionnellement active d'une enzyme de venin non-*Polistinae.*

12. Protéine de fusion selon la revendication 11, comprenant en outre un site de clivage pour une protéase spécifique.

13. Hyaluronidase de venin de *Polistinae* recombinante, ou fragment immunomodulateur de celle-ci, selon la revendication 9, qui comprend une séquence polyhistidine.

14. Composition pharmaceutique pour moduler une réponse immune vis-à-vis d'un immunogène chez un mammifère comprenant la hyaluronidase de venin de *Polistinae* recombinante, ou un fragment immunomodulateur de celle-ci, selon l'une quelconque des revendications 8 à 13, et un support acceptable sur le plan pharmaceutique.

15. Utilisation de la hyaluronidase de venin de *Polistinae* recombinante, ou d'un fragment immunomodulateur de celle-ci, selon l'une quelconque des revendications 8 à 13, pour la fabrication d'un médicament pour moduler une réponse immune vis-à-vis d'un immunogène chez un mammifère.

16. Composition selon la revendication 14 ou utilisation selon la revendication 15, dans laquelle la réponse immune est une condition allergique spécifique d'un allergène de venin de vespidé.

17. Composition ou utilisation selon la revendication 16, dans laquelle l'allergène de venin de vespidé est une hyaluronidase.

papla, cDNA and translated amino acid sequence:

```
 I  C  F  L  L  D  D  S  T  T  F  R  N  G  T  L  N  R  G  M
ATTTGCTTCTTGTTAGATGATTCGACGACATTTAGAAATGGTACCTTGAATAGAGGCATG    60

 S  P  D  C  T  F  N  E  K  D  I  V  F  Y  V  Y  S  R  D  K
TCTCCGGATTGTACTTTTAATGAGAAAGATATAGTATTCTATGTTTACTCAAGGGATAAG   120

 R  D  G  I  I  L  K  K  E  T  L  T  N  Y  D  L  F  T  K  S
CGAGATGGTATTATTCTTAAGAAAGAAACTTTAACGAATTACGATCTGTTTACAAAGTCT   180

 T  I  S  K  Q  V  V  F  L  I  H  G  F  L  S  T  G  N  N  E
ACAATATCAAAACAAGTTGTATTTCTTATACATGGTTTCCTTTCAACTGGGAATAATGAA   240

 N  F  V  A  M  S  K  A  L  I  E  K  D  D  F  L  V  I  S  V
AACTTCGTTGCTATGTCGAAAGCTTTAATAGAAAAAGATGATTTTCTTGTAATTTCGGTC   300

 D  W  K  K  G  A  C  N  A  F  A  S  T  K  D  A  L  G  Y  S
GACTGGAAGAAGGGTGCTTGTAATGCTTTTGCTTCAACAAAGGATGCTTTGGGTTATTCC   360

 K  A  V  G  N  T  R  H  V  G  K  F  V  A  D  F  T  K  L  L
AAAGCCGTTGGAAACACACGTCACGTTGGAAAATTTGTAGCTGATTTTACAAAACTACTT   420

 V  E  K  Y  K  V  L  I  S  N  I  R  L  I  G  H  S  L  G  A
GTAGAAAAATATAAAGTGCTGATATCAAATATACGATTGATCGGGCATAGTTTGGGCGCG   480

 H  T  S  G  F  A  G  K  E  V  Q  K  L  K  L  G  K  Y  K  E
CATACTTCAGGTTTTGCGGGAAAAGAAGTTCAAAAGTTAAAATTAGGAAAATACAAGGAA   540

 I  I  G  L  D  P  A  G  P  Y  F  H  R  S  D  C  P  D  R  L
ATTATCGGGCTTGATCCTGCTGGACCGTATTTTCATCGGAGTGACTGTCCGGACAGACTT   600

 C  V  T  D  A  E  Y  V  Q  V  I  H  T  S  I  I  L  G  V  Y
TGCGTAACAGACGCAGAATATGTTCAAGTTATACATACATCAATCATATTAGGAGTATAT   660

 Y  N  V  G  S  V  D  F  Y  V  N  Y  G  K  N  Q  P  G  C  N
TATAATGTTGGTAGCGTTGATTTCTACGTGAATTATGGAAAAAATCAACCTGGTTGCAAT   720

 E  P  S  C  S  H  T  K  A  V  K  Y  L  T  E  C  I  K  H  E
GAACCATCCTGCTCTCATACGAAAGCCGTGAAATATCTGACTGAGTGCATAAAACATGAA   780

 C  C  L  I  G  T  P  W  K  K  Y  F  S  T  P  K  P  I  S  Q
TGTTGTTTAATTGGAACACCATGGAAGAAATATTTCAGCACTCCAAAACCAATTTCCCAG   840

 C  R  G  D  T  C  V  C  V  G  L  N  A  K  S  Y  P  A  R  G
TGCAGAGGAGACACCTGTGTTTGCGTTGGATTGAATGCAAAAAGTTATCCTGCTAGAGGC   900

 A  F  Y  A  P  V  E  A  N  A  P  Y  C  H  N  E  G  I  K  L
GCATTTTATGCACCGGTTGAAGCAAATGCACCTTATTGCCATAACGAGGGGATTAAACTT   960

 *
TAATTATAAACAAAAGTCAATGTACACAAAAATGTATCTATTGATGAATATTAAATGAAT  1020

AAACGAACAGTCAAATAAAAAAAAAAAA    1048
```

