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(54) Title: HISTAMINE DERIVATIVES AND METHODS FOR THEIR USE AS IMMUNOMODULATORS (57) Abstract The present invention provides methods for using histamine derivatives as immunomodulators and in immunotherapeutics. More specifically the present invention provides methods for inhibiting at least a portion of an antigen specific antibody response and/or a portion of a T cell proliferative response by the immune system of a mammal comprising administering to said mammal an effective amount of a composition comprising at least one histamine derivative having binding specificity for at least one histamine receptor. The invention also pertains to methods of treating sensitivity to a particular antigen and methods of treating T cell mediated disease in a person by administering to the person a therapeutically effective amount of a composition comprising at least one histamine derivative having binding specificity for at least one histamine receptor, optionally administering to the person the antigen or an immunogenic portion thereof and further optionally administering to the person a peptide comprising at least one T cell epitope of said antigen.		

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HISTAMINE DERIVATIVES AND METHODS FOR THEIR
USE AS IMMUNOMODULATORS

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FIELD OF THE INVENTION

The invention relates generally to methods for modulating the immune system of mammals and more particularly to methods of modulating the immune system using compositions comprising histamine derivatives.

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BACKGROUND OF THE INVENTION

With the increased level of understanding concerning the immune response process in mammals, there is a growing awareness that certain molecules play a significant role in immune modulation. Unfortunately, these molecules are generally nonspecific as to their effects on single cell types in a mixture of cells. A critical need exists for agonists that are effect or cell specific.

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Histamine is a small molecule that has been shown to have a significant role in the immune response process in mammals. However, its ubiquitous effects on many cells that have receptors for histamine limits its possible immunotherapeutic use. Histamine derivatives that are tissue directed or effect specific would significantly aid in determining the role of histamine in immune modulation and produce valuable immunotherapeutics.

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Histamine can substantially modulate models of immune responses in mammals, particularly models of delayed hypersensitivity and T and B cell functions. Histamine is synthesized during different phases of response to antigen and is able directly or indirectly to effect further responses to antigen. It is possible that the concentration of histamine in tissue during inflammation and immune response can modify the function of a number of lymphoid cells. Although these effects may be substantial, the direct effect on single cell types in a mixture of cells cannot be determined unless the agonists are effect or cell specific. Ubiquitous effects of agonists on all cells that have receptors for histamine would limit any immunotherapeutic use of histamine. See Khan, et al., *Clin Immunol. Rev.* (1985) 4:1 Melmon, et al., *Am. J. Med* (1981) 71:100: and Roclin et al., *Cell Immunol.* (1978) 37:162.

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Histamine is an autacoid as are catecholamines, prostaglandins and some peptides, e.g., bradykinin and probably lymphokines. Autacoids differ from hormones in that they are made at their local sites of action and they can be made in a variety of tissues. Autacoids play an important role in mediating inflammation.

- 5 During inflammation, certain events may occur which include: protein denaturation, lowering of local pH, release of "new peptides" and lysosomal enzymes, and the like. Such events create a setting in which the immune system should not overreact to the new products. Yet, despite the ability of inflammation to generate likely immunogens, the inflammatory process usually is not accompanied or followed by
- 10 grossly abnormal immune responses. Autacoids appear to somehow modulate this response.

Autacoids affect natural suppressor cells, T cell subsets and B cells during various stages of immunity. Receptors for autacoids are non-randomly distributed (in number and affinity for agonist) on cells that carry out immune functions.

- 15 Precursor B cells do not appear to have histamine and catecholamine receptors, while B cells committed to produce antibodies do. T suppressor (T_S) cells modulate the CAMP responses of T helper (T_H) and T cytotoxic (T_C) cells to histamine. Mitogens alter responsiveness of these cells to histamine. In some cells biologic response is inhibitory (e.g., reduced release of antibody from B cells; inhibition of lymphokine
- 20 release or lysis of target cells by T effector cells and inhibition of release of histamine from mast cells); in others the response enhances immune function (e.g., enhanced suppression by natural suppressor and T_S cells or T helper (T_H) cell proliferation. The autacoids seem to be enhancing selected early events in immune response (e.g., enhanced suppressor function) while inhibiting later phases of phenotypic
- 25 manifestations (e.g., release of lymphokines or antibodies) of immunity.

- The appearance of naturally occurring suppressor cells in the spleens of neonatal or irradiated mice may have a key role in induction of immune tolerance. See Strober et al., *Ann. Rev. Immunol.* (1984) 2:219; Hertel-Wulff et al., *J. Immunol.* (1984) 133:2791; Okada et al., *J. Expt. Med.* (1982) 156:522; and Okada et al., *J.*
- 30 *Immunol.* (1982) 129:1892. These cells are related to NK cells in terms of their surface phenotype but differ in function. The natural suppressor cells appear briefly during the early maturation of lymphoid tissue but can be induced in adults by total lymphoid irradiation. The cells have the unique feature of inhibiting the antigen-specific cytolytic arm of alloreactive immune response but leave the antigen-specific
- 35 suppressive arm intact. In this way, alloreactions in the regulatory milieu of natural suppressor (NS) cells generate large numbers of antigen-specific suppressor cells that

in turn maintain tolerance in vivo. Thus, the natural suppressor cells may play an important role in preventing the development of host versus graft and graft versus host diseases in allogenic bone marrow chimeras, and in immune tolerances in the neonatal and total lymphoid irradiated (TLI) mice.

5 Histamine activates human T_S cells and enhances the suppressive ability of murine NS cells in vitro. See, Khan et al., *J. Immunol.* (1985) 134:4100 and Sansoni et al., *J. Clin. Invest.* (1985) 75:650. After pretreatment of both human T_S cells (Leu2., 9.3) with histamine, both phytohemagglutinin-induced T_H cell proliferation and pokeweed mitogen-induced B cell differentiation were inhibited. The effects
10 were mediated via H₂ receptors. The enhancement of natural suppressor function is via H₁ receptors. Natural suppressor cells can be propagated and cloned in long-term tissue culture and cause nonspecific suppression in both in vitro and in vivo models of mixed leukocyte reactions.

Therefore, methods using histamine derivatives that have little or no systemic
15 effects in immune modulation and immunotherapeutics would be advantageous.

SUMMARY OF THE INVENTION

The present invention provides methods for inhibiting at least a portion of an antigen specific antibody response and/or a portion of a T cell proliferative response
20 by the immune system of a mammal comprising administering to the mammal an effective amount of a composition comprising at least one histamine derivative having binding specificity for at least one histamine receptor and optionally administering a predetermined antigen or immunogenic portion thereof.

The invention also provides methods of treating sensitivity to a particular
25 antigen and methods of treating T cell mediated diseases in a person by administering to the person a therapeutically effective amount of a composition comprising at least one histamine derivative having binding specificity for at least one histamine receptor, e.g. H₁, H₂, H₃, or H_x and a pharmaceutically acceptable carrier or diluent. In one variation of this embodiment, the histamine derivative is administered
30 simultaneously or sequentially with a predetermined antigen or an immunogenic portion thereof to which the person is sensitive in order to specifically inhibit the antigen specific antibody response and/or the T cell proliferative response to said predetermined antigen or immunogenic portion thereof. In another variation, the histamine derivative is administered to the person simultaneously or sequentially with
35 a peptide having T cell stimulating activity derived from a predetermined antigen such as a protein allergen or autoantigen to which the person may be sensitive and

optionally, administered simultaneously or sequentially with the predetermined antigen or immunogenic portion thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

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Fig. 1a is a graphic representation depicting the results of an ELISA assay showing the total anti h-Mb IgG antibody response in two groups of 2-6 DBA/2 mice which had been treated with either saline PBS or 36 mg/kg of Compound 3 intravenously one day before (day-1) antigen treatment with 100 ug h-Mb in Complete Freund's Adjuvant (CFA) injected intravenously and two days following h-Mb antigen treatment.

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Fig. 1b is a graphic representation depicting the results of an ELISA assay showing the anti h-Mb IgG₁ antibody response in two groups of 2-6 DBA/2 mice which had been treated with either saline PBS or 36 mg/kg of Compound 3 intravenously one day before (day-1) antigen treatment with 100 ug h-Mb in Complete Freund's Adjuvant (CFA) injected intravenously and two days following h-Mb antigen treatment (day+2).

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Fig. 1c is a graphic representation depicting the results of an ELISA assay showing the anti h-Mb IgG₂ antibody response in two groups of 2-6 DBA/2 mice which had been treated with either saline PBS or 36 mg/kg of Compound 3 intravenously one day (day -1) before antigen treatment with 100 ug h-Mb in Complete Freund's Adjuvant (CFA) injected intravenously and two days following h-Mb antigen treatment (day+2).

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Fig. 1d is a graphic representation depicting the results of an ELISA assay showing the anti h-Mb IgG_{2b} antibody response in two groups of 2-6 DBA/2 mice which had been treated with either saline PBS or 36 mg/kg of Compound 3 intravenously one day (day -1) before antigen treatment with 100 ug h-Mb in Complete Freund's Adjuvant (CFA) injected intravenously and two days following h-Mb antigen treatment (day+2).

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Fig. 2 is a graphic representation depicting a T cell proliferative response assay showing the effect of Compound 3 on spW-Mb specific T cell proliferation in mice; Two groups of 4 DBA/2 mice were given either 32 mg/kg Compound 3 in

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buffer or PBS control (on day (-2) and day (-1) intravenously), the mice were primed with 100 ug spW-Mb intravenously on day 0, lymph nodes were pooled and harvested on day 8.

5 Fig. 3 is a graphic representation of an ELISA assay showing the IgG anti h-Mb antibody response in mice which had been treated subcutaneously (sc) with 35 mg/Kg Compound 1 or Compound 3 or saline (PBS) control on day-1 and day 2, and 100 ug of h-Mb in complete Freund's Adjuvant (CFA) on day 0 and 100 ug of h-Mb in Incomplete Freund's Adjuvant (IFA) on day 21. Mice were bled on day 33 and
10 sera was assayed for h-Mb specific IgG. The mean antibody binding from 5 mice is shown.

 Fig. 4 is a graphic representation of an ELISA assay showing the IgG anti h-Mb antibody response in mice which had been treated subcutaneously (sc) with 35
15 mg/Kg Compound 1 or Compound 3 or saline (PBS) control on day-1 and day 2, and 100 ug of h-Mb in complete Freund's Adjuvant (CFA) on day 0 and 100 ug of h-Mb in Incomplete Freund's Adjuvant (IFA) on day 21. Mice were bled on day 33 and sera was assayed for h-Mb specific IgG2a. The mean antibody binding from 5 mice
20 is shown.

