PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C07K 19/00, 16/46, A16K 39/395

A1

(11) International Publication Number: **WO 95/26365**

(43) International Publication Date: 5 October 1995 (05.10.95)

(21) International Application Number: PCT/US95/03741

(22) International Filing Date: 24 March 1995 (24.03.95)

(30) Priority Data:

08/218,461	28 March 1994 (28.03.94)	US
08/328,912	25 October 1994 (25.10.94)	US

(60) Parent Applications or Grants
(63) Related by Continuation

US	08/218,461 (CIP)
Filed on	28 March 1994 (28.03.94)
US	08/328,912 (CIP)
Filed on	25 October 1994 (25.10.94)

(71) Applicant *(for all designated States except US)*: UNITED BIOMEDICAL, INC. [US/US]; 25 Davids Drive, Hauppauge, NY 11788 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: WANG, Chang, Yi [US/US]; 47 Snake Hill Road, Cold Spring Harbor, NY 11724 (US).

(74) Agent: LIN, Maria, C., H.; Morgan & Finnegan, 345 Park Avenue, New York, NY 10154 (US).

(81) Designated States: AU, CA, CN, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: SYNTHETIC PEPTIDE BASED IMMUNOGENS FOR THE TREATMENT OF ALLERGY

(57) Abstract

The present invention relates to a method for eliciting the production in healthy mammals, including humans, of high titer antibodies to an effector site in human IgE heavy chain, i.e. a site in the CH4 domain of the ∈-chain, by the use of compositions of synthetic peptide immunogens in either a radially branching multimeric form (such as branching octameric or hexadecameric peptides) or a linearly arranged monomeric form, to inhibit mast cell activation and reduce allergen-induced IgE production. It also relates to the use of such "optimally" designer, carrier protein free, IgE ∈-chain related immunogens as key components in a synthetic vaccine to provide an immunotherapy for the treatment of allergy. The subject peptides contain immune stimulator sequences, including a built-in helper T cell epitope tandemly linked in a specific orientation, to aid in stimulating the immune response towards the IgE CH4 domain.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**GB**	United Kingdom	**MR**	Mauritania
AU	Australia	**GE**	Georgia	**MW**	Malawi
BB	Barbados	**GN**	Guinea	**NE**	Niger
BE	Belgium	**GR**	Greece	**NL**	Netherlands
BF	Burkina Faso	**HU**	Hungary	**NO**	Norway
BG	Bulgaria	**IE**	Ireland	**NZ**	New Zealand
BJ	Benin	**IT**	Italy	**PL**	Poland
BR	Brazil	**JP**	Japan	**PT**	Portugal
BY	Belarus	**KE**	Kenya	**RO**	Romania
CA	Canada	**KG**	Kyrgystan	**RU**	Russian Federation
CF	Central African Republic	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CG	Congo			**SE**	Sweden
CH	Switzerland	**KR**	Republic of Korea	**SI**	Slovenia
CI	Côte d'Ivoire	**KZ**	Kazakhstan	**SK**	Slovakia
CM	Cameroon	**LI**	Liechtenstein	**SN**	Senegal
CN	China	**LK**	Sri Lanka	**TD**	Chad
CS	Czechoslovakia	**LU**	Luxembourg	**TG**	Togo
CZ	Czech Republic	**LV**	Latvia	**TJ**	Tajikistan
DE	Germany	**MC**	Monaco	**TT**	Trinidad and Tobago
DK	Denmark	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**US**	United States of America
FI	Finland	**ML**	Mali	**UZ**	Uzbekistan
FR	France	**MN**	Mongolia	**VN**	Viet Nam
GA	Gabon				

SYNTHETIC PEPTIDE BASED IMMUNOGENS FOR THE TREATMENT OF ALLERGY

CROSS REFERENCE TO RELATED APPLICATION

This is a continuation-in-part application of pending Application Serial No. 08/218,461 filed March 28, 1994 which is a continuation of pending application Serial No. 08/060,798 filed May 10, 1993 which is a continuation-in-part of pending application Serial No. 07/847,745, filed March 6, 1992, now abandoned, which was a continuation-in-part of application Serial No. 07/637,364, filed January 4, 1991, now abandoned.

FIELD OF THE INVENTION

The present invention relates to the use of a composition of a synthetic peptide, in a linear or radially branching multimeric form, as an immunogen for eliciting the production in healthy mammals, including humans, of high titer antibodies to the effector site on the CH4 domain of the ϵ-chain of the human IgE heavy chain, and to the use of the composition as a vaccine to provide an immunotherapy for the treatment of allergy.

BACKGROUND OF THE INVENTION

Immunotherapy for the prevention of IgE-mediated allergic responses, such as asthma and hay fever, as known and practiced since early in this century, has been by desensitization or hyposensitization, wherein a gradually increasing amount of an allergen is given to a patient to reduce the effects of subsequent exposure to that allergen[1]. Limitations to such an allergen-based immunotherapy include difficulties in identifying the allergen involved and, if an allergen is identified, the adverse reactions frequently caused by the use of the identified allergen[2].

Other treatments for the relief of allergies employ drugs to block the cascade of cellular events that

is responsible for allergic reactions. These drugs include anti-histamines, decongestants, β_2 agonists, and corticosteroids. Anti-histamines, decongestants, and β_2 agonists act on events downstream of IgE in the allergic cascade, making them palliative remedies which address only the allergy symptoms. Preventative treatments must act on cellular events closer to the initiation of IgE-mediated allergic reactions. These palliatives provide relief that is short term and partial. Moreover, the relief of symptoms is frequently accompanied by adverse side effects, e.g. anti-histamines may cause restlessness or drowsiness, and β_2 agonists have sometimes been associated with increased morbidity in asthmatic patients.

Corticosteroids are powerful immunosuppressants and are highly efficacious for the treatment of allergic symptoms. However, they stimulate adverse hormonal activities and may cause an undesirably broad immunosuppression.

To avoid the shortcomings of the known therapeutic drugs, it would be more desirable to prevent allergic responses by selective suppression targeted to IgE. This may be accomplished either by suppressing IgE synthesis, such as is achieved by the inconvenient desensitization method; or by blocking the process by which IgE-allergen complexes stimulate the degranulation of mast cells and basophils with the concomitant release of the chemical mediators of hypersensitivity.

At a more fundamental level, Stanworth et al.[3-7] and others[8-13] have used synthetic IgE ∈-chain peptides and the corresponding antibodies to study the role of cytophilic peptides in cell signaling processes, in an attempt to elucidate the molecular basis for the immunological triggering of mast cells and basophils.

Among the many IgE peptides studied over the past two decades (Table 1), a potential effector site within the Fc CH4 domain of the human ∈-chain (Lys_{497}-

Phe_{506}, shown in Table 2 by double underlining) was the decapeptide. It was synthesized and used for structure/activity studies[3]. This IgE CH4 domain-derived decapeptide was found to be capable of activating dose-dependent histamine release from isolated rat peritoneal mast cells in a non-cytolytic manner resembling the IgE-mediated mast cell triggering process[4]. Precise structural requirements for this peptide effector site were deduced through structure-activity studies using multiple synthetic analogues of the ϵ-chain decapeptide[3,4,5].

Anti-IgE CH4 peptide antibodies derived from immunizations with ϵ chain-related "peptide-carrier protein conjugates" were also used for structure action studies on the degranulation of IgE-sensitized cells, by observing inhibitory activities[5,11,12].

The feasibility of using a peptide based vaccine to provide immunotherapy to patients with IgE-mediated sensitivities has been suggested by Stanworth et al.[14,15]. He used the previously identified ϵ-chain decapeptide with a sequence of Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe-NH_2[3] (SEQ ID NO:1) conjugated to a "carrier protein", such as keyhole limpet hemocyanin (KLH) or the purified protein derivative (PPD) of tuberculin, and found that the "peptide-carrier protein" conjugates elicited decapeptide-specific antibodies. For example, a rabbit anti-peptide serum, selected on the basis of its better-than-average anti-peptide titer, reduced the decapeptide-induced histamine release from rat peritoneal mast cells in a titer-dependent fashion. This inhibitory activity was further confirmed by *in vivo* tests in a rat passive cutaneous anaphylaxis (PCA) model system. The effect of this rabbit anti-peptide serum on anaphylaxis was assessed, by measurement of the area of blueing and by an estimate of color intensity when given to rats which had been previously sensitized by multiple allergen

application prior to anaphylactic challenge with the allergen.

In the same study, results obtained in rats using immunogens containing such "decapeptide-protein carrier conjugates" gave preliminary indications of feasibility for their use as a vaccine for the treatment of allergy.

However, this strategy has met with considerable difficulties. The major deficiencies of this prototype "decapeptide-protein carrier conjugate" vaccine include a less-than-optimal immune stimulatory capability and manufacturing difficulties stemming from the poorly defined composition of the carrier protein and the non-uniformity of the conjugation reaction. It has also been found that the resultant antisera raised by such peptide-protein conjugates frequently contain more antibodies directed at the epitopes on the protein carrier, e.g. Keyhole Limpet Hemocyanin (KLH), than to the target-peptide[5].

TABLE 1

IgE HEAVY CHAIN PEPTIDES USED IN STRUCTURE-ACTIVITY STUDIES

Amino Acid Res. Nos. and Peptide Sequence	Domain	Structure-Activity Studies	References
Hu ∈ 497-506 RTKGSGFFVF	CH4	Involved in non-antigen receptors in mast cell signalling processes	Stanworth, Mol. Immunol., 21:1183-1190, 1984(4) Stanworth and Burt, Mol. Immunol., 23:1231-1235, 1986(5)
Hu ∈ 301-376	CH2/CH3	Blocking of passive sensitization of human mast cells and basophils	Helm et al., PNAS, 86:9465-9469, 1989(13)
Hu ∈ 363-376	CH3	Not essential for binding of the peptide to Fc ϵ-chain receptor I.	ibid.
Hu ∈ 367-376	CH3	Binding to the low affinity IgE receptor (CD23)	Chretien et al., J. Immunol., 141:3128-3134, 1988(11)
E. coli-derived human Fc∈ fragment	CH2/CH3/CH4	Expression in E. coli and comparison to cell- binding activity of native human IgE myeloma. Recombinant Fc had 20% of native binding activity.	Kenten et al., PNAS 81:2955-2959, 1984(12)
E. coli-derived human Fc∈ fragment	CH2/CH3/CH4	Monomeric form was inactive. The Fc-like dimeric form displayed only ¼ of the cell-binding activity of native IgE.	Coleman et al., Eur. J. Immunol., 15:966-969, 1985(13)
Rat ∈ 414-428(p129)* 459-472(p124) 491-503(p128) 542-557(p123)	CH3/CH4	Inhibiting binding of rat IgE to mast cells by 20-50% at concentrations between 10^{-4}-10^{-5}M. *Even the most active peptide(p129) was found 1000-times less active than the active rat IgE.	Burt and Stanworth, Eur. J. Immunol., 17:437-440, 1987(9)
378-396(p130) 522-535(p122) 560-571(p131)	CH3/CH4	No inhibition	

Amino Acid Res. Nos. and Peptide Sequence	Domain	Structure-Activity Studies	References
rat ε 459-472 (YVFLPPEEEEKDKD) 542-547 (HEAKRELERTISK)	CH4	Contribute to the heat-sensitive region of the IgE molecule which is cytophilic for mast cells and basophils.	Stanworth and Burt, Mol Immunol., 23:1231-1235, 1986(5)
Hu ε pentapeptide 330-334 (DSDPR)	CH2	Nonspecific inhibition of IgE antibody mediated PK reactions in human.	Hamberger, Science, 189:389-390, 1975(8)
Mouse ε peptides	CH2/CH3/CH4	Structure-function relationships defined by sequence directed antibodies	Robertson and Liu., Mol. Immunol., 25:103-113, 1988(12)
167-180 (P1)	CH2	Anti-p1 displays low binding to IgE.	
207-218 (P2)	CH2/CH3	Hydrophobic and conformational. Anti-p2 does not bind IgE. Inaccessible.	
237-251 (P3)	CH3	Most proximal to IgE-receptor recognition site; anti-p3 showed least IgE binding activity.	
291-305 (P4)	CH3	Anti-p4 stimulated serotonin release.	
338-352 (P5)	CH4	Anti-p5 stimulated serotonin release.	
372-385 (P6)	CH4	Anti-p6 binds best to IgE (in either free or receptor bound forms), however, not effective in crosslinking of IgE-receptor complex.	

It is known to those of skill in the art, small peptides are poor immunogens. To make small peptides immunogenic, they are usually joined to large carrier proteins by chemical conjugation or by gene fusion. These processes, however, generally produce unpredictable conformational changes in a peptide. Further, the immune response is frequently misdirected to the immunodominant carrier. Consequently, the development of a potent vaccine to provide long-lasting relief from allergies awaits further immunogen design.

In Table 2, the amino acid sequences for the CH2 to CH4 domains of rat IgE ∈-chain[16] and mouse ∈-chain[17] are aligned with the amino acid sequence for human ∈-chain[18] (SEQ ID NOS:2-4) to provide a guide for IgE-related peptide fragments previously reported. It is to be noted that in human IgE ∈ heavy chain, L next to Q at position 252 is not present in the original IgE myeloma ND sequence. Gaps, indicated by dashes, have been introduced to maximize homology. Matches of homologous residue positions are boxed. The positions on the ∈ sequences which have been studied for structural activity (Table 1) are underlined in Table 2. The structurally active IgE CH4 decapeptide sequence in the human IgE CH4 domain is double underlined (SEQ ID NO:1). The amino acid code used in the Table is: A, alanine; R, arginine; N, asparagine; D, aspartic acid; C, cysteine; Q, glutamine; E, glutamic acid; G, glycine; H, histidine; I, isoleucine; L, leucine; K, lysine; M, methionine; F, phenylalanine; P, proline; S, serine; T, threonine; W, tryptophan; Y, tyrosine; V, valine.