Note: The amino acid sequence of ICFLL·····GTLNR represents a portion of the leader sequence, as venom protein has the sequence of GMSPD·····.

Figure 1

A

papla intron 1, (between nucleotides 111-112; see papla file):

AGGTAATAATCTCGATTCTATGCGTACGCGATTTTGTTGATTATTTTTCAAGAAAATGTA 60

AGAAAAATTTTTAAAAATATATTACTGAAGTATGAAATAAAAACTTTATACTTT 114

B

papla intron 2, (between nucleotides 720-721; see papla file):

GGTAATATTTTTATATTAAAATGAACAATTCTATGGAATAGAAATAGTACAAGCATCGAT 60

TATATCCTATGCCTTGTTATATGATTTCGGAGTTAGACACTATTATTTTTAAATAATTTT 120

TACATTA 127

Figure 2

Vespid plas:
- wfh, white face hornet (D. maculata); vv, yellow jacket (V. vulgaris); pa, wasp (P. annularis):

```
        1                                                    50
wfh     --FSVCPFSN DTVKMIFLTR ENRK.HDFYT LDTMNRHNEF KKSIIKRPVV
vv      --GPKCPFNS DTVSIIIETR ENRN.RDLYT LQTLQNHPEF KKKTITRPVV
pa      GMSPDCTFNE KDIVFYVYSR DKRDGIILKK E.TLTNYDLF TKSTISKQVV

        51                                                  100
wfh     F.ITHGFTSS ATEKNFVAMS EALMHTGDFL IIMVDWRMAA CTDEYPGLKY
vv      F.ITHGFTSS ASETNFINLA KALVDKDNYM VISIDWQTAA CTNEAAGLKY
pa      FLI.HGFLST GNNENFVAMS KALIEKDDFL VISVDWKKGA C.NAFASTKD

        101                                                 150
wfh     .MFYKAAVGN TRLVGNFIAM IAKKLVEQYK VPMTNIRLVG HSLGAHISGF
vv      .LYYPTAARN TRLVGQYIAT ITQKLVKHYK ISMANIRLIG HSLGAHASGF
pa      ALGYSKAVGN TRHVGKFVAD FTKLLVEKYK VLISNIRLIG HSLGAHTSGF

        151                                                 200
wfh     AGKRVQELKL GKFSEIIGLD PAGPSFKKND CSERICETDA HYVQILHTSS
vv      AGKKVQELKL GKYSEIIGLD PARPSFDSNH CSERLCETDA EYVQIIHTSN
pa      AGKEVQKLKL GKYKEIIGLD PAGPYFHRSD CPDRLCVTDA EYVQVIHTSI

        201                                                 250
wfh     NLGTERTLGT VDFYINNGSN QPGCRYIIGE TCSHTRAVKY FTECIRRECC
vv      YLGTEKTLGT VDFYMNNGKN QPGCGRFFSE VCSHSRAVIY MAECIKHECC
pa      ILGVYYNVGS VDFYVNYGKN QPGCNEPS.. .CSHTKAVKY LTECIKHECC

        251                                                 300
wfh     LIGVPQSK.. .SPQPVSKCT RNECVCVGLN AKKYPKRGSF YVPVEAEAPY
vv      LIGIPKSK.. .SSQPISSCT KQECVCVGLN AKKYPSRGSF YVPVESTAPF
pa      LIGTPWKKYF STPKPISQCR GDTCVCVGLN AKSYPARGAF YAPVEANAPY

        301
wfh     CNNNGKII
vv      CNNKGKII
pa      CHNEGIKL
```