 Fig. 5 is a graphic representation of an ELISA assay showing the IgG anti h-Mb antibody response in mice which had been treated subcutaneously (sc) with 35 mg/Kg Compound 1 or Compound 3 or saline (PBS) control on day-1 and day 2, and 100 ug of h-Mb in complete Freund's Adjuvant (CFA) on day 0 and 100 ug of h-Mb
25 in Incomplete Freund's Adjuvant (IFA) on day 21. Mice were bled on day 33 and sera was assayed for h-Mb specific IgG2b. The mean antibody binding from 5 mice is shown.

 Fig. 6 is a graphic representation of an ELISA assay showing the effect of
30 Compound 1 and Compound 3 on h-Mb specific T cell proliferation, 3 mice were given either 35 mg/kg or PBS (control on day -2 and day -1 intravenously, the mice were primed with 100ug h-Mb/CFA subcutaneously on day 0. Lymph nodes were pooled and harvested on day 7, proliferation of lymph node T cells is shown.

35 Fig. 7 is a graphic representation showing the effect of Compound 1 only, Compound 3 only, or Compound 1 and Compound 3 together on the incidence of

diabetes in NOD mice, 10 mice were treated subcutaneously with 35 mg/Kg Compound 1 only, Compound 3 only, Compound 1 and Compound 3 together, or saline (PBS) on days 90 and 91 of life. The incidence of diabetes was measured by serum glucose levels.

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Fig. 8 is a graphic representation showing the effect of Compound 1 only, Compound 3 only, or Compound 1 and Compound 3 together on the incidence of diabetes in NOD mice, 10 mice were treated subcutaneously with 35 mg/Kg Compound 1 only, Compound 3 only, Compound 1 and Compound 3 together, or
10 saline (PBS) on day 76 of life at each data point shown. The incidence of diabetes was measured by serum glucose levels.

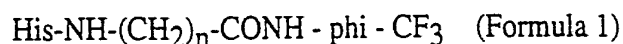
Fig. 9 is a graphic representation of the effect of Compound 1 or Compound 3 on the IgM response of mice which were treated subcutaneously with 35 mg/Kg
15 Compound 1, Compound 3 or PBS (Control) on day 0 and day 2 and 100 ug of h-Mb/CFA on day 0, the mice were bled on day 7 and sera was assayed for h-Mb specific IgM. The mean antibody binding from 5 mice is shown.

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DETAILED DESCRIPTION OF INVENTION

The present invention provides methods for using histamine derivatives (also known as histamine congeners) as immunomodulators and in immunotherapeutics. Histamine derivatives useful in methods of the invention have the following general formula:

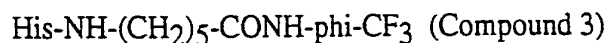
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wherein $n=2-10$, preferably 3-6, most preferably 5.

A histamine derivative of particular interest has the following formula:

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Histamine derivatives useful in methods of the inventions may be synthesized by various methods according to procedures well known in the art. A general discussion of synthesis of histamine derivatives can be found in U.S. Pat. No. 4,996,221 incorporated herein by reference.

The histamine derivatives may be purified by conventional purification techniques, such as crystallization, or by chromatographic techniques, such as column chromatography, high performance liquid chromatography, preparative thin-layer chromatography, or the like.

5 It is understood that the subject invention includes methods of using derivatives of histamine wherein histamine is connected by a linking group to an amino acid of poly(amino acid) molecule thereby defining a conjugate. The histamine derivative may be linked to a carrier such as polypeptides, proteins, glycoproteins or derivatives thereof (all included within the name poly(amino acid)).

10 The conjugates may serve a variety of functions, changing the physiological character of the histamine derivative, acting as immunogens, providing for cell specific binding and the like. Depending on the purpose of the conjugate, the nature of the histamine derivative may be modified to lesser or greater degrees by adding additional functionalities, substituting groups or the like. Particularly for the
15 production of antibodies from immunogens, a group may be substituted for another group, e.g., methyl or trifluoromethyl with carboxyl. Also, in the case of immunogens, substitution at histamine or intermediate the ends of the histamine derivative may be desirable.

20 The conjugates may be bonded through a wide variety of functionalities to form amide, methyleneamine, thioether, disulfide, sulfonamide, azo, amidine, etc. The particular functionality chosen will depend upon the purpose of the conjugate, ease of synthesis, stability of the linking functionality, affect of the linking group on the physical, chemical like.

25 The number of histamine derivatives per carrier may be one, or any number greater than one. The number of histamine derivatives per carrier molecule is dependent upon the number of appropriate functional groups in the carrier and the stoichiometry used during the coupling reaction.

30 Synthesis routes are well known in the art. One method would involve the preparation of appropriate histamine derivatives where the extended amine side chain or other location on histamine has a suitable functional group. One or more functionalized histamine derivatives are then, in turn, coupled to appropriate side chains of the carrier. Alternatively, a method of synthesis may involve the initial modification of the carrier by coupling the derivative group moiety containing a further functional group reactive with histamine directly to the carrier side chain.
35 The resulting carrier derivative is then coupled directly to the histamine, for example, by a reductive amination reaction to produce the conjugate.

Compound 3 has been shown to have H₂ receptor activity but not H₁ receptor activity, and further has some H_x activity. As referred to herein, a compound which has receptor activity can bind to a specific receptor. It is believed that compounds having H₁ receptor activity enhance the suppressive capacity of the natural suppressor cells via H₁ receptor mediated mechanisms. It is believed that compounds having H₂ receptor activity stimulate intracellular accumulations of cAMP.

H_x receptors are believed to be found only on human monocytes, neutrophils, and HL-60 cells that have been transformed. The H_x receptor system is believed to play a role in mediating certain immune responses such as suppressing IL-4 secretion from T helper cells, inhibiting natural T-killer cell activity, enhancing suppression caused by natural suppressor cells and increasing the calcium flux in HL-60 cells and human peripheral blood lymphocytes (PBL). Therefore, compounds having H_x receptor activity are believed to have an effect on these processes.

In order to take advantage of non-overlapping properties of specific histamine derivatives to achieve a desired effect, it may be beneficial to provide a composition for use in the methods of the invention comprising two or more histamine derivatives. Preferably such a composition comprising two or more compounds is selected from the group consisting of:

His-NH-(CH₂)₅-CONH-phi-CF₃ (Compound 3);
His-NH-CHCH₃-(CH₂)₃-CONH-phi-CF₃ (compound 2);
and His-NH-CHCH₃-(CH₂)₄ CONH-phi-CF₃ (Compound 1).

Table 1 summarizes the histamine receptor activity of various derivatives of histamine.

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Table 1

Compound #	H ₁	H ₂	H ₃	H _x
1	++	++	Anti H ₃	+++
2	+++	-	NT	+
3	-	+++	NT	++
Histamine	+	+	+	Anti H _x

NT = not tested

The histamine derivatives of Formula 1 are useful in methods of inhibiting at least a portion of an antigen specific antibody response by the immune system of a mammal. In particular, administration of an effective amount of at least one histamine derivative having binding specificity for at least one histamine receptor inhibits the production of IgG antibodies. It further appears that the production of IgM antibodies are not inhibited. The inhibition is dependent on the dose of the histamine derivative, can endure for extended periods of time, can be prolonged by repeated dosing of the histamine derivative, and moreover, can reverse an already established response to antigen. Administration of at least one histamine derivative of Formula 1 in accordance with the invention results in inhibition of an antigen specific antibody response by the immune system of a mammal of at least about 60% inhibition of the production of total IgG₁ antibodies, of which at least 40% of the production of IgG antibodies are inhibited, at least 40% of the production of IgG_{2a} antibodies are inhibited and at least 40% of the production of IgG_{2b} antibodies are inhibited.

Furthermore, at least one histamine derivative of Formula 1 is useful in methods of inhibiting at least a portion of an antigen specific T cell proliferative response by the immune system of a mammal. In particular, administration of an effective amount of such a histamine derivative inhibits T cell proliferation by at least 60% and preferably by at least 80%. The inhibition is dependent on the dose of the histamine derivative, can endure for extended periods of time, and can be prolonged by repeated dosing of the histamine derivative.

The manner in which a composition comprising at least one histamine derivative of Formula 1 is administered to a mammal varies widely in accordance with methods well known in the art. The composition is preferably administered with a physiologically suitable or pharmaceutically acceptable carrier or diluent. The carrier may be any physiologically acceptable buffer as is known in the art and includes but is not limited to phosphate buffered saline (PBS). Suitable methods of administration include but are not limited to: orally, parenterally, by injections or the like. Pharmaceutically effective concentrations and dosages of compositions comprising at least one histamine derivative will vary widely, depending upon the purpose, host and particular derivative employed. Concentrations may vary from less than 10^{-1} M, and preferably less than or equal to 10^{-3} M. Suitable single pharmaceutically effective dosages of such compositions range from about 0.5mg/kg body weight to about 100 mg/kg. A preferred range is from about 1 mg/kg to 50mg/kg. Suitable pharmaceutically effective daily total dosages range from about 1 mg/kg to 100 mg/kg.

The present invention also provides methods using the histamine derivatives of the Formula 1 for inhibiting at least a portion of an antigen specific antibody response to a predetermined antigen, such as a protein allergen or autoantigen, by the immune system of a mammal sensitive to the predetermined antigen (i.e., the mammal has been producing antibodies to the predetermined antigen). According to this embodiment, an effective amount of a composition comprising at least one histamine derivative of Formula 1 is administered to a mammal sensitive to the predetermined antigen or immunogenic portion thereof (i.e., a portion of an antigen capable of eliciting an immune response) in order to at least partially inhibit further production of antibodies to the predetermined antigen by the immune system of the mammal.

In one variation of the above described embodiment, the predetermined antigen or an immunogenic portion thereof is administered to a mammal sensitive to the predetermined antigen or immunogenic portion thereof, in conjunction with at least one histamine derivative of Formula 1 to specifically inhibit the antigen specific antibody response to the predetermined antigen or immunogenic portion thereof (i.e., further production of antibodies specifically reactive to the predetermined antigen or immunogenic portion thereof is at least partially inhibited). The predetermined antigen or immunogenic portion can be administered to the mammal simultaneously or sequentially with a composition comprising at least one of the above histamine derivatives. As used herein, antigens include but are not limited to protein allergens.

autoantigens or at least an immunogenic portion of either antigen capable of eliciting an immune response.

