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(54) Title: CELLULAR IMMUNE RESPONSE-SPECIFIC ANTIGENS AND USES THEREFOR

(57) Abstract

A CMI-specific immunogenic composition, comprising: (a) a non-naturally occurring conjugate of a primary epitope which is substantially the same as a T cell epitope of a cancer-, microbial-, parasitic-, or virally infected cell-associated antigen, and of an immunomodulatory peptide, or (b) a mixture of (1) primary antigen bearing a primary epitope which is substantially the same as a T-cell epitope of a cancer-, microbial-, parasitic-, or virally infected cell-associated antigen, (2) an immunomodulatory peptide, where the conjugate of (a) and the immunomodulatory peptide of (b) have a molecular weight of less than 5,000 daltons, where said immunomodulatory peptide of (a) or (b) comprises an allopeptide moiety of at least five amino acids whose amino acid sequence corresponds essentially to that of a polymorphic region of an MHC-encoded polymorphic Class I or Class II antigen. The composition modulates a stronger cellular immune response than the humoral immune response and is useful for treatment of tumors.

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CELLULAR IMMUNE RESPONSE-SPECIFIC ANTIGENS AND USES THEREFOR

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to the field of immunology, and, more particularly, to improved vaccines which preferentially elicit a cellular rather than a humoral immune system-mediated response. These improved vaccines are particularly useful in the treatment of human and animal tumors.

Description of the Background Art

A. The Immune System.

The ability of vertebrates to protect themselves against infectious microbes, toxins, viruses, or other foreign macromolecules is referred to as immunity. The art distinguishes between natural, and acquired or specific immunity (Abbas, et al., Cellular and Molecular Immunology, W. B. Saunders Company, 1991; Hood, et al., Immunology, 2nd Edition, The Benjamin/Cummings Publishing Company Inc., 1984).

Natural immunity is comprised of defense mechanisms which are active before exposure to microbes or foreign macromolecules, are not enhanced by such exposure, and do not distinguish among most substances foreign to the body. Effectors of natural immunity are physical barriers such as skin or mucous membranes, phagocytic cells such as macrophages or neutrophils, a class of lymphocytes termed natural killer cells, and the complement system. Complement is a serum protein complex that is destructive to certain bacterial and other cells sensitized by specific, complement-fixing antibodies; its activity is effected by a series of interactions resulting in proteolytic cleavages and which can follow one or the other of at least two pathways (Illustrated Stedman's Medical Dictionary, 24th Edition, Williams and Wilkins, Baltimore/London, 1982).

Acquired or specific immunity comprises defense mechanisms which are induced or stimulated by exposure to foreign substances.

In vertebrates, the mechanisms of natural and specific immunity cooperate within a system of host defenses, the immune system, to eliminate foreign invaders. In addition to microbes, cancer cells, parasites and virus-infected cells, the immune system also recognizes and eliminates cells or tissues transplanted into a subject from a genetically different individual of the same species (allografts) or from a different species (xenografts).

The events by which the mechanisms of specific immunity become engaged in the defense against invading microorganisms cancer cells, etc. are termed immune responses. Vertebrates have two basic immune responses: humoral and cellular. Humoral immunity is provided by B lymphocytes, which, after proliferation and differentiation, produce antibodies which circulate in the blood and lymphatic fluid. Cellular immunity is provided by the T cells of the lymphatic system. The cellular immune response is particularly effective against fungi, parasites, intracellular viral infections, cancer cells and foreign matter, whereas the humoral response primarily defends against the extracellular phases of bacterial and viral infections.

An "antigen" is a foreign substance which is recognized (specifically bound) by an antibody or a T-cell receptor, regardless of whether it can induce an immune response. Foreign substances inducing specific immunity are termed "immunizing antigens", or "immunogens". An "hapten" is an antigen which cannot, by itself, elicit an immune response (though a conjugate of several molecules of the hapten, or of the hapten to a macromolecular carrier, might do so). Since the present application is concerned with eliciting immune response, the term "antigen" will refer to immunizing antigens unless otherwise stated.

The time course of an immune response is subdivided into the cognitive phase, wherein specific lymphocytes recognize the foreign antigen; the activation phase, wherein specific lymphocytes respond to the foreign antigen; and the effector

phase, within which antigen-activated lymphocytes mediate the processes required to eliminate the antigen. Lymphocytes are immune cells that are specialized in mediating and directing specific immune responses (Abbas, et al., loc. cit.; Hood, et al., loc. cit.).

The immune system has evolved so that it is able to recognize surface features of macromolecules that are not normal constituents of the host. A foreign molecule which is recognized by the immune system (e.g., bound by antibodies), regardless of whether it can itself elicit is called an "antigen", and the portion of the antigen to which an antibody binds is called the "antigenic determinant", or "epitope". When the antigen is a polypeptide, it is customary to classify epitopes as being linear (i.e., composed of a contiguous sequence of amino acids along the polypeptide chain) or nonlinear (i.e., composed of amino acids brought into proximity as a result of the folding of the polypeptide chain). (The nonlinear epitopes are also called "conformational" because they arise through the folding of the polypeptide chain into a particular conformation.)

To cope with the immense variety of epitopes encountered, the immune system of a mammalian individual contains an extremely large repertoire of lymphocytes. Each lymphocyte clone of the repertoire contains surface receptors specific for one epitope. It is estimated that the mammalian immune system can distinguish at least 10^8 distinct antigenic determinants (Abbas, et al., loc. cit., p.8).

An initial or primary immune response to a foreign antigen enhances the ability of the immune system to respond again to that antigen (in a secondary immune response). This feature of specific immunity is called immunologic memory. Secondary immune responses are often more effective than primary responses.

The lymphocytes in an individual specifically respond to foreign antigens but are usually unresponsive to the potentially antigenic substances native to that individual.

Immunologic unresponsiveness is referred to as tolerance. Self-tolerance is acquired at an early developmental stage when potentially self-recognizing lymphocytes come into contact with self-antigens and are prevented from developing to a stage at which they would be able to respond positively to self antigens (Abbas, et al., loc., cit.)

Lymphocytes are the agents of antigenic specificity in the immune response. They divide into two groups. One group, the "B-lymphocytes" or "B-cells", play a central role in the production of antibodies. Antibodies (immunoglobulins, Ig's) are proteins capable of binding antigens, and exerting effector functions that are involved in the elimination of foreign antigens. The other group consists of T-lymphocytes or T-cells that perform a variety of functions including help for B-cells, production of delayed-type hypersensitivity reactions, and specific killing of virus-infected cells (Bjorkman, et al., Annu. Rev. Biochem., 59, 253, 1990).

Normally, immune responses progress toward effector mechanisms characteristic of both B and T-lymphocytes. However, in the course of most immune responses, either B or T lymphocytes assume a dominant role, with less substantial participation of the respective other type of lymphocyte. Immune responses whose effector mechanisms are mediated preponderantly through B-cells and antibodies are termed humoral immune responses. Those responses wherein T-cells mediate the more important effector functions are referred to as cell-mediated or cellular immune responses.

B-cells constitute the population of lymphocytes central to humoral immune responses. Each clone of B-lymphocytes expresses membrane immunoglobulins (membrane Ig's, surface-bound antibody molecules) that function as antigen receptors with one unique epitope specifically per B-lymphocyte clone. These membrane Ig molecules (antigen receptors) are the sole source of B-cell specificity (Bjorkman, et al., loc. cit.). Antigens that contain an epitope complementary to the membrane Ig will bind to the antigen receptor. Such antigens are also

referred to as cognate antigens of the antibody. On protein antigens, antibodies can bind linear determinants (epitopes formed by adjacent amino acid residues in the covalent sequence), or conformational determinants, which are formed by amino acid residues from separate portions of the linear polypeptide that are specially juxtaposed by polypeptide folding (Abbas, et al., loc. cit.). Binding to the antigen receptor (membrane Ig) will result in differentiation and clonal proliferation of the B-lymphocyte. Some of its progeny will differentiate into mature plasma cells which are specialized in the synthesis of antibodies corresponding in epitope specificity to the membrane Ig by which the B-lymphocyte had initially bound the antigen.

By an effector mechanism typical of humoral immune responses, antibodies will bind to cognate epitopes on the surface of invading target cells, e.g., bacteria. Following antibody binding, the components of the complement system will sequentially attach to the target cell-antibody complex, resulting ultimately in the rupture of the target cell membrane and killing of the target cell. By another antibody-mediated effector mechanism, target antigens are bound and cross-linked (opsonized) by antibodies, and are thus prepared for ingestion and subsequent destruction by phagocytes of reticuloendothelial origin, such as granulocytes or macrophages.

The antibody itself is an oligomeric molecule, classified, according to its structure, into a class (e.g., IgG) and subclass (e.g., IgG1). IgG molecules are the most important component of the humoral immune response and are composed of two heavy (long) and two light (short) chains, joined by disulfide bonds into a "Y" configuration. The molecule has both variable regions (at the arms of the "Y") and a constant region (the hinge and base of the "Y"). The regions are so named because antibodies of a particular subclass, produced by a particular individual in response to different antigens, will differ in the variable region but not in the constant region. The variable regions themselves are composed of both a

relatively invariant framework, and of hypervariable loops, which confer on the antibody its specificity for a particular epitope. An antibody binds to an epitope of an antigen as a result of molecular complementarity. The portions of the antibody which participate directly in the interaction is called the "antigen binding site", or "paratope". The antigens bound by a particular antibody are called its "cognate antigens".

An antibody of one animal will be seen as a foreign antigen by the immune system of another animal, and will therefore elicit an immune response. Some of the resulting antibodies will be specific for the unique epitopes (idiotype) of the variable region of the immunizing antibody, and are therefore termed anti-idiotypic antibodies. These often have immunological characteristics similar to those of an antigen cognate to the immunizing antibody. Anti-isotypic antibodies, on the other hand, bind epitopes in the constant region of the immunizing antigen.

The typical effector phase of cell-mediated or cellular immune responses involves lysis or killing of target cells by cytotoxic or cytolytic T-lymphocytes (CTLs) through direct cell-to-cell contact. Molecules from two diverse families of cell-surface glycoproteins, the T-cell receptors (TCRs) and the major histocompatibility complex (MHC) type I glycoproteins, are the key elements of specificity in the CTL response to foreign antigens. T-cell receptors (TCRs) recognize short, linear peptide determinants of 8-24 amino acids, the generation of which usually requires unfolding and proteolytic fragmentation ("processing") of the antigenic protein (Allen, Immunology Today, 8., 270, 1987; Unanue, et al., Science, 236, 551, 1987; Bjorkman, et al., loc. cit.; Braciale, et al., Immunol. Rev., 98, 95, 1987; Unanue, Annu. Rev. Immunol. 2, 395, 1984; Ziegler, et al., J. Immunol., 127, 1869, 1981; Shimonkevitz, et al., J. Exp. Med., 158, 303, 1983; Zweerink, et al., Eur. J. Immunol., 7, 630, 1977). They can also recognize oligosaccharide determinants. (Menningsson, et al.,

Cancer Immunol. Immunother., 25, 231, 1989; Fung, et al., Cancer Res., 50, 4308, 1990). Unlike antibodies, T-cell receptors cannot recognize conformational epitopes.

The second difference in antigen recognition by antibodies and T-cell receptors is the involvement of a third molecule that performs the role of presenting the antigen to the T-cell receptor. For B-cells, such molecules are not necessary, as the membrane Ig (antibody) forms a stable bimolecular complex with the antigenic protein. For T-cells, the antigenic peptide must be bound by an MHC glyco-protein, and it is this complex of MHC molecule plus peptide that forms the structure recognized by the T-cell receptor. MHC glycoproteins are thus peptide-binding proteins which function as antigen-presenting molecules (*Bjorkman, et al., loc. cit.).

Cytotoxic T-lymphocytes, as the main effector cells of the cell-mediated immune response, recognize peptides bound to MHC Class I proteins, and are capable of killing virus-infected cells and cancer cells (Zinkernagel, et al., Nature, 248, 701, 1974; Rouse, et al., Rev. Infect. Dis., 10, 16, 1988; Lukacher, et al., J. Exp. Med., 160, 814, 1984; McMichael, et al., N. Engl. J. Med., 309, 13, 1983; Wraith , et al., J. Gen. Virol., 68, 433, 1987; Cerundolo, et al., Eur. J. Immunol., 17, 173, 1987; Kast, et al., Cell, 59, 603, 1989). CTLs generally display a cell surface marker termed CD8 (Abbas, et al., loc. cit., p. 310). The CD designation refers to a nomenclature system of cell surface markers whereby a surface marker characteristic of a cell lineage or differentiation stage which has a defined structure and is recognized by a group ("cluster") of monoclonal antibodies, is called a member of a cluster of differentiation (CD; Abbas, et al., loc. cit., pp. 19; 398-401).

Another important set of T-lymphocytes is generally CD8-negative and displays the CD4 marker. These T-cells participate in the cognitive and activation phases of both the humoral and cell-mediated immune responses and are referred to as T-helper cells. T-helper cells, through their T-cell

receptors, recognize foreign antigens bound to MHC Class II molecules on the surface of accessory cells such as mononuclear phagocytes (macrophages), follicular dendritic cells of the spleen and lymph nodes, Langerhans cells of the epidermis, or venular endothelial cells (Abbas, et al., loc. cit., p. 122-3).

One of two distinct subsets of T-helper lymphocytes, termed T_H1 and T_H2, direct the immune system toward either a primarily cell-mediated or humoral type of response, respectively (Mosmann, et al., Adv. Immunol., 46, 111, 1989). The T_H1 and T_H2 cells are categorized by their different functions and by the constellation of immunological mediators (cytokines) they produce. Generally, T_H1 cells secrete interferon-γ and interleukin-2 (IL-2), and contribute to cell-mediated immune responses such as delayed-type hypersensitivity (DTH) and macrophage activation. T_H2 cells produce interleukin-4 (IL-4), interleukin-5 (IL-5), and interleukin-10 (IL-10) and help B-cells to generate antibody response (Mosmann, et al., J. Immunol., 136, 2348, 1986; Cherwinski, et al, J. Exp. Med., 166, 1229, 1987; Fiorentino, et al., J. Exp. Med. 170, 2081, 1989).

The tendency for either the cell-mediated or humoral immune response to predominate is believed to be a consequence of cross-regulation (Parish, Transplant. Rev., 13, 35, 1972; Mosmann, Ann. New York Acad. Sci., 337, 19___). Thus T_H1 cells would inhibit the elicitation of T_H2 responses, e.g., by secretion of interferon-γ. Conversely, T_H2-cells could inhibit the generation of T_H1-responses by producing cytokines such as IL-4 and IL-10 (Salk, et al., Science, 260, 1270, 1993).