Table 2

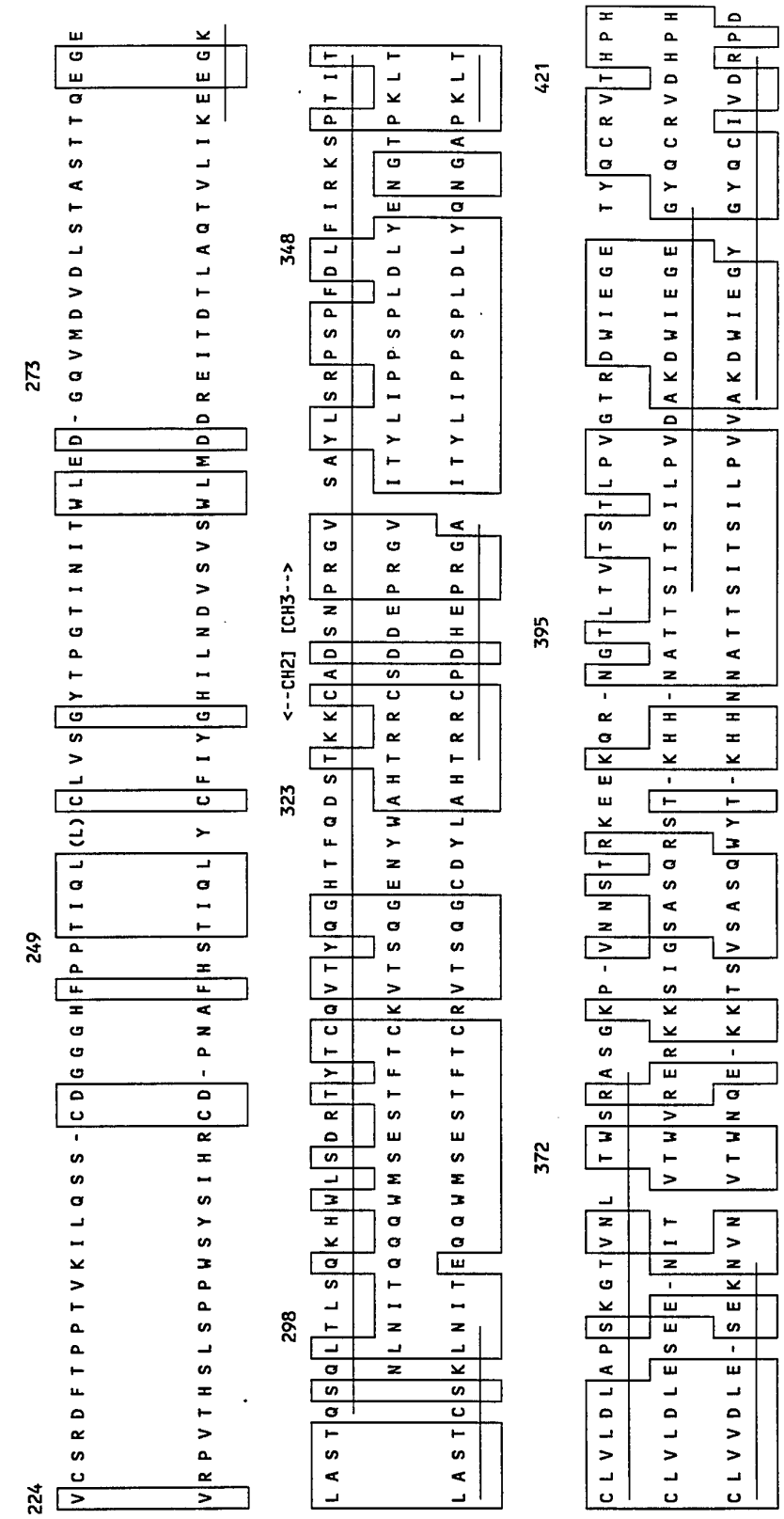
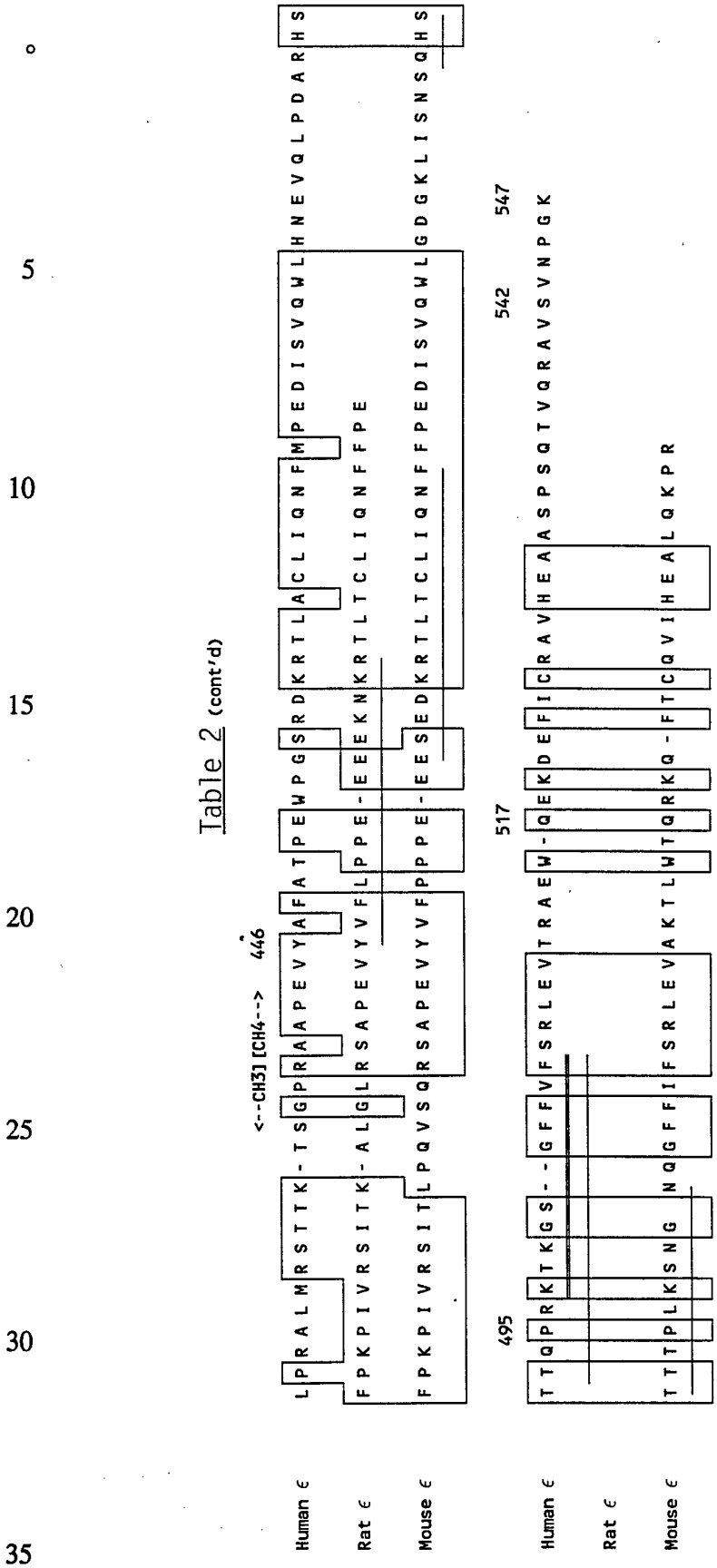

Sequence
Human ε (SEQ ID NO:2)
Rat ε (SEQ ID NO:3)
Mouse ε (SEQ ID NO:4)
224
249
273
298
323
348
372
395
421
<--CH2] [CH3-->

Table 2 (cont'd)



LIST OF REFERENCES

1. Noon L. "Prophylactic inoculation against hay fever." Lancet, i:1572-1573 (1911).
2. World Health Organization and International Union of Immunological Societies Working Group Report: Current status of allergen immunotherapy. Lancet, i:259-261 (1989).
3. Stanworth, Kings, Roy, et al. Biochem. J., 180:665-668 (1979).
4. Stanworth. Mol. Immunol., 21:1183-1190 (1984).
5. Stanworth, and Burt. Mol. Immunol., 23:1231-1235 (1986).
6. Burt, and Stanworth. Eur. J. Immunol., 17:437-440 (1987).
7. Stanworth. Mol. Immunol., 25:1213-1215 (1988).
8. Hamburger. Science, 189:389-390 (1975).
9. Kenten, Helm, Ishizaka, et al. Proc. Natl. Acad Sci., USA, 81:2955-2956 (1984).
10. Coleman, Helm, Stanworth, and Gould. Eur. J. Immunol., 15:966-969 (1985).
11. Chretien, Helm, Marsh, et al. J. Immunol., 141:3128-3134 (1988).
12. Robertson, and Lin. Mol. Immunol., 25:103-113 (1988).
13. Helm, Kebo, Vercelli, et al. Proc. Natl. Acad. Sci., USA 86:9465-9469 (1989).
14. Stanworth, Jones, Lewin, and Nayyar. Allergy treatment with a peptide vaccine. Lancet, 336:1279-1281 (1990).
15. Stanworth, Lewin, Nayyar, and Jones. Immunoreactive peptides and antibodies and their use in anti-allergy treatment. EPO 403 312 A1 (1990).
16. Kindsroger et al., DNA 1:335-343 (1982).
17. Ishida et al., EMBO 1:1117-1123 (1982).
18. Dorrington and Bennich, Immunol. Rev., 41:3-25 (1978).

19. Brett et al. Eur. J. Immunol., 23:1608-1614 (1993).
20. Weismuller et al., Int. J. Peptide Res., 40:255-260 (1992).
21. Celis et al., J. Immunol., 140:1808-1815 (1988).
22. Demotz et al., J. Immunol., 142:394-402 (1989).
23. Chong et al., Infect. Immun., 60:4640-4647 (1992).
24. Grant ed., Synthetic Peptides: A User's Guide, W.H.Freeman & Co., New York, NY, (1992) pp. 382.
25. O'Hagan et al., Vaccine, 9:768-771 (1991).
26. Eldridge et al., Molec. Immunol., 28:287-294 (1991).
27. MacGlashan et al., J. Immunol., 136:2231-2239 (1986).

OBJECTS OF THE INVENTION

It is an objective of the present invention to employ a group of IgE ϵ-chain based peptide immunogens chemically synthesized in either a radially branching form or a linear T helper epitope containing form, to elicit high titer antibodies to the decapeptide effector site of the CH4 domain of the human ϵ-chain, when introduced to mammals, including humans.

Another objective is to design optimal peptide immunogens, with specific amino acid sequences taken from the human IgE heavy chain CH4 domain (IgE CH4) attached to peptides containing promiscuous human helper T cell epitopes in a specific orientation which, when introduced into mammals, including humans, will stimulate production of high titers of efficacious antibodies to the effector site on human IgE CH4. These antibodies should inhibit mast cell activation, reduce the release of chemical mediators such as histamines that are responsible for allergy symptoms, depress IgE-mediated passive cutaneous anaphylaxis (PCA) reaction, and suppress allergen-induced IgE production by B lymphocytes.

A further objective is to develop an effective IgE ϵ-chain peptide-based vaccine, employing compositions containing such branching multimeric or linear immunogens, to provide immunotherapy for the treatment of allergic reactions.

SUMMARY OF THE INVENTION

According to the present invention, peptide immunogens are made by solid phase synthesis. The peptide immunogens comprise a series of radially branched multimeric peptides containing a ten amino acid IgE CH4 peptide (SEQ ID NO:1), or an immunogenic analog thereof; a series of multimeric branched peptides containing the IgE CH4 peptide (SEQ ID NO:1) or an immunogenic analog thereof together with a helper T-cell epitope (Th epitope); and a

series of linear monomeric peptides containing the IgE CH4 peptide (SEQ ID NO:1) or an immunogenic analog thereof together with a portion of a helper T-cell epitope (Th epitope). The IgE CH4 peptide is taken from the Fc region of the IgE heavy chain, i.e. ϵ-chain CH4 domain (IgE CH4). Of the three series of peptide immunogens, the linear peptides are preferred. Compositions containing these peptides are used to immunize healthy mammals, e.g. guinea pigs, rats, and humans, to elicit the production of high titer antisera specific for the IgE CH4 effector site (SEQ ID NO:1) and free of irrelevant antibodies.

According to the present invention, vaccines containing the synthetic peptides as the key immunogen may also be prepared with an effective amount of a multimeric-branching peptide or a linear peptide in the presence of a proper adjuvant and/or delivery vehicle. It is expected that such vaccine compositions will elicit a more focused anti-IgE peptide response than those of the peptide-carrier protein conjugates currently used by Stanworth et al.[14], thus providing a better immunotherapy for the treatment of allergy.

DETAILED DESCRIPTION OF THE INVENTION

This invention is directed to the use of a novel group of peptide-based immunogens for the generation of high titer antibodies to an effector site on the CH4 domain of human IgE ϵ heavy chain (SEQ ID NO:1) in healthy mammals, including humans, for the treatment of IgE-mediated allergic diseases.

It is generally accepted that allergy symptoms, the immediate result of IgE-dependent hypersensitivities, are caused by chemical mediators released by mast cells and basophils. The release is triggered when a mast cell or basophil that has been sensitized with surface-bound IgE binds to an allergen for which the surface-bound IgE is specific. The triggering is actuated by the binding of

the allergen to the Fab' portion of the surface-bound IgE in an antigen-antibody type interaction. The allergen/antibody binding crosslinks the bivalent surface-bound IgE and induces a conformational change in the distal Fc region of IgE, the region of IgE in direct contact with a high affinity Fc receptor on the cell surface. By a mechanism as yet not precisely understood, the conformational change activates the cell-IgE-allergen complex with the resultant release of mediators, including histamine, by the cell. Effector site(s) on IgE are believed to participate in the triggering event. The presence of specific anti-IgE antibodies directed against such "effector sites", through either active or passive immunization, may lead to inhibition of the cell activation process in hosts suffering from allergic reactions by interfering with the interaction between the IgE "effector sites" and the cell surface.

Such interventions through the use of specific anti-IgE antibodies, i.e. a kind of immunotherapy, can be achieved either passively, through prophylactic treatment with specific "site-directed" antibodies to IgE, or, more preferably, actively, by providing the host with a vaccine comprised of site-directed peptide immunogens, to elicit the production by the host of its own site-directed anti-IgE antibodies. It is believed that active immunization will provide a more effective and longer lasting protection.

Among the sites from the Fc region of circulating IgE that have been studied for functional activity, a region on the CH4 domain of the IgE molecule (Lys_{497}-Phe_{506}) has been identified as a conformational effector involved in the triggering of mast cells and basophils[3-8,14]. See Table 1 and the areas underlined in Table 2. A decapeptide derived from this site with the sequence Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe-NH2 (SEQ

ID NO:1) was found to approximate the conformation of this effector site. This is evidenced by the ability of the decapeptide to elicit dose-dependent histamine release from rat mast cells in a manner resembling the immunological triggering process[4].

Stanworth et al.[14,15] demonstrated the feasibility of providing immunotherapy to patients with IgE-mediated allergic reactions through the use of experimental vaccines by using the IgE CH4 decapeptide (SEQ ID NO:1) coupled to a carrier protein, keyhole limpet hemocyanin (KLH) as an immunogen. Animal immune sera obtained from such immunizations were found by Stanworth et al.[14,15] to moderately reduce the decapeptide-induced histamine release from rat peritoneal mast cells in a titer-dependent fashion. Inhibitory activity by the immune sera generated was further confirmed by *in vivo* passive cutaneous anaphylaxis (PCA) tests under conditions of multiple allergen application.

A major deficiency of the prototype "IgE CH4 peptide" vaccine developed by Stanworth et al is its weak immunogenicity, a problem inherently associated with almost all self-antigens.