Another embodiment of the present invention provides methods for treating sensitivity to an antigen, such as a protein allergen or autoantigen utilizing peptides having T cell stimulating activity. According to the method, a peptide having T cell stimulating activity and derived from a protein allergen or other antigen is administered to a person sensitive to the protein allergen or autoantigen from which the peptide is derived simultaneously or sequentially with a histamine derivative of Formula 1 of the present invention. T cell stimulating activity is defined herein as induction of T cell proliferation, lymphokine secretion and/or T cell anergy/tolerization. Such T cell stimulating activity can be tested by culturing T cells obtained from a person sensitive to a protein allergen or other antigen with a peptide derived from the protein allergen or other antigen and determining the presence of proliferation by the T cells in response to the peptide.

Peptides useful in methods of treating sensitivity to a protein allergen or other antigen have T cell stimulating activity and preferably have human T cell stimulating activity and accordingly comprise at least one T cell epitope of a protein allergen or other protein antigen. Peptides derived from protein allergens preferably do not bind immunoglobulin E (IgE) or bind IgE to a substantially lesser extent than the protein allergen from which the peptide is derived binds IgE.

T cell epitopes are believed to be involved in initiation and perpetuation of the immune response to a protein allergen or other protein antigen which is responsible respectively for the clinical symptoms of allergy or other diseases. These T cell epitopes are thought to trigger early events at the level of the T helper cell by binding to an appropriate HLA molecule on the surface of an antigen presenting cell and stimulating the relevant T cell subpopulation. These events lead to T cell proliferation, lymphokine secretion, local inflammatory reactions, recruitment of additional immune cells to the site, and activation of the B cell cascade leading to production of antibodies. One isotype of these antibodies, IgE, is fundamentally important to the development of allergic symptoms and its production is influenced early in the cascade of events, at the level of the T helper cell, by the nature of the lymphokines secreted. A T cell epitope is the basic element or smallest unit of recognition by a T cell receptor, where the epitope comprises amino acids essential to receptor recognition and may be contiguous and/or non-contiguous in the amino acid sequence of the protein. Amino acid sequences which mimic those of the T cell

epitopes and which modify the allergic response to protein allergens are within the scope of this invention.

Administration of these peptides may tolerize or anergize appropriate T cell subpopulations such that they become unresponsive to the protein allergen or other antigen and do not participate in stimulating an immune response upon such exposure. In addition, administration of a peptide comprising at least one T cell epitope of a protein allergen may modify the lymphokine secretion profile as compared with exposure to the naturally-occurring protein allergen or portion thereof. Furthermore, exposure to the peptide may influence T cell subpopulations which normally participate in the response to the allergen such that these T cells are drawn away from the site(s) of normal exposure to the allergen (e.g., nasal mucosa, skin, and lung) towards the site(s) of therapeutic administration of the peptide. This redistribution of T cell subpopulations may ameliorate or reduce the ability of a person's immune system to stimulate the usual immune response at the site of normal exposure to the allergen, resulting in a diminution in allergic symptoms. The purpose behind administering these peptides is to have the effects caused by the administration of these peptides outlast the immunomodulating effects caused by administration of the histamine derivative.

When peptides are derived from protein allergens, they can comprise at least one T cell epitope of a protein allergen such as a protein allergen selected from the group consisting of: a protein allergen of the genus Dermatophagoides; a protein allergen of the genus Felis; a protein allergen of the genus Ambrosia; a protein allergen of the genus Lolium; a protein allergen of the genus Cryptomeria; a protein allergen of the genus Alternaria; a protein allergen of the genus Alder; a protein allergen of the genus Betula; a protein allergen of the genus Quercus; a protein allergen of the genus Olea; a protein allergen of the genus Artemisia; a protein allergen of the genus Plantago; a protein allergen of the genus Parietaria; a protein allergen of the genus Canine; a protein allergen of the genus Blattella; a protein allergen of the genus Apis; and a protein allergen of the genus Periplaneta.

Peptides useful in methods of the invention can be derived from protein antigens other than protein allergens where enhancement or depression of an antigen specific immune response is desired. For example, peptides having human T cell stimulating activity of a known autoantigen involved in the pathogenesis of an autoimmune disease or peptides comprising at least one T cell epitope of a known autoantigen can be administered to decrease the antibody response to the autoantigen, to interfere with efficacy and/or decrease immune complex related side effects. In

order to preserve the T cell reactivity of the autoantigen, peptides derived from the autoantigen having human T cell stimulating activity could be defined by standard T cell biology techniques, or if desired, precise T cell epitopes can be defined by fine mapping techniques and a peptide comprising at least one T cell epitope produced.

- 5 — Peptides which stimulate T cells and do not have undesired properties of the autoantigen (e.g., do not bind autoantibodies in a substantial percentage of persons sensitive to the autoantigen) are selected for use in methods of treatment as immunotherapeutics. Autoantigens useful in methods of the present invention include insulin, glutamic acid decarboxylase (64K), PM-1 and carboxypeptidase H in
10 diabetes; myelin basic protein in multiple sclerosis; rh factor in erythroblastosis fetalis; acetylcholine receptors in myasthenia gravis; thyroid receptors in Graves' Disease; basement membrane proteins in Good Pasture's syndrome; and thyroid proteins in thyroiditis.

The present invention also provides methods of treating sensitivity in a person
15 who is already producing antibodies to such antigen, such as a protein allergen or autoantigen and methods of inhibiting at least a portion of an antigen specific antibody response by the immune system of an individual who has not already been producing antibodies to the antigen, whereby a therapeutically effective amount of a therapeutic composition comprising at least one histamine derivative of Formula 1
20 and a pharmaceutically acceptable carrier or diluent is administered to such person. Subsequently, or simultaneously therewith, the antigen, or an immunogenic portion thereof is optionally administered to the person to specifically inhibit the antigen specific immune response by the person to the antigen or immunogenic portion thereof. In addition, a peptide having T cell stimulating activity and derived from the
25 antigen can be administered to the person to desensitize the person to the antigen. Preferably, the peptide comprises at least one T cell epitope of the antigen. Such peptides are administered to persons sensitive to an allergen or other protein antigen from which the peptide is derived, at dosages and for lengths of time effective to reduce sensitivity of the person to the allergen or other antigen.

30 Peptides having T cell stimulating activity can be administered in the form of a therapeutic composition including a physiologically acceptable vehicle. For example, the peptide can be administered in combination with an appropriate diluent, a carrier, and/or an adjuvant, where appropriate. Pharmaceutically acceptable diluents include saline and aqueous buffer solutions. Pharmaceutically acceptable
35 carriers include polyethylene glycol (Wie et al., International Archives of Allergy and Applied Immunology 64: 84-99 (1981)) and liposomes (Strejan et al., Journal of

Neuroimmunology 7: 27 (1984)). Pharmaceutically acceptable adjuvants include alum. Such compositions will generally be administered by injection, oral administration (e.g., as in the form of a capsule), inhalation, transdermal application or rectal administration.

5 Another embodiment of the present invention provides methods of treating T cell mediated diseases or a method of treating graft rejection in a person comprising administering to the person a therapeutically effective amount of a therapeutic composition comprising at least one histamine derivative of Formula 1 and a pharmaceutically effective carrier or diluent.

10 In one variation of the above described embodiment, the antigen responsible for the T cell mediated disease or an immunogenic portion thereof is administered to the person subsequently or simultaneously with the histamine derivative of Formula 1 to specifically inhibit the T cell proliferative response of the antigen or immunogenic portion thereof.

15 In addition, a peptide having T cell stimulating activity and derived from the antigen responsible for the T cell mediated disease can be administered to the person to desensitize the person to the antigen. Preferably the peptide comprises at least one T cell epitope of the antigen. Such peptides are administered to persons at dosages and for lengths of time effective to tolerize the person to the antigen. Peptides having
20 T cell stimulating activity can be administered as discussed above. T cell mediated diseases which can be treated include but are not limited to diabetes, T cell leukemia, endotoxin induced food poisoning and mycosis fungoides.

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

EXAMPLE 1

The following experiment shows the inhibition of the production of total IgG antibodies in mice as well as the inhibition of the production of the IgG antibody subclasses: IgG₁, IgG_{2a}, and IgG_{2b}, which comprise at least a portion of the total IgG response, as a result of administering to the mice Compound 3 in accordance with the invention and challenging the mice with a T cell mediated antigen, horse myoglobin (h-Mb), which elicits an IgG antibody response.

Anti-h-Mb Elisa

Elisa plates were coated with 10µg/ml commercially available h-Mb in PBS 50 µl/ well overnight at 4° C. The plates were washed with PBS once. The plates were then coated with 50 µl BBA (1mg/ml) and washed with PBS once. The plates were then coated with test serum diluted in PBS-Tween, 50 µl/well and incubated at 4° C overnight. The plates were then washed with PBS-Tween three times. Anti-IgG (1:2000) was added and diluted with PBS-Tween. For the IgG antibody subclasses, anti-IgG₁, anti-IgG_{2a}, anti-IgG_{2b} was diluted and added to selected wells. Incubation was at 4° C for two hours. Washing was with PBS-Tween 2 times with 5 minutes between each wash. A 1 mg/ml OPD in citrate buffer solution pH5 was prepared, and 10 µl of H₂O₂ was added to 10 ml of the buffer solution. 50 µl of the buffer was added to each well and the plates were placed in the dark for about 10 minutes. The reaction was stopped with 50 µl of 5N H₂SO₄ in each well. The plates were then read on an Elisa reader.

Protocol for Immunization

The following immunization protocol was repeated several times. The results have been collected in Figs. 1a - d.

This experiment was done using two groups of 2 - 6 DBA/2 mice (average weight 22 grams). One group of mice were given one treatment of Compound 3 intravenously one day before antigen treatment (day-1) and two days following antigen treatment (day+2). The dose of Compound 3 was approximately 36 mg/kg in buffer. The other group of mice was given a saline control in buffer. On day 0 the two groups of mice were injected with 100 µg h-Mb in Complete Freund's Adjuvant (CFA) intravenously (i.v.). The mice were then boosted fourteen days later with 100

µg of h-Mb in Incomplete Freund's Adjuvant (IFA). The mice were bled 30 days later.