Figure 3

<u>Pahya</u>, cDNA and translated amino acid sequence:

Y V S L S P D S V F N
TATGTGTCATTGTCCCCCGACTCAGTATTTAA 480

I I T D D I S H Q I L S R S N C E R S K
TATCATCACCGATGACATCTCCCACCAAATTCTTTCCAGATCGAATTGTGAAAGATCCAA 540

R P K R V F S I Y W N V P T F M C H Q Y
AAGACCGAAAAGGGTCTTCAGCATTTATTGGAACGTTCCTACCTTTATGTGCCACCAATA 600

G M N F D E V T D F N I K H N S K D N F
TGGCATGAATTTCGACGAGGTGACAGATTTTAATATCAAACATAATTCTAAGGACAATTT 660

R G E T I S I Y Y D P G K F P A L M P L
TCGCGGTGAAACTATATCAATTTATTACGATCCTGGAAAATTTCCAGCATTGATGCCACT 720

K N G N Y E E R N G G V P Q R G N I T I
AAAAAATGGTAATTATGAGGAAAGAAACGGAGGGGTTCCTCAGCGAGGTAACATCACGAT 780

H L Q Q F N E D L D K M T P D K N F G G
ACATTTGCAACAATTTAACGAAGATTTGGATAAAATGACACCGGATAAAAATTTCGGTGG 840

I G V I D F E R W K P I F R Q N W G N T
TATCGGTGTAATCGATTTCGAAAGATGGAAACCGATTTTCCGACAGAATTGGGGTAACAC 900

E I H K K Y S I E L V R K E H P K W S E
GGAAATACATAAGAAATATTCTATTGAACTCGTTCGGAAAGAACATCCAAAGTGGAGCGA 960

S M I E A E A T K K F E K Y A R Y F M E
ATCGATGATCGAAGCGGAAGCTACGAAAAAGTTCGAGAAATATGCGAGATATTTCATGGA 1020

E T L K L A K K T R K R A K W G Y Y G F
AGAAACTTTGAAATTGGCAAAAAAGACTAGGAAAAGGGCTAAGTGGGGTTATTACGGATT 1080

P Y C Y N V T P N N P G P D C D A K A T
TCCTTACTGCTATAACGTAACACCGAATAATCCTGGCCCGGATTGCGATGCTAAAGCGAC 1140

I E N D R L S W M Y N N Q E I L F P S V
AATCGAGAACGATAGACTGTCGTGGATGTACAATAATCAAGAAATACTTTTTCCATCCGT 1200

Y V R H E Q K P E E R V Y L V Q G R I K
CTACGTGAGACATGAACAAAAACCGGAGGAAAGGGTTTACCTAGTGCAAGGTAGAATTAA 1260

E A V R I S N N L E H S P S V L A Y W W
AGAAGCTGTTAGGATATCGAATAATTTAGAACATTCACCTAGTGTGCTTGCTTATTGGTG 1320

Y V Y Q D K M D I Y L S E T D V E K T F
GTACGTGTATCAGGACAAGATGGACATTTACCTAAGCGAGACCGACGTGGAAAAGACTTT 1380

Q E I V T N G G D G I I I W G S S S D V
CCAAGAGATAGTGACTAATGGTGGGGATGGTATCATAATATGGGGTAGCTCGTCCGATGT 1440

N S L S K C K R L R E Y L L N T L G P F
TAACAGCCTAAGTAAATGTAAGAGATTGAGAGAGTACCTGTTAAACACTTTAGGACCGTT 1500

A V N V T E T V N G R S S L N F *
CGCGGTTAATGTAACAGAAACTGTCAACGGAAGATCATCCCTAAACTTCTAAAATAATCG 1560

ATAACGCCTAATCACGTCGATGATGATTATTAGGGTGTTCTTCGGTGATTGGTTTGATCT 1620

CACTGAAAAGACTTTTCGTTAAAAAACAAAAAGATAAATGTAATTTATAAGTTAAAAAAA 1680

CCTATACGACCAAAGAAAGAAAGAAAAAAAAAAAAAAAAAA

Note: The amino acid sequence of YVSLSP·····RSNCER represents a portion of the leader sequence as the venom protein has the sequence of SKRPKR·····.