In the present invention, specific immunogens are provided wherein synthetic immune stimulatory elements are linked to the CH4 decapeptide of IgE (SEQ ID NO:1) in a specific orientation such that potent antibodies directed to this effector site on IgE can be broadly generated in a genetically diverse host population. In turn, these antibodies block the stimulatory action of IgE on mast cells and basophils, thus resulting in an effective treatment to prevent IgE-mediated allergic diseases.

The peptide immunogens of the present invention are capable of eliciting antibodies with serological cross-reactivity with the target amino acid sequence of

the Fc region of IgE (SEQ ID NO:1) while being substantially incapable of mediating non-cytolytic histamine release.

The initial dose, e.g. 0.2-2.5 mg; preferably 1 mg, of immunogen is to be administered by injection, preferably intramuscular, followed by repeat (booster) doses. Dosage will depend on the age, weight and general health of the patient as is well known in the therapeutic arts.

While there is no particular limitation to the species of mammals suitable for the production of antibodies, it is generally preferred to use mice, rabbits, guinea pigs, pigs, goats, rats or sheep, etc. as the hosts.

For active immunization, the term "immunogen" referred to herein relates to synthetic peptides which are capable of inducing antibodies against the IgE CH4 decapeptide (SEQ ID NO:1), which antibodies lead to the suppression of IgE-mediated basophil and mast cell degranulation. The immunogen of this invention included multimeric peptides or its analogs with a branching lysyl core matrix structure.

These branched multimeric peptides have the capability of independently eliciting an immune response in a host animal. The analogs of IgE CH4 decapeptide (SEQ ID NO:1) include the synthetic peptide analogs described by Stanworth et al.[3,4,5], which are incorporated herein by reference. To be suitable, the molecular weight of the immunogen should be higher than 5,000 and preferably be higher than 10,000. The repeating branch unit for the peptide should be equal to or higher than 4.

Bifunctional amino acids such as lysine followed by attachment to an amino acid with a preferably non-charged side chain, such as Gly or Ala, are useful in the making of the core matrix structure. By inserting an

amino acid in one additional coupling cycle in between two di-Boc-Lysine coupling cycles, the amino acid acts as a spacer in between the peptide branches to allow maximum freedom to attain the conformation necessary for optimal presentation.

The immunogen referred to in the present invention also included linear peptides which contain promiscuous helper T cell epitopes (Th epitopes). These Th epitopes were covalently attached in a defined fashion to the decapeptide effector sequence (SEQ ID NO:1), with or without a spacer, so as to be adjacent to the N terminus of the decapeptide, in order to evoke efficient antibody responses. The immunogen may also be comprised of an immune stimulatory sequence corresponding, for example, to a domain of an invasin protein from the bacteria *Yersinia* spp[19]. The invasin domain may also be attached through a spacer to a Th epitope.

The "immunogen" of the present invention minimizes the generation of irrelevant antibodies, thus eliciting a more focused immune response to the "target sequence", i.e., the desired IgE CH4 cross-reactivity (SEQ ID NO:1), without producing undesirable side effects which may complicate the immunotherapy process for the treatment of allergy.

However, when a short target sequence, such as the 10 amino acid IgE CH4 segment Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe (SEQ ID NO:1), is used to design a carrier protein-free immunogen, one faces serious challenges. A short peptide antigen is usually a T cell-dependent antigen, i.e. the presence of a T helper epitope is required to render a short "target" peptide immunogenic. The short IgE CH4 decapeptide (SEQ ID NO:1) or an immunogenic analog thereof does not contain a T helper cell epitope. The branched multimeric and linear immunogens comprising the short IgE CH4 decapeptide are

designed herein to provide for artificially built-in functional helper T-cell epitopes.

The peptides immunogens of this invention are represented by the formula

$(A)_n$-$(Th)_m$-$(B)_o$-(IgE CH4 peptide)$_p$

wherein

A is an amino acid, α-NH_2, a fatty acid, a derivative of a fatty acid, or an invasin domain;

B is an amino acid;

Th is a helper T cell epitope or an immune enhancing analog or segment thereof;

IgE CH4 peptide is Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe (SEQ ID NO:1) or an immunogenic analog thereof;

n is from 1 to 10;

m is from 1 to 4;

o is from 0 to 10; and

p is from 1 to 3.

The peptide immunogens of the present invention comprise from about 20 to about 100 amino acid residues, preferably from about 20 to about 50 amino acid residues and more preferably from about 20 to about 35 amino acid residues.

When A is an amino acid, it can be any non-naturally occurring or any naturally occurring amino acid. Non-naturally occurring amino acids include, but are not limited to, ß-alanine, ornithine, norleucine, norvaline, hydroxyproline, thyroxine, γ-amino butyric acid, homoserine, citrulline and the like. Naturally-occurring amino acids include alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine. Moreover, when n is

greater than one, and two or more of the A groups are amino acids, then each amino acid is independently the same or different.

When A is a fatty acid, such as stearic acid or palmitic acid or a fatty acid derivative, such as a tripalmitoyl cysteine (Pam_3Cys) group, it acts as an adjuvant by enhancing the immunostimulating properties of the Th epitope[20]. When A is a fatty acid or its derivative it is usually located at the amino terminus of the peptide. Furthermore, when one of A is a fatty acid, there are 2 or 3 additional amino acid A moieties. The fatty acids useful in the invention have a hydrocarbon chain of 8 to 24 carbon atoms which may be saturated or unsaturated.

When A is an invasin domain, it is an immune stimulatory epitope from the invasin protein of a *Yersinia* species. This immune stimulatory property results from the capability of this invasin domain to interact with the ß1 integrin molecules present on T cells, particularly activated immune or memory T cells. The specific sequence for an invasin domain found to interact with the $\beta 1$ integrins has been described by Brett et al [19]. In a preferred embodiment, the invasin domain (Inv) for linkage to a promiscuous Th epitope has the sequence:

Thr-Ala-Lys-Ser-Lys-Lys-Phe-Pro-Ser-Tyr-Thr-Ala-Thr-Tyr-Gln-Phe (SEQ ID NO: 25)

or is an immune stimulatory analog thereof from the corresponding region in another *Yersinia* species invasin protein. Such analogs may contain substitutions, deletions or insertions to accommodate strain to strain variation, provided that the analogs retain immune stimulatory properties.

In one embodiment, n is 4 and A is α-NH_2, lysine, lysine and lysine in that order. In another embodiment n is 1 and A is α-NH_2. In yet another

o

embodiment, n is 4 and A is α-NH_2, an invasin domain (Inv), glycine and glycine in that order.

B comprises naturally occurring or the non-naturally occurring amino acids as described above. Each B may be independently the same or different. When B is lysine, a branched polymer can be formed. For example, if o is 7 and all seven B groups are lysine then a branching K core (K_4K_2K) is formed when the peptide synthesis is conducted without protecting the lysyl side chain ϵ-amino group. Peptides with a K core have eight branch arms, with each branch arm being identical and represented as "$(A)_n$-$(Th)_m$-" or "(IgE CH4 peptide with built-in-Th)-". In addition, the amino acids of B can form a flexible hinge, or spacer, to enhance the immune response to the Th epitope and IgE CH4 decapeptide or an analog thereof. Examples of sequences encoding flexible hinges can be found in the immunoglobulin heavy chain hinge region. Flexible hinge sequences are often proline rich. One particularly useful flexible hinge is provided by the sequence Pro-Pro-Xaa-Pro-Xaa-Pro (SEQ ID NO:24), where Xaa is any amino acid, preferably aspartic acid. Immunogenicity can also be improved through the addition of spacer residues (e.g. Gly-Gly) between the promiscuous Th epitope and the IgE CH4 decapeptide or an analog thereof. In addition to physically separating the Th epitope from the B cell epitope (i.e., the IgE CH4 decapeptide site or an analog thereof), the glycine residues can disrupt any artifactual secondary structures created by the joining of the Th epitope with the IgE CH4 decapeptide (SEQ ID NO:1) or an analog thereof and thereby eliminate interference between the T and/or B cell responses. Thus, the conformational separation between the helper cell and the antibody eliciting domains permits more efficient interactions between the presented immunogen and the appropriate Th and B cells.

Th is a Th epitope comprising natural or non-natural amino acids. A Th epitope may consist of a continuous or discontinuous epitope; not every amino acid of Th is necessarily part of the epitope. Th epitopes, including analogs and segments thereof, to be suitable for the present invention are capable of enhancing or stimulating an immune response to the IgE CH4 decapeptide (SEQ ID NO:1) or an analog thereof. Th epitopes that are immunodominant and promiscuous are highly and broadly reactive in animal and human populations with widely divergent MHC types[21-23]. The Th domain suitable for the present invention has from about 10 to about 50 amino acids and preferably from about 10 to about 30 amino acids. When multiple Th epitopes are present (i.e. $m \geq 2$), then each Th epitope may be independently the same or different.

Th epitope analogs include substitutions, additions, deletions and insertions of from one to about 10 amino acid residues in the Th epitope. Th segments are contiguous portions of a Th epitope that are sufficient to enhance or stimulate an immune response to the IgE CH4 decapeptide (SEQ ID NO:1) or an analog thereof.

Th epitopes of the present invention include hepatitis B surface and core antigen helper T cell epitopes (HB_sTh and HB_cTh), pertussis toxin helper T cell epitopes (PT Th), tetanus toxin helper T cell epitopes (TT Th), measles virus F protein helper T cell epitopes (MV_F Th), *Chlamydia trachomatis* major outer membrane protein helper T cell epitopes (CT Th), diphtheria toxin helper T cell epitopes (DT Th), *Plasmodium falciparum* circumsporozoite helper T cell epitopes (PF Th), *Schistosoma mansoni* triose phosphate isomerase helper T cell epitopes (SM Th), *Escherichia coli* TraT helper T cell epitopes (TraT Th) and immune-enhancing analogs and segments of any of these Th epitopes. Examples of Th

epitope sequences are provided below:

HB_s Th: Phe-Phe-Leu-Leu-Thr-Arg-Ile-Leu-Thr-Ile-Pro-Gln-Ser-Leu-Asp (SEQ ID NO:5)

PT_1 Th: Lys-Lys-Leu-Arg-Arg-Leu-Leu-Tyr-Met-Ile-Tyr-Met-Ser-Gly-Leu-Ala-Val-Arg-Val-His-Val-Ser-Lys-Glu-Glu-Gln-Tyr-Tyr-Asp-Tyr (SEQ ID NO:6)

TT_1 Th: Lys-Lys-Gln-Tyr-Ile-Lys-Ala-Asn-Ser-Lys-Phe-Ile-Gly-Ile-Thr-Glu-Leu (SEQ ID NO:7)

TT_2 Th: Lys-Lys-Phe-Asn-Asn-Phe-Thr-Val-Ser-Phe-Trp-Leu-Arg-Val-Pro-Lys-Val-Ser-Ala-Ser-His-Leu (SEQ ID NO:8)

PT_{1A} Th: Tyr-Met-Ser-Gly-Leu-Ala-Val-Arg-Val-His-Val-Ser-Lys-Glu-Glu (SEQ ID NO:9)

TT_3 Th: Tyr-Asp-Pro-Asn-Tyr-Leu-Arg-Thr-Asp-Ser-Asp-Lys-Asp-Arg-Phe-Leu-Gln-Thr-Met-Val-Lys-Leu-Phe-Asn-Arg-Ile-Lys (SEQ ID NO:10)

PT_2 Th: Gly-Ala-Tyr-Ala-Arg-Cys-Pro-Asn-Gly-Thr-Arg-Ala-Leu-Thr-Val-Ala-Glu-Leu-Arg-Gly-Asn-Ala-Glu-Leu (SEQ ID NO:11)

MV_{F1} Th: Ser-Glu-Ile-Lys-Gly-Val-Ile-Val-His-Arg-Leu-Glu-Gly (SEQ ID NO:12)

and

Leu-Ser-Glu-Ile-Lys-Gly-Val-Ile-Val-His-Arg-Leu-Glu-Gly-Val (SEQ ID NO:61)

HB_c Th: Val-Ser-Phe-Gly-Val-Trp-Ile-Arg-Thr-Pro-Pro-Ala-Tyr-Arg-Pro-Pro-Asn-Ala-Pro-Ile-Leu (SEQ ID NO:14)

MV_{F2} Th: Gly-Ile-Leu-Glu-Ser-Arg-Gly-Ile-Lys-Ala-Arg-Ile-Thr-His-Val-Asp-Thr-Glu-Ser-Tyr (SEQ ID NO:26)

TT_4 Th: Trp-Val-Arg-Asp-Ile-Ile-Asp-Asp-Phe-Thr-Asn-Glu-Ser-Ser-Gln-Lys-Thr (SEQ ID NO:27)

TT_5 Th: Asp-Val-Ser-Thr-Ile-Val-Pro-Tyr-Ile-Gly-Pro-Ala-Leu-Asn-His-Val (SEQ ID NO:28)

CT Th: Ala-Leu-Asn-Ile-Trp-Asp-Arg-Phe-Asp-Val-Phe-Cys-Thr-Leu-Gly-Ala-Thr-Thr-Gly-Tyr-Leu-Lys-Gly-Asn-Ser (SEQ ID NO:29)

DT_1 Th: Asp-Ser-Glu-Thr-Ala-Asp-Asn-Leu-Glu-Lys-Thr-Val-Ala-Ala-Leu-Ser-Ile-Leu-Pro-Gly-Ile-Gly-Cys (SEQ ID NO:30)

DT_2 Th: Glu-Glu-Ile-Val-Ala-Gln-Ser-Ile-Ala-Leu-Ser-Ser-Leu-Met-Val-Ala-Gln-Ala-Ile-Pro-Leu-Val-Gly-Glu-Leu-Val-Asp-Ile-Gly-Phe-Ala-Ala-Thr-Asn-Phe-Val-Glu-Ser-Cys (SEQ ID NO:31)

PF Th: Asp-Ile-Glu-Lys-Lys-Ile-Ala-Lys-Met-Glu-Lys-Ala-Ser-Ser-Val-Phe-Asn-Val-Val-Asn-Ser (SEQ ID NO:32)

SM Th: Lys-Trp-Phe-Lys-Thr-Asn-Ala-Pro-Asn-Gly-Val-Asp-Glu-Lys-Ile-Arg-Ile (SEQ ID NO:33)

$TraT_1$ Th: Gly-Leu-Gln-Gly-Lys-Ile-Ala-Asp-Ala-Val-Lys-Ala-Lys-Gly (SEQ ID NO:34)

$TraT_2$ Th: Gly-Leu-Ala-Ala-Gly-Leu-Val-Gly-Met-Ala-Ala-Asp-Ala-Met-Val-Glu-Asp-Val-Asn (SEQ ID NO:35)

$TraT_3$ Th: Ser-Thr-Glu-Thr-Gly-Asn-Gln-His-His-Tyr-Gln-Thr-Arg-Val-Val-Ser-Asn-Ala-Asn-Lys (SEQ ID NO:36)

Preferably, the Th epitope is HB_s Th, PT_1 Th, PT_2 Th, TT_1 Th, TT_3 Th, or MV_{F1} Th.