All sera was tested in the above described Elisa for IgG and IgG subclass antibody response to h-Mb. As shown in Figs. 1a-d mice immunized with

- 5 Compound 3 had a much lower total IgG response to h-Mb compared to the saline control mice where there was a significant total IgG response. Furthermore, the mice immunized with Compound 3 showed a lower IgG₁, IgG_{2a}, and IgG_{2b} response than did the saline control mice in all of those same IgG subclasses.

10 TABLE 2

15	Group 1	5 mice	Day 0, Day 14	100 µg h-Mb
			Day-1 & Day+2	32 mg/kg Compound 3
15	Group 2	5 mice	Day 0, Day 14	100 µg h-Mb
			Day-1 & Day+2	PBS Control

20 EXAMPLE 2

- The following experiment shows T cell proliferation in mice in response to treatments with Compound 3 and challenge with commercially available sperm whale myoglobin (SpW-Mb) antigen. In this model the response to spW-Mb is mediated through CD4+ T cells.
- 25

T Cell Proliferation Assay

- Two groups of DBA/2 mice were tested. One group of four mice was a control. The other group of four mice was treated with Compound 3. Both groups of mice were immunized using approximately 100 µg per mouse of SpW-Mb plus CFA intravenously on day 0. On day-1 and day+2 one group of mice was given approximately 32 mg/kg Compound 3 in buffer i.v.
- 30

- On day 8, the mice were killed and the draining lymph nodes were removed and a single cell suspension was made. The cells were then plated out in a 96 well U-bottom plate at a concentration of 4×10^6 cell/ml in media containing 1% mouse
- 35

serum. The cells were then challenged in vitro with antigen diluted at 1, 5, and 25 μ m in media without serum. The plates were then placed in the incubator at 37 C. On day 4 the wells were pulsed with tritiated thymidine and on day 5 the plates were harvested. The results are shown in Fig. 2.

5

EXAMPLE 3

Monoclonal Affinity Purification of Fel d I

10 Native Fel d I protein was purified from an extract of house dust as described by Chapman et al. Briefly, house dust (from vacuum containers used in homes with multiple cats) was extracted with PBS, then lyophilized and redissolved in water. The extract was applied to a column coupled with anti-Fel d I monoclonal antibody (hybridomas 6F9 and 1G4 were both provided by M. Chapman). The Fel d I was
15 eluted from the column with 100 mM glycine pH 2.5 and was neutralized.

Direct Binding ELISA

For the IgG assay Fel d I was coated onto Immulon 2 (Dynatech, Chantilly, VA) 96-well plates by incubation of 50 μ l/well of 2 μ g/ml Fel d I in PBS overnight at
20 4°C. The wells were incubated with 0.5% gelatin in PBS at 37°C for one hour. Plates were washed three times with PBS-T (1 X PBS + 0.05% Tween 20). Sera were diluted in PBS-T. After incubation at room temperature for one hour and washing with PBS-T, the bound mouse antibody was detected by incubation with biotinylated goat anti-mouse IgG (Southern Biotechnology Associates, Birmingham,
25 AL). Streptavidin conjugated to horseradish peroxidase (Southern Biotechnology Associates) was added to detect antigen bound biotinylated antibody-complexes. TMB peroxidase substrate (Dirkegaard and Perry, Gaithersburg, MD) was used according to the directions supplied and resulting O.D. (450 nm) values were determined using an ELISA reader (Bio-Tek model #310, Winooski, VT). The
30 serum titer is determined by 25% of the positive control.

H-Mb ELISA is carried out similarly, using isotype specific polyclonal reagents. Antigen bound IgE was detected similarly, but using biotinylated EM95-1, a rat monoclonal antibody specific for mouse IgE. Biotinylated goat anti-rat IgG (Dirkegaard and Perry) was used as an added signal amplification step in the IgE
35 ELISA. Antigen bound IgM was detected similarly, but using anti-mouse IgM.

Culture Conditions for Proliferation Assays

The inguinal, paraaortic, and popliteal lymph nodes were removed from the animals seven days after antigen challenge. The cells from these organs were suspended by being forced through a stainless steel mesh with a glass pestal. The
5 cells were washed two times in RPMI 1640 with 1% FCS before being cultured. All cells were cultured at 37°C in 5% CO₂ in RPMI-1640 with 10% FCS (#F4884, Sigma, St. Louis, MO), 100 U/ml penicillin G, 10 µg/ml streptomycin, 10 mM glutamine, and 5 x 10⁻⁵ M 2-ME. Cells were cultured in triplicate 0.2 ml wells for in
10 96 well plates at 4 x 10⁶ cells/ml. Proliferation was measured by tritiated thymidine incorporation on Day 7.

The Effect of Histamine Congeners on Antibody Isotype

Recent work has demonstrated the ability of helper T cell subsets to augment different antibody isotypes. Murine TH₁ cells appear to stimulate the production of
15 IgG₂ while TH₂ cells stimulate the production of IgG1 and IgE. Experiments are addressing whether histamine congeners can specifically effect different populations of helper T cell functions. Two different histamine congeners (Compound 1 and Compound 3 discussed earlier) have been compared for their ability to effect the antibody response to Horse myoglobin (H-Mb).

20 The IgM response specific for H-Mb was assayed on day 7 following an antigen priming with a day 0 and day 2 drug treatment (Fig. 9). There was no detectable effect of 35 mg/Kg histamine congener on the antigen specific IgM made in response to the H-Mb priming.

A separate group of mice were treated with 35 mg/Kg histamine congener on
25 day (-1) and day 2. These mice received H-Mb on day 0 and day 21. Sera (day 33) from these mice were assayed for H-Mb specific IgG. Fig. 3 demonstrates the ability of Compound 1 and Compound 3 to inhibit the production of H-Mb specific IgG. The same bleeds were assayed for H-Mb specific IgG2a (Fig. 4) and IgG2b (Fig. 5). Compound 3 appears to decrease the H-Mb specific IgG2a and IgG2b. This implies
30 that the target of Compound 3 activity may be part of the TH₁ pathway. In contrast, Compound 1 does not effect the H-Mb specific IgG2a or IgG2b. The decrease in IgG found after Compound 1 treatment (Fig. 3) may reflect an effect on IgG1. The ability of Compound 1 to decrease IgG and IgE responses to Fel d I (data not shown) suggests that its target may be the TH₂ pathway.

The Effect of Histamine Congeners on T Cell Proliferation

Receptors for the Histamine congeners are present on most cells involved in the immune response. Experiments have been conducted to define the target of action of compound 1 and Compound 3. Mice were primed with Mb and treated with drug on day (-2) and (-1) (iv). Draining lymph nodes were harvested and antigen specific proliferation was measured (Fig. 6). Antigen specific T cell proliferation is decreased by treatment of the mice with Compound 3. The data implies that the stimulation of specific T cell activation is affected by Compound 3. This appears to be consistent with the antibody isotype effect of Compound 3. The TH₁ activity is responsible for the majority of T cell proliferation in vitro.

Example 4

The Effects of Histamine Autacoids on an Autoimmune Disease Model

Insulin-dependent diabetes mellitus (IDDM or Type I Diabetes Mellitus) is an autoimmune disease and involves lymphocyte dependent inflammatory destruction of the insulin-producing beta cells in pancreatic islets of Langerhans. T lymphocytes have been implicated in the destruction of the pancreatic cells and autoantibody production is associated with the development of insulinitis, the inflammatory lesion of IDDM. A strain of mouse (non obese diabetic mice referred to as NOD mice) develops a type of pancreatic lesion that closely resembles Type I diabetes in man. Many experiments using immunosuppressive drugs that showed efficacy in NOD mice predicted the value of immunosuppressives in people susceptible to type I diabetes. Unfortunately, the immunosuppressives available for the treatment of Type I disease in susceptible patients are efficacious but only during the time they are given. Those agents, with the possible exception of anti-CD4 monoclonal antibody, are too toxic to give continuously for prolonged periods.

The experiments described herein focus on the effects of compounds 1 and 3 on the progress of hyperglycemia and the development of insulinitis. The experiments focus on the following issues: 1. Whether short courses (two doses) of either drug or the drugs combined alter the onset of hyperglycemia and occurrence of death; 2. Whether protracted dosing of drug at weekly intervals alter the onset of IDDM in any more definitive way than short courses; and 3. Whether intermittent dosing of the two drugs retard or modify the onset or severity of the inflammatory lesion of the pancreas. It is believed that the immunosuppressive effects of the autacoid (histamine congeners) could influence the course of the disease because Compound 3

is capable of inhibiting T Cell proliferation to antigen. It should thus be able to limit a T cell-mediated cytolytic event.

The effects of short term treatment with compounds 1, 3 or 1 plus 3 were tested in groups of 10 female NOD mice. The drug (35 mg/Kg was given subcutaneously) for 2 consecutive days for a cumulative dose of 70 mg/Kg of compounds 1 or 3 and 140 mg/Kg of combined compounds 1 and 3. The drug was given on days 90 and 91 (Fig. 7). Suppression of the appearance of the hyperglycemia was most pronounced in the groups treated with Compound 3 or 1 plus 3. It is quite likely that the apparent effects of Compound 1 plus 3 is caused by the additive H₂ effects of the two drugs.

The effects of long term treatment with histamine congeners on the development of hyperglycemia in NOD mice was tested in groups of 10 mice per group. The drugs were administered subcutaneously as Compound 1, or 3 or 1 plus 3 in the same doses used above. Drug or control treatment was started on day 76 and repeated at the same dose/Kg 14 days later and then at weekly intervals. Fig. 8 shows the absence of any disease in the group treated with compounds 1 plus 3 up to day 140.

In summary, the Examples show that histamine congeners are potent immunosuppressants and each has a different potential mechanism of immunomodulation. Compound 1 suppresses T cell dependent IgE, IgG1 (but not IgM, IgG2a or IgG2b) antibody responses. In various antigenic responses, Compound 3 suppresses IgG1, IgG2a and IgG2b but not IgM responses. The effects of Compound 3 on IgE will be tested. Only Compound 3 appears to directly suppress T cell proliferation to specific antigen at the doses tested. The suppression of antibody production is transferable and also can be seen after the response to the antigen is established.