B. Polymorphism

In a population of individual animals of a given species, some genes have only one normal nucleic acid sequence; variant sequences are uncommon mutations and may result in a disease state. Such genes are said to be nonpolymorphic, and the normal sequence will usually be present in both chromosomes of the appropriate chromosome pair in he diploid cells of that animal.

In other genes, the nucleic acid sequences may vary at a relatively high frequency amonog normal individuals in the population, i.e., so that at least 1% of the individuals express a gene that differs from the homologous gene in the remaining members to the population. Such genes are said to be polymorphic, and the various variants of the polymorphic gene are called alleles. Some alleles will be common; others, rare. An individual who has the same allele at a genetic locus on both chromosomes of a pair is homozygous for it; one who has two different alleles at that locus, one for each chromosome, is heterozygous for it.

One may also speak of the encoded proteins, as well as their genes, as being subject to allelic variation, and thus of the proteins as being polymorphic too. By extension, particular residues, or regions of residues, in a polymorphic protein may be termed "polymorphic" as well. Such terminology is useful if a polymorphic protein can be characterized as having, over a particular population, certain residues which are typically invariant and others which are more variable.

3. *The Major Histocompatibility Complex*

Grafts from an individual to himself (autografts, i.e., of autologous cells), and from one individual to another, genetically identical individual (syngeneic grafts) almost invariably succeed. Grafts between genetically dissimilar individuals of the same species (allogeneic grafts), or between individuals of different species (xenogeneic grafts), are unsuccessful unless the immune response to the cell surface antigens that distinguish donor from host is abated, e.g,, by use of immunosuppressants. The tissue antigens that induce an immune response in other individuals are called histocompatibility or transplantation antigens, and the genes that specify their structure and synthesis are called histocompatibility genes. The histocompatibility antigens are further classified as major or minor, depending on the speed and severity of the graft rejection.

When a histocompatibility antigen is administered to an allogeneic individual it is referred to as an "alloantigen", the elicited antibodies as "alloantibodies", and the epitopes of the alloantigen which were recognized by those antibodies as "alloepitopes".

Usually, the alloepitopes of an alloantigen will correspond to polymorphic sites, as it is as these sites that the alloantigen normally differs from the cognate autologous protein. A peptide fragment of an alloantigen, which presents at least one alloepitope, is an "allopeptide".

When a histocompatibility antigen is administered to a xenogeneic individual, one may similarly speak of xenoantigens, xenoantibodies, xenoepitopes and xenopeptides.

Laboratory animals, such as mice, can be inbred until all mice of the particular inbred strain are genetically identical (syngeneic) to each other, and are homozygous at all genetic loci. Consequently, each inbred strain can exhibit, for a given polymorphic gene, only one allele from the original population. However, absent selective pressure, it is a matter of chance as to which alleles of the parental animals are fixed in the inbred strain. Consequently, mice of different inbred strains, even when bred from the same parents, are likely to express different combinations of alleles of their various polymorphic proteins, and therefore to be allogeneic to each other.

While there are numerous histocompatibility loci in mice, one complex, initially denoted H-2, encodes cell surface antigens leading to rapid graft rejection. The greater gene complex of which the H-2 complex is a part, which also encodes other proteins involved in self-nonself recognition, has come to be known as the major histocompatibility complex. Mouse MHC, which is located on chromosome 17, is divided into an H-2 complex and a Tla complex. The H-2 complex and a Tla complex. The H-2 complex, in turn, is divided into K, I, S and D regions, while the Tla complex includes Qa-2,3, Tla and Qa-1 regions. The I region is divided into B, J, E and C

subregions.

The MHC encodes three classes of genes. The class I gene products include he transplantation antigens K, D and L (K is encoded in the K region, and D and L, encoded in the D region) which are responsible for rapid graft rejection, and the hematolymphoid differentiation antigens Qa-1, Qa-2,3 and TL. The transplantation antigens are found on almost all nucleated somatic cells. Class I MHC molecules are involved in the recognition of target cells by cytotoxic T cells. Typical target cells are virally infected tissue cells, and graft cells expressing non-self MHC antigen.

The class II genes -- Aα, Aβ, Eα and Eβ -- are located in the I region, and hence the encoded antigens are also known as Ia antigens. Class II molecules are found on macrophages Langerhans cells of the skin, and certain other cells. They present antigen to T helper cells, which in turn facilitates the proliferation and maturation of B cells. The Aα and Aβ genes encode the two chains of the "I-A" class II molecule, and the Eα and Eβ genes encode the two chains of the "I-E" class II molecule.

Certain complement components (Class III) are also genetically associated with the MHC, but are not, properly speaking, MHC genes.

The MHC of humans is denoted the human HLA complex and is located on chromosome 6. The three class I transplantation antigens are denoted A, B and C; the three class II antigens are known as SB, DC, and DR; and there are also class III genes. The mouse I-A and I-E molecules are homologous to HLA-DC and HLA-DR, respectively.

Several MHC genes are polymorphic. The total set of MHC alleles present on one chromosome of an individual animal is its MHC haplotype; heterozygous individuals have two MHC haplotypes.

All class I MHC proteins contain two polypeptide chains: an MHC-encoded α or heavy chain, and a non-MHC-encoded β or light chain. The light chain, also known as β_2-microglobulin,

is non-polymorphic in humans and dimorphic in mice. The polymorphic heavy chain may be divided into an amino terminal extra cellular region, a transmembrane region, and a carboxy terminal cytoplasmic region. At the amino terminal, the first about 180 residues fold into two homologous segments of about 90 amino acid residues each, referred to as α_1 and α_2. These two domains interact to form a platform of an eight-stranded, β-pleated sheet supporting two parallel strands of α-helix. Four β-strands and one α-helix are formed from the residues of α_1, the others from those of α_2. The two α-helices form the sides of a cleft whose floor is formed by the strands of the β-pleated sheet. This cleft is about 25Åx10Åx11Å. Studies of MHC Class I: peptide complexes reveal that about 70% of the time, the cleft holds a 9 a.a. peptide; about 20% of the time, an 8 a.a. peptide, and about 10% of the time, a 10 a.a. protein. Complexing of peptides larger than 13 a.a. is very rare.

Grandea and Bevan, J. Immunol., 151:3481-87 (1993) have shown, in a transgenic mouse model, that even a small variance from self in an MHC Class I molecule (H-2K^b), even though outside the peptide binding groove (though at a residue involved in TCR binding), can be antigenic. A transgenic mouse which expressed a mutant of H-2K^b, E65, mounted a weak alloreactive response to the mutant H-2K^b (D65) (a highly conservative difference) and a stronger response to wild type H-2K^b (Q65).

The degree of polymorphism of MHC Class I proteins depends on the gene in question. In mouse lines, more than 50 alleles have been serologically identified at both the H-2K and H-2D loci. The H2-L locus is less polymorphic. In humans the HLA-A and HLA-B loci are more polymorphic than the HLA-C locus. Comparison of the sequences of a large number of amino acid sequences of human and murine class I MHC heavy chains indicates that virtually all of the polymorphic residues are located either in the α-helical sides of the cleft, or on the β-strands that form its floor, and are oriented such that the

amino acid side chains point into the cleft or toward the top of the helices. Other polymorphic residues of MHC molecules form contacts with T cell antigen receptors.

It is been proposed that the polymorphism of the MHC Class I antigen binding domains evolved so that pathogens could not readily escape T cell recognition among all individuals of a population, merely by mutating their surface antigens until they were no longer capable of binding to MHC molecules.

Each Class II molecule is a heterodimer, consisting of two MHC-encoded polypeptides. Aα and Aβ genes are highly polymorphic, but Eα is not. Each class II polypeptide is composed of two external domains (α and α_2, or β, and β_2, each about 90 amino acids residues long), a transmembrane domain (about 30 residues), and a cytoplasmic domain (about 10-15 residues). The antigen binding domains of these molecules can accommodate peptides of up to about 24 amino acids. Larger peptides will be partially exposed to proteolytic attack and trimmed down in that manner.

For more information on the MHC, See generally Klein, Biology of the Mouse Histocompatibility-2 Complex (Springer-Verlag, 1975); Snell, et al., Histocompatibility (Academic Press, 1976); Gotze, The Major Histocompatibility System in Man and Animals, (Springer-Verlag, 1977).

D. Vaccinological Control of the Nature of the Immune Response

Vaccines are preparations administered to animals or humans to effect the prophylaxis, cure or alleviation of disease states through induction of specific immunity. Prophylactic vaccines are given to healthy individuals with the intention of preparing or priming the immune system for more effective defense against infections in the future. In the event of an infection or infestation, the immune system of vaccinated individual can mount a secondary immune response and can more rapidly recognize and eliminate the respective pathogens. Therapeutic vaccines are given to diseased individuals with the intent of stimulating or modulating the

immune system which of itself has either mounted an insufficiently effective immune response or has altogether failed to respond.

In the design of prophylactic or therapeutic vaccines, it is important to choose preparations that will elicit the type of immune response most capable of either providing first-line protection, or effecting speedy recovery (Rouse, et al., Rev. Infect. Dis., 10, 16, 1988; Pfeiffer, et al., Immunol. Rev., 123, 65, 1991).

In many diseases, the causative pathogens or toxins (e.g., influenza, polio, and rabies viruses; pneumococcus bacteria; diphtheria and tetanus toxins) can be effectively targeted and neutralized in the extracellular fluid by the mechanisms of humoral immunity through antibodies that bind to the pathogens or toxins and thereby lead to their inactivation of destruction (Plotkin, et al., Vaccines, Saunders, Philadelphia, 1988; Salk, et al., loc. cit.). In these cases, vaccination with preparations that elicit a humoral immune response, presumably mediated by T_H2 cells , is generally sufficient for protection.

On the other hand, for many intracellular infections, for recovery from viral infections (e.g., ref. 50), and for targeted killing of cancer cells (ref. 52), it is cell-mediated immunity that protects the organism against the invaders (Hood, et al., loc. cit., p. 3). Applicable situations include vaccines against the fungal infection of candidiasis (ref. 45) bacterial infections of tuberculosis and leprosy (refs. 48-49), the spirochetal infection of syphilis, the protozoan infections of cutaneous and visceral leishmaniasis (ref. 46-47) and schistosomiasis (ref 43-44), and infection with the parasitic nematode Trichinella spiralis (Ellner, in Textbook of Internal Medicine, W. W. Kellev, Ed., Lippincott, Philadelphia, 1989; pp. 569-77; Fitzgerald, Infect. Immun., 60, 3475, 1992; Bretscher, et al., Science, 257, 539, 1992; Finkelman, et al., Immunoparasitology Today, A62, 1991; Pond, et al., J. Immunol., 143, 4232, 1989). T_H2 responses might actually exacerbate the development of these diseases (refs. 43, 51).

A special application would be vaccination against human T-lymphotropic virus infections (HTLV, HIV, AIDS) where it has been shown that a T_H2-driven humoral immune response is often associated with progression of the AIDS into a terminal stage whereas a cell-mediated (T_H1-driven) immune response appears to maintain infected individuals in a symptom-free stage with a normal quality of life (Clerici, et al., Immunology Today, 14, 107, 1993; Levy J. Am. Med. Assoc., 259, 3037, 1988; ibid., 261, 2997, 1988).

The art is aware of experimental approaches for controlling the choice between humoral and cellular vaccination responses through variation of vaccination conditions.

Thus, it has been well known in the art that injections of small amounts of immunizing antigens will preferentially elicit delayed-type hypersensitivity responses, indicative of cell-mediated immunity, whereas vaccination with larger amounts of antigen will result in a more pronounced humoral immune response as reflected by high antibody titers (Wortis, et al., Immunology, 14, 69, 1968; Parish, loc. cit.; Lagrange, et al., J. Exp. Med., 139, 528, 1974; Bretscher, et al., Science, 257, 539, 1992; Clerici, et al., IX International Conference on AIDS, Berlin, 7-11 June 1993, Abstract 3279); Clerici, et al., Immunol. Today, 14, 107, 1993; Clerici, et al., Eur. J. Immunol., 21, 1345, 1991. However, it is difficult to avoid a high IgG response, and achieve a high and prolonged cellular response, by this method, and depending on the antigen, small doses may be insufficient to elicit a sufficiently strong CMI response to be useful.

For many years, live, attenuated vaccines have been used to induce immunity against viral infections such as influenza and polio. These preparations contain live virions which cause mild, subclinical infections of the vaccinated individuals. In the course of such infections, viral vectors will enter certain host cells and code for the synthesis of virus-specific proteins (Zweerink, et al., Eur. J. Immunol., 7, 630, 1977). These endogenously produced antigenic proteins will be

processed into smaller peptides and presented in the context of MHC Class I and II antigens, thereby recruiting T_H1 cells and eliciting cell-mediated immune responses. However, this strategy requires that, for each pathogen of interest, one identify and follow an attenuation procedure, which may be time-consuming, laborious, or expensive.

More recently, recombinant vaccinia virus constructs have been used for immunization. These have been prepared and experimentally used against hepatitis B (Moss, et al., Nature, 311, 67, 1984), herpes simplex virus (Wacchsman, et al., Biosci. Rep. 8, 323; 334, 1988), parainfluenza type 3 (Spriggs, et al., J. Virol., 62, 1293, 1988), and Lassa fever virus (Fisher-Hoch, et al., Proc. Natl. Acad. Sci. USA, 86, 317, 1989). Vaccinia virus constructs comprising gene for cancer-associated antigens have also been prepared (Lathe, et al., Nature, 326, 878, 1987; Bernards, et al., Proc. Natl. Acad. Sci. USA, 84, 6854, 1987; Estin, et al., Proc. Natl. Acad. Sci. USA, 85, 1052, 1988). This method requires that a gene encoding the antigen of interest be available, and be cloned into a Vaccinia construct.

While live vaccines have been well established, e.g., for prophylaxis against polio and influenza, their successful use against intracellular bacteria or parasites has not been broadly established. Also, the use of live attenuated virus, or antigens that comprise replicating viral vectors such as Vaccinia in conjunction with genetic information for T_H1-eliciting epitopes of cancer cells or HIV-infected cells, poses grave risks for the recipients due to potential pathogenicity of the vectors used (Donnelly, et al., loc. cit.; Redfield, et al., N. Engl. J. Med., 316, 673, 1987; Mascola, et al., Arch. Intern. Med., 149, 1569, 1989).