In the monomeric linear peptides of this invention, as described by the Formula $(A)_n$-$(Th)_m$-$(B)_o$-(IgE CH4 peptide), the Th epitope is covalently attached through spacer B to the N terminus of the IgE CH4 decapeptide (SEQ ID NO:1). The IgE CH4 peptide is Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe (SEQ ID NO:1), a decapeptide. The IgE CH4 peptide may be replaced by an immunogenic analog. The immunogenic analogs thereof may contain a substitution, addition, deletion, or insertion of from one to about four amino acid residues provided that the analog is capable of eliciting an immune response crossreactive with the IgE CH4 decapeptide (SEQ ID NO:1). The substitutions, additions, and insertions may be made with natural or non-natural amino acids as defined herein. Immunogenic analogs of the IgE CH4 peptide (SEQ NO:1) have been identified by Stanworth et al.[3,4,5] and are incorporated herein by reference.

Accordingly, preferred peptide immunogens of this invention are monomeric peptides containing IgE CH4 decapeptide (SEQ ID NO:1) or an immunogenic analog thereof and Th. More specifically, preferred peptide immunogens are those linear constructs containing IgE CH4 (SEQ ID NO:1) or an immunogenic analog thereof; a spacer (e.g Gly-Gly); a Th epitope selected from the group consisting HB_s Th, PT_1 Th, PT_2 Th, TT_1 Th, TT_3 Th, and MV_{F1} Th (SEQ ID NOS:5,6,11,7,10,61, respectively); and optionally the Inv domain (SEQ ID NO:25). Preferred peptide immunogen compositions include, for example, Peptide Nos. 19-23 and 28 (Tables 5 and 6, SEQ ID NOS:51-55,62).

The peptide immunogens of this invention may be made by chemical synthesis well known to the ordinarily skilled artisan. See, for example, Grant, ed. Synthetic Peptides[24]. Hence, peptide immunogens may be synthesized using the automated Merrifield techniques of solid phase synthesis with the α-NH_2 protected by either t-Boc or F-moc chemistry using side chain protected amino acids on, for example, an Applied Biosystems Peptide Synthesizer Model 430A or 431. To synthesize a K core moiety on which to synthesize peptide branches, Di-α,ϵ(t-Boc) lysine residues are used in place of t-Boc lysine with a 2,4-dichlorobenzyl protecting ϵ-amino group.

When A is a fatty acid, it may be added easily to the N-terminus of the resin bound peptide by the well known carbodiimide method. To add Pam_3Cys, the lipoamino acid S-[2,3-Bis(palmitoyloxy)-(2R)-propyl-N-palmitoyl-(R)-cysteine (Pam_3Cys) is chemically synthesized. Pam_3Cys may then be coupled to the N terminus of a peptide by solid-phase synthesis using Pam_3Cys-OH in the final coupling step to link the lipoamino acid to a resin-bound peptide chain.

To improve the solubility of the final coupled lipopeptide product, the solid-phase peptide can be elongated with additional serine and lysine residues at the N-terminus.

After complete assembly of the desired peptide immunogen, the resin is treated according to standard procedures to cleave the peptide from the resin and deblock the functional groups on the amino acid side chains. The free peptide is purified by HPLC and characterized biochemically, for example, by amino acid analysis or by sequencing. Purification and characterization methods for peptides are well known to one of ordinary skill in the art.

Other chemical means to generate linear Th-IgE

CH4 decapeptide constructs of the invention include the ligation of the haloacetylated and the cysteinyl peptide through the formation of a "thioether" linkage. For example, cysteine can be added to the C terminus of a Th-containing peptide and the thiol group of cysteine is used to form a covalent bond to an electrophilic group such as an N^{α} chloroacetyl-modified or a maleimide-derivatized α- or ϵ-NH_2 group of a lysine residue that is attached to the N-terminus of the IgE CH4 decapeptide (ID SEQ NO:1) or an immunogenic analog thereof.

The subject peptides can also be polymerized. Polymerization can be accomplished for example by reaction between glutaraldehyde and the -NH_2 groups of the lysine residues using routine methodology. The linear "A-Th-spacer-IgECH4" peptide constructs (e.g., Peptide Nos. 19-23 and 28, SEQ ID NOS:51-55 and 62) may also be polymerized or co-polymerized by utilization of an additional cysteine added to the N-terminus of the linear "A-Th-spacer-IgECH4" construct. The thiol group of the N-terminal cysteine may be used for the formation of a "thioether" bond with a halochloroacetyl-modified or a maleimide-derivatized α- or ϵ-NH_2 group of a lysine residue that is attached to the N-terminus of a branched poly-lysyl core molecule (e.g., K_2K, K_4K_2K, $K_8K_4K_2K$).

Alternatively, the longer linear peptide immunogens may be synthesized by well known recombinant DNA techniques. Any standard manual on DNA technology provides detailed protocols to produce the peptides of the invention. To construct a gene encoding a peptide of this invention, the amino acid sequence is reverse translated into a nucleic acid sequence, and preferably using optimized codon usage for the organism in which the gene will be expressed. Next, a synthetic gene is made, typically by synthesizing overlapping oligonucleotides which encode the peptide and any regulatory elements, if

necessary. The synthetic gene is inserted in a suitable cloning vector and recombinants are obtained and characterized. The peptide is then expressed under suitable conditions appropriate for the selected expression system and host. The peptide is purified and characterized by standard methods.

The efficacy of the peptide immunogen of the present invention may be established by injecting the immunogen into an animal, and then monitoring the humoral immune response to IgE CH4 decapeptide (SEQ ID NO:1) or an immunogenic analog thereof, as detailed in the Examples. Suitable animals include mice, rats, rabbits, guinea pigs, pigs, goats, sheep, or the like.

Another aspect of this invention provides a vaccine composition comprising an effective amount of one or more of the peptide immunogens of this invention in a pharmaceutically acceptable delivery system. Such vaccine compositions are used for prevention of atopic allergic reactions including allergic rhinitis, those of food allergies, asthma, anaphylaxis, and other IgE-mediated hypersensitive reactions such as virally-induced asthma.

Accordingly, the subject peptide immunogens can be formulated as a vaccine composition using adjuvants, pharmaceutically-acceptable carriers or other ingredients routinely provided in vaccine compositions. Such formulations are readily determined by one of ordinary skill in the art and include formulations for immediate release and/or for sustained release, and for induction of systemic immunity and/or induction of localized mucosal immunity, which may be accomplished by, for example, immunogen entrapment by microparticles. The formulation may also include adjuvants or emulsifiers such as alum, incomplete Freund's adjuvant, liposyn, saponin, squalene, L121, emulsigen and ISA 720 and the like.

The vaccine of the present invention may be

administered by any convenient route including subcutaneous, oral, intramuscular, or other parenteral or enteral route. It may be administered as a single dose or in multiple doses. Immunization schedules are readily determined by the ordinarily skilled artisan.

The vaccine compositions of the instant invention contain an effective amount of one or more of the synthetic peptide immunogens containing the IgE CH4 decapeptide or its immunogenic analog and a pharmaceutically acceptable carrier. The dosage unit form may be formulated to contain about 0.5 μg to about 1 mg of each peptide per kg body weight. When delivered in multiple doses, the effective dose may be conveniently divided to contain the appropriate amounts per unit dosage form.

The vaccine compositions of the present invention may be formulated to contain a cocktail of two or more of the subject peptide immunogens to enhance immunoefficacy in a broader population and thus provide a better immune response against IgE CH4 decapeptide. For example, a cocktail of Peptide Nos. 19, 20, 21, 23, and 4 is useful. The composition may also be formulated to comprise lipopeptides to provide a built-in adjuvant. The immune response to synthetic IgE CH4 decapeptide-containing immunogens may also be improved by delivery through entrapment in or on biodegradable microparticles of the type described by O'Hagan *et al*[25]. The immunogens can be encapsulated with or without adjuvant, including covalently attached Pam_3Cys, and such microparticles may carry an immune stimulatory adjuvant such as Freund's Incomplete Adjuvant or alum. The microparticles function to potentiate immune responses to the immunogen, including localized mucosal immunity. Such localized immunity is especially desirable, for example, for mucosally localized allergic reactions. Vaccine compositions in

microparticular form may also be formulated to provide time-controlled release for sustained or periodic responses, for oral administration, and for topical administration[25-26].

Examples of specific peptide immunogens are provided herebelow to illustrate the present invention and are to be used to limit the scope of the invention.

EXAMPLE 1

SYNTHESIS OF OCTAMERIC PEPTIDE IMMUNOGENS

The following multimeric peptides were synthesized:

Peptide No. 1

[LysThrLysGlySerGlyPhePheValPheGlyProGlyLysThrLysGlySerGly PhePheValPheGlyLysMet]$_8$Lys$_4$Lys$_2$Lys, (SEQ ID NO:23)

Peptide No. 2

[LysThrLysGlySerGlyPhePheValPheGlyProGlyLysThrLysGlySerGly PhePheValPheGlyProGlyLysThrLysGlySerGlyPhePheValPheGlyLys Met]$_8$Lys$_4$Lys$_2$Lys, (SEQ ID NO:13)

The synthesis of the multimeric peptides proceeds by the limited sequential propagation of a trifunctional amino acid to serve as a low molecular weight matrix core is the basis for the formation of a branching multimeric peptide antigen system. The trifunctional amino acid, Boc-Lys(Boc), or di-(Boc)-Lys is most suitable since both N^{α}- and N^{ϵ}- amino acid groups are available as reactive ends. Thus, sequential propagation of di-(Boc)-Lys will generate 2^n reactive ends.

For example, the first coupling of di-(Boc)-Lys onto a solid phase resin will produce two reactive amino ends to bind two peptide chains. Sequential generations of a second, third, and fourth step with di-(Boc)-Lys will therefore generate respectively tetravalent, octavalent, and hexadecavalent ends for binding multimeric peptide chains antigens. Such multimeric peptides are useful as

immunogens. Branched octameric Peptide Nos. 1 and 2 as described above were synthesized for use as immunogens. The branched antigens contain a small heptalysyl core surrounded by a layer of high density of uniform peptide-antigens around the core matrix. This design differs from the conventional peptide-carrier conjugate antigen which contains a large protein carrier such as PPD or KLH and a small peptide antigen randomly distributed on the surface of the protein carrier in many undefined forms.

The synthesis of the octameric peptide immunogens employs a combination of Boc-amino acid resin-bound benzhydrylamide and tBoc-chemistry. For example, an 8-branched heptalysyl core resin was prepared by coupling di-t-Boc Lys onto an extra low loading of 0.14 mmole/g MBHA (4-methylbenzhydrylamine) resin on a Biosearch 9500 instrument. Two coupling cycles of di-(Boc)-Lys for each was followed by two capping reactions using 0.3 M acetylimidazole in DMF dimethylformamide.

Another two di-(Boc)-Lys couplings were added onto the first di-(NH_2) Lys-resin. The substitution level of synthetic octameric resin was then determined by the ninhydrin test and found to have an appropriate level of free -NH_2 groups, based on the theoretical coupling yield, and was used thereafter for the synthesis of octameric peptide immunogen according to the standard t-Boc procedure.

Acid-labile tert-butyloxycarbonyl (t-Boc) was used for the protection of N-α amino acid. The following functional side-chain protecting groups were used: O-benzyl for Thr, Ser, Glu and Tyr; N^{δ}-tosyl for Arg; BOM, i.e., BOC-N^{im}-Benzyloxymethyl for His, N^{ϵ}-dichlorobenzyloxycarbonyl for Lys; S-4-methylbenzyl- for Cys; O-cyclohexyl for Asp and CHO for Trp.

The successive amino acids of Peptides No. 1 and No. 2 were added from the C- to N- terminus as dictated by

the sequences of Peptide Nos. 1 and 2 (SEQ ID NOS:23,13). The resultant octameric peptidyl resins for Peptide No. 1 and Peptide No. 2 were cleaved by anhydrous HF at 0°C for 1 hr in the presence of 10% v/v anisole. The released multimeric antigens were extracted with acetic acid, washed twice with ether and lyophilized to dryness. The lyophilized multimeric peptides were used as immunogens.

EXAMPLE 2
ACTIVE IMMUNIZATION WITH BRANCHED OCTAMERIC PEPTIDE IMMUNOGENS USING CFA AND IFA AS ADJUVANTS

(a) Immunization procedure

Groups of Guinea Pigs (N=3 per group) were immunized with each of the two IgE CH4-related multimeric peptide immunogens (Peptide Nos. 1 and 2) and with Peptide No. 3 (SEQ ID NO:1) conjugated to KLH, according to the following protocol: Each animal was injected subcutaneously with a mixture (200 μL) of the peptide-based immunogen or conjugate (100 μg/mL) emulsified with an equal volume of complete Freund's adjuvant (CFA). Subcutaneous injections of the peptide-based immunogen mixed with incomplete Freund's adjuvant (IFA) were repeated at days 21, 42, and 63.

(b) Assay of Guinea Pigs immune sera by measuring their Anti-IgE CH4 related peptide response

Anti-peptide antibody activity is determined by ELISA (enzyme-linked immunosorbentassay) using 96-well flat bottom microtiter plates which were coated with the corresponding immunogen. Aliquots (100 μL) of a peptide immunogen solution at a concentration of 5 μg/mL were incubated for 1 hour at 37°C. The plates were blocked by another incubation at 37°C for 1 hour with a 3% gelatin/PBS solution. The blocked plates were then dried and used for the assay. Aliquots (100 μL) of the test guinea pig sera, starting with a 1:10 dilution in a sample dilution buffer and ten-fold serial dilutions thereafter,

were added to the peptide coated plates. The plates were incubated for 1 hour at 37°C. Normal guinea pig serum was used as a control.

The plates were washed six times with 0.05% PBS/Tween® buffer. 100 μL of horseradish peroxidase labelled goat-anti-guinea pig antibody was added at a dilution of 1:1,000 in conjugate dilution buffer (Phosphate buffer containing 0.5M NaCl, and normal goat serum). The plates were incubated for 1 hour at 37°C before being washed as above. Aliquots (100 μL) of o-phenylenediamine substrate solution were then added. The color was allowed to develop for 5-15 minutes before the enzymatic color reaction was stopped by the addition of 50 μL 2N H_2SO_4. The A_{492nm} of the contents of each well was read in a plate reader.

The immunogens, Peptide No. 1 and its closely related derivative Peptide No. 2, both in branching multimeric form, were found to be effective in eliciting antibodies specific to the IgE CH4 target sequence (SEQ ID NO:1) through an ELISA inhibition assay. The results, when compared to a control immunogen, the KLH conjugate of monomeric Peptide No. 3 (IgE CH4 decapeptide SEQ ID NO:1) showed that these two multimeric peptide antigens generated a higher level of antibody titers than the KLH conjugate.