Histamine congeners have similar immunosuppressive effects in the mouse model of human IDDM. Congener treatment delays the onset of hyperglycemia and insulinitis in NOD mice. Since treatment with compounds 1 and 3 but not Compound 1 alone results in the greatest effect so far determined, these results indicate that: H₁ and H₂ receptor effects may be needed and cooperative in the effects on the NOD mouse or the apparent cooperation may simply be additive H₂ effects contributed separately by the two drugs; dose dependence and blocking effects of H₁ or H₂ blockers will be studied to determine the pharmacologic mechanism by which the experiments work; the cellular mechanism by which the autacoids work are not

established by the selectivity of the effects on isotypes and actions of T cell proliferation suggest the target for Compound 3 could be a TH₁ cell.

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

Claims

1. A method for inhibiting at least a portion of an antigen specific antibody response by the immune system of a mammal comprising :
- 5 1) administering to said mammal an effective amount of a composition comprising at least one histamine derivative having binding specificity for at least one histamine receptor, said histamine derivative having the formula:
- 10
$$\text{His-NH-(CH}_2\text{)}_n\text{-CONH-}\phi\text{-CF}_3$$
 wherein $n=2-10$; and
- 2) administering to said mammal an antigen or an immunogenic portion thereof to specifically inhibit the antigen specific antibody response to said antigen or
- 15 said immunogenic portion thereof.
2. The method of claim 1 wherein $n=5$.
3. The method of claim 1 wherein the production of IgG antibodies by the immune system of the mammal is inhibited by at least about 60%.
- 20 4. The method of claim 1 wherein the composition comprises said at least one histamine derivative administered to the mammal in a dosage of 1-100 mg/kg.
- 25 5. The method of claim 4 wherein the composition comprises said at least one histamine derivative administered to the mammal in a dosage of less than or equal to 50 mg/kg.
6. A method for inhibiting at least a portion of an antigen specific antibody response to a predetermined antigen by the immune system of a mammal sensitive to said predetermined antigen and which has been producing antibodies to said predetermined antigen comprising administering to said mammal an effective amount of a composition comprising at least one histamine derivative having binding specificity for at least one histamine receptor, said histamine derivative having the
- 30 formula:
- 35
$$\text{His-NH-(CH}_2\text{)}_n\text{-CONH-}\phi\text{-CF}_3$$
 wherein $n = 2 - 10$ and wherein further production of antibodies to said predetermined antigen is at least partially inhibited.
- 40 7. The method of claim 6 wherein $n = 5$.
8. The method of claim 6 further comprising the step of administering to said mammal said predetermined antigen or an immunogenic portion thereof to

specifically inhibit the antigen specific antibody response to said predetermined antigen or said immunogenic portion thereof.

5 9. The method of claim 6 wherein the predetermined antigen is a protein allergen.

10. The method of claim 8 wherein the predetermined antigen is a protein allergen.

10 11. The method of claim 9 further comprising the step of administering to said mammal a peptide derived from said protein allergen, said peptide comprising at least one T cell epitope of said protein allergen.

15 12. The method of claim 10 further comprising the step of administering to said mammal a peptide derived from said protein allergen, said peptide comprising at least one T cell epitope of said protein allergen.

20 13. A method of inhibiting at least a portion of an antigen specific T cell proliferative response by the immune system of a mammal comprising :

1) administering to said mammal an effective amount of a composition comprising at least one histamine derivative having binding specificity for at least one histamine receptor, said histamine derivative having the formula:

25
$$\text{His-NH-(CH}_2\text{)}_n\text{-CONH-phi-CF}_3$$

wherein $n=2-10$; and

30 2) administering to the mammal an antigen or an immunogenic portion thereof to specifically inhibit the T cell proliferative response to said antigen or immunogenic portion thereof.

14. The method of claim 13 wherein $n=5$.

35 15. A method of treating sensitivity to a predetermined antigen in a person which has been producing antibodies to said predetermined antigen comprising:

40 administering to the person a therapeutically effective amount of a therapeutic composition comprising at least one histamine derivative having binding specificity for at least one histamine receptor, said histamine derivative having the formula:

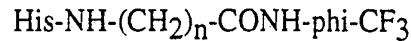
$$\text{His-NH-(CH}_2\text{)}_n\text{-CONH-phi-CF}_3$$

45 wherein $n=2-10$ and a pharmaceutically acceptable carrier or diluent.

16. The method of claim 15 wherein $n=5$.
17. The method of claim 15 further comprising the step of administering to said individual said antigen or an immunogenic portion thereof to specifically inhibit the antigen specific antibody response to said antigen or immunogenic portion thereof.
18. The method of claim 15 wherein said antigen is an autoantigen or a protein allergen.
19. The method of claim 17 wherein said antigen is an autoantigen or a protein allergen.
20. The method of claim 15 further comprising the step of administering to said person a peptide derived from said antigen, said peptide comprising at least one T cell epitope.
21. The method of claim 17 further comprising the step of administering to said person a peptide derived from said antigen, said peptide comprising at least one T cell epitope.
22. The method of claim 18 wherein the protein allergen is selected from the group consisting of: a protein allergen of the genus Dermatophagoides; a protein allergen of the genus Felis; a protein allergen of the genus Ambrosia; a protein allergen of the genus Lolium; a protein allergen of the genus Cryptomeria; a protein allergen of the genus Alternaria; a protein allergen of the genus Alder; a protein allergen of the genus Betula; a protein allergen of the genus Quercus; a protein allergen of the genus Olea; a protein allergen of the genus Artemisia; a protein allergen of the genus Plantago; a protein allergen of the genus Parietaria; a protein allergen of the genus Canine; a protein allergen of the genus Blattella; a protein allergen of the genus Apis; and a protein allergen of the genus Periplaneta.
23. A method of treating T-cell mediated disease in a person comprising:
- 1) administering to the person a therapeutically effective amount of a therapeutic composition comprising at least one histamine derivative having binding specificity for at least one histamine receptor, said histamine derivative having the formula:
- $$\text{His-NH-(CH}_2\text{)}_n\text{-CONH-phi-CF}_3$$
- wherein $n=2-10$ and a pharmaceutically acceptable carrier or diluent; and
- 2) administering to the person the antigen responsible for the T-cell mediated disease or an immunogenic portion thereof to specifically inhibit the T cell proliferative response to said antigen or said immunogenic portion thereof.

24. A method of treating T-cell mediated disease in a person comprising:

- 1) administering to the person a therapeutically effective amount of a therapeutic composition comprising at least one histamine derivative having binding specificity for at least one histamine receptor, said histamine derivative having the formula:

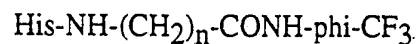


wherein $n=2-10$ and a pharmaceutically acceptable carrier or diluent; and

2) administering to the person a peptide derived from the antigen responsible for the T cell mediated disease, said peptide comprising at least one T cell epitope of said antigen.

25. A method of treating T-cell mediated disease in a person comprising:

1) administering to the person a therapeutically effective amount of a therapeutic composition comprising at least one histamine derivative having the formula:



wherein $n=2-10$ and a pharmaceutically acceptable carrier or diluent; and

2) administering to the person the antigen responsible for the T cell mediated disease or an immunogenic portion thereof to specifically inhibit the T cell proliferative response to the antigen or the immunogenic portion thereof.

26. The method of claim 23 further comprising the step of administering to the person a peptide derived from the antigen responsible for the T cell mediated disease, said peptide comprising at least one T cell epitope of said antigen.

27. The method of claim 23 wherein $n=5$.

28. The method of claim 24 wherein $n=5$.

29. The method of claim 25 wherein $n=5$.

30. The method of claim 23 wherein the T cell mediated disease is selected from the group consisting of: diabetes; T cell leukemia; endotoxin induced poisoning; and mycosis fungoides.

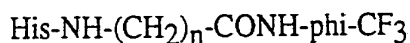
31. The method of claim 24 wherein the T cell mediated disease is selected from the group consisting of: diabetes; T cell leukemia; endotoxin induced poisoning; and mycosis fungoides.

5 32. The method of claim 25 wherein the T cell mediated disease is selected from the group consisting of: diabetes; T cell leukemia; endotoxin induced poisoning; and mycosis fungoides.

10 33. A method of treating graft rejection in a person, comprising:

1) administering to the person a therapeutically effective amount of a therapeutic composition comprising at least one histamine derivative having binding specificity for at least one histamine receptor, said histamine derivative having the formula:

15



20

wherein $n=2-10$ and a pharmaceutically acceptable carrier or diluent; and

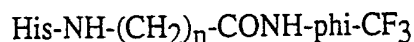
2) administering to the person a peptide derived from the antigen responsible for the rejection, said peptide comprising at least one T cell epitope of the antigen.

25 34. The method of claim 33 wherein $n=5$.

30 35. A method of treating a person by inhibiting an antigen specific antibody response to an antigen comprising:

1) administering to said person a therapeutically effective amount of a composition comprising at least one histamine derivative having the formula:

35



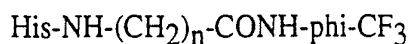
wherein $n=2-10$ and a pharmaceutically acceptable carrier or diluent; and

40

2) administering to said person said antigen or immunogenic portion thereof to specifically inhibit the antigen specific antibody response to said antigen or said immunogenic portion thereof.

36. A method of treating a person by inhibiting an antigen specific antibody response to an antigen comprising:

1) administering to said person a therapeutically effective amount of a composition comprising at least one histamine derivative having the formula:



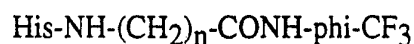
5 wherein $n=2-10$ and a pharmaceutically acceptable carrier or diluent; and

2) administering to said person a peptide derived from said antigen said peptide comprising at least one T cell epitope.

10 37. A method of treating a person by inhibiting an antigen specific antibody response to an antigen comprising:

1) administering to said person a therapeutically effective amount of a composition comprising at least one histamine derivative having the formula:

15



wherein $n=2-10$ and a pharmaceutically acceptable carrier or diluent;

20 2) administering to said person said antigen or immunogenic portion thereof to specifically inhibit the antigen specific antibody response to said antigen or said immunogenic portion thereof; and

3) administering to said person a peptide derived from said antigen, said peptide comprising at least one T cell epitope.