Another principle suggested in the art for directing a vaccination response toward control by the T_H1 or T_H2 helper subsets is the inclusion of immunologic mediators or cytokines in appropriate vaccine preparations. Thus, admixture of interferon-γ would be expected to result in a predominantly

T_H1-mediated, cellular response, while anti-γIFN switches T_H1 to T_H2 and anti-IL4 switches T_H2 to T_H1 (Bretscher, et al.). Similarly, interleukin-12 (IL-12) has been reported to convert the antigen-dependent immune response from a T_H2-mediated into a T_H1-mediated mode (Hsieh, et al., Science , 260, 547, 1993; Scott, Science, 260, 496, 1993). Interleukin-10 (IL-10) has been shown to suppress T_H1-functions and could be used to direct immune responses toward mediation by T_H2-cells (Mosmann, Ann. New York Acad. Sci., loc. cit.). Conversely, administration of anti-IL-10 antibody has been shown to abrogate mediation by the T_H2-subset and thus favor a T_H1-directed response (Mosmann, loc. cit.). Conceivably, the dose of cytokine required for reliable control of the vaccination response would vary among individuals and would be complicated to assess for each case. Also, cytokines such as interferon-γ and IL-2 have powerful pharmacologic effects (e.g., decreases vascular permeability) which could result in adverse reactions, depending on individual susceptibility (Abbas, et al., loc. cit., p. 239).

It may be possible to introduce soluble antigenic proteins or peptides into the MHC Class I restricted antigen presentation pathway of accessory cells. Experimentally, this type of targeting has been achieved by vaccination in conjunction with saponin adjuvants such as ISCOMs and QS-21, by administering conjugates consisting of antigenic peptides and the terminal lipopetide of Braun's lipoprotein, and by the use of vaccines comprising fusion proteins of bacterial toxins.

Generally, adjuvants are substances admixed with an immunogen in order to elicit a more marked immune response. The saponin adjuvant QS 21 has been shown to introduce soluble protein antigens into the processing pathway leading to Class I MHC-restricted antigen presentation, resulting in a primarily cell-mediated immune response. Control vaccinations without the adjuvant resulted in humoral immune responses (Newman, et al., J. Immunol., 148, 2357, 1992). A similar adjuvant principle termed ISCOMs (Morein, et al., Nature, 308 457, 1984;

Jones, et al., Scand. J. Immunol., 27, 645, 1988) elicits HIV-specific or influenza-specific $CD8^+$ MHC Class I restricted cytolytic T-lymphocytes, indicating involvement of T_H1-cells, while vaccinations with the same soluble protein antigens of HIV or influenza in the absence of adjuvant resulted in T_H2-controlled (antibody) responses (Takahashi, et al., Nature, 344, 873, 1990).

Conjugates have been synthesized of virus-derived peptides with tripalmitoyl-S-glyceryl-cysteinyl-serylserine (P_3CSS; Deres, et al., Nature, 342, 561, 1989). These conjugates are capable of inducing, in vivo, influenza-virus specific cytolytic T-lymphocytes of the same high affinity as does the infectious virus (Deres, et al., loc. cit.).

The art is further aware of fusion proteins, consisting of the binding and translocation domains of Pseudomonas exotoxin A fused to peptide epitopes, e.g., from influenza A matrix protein or nucleoprotein (Donnelly, et al., Proc. natl. Acad. Sci. USA, 90, 3530, 1993). Such fusion proteins are internalized and processed by MHC Class I positive target cells, resulting in the sensitization of such target cells for lysis by peptide-specific, cytolytic T-lymphocytes.

Thus, the art has recognized the need for prophylactic and therapeutic vaccination modalities that would prime an individual for a cell-mediated rather than a humoral immune response. This is especially applicable to prophylaxis against intracellular bacterial or parasitic infections such as tuberculosis, leprosy, syphilis, leishmaniasis, schistosomiasis, and malaria (Süss, et al., J. Immunol., 149, 1334, 1992). In addition to these needs in the area of prophylaxis, there is also a pressing need for therapeutic vaccination modalities that would elicit cell-mediated immune responses against tumor antigens in cancer patients or against HIV-infected cells in HIV-infected individuals. Of greatest importance are the observations that antibody responses not only have no therapeutic effects in AIDS or cancer, but may even contribute to progression of disease by shielding or

protecting virus-infected cells or cancer cells against effectors of cell-mediated immunity (Hellström, K. E., et al. in The Biologic Therapy of Cancer, Eds. V. T. DeVita, Jr., et al., J.B. Lippincott, Philadelphia, 1991; pp 35-52; Clerici, et al., loc. cit.). Therefore, suitable therapeutic vaccines against AIDS or cancer should not only elicit a cell-mediated immune response where no immune response exists, but should also be capable of converting or re-directing an existing humoral response into a cell-mediated response.

There remains at this time an unfulfilled need for a generic and safe vaccination modality capable of selectively and strongly eliciting cell-mediated immune response in prophylactic and therapeutic vaccinations of humans and other mammals. Particularly in AIDS and cancer, therapeutic vaccines are required which can downregulate an ongoing, T_H2-directed humoral response and replace it by a T_H1-directed, cellular response (Nardelli, et al. 1992).

E. Immune Response to Alloantigens

One method of stimulating "helper" T-cells involves the use of alloantigens. Cellular alloantigens stimulate very strong T-cell mediated immune reactions. In fact, allo-immune reactions are the strongest proliferative T-cell responses known because such a large number of T-helper cells (TH) proliferate and secrete cytokines in response to foreign alloantigens. However, alloantigens, whether in the form of a graft, or of injected cells, strongly induce alloantibodies as well as cellular immune responses, and therefore would not have been considered a viable approach to highly selective elicitation of a strong CMI response.

Singh et al. (*J. Immunol.* 137:2311-2318, 1986) were the first to show that alloantigenic synthetic peptides of class I antigens can be used to stimulate strong allo-immune reactions. T-cells of allostrain mice that were previously primed with H-2K^b alloantigen bearing cells or with the synthetic (61-69)-H-2K^b peptide undergo extensive in vitro proliferation in response to the synthetic (61-69)-H-2K^b

peptide presented on syngeneic antigen presenting cells (APC's). They further showed that the APC bound 61-69-H-2K^b peptide activates only helper T-cells (TH) and not cytotoxic T-cells. However, there was no response to the soluble (61-69) H-2K^b. When coupled to a protein carrier (KLH), the peptide induced allo-antibodies in allostrain mice.

Clerici et al. (1990) discloses that defective CD4 T helper cell function, e.g., to influenza A virus, in HIV$^+$ patients can be restored by co-stimulation with HLA alloantigens and influenza antigens. Clerici suggests that the use of HLA alloantigen in combination with specific pathogen antigens could be useful for treating HIV$^+$ patients exhibiting selective defects in Th function. Clerici used whole HLA antigen in his experiments. Used in vivo, there would be the risk of raising alloantibodies. Indeed, these alloantibodies might cross-react with autologous HLA antigens, initiating an autoimmune response.

SUMMARY OF THE INVENTION

While the humoral immune response is one of the body's major defenses, as explained above, it can at times be disadvantageous.

Applicants have found that the immune response to an epitope of interest (the "primary epitope") may be modulated, so as to strongly favor a cellular immune response over a humoral immune response, by simultaneously presenting a low molecular weight (<5,000 Da) immunomodulatory peptide comprising an allopeptide moiety corresponding essentially with a polymorphic region of a polymorphic MHC polypeptide, especially an MHC Class I antigen. Preferably, the humoral immune response is essentially absent.

In one embodiment, the epitope of interest is conjugated, directly or indirectly, covalently or noncovalently, to the immunomodulatory peptide to form a chimeric molecule.

In a second embodiment, the epitope of interest is borne by an antigen of interest, which may be an immunogen or a hapten, and the alloepitope (or xenoepitope) by an immunomodulatory peptide, and the antigen of interest is mixed with the immunomodulatory peptide to form an unconjugated mixture which is CMI-specific. We have discovered that the mixture of an immunomodulatory peptide with a hapten can elicit a CMI response even though the hapten alone has no effect.

"CMI-specific antigen" is used herein to refer to both the antigenic conjugate of the first embodiment and the antigenic mixture of the second embodiment, the terms "conjugate" and "mixture" being used when necessary to distinguish them.

In a third embodiment, the immunomodulatory peptide is administered first to the subject, to shift the subject's immune system to a T_H1 mode, and then, while this "imprint" is still effective, the antigen of interest is administered.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 plots variability index versus residue number for published alleles of human MHC Class I antigens, namely (A) HLA-A, (B) HLA-B, and (C) HLA-C.

Figure 2 is a similar plot for published alleles of mouse MHC Class I antigens, namely (B) H-2K and (C) H-2D. Plot (A) is for the aggregate of published mouse MHC Class I antigens, including both H-2K an H-2D.

Figure 3 provides similar plots for (A) all MHC Class I antigens of all species tabulated, and (B) for all human MHC Class I antigens (i.e., Figs. 2A and 3B are at the same phylogenetic level).

Figures 4-7 show the result of applying a 10-a.a averaging window to the variability indexes for mouse H2D (Figure 4), mouse H2K (Figure 5), Human HLA-A (Figure 6), and human HLA-B (Figure 7).

Figure 8-12 show, analogously to Figures 1-3, variability plots for MHC Class II proteins (Figure 8A, all A chains; 8B, all human A chains; 8C, human DQ locus A chain; 9A, mouse A chains; 9B, mouse A locus A chains; 10A, all B chains; 10B, human B chains, 11A, DR locus B chains; 11B, DQ locus B chains; 11C, DP locus B chains; 12A, mouse B chains; 12B, mouse A locus B chains; 12C, mouse E locus B chains.)

Figures 13 and 14 show the effect of $H2K^K$ peptides on BP12-7 induced DTH response in CBA mice, as measured by increase of footpad thickness after 24 (dark bar) or 48 (light bar) hours. Figure 13 refers to peptides BP1-82, -81, -79, -76, -68, -78, -77, and -32. Figure 14 refers to BP1-82, -81, -62 and -32.

In Figure 13, BP1-7 is the human MUC-1 peptide, BP1-82, -81, -68, -32 are based on the mouse MHC class I polymorphic region 62-69 (also including conserved residue 61); BP1-79 and -78 are based on another polymorphic region, 92-102 (or 94-102); and BP1-77 and -76 are based on a third polymorphic region, 75-83. The induced DTH response was greatest for the 61-69 derived allopeptides.

In Figure 14, BP1-82, -81, -62 and -32 explore the effect of fine changes in the sequence of the 62-69 region.

Figures 1-12 are based on MHC Class I and II sequences tabulated in Kabat, et al., Sequences of Proteins of Immunologic Interest (5th ed., 1991; US-HHS).

Polymorphism is variability at a single locus in a single species of animal. The figures also examine interlocus variability (aggregated variability of, e.g., all mouse MHC Class I loci) or interspecies variability (aggregated variability of, e.g., all mammalian MHC Class I loci).

It will be evident from a study of Figures 1-3 that while the α_3 domain and the membrane and cytoplasmic domains are not significantly polymorphic, they do show substantial interlocus and interspecies variability.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention relates to the use of "cell-mediated immune response-specific antigen" conjugates or mixtures as vaccines. When such antigens are administered to a human or other vertebrate animal subject (especially mammal or bird) to which the antigen is foreign, an immune response is elicited, for which the cell-mediated immune response predominates over the humoral immune response.

Preferably, the presence of the immunomodulatory peptide results in at least a 20%, more preferably at least a 50%, still more preferably at least a 100%, enhancement in the cellular immune response relative to that obtained with the primary epitope (and the immunogenic carrier, if any) alone. Desirably, the presence of the immunomodulatory peptide results in at least a 20%, more preferably at least a 50% more preferably at least a 90%, reduction in the humoral immune response relative to that obtained with the primary epitope (with the immunogenic carrier, if any) alone.

Primary Epitope

The primary epitope may be any T cell epitope which is at

least substantially the same as a T-cell epitope of an antigen including a hapten) which is associated with a disease or adverse condition to a degree such that it could be prophylactically or therapeutically useful to stimulate or enhance a cellular immune response to that epitope. Such diseases and conditions include, but are not limited to parasitic diseases such as schistosomiasis and leishmania, fungal infections such as candidiasis, bacterial infections such as leprosy, viral infections such as HIV infections, and cancers, especially solid tumors. Of course, the greater the degree of specificity of the epitope for the associated disease or adverse condition, the more likely it is that the stimulation of an immune response to that epitope will be free of adverse effects.

An epitope may be deemed associated with a cell (e.g., a cell of a cancer, a microbe, or a parasite) because an antigen bearing the epitope is produced by that cell. If so, the antigen may be a surface antigen, an intracellular antigen or a "shed" antigen. An epitope may be deemed virus-associated because it is encoded by the viral genome or because it is specifically produced or overproduced by a virus-infected cell in response to the viral infection.

An epitope may also be deemed associated with a disease if it is borne by a biochemical produced by other cells of the subject in specific, but non-immunological, response to the disease, e.g., an angiogenic factor which is expressed by other cells as a result of regulatory substances secreted by a tumor.

Chemically speaking, the epitope may have, inter alia, a carbohydrate, peptide or glycopeptide structure. When the primary epitope is a peptide, it is "at least substantially the same" as a naturally occurring epitope if it is (a) identical to the latter, or (b) differs from the latter only by (1) a single substitution, and/or (2) one or more conservative substitutions, and in either of cases (1) or (2) is immunologically cross-reactive with the naturally occurring epitope. It is well known in the art that modified epitopes

which are cross-reactive with a natually occurring epitope may be obtained by random or nonrandom mutagenesis of the latter. For example, Geysen, et al., Proc. Nat. Acad. Sci. (USA), 81:3998-4002 (1984). has taken B-cell epitopes and prepared a set of all the peptides which differ from the original epitope by a single amino acid substitution. It is also known that one may randomly mutate one or more residues of an epitope so that any of the twenty possible amino acids, or a selected set (such as all conservative replacements), can occur at that residue position, and screen for mutants with a desired immunological activity. Parmley and Smith, Gene, 73:305-18 (1988); Devlin, et al., Science, 25:49:404-6 (1990); Scott and Smith, Science, 249:386-90 (1990); Greenwood, et al., J. Mol. Biol., 220:821-7 (1991); Cwirla, et al., Proc. Nat. Acad. Sci. (USA), 87:6378-82 (1990); Stephen and Lane, J. Mol. Biol., 225:577-83 (1992); Barrett, et al., Anal. Biochem., 204:357-64 (1992); Ladner, USP 5,223,409.