The successful results of these immunization experiments indicated the generation of a Th epitope in the multimeric system as a result of insertion of Gly-Lys-Met at the C-terminus of the peptide sequence (see SEQ ID NOS:23 and 13, Peptide Nos. 1 and 2) and indicated the importance of certain orientations for effective presentation to the immune system. Other experiments showed that merely making 8- or even 16-branched IgE peptide immunogens containing the IgE CH4 decapeptide (SEQ ID NO:1) or multiple repeats thereof, in other

orientations, were not effective in the induction of anti-IgE CH4 responses. In fact, out of a total of 19 branched multimeric constructs, Peptide Nos. 1 and 2 were the only ones to display enhanced immunogenicity. In this respect, the high immunogenicity observed with multimeric Peptide Nos. 1 and 2 required experimentation and was not predictable by one skilled in the art.

In addition, the results obtained suggest that a spacer sequence, i.e., Gly-Pro-Gly, incorporated between the short IgE CH4 segments, is necessary to allow free presentation of the epitopes conferred by the subunit sequence. The insertion of a spacer, i.e., Gly-Lys-Met, at the C-terminus prior to linkage to the branched lysine core resin was also found to be necessary for the immunogenicity of multimeric branched IgE CH4 decapeptide (SEQ ID NO:1) synthetic constructs.

EXAMPLE 3

IMMUNIZATION OF RATS WITH LINEAR IMMUNOGENS (SEQ ID NOS:15-22)

A. Immunogen preparation: Peptide immunogens A-H (Table 3) are synthesized by solid phase synthesis using F-moc chemistry on an Applied Biosystems Peptide Synthesizer Model 430A or 431 according to manufacturer's instructions. After complete assembly of the peptide, the resin is treated according to standard procedures to cleave the peptide from the resin and deprotect the functional groups on amino acid side chains. The structure of the peptide immunogens from the amino terminus to the carboxyl terminus is as follows: Peptide immunogen A is a linear peptide with three domains: 3 lysine residues (3K), the hepatitis B surface antigen helper T cell epitope (HB_sTh epitope) and IgE CH4 peptide. Peptide immunogen A is thus represented as 3K-HB_sTh-IgE CH4 peptide. The actual sequences for Peptide immunogen A and for Peptide immunogens B-H are shown in Table 5 (SEQ

ID NOS:15-22).

For immunizations at weeks 0, 2 and 5, each peptide immunogen is dissolved and combined with an adjuvant solution (Complete Freund's Adjuvant, Incomplete Freund's Adjuvant, or 0.2% Alum) to result in a final concentration of 0.5 mg/ml. The solution is stored at 4°C until use and vortexed for 3 to 5 min prior to injection. Each rat receives 100 μg per injection.

B. Immunization schedule and serum collection: Sprague-Dawley rats (n=5) are immunized subcutaneously (s.c.). Booster injections are given s.c. at weeks 2 and 5. Blood is collected at weeks 3, 6, 7 and 11.

Blood collection from the middle caudal artery is performed following anesthesia of the rats by intraperitoneal injection of 1 mL of sodium pentobarbital (64.8 mg/mL; Anthony Products Co., Accadia, CA) diluted 1 to 10 in 0.9% NaCl. The tails are kept in 48°C ± 0.5°C water for 2 min and rapidly massaged with paper towels (i.e., milked). Blood is collected immediately into a 5 mL syringe outfitted with a 23 gauge needle. Typically, 2 to 2.5 mL of blood is obtained. The serum is collected by centrifugation for 25 min at 3000 rpm. The serum is aliquoted in 300 μL volumes and stored frozen until used for ELISA assays.

TABLE 3

Sequences of Peptide Immunogens A-H

	Peptide Immunogen	Amino Acid Sequence
A	3K-HB_sTh-IgECH4	Lys-Lys-Lys-Phe-Phe-Leu-Leu-Thr-Arg-Ile-Leu-Thr-Ile-Pro-Gln-Ser-Leu-Asp-Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe (SEQ ID NO:15)
B	PT_1Th-IgECH4	Lys-Lys-Leu-Arg-Arg-Leu-Leu-Tyr-Met-Ile-Tyr-Met-Ser-Gly-Leu-Ala-Val-Arg-Val-His-Val-Ser-Lys-Glu-Glu-Gln-Tyr-Tyr-Asp-Tyr-Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe (SEQ ID NO:16)
C	PT_{1A}Th-IgECH4	Tyr-Met-Ser-Gly-Leu-Ala-Val-Arg-Val-His-Val-Ser-Lys-Glu-Glu-Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe (SEQ ID NO:17)
D	TT_1Th-IgECH4	Lys-Lys-Gln-Tyr-Ile-Lys-Ala-Asn-Ser-Lys-Phe-Ile-Gly-Ile-Thr-Glu-Leu-Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe (SEQ ID NO:18)
E	TT_2Th-IgECH4	Lys-Lys-Phe-Asn-Asn-Phe-Thr-Val-Ser-Phe-Trp-Leu-Arg-Val-Pro-Lys-Val-Ser-Ala-Ser-His-Leu-Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe (SEQ ID NO:19)

F	TT_3Th-IgECH4	Tyr-Asp-Pro-Asn-Tyr-Leu-Arg-Thr-Asp-Ser-Asp-Lys-Asp-Arg-Phe-Leu-Gln-Thr-Met-Val-Lys-Leu-Phe-Asn-Arg-Ile-Lys-Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe (SEQ ID NO:20)
G	PT_2Th-IgECH4	Gly-Ala-Tyr-Ala-Arg-Cys-Pro-Asn-Gly-Thr-Arg-Ala-Leu-Thr-Val-Ala-Glu-Leu-Arg-Gly-Asn-Ala-Glu-Leu-Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe (SEQ ID NO:21)
H	MV_{F1}Th-IgECH4	Ser-Glu-Ile-Lys-Gly-Val-Ile-Val-His-Arg-Leu-Glu-Gly-Val-Leu-Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe (SEQ ID NO:22)

EXAMPLE 4

IMMUNIZATION OF RATS WITH LINEAR IMMUNOGENS (SEQ ID NOS:37-50)

Linear peptide immunogens represented as A-Th-GG-IgE CH4, where A may be either NH_2-, Lys-Lys (2K), Lys-Lys-Lys (3K), or an invasin domain (Inv) (SEQ ID NO:25), Th is a T helper peptide, GG is a Gly-Gly spacer, and IgE CH4 is the target decapeptide (SEQ ID NO:1), are synthesized as described in Example 3. These peptide immunogens are shown in Table 4 as Peptide Immunogens Nos. 4-17 (SEQ ID NOS:37-50). The synthesized and cleaved peptides are used to immunize rats to test for efficacy.

Efficacy is evaluated on groups of five rats by the experimental immunization protocol outlined below.

Experimental Design:

Immunogen: Peptide Nos. 4-17 (1 per trial)
Dose: 100 μg per immunization
Route: intramuscular

Adjuvant: Freund's Complete/Incomplete
Dose Schedule: week 0 (FCA), 3 and 6 weeks (IFA)
Bleed Schedule: weeks 0, 3, 6, 8, 10
Species: Sprague-Dawley rats
Group size: 5
Assay: ELISA for anti-peptide activity, solid-phase immunosorbent is monomeric Peptide No. 3 of the IgE CH4 decapeptide sequence (SEQ ID NO:1).

Blood is collected, processed into serum, and stored prior to titering by ELISA as described in Example 2, with the exception of using horseradish peroxidase-labelled goat anti-rat IgG antibody instead of goat anti-guinea pig IgG as the tracer.

TABLE 4

Sequences of Peptide Immunogens Nos. 4-17

Peptide Immunogen		Amino Acid Sequence
4	TT_1Th-GG-$IgECH_4$	Lys-Lys-Gln-Tyr-Ile-Lys-Ala-Asn-Ser-Lys-Phe-Ile-Gly-Ile-Thr-Glu-Leu-Gly-Gly-Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe (SEQ ID NO.37)
5	TT_2Th-GG-$IgECH_4$	Lys-Lys-Phe-Asn-Asn-Phe-Thr-Val-Ser-Phe-Trp-Leu-Arg-Val-Pro-Lys-Val-Ser-Ala-Ser-His-Leu-Gly-Gly-Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe (SEQ ID NO:38)
6	$PT_{1A}Th$-GG-$IgECH_4$	Tyr-Met-Ser-Gly-Leu-Ala-Val-Arg-Val-His-Val-Ser-Lys-Glu-Glu-Gly-Gly-Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe (SEQ ID NO:39)

7	$MV_{F2}Th$-GG-$IgECH_4$	Gly-Ile-Leu-Glu-Ser-Arg-Gly-Ile-Lys-Ala-Arg-Ile-Thr-His-Val-Asp-Thr-Glu-Ser-Tyr-Gly-Gly-Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe (SEQ ID NO:40)
8	TT_4Th-GG-$IgECH_4$	Trp-Val-Arg-Asp-Ile-Ile-Asp-Asp-Phe-Thr-Asn-Glu-Ser-Ser-Gln-Lys-Thr-Gly-Gly-Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe (SEQ ID NO:41)
9	TT_5Th-GG-$IgECH_4$	Asp-Val-Ser-Thr-Ile-Val-Pro-Tyr-Ile-Gly-Pro-Ala-Leu-Asn-His-Val-Gly-Gly-Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe (SEQ ID NO:42)
10	CTTh-GG-$IgECH_4$	Ala-Leu-Asn-Ile-Trp-Asp-Arg-Phe-Asp-Val-Phe-Cys-Thr-Leu-Gly-Ala-Thr-Thr-Gly-Tyr-Leu-Lys-Gly-Asn-Ser-Gly-Gly-Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe (SEQ ID NO:43)
11	DT_1Th-GG-$IgECH_4$	Asp-Ser-Glu-Thr-Ala-Asp-Asn-Leu-Glu-Lys-Thr-Val-Ala-Ala-Leu-Ser-Ile-Leu-Pro-Gly-Ile-Gly-Cys-Gly-Gly-Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe (SEQ ID NO:44)
12	DT_2Th-Gg-$IgECH_4$	Glu-Glu-Ile-Val-Ala-Gln-Ser-Ile-Ala-Leu-Ser-Ser-Leu-Met-Val-Ala-Gln-Ala-Ile-Pro-Leu-Val-Gly-Glu-Leu-Val-Asp-Ile-Gly-Phe-Ala-Ala-Thr-Asn-Phe-Val-Glu-Ser-Cys-Gly-Gly-Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val- (SEQ ID NO:45)
13	PFTh-GG-$IgECH_4$	Asp-Ile-Glu-Lys-Lys-Ile-Ala-Lys-Met-Glu-Lys-Ala-Ser-Ser-Val-Phe-Asn-Val-Val-Asn-Ser-Gly-Gly-Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe (SEQ ID NO:46)
14	SMTh-GG-$IgECH_4$	Lys-Trp-Phe-Lys-Thr-Asn-Ala-Pro-Asn-Gly-Val-Asp-Glu-Lys-Ile-Arg-Ile-Gly-Gly-Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe (SEQ ID NO:47)

15	$TraT_1Th$-GG-$IgECH_4$	Gly-Leu-Gln-Gly-Lys-Ile-Ala-Asp-Ala-Val-Lys-Ala-Lys-Gly-Gly-Gly-Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe (SEQ ID NO:48)
16	$TraT_2Th$-GG-$IgECH_4$	Gly-Leu-Ala-Ala-Gly-Leu-Val-Gly-Met-Ala-Ala-Asp-Ala-Met-Val-Glu-Asp-Val-Asn-Gly-Gly-Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe (SEQ ID NO:49)
17	$TraT_3Th$-GG-$IgECH_4$	Ser-Thr-Glu-Thr-Gly-Asn-Gln-His-His-Tyr-Gln-Thr-Arg-Val-Val-Ser-Asn-Ala-Asn-Lys-Gly-Gly-Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe (SEQ ID NO:50)

EXAMPLE 5

IMMUNIZATION OF RATS WITH LINEAR IMMUNOGENS (SEQ ID NOS:51-56,62) AND LINEAR IMMUNOGENS OF REVERSE POLARITY (SEQ ID NOS:57-60)

Peptide immunogens Nos. 18-23 (ID SEQ ID NOS:51-56) as shown in Table 5, were synthesized as described in Example 3. The formula for peptide immunogens Nos. 18-23 may be represented as A-Th-GG-IgECH4, wherein A is either the N terminus, Lys-Lys (2K), Lys-Lys-Lys (3K), or the invasin domain (Inv)(SEQ ID NO:25) separated from the Th sequence by a spacer GG; Th is selected from the group consisting of HB_s Th, PT_1 Th, PT_2 Th, MV_{F1} Th, or TT_3 Th; GG is a Gly-Gly spacer; and IgECH4 is the IgE CH4 decapeptide (SEQ ID NO:1).

Peptide immunogens with SEQ ID NOS:57-60, also shown in Table 5, as Peptide Nos. 24-27, were synthesized in an identical fashion to the Peptide Nos. 18-23. These peptides may be represented as IgECH4-GG-Th. These peptides are equivalent to Peptide Nos. 19,20,21,23 (Table 5) in terms of IgECH4 decapeptide, spacer, and Th sequences except that the decapeptide/Th polarity was reversed, i.e., the IgE CH4 decapeptide (SEQ ID NO:1) was on the N terminus while Th was located on the C terminus.

These peptide immunogens were used to immunize rats as described in the experimental protocol below, for comparison and demonstration of efficacy.

Experimental Design:

Immunogen: Peptide Nos. 18-28 (1 per group) (SEQ ID NOS:51-60 and 62)
Dose: 100 μg per immunization
Route: intramuscular
Adjuvant: Freund's Complete/Incomplete for Peptide Nos. 18-27, 0.4% Alum for

Peptide No. 28
Dose Schedule: week 0 (FCA), 3 and 6 weeks (IFA) for Peptide Nos. 8-27, Alum for Peptide No. 28 on weeks 0, 3, and 6
Bleed Schedule: weeks 0, 3, 6, 8, 10
Species: Sprague-Dawley rats
Group size: 5 for Peptide Nos. 27-28, 4 for Peptide No. 28
Assay: ELISA for anti-peptide activity, solid-phase substrate is Peptide No. 3 (SEQ ID NO:1).

Blood was collected, processed into serum, and stored prior to titering by ELISA as described in Example 2 with the exception of substituting horseradish peroxidase-labelled goat anti-rat IgG antibody for anti-guinea pig IgG as the tracer. All sera were assayed by anti-peptide ELISA and those samples which gave A_{492nm} values of ≥ 0.2 at a 1:100 dilution were recorded as seropositive.

The immunopotencies of Peptide immunogens Nos. 18-28 (SEQ ID NOS:51-60, and 62) were evaluated by the anti-peptide ELISA and are shown in Table 6 as the number of rats in each group of 4 or 5 that converted to seropositive reactivity for IgE CH4 Peptide No. 3 on weeks 6 and 8 (i.e., A_{492mm} ≥ 0.2 at a 1:100 dilution), in response to the experimental immunizations.