25

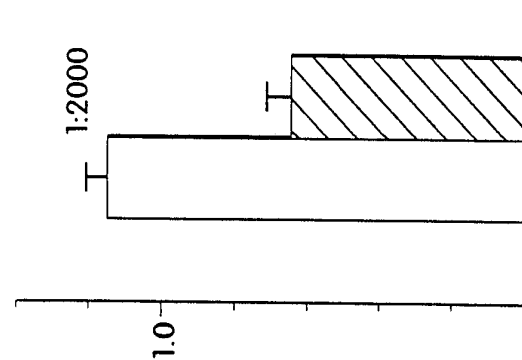


Fig. 1d

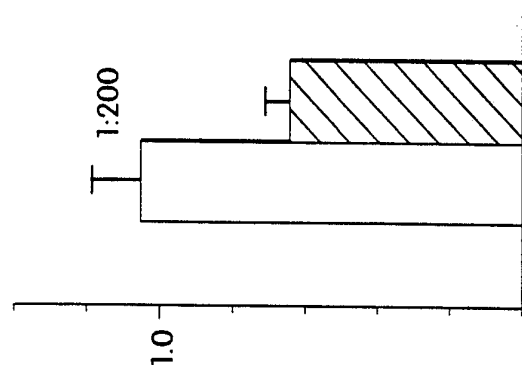
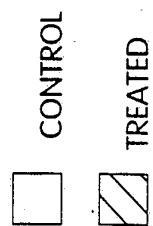


Fig. 1c

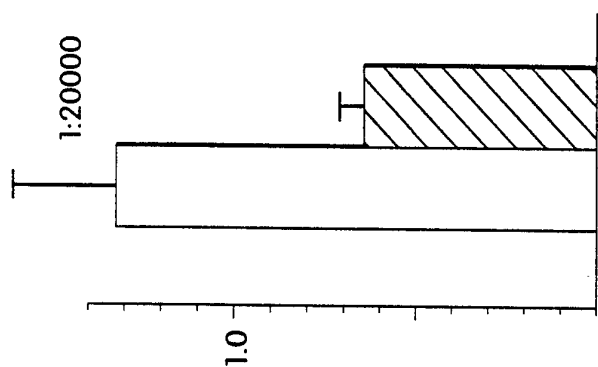


Fig. 1b

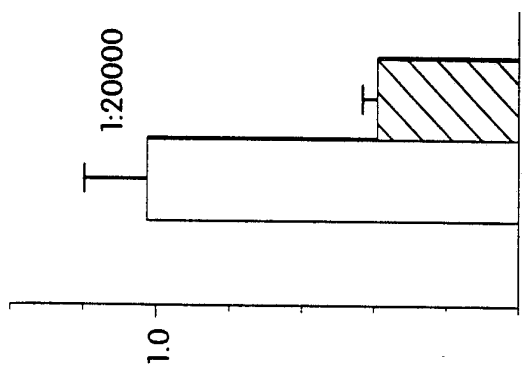


Fig. 1a

O.D.