The epitope must, of course, be one amenable to recognition by T-cell receptors so that a cellular immune response can occur. For peptides, the T-cell epitopes are likely to be linear sequences of about 5-24, more often 8-24 amino acids. The carbohydrate T-cell epitopes may be as small as a single sugar unit (e.g., Tn), or as large as five sugars, with 2-5 preferred.

In one embodiment, the epitope is a parasite-associated epitope, such as an epitope associated with leishmania, malaria, trypanosomiasis, babesiosis,or schistosomiasis. Suitable parasite-associated epitopes include, but are not limited to, the following.

Parasite	Epitope	References
Plasmodium Falciparum (Malaria	(NANP)3	Good et al (1986) J. Exp. Med. 164:655
	Circumsporoz. protein AA 326-343	Good et al (1987 Science 235:1059

Leishmania donovani	Repetitive peptide	Liew et al (1990) J. Exp. Med. 172:1359
Leishmani major	EAEEAARLQA (code)	This application
Toxoplasma gondii	P30 surface protein	Darcy et al (1992) J. Immunolog. 149:3636
Schistosoma mansoni	Sm-28GST antigen	Wolowxzuk et al (1991) J. Immunol 146:1987

In another embodiment, the epitope is a viral epitope, such as an epitope associated with human immunodeficiency virus (HIV), Epstein-Barr virus (EBV), or hepatitis. Suitable viral epitopes include, but are not limited to:

Virus	Epitope	Reference
HIV gp120	V3 loop, 308-331	Jatsushita, S. et al (1988) J. Viro. 62:2107
HIV GP120	AA 428-443	Ratner et al (1985) Nature 313:277
HIV gp120	AA 112-124	Berzofsky et al (1988) Nature 334:706
HIV	Reverse transcriptase	Hosmalin et al (1990) PNAS USA 87:2344
Flu	nucleoprotein AA 335-349, 366-379	Townsend et al (1986) Cell 44:959
Flu	haemagglutinin AA48-66	Mills et al (1986) J. Exp. Med. 163:1477
Flu	AA111-120	Hackett et al (1983) J. Exp. Med 158:294
Flu	AA114-131	Lamb, J. and Green N. (1983) Immunology 50:659
Epstein-Barr	LMP43-53	Thorley-Lawson et al (1987) PNAS USA 84:5384
Hepatitis B	Surface Ag AA95-109; AA 140-154	Milich et al (1985) J. Immunol. 134:4203
	Pre-S antigen AA 120-132	Milich, et al. (1986) J. Exp. Med. 164:532
Herpes simplex	gD protein AA5-23	Jayaraman et al (1993) J. Immunol. 151:5777
	gD protein AA241-260	Wyckoff et al (1988) Immunobiology 177:134

Rabies	glycoprotein AA32-44	MacFarlan et al (1984) J. Immunol 133:2748

The epitope may also be associated with a bacterial antigen. Suitable epitopes include, but are notlimited to:

Bacteria	Epitope ID	Reference
Tuberculosis	65Kd protein AA112-126 AA163-184 AA227-243 AA242-266 AA437-459	Lamb et al (1987) EMBO J. 6:1245
Staphylococcus	nuclease protein AA61-80	Finnegan et al (1986) J. Exp. Med 164:897
E. coli	heat stable enterotoxin	Cardenas et al (1993) Infect Immunity 61:4629
	heat labile entertoxin	Clements et al (1986) Infect. Immunity 53:685
Shigella sonnei	form I antigen	Formal et al (1981) Infect. Immunity 34:746

In another embodiment, the epitope is associated with a cancer, including but not limited to cancers of the respiratory system (lung, trachea, larynx), digestive system (mouth, throat, stomach, intestines) excretory system (kidney, bladder, colon, rectum), nervous system (brain), reproductive system (ovary, uterus, cervix), glandular system (breast, liver, pancreas, prostate), skin, etc. The two main groups of cancers are sarcomas, which are of mesenchymal origin and affect such tissues as bones end muscles, and carcinomas, which are of epithelial origin and make up the great majority of the glandular cancers of breasts, stomach, uterus, skin and tongue. The sarcomas include fibrosarcomas, lymphosarcomas, osteosarcomas, chondrosarcomas, rhabdosarcomas and liposarcomas. The carcinomas include adenocarcinomas, basal cell carcinomas and squamous carcinomas.

Cancer-associated epitopes include, but are not limited to, peptide epitopes such as those of mutant p53, the point mutated Ras oncogene gene product, her 2/neu, c/erb2, and the MUC1 core protein, and carbohydrate epitopes such as sialyl Tn (STn), TF, Tn, CA 125, sialyl Le^x, sialyl Le^a and P97.

In a preferred embodiment, the primary epitope is a T-cell epitope of a cancer-associated mucin. Mucins are glycoproteins characterized by high molecular weight (>1,000,000 daltons) and extensive glycosylation (over 80%). Mucins may be expressed extracellularly, or as an integral cell membrane glycoprotein with distinct external, transmembrane, and cytoplasmic domains. Cell membrane mucins exist as flexible rods and protrude relatively great distances from the cell surface forming an important component of the glycocalyx (Jentoff, 1990) and the terminal carbohydrate portions thereof are probably the first point of contact with antibodies and cells of the immune system.

Abberant or cancer-associated mucins are known to be relatively less glycosylated (Hull et al, 1989) and hence antigenically different from their normal cell counterpart mucins exposing normally cryptic carbohydrate- (Hanish et al, 1989; Torben et al,1990; Samuel et al, 1990), peptide- (Burchell et al, 1987) and perhaps even glycopeptide-epitopes. Therefore, because cell surface mucins protrude, they themselves may serve as targets for immune attack (Henningson, et al., 1987; Fung, et al., 1990; Singhal, et al., 1991; Jerome et al., 1991; Oncogen, EP 268,279; Biomembrane Institute, WO89/08711; Longenecker, USP 4,971,795). Under some circumstances, cancer-associated cell membrane mucins can actually "mask" other cell surface antigens and protect cancer cells from immune attack (Codington et al, 1983; Friberg, 1972; Miller et al, 1977).

The mucine T-cell epitope may be a core peptide, a carbohydrate, or a glycopeptide. Non-limiting examples of mucins which may carry T-cell epitopes are the human tumor associated Thomsen-Friedenreich antigen, (MacLean, 1992), epiglycanin-related glycoprotein (Codington, 1984) ovine submaillary mucin, bovine submaxillary mucin, breast tumor mucins (e.g., human polymorphic epithelial mucin, including breast tumor mucins, Gendler, 1988, 1990; breast cancer epithelial tumor antigen, Hareuveni, 1990, breast carcinoma,

Hull, 1989), mammary tumor mucins (e.g., such as murine mammary adenocarcinoma, Fung, 1990) carcinoma mucins such as mucins arising from the kidney (e.g., renal cell carcinoma), ovary (e.g., ovarian carcinoma-associated sebaceous gland antigen, Layton, 1990), bladder, colon (e.g., Sialosyl-Tn in colorectal cancer, Itzkowitz, 1990) pancreatic tumor mucin (Lan, 1990), gallbladder, bladder, colon (e.g., malignant colon mucosa mucins, Torbin, 1980) and some lung tissues, melanoma mucins (e.g., melanoma-associated antigen, Kahn, 1991) epithelial tumor cell mucins, leukemia associated mucins, carcinoembryonic antigen, or any other mucin associated with abnormal cells according to known characteristics of cancer associated mucins or abnormal mucins, such as abberant glycosylation (Hakomori, 1989, and Singhal, 1990).

In an especially preferred embodiment, the tumor-associated mucin-derived peptide is the MUC1 peptide. The human MUC1 gene product has been referred to by various names, including MAM6, milk mucin; human milk fat globule antigen (HMFG); human mammary epithelial antigen, CA 15-3, CA 27.29; episialin; and polymorphic epithelial mucin (PEM) (reviewed in Taylor-Papadimitriou et al, 1988). This mucin is strongly expressed on human breast (Gendler et al, 1988), pancreatic (Lan et al, 1990) and certain ovarian cancer cells (Layton et al, 1990); See also refs. 4-6. Although the MUC1 encoded mucins expressed on various cancers contain the same tandem repeat core peptide sequence, glycosylation differences do exist (Gendler et al, 1988; Lan et al, 1990). Because of underglycosylation in cancer cells[7], MUC-1 molecules on cancer cells express cryptic epitopes which are not expressed (i.e, are cryptic) on normal epithelial cells[8-11].

The peptide moiety may be a consensus peptide, e.g., a peptide, distinct from known cancer associated mucin core peptide sequences, but derived from a combination of known cancer associated mucin core peptide sequences. Such consensus peptides, may be derived by molecular modeling, optionally combined with hydrophobicity analysis and/or fitting to model

helices, as non-limiting examples. Such modeling can be accomplished according to known method steps using known modeling algorithms, such as, but not limited to, ECEPP, INSIGHT, DISCOVER, CHEM-DRAW, AMBER, FRODO and CHEM-X. Such algorithms compare peptides to determine probable suitable alternative consensus polypeptide fragments.

Such consensus peptides or fragments of cancer associated mucin core peptides may then be synthesized or produced recombinantly, in order to provide abberant mucin polypeptides according to the present invention which preferentially induce a cellular specific immune response to cancer mucins on abberant cells.

We have shown that cancer vaccines composed of synthetic peptide antigens which mimic cryptic MUC-1 peptide sequences on cancer cells are able to induce effective anti-cancer immunotherapy against MUC-1 expressing tumor cells in a murine model[12]. Finn and co-workers have shown that cancer patients are able to produce specific non-MHC restricted cytotoxic T-lymphocytes (CTL) which recognize peptide epitopes expressed on MUC-1 molecules on cancer cells[53-55]. Indeed the MUC1 sequence SAPDTRP has been shown to be both a T-and a B- cell epitope. We have recently demonstrated that the immunization of chimpanzees with synthetic MUC-1 antigens induces the development of specific antibodies and CMI against MUC-1.

MUC1 is the first cancer-associated mucin gene to be cloned and mapped (Gendler et al, 1990), and has recently been transfected into a murine mammary cell line, 410.4 (Lalani et al, 1991). MUC1 transfected 410.4 cells express the MUC1 gene product on the cell surface. The pattern of glycosylation is similar to, but different from, malignant cell derived mucins expressing the same cryptic peptide epitopes as expressed by human cancer associated MUC1 (Taylor-Papadimitriou et al, 1988). Lalani and co-workers (1991) have examined the immunogenicity of the 410.4 transfectants in mice. These workers demonstrated that mice which rejected a low dose of transfected 410.4 cells did not develop tumors after a

subsequent transplant of a high dose of transfected 410.4 cells although no effect on tumor development of untransfected wild type 410.4 cells was seen (Taylor-Papadimitriou et al, 1988).

Tumor-associated carbohydrate epitopes are also of interest. For example, any of three types of tumor-associated carbohydrate epitopes which are highly expressed in common human cancers may be presented. These particularly include the lacto series type 1 and type 2 chains, cancer associated ganglio chains, and neutral glycosphingolipids. Examples of the lacto series Type 1 and Type 2 chains are as follows: Lewis a, dimeric Lewis a, Lewis b, Lewis b/Lewis a, Lewis x, Lewis, y, Lewis a/Lewis x. dimeric Lewis x, Lewis y/Lewis x, trifucosyl Lewis y, trifucosyl Lewis b, sialosyl Lewis x, sialosyl Lewis y, sialosyl dimeric Lewis x, Tn, sialosyl Tn, sialosyl TF, TF. Examples of cancer-associated ganglio chains are as follows: GM3. GD3, GM2, GM4, GD2, GM1, GD-1a, GD-1b. Neutral sphingolipids include globotriose, globotetraose, globopentaose, isoglobotriose, isoglobotetraose, mucotriose, mucotetraose, lactotriose, lactotetraose, neolactotetraose, gangliotriose, gangliotetraose, galabiose, and 9-O-acetyl-GD3.

An antigen of interest may include both B-cell and T-cell epitopes. In one embodiment of the invention, both T and B cell epitopes are presented, and the immunomodulatory peptide ensurees that the immune rsponse is primarily directed against the T cell epitope.

In an alternative embodiment, only T cell epitopes of the antigen are presented. While the immunomodulatory peptides of the present invention shift the immune response toward T_H1 and away from T_H2, it may nonetheless be advantageous to use, as the primary epitope, a sequence of the antigen which provides a complete T-cell epitope but not a complete B-cell epitope. For example, in the case of MUC-1, SAPDTRP is a B-cell epitope, while PDTRP is merely a T-cell epitope. If SAPDTRP invoked an undesirable humoral response, despite the presence of the immunomodulatory peptide, PDTRP could be used instead.

The CMI-specific antigen of the present invention may

present a single copy of an epitope of interest, multiple copies of the same epitope, or a plurality of different epitopes. If multiple epitopes ae prescribed, they may be linked directly or indirectly, and covalently or noncovalently.

When the epitope is a peptide epitope, its sequence may be modified, to increase the specificity of the immune response, or the predominance of the CMI response over the cellular immune response. Amino acid sequence modifications are discussed in detail in a later section.

An T-cell peptide epitope is usually at least five and up to about 24 amino acids (the longest peptide which can fit in the Class II groove). Of course, the epitope may be presented as a moiety of a larger antigen, which will be processed by the immune system to properly present the T-cell epitope.

Many T-cell epitopes are known. Several techniques of identifying additional T-cell epitopes are recognized by the art. In general, these involve preparing a molecule which potentially provides a T-cell epitope and characterizing the immune response to that molecule. Methods of characterizing the immune response are discussed in a later section.

One method of identifying peptide T-cell epitopes is by systematic testing of peptide fragments. This can be done in different ways. For example, one may prepare a series of twenty-mer peptides with progessive overlap of 5 amino acids, e.g., residues 1-20, 16-35, 31-50, etc., of original polypeptide. The length of the peptides, and the degree of overlap, is up to the practitioner. The overlap should, however, be at least five amino acids since that is the smallest T-cell epitope size.

Fragments are readily prepared if the amino acid sequence of the peptide is known; a coding sequence may then be constructed for any desired fragment, and the fragment produced by recombinant DNA techniques. If the fragment is small, it may also be prepared by liquid or solid phase peptide synthesis.