Figure 4

pahya, intron sequence, (between nucleotides 733 and 734)

ATTTTTCTACTACAGTTCTTTTTATCTCTCTATCATTGATGATAAATCGTTTAAATCGAT
60

CTATTGTAAATTATCTATCGATTGTTTAGGCAAA 94

Figure 5

Vespid hyas:

```
     1                                                          50
wfh  SERPKRVFNI YWNVPTFMCH QYGLYFDEVT N.FNIKHNSK DDFQGDKISI
vv   SERPKRVFNI YWNVPTFMCH QYDLYFDEVT N.FNIKRNSK DDFQGDKIAI
pa   SKRPKRVFSI YWNVPTFMCH QYGMNFDEVT D.FNIKHNSK DNFRGETISI
bv   NNKTVREFNV YWNVPTFMCH KYGLRFEEVS EKYGILQNWM DKFRGEEIAI

     51                                                        100
wfh  FYDPGEFPAL LPLKEGNYKI RNGGVPQEGN ITIHLQRFIE NLDKTYPNRN
vv   FYDPGEFPAL LSLKDGKYKK RNGGVPQEGN ITIHLQKFIE NLDKIYPNRN
pa   YYDPGKFPAL MPLKNGNYEE RNGGVPQRGN ITIHLQQFNE DLDKMTPDKN
bv   LYDPGMFPAL LKDPNGNVVA RNGGVPQLGN LTKHLQVFRD HLINQIPDKS

     101                                                       150
wfh  FNGIGVIDFE RWRPIFRQNW GNMMIHKKFS IDLVRNEHPF WDKKMIELEA
vv   FSGIGVIDFE RWRPIFRQNW GNMKIHKNFS IDLVRNEHPT WNKKMIELEA
pa   FGGIGVIDFE RWKPIFRQNW GNTEIHKKYS IELVRKEHPK WSESMIEAEA
bv   FPGVGVIDFE SWRPIFRQNW ASLQPYKKLS VEVVRREHPF WDDQRVEQEA

     151                                                       200
wfh  SKRFEKYARL FMEETLKLAK KTRKQADWGY YGYPYCFNMS PNNLVPDCDA
vv   SKRFEKYARF FMEETLKLAK KTRKQADWGY YGYPYCFNMS PNNLVPECDV
pa   TKKFEKYARY FMEETLKLAK KTRKRAKWGY YGFPYCYNVT PNNPGPDCDA
bv   KRRFEKYGQL FMEETLKAAK RMRPAANWGY YAYPYCYNLT PNQPSAQCEA

     201                                                       250
wfh  TAMLENDKMS WLFNNQNVLL PSVYIRHELT PDQRVGLVQG RVKEAVRISN
vv   TAMHENDKMS WLFNNQNVLL PSVYVRQELT PDQRIGLVQG RVKEAVRISN
pa   KATIENDRLS WMYNNQEILF PSVYVRHEQK PEERVYLVQG RIKEAVRISN
bv   TTMQENDKMS WLFESEDVLL PSVYLRWNLT SGERVGLVGG RVKEALRIAR

     251                                                       300
wfh  NLKHS.PKVL SYWWYVYQDD TNTFLTETDV KKTFQEIAIN GGDGIIIWGS
vv   NLKHS.PKVL SYWWYVYQDE TNTFLTETDV KKTFQEIVIN GGDGIIIWGS
pa   NLEHS.PSVL AYWWYVYQDK MDIYLSETDV EKTFQEIVTN GGDGIIIWGS
bv   QMTTSRKKVL PYYWYKYQDR RDTDLSRADL EATLRKITDL GADGFIIWGS

     301                                                  348
wfh  SSDVNSLSKC KRLREYLLTV LGPITVNVTE TVN------- ---------
vv   SSDVNSLSKC KRLQDYLLTV LGPIAINVTE AVN------- ---------
pa   SSDVNSLSKC KRLREYLLNT LGPFAVNVTE TVNGRSSLNF ---------
bv   SDDINTKAKC LQFREYLNNE LGPAVKRIAL NNNANDRLTV DVSVDQV*
```

Figure 6

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- US 5593877 A **[0003] [0005] [0063] [0077] [0136] [0137] [0143] [0145]**
- US 4822608 A **[0010]**
- US 5827829 A **[0010]**
- DK 9900136 W **[0051]**
- WO 5593877 A **[0084]**
- CA 2012311, Hartmut **[0087] [0094]**
- WO 9528494 A **[0088]**
- WO 9421807 A **[0090]**
- WO 9205263 A **[0090]**
- WO 9518863 A **[0092]**
- WO 9617823 A **[0092]**
- US 5459127 A **[0092]**
- WO 9521931 A **[0093] [0093]**
- WO 9625508 A **[0093]**
- US 5204253 A **[0094]**
- US 5853663 A **[0094]**
- US 5885795 A **[0094]**
- US 5702384 A **[0094]**
- US 5580859 A **[0094]**
- US 5589466 A **[0094]**
- WO 9901157 A **[0094]**
- WO 9901158 A **[0094]**
- WO 9901175 A **[0094]**
- US 08474853 B **[0143]**
- EP 99950198 A **[0152]**