The peptide immunogens of this Example of polarity Th-GG-IgECH4 (Peptide Nos. 18-23 and 28, SEQ ID NOS:51-56 and 62) showed significant efficacy for the induction of antibodies to the IgE CH4 decapeptide (Peptide No. 3, SEQ ID NO:1). All 6 groups of rats immunized with the peptide immunogens of this polarity (Peptide Nos. 18-23, 28) showed significant conversion to seropositivity compared to the control. Prevalences of

seroconversion for the groups varied from 1/5 to 5/5 by week 6 and seroconversion prevalences continued to increase between weeks 6 and 8 in response to the third dose of immunogens. Peptide immunogen No. 18 containing the HB_s Th peptide sequence, Peptide immunogen No. 19 with the MV_{F1} Th peptide and Peptide No. 28 containing the PT_1Th peptide sequence were the most effective, with seroconversion prevalences of 4/5, 5/5 and 4/4, respectively, by week 8. Comparison of the immunogenicities of Peptide immunogens Nos. 21 and 22 (SEQ ID NOS:54,55) demonstrates that the Inv domain peptide provided significant improvement by week 8 to the immune stimulatory capability of the PT_2 Th-containing peptide (Table 6).

In contrast, the analogous peptide immunogens with reversed Th polarity (Peptide immunogens Nos. 24-27, SEQ ID NOS:57-60) failed to display significant immunopotency for the seroconversion of rats. This poor immunopotency shows that a Th-GG-IgECH4 amino to carboxyl terminus polarity is critical to the immunogenicity of the linear peptide immunogens of the invention. A determination of efficacy for one orientation of target peptide and Th over the other was not predictable by one skilled in the art and is unexpected.

TABLE 5

Sequences of Peptide Immunogens Nos. 18-28

Peptide Immunogen		Amino Acid Sequence
18	3K-HB_sTh-GG-$IgECH_4$	Phe-Phe-Leu-Leu-Thr-Arg-Ile-Leu-Thr-Ile-Pro-Gln-Ser-Leu-Asp-Gly-Gly-Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe (SEQ ID NO:51)

19	$MV_{F1}Th$-GG-$IgECH_4$	Leu-Ser-Glu-Ile-Lys-Gly-Val-Ile-Val-His-Arg-Leu-Glu-Gly-Val-Gly-Gly-Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe (SEQ ID NO:52)
20	PT_1Th-GG-$IgECH_4$	Lys-Lys-Leu-Arg-Arg-Leu-Leu-Tyr-Met-Ile-Tyr-Met-Ser-Gly-Leu-Ala-Val-Arg-Val-His-Val-Ser-Lys-Glu-Glu-Gln-Tyr-Tyr-Asp-Tyr-Gly-Gly-Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe (SEQ ID NO:53)
21	PT_2Th-GG-$IgECH_4$	Gly-Ala-Tyr-Ala-Arg-Cys-Pro-Asn-Gly-Thr-Arg-Ala-Leu-Thr-Val-Ala-Glu-Leu-Arg-Gly-Asn-Ala-Glu-Leu-Gly-Gly-Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe (SEQ ID NO:54)
22	Inv-GG-PT_2Th-GG-$IgECH_4$	Thr-Ala-Lys-Ser-Lys-Lys-Phe-Pro-Ser-Tyr-Thr-Ala-Thr-Tyr-Gln-Phe-Gly-Gly-Gly-Ala-Tyr-Ala-Arg-Cys-Pro-Asn-Gly-Thr-Arg-Ala-Leu-Thr-Val-Ala-Glu-Leu-Arg-Gly-Asn-Ala-Glu-Leu-Gly-Gly-Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe (SEQ ID NO:55)
23	TT_3Th-GG-$IgECH_4$	Tyr-Asp-Pro-Asn-Tyr-Leu-Arg-Thr-Asp-Ser-Asp-Lys-Asp-Arg-Phe-Leu-Gln-Thr-Met-Val-Lys-Leu-Phe-Asn-Asp-Arg-Phe-Leu-Gln-Thr-Met-Val-Lys-Leu-Phe-Asn-Arg-Ile-Lys-Gly-Gly-Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe (SEQ ID NO:56)
24	$IgECH_4$-GG-$MV_{F1}Th$	Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe-Gly-Gly-Leu-Ser-Glu-Ile-Lys-Gly-Val-Ile-Val-His-Arg-Leu-Glu-Gly-Val (SEQ ID NO:57)
25	$IgECH_4$-GG-PT_1Th	Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe-Gly-Gly-Lys-Lys-Leu-Arg-Arg-Leu-Leu-Tyr-Met-Ile-Tyr-Met-Ser-Gly-Leu-Ala-Val-Arg-Val-His-Val-His-Lys-Glu-Glu-Gln-Tyr-Tyr-Asp-Tyr (SEQ ID NO:58)

26	$IgECH_4$-GG-PT_2Th	Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe-Gly-Gly-Gly-Ala-Tyr-Ala-Arg-Cys-Pro-Asn-Glu-Thr-Arg-Ala-Leu-Thr-Val-Ala-Glu-Leu-Arg-Gly-Asn-Ala-Glu-Leu (SEQ ID NO:59)
27	$IgECH_4$-GG-TT_3Th	Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe-Gly-Gly-Tyr-Asp-Pro-Asn-Tyr-Leu-Arg-Thr-Asp-Ser-Asp-Lys-Asp-Arg-Phe-Leu-Gln-Thr-Met-Val-Lys-Leu-Phe-Asn-Asp-Arg-Phe-Leu-Gln-Thr-Met-Val-Lys-Leu-Phe-Asn-Arg-Ile-Lys (SEQ ID NO:60)
28	PT_1Th-$IgECH_4$	Lys-Lys-Leu-Arg-Arg-Leu-Leu-Tyr-Met-Ile-Tyr-Met-Ser-Gly-Leu-Ala-Val-Arg-Val-His-Val-Ser-Lys-Glu-Glu-Gln-Tyr-Tyr-Asp-Tyr-Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe (SEQ ID NO:62)

TABLE 6

Peptide Immunogen	Animals Seroconverted/group*	
	Week 6	Week 8
18 $3K\text{-}HB_sTh\text{-}GG\text{-}IgECH_4$ (SEQ ID NO:51)	4	4
19 $MV_{F1}Th\text{-}GG\text{-}IgECH_4$ (SEQ ID NO:52)	5	5
20 $PT_1Th\text{-}GG\text{-}IgECH_4$ (SEQ ID NO:53)	2	3
21 $PT_2Th\text{-}GG\text{-}IgECH_4$ (SEQ ID NO:54)	1	1
22 $Inv\text{-}GG\text{-}PT_2\text{-}GG\text{-}IgECH_4$ (SEQ ID NO:55)	1	3
23 $TT_3Th\text{-}GG\text{-}IgECH_4$ (SEQ ID NO:56)	3	3
24 $IgECH_4\text{-}GG\text{-}MV_{F1}Th$ (SEQ ID NO:57)	0	0
25 $IgECH_4\text{-}GG\text{-}PT_1Th$ (SEQ ID NO:58)	1	1
26 $IgECH_4\text{-}GG\text{-}PT_2Th$ (SEQ ID NO:59)	0	0
27 $IgECH_4\text{-}GG\text{-}TT_3Th$ (SEQ ID NO:60)	0	0
28 $PT_1Th\text{-}IgECH_4$ (SEQ ID NO:62)	4	4
Control Immunization (No peptide)	0	0

*5 animals per group for Peptide Nos. 18-27, 4 animals for Peptide No. 28

EXAMPLE 6

COCKTAIL OF LINEAR IMMUNOGENS FURTHER BROADENS THE RESPONSIVE POPULATION

Establishing the relative efficacies of the many different linear constructs containing IgE CH4 decapeptide and Th (Examples 3-5) permits selection of useful peptide immunogens to formulate a cocktail vaccine composition. Individual Th-GG-IgECH4 constructs carrying immunodominant promiscuous Th peptides derived from measles virus F protein, tetanus toxin and pertussis toxin (Peptide Nos. 19-23) were proven by the study of Example 5 to be efficacious in eliciting antibody responses to the IgECH4 decapeptide (SEQ ID NO:1). A formulation containing a mixture of these linear peptides may provide a desired maximum immunogenicity in a genetically diverse population.

The immunopotency of such a composition formulated to contain a mixture of synthetic peptides with the preferred "A-Th-GG-IgECH4" polarity, Peptide immunogens Nos. 19, 20, 21, 23 (Table 5) and Peptide immunogen No. 4 (Table 4, Example 4) were evaluated in rats by the protocol described in Example 5. Each animal in a group of 5 rats were immunized with 100 μg doses of an equimolar formulation of the 5 peptides, i.e. 20 μg of each peptide. **The number of rats that converted to seropositive reactivity by weeks 5 and 8 were 5 out of 5 (i.e., 100%) at both time intervals.**

The results demonstrate that a vaccine comprising a cocktail of the peptide immunogens of the present invention provides improved immunogenicity. It also indicates the potential for this mixture, and of like cocktails composed of individually efficacious peptides, to induce immunotherapeutic antibody responses in the genetically diverse human population.

EXAMPLE 7

IMMUNIZATIONS WITH COCKTAILS OF EFFICACIOUS LINEAR IMMUNOGENS

Establishing the relative efficacies of the many different linear constructs containing IgE CH4 decapeptide and Th (Examples 3-5) permits selection of useful peptides for a cocktail of immunogens. Individual constructs carrying a Gly-Gly spacer and promiscuous Th peptides derived from measles virus F protein, hepatitis B surface antigen, tetanus toxin and pertussis toxin in the immunogen cocktail are demonstrated to be efficacious (Table 6). A mixture of these linear peptide immunogens with specific polarity with proven efficacy may thus provide maximum immunogenicity in a genetically diverse population. The protocol below has been designed to demonstrate efficacy for compositions of the invention formulated as mixtures of synthetic peptide immunogens containing preferred "A-Th-GG-IgECH4" constructs.

Experimental Design:

Immunogens:	(1) Cocktail 1: Peptide Nos. 18, 19, 20 (2) Cocktail 2: Peptide Nos. 18, 19, 22 (3) Positive Control- KLH conjugate of Peptide 3 (One immunogen per group of rats)
Dose:	Molar equivalents of each synthetic peptide or IgE CH4 equivalent, to equal either 100 μg or 33.3 μg of peptide per immunization
Route:	intramuscular
Adjuvants:	(1) Freund's Complete/Incomplete (2) 0.4% Alum (Aluminum hydroxide) (One of either adjuvant per immunogen per group)
Dose Schedule:	week 0, 2 and 4 weeks

	(CFA/IFA groups receive CFA week 0, IFA weeks 2 and 4. Alum groups receive Alum formulations for all 3 doses)
Bleed Schedule:	weeks 0, 3, 6 and 8
Species:	Sprague-Dawley rats/group
Group size:	5, 6 groups
Assay:	ELISA for anti-peptide activity, solid-phase immunosorbent is Peptide No. 3 (SEQ ID NO:1).

Blood is collected, processed into serum, and stored prior to titering by ELISA as described in Example 5.

This experiment is designed to demonstrate improved performance of the immunogens of the present invention as compared to the known immunogens of the prior art[14,15]. The results are useful for the evaluation of two mixtures of efficacious peptide immunogens, each containing three Th peptides, demonstrate the usefulness of the immune stimulatory Inv domain (cocktail 2 contains Inv, cocktail 1 does not), and the efficacy of the adjuvant, Alum, in a vaccine composition of the invention.

EXAMPLE 8

CLINICAL TRIAL USING COCKTAILS OF IMMUNOGENS

Establishing the relative efficacies of the many different constructs containing IgE CH4 decapeptide and Th (Examples 3-5) permits selection of representative peptides for a cocktail of immunogens. Individual constructs carrying a Gly-Gly spacer and Th peptide sequences from measles virus F, hepatitis B surface antigen, tetanus toxin and pertussis toxin in the immunogen cocktail are of demonstrated efficacy (Table 6) and are promiscuous for multiple human HLA DR antigens, so

as to provide maximum immunogenicity in a genetically diverse human population. Moreover, because these Th peptides are derived from children's vaccines, childhood vaccinations are a potential source of Th memory in an immunized human population. Thus, children's vaccines have the potential to afford enhanced immunopotency to anti-allergy vaccines comprised of mixtures of such Th peptides. The clinical protocol below has been designed to demonstrate efficacy for compositions of the invention formulated as a mixture of such linear "A-Th-Spacer-IgE Ch4 decapeptide" peptide immunogens, in a widely acceptable adjuvant, Alum.

Experimental Design:

Subjects:	Hay fever patients
Season & Duration:	Hay fever seasons, 8 weeks
Groups:	4 groups, 1 group/immunogen/dose N=15 per group, 12 receive immunogen, 3 receive placebo
Immunogen:	Cocktail 1: Peptide Nos. 18, 19, 20, 23
Adjuvant:	0.2% Alum
Dose:	Molar equivalents of each synthetic peptide to equal 500 μg or 125 μg of peptide per dose
Route:	intramuscular
Dose Schedule:	week 0, and 4 weeks
Evaluation schedule:	weeks 0, 4, and 6

Blood is collected, processed into serum, and stored prior to titering by ELISA as described in Example 5.

Efficacy and safety of the vaccine composition "cocktail 1" are evaluated serologically, by skin reaction tests, the rate of patient usage of hay fever medication, physical examination of patients for allergic symptoms and adverse reactions, and interviewing the patients to obtain their subjective assessments of the effect of using the

products. Serological evaluations include the aforementioned ELISA for antipeptide titer, and a standard automated spectrofluorimetric assay to determine reduction in histamine levels[15] as well as to ascertain that the products do not trigger histamine release. The skin test is an intradermal test in which a standardized solution of allergens is injected into the upper layers of the skin. Reactions to the allergens are quantitated by determining the area of the typical "wheal and flare" produced in response to the allergens. The expected results include significant improvement in allergic symptoms at the endpoint of the study, and no evidence of histamine release triggered by the vaccine composition of the invention.

This experiment is designed to demonstrate the clinical efficacy of the invention. The results provide an evaluation of a mixture of "A-Th-Spacer-IgE CH4 decapeptide" immunogens containing four Th peptide sequences formulated with a pharmaceutically acceptable adjuvant, Alum.

EXAMPLE 9
IN VITRO ASSAY DEMONSTRATES EFFICACY OF IgE CH4 DECAPEPTIDE-SPECIFIC ANTIBODIES

Passively-sensitized human basophils are used in a well-known histamine-release assay for an in vitro evaluation of antibodies induced by immunizations with IgE CH4 decapeptide immunogens. Human basophils are prepared from the venous blood of volunteers and then passively sensitized with IgE specific for benzylpenicilloyl-human serum albumin conjugate (BPO-HSA) that is prepared from the blood of donors hyperimmunoglobulemic for BPO-HSA-specific IgE. Histamine release by the sensitized basophils is affected by the addition of either BPO-HSA or IgE CH4 Peptide No. 3 (SEQ ID NO:1). Prior to the addition of the agents to induce histamine release, the basophils are combined with serial dilutions of antiserum to IgE CH4 decapeptide (SEQ ID NO:1) or pre-immune control serum. Samples are analyzed for histamine release by the automated fluorescence technique. The percentage of histamine release is calculated from the ratio of sample to total basophil histamine content after spontaneous release is subtracted from both[27]. The capacity of the experimental antiserum to inhibit histamine release is demonstration of in vitro efficacy.