If no sequence information is available, a polypeptide

antigen may be fragmented with site-specific cleavage agents, such as cyanogen bromide, iodosobenzoic acid, and trypsin. Larger fragments may be obtained by using agents with rarer substrates, or by using the agents in low concentrations, at lower temperatures, or shorter reaction times. Smaller fragments may be obtained by using combinations of agents simultaneously, or by using high concentrations, higher temperatures, or longer reaction times, and optionally using chaotropic agents to help unfold the peptide. The fragments, large or small, are screened. Positive fragments may be fragmented further to localize the epitope.

The number of fragments to be screened may be reduced, if the amino acid sequence is known, by using the amino acid sequence to predict which fragments are likely to act as T-cell epitopes. In general, these predictive methods work by assembling a database of known T cell antigenic sites (and perhaps a second database of sequences known not to be T cell epitopes) and comparing these sites with (a) known 3-D structures of the proteins in question, and/or (b) the known amino acid sequence, especially in the vicinity of the site.

Margalit, et al., "Prediction of Immunodominant Helper T Cell Antigenic Sites from the Primary Sequence", J. Immunol., 138=2213-29 (1987) has identified (using the AMPHI program), a subset of T cell epitopes which are characterized by the presence of an amphipthic structure. In the Margalit algorithm, the amino acid sequence was converted into sequence of hydrophobicity values (the preferred scale was that of Fauchere-Pliska) and this sequence was divided into overlapping blocks (preferably of length 11). The blocks were examined for periodicity in hydrophobicity consistent with a regular amphipathic helical structure; the preferred power spectrum procedure is a least squares fit of a sinusoid. Margalit preferred to look for a segment of several conservative blocks which has an amphipathic score (the sum of the amphipathic indices of the blocks) of greater than 4.

Other algorithms may be developed by study of the T-cell

epitope database.

When testing a large number of fragments for T cell epitope activity, it is possible to use a divide-and-conquer strategy to minimize the number of test animals or cultures required. The fragments may be divided into two or more known groups and all fragments of a group administered to a single animal or culture. If no immune response is observed then all fragments if the mixture is positive, it may then be divided into smaller subgroups and the process repeated.

The fragments may be tested against T cells of a single haplotype, or of several different haplotypes.

Once an epitope is identified, functionally equivalent epitopes may be identified by a combination of knowledge of amino acid similarities and systematic variation of the sequence of the epitope.

Immunomodulatory Peptide

The second component of the CMI-specific antigen of the present invention is an immunomodulatory peptide moiety which causes the immune response to the first component to shift in favor of the CMI response. This immunomodulatory peptide may be a moiety of a larger peptide, or a separate molecule.

The immunomodulatory peptide of the present invention has a molecular weight of less than 5,000 daltons, the conventionally accepted minimum size for a peptide to be immunogenic in its own right. It will be appreciated, however, that it is likely that the immunomodulatory peptides of the present invention, by virtue of their similarity to a polymorphic region of a subject's MHC Class I or II antigen, become processed by the subject's antigen presenting cells despite their size.

The instant immunomodulatory peptides may further be characterized as comprising an amino acid sequence (the "allopeptide moiety"), at least five amino acids long, which corresponds essentially to a "polymorphic region", as hereafter defined, of a polymorphic MHC-encoded Class I or Class II

antigen, preferably the heavy chain of MHC Class I, and more particularly that of the α_1 or α_2 domain of that chain.

The term "allopeptide" moiety is used above purely for convenience, as it will appreciated that whether a peptide sequence is syngeneic, alllogeneic, or xenogeneic will depend on the subject to which it is administered.

A polymorphic residue of a polymorphic MHC Class I molecule is a residue whose variability index, among the known alleles of that molecule, is greater than unity. The variability index is obtained by dividing the total number of known sequences by the number of sequences in which the consensus (modal) amino acid appears at that position, and then multiplying by the total number of different amino acids found at that site. It will be appreciated that, as new alleles of these molecules are discovered, a residue which initially appeared to be invariant within the allelic population may in fact turn out to be polymorphic, and it is applicant's intent that such residues then be considered to be embraced by the term "polymorphic residue" as used in this specification, including the appended claims.

The term "polymorphic region" is potentially open to several interpretations. In the strictest sense, it is a stretch of two or more consecutive amino acids in an MHC Class I molecule such that all of the component amino acids are polymorphic. However, this interpretation is stricter than the common usage in the art, which recognizes that a polymorphic region may usefully be defined so as to include occasional residues which do not experience allelic variation.

In determining the bounds of a polymorphic region, the person of ordinary skill in the art would consider both the variability index of each residue, and the proximity of the residue to polymorphic residues, especially those with a high variability index.

A non-exhaustive definition of what the art would consider to be a polymorphic region would be a contiguous sequence of amino acids, characterized as: (a) beginning and ending with

a polymorphic residue; (b) comprising at least one cluster (two or more adjacent residues) of polymorphic residues; (c) wherein any component cluster of three or more non-polymorphic residues is adjacent to at least one, at least equally long component cluster of polymorphic residues; (d) wherein the biggest cluster of non-polymorphic residues is not longer than four residues; and (e) wherein more than half of the residues are polymorphic. Based on this definition, HLA-A would include at least the following polymorphic regions: 30-31, 43-44, 62-83, 89-109, 142-171. Similarly, H2K would include 22-24, 49-56, 62-83, 89-90, 95-108 and 162-180.

However, criteria (c) and (d) might be relaxed if extending the region would raise the mean variability index (MVI) for the region. For example, the MVI for residues 89-109 of HLA-A is 2.719; this increases to 2.723 for 89-114 and to 2.825 for 89-116.

It will be appreciated that a polymorphic region, such as (62-83)H2K, may include subsequences which likewise can be considered polymorphic, e.g., (62-69) H2K or (75-83) H2K.

Similarly, the allopeptide may correspond essentially to a polymorphic region of a polymorphic class II polypeptide. The following regions of the human and mouse class II polypeptides are considered polymorphic for the purposes of this application (to the extent they are inconsistent with the above definition, they may be considered alternative regions):

Human HLA Class II Alpha chains
Human DR locus alpha 1 domain variable regions, AA 44-53 and AA 58-66
Human DQ locus alpha 1 domain variable regions, AA 44-53 and AA 58-75

Murine H2 Class II Alpha chains
Murine A locus Alpha 1 domain variable regions, AA 65-79

Human HLA Class II Beta chains
Human DR locus Beta 1 domain variable regions, AA 9-14, AA 25-38 and AA 67-78
Human DQ locus Beta 1 domain variable regions, AA 52-60, AA 66-78 and AA 84-90
Human DP locus Beta 1 domain variable regions, AA 57-90

Murine H2 Class II Beta chains
Murine A locus Beta 1 domain variable regions, AA 9-19 and A 85-100
Murine E locus Beta 1 domain variable regions, AA 23-38 and AA 71-92

The allopeptide moiety will be considered to correspond essentially to a polymorphic region of a polymorphic MHC-encoded polypeptide if it is (a) identical in sequence to that region as it appears in a single allelic form of an MHC-encoded histocompatibility antigen, (b) a hybrid of the sequences for that region of two or more allelic forms of the same MHC-encoded histocompatibility antigen, (c) differs from a sequences covered by (a) or (b) above only by conservative (more preferably, highly conservative) substitutions of amino acids, as later defined, or (d) is an "MHC-like" sequence as defined below.

An example of a potential peptide moiety of type (a) would be the sequence RETGKAKG (BP1-23), which is the sequence of the polymorphic region 62-69 of the H2K protein for the "b" allele. An example of a potential allopeptide of type (b) would be EETRKVKS, see Table 1002K. An example of a potential allopeptide moiety of type (c) would be DETKLARG, based on Table 1002K, with the underlined residues being conservative substitutions for residues appearing in that table at the appropriate positions.

An "MHC-like" sequence is an amino acid sequence wherein, at at least 30% of the residues, or for at least three residues, whichever is greater, the residue chosen is the same as, or a highly conservative substitution for, a corresponding non-polymorphic residue of a particular MHC-encoded polypeptide, but where, at the other residue positions, there is no constraint, i.e., the residue may be any genetically encoded amino acid. An example appears in Table 1004, Part B.

The peptide moiety preferably corresponds essentially to a polymorphic region of at least nine amino acids, and more advantageously of up to 24 amino acids. While the allopeptide moiety may correspond essentially to a still longer polymorphic

region, it will be appreciated that an immunomodulatory peptide which is longer than 24 amino acids will be processed by the immune system so as to trim it to that length, so that it will fit in the antigen presenting site of an MHC Class II antigen.

The polymorphic region in question may be such a region in the antigen-binding domain of any MHC histocompatibility antigen of any animal species, including without limitation, polymorphic regions of the mouse H-2K, H-2D or H-2L, or the human HLA-A, -B, or -C loci. H-2K, H-2D, HLA-A and HLA-B are preferred.

The immunomodulatory peptides of the present invention may comprise two or more allopeptide moieties, which may be the same or different, and which may be joined directly or through an amino acid or peptide spacer. It may further comprise one or unrelated promiscuous TH epitopes, such as that of tetanus toxin (YSYFPSV). The immunomodulatory peptides may also comprise other amino acid sequences which do not substantially interfere with the desired immunomodulatory effect, especially conserved residues of relevant MHC proteins, and most especially those likely to be involved in peptide binding or TCR interaction. For example, an immunomodulatory peptide comprising the polymorphic region 62-69 preferably also comprises the conserved residue 61.

For example, the immunomodulatory peptide sequence may be:

61		64				68
Asn	-Xaa -Xaa	-Thr	-Xaa -Xaa	-Xaa	-Lys	-Xaa
Glu		Arg				
		Asn				
		Leu				
		Gln				
		Phe				

where Xaa is preferably an amino acid found in that position in at least one allele of MHC Class I. Residues 61, 64 and 68 are conserved, even across species boundaries, while the other residues in the region 61-69 are hypervariable.

Silver, et al., Nature, 360:367-369 (1992) sets forth the atomic structure of a human MHC Class I protein (HLA-Aw68)

presenting the influenza peptide Np 91-99. Solvent accessibility was determined using the program access with a 1.4Å probe, and TCR accessibility, with a 1.9Å probe. Conserved and polymorphic residues were characterized in accordance with Porham, et al., Proc. Nat. Acad. Sci. (USA), 85:4005-9 (1988). Of the 12 polymorphic residues facing into the binding site, 8 contacted the peptide directly (9, 45, 66, 70, 74, 77, 95, 116) and four do not (67, 97, 114, 156). Eleven of the 12 (66 is the exception) were nonetheless completely buried by the bound peptide. Of the six polymorphic residues facing more directly toward solvent (62, 65, 69, 76, 80, 163), and most likely involved in TCR recognition, four also contact the peptide (62, 69, 80, 163). For MHC Class II structure, see Brown, et al., Nature, 364:33 (1993).

In Tables 1001 et seq., we provide, several human and murine loci, the amino acids found at each position, the frequency of occurrence (among alleles sequenced to date), and the variability index (=total number of observations* number of different amino acids at position/frequency of most common ("consensus") amino acid at that position).

Table 1001 shows known sequence variations of Human HLA-class I molecules, residues 61-83 of alpha 1 domain. The human A,B,C and miscellaneous loci are each tabulated separately (tables 1001 A, 1001B, 1001C and 1001Z, respectively). For each loci the consensus amino acid at each position is noted along with its frequency of observation. All other amino acids observed at that position are listed at the bottom. The variability index is determined by dividing the total number of known sequences by the number of times the consensus amino acid appears, this is then multiplied by the total number of known sequences by the number of times the consensus amino acid appears, this is then multiplied by there total number of different amino acids observed at that site. An example of this for the A locus residue number 66 is as follows; 27/15=1.8, 1.8X2=3.6. For invariant residues the index is equal to 1. These observations are compiled from Sequences of

Proteins of Immunologic Interest, Vol. II, Fifth Edition, Published by US Dept. of Health and Human Services 1991, Ed. E.A. Kabat, et al.

Table 1002 shows known sequence variations of Murine MHC class I molecules, residues #61-83 of the alpha 1 domain. The murine H-2K, H-2D and miscellaneous loci are tabulated separately (tables 1002K, 1002D, and 1002Z, respectively) (reference as per Table 1).

Table 1003 shows known sequence variations of MHC class I molecules of miscellaneous species, residues #61-83 of the alpha-1 domain. These species include chimpanzee and other primates, swine, bovine, feline, rabbit, syrian hamster, chicken, and frog sequences.

Finally, Table 1004 presents a summary of all of the observed amino acids of MHC class I antigens at residues #61-83 (all species). These include all of the data from Table 1-3. In part B of this table is the listing of residues that show an overall consensus throughout the phylogenetic tree. Key essentially invariant residues are listed for positions 61, 64, 68, 72, 75, 78, 79 and 81. All highly variable residues are listed as X. The residues that have been observed at each location of X are listed in the table Part A, but any amino acid may be substituted at the variable residues.

Tables 2001 (Human MHC Class 1A), 2002 (Human MHC Class 1B), 2003 (Mouse MHC Class 1K) and 2004 (Mouse MHC Class 1D) set forth, for amino acids 1-182 of each polypeptide, both the variability index for that amino acid, and the mean variability index for the ten amino acid region beginning with that amino acid. This is based on the same data used to compile Table 1001 et seq.

Presentation of the CMI-Specific Antigen

In a preferred embodiment, the CMI-specific antigen takes the form of a mixture of a first molecule (the primary antigen) bearing the primary epitope, and, as a second and unlinked molecule, the immunomodulatory peptide. The first molecule may be a naturally occurring molecule, such as a naturally

occurring antigen, a fragment of such an antigen, or an artificial or semi-artificial construct (such as a hapten joined to an immunogenic carrier). When the first molecule is small, it will not be immunogenic in its own right. However, we have found, surprisingly, that a cellular immune response may be obtained against a simple mixture of an immunomodulatory peptide with a hapten molecule. The proportion of the primary antigen in the mixture may be adjusted, if desired, to take advantage of the aforestated effect of dosage on the extent to which a CMI response predominates.

If desired, the mixture may include a plurality of different immunomodulatory peptides. If each corresponds to a different allele of the same polymorphic locus, at least one will be allogeneic to the subject to whom the mixture may be administered. The mixture may include peptides corresponding to several different MHC polymorphic loci.

If a plurality of immunomodulatory peptides are to be administered (whether as discrete molecules or as moieties of a corresponding plurality of primary epitope/immunomodulatory peptide conjugates), the desired peptides may either be individually prepared and then mixed together, or prepared simultaneously. If the number of different immunomodulatory peptides is large, the latter may be convenient. Techniques for simultaneous synthesis of a multiplicity of peptides are described in Rutter, USP 5,010,175; Houghten, et al, Proc. Nat. Acad. Sci. (USA), 82:5131-35 (1985; Geysen, et al., Proc. Nat. Acad Sci. USA, 81:3998-4002 (1984); WO86/06487; WO86/00991.