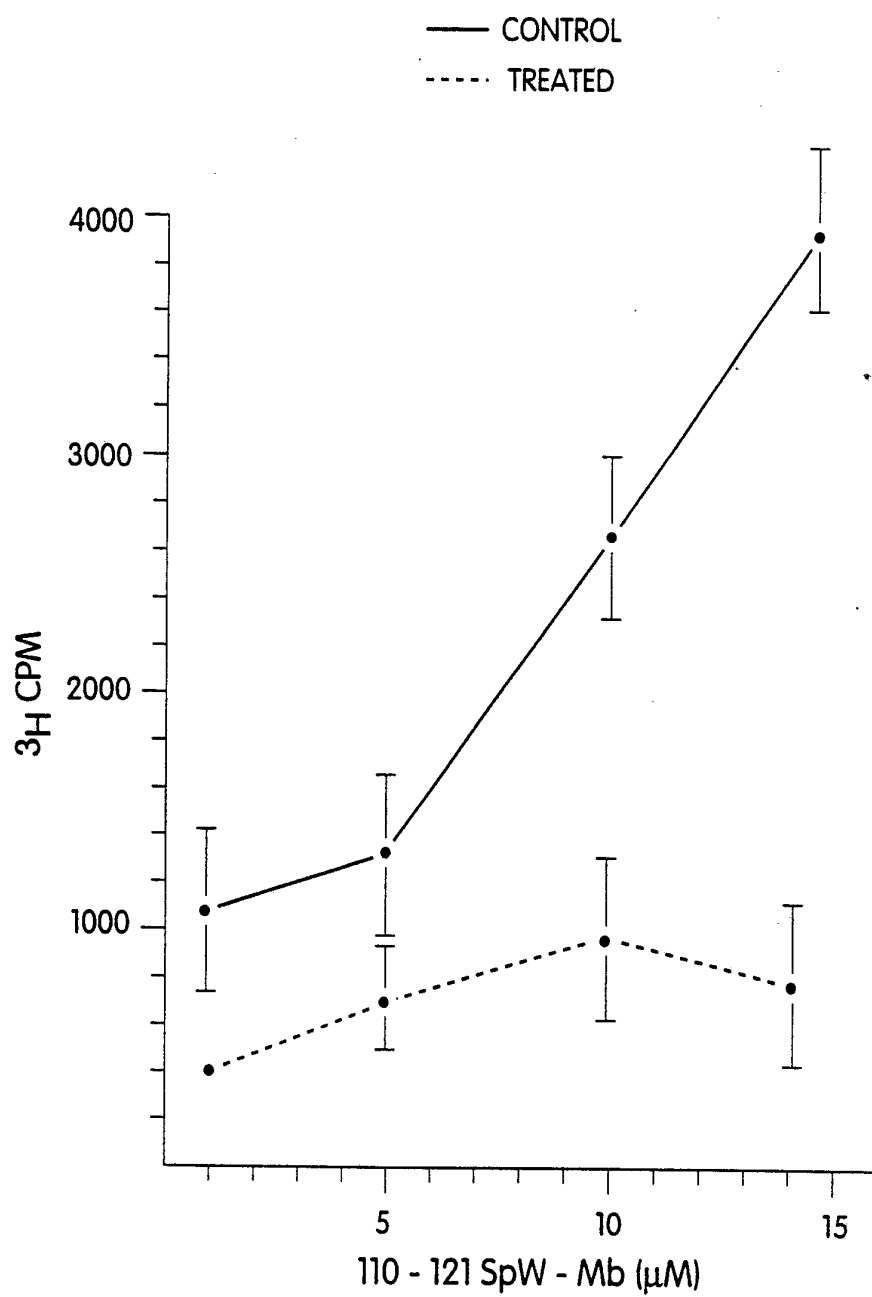


Fig. 2

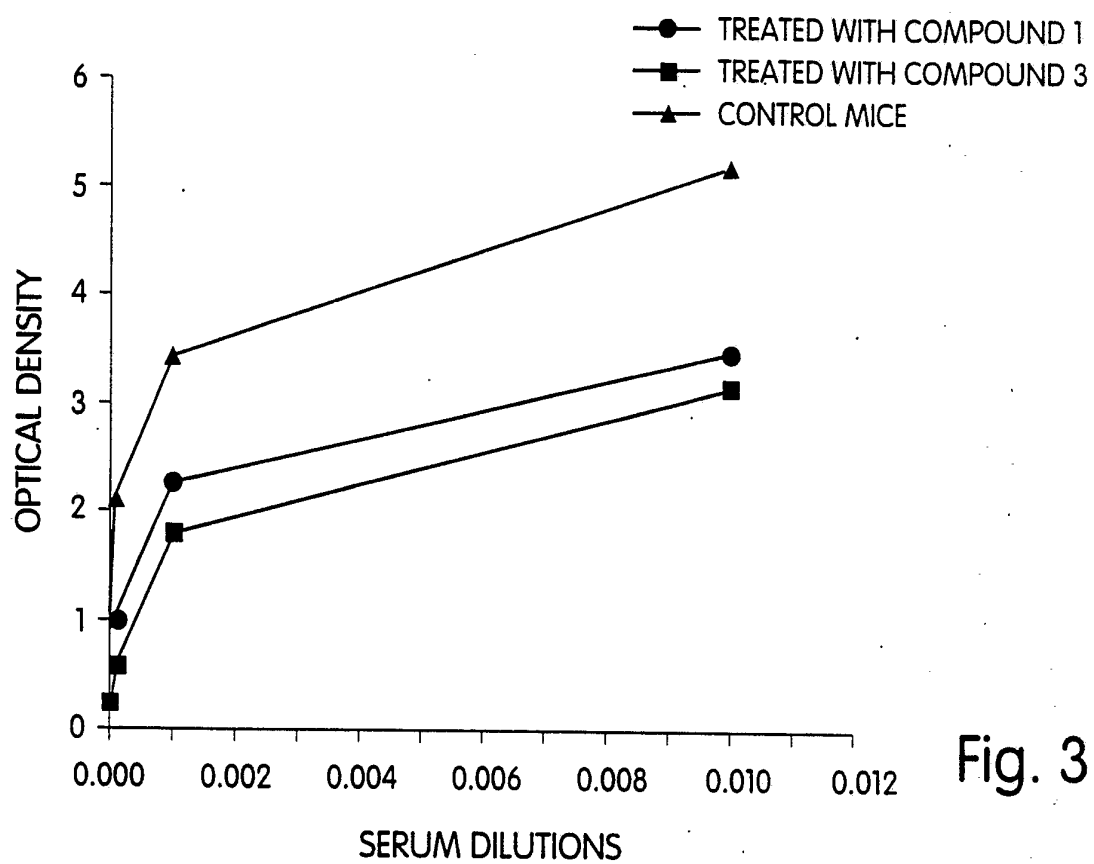


Fig. 3

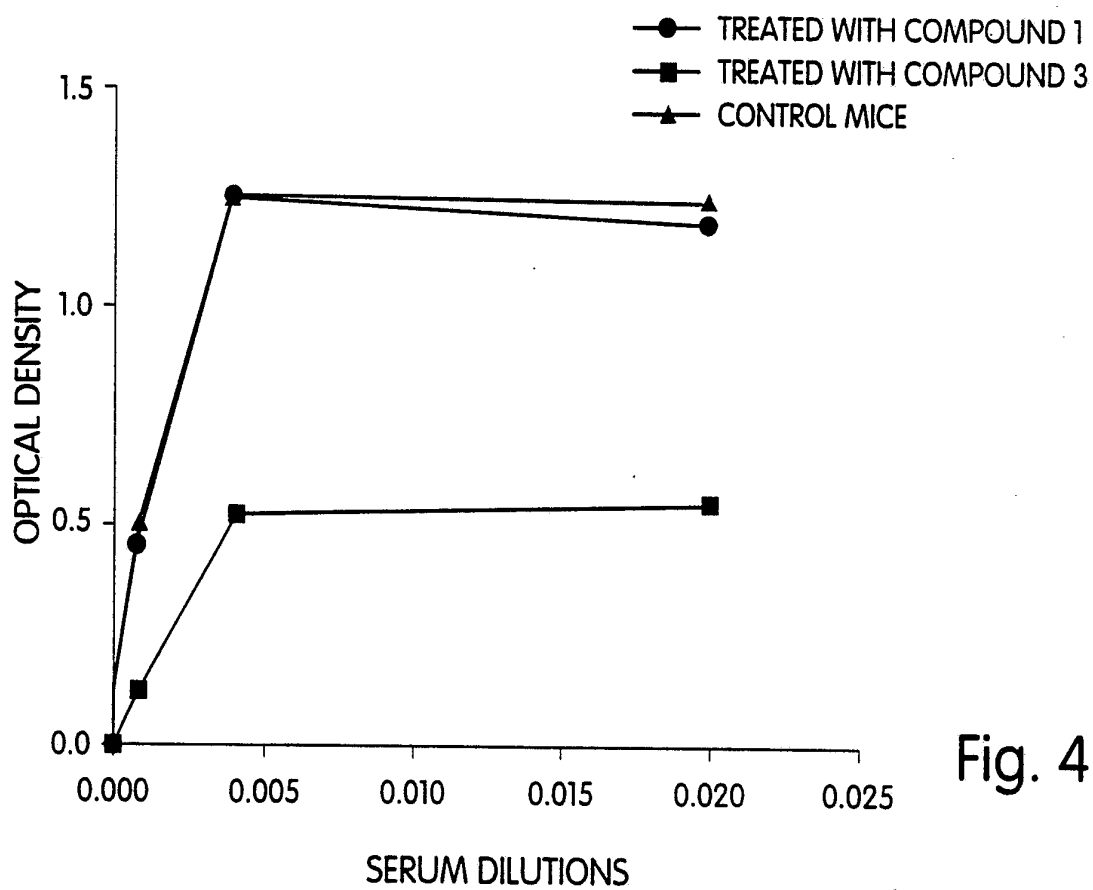


Fig. 4

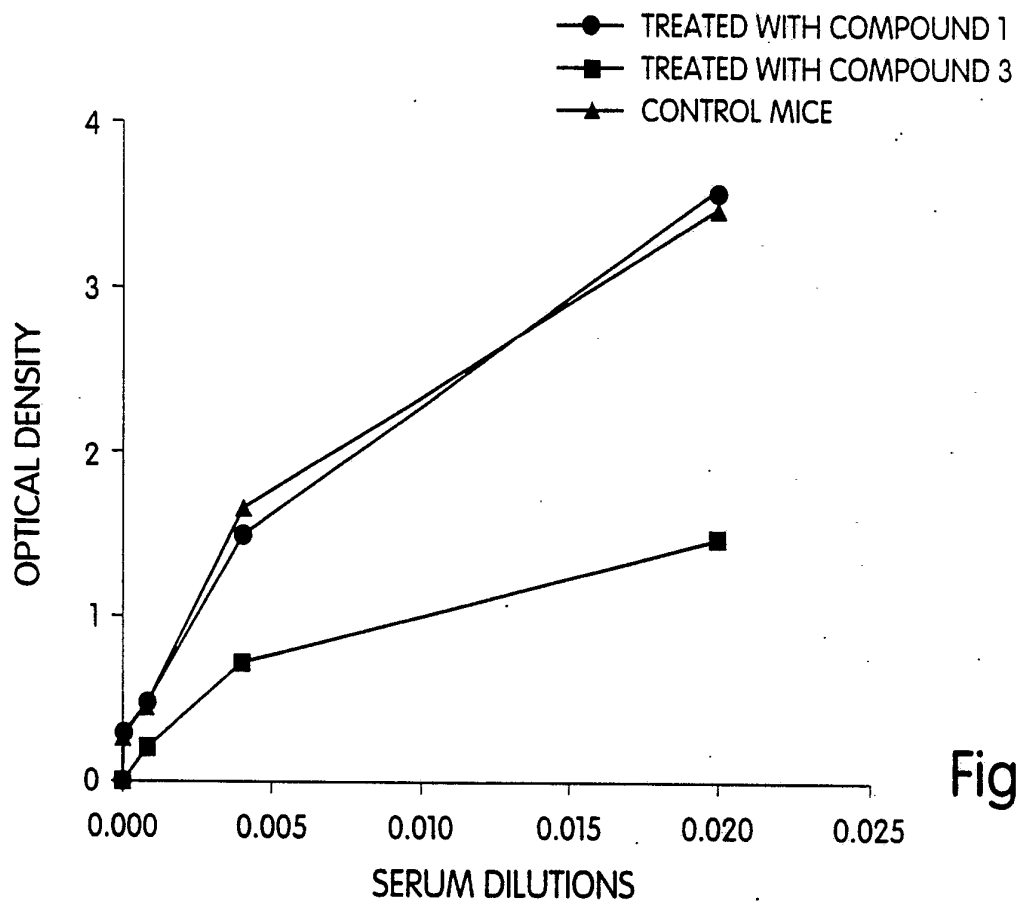


Fig. 5

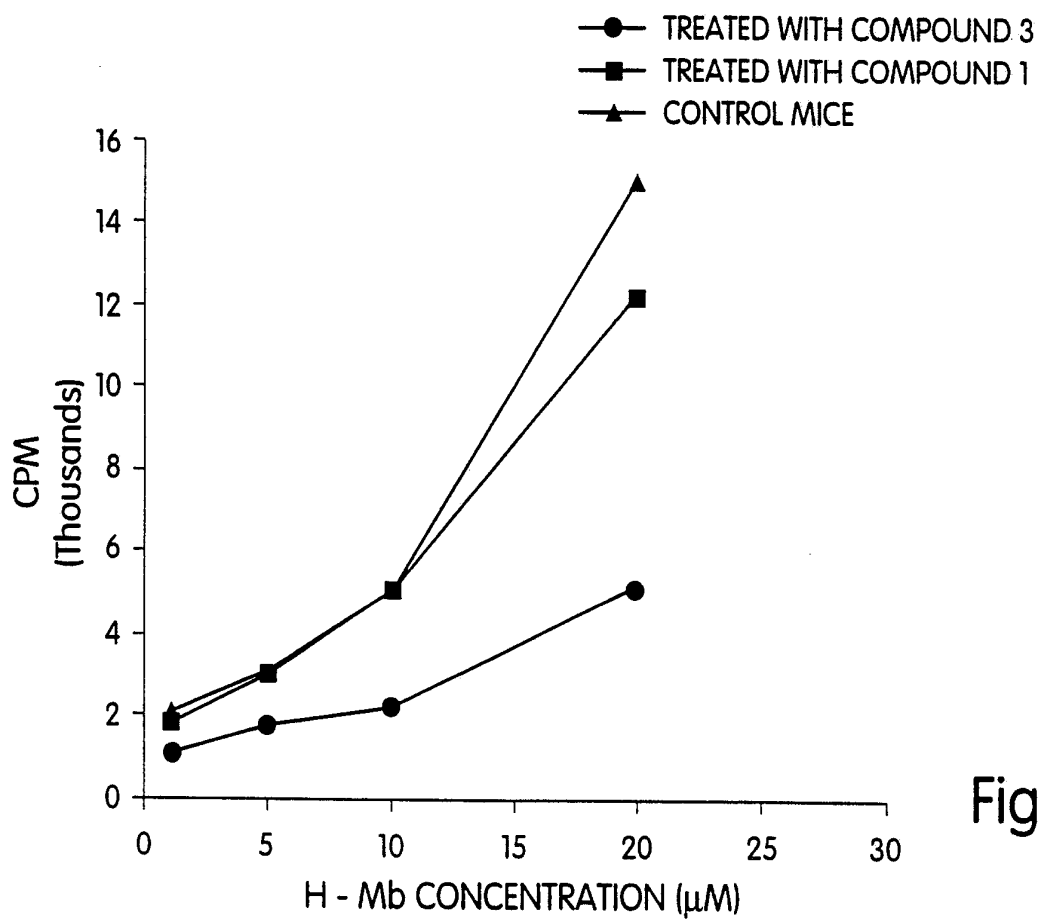
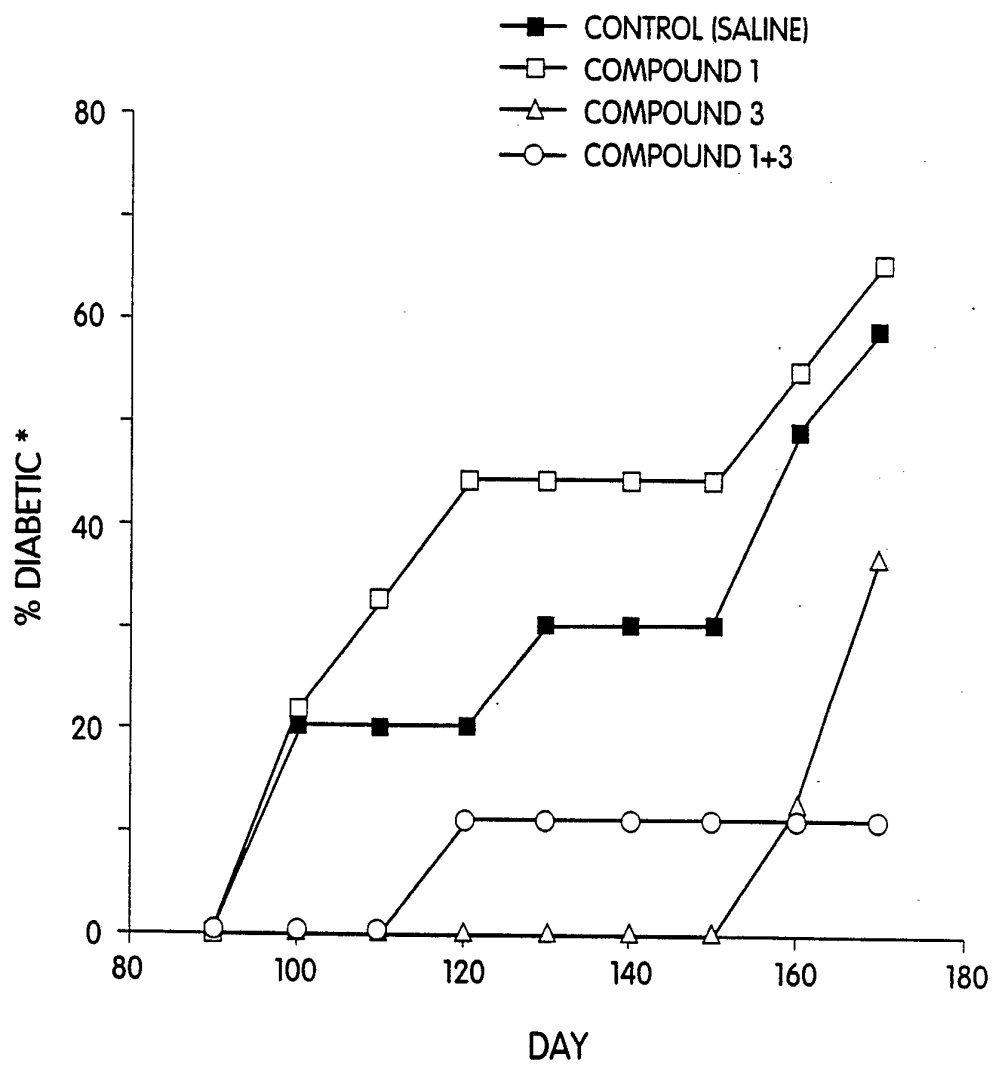
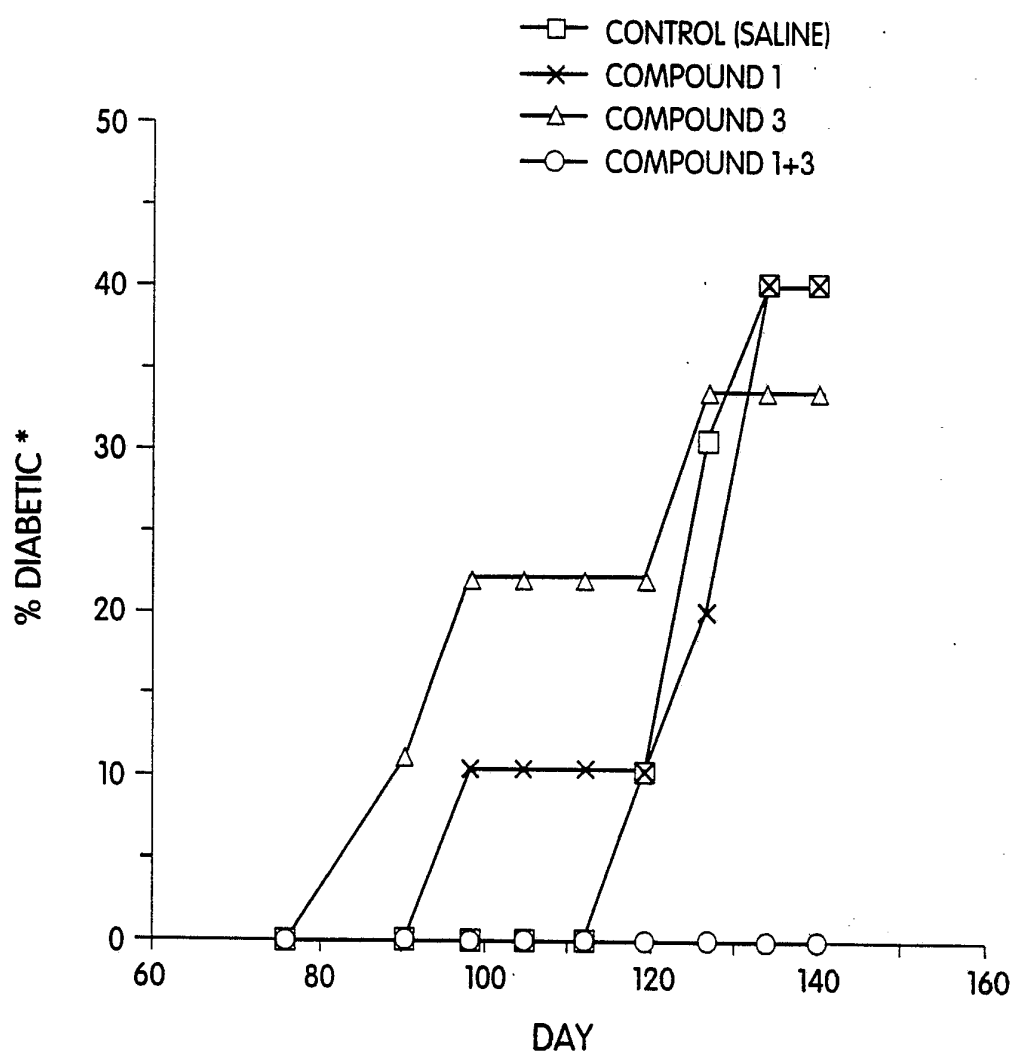