The ability of the IgE CH4 Peptide No. 3 (SEQ ID NO:1) to induce histamine release in a concentration-dependent manner was demonstrated by this assay. The results, presented in Table 7, showed that the IgE CH4 Peptide No. 3 (SEQ ID NO:1) induced histamine release by human basophils and served to validate the relevance of SEQ ID NO:1 and corresponding antibodies for the human allergic response.

TABLE 7

Inducer	% Net Histamine Release*
Peptide No. 3	
150 μg/mL (1.3×10^{-4}M)	30%
60 μg/mL (7×10^{-5}M)	13
6 μg/mL (7×10^{-6}M)	2
BPO-HSA	
0.1 μg/mL	63%

* Corrected by subtraction of spontaneous histamine release, 9%

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANTS: United Biomedical, Inc. & WANG, Chang Yi

(ii) TITLE OF INVENTION: SYNTHETIC PEPTIDE BASED IMMUNOGENS FOR THE TREATMENT OF ALLERGY

(iii) NUMBER OF SEQUENCES: 62

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Maria C.H. Lin
(B) STREET: 345 Park Avenue
(C) CITY: New York
(D) STATE: NY
(E) COUNTRY: USA
(F) ZIP: 10154

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: WordPerfect 5.1

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE: 24-MAR-1995
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/328,912
(B) FILING DATE: 25-OCT-1994

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/218,461
(B) FILING DATE: 28-MAR-1994

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/060,798
(B) FILING DATE: 10-MAY-1993

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 07/847,745
(B) FILING DATE: 06-MAR-1992

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 07/637,364
(B) FILING DATE: 04-JAN-1991

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Maria C.H. Lin
(B) REGISTRATION NUMBER: 29,323
(C) REFERENCE/DOCKET NUMBER: 1151-4061US4

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 212-758-4800
(B) TELEFAX: 212-751-6849

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10
(B) TYPE: amino acid
(C) STRANDEDNESS: not applicable
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(x) PUBLICATION INFORMATION:
(A) AUTHORS: Stanworth et al.
(B) TITLE: The Role Of Non-Antigen Receptors In Mast Cell Signalling Processes
(C) JOURNAL: Molecular Immunology
(D) VOLUME: 21
(E) ISSUE: 12
(F) PAGES: 1183-1190
(G) DATE: 1984
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES: 497 to 506

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

Lys Thr Lys Gly Ser Gly Phe Phe Val Phe
1 5 10

(3) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 325
(B) TYPE: amino acids
(C) STRANDEDNESS: not applicable
(D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE: Poylpeptide ε-chain of human IgE

(x) PUBLICATION INFORMATION:
(A) AUTHORS: Dorrington and Bennich
(B) TITLE:
(C) JOURNAL: Immunology Review
(D) VOLUME: 41

(E) ISSUE:
(F) PAGES: 3-25
(G) DATE: 1978

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

```
Val Cys Ser Arg Asp Phe Thr Pro Pro Thr Val Lys Ile Leu Gln
 5                  10                  15
Ser Ser Cys Asp Gly Gly Gly His Phe Pro Pro Thr Ile Gln Leu
            20                  25                  30
Leu Cys Leu Val Ser Gly Tyr Thr Pro Gly Thr Ile Asn Ile Thr
        35                  40                  45
Trp Leu Glu Asp Gly Gln Val Met Asp Val Asp Leu Ser Thr Ala
    50                  55                  60
Ser Thr Thr Gln Glu Gly Glu Leu Ala Ser Thr Gln Ser Gln Leu
65                  70                  75
Thr Leu Ser Gln Lys His Trp Leu Ser Asp Arg Thr Tyr Thr Cys
            80                  85                  90
Gln Val Thr Tyr Gln Gly His Thr Phe Gln Asp Ser Thr Lys Lys
        95                  100                 105
Cys Ala Asp Ser Asn Pro Arg Gly Val Ser Ala Tyr Leu Ser Arg
    110                 115                 120
Pro Ser Pro Phe Asp Leu Phe Ile Arg Lys Ser Pro Thr Ile Thr
125                 130                 135
Cys Leu Val Leu Asp Leu Ala Pro Ser Lys Gly Thr Val Asn Leu
            140                 145                 150
Thr Trp Ser Arg Ala Ser Gly Lys Pro Val Asn Asn Ser Thr Arg
        155                 160                 165
Lys Glu Glu Lys Gln Arg Asn Gly Thr Leu Thr Val Thr Ser Thr
    170                 175                 180
Leu Pro Val Gly Thr Arg Asp Trp Ile Glu Gly Glu Thr Tyr Gln
185                 190                 195
Cys Arg Val Thr His Pro His Leu Pro Arg Ala Leu Met Arg Ser
            200                 205                 210
Thr Thr Lys Thr Ser Gly Pro Arg Ala Ala Pro Glu Val Tyr Ala
        215                 220                 225
Phe Ala Thr Pro Glu Trp Pro Gly Ser Arg Asp Lys Arg Thr Leu
    230                 235                 240
Ala Cys Leu Ile Gln Asn Phe Met Pro Glu Asp Ile Ser Val Gln
245                 250                 255
Trp Leu His Asn Glu Val Gln Leu Pro Asp Ala Arg His Ser Thr
            260                 265                 270
Thr Gln Pro Arg Lys Thr Lys Gly Ser Gly Phe Phe Val Phe Ser
        275                 280                 285
Arg Leu Glu Val Thr Arg Ala Glu Trp Gln Glu Lys Asp Glu Phe
    290                 295                 300
Ile Cys Arg Ala Val His Glu Ala Ala Ser Pro Ser Gln Thr Val
305                 310                 315
Gln Arg Ala Val Ser Val Asn Pro Gly Lys
            320                 325
```

(4) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175
(B) TYPE: amino acids
(C) STRANDEDNESS: not applicable
(D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE: Polypeptide ε-chain of rat IgE

(x) PUBLICATION INFORMATION:
(A) AUTHORS: Kindsrogel et al.
(B) TITLE:
(C) JOURNAL: DNA
(D) VOLUME: 1
(E) ISSUE:
(F) PAGES: 335-343
(G) DATE: 1982

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

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

(5) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 315
(B) TYPE: amino acids
(C) STRANDEDNESS: not applicable
(D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE: Polypeptide ε-chain of mouse IgE

(x) PUBLICATION INFORMATION:
(A) AUTHORS: Ishida et al.
(B) TITLE:
(C) JOURNAL: EMBO
(D) VOLUME: 1
(E) ISSUE:
(F) PAGES: 1117-1123
(G) DATE: 1982

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

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

Gln Phe Thr Cys Gln Val Ile His Glu Ala Leu Gln Lys Pro Arg
305 310 315

(6) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15
(B) TYPE: amino acid
(C) STRANDEDNESS: not applicable
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

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

Phe Phe Leu Leu Thr Arg Ile Leu Thr Ile Pro Gln Ser Leu Asp
1 5 10 15

(7) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28
(B) TYPE: amino acid
(C) STRANDEDNESS: not applicable
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

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

Lys Lys Leu Arg Arg Leu Leu Tyr Met Ile Tyr Met Ser Gly Leu
1 5 10 15
Ala Val Arg Val His Val Ser Lys Glu Glu Gln Tyr Tyr Asp Tyr
20 25 30

(8) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17
(B) TYPE: amino acid
(C) STRANDEDNESS: not applicable
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

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

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

(9) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22
(B) TYPE: amino acid
(C) STRANDEDNESS: not applicable
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

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

Lys Lys Phe Asn Asn Phe Thr Val Ser Phe Trp Leu Arg Val Pro
1 5 10 15
Lys Val Ser Ala Ser His Leu
20

(10) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15
(B) TYPE: amino acid
(C) STRANDEDNESS: not applicable
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

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

Tyr Met Ser Gly Leu Ala Val Arg Val His Val Ser Lys Glu Glu
1 5 10 15

(11) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27
(B) TYPE: amino acid
(C) STRANDEDNESS: not applicable
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

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

Tyr Asp Pro Asn Tyr Leu Arg Thr Asp Ser Asp Lys Asp Arg Phe
1 5 10 15
Leu Gln Thr Met Val Lys Leu Phe Asn Arg Ile Lys
20 25

(12) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: amino acid
(C) STRANDEDNESS: not applicable
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

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

Gly Ala Tyr Ala Arg Cys Pro Asn Gly Thr Arg Ala Leu Thr Val
1 5 10 15
Ala Glu Leu Arg Gly Asn Ala Glu Leu
20

(13) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15
(B) TYPE: amino acid
(C) STRANDEDNESS: not applicable
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

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

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

(14) INFORMATION FOR SEQ ID NO:13:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39
(B) TYPE: amino acids
(C) STRANDEDNESS: not applicable
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE:
(A) DESCRIPTION: peptide

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

Lys Thr Lys Gly Ser Gly Phe Phe Val Phe Gly Pro Gly Lys Thr
5 10 15
Lys Gly Ser Gly Phe Phe Val Phe Gly Pro Gly Lys Thr Lys Gly
20 25 30
Ser Gly Phe Phe Val Phe Gly Lys Met
35

(15) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: amino acids
(C) STRANDEDNESS: not applicable
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE:
(A) DESCRIPTION: peptide

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

Val Ser Phe Gly Val Trp Ile Arg Thr Pro Pro Ala Tyr Arg Pro
1 5 10 15
Pro Asn Ala Pro Ile Leu
20

(16) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28
(B) TYPE: amino acid
(C) STRANDEDNESS: not applicable
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

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

Lys Lys Lys Phe Phe Leu Leu Thr Arg Ile Leu Thr Ile Pro Gln
1 5 10 15
Ser Leu Asp Lys Thr Lys Gly Ser Gly Phe Phe Val Phe
20 25

(17) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38
(B) TYPE: amino acid
(C) STRANDEDNESS: not applicable

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

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

```
Lys Lys Leu Arg Arg Leu Leu Tyr Met Ile Tyr Met Ser Gly Leu
1               5                  10                  15
Ala Val Arg Val His Val Ser Lys Glu Glu Gln Tyr Tyr Asp Tyr
            20                  25                  30
Lys Thr Lys Gly Ser Gly Phe Phe Val Phe
            35                  40
```

(18) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25
(B) TYPE: amino acid
(C) STRANDEDNESS: not applicable
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

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

```
Tyr Met Ser Gly Leu Ala Val Arg Val His Val Ser Lys Glu Glu
1               5                  10                  15
Lys Thr Lys Gly Ser Gly Phe Phe Val Phe
            20                  25
```

(19) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27
(B) TYPE: amino acid
(C) STRANDEDNESS: not applicable
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

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

```
Lys Lys Gln Tyr Ile Lys Ala Asn Ser Lys Phe Ile Gly Ile Thr
1               5                  10                  15
Glu Leu Lys Thr Lys Gly Ser Gly Phe Phe Val Phe
            20                  25
```

(20) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 32
(B) TYPE: amino acid
(C) STRANDEDNESS: not applicable
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

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

Lys Lys Phe Asn Asn Phe Thr Val Ser Phe Trp Leu Arg Val Pro
1 5 10 15
Lys Val Ser Ala Ser His Leu Lys Thr Lys Gly Ser Gly Phe Phe
20 25 30
Val Phe

(21) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37
(B) TYPE: amino acid
(C) STRANDEDNESS: not applicable
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

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

Tyr Asp Pro Asn Tyr Leu Arg Thr Asp Ser Asp Lys Asp Arg Phe
1 5 10 15
Leu Gln Thr Met Val Lys Leu Phe Asn Arg Ile Lys Lys Thr Lys
20 25 30
Gly Ser Gly Phe Phe Val Phe
35

(22) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34
(B) TYPE: amino acid
(C) STRANDEDNESS: not applicable
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

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

Gly Ala Tyr Ala Arg Cys Pro Asn Gly Thr Arg Ala Leu Thr Val
1 5 10 15

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

(23) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25
(B) TYPE: amino acid
(C) STRANDEDNESS: not applicable
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

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

Ser Glu Ile Lys Gly Val Ile Val His Arg Leu Glu Gly Val Leu
1 5 10 15
Lys Thr Lys Gly Ser Gly Phe Phe Val Phe
20 25

(24) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26
(B) TYPE: amino acids
(C) STRANDEDNESS: not applicable
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

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

Lys Thr Lys Gly Ser Gly Phe Phe Val Phe Gly Pro Gly Lys Thr
5 10 15
Lys Gly Ser Gly Phe Phe Val Phe Gly Lys Met
20 25

(25) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6
(B) TYPE: amino acids
(C) STRANDEDNESS: not applicable
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: linking group

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

Pro Pro Xaa Pro Xaa Pro
5

(2) INFORMATION FOR SEQ ID NO:25:

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

(ii) MOLECULE TYPE: peptide

Thr Ala Lys Ser Lys Lys Phe Pro Ser Tyr Thr Ala Thr Tyr Gln Phe
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:26:

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

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Gly Ile Leu Glu Ser Arg Gly Ile Lys Ala Arg Ile Thr His Val Asp
1 5 10 15
Thr Glu Ser Tyr
20

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Trp Val Arg Asp Ile Ile Asp Asp Phe Thr Asn Glu Ser Ser Gln Lys
1 5 10 15
Thr

(2) INFORMATION FOR SEQ ID NO:28:

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

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Asp Val Ser Thr Ile Val Pro Tyr Ile Gly Pro Ala Leu Asn His Val
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Ala Leu Asn Ile Trp Asp Arg Phe Asp Val Phe Cys Thr Leu Gly Ala
1 5 10 15
Thr Thr Gly Tyr Leu Lys Gly Asn Ser
20 25

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Asp Ser Glu Thr Ala Asp Asn Leu Glu Lys Thr Val Ala Ala Leu Ser
1 5 10 15
Ile Leu Pro Gly Ile Gly Cys
20

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

```
Glu Glu Ile Val Ala Gln Ser Ile Ala Leu Ser Ser Leu Met Val Ala
1               5                  10                  15
Gln Ala Ile Pro Leu Val Gly Glu Leu Val Asp Ile Gly Phe Ala Ala
            20                  25                  30
Thr Asn Phe Val Glu Ser Cys
        35
```

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

```
Asp Ile Glu Lys Lys Ile Ala Lys Met Glu Lys Ala Ser Ser Val Phe
1               5                  10                  15
Asn Val Val Asn Ser
            20
```

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

```
Lys Trp Phe Lys Thr Asn Ala Pro Asn Gly Val Asp Glu Lys Ile Arg
1               5                  10                  15
Ile
```

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Gly Leu Gln Gly Lys Ile Ala Asp Ala Val Lys Ala Lys Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Gly Leu Ala Ala Gly Leu Val Gly Met Ala Ala Asp Ala Met Val Glu
1 5 10 15
Asp Val Asn