Alternatively, one or more immunomodulatory peptides may be conjugated, directly or indirectly, covalently or noncovalently to the primary epitope. Such a conjugate is considered "non-naturally occurring" for purpose of the appended claims if the components of the conjugate are not so conjugated in nature, even if the components themselves are all naturally occurring.

In one embodiment, the primary epitope is a peptide, and the conjugate is a chimeric peptide.

If desired, the CMI-specific antigen conjugate may include multiple copies of the primary epitope and/or the immunomodulatory peptide. These may be conjugated linearly, head-to-tail,with or without an intervening linker, or to a central structure such as a branched lysine core (see Posnett, et al., J. Biol. Chem., 263:1719-25, 1988) or a macromolecular carrier (such as albumin, keyhole (limpet hemocyanin, or dextran).

The immunogenic compositions of the present invention may include, beside the CMI-specific antigen, an adjuvant that favors a T_H1 response, such as saponin. Muramyl peptides induce an enhanced T cell response, but also amplify the humoral response. It is possible, however, that in the presence of the immunomodulatory peptides of the present invention, the potentiation of the humoral immune response by certain adjuvants will be muted, or at least kept within tolerable limits.

Amino Acid Sequence Modifications

When a CMI-specific antigen of the present invention, including its primary epitope and (or alloepitope comprised an amino acid sequence, this sequence may be one which is naturally occurring, or a modified sequence.

One class of allowable modifications of the amino acid sequence of a peptide moiety are amino acid substitutions. Conservative substitutions replace an amino acid with another of like size, charge and polarity; these are less likely to substantially alter the conformation of the peptide. The types of substitutions which may be made in the protein or peptide molecule of the present invention may be based on analysis of the frequencies of amino acid changes between a homologous protein of different species, such as those presented in Table 1-2 of Schulz et al., supra and Figs. 3-9 of Creighton, supra. Based on such an analysis, conservative substitutions are defined herein as exchanges within one of the following five groups:

TABLE V

1. Small aliphatic, nonpolar or slightly polar residues: Ala, Ser, Thr (Pro, Gly);
2. Polar, negatively charged residues and their amides: Asp, Asn, Glu, Gln;
3. Polar, positively charged residues: His, Arg, Lys;
4. Large aliphatic, nonpolar or slightly polar residues: Met, Leu, Ile, Val (Cys); and
5. Large aromatic residues: Phe, Tyr, Trp.

Groups 1-3 are somewhat related and mutations within that set may be considered semi-conservative. Similarly, mutations within groups 4-5 may be considered semi-conservative.

Residues Pro, Gly and Cys are parenthesized because of their special role in protein architecture. Pro imparts rigidity to the peptide chain, and has a tendency to interfere with alpha helix formation. Gly imparts flexibility to the peptide chain, and is often found in "loops" between alpha helices or beta strands. The thiol groups of cysteine residues can be oxidized to form disulfide bonds between nonadjacent cysteinyl residues.

Within the foregoing groups, the following substitutions are considered "highly conservative":

Asp/Glu
His/Arg/Lys
Phe/Tyr/Trp
Met/Leu/Ile/Val

Insertions or deletions of amino acids may also be made. Insertions and deletions are most likely to be tolerated at the amino or carboxy termini, at interdomain segments, at loops, or at other regions of relatively high mobility (e.g., areas which are not well resolved upon X-ray diffraction analysis). However, when the exact effect of the substitution, deletion, or insertion is to be confirmed one skilled in the art will appreciate that the effect of the mutation will be evaluated by routine screening assays, to confirm the ability to

preferentially induce a predominantly cellular immune response, otherwise analogous to that elicited by the antigen of interest. Such screening may be coupled with random mutagenesis so as to simultaneously screen a large number of mutants. See e.g., Ladner, USP 5, 223,409.

Such modifications may be made as part of an organic synthesis of a peptide, or by recombinant methods. The latter involve site-specific mutagenesis of the peptide molecule-encoding nucleic acid, expression of the mutant nucleic acid in recombinant cell culture, and, optionally, purification from the cell culture, for example, by immunoaffinity chromatography using a specific antibody on a chemically derivatized column or immobilized membranes or hollow fibers (to absorb the mutant by binding to at least one epitope).

Standard reference works setting forth the general principles of recombinant DNA technology include Watson, J.D. et al., *Molecular Biology of the Gene*, Volumes I and II, The Benjamin/Cummings Publishing Company, Inc., publisher, Menlo Park, CA (1987); Darnell, J.E. et al., *Molecular Cell Biology*, Scientific American Books, Inc., publisher, New York, NY (1986); Lewin, B.M., *Genes III*, John Wiley & Sons, publishers, New York, NY (1989); Old, R.W., et al., *Principles of Gene Manipulation: An Introduction to Genetic Engineering*, 2d edition, University of California Press, publisher, Berkeley, CA (1981); Ausubel et al, eds., *Current Protocols in Molecular Biology*, Wiley Interscience, publisher, New York, NY (1987, 1992); and Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory, publisher, Cold Spring Harbor, NY (1989), the entire contents of which references are herein incorporated by reference.

When the primary epitope is a carbohydrate, it may be isolated from nature, or synthesized by suitable glycosylation reactions as known in the art. When the primary epitope is a glycopeptide, the peptide may be synthesized and then glycosylated, either biologically (by expression in a suitable

host cell) or chemically.

Characterizing the Immune Response

The cell-mediated immune response may be assayed in vitro or in vivo. The conventional in vitro assay is a T cell proliferation assay. A blood sample is taken from an individual who suffers from the disease of interest, associated with that disease, or from a vaccinated individual. The T cells of this individual should therefore be primed to respond to a new exposure to that antigen by proliferating. Proliferation requires thymidine because of its role in DNA replication.

Generally speaking, T cell proliferation is much more extensive than B cell proliferation, and it may be possible to detect a strong T cell response in even an unseparated cell population. However, purification of T cells is desirable to make it easier to detect a T cell response. Any method of purifying T cells which does not substantially adversely affect their antigen-specific proliferation may be employed. In our preferred procedure, whole lymphocyte populations would be first obtained via collection (from blood, the spleen, or lymph nodes) on isopycnic gradients at a specific density of 10.7, ie Ficoll-Hypague or Percoll gradient separations. This mixed population of cells could then be further purified to a T cell population through a number of means. The simplest separation is based on the binding of B cell and monocyte/macrophage populations to a nylon wool column. The T cell population passes through the nylon wool and a >90% pure T population can be obtained in a single passage. Other methods involve the use of specific antibodies to B cell and or monocyte antigens in the presence of complement proteins to lyse the non-T cell populations (negative selection). Still another method is a positive selection technique in which an anti-T cell antibody (CD3) is bound to a solid phase matrix (such as magnetic beads) thereby attaching the T cells and allowing them to be separated (e.g., magnetically) from the non-T cell population. These may

be recovered from the matrix by mechanical or chemical disruption.

Once a purified T cell population is obtained it is cultured in the presence of irradiated antigen presenting cells (splenic macrophages, B cells, dendritic cells all present). (These cells are irradiated to prevent them from responding and incorporating tritiated thymidine). The viable T cells (100,000-400,000 per well in 100μl media supplemented with IL2 at 20 units) are then incubated with test peptides or other antigens for a period of 3 to 7 days with test antigens at concentrations from 1 to 100μg/mL.

At the end of the antigen stimulation period a response may be measured in several ways. First the cell free supernatants may be harvested and tested for the presence of specific cytokines. The presence of α-interferon, IL2 or IL12 are indicative of a Th helper type 1 population response. The presence of IL4, IL6 and IL10 are together indicative of a T helper type 2 immune response. Thus this method allows for the identification of the helper T cell subset.

A second method termed blastogenesis involves the adding tritiated thymidine to the culture (e.g., 1μcurie per well) at the end of the antigen stimulation period, and allowing the cells to incorporate the radiolabelled metabolite for 4-16 hours prior to harvesting on a filter for scintillation counting. The level of radioactive thymidine incorporated is a measure of the T cell replication activities. Negative antigens or no antigen control wells are used to calculated the blastogenic response in terms of a stimulation index. This is CPM test/CPM control. Preferably the stimulation index achieved is at least 2, more preferably at least 3, still more preferably 5, most preferably at least 10.

CMI may also be assayed in vivo in a standard experimental animal, e.g., a mouse. The mouse is immunized with a priming antigen. After waiting for the T cells to respond, the mice are challenged by footpad injection of the test antigen. The DTH response (swelling of the test mice is compared with that

of control mice injected with, e.g., saline solution. Preferably, the response is at least .10 mm, more preferably at least .15 mm, still more preferably at least .20 mm, most preferably at least .30 mm.

The humoral immune response, in vivo, is measured by withdrawing blood from immunized mice and assaying the blood for the presence of antibodies which bind an antigen of interest. For example, test antigens may be immobilized and incubated with the samples, thereby capturing the cognate antibodies, and the captured antibodies then measured by incubating the solid phase with labeled anti-isotypic antibodies. Preferably, the humoral immune response is no stronger than that represented by an antibody titer of 1/80.

Subjects

The recipients of the vaccines of the present invention may be any vertebrate animal which can acquire specific immunity via a cellular immune response, where said response is mediated by an MHC protein. MHC proteins have been identified in mammals, birds, bony fish, frogs and toads. Among mammals, the preferred recipients are mammals of the Orders Primata (including humans, apes and monkeys), Arteriodactyla (including horses, goats, cows, sheep, pigs), Rodenta (including mice, rats, rabbits, and hamsters), and Carnivora (including cats, and dogs). Among birds, the preferred recipients are turkeys, chickens and other members of the same order. The most preferred recipients are humans.

Pharmaceutical Compositions

Pharmaceutical preparations of CMI-specific antigens of the present invention, suitable for inoculation or for parenteral administration, include sterile aqueous or non-aqueous solutions, suspensions, and emulsions, which may contain auxiliary agents or excipients which are known in the art. See, e.g., Berkow et al, eds., *The Merck Manual*, 15th edition, Merck and Co., Rahway, N.J., 1987; Goodman et al.,

eds., *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 8th edition, Pergamon Press, Inc., Elmsford, N.Y., (1990); *Avery's Drug Treatment: Principles and Practice of Clinical Pharmacology and Therapeutics*, 3rd edition, ADIS Press, LTD., Williams and Wilkins, Baltimore, MD. (1987), Katzung, ed. *Basic and Clinical Pharmacology*, Fifth Edition, Appleton and Lange, Norwalk, Conn. (1992), which references and references cited therein, are entirely incorporated herein by reference. A composition may also include other immunomodulators, such as cytokines which accentuate a CMI response or inhibit a humoral immune response, or inhibitory antibodies against such cytokines.

A pharmaceutical composition according to the present invention may further comprise at least one cancer chemotherapeutic compound, such as one selected from the group consisting of an anti-metabolite, a bleomycin peptide antibiotic, a podophyllin alkaloid, a *Vinca* alkaloid, an alkylating agent, an antibiotic, cisplatin, or a nitrosourea. A pharmaceutical composition according to the present invention may further or additionally comprise at least one viral chemotherapeutic compound selected from gamma globulin, amantadine, guanidine, hydroxybenzimidazole, interferon-α, interferon-β, interferon-γ, thiosemicarbarzones, methisazone, rifampin, ribvirin, a pyrimidine analog, a purine analog, foscarnet, phosphonoacetic acid, acyclovir, dideoxynucleosides, or ganciclovir. See, e.g., Katzung, *supra*, and the references cited therein on pages 798-800 and 680-681, respectively, which references are herein entirely incorporated by reference.

Anti-parasitic agents include agents suitable for use against arthropods, helminths (including roundworms, pinworms, threadworms, hookworms, tapeworms, whipworms, and Schistosomes), and protozoa (including amebae, and malarial, toxoplasmoid, and trichomonad organisms). Examples include thiabenazole, various pyrethrins, praziquantel, niclosamide, mebendazole, chloroquine HCl, metronidazole, iodoquinol, pyrimethamine, mefloquine HCl, and hydroxychloroquine HCl.

Pharmaceutical Purposes

The term "protection", as in "protection from infection or disease", as used herein, encompasses "prevention," "suppression" or "treatment." "Prevention" involves administration of a Pharmaceutical composition prior to the induction of the disease. "Suppression" involves administration of the composition prior to the clinical appearance of the disease. "Treatment" involves administration of the protective composition after the appearance of the disease. It will be understood that in human and veterinary medicine, it is not always possible to distinguish between "preventing" and "suppressing" since the ultimate inductive event or events may be unknown, latent, or the patient is not ascertained until well after the occurrence of the event or events. Therefore, it is common to use the term "prophylaxis" as distinct from "treatment" to encompass both "preventing" and "suppressing" as defined herein. The term "protection," as used herein, is meant to include "prophylaxis." See, e.g., Berker, *supra*, Goodman, *supra*, Avery, *supra* and Katzung, *supra*, which are entirely incorporated herein by reference, including all references cited therein. The "protection" provided need not be absolute, i.e., the disease need not be totally prevented or eradicated, provided that there is a statistically significant improvement ($p=0.05$) relative to a control population. Protection may be limited to mitigating the severity or rapidity of onset of symptoms of the disease.

Pharmaceutical Administration

At least one CMI-specific antigen of the present invention may be administered by any means that achieve the intended purpose, using a pharmaceutical composition as previously described.

For example, administration of such a composition may be by various parenteral routes such as subcutaneous, intravenous, intradermal, intramuscular, intraperitoneal, intranasal, transdermal, or buccal routes. Parenteral administration can

be by bolus injection or by gradual perfusion over time. A preferred mode of using a pharmaceutical composition of the present invention is by subcutaneous, intramuscular or intravenous application. See, e.g., Berker, *supra*, Goodman, *supra*, Avery, *supra* and Katzung, *supra*, which are entirely incorporated herein by reference, including all references cited therein.

A typical regimen for preventing, suppressing, or treating a disease or condition which can be alleviated by a cellular immune response by active specific cellular immunotherapy, comprises administration of an effective amount of a pharmaceutical composition as described above, administered as a single treatment, or repeated as enhancing or booster dosages, over a period up to and including between one week and about 24 months.