Non-patent literature cited in the description

- **GOLDEN et al.** *Am. Med. Assoc.,* 1989, vol. 262, 240 **[0002]**
- **HOFFMAN ; JACOBSON.** *Ann. Allergy.,* 1984, vol. 52, 276 **[0002]**
- **AKRE et al.** Yellowjackets of America North of Mexico. *Agriculture Handbook No. 552, US Department of Agriculture,* 1980 **[0003]**
- **KING et al.** *Biochemistry,* 1978, vol. 17, 5165 **[0003]**
- **KING et al.** *Mol. Immunol.,* 1983, vol. 20, 297 **[0003]**
- **KING et al.** *Arch. Biochem. Biophys.,* 1984, vol. 230, 1 **[0003]**
- **KING et al.** *J. Allergy and Clin. Immunol.,* 1985, vol. 75, 621 **[0003]**
- **KING.** *J. Allergy Clin. Immunol.,* 1987, vol. 79, 113 **[0003]**
- **HOFFMAN.** *J. Allergy and Clin. Immunol.,* 1985, vol. 75, 611 **[0003]**
- **PEREZ et al.** *J. Biol. Chem.,* 1990, vol. 265, 16210 **[0004]**
- **ANSARI et al.** *Biochemistry,* 1989, vol. 26, 8665 **[0004]**
- **SILVANOVICH et al.** *J. Biol. Chem.,* 1991, vol. 266, 1204 **[0004]**
- **BREITENEDER.** *EMBO J.,* 1989, vol. 8, 1935 **[0004]**
- **VALENTA et al.** *Science,* 1991, vol. 253, 557 **[0004]**
- **RAFNAR et al.** *J. Biol. Chem.,* 1991, vol. 266, 1229 **[0004]**
- **GRIFFITH et al.** *Int. Arch. Allergy Appl. Immunol.,* 1991, vol. 96, 296 **[0004]**
- **CHUA et al.** *J. Exp. Med.,* 1988, vol. 167, 175 **[0004]**
- **GRIFFITH et al.** *Gene,* 1992, vol. 113, 263 **[0004]**
- **ARUDA et al.** *J. Exp. Med.,* 1990, vol. 172, 1529 **[0004]**
- **HAN et al.** *J. Allergy Clin. Immunol.,* 1991, vol. 87, 327 **[0004]**
- **SAMBROOK ; FRITSCH ; MANIATIS.** Molecular Cloning: a Laboratory Manual. Cold Spring Harbor Laboratory Press, 1989 **[0061]**
- DNA Cloning: a Practical Approach. 1985, vol. I and II **[0061]**
- Oligonucleotide Synthesis. 1984 **[0061]**
- Nucleic Acid Hybridization. 1985 **[0061]**
- Transcription And Translation. 1984 **[0061]**
- Animal Cell Culture. 1986 **[0061]**
- Immobilized Cells And Enzymes. IRL Press, 1986 **[0061]**
- **B. PERBAL.** *a Practical Guide To Molecular Cloning,* 1984 **[0061]**
- **FROHMAN et al.** *Proc. Nat. Acad. Sci. USA,* 1998, vol. 85, 8998-9002 **[0066]**
- **FROHMAN.** *Amplifications: A Forum for PCR Users,* 1990, vol. 5, 11 **[0066]**
- **BENOIST ; CHAMBON.** *Nature,* 1981, vol. 290, 304-310 **[0084]**
- **YAMAMOTO et al.** *Cell,* 1980, vol. 22, 787-797 **[0084]**
- **WAGNER et al.** *Proc. Natl. Acad. Sci. U.S.A.,* 1981, vol. 78, 1441-1445 **[0084]**
- **BRINSTER et al.** *Nature,* 1982, vol. 296, 39-42 **[0084]**
- **VILLA-KAMAROFF et al.** *Proc. Natl. Acad. Sci. U.S.A.,* 1978, vol. 75, 3727-3731 **[0084]**