(2) INFORMATION FOR SEQ ID NO:36:

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

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Ser Thr Glu Thr Gly Asn Gln His His Tyr Gln Thr Arg Val Val Ser
1 5 10 15
Asn Ala Asn Lys
20

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

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

Leu Gly Gly Lys Thr Lys Gly Ser Gly Phe Phe Val Phe
20 25

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Lys Lys Phe Asn Asn Phe Thr Val Ser Phe Trp Leu Arg Val Pro Lys
1 5 10 15
Val Ser Ala Ser His Leu Gly Gly Lys Thr Lys Gly Ser Gly Phe Phe
20 25 30
Val Phe

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Tyr Met Ser Gly Leu Ala Val Arg Val His Val Ser Lys Glu Glu Gly
1 5 10 15
Gly Lys Thr Lys Gly Ser Gly Phe Phe Val Phe
20 25

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

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

Thr Glu Ser Tyr Gly Gly Lys Thr Lys Gly Ser Gly Phe Phe Val Phe
20 25 30

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Trp Val Arg Asp Ile Ile Asp Asp Phe Thr Asn Glu Ser Ser Gln Lys
1 5 10 15
Thr Gly Gly Lys Thr Lys Gly Ser Gly Phe Phe Val Phe
20 25

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Asp Val Ser Thr Ile Val Pro Tyr Ile Gly Pro Ala Leu Asn His Val
1 5 10 15
Gly Gly Lys Thr Lys Gly Ser Gly Phe Phe Val Phe
20 25

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Ala Leu Asn Ile Trp Asp Arg Phe Asp Val Phe Cys Thr Leu Gly Ala
1 5 10 15

Thr Thr Gly Tyr Leu Lys Gly Asn Ser Gly Gly Lys Thr Lys Gly Ser
20 25 30
Gly Phe Phe Val Phe
35

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Asp Ser Glu Thr Ala Asp Asn Leu Glu Lys Thr Val Ala Ala Leu Ser
1 5 10 15
Ile Leu Pro Gly Ile Gly Cys Gly Gly Lys Thr Lys Gly Ser Gly Phe
20 25 30
Phe Val Phe
35

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

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

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

```
Asp Ile Glu Lys Lys Ile Ala Lys Met Glu Lys Ala Ser Ser Val Phe
1               5                  10                  15
Asn Val Val Asn Ser Gly Gly Lys Thr Lys Gly Ser Gly Phe Phe Val
            20                  25                  30
Phe
```

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

```
Lys Trp Phe Lys Thr Asn Ala Pro Asn Gly Val Asp Glu Lys Ile Arg
1               5                  10                  15
Ile Gly Gly Lys Thr Lys Gly Ser Gly Phe Phe Val Phe
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

```
Gly Leu Gln Gly Lys Ile Ala Asp Ala Val Lys Ala Lys Gly Gly Gly
1               5                  10                  15
Lys Thr Lys Gly Ser Gly Phe Phe Val Phe
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

```
Gly Leu Ala Ala Gly Leu Val Gly Met Ala Ala Asp Ala Met Val Glu
1               5                  10                  15
Asp Val Asn Gly Gly Lys Thr Lys Gly Ser Gly Phe Phe Val Phe
            20                  25                  30
```

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

```
Ser Thr Glu Thr Gly Asn Gln His His Tyr Gln Thr Arg Val Val Ser
1               5                  10                  15
Asn Ala Asn Lys Gly Gly Lys Thr Lys Gly Ser Gly Phe Phe Val Phe
            20                  25                  30
```

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

```
Phe Phe Leu Leu Thr Arg Ile Leu Thr Ile Pro Gln Ser Leu Asp Gly
1               5                  10                  15
Gly Lys Thr Lys Gly Ser Gly Phe Phe Val Phe
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

```
Leu Ser Glu Ile Lys Gly Val Ile Val His Arg Leu Glu Gly Val Gly
1               5                  10                  15
Gly Lys Thr Lys Gly Ser Gly Phe Phe Val Phe
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

```
Lys Lys Leu Arg Arg Leu Leu Tyr Met Ile Tyr Met Ser Gly Leu Ala
1               5                  10                  15
Val Arg Val His Val Ser Lys Glu Glu Gln Tyr Tyr Asp Tyr Gly Gly
            20                  25                  30
Lys Thr Lys Gly Ser Gly Phe Phe Val Phe
        35                  40
```

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

```
Gly Ala Tyr Ala Arg Cys Pro Asn Gly Thr Arg Ala Leu Thr Val Ala
1               5                  10                  15
Glu Leu Arg Gly Asn Ala Glu Leu Gly Gly Lys Thr Lys Gly Ser Gly
            20                  25                  30
Phe Phe Val Phe
        35
```

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 54 amino acids
(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

```
Thr Ala Lys Ser Lys Lys Phe Pro Ser Tyr Thr Ala Thr Tyr Gln Phe
1               5                  10                  15
Gly Gly Gly Ala Tyr Ala Arg Cys Pro Asn Gly Thr Arg Ala Leu Thr
            20                  25                  30
Val Ala Glu Leu Arg Gly Asn Ala Glu Leu Gly Gly Lys Thr Lys Gly
        35                  40                  45
Ser Gly Phe Phe Val Phe
    50
```

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

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

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

```
Lys Thr Lys Gly Ser Gly Phe Phe Val Phe Gly Gly Leu Ser Glu Ile
1               5                  10                  15
Lys Gly Val Ile Val His Arg Leu Glu Gly Val
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

```
Lys Thr Lys Gly Ser Gly Phe Phe Val Phe Gly Gly Lys Lys Leu Arg
1               5                  10                  15
Arg Leu Leu Tyr Met Ile Tyr Met Ser Gly Leu Ala Val Arg Val His
            20                  25                  30
Val His Lys Glu Glu Gln Tyr Tyr Asp Tyr
        35                  40
```

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

```
Lys Thr Lys Gly Ser Gly Phe Phe Val Phe Gly Gly Gly Ala Tyr Ala
1               5                  10                  15
Arg Cys Pro Asn Glu Thr Arg Ala Leu Thr Val Ala Glu Leu Arg Gly
            20                  25                  30
Asn Ala Glu Leu
        35
```

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 52 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

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

(2) INFORMATION FOR SEQ ID NO:61:

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

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

```
Leu Ser Glu Ile Lys Gly Val Ile Val His Arg Leu Glu Gly Val
1               5                  10                  15
```

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

```
Lys Lys Leu Arg Arg Leu Leu Tyr Met Ile Tyr Met Ser Gly Leu Ala
1               5                  10                  15
Val Arg Val His Val Ser Lys Glu Glu Gln Tyr Tyr Asp Tyr Lys Thr
            20                  25                  30
Lys Gly Ser Gly Phe Phe Val Phe
        35                  40
```

I claim:

1. A peptide immunogen represented by the formula:

$$(A)_n\text{-}(Th)_m\text{-}(B)_o\text{-}(\text{IgE CH4 peptide})_p$$

wherein: A is an amino acid, α-NH_2, a fatty acid or a derivative thereof, or an invasin;
B is an amino acid;
Th is a helper T cell epitope, an analog or segment thereof;
IgE CH4 peptide is SEQ ID NO:1 or an immunogenic analog thereof;
n is from 1 to 10;
m is from 1 to 4;
o is from 0 to 10; and
p is from 1 to 3.

2. The peptide immunogen of Claim 1 wherein p is 1.

3. The peptide immunogen of Claim 1 wherein Th is selected from the group consisting SEQ ID NOS:5-12, 14, 26-36, 61 and an immunogenic analog or segment thereof.

4. The peptide immunogen of Claim 2 wherein Th is selected from the group consisting SEQ ID NOS:5-12, 14, 26-36, 61 and an immunogenic analog or segment thereof.

5. The peptide immunogen of Claim 1 selected from the group consisting SEQ ID NOS:13, 15-23, 37-50, 51-56 and 62.

6. The peptide immunogen of Claims 3 selected from the group consisting SEQ ID NOS:51-56 and 62.

7. The peptide immunogen of Claim 1 wherein A is a fatty acid.

8. The peptide immunogen of Claim 2 wherein A is a fatty acid.

9. The peptide immunogen of Claim 1 wherein A is a fatty acid derivative.

10. The peptide immunogen of Claim 2 wherein A is a fatty acid derivative.

11. The peptide immunogen of Claim 9 wherein the fatty acid derivative is Pam_3Cys.

12. The peptide immunogen of Claim 10 wherein the fatty acid derivative is Pam_3Cys.

13. A vaccine composition comprising an effective amount of a peptide immunogen of Claim 1 in a pharmaceutically acceptable delivery system.

14. A vaccine composition comprising an effective amount of a peptide immunogen of Claim 2 in a pharmaceutically acceptable delivery system.

15. A vaccine composition comprising an effective amount of a peptide immunogen of Claim 3 in a pharmaceutically acceptable delivery system.

16. A vaccine composition comprising an effective amount of a peptide immunogen of Claim 4 in a pharmaceutically acceptable delivery system.

17. A vaccine composition comprising an effective amount of a peptide immunogen of Claim 5 in a pharmaceutically acceptable delivery system.

18. A vaccine composition comprising an effective amount of a peptide immunogen of Claim 6 in a pharmaceutically acceptable delivery system.

19. A vaccine composition comprising an effective amount of a peptide immunogen of Claim 7 in a pharmaceutically acceptable delivery system.

20. A vaccine composition comprising an effective amount of a peptide immunogen of Claim 8 in a pharmaceutically acceptable delivery system.

21. A vaccine composition comprising an effective amount of a peptide immunogen of Claim 9 in a pharmaceutically acceptable delivery system.

22. A vaccine composition comprising an effective amount of a peptide immunogen of Claim 10 in a pharmaceutically acceptable delivery system.

23. A vaccine composition comprising an effective amount of a peptide immunogen of Claim 11 in a pharmaceutically acceptable delivery system.

24. A vaccine composition comprising an effective amount of a peptide immunogen of Claim 12 in a pharmaceutically acceptable delivery system.

25. A vaccine composition comprising an effective amount of a mixture of peptide immunogens of Claim 9 in a pharmaceutically acceptable delivery system.

26. A vaccine composition comprising an effective amount of a mixture of peptide immunogens of Claim 10 in a pharmaceutically acceptable delivery system.

27. A vaccine composition comprising an effective amount of a mixture of peptide immunogens of Claim 11 in a pharmaceutically acceptable delivery system.

28. A vaccine composition comprising an effective amount of a mixture of peptide immunogens of Claim 12 in a pharmaceutically acceptable delivery system.

29. A method of treating allergic reactions by administering an effective amount of a vaccine composition according to Claim 13.

30. A method of treating allergic reactions by administering an effective amount of a vaccine composition according to Claim 14.

31. A method of treating allergic reactions by administering an effective amount of a vaccine composition according to Claim 15.

32. A method of treating allergic reactions by administering an effective amount of a vaccine composition according to Claim 16.

33. A method of treating allergic reactions by administering an effective amount of a vaccine composition according to Claim 17.

34. A method of treating allergic reactions by administering an effective amount of a vaccine composition according to Claim 18.

35. A method of treating allergic reactions by administering an effective amount of a vaccine composition according to Claim 19.

36. A method of treating allergic reactions by administering an effective amount of a vaccine composition according to Claim 20.

37. A method of treating allergic reactions by administering an effective amount of a vaccine composition according to Claim 21.

38. A method of treating allergic reactions by administering an effective amount of a vaccine composition according to Claim 22.

39. A method of treating allergic reactions by administering an effective amount of a vaccine composition according to Claim 23.

40. A method of treating allergic reactions by administering an effective amount of a vaccine composition according to Claim 24.

41. A method of treating allergic reactions by administering an effective amount of a vaccine composition according to Claim 25.

42. A method of treating allergic reactions by administering an effective amount of a vaccine composition according to Claim 26.

43. A method of treating allergic reactions by administering an effective amount of a vaccine composition according to Claim 27.

44. A method of treating allergic reactions by administering an effective amount of a vaccine composition according to Claim 28.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/03741

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07K 19/00, 16/46; A16K 39/395
US CL :424/275.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/275.1; 530/402, 406, 324

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Medline, Derwent
search terms allergy, treatment, IgE, anti-IgE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	EP, A1 0 403 312 , (STANWORTH ET AL.) 19 December 1990, see claims 1 and 11.	1, 2, 13, 14, 29, 30 ------- 1-44
Y	Molecular Immunology, Volume 30, Number 11, issued 1993, Shaw et al., "Influence of the T-Helper Epitope on the Titre and Affinity of Antibodies to B-Cell Epitopes after Co-immunization", pages 961-968, see table 1.	4, 16, 32
Y	Infection and Immunity, Volume 60, Number 11, issued November 1992, Chong et al., "Identification of T- and B-Cell Epitopes of the S2 and S3 Subunits of Pertussis Toxin by Use of Synthetic Peptides", pages 4640-4647, see abstract and table 1.	3-6,15-18, 31-34

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 26 MAY 1995	Date of mailing of the international search report 19 JUN 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer LAWRENCE J. CARROLL, II Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/03741

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Journal of Experimental Medicine, Volume 172, issued July, 1990, Su et al., "Identification and Characterization of T Helper Cell Epitopes of the Major Outer Membrane Protein of Chlamydia trachomatis", pages 203-212, see abstract and page 208.	3-5, 15-17, 31-33
Y	Journal of Immunology, Volume 152, issued 1994, Reynolds et al., "T and B Epitope Determination and Analysis of Multiple Antigenic Peptides for the Schistosoma mansoni Experimental Vaccine Triose-Phosphate Isomerase", pages 193-200, see figure 2.	3-5, 15-17, 31-33
Y	Nature, Volume 336, Number 22, issued 1988, Sinigaglia et al., "A Malaria T-cell epitope recognised with most mouse and human MHC class II molecules", pages778-780, see table 2.	3-5, 15-17, 31-33
Y	Vaccine, Volume 11, Number 13, issued 1993, Russell-Jones et al., " Peptide sequences with strong stimulatory activity for lymphoid cells: implications for vaccine developement", pages 1310-1315, see table 1.	3-5, 15-17, 31-33
Y	Journal of Immunology, Volume 151, Number 11, issued 1993, Reece et al., "Mapping the Major Human T Helper Epitopes of Tetanus Toxin", pages 6175-6184, see entire document.	3-6, 15-18, 31-34
Y	International Journal ofPeptide and Protein Research, Volume 40, issued 1992, Wiesmuller et al., "Solid phase synthesis of lipopeptide vaccines eliciting epitope-specific B-, T-helper and T-killer cell response", pages 255-260, see entire document.	1-44
Y	Molecular Immunology, Volume 29, Number 5, issued 1992, Partidos et al., "The Effect of Orientation of Epitopes on the Immunogenicity of Chimeric Synthetic Peptides Representing Measles Virus Protein Sequences", pages 651-658, see entire document	1-44