It is understood that the effective dosage will be dependent upon the age, sex, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired. The ranges of effective doses provided below are not intended to limit the invention and represent preferred dose ranges. However, the most preferred dosage will be tailored to the individual subject, as is understood and determinable by one of skill in the art, without undue experimentation. See, e.g., Berkow et al, eds., *The Merck Manual*, 15th edition, Merck and Co., Rahway, N.J., 1987; Goodman et al., eds., *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 8th edition, Pergamon Press, Inc., Elmsford, N.Y., (1990); *Avery's Drug Treatment: Principles and Practice of Clinical Pharmacology and Therapeutics*, 3rd edition, ADIS Press, LTD., Williams and Wilkins, Baltimore, MD. (1987), Ebadi, *Pharmacology*, Little, Brown and Co., Boston, (1985); Chabner et al., *supra*; De Vita et al., *supra*; Salmon, *supra*; Schroeder et al., *supra*; Sartorelli et al., *supra*; and Katsung, *supra*, which references and references cited therein, are entirely incorporated herein by reference.

The total dose required for each treatment may be administered by multiple doses or in a single dose. A cancer-associated mucin polypeptide may be administered alone or in conjunction with other therapeutics directed to cancer or viral related pathologies, as described herein.

Generally speaking, the dosage for a human adult will be from about 0.01 μg to about 1 mg. However, the dosage should be a safe and effective amount as determined by conventional methods, using existing vaccines as a starting point.

Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions, which may contain auxiliary agents or excipients which are known in the art. See, e.g., Berker, *supra*, Goodman, *supra*, Avery, *supra* and Katzung, *supra*, which are entirely incorporated herein by reference, included all references cited therein.

The antigen may be delivered in a manner which enhance, e.g., delivering the antigenic material into the intracellular compartment such that the "endogenous pathway" of antigen presentation occurs. For example, the antigen may be entrapped by a lipsome (which fuses with the cell), or incorporated into the coat protein of a viral vector (which infects the cell).

Another approach, applicable when the antigen is a peptide, is to inject naked DNA encoding the antigen into the host, intramuscularly. The DNA is internalized and expressed.

It is also possible to prime autologous PBLs with the compositions of the present invention, confirm that the PBLs have manifested the desired T_H1 response, and then administer the PBLs, or a subset thereof, to the subject.

EXAMPLE I

CMI-Specific Response to Cancer-Associated Peptide Epitope

We have shown that immunization with synthetic MUC-1 peptide vaccines conjugated to KLH induce specific anti-MUC-1 antibody as well as DTH reactions in mice[12]. In order to attempt to replace KLH, we tested synthetic MHC peptides as

potential carriers for MUC-1. We used MHC allopeptides because they have been shown to be potent stimulators of both antibody as well as CD4+ T cell proliferative responses[17, 19, 31]. We synthesized peptide molecules which encompassed both MUC-1 peptide sequences plus alloantigenic (H-2) synthetic Class I peptides as part of a single chimeric peptide molecule (MUC-1-MHC). Surprisingly we found that immunization of mice with MUC-1-MHC chimeric molecules or even simple mixtures of MUC-1 plus allopeptides induced DTH reactions to MUC-1 without any detectable antibody production, suggesting the stimulation of TH1 responses in preference to TH2 responses.

MATERIALS AND METHODS

Synthetic Peptides and Conjugates

All antigens including their conjugates used in this study were obtained from Biomira Inc. (Edmonton, Canada). The various synthetic peptides were designed to contain the B and T epitopes of the MUC-1 core peptides PDTRP and/or $H2K^b$61-69 peptide ERETQKAKG (Figure 1). Various linear sequences and their conjugates to HSA were used for serological and delayed type hypersensitivity (DTH) assay. Those linear sequences were also conjugated to KLH and used as immunogens. BP1-28 and BP1-29 which were chimeric peptides containing both MUC-1 and $H2K^b$61-69 sequence were used to determine whether $H2K^b$61-69 peptide could replace KLH as a carrier to induce DTH response and antibody production. In BP1-28, $H2K^b$61-69 (ERETQKAKG) is linked to the C-end of peptide, while in BP1-29, $H2K^b$61-69 sequenced linked to N-end of peptide (Table 1).

Mice

All CAF/1 and C57/B6 mice (female, 8-12 weeks of age) were obtained from the University of Alberta Medical Laboratory Animal Service.

Antigen Preparation and Immunization

Freeze dried synthetic peptides (SP) were dissolved in

phosphate buffer saline (PBS), pH 7.4, and mixed with Ribi adjuvant (Ribi ImmunoChem Research Inc., Hamilton, Montana). The mixture was vortexed vigorously for 10 minutes. Each immunization consisted of 10 or 50 μg of peptide plus 4.5 μg adjuvant in total volume of 200 μL was injected subcutaneously into both inguinal areas. For induction of DTH response, the mice were immunized seven days before footpad challenge. For antibody, the test mice were immunized and bled at the two week interval.

Antibody Treatment of Mice

The mice were injected intravenously with XMG1.2 antibody (1 mg per mouse) on day 0, 4 and 10 after immunization. The mice in control group were injected with normal rat IgG on the same schedule (200 μL). The schedule of DTH test and bleeding were same as the above.

DTH Assay

DTH assay were conducted as previously described. For the footpad challenged antigen, the dose of synthetic peptides was 50 μg/ mouse in 50 μL of PBS. Footpads were measured at the 24th and 48th hour after footpad injection.

ELISA Assay

The mice were bled by the tail at the 14th day after immunization. The serum was incubated for one hour with different test antigens which were fixed in the 96 microwell plates and blocked 5% FCS-RPMI. The antibody tithes were then detected by goat-anti-mouse IgG and peroxidase (Kirkegaard and Perry Laboratories Inc).

Cytokine Assay

Cytokine assays for IL-2, IL-4, IL-10 and INFgamma were conducted as described by Lin and co-workers[32].

RESULTS

Comparison of Synthetic MUC-1-KLH Conjugates With MUC-1-

$H2K^b$61-69 Chimeric Synthetic Peptides for the Induction of DTH to MUC-1-HSA

In a previous publication, we demonstrated that unconjugated MUC-1 peptides were not immunogenic but following conjugation to KLH, synthetic MUC-1 peptides were effective immunogens for the induction of DTH reactions to not only the synthetic peptides themselves but also natural forms of MUC-1 represented on the cell surface of human MUC-1 transfected syngeneic tumor cells. Conversely, we found that immunization with syngeneic tumor cells expressing human MUC-1 induced DTH reactions when challenged in the footpad with the appropriate synthetic MUC-1 peptides. We compared MUC-1-KLH conjugates with MUC-1 peptides made chimeric with the $H2K^b$61-69 sequence in order to test whether the allopeptide sequence might be able to replace KLH as a carrier for the MUC-1 peptide. In confirmation of our previous results, Table 2 demonstrates that the MUC-1-KLH conjugates were able to induce specific DTH reactions to MUC-1 conjugated to HSA in both strains of mice tested. The non-conjugated chimeric MUC-1-$H2K^b$ molecule was able to induce MUC-1 specific DTH reactions in allogeneic CAF1 ($H2K^a/H2^d$)mice. In contrast, DTH reactions were not induced in syngeneic C57/BL6 ($H2K^b$) mice. However, following conjugation to KLH, the MUC-1-$H2K^b$ molecule induced DTH reactions in C57/BL6($H2K^b$) mice. As in our previous studies, simply immunizing with the unconjugated MUC-1 synthetic peptide alone was ineffective for inducing DTH reactions in either strain of mice.

Comparison of Synthetic MUC-1-KLH Conjugates with MUC-1 $H2K^b$61-69 Chimeric Synthetic Peptides for the Induction of IgG to MUC-1

We have previously shown that immunization with MUC-1-KLH conjugates induce both DTH reactions as well as specific IgG antibody responses to synthetic MUC-1 and natural MUC-1 mucin expressed on the surface of syngeneic tumor cells. Thus, we compared the IgG response of CAF1 mice which were immunized with MUC-1-KLH conjugates with those immunized with MUC-1-

$H2K^b61$-69 chimeric synthetic peptides (Table 3). As expected, the MUC-1-KLH conjugate induced high titers of IgG antibody tested on the various MUC-1 peptides but not on non-MUC-1 peptides. The chimeric synthetic peptides did not induce any detectable IgG antibodies to MUC-1 antigen. Surprisingly, no detectable anti-MUC-1 antibodies were induced even following immunization with KLH conjugates of the chimeric peptide while high tithes of anti-KLH antibodies were induced. We conclude that the allo-$H2K^b61$-69 sequence attached to MUC-1 preferentially stimulated DTH with no detectable antibody, consistent with the hypothesis that the presence of the allopeptide favors the induction of TH1 responses to MUC-1.

The Effects of Anti-Gamma Interferon (anti-gINF) Injections on the DTH Response to MUC-1-KLH Conjugates and Chimeric MUC-1-$H2K^b61$-69-KLH Conjugates

Table 4 illustrates that anti-gINF rat monoclonal antibody injected intravenously at the time of antigen priming into the DTH test mice completely abolished the DTH responses against MUC-1 in mice immunized with either MUC-1-KLH or MUC-1 chimeric peptide conjugated to KLH confirming that the DTH to MUC-1 is mediated by a TH1 cytokine.

The Effect of Mixing Various $H2K^b61$-69 Synthetic Allopeptides with MUC-1 Synthetic Peptide on DTH Responses in Various Inbred Strains of Mice

In Table 5 we compare the DTH responses induced by chimeric peptides with those induced by simple mixtures of $H2K^b61$-69 with synthetic MUC-1 peptides. Surprisingly, we found that the mixtures were just as effective as the chimeric peptides indicating that linked recognition or hapten carrier types of effects were not essential for this type of enhanced DTH reactivity to MUC-1. It is also noteworthy that in every case where an allopeptide was mixed with the MUC-1 peptide, DTH reactions were noted to the MUC-1 peptide. However, when the 61-69 MHC peptide-MUC-1 mixtures were injected into strains of

mice which were syngeneic to the MHC peptide no detectable DTH was noted to the MUC-1 synthetic peptide. Furthermore, mixing a known TH1 synthetic peptide sequence[15, 16] from OVA (BP1-37) did not induce a DTH to MUC-1. Using the same strain combinations and allopeptides, we were unable to induce detectable anti-MUC-1 antibody following three immunizations in RIBI adjuvant (data not shown) which are conditions which induce high titers of IgG against MUC-1-KLH conjugates (See Table 1).

The Effects of Changing Amino Acid Number 65 in the $H2K^b$61-69 Synthetic Peptide on Induction of DTH Responses to MUC-1 in C57/B6 ($H2K^b$) Mice

Grandea and Bevan[20] showed that transgenic mice that express a glutamic acid at position 65 (E65) in place of glutamine show strong alloreactivity to wild type K^b. Based on this model we generated two synthetic peptides with substitutions at position 65: glutamic acid (E65) and aspartic acid (D65) in place of glutamine and tested their ability to generate DTH reactions to MUC-1 in B6 mice. Table 7 demonstrates that these single amino acid substitutions were sufficient to induce DTH reactions to MUC-1 which were comparable to those induced by the $H2K^k$ (BP1-62) allopeptide while the syngeneic $H2K^b$ (BP1-32) peptide did not induce significant DTH reactions.

T-Cell Proliferation Against MUC-1 Synthetic Peptide in CBA Mice Immunized With Mixtures of Allo-$H2K^b$61-69 Plus MUC-1 Peptide

In order to compare antigen specific T cell profileration against KLH and MUC-1, we immunized two groups of CBA mice. The first group of mice received MUC-1-KLH plus $H2K^b$61-69 allopeptide. The second group was immunized with MUC-1-KLH plus syngeneic peptide derived from the 61-69 region. Ten days following subcutaneous immunization, T cells were prepared from draining inguinal lymph nodes and added in culture with antigens at three concentrations of lymph node T cells. Table

6 demonstrates that higher T cell proliferation was noted against KLH and MUC-1 in mice that received the allopeptide MUC-1 mixtures as compared to lower responses in mice that received the syngeneic peptide plus MUC-1 vaccine. Furthermore, a higher level of gamma-interferon was noted at the higher dose level in the allopeptide immunized mice as compared to syngeneic peptide immunized mice. Thus, allopeptide appears to enhance T cell proliferation, not only against the MUC-1 peptide, but also the KLH carrier.

Up to now all experiments were conducted with human MUC-1 peptide sequence, which is a xenogeneic antigen in mice. Therefore we synthesized murine (syngeneic) MUC-1 peptide and tested it with allopeptides in B6 and CBA mice. Table 7 shows that combining it with an allogeneic MHC peptide, but not a syngeneic MHC peptide, results in significant DTH reactions to mouse MUC-1.

Table 101 shows that a mixture of allogeneic peptides can also stimulate a cellular immune response.

In the experiment discussed in Table 102, the efficacy of 24 mer allogeneic peptides BP1-87 (HLA A2-1, a.a. 61-84) and BP1-88 (HLA Aw 68.1; a.a. 61-84) was compared with that of 9 mer allogeneic peptides BP1-57 (HLA A2, a.a. 61-69) and BP1-85 (HLA Aw68.1; a.a.61-69, as well as a mixture of all four peptides. At a given dosage, the 24-mer peptides had a higher stimulation index than the 9-mer peptides. The four peptide mixture was more effective than BP1-87, but not BP1-85. In general, the stimulation index was higher when a lower dosage was used.

EXAMPLE 2

Primary doses (Group 3, 1 μg; Group 4, 25 μg) of STn-KLH with a Detox (Lecithin) adjuvant Detox (Ribi Immunochemical, Hamilton, MT) (one standard human dose is 25 μg MPL monophosphoryl lipid A, 250μg cell wall skeletons of Mycobacterium phlei, .375 mg Tween -80, 8.1 mg squalene oil, 1.62 mg lecithin, in final volume of .5 mL) were injected on day 0 into footpad of C57 Black mice (5 mice per group). After

3, 7 and 14 days the group of mice were sacrificed and draining lymph node cells were harvested for cellular immune response studies.

Lymph node cells (group pools) were plated at 400,000 per well in triplicate in the presence of stimulating antigens at 10 μg/ML. Cells were incubated for 48 hrs., and then pulsed with 1 μG of 3H-thymidine for 6 hours.

The stimulating antigens were STn-KLH, KLH, STn-HSA, or medium (control), alone or in the presence of BP1-32 (syngeneic peptide), BP1-56 (allogeneic peptide), or BP1-68 (allogeneic peptide).

Table 201 shows the CPM ± S.D. data. Table 202 shows the stimulation indexes for group 4.