Fig. 6



* # MICE >400 mg/dl GLUCOSE / TOTAL # IN GROUP x100

Fig. 7



* # MICE >400 mg/dl GLUCOSE / TOTAL # IN GROUP x100

Fig. 8

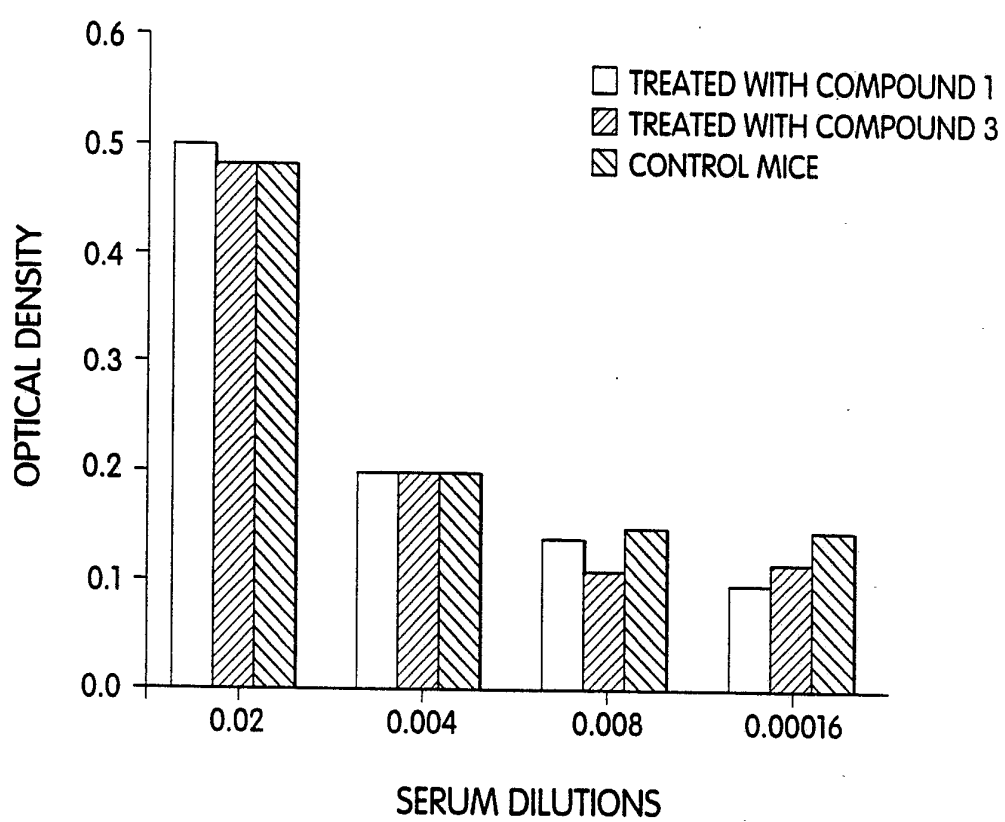


Fig. 9

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 93/00659

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC Int.Cl. 5 A61K31/415; A61K39/35; A61K39/39		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
Int.Cl. 5	A61K	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
Y	US,A,4 996 221 (K.L. MELMON) 26 February 1991 cited in the application see column 7, line 33 - line 68; claims 9,10 ---	1-37
Y	AGENTS ACTIONS SUPPL. vol. 30, 1991, NEW PERSPECTIVES IN HISTAMINE RES. pages 365 - 379 M.M. KHAN 'Histamine and its congener derivatives as immune modulators.' see the whole document --- -/--	1-37
¹⁰ Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "&" document member of the same patent family		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search 29 APRIL 1993		Date of Mailing of this International Search Report APR 30 1993
International Searching Authority EUROPEAN PATENT OFFICE		Signature of Authorized Officer ORVIZ DIAZ P.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	J. PHARMACOL. EXP. THER. vol. 253, no. 3, 1990, pages 1245 - 1252 R. QIU 'Effects of histamine-trifluoromethyl-toluidide derivative (HTMT) on intracellular calcium in human lymphocytes.' see the whole document ---	1-37
Y	PROC. WEST. PHARMACOL. SOC. vol. 30, 1987, pages 383 - 387 M.M. KHAN 'Receptor and tissue specific derivatives of histamine: novel immune modulators.' see the whole document ---	1-37
Y	TRENDS PHARMACOL. SCI. vol. 8, no. 11, 1987, pages 437 - 441 K.L. MELMON 'Histamine and its lymphocyte-selective derivatives as immune modulators.' see the whole document ---	1-37
Y	J. MED. CHEM. vol. 30, no. 11, 1987, pages 2115 - 2120 M.M. KHAN 'Congener derivatives and conjugates of histamine: synthesis and tissue receptor selectivity of the derivatives.' see the whole document ---	1-37
Y	J. IMMUNOL. vol. 137, no. 1, 1986, pages 308 - 314 M.M. KHAN 'The effects of derivatives of histamine on natural suppressor cells.' see the whole document ---	1-37
Y	EP,A,0 094 575 (UNIVERSITY PATENTS, INC) 23 November 1983 see claims -----	1-37

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 93/ 00659

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

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

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
REMARK: Although the claims are directed to a method of treatment of the human or animal body the search has been carried out and based on the alleged effects of the compositions.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

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

US 9300659
SA 69820

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

29/04/93

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A-4996221	26-02-91	None	

EP-A-0094575	23-11-83	US-A- 4521405	04-06-85
		DE-A- 3377619	15-09-88
		JP-A- 59059631	05-04-84
		US-A- 4705685	10-11-87
		US-A- 4704273	03-11-87