- **DEBOER et al.** *Proc. Natl. Acad. Sci. U.S.A.,* 1983, vol. 80, 21-25 **[0084]**
- Useful proteins from recombinant bacteria. *Scientific American,* 1980, vol. 242, 74-94 **[0084]**
- **WU et al.** *J. Biol. Chem.,* 1992, vol. 267, 963-967 **[0087] [0094]**
- **WU ; WU.** *J. Biol. Chem.,* 1988, vol. 263, 14621-14624 **[0087] [0094]**
- **MILLER ; ROSMAN.** *BioTechniques,* 1992, vol. 7, 980-990 **[0089]**
- **KAPLITT et al.** *Molec. Cell. Neurosci.,* 1991, vol. 2, 320-330 **[0090]**
- **STRATFORD-PERRICAUDET et al.** *J. Clin. Invest.,* 1992, vol. 90, 626-630 **[0090]**
- **LA SALLE et al.** *Science,* 1993, vol. 259, 988-990 **[0090]**
- **SAMULSKI et al.** *J. Virol.,* 1987, vol. 61, 3096-3101 **[0090]**
- **SAMULSKI et al.** *J. Virol.,* 1989, vol. 63, 3822-3828 **[0090]**
- **LEBKOWSKI et al.** *Mol. Cell. Biol.,* 1988, vol. 8, 3988-3996 **[0090]**
- **FELGNER.** *Proc. Natl. Acad. Sci. U.S.A.,* 1987, vol. 84, 7413-7417 **[0092]**
- **FELGNER ; RINGOLD.** *Science,* 1989, vol. 337, 387-388 **[0092]**
- **MACKEY et al.** *Proc. Natl. Acad. Sci. U.S.A.,* 1988, vol. 85, 8027-8031 **[0092]**
- **ULMER et al.** *Science,* 1993, vol. 259, 1745-1748 **[0092]**
- **SANFORD.** *TIB-TECH,* 1988, vol. 6, 299-302 **[0094]**
- **FYNAN et al.** *Proc. Natl. Acad. Sci. U.S.A.,* 1993, vol. 90, 11478-11482 **[0094]**
- **YANG et al.** *Proc. Natl. Acad. Sci. U.S.A.,* 1990, vol. 87, 1568-9572 **[0094]**
- **WILLIAMS et al.** *Proc. Natl. Acad. Sci. USA,* 1991, vol. 88, 2726-2730 **[0094]**
- **CURIEL et al.** *Hum. Gene Ther.,* 1992, vol. 3, 147-154 **[0094]**
- **WU ; WU.** *J. Biol. Chem.,* 1987, vol. 262, 4429-4432 **[0094]**
- **MIR et al.** *C.P. Acad. Sci.,* 1998, vol. 321, 893 **[0094]**
- **MANIATIS, T.** Molecular Cloning, A Laboratory Manual. Cold Spring Harbor Laboratory, 1990 **[0106]**
- **HUTCHINSON, C. et al.** *J. Biol. Chem.,* 1978, vol. 253, 6551 **[0107]**
- **ZOLLER ; SMITH.** *DNA,* 1984, vol. 3, 479-488 **[0107]**
- **OLIPHANT et al.** *Gene,* 1986, vol. 44, 177 **[0107]**
- **HUTCHINSON et al.** *Proc. Natl. Acad. Sci. U.S.A.,* 1986, vol. 83, 710 **[0107]**
- Using PCR to Engineer DNA. **HIGUCHI.** PCR Technology: Principles and Applications for DNA Amplification,. Stockton Press, 61-70 **[0107]**
- **MOSSMAN.** *J. Immunol. Methods,* 1983, vol. 65, 55-63 **[0113]**
- **NIKS ; OTTO.** *J. Immunol. Methods,* 1990, vol. 130, 140-151 **[0113]**
- **FEHLNER et al.** *J. Immunol.,* 1991, vol. 146, 799 **[0114]**
- **CZERKINSKY et al.** *J. Immunol. Methods,* 1988, vol. 110, 29 **[0114]**
- **BRENNER et al.** *Biotechniques,* 1989, vol. 7, 1096 **[0114]**
- **KASAIAN ; BIRON.** *J. Immunol.,* 1989, vol. 142, 1287 **[0114]**
- **PURKESON ; ISAKSON.** *J. Exp. Med.,* 1992, vol. 175, 973-982 **[0114]**
- **BRINE et al.** *Proc. Natl. Acad. Sci. U.S.A.,* 1993, vol. 90, 7608-12 **[0125]**
- **HOYNE et al.** *J. Exp. Med.,* 1993, vol. 178, 1783-88 **[0126]**