Hypothetical Example=HIV

The immunodominant antigenic moiety of HIV is the gp120 region three (Vs), that exists as a disulfide linked loop of approximately 30 amino acids (exact size is isolate specific). The sequence of the central amino acids numbered 307-321 of the V3 loop from the isolate 111b is SIRIQRGPGRAFVTIG (Guodsmit et al (1988) PNAS 85:4478-4482). The strong Th2 immunodominance of this and similar V3 sequence epitopes are thought to be specificaly related to the switch of the human immune response from a clinically benificial Th1 based CMI response to a clinically deleterious Th2 response. This epitope and other HIV epitopes are therefore appropriate targets for immunomodulation towards the Th1 response. A protocol for evaluationg this model is siilar to the protocols for MUC1 related peptide antigens. Indeed this antigen structure, which repeats all across the surface of the virion, shows several similarities to the MUC1 structure at this region (RGPGRA between O linked glycosylation sites at Serine 306 and Threonine at 319), and these epitopes are noted for forming overlapping T cell and B cell epitopes. These structural characteristics, along with the repetitive nature of these epitopes in the format they are presented to the immune system may be a key to the noted Th2 immunodominance of these

epitopes. To asses MHC peptide immunomodulatory activities, animals are vaccinated with V3 sequence peptides either conjugated to carriers or in soluble form, and these are either mixed with MHC polymorphic peptides, or not, for comparative data on immune response characterization. The specificity of these response are further controlled via the use of MHC syngeneic peptides (nonimmunomodulatory) and allogeneic peptides. In a C57/bl mouse the Class I sequences from H2Kb and H2Db are syngeneic while the H2Kd and H2Kd sequences from amino acids 61-83 are allogeneic and are therefore expected to be immunomodulatory towards the favored Th1 immune response. Animals could be injected either subcutaneously or intra footpad and regional lymph nodes harvested for in vitro analysis of the blastogenic responses and for determining cytokine production patterns typical of Th1 or Th2 responses. In addition with footpad injections specific DTH responses may be measured for typical CMI responses. Also at specified intervals (days 12, 26, 38 etc) serum samples are collected for analysis of antibodies specific to the V3 sequence. These can be titered by standard methods (ELISA titration) for comparison between groups to establish relative titers to the target epitope.

Hypothetical Example: Leishmania

Studies to evaluate the effects of immunomodulatory MHC peptides on the anti Leishmania major parasite specific antigens can also be performed in manners similar to the MUC1 and HIV systems described elsewhere. The T helper cell characteristics of the immune response towards Leishmania antigens are critical to determine disease course. A cellular Th1 response is noted to be host protective while antibody dominated Th2 responses have been associated with disease exacerbation (Liew et al (1990 J Exp Med. 172:1359-1365). On the parasite surface there is a highly repetitive tandem repeating protein sequence noted for its ability to induce either Th1 type protective responses in the H2 Kb strain C57/bl or Th2 type responses in the H2Kd strain, Balb/c. Thus it is

possible to evaluate immunomodulation in a genetically susceptible Th2 responder using Class I peptides from H2 Kb 61-83. These studies involve vaccination with the repetitive peptide sequence EAEEAARLQA either in the presence of absence of either syngeneic (control) or allogeneic (immunomodulatory) class I peptides. Immune responses are then characterized via the noted methods for antibody titration, DTH responses, blastogenic responses and cytokine production profiles. A switching of the genetically determined T helper response to Th1 would be expected to result in host protection from lethal challenge.

Remarks

All references cited herein, including journal articles or abstracts, published or corresponding U.S. or foreign patent applications, issued U.S. or foreign patents, or any other references, are entirely incorporated by reference herein, including all data, tables, figures, and text presented in the cited references. Additionally, the contents of the references cited within the references cited herein are also entirely incorporated by reference.

Reference to known method steps, conventional methods steps, known methods or conventional methods is not in any way an admission that any aspect, description or embodiment of the present invention is disclosed, taught or suggested in the relevant art.

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art (including the contents of the references cited herein), readily modify and/or adapt for various applications such specific embodiments, without undue experimentation, without departing from the generic concept of the present invention. Therefore, such adaptations and modifications are intended to be comprehended within the meaning and range of equivalents of the disclosed embodiments, based on the teaching and guidance

presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance presented herein.

For immunological techniques generally, see Coligan, et al, Current Protocols in Immunology (NIH: 994); Harlow and Lane, Antibodies:A laboratory Manual (Cold Spring Harbor Lab.: 1988).

Table 1. Synthetic peptides used in this study:

NAME	SEQUENCE
BP1-7	GVTSAPDTRPAPGSTA (16 mer of MUC-1)
BP1-23	PDTRPAPGSTAPPAHGVTSA (20 mer of MUC-1)
BP1-28	PDTRPAPGSTAPPAHGUTSAERETQKAKG (20 mer of MUC-1-H2K^{b}61-69 Chimeric)
BP1-29	ERETQKAKGPDTRAPAPGSTAPPAHGVTSA (H2K^{b}61-69/20 mer of MUC-1 Chimeric)
BP1-32	ERETQKAKG (H2K^{b}61-69)
BP1-56	EEQTQRVKS (H2Kd V67 61-69)
BP1-57	DGETRKVKA (Human A2 61-69)
BP1-76	RVSTRTAQR (H2Kd 75-83)
BP1-77	RVDLRTLLG (H2Kb 75-83)
BP1-78	TIQVISGCE (H2Kb 92-102)
BP1-79	TFQRMFGCD (H2Kd 94-102)
BP1-81	ERETEKAKG (H2K^{b}65E 61-69)
BP1-82	ERETDKAKG (H2K^{b}65D 61-69)
BP1-85	DRNTRNVKA (HLA AW 68.1 61-69)
BP1-87	DGETRKVKAHSQTHRVDLGTLRGY (HLA A2.1 61-84)
BP1-88	DRNTRNVKAQSQTDRVDLGTLRGY (HLA AW68.1 61-84)
BP1-62	ERNTQIAKG (H2K^{k}61-69)
BP1-63	ERETRRAKG (H2D^{d}61-69)
BP1-68 (1-56)	EEQTQRAKS (H2K^{d}61-69)
BP1-69	EEQTQRAKSPDTRPAPGSTAPPAHGVTSA (H2K^{d}61-69-20 mer of MUC-1 Chimeric)
BP1-20	DSTSSPVVHSGTSSPATSAPEDSTS mouse MUC-1

Anti g-IFN MAb (XMG1.2) used in this study was kindly provided by Dr. Tim Mosmann[13].

Table 2. Comparison of synthetic MUC-1-KLH conjugates with MUC-1-H2K^{b}61-69 chimeric synthetic peptides for the induction of DTH to MUC-1 HSA.

IMMUNIZATION	FOOTPAD CHALLENGE	MOUSE STRAIN TESTED CAF1 (H2K^A/H2K^d)	C56/BL (H2K^B)
MUC-1[a]	MUC-1-HSA	3±4[b]	6±2
MUC-1KLH	MUC-1-HSA	28±9	14±5
MUC-1-KLH	HSA	5±4	NT[c]
MUC-1-H2K^{b}61-69[d]	MUC-1-HSA	24±2	NT[c]
H2K^{b}61-69-MUC-1[e]	MUC-1-HSA	26±4	6±2
H2K^{b}61-69-MUC-1-KLH	MUC-1-HSA	34±7	23±9
KLH	KLH	34±5	32±7
KLH	MUC-1-HSA	5±4	5±4

[a] MUC-1 refers to the 16 mer BP1-7 synthetic peptide listed in Table 1

[b] Millimetre footpad swelling ± S.D.[12]

[c] Not tested

[d] Refers to the BP1-28 peptide listed in Table 1

[e] Refers to the BP1-29 peptide listed in Table 1

Table 3. IgG antibody response following immunization with various MUC-1 synthetic peptides in CAF1 mice.

Immunogen	ELISA TEST ANTIGEN					
	BP1-9[a]	MUC-1	MUC-1-H2K^b-HSA	MUC-1-HSA	H2K^b-H2K^b-HSA	KLH
MUC-1-KLH	0/5	4/4[b]	5/5	5/5	0/5	5/5
	14.3 ± 0.0[c]		13.3 ± 2.0	13.9 ± 0.9		>14
MUC-1-H2K^b-KLH	0/5	0/5	0/5	--	0/5	5/5 >14
H2K^b-MUC-1-KLH	0/5	0/5	0/5	--	0/5	--
H2K^b:KLH	0/5	0/5	--	4/5 8.8 ± 3.8	--	5/5 >14
MUC-1-H2K^b	0/5	0/5	--	--	--	--
H2K^b-MUC-1	0/50	0/5	--	--	--	--

[a] Negative control 16 mer
[b] Response to test peptide; total positive/total tested
[c] Mean $\log_2$ titre ± S.D.

Table 4. Inhibition of anti-MUC-1 DTH response following *in vivo* injection of anti-gINF.

IMMUNOGEN	TREATMENT		
	PBS	RAT IgG	MAb XMG1.2[a]
KLH	36 ± 7[b]	--	--
MUC-1-KLH	22 ± 10	30 ± 6	4 ± 2
MUC-1-H2K^{b}61-69-KLH	21 ± 10	30 ± 7	3 ± 8

[a] Rat anti-mouse interferon gamma
[b] mm Footpad swelling ± S.D.

Table 5. DTH response of various strains of mice to BP1-7 HSA Following Immunization With MHC Peptide Plus MUC-1 Peptide Mixtures.

Immunogen	MOUSE STRAIN C57/B6(b)2	BALB/c(d)	CBA(k)	DBA/1J(q)
BP1-7 + PB1-32 (b)	9 ± 2[1]	15 ± 7	24 ± 5	25 ± 12
BP1-7 + PB1-56 (d)	21 ± 2	2 ± 4	23 ± 7	35 ± 7
BP1-7 + BP1-62 (k)	27 ± 6	14 ± 9	26 ± 8	26 ± 8
BP1-61[3]	25 ± 5	3 ± 4	20 ± 10	37 ± 5
PBS	6 ± 4	-	-	8 ± 3
BP1-7 + BP1-37[4]	-	-	-	-

[1] mm footpad swelling ± S.D.
[2] H2K haptotype of mouse strain or allopeptide
[3] BP1-61 is a chimeric $H2K^d$-MUC-1 synthetic peptide
[4] BP1-37 is a synthetic TH1 OVA epitope in BALB/c mice[15, 16]

Table 6. Antigen stimulated T cell proliferation *in vitro* following immunization of CBA mice with MUC-1-KLH plus allogeneic or syngeneic MHC.

NUMBER OF T CELLS PER WELL	ALLOGENEIC MHC IMMUNIZED MICE KLH	MUC-1	SYNGENEIC MHC IMMUNIZED MICE KLH	MUC-1
2×10^5	13[a]	2	4	2
5×10^5	188	5	44	2
10^6	579	23(243)[b]	18	1(40)[b]

[a] Stimulation index
[b] INFg (pg/mL) produced in culture

Effects of H2K Synthetic Peptides on the DTH to Synthetic Mouse MUC-1 in B6 and CBA mice.

Table 7.

Immunogen	MOUSE STRAIN C57/B6	CBA
BP1-20 - JKG	17±4	15±4
BP1-62 + BP1-20	18±4	1±4
BP1-32 + BP1-20	1±4	10±4

Table 101

In Vitro Proliferation of 7 day Cultures - 3H Thymidine

Groups #BC	CPM-1	CPM-2	CPM-3	CPM-4	CPM-5	Avg. (Mean)	S.D.	S.I.
No treatment, cells only, 4 x 10^5	1001	495	589			695		1.0
Human PBL Response Mixture of BP1-32, 1-56, 1-57 & 1-62 added at various concentrations								
50 ug	2221	2678	2611	2755	2379	2529	222	3.9
20 ug	1909	1961	1582	2320	1607	1876	302	2.9
10 ug	1710	1748	1332	1462	1291	1509	211	2.3
1 ug	781	1358	882	1148	985	1031	228	1.6

Supporting data to show that Human PBL's (peripheral blood lymphocytes) in response to synthetic allopeptides. A mixture of synthetic allopeptides were incubated with the PBL's and 7 days later ^{3}H-thymidine was used for incorporation studies. The proliferative response suggests a typical TH1 response.

Table 102

Controls	CPM1	CPM2	CPM3	CPM4	CPM5	AVG.	S.D.	S.I.
Cells only, 4 x 10^5/well	463	301	342			369	84	1.0
PHA	38462	28636	31457	29509	28636	31340	4145	84.9
BP1-57								
50 ug	1142	270	3821	306	325	1173	1525	3.2
20 ug	866	631	260	1276	1382	883	462	2.4
2 ug	4108	1929	769	714	3336	2171	1521	5.9
BP1-85								
50 ug	1286	910	530	605	2814	1229	935	3.3
20 ug	446	1093	2660	683	615	1099	904	3.0
2 ug	3065	1173	1704	1372		1829	853	5.0
BP1-87								
50 ug	1590	650	550	2225	3502	1703	1221	4.6
20 ug	1276	2260	2581	1390	702	1642	766	4.4
2 ug	606	659	5797	797	2638	2099	2234	5.7
BP1-88								
50 ug	2706	8219	1066	621	7343	3991	3559	10.8
20 ug	2101	468	2295	2628	1274	1753	875	4.8
2 ug	520	510	424			485	53	1.3
Mixture of 57, 85, 87, 88								
50 ug	1730	4543	1844	1107	5237	2892	1861	7.8
20 ug	4659	2195	1778	812		2361	1638	6.4
2 ug	646	697				672	36	1.8

Table 201: Influence of Syngeneic and Allogeneic Peptides (20 μg/mL) on Lump Node Cell Proliferation in Response to Carbohydrate

GROUP	DAY 0 IMMUNIZATION IN VIVO		DAY 7 + 64h IN VITRO* STn-KLH	KLH	STn-HSA	MEDIUM
3	STn-KLH 1 μg + Detox (Lecith)	---	3324 ± 291	9755 ± 647	1537 ± 183	386 ± 95
		+BP1-32	3823 ± 1073	10610 ± 531	2171 ± 432	506 ± 71
		+BP1-56	3364 ± 736	10379 ± 408	1533 ± 60	423 ± 55
		+BP1-68	3082 ± 722	7401 ± 1064	1244 ± 46	439 ± 166
4	STn-KLH 25 μg + Detox (Lecith)	---	2477 ± 100	6406 ± 1007	2642 ± 552	1484 ± 242
		+BP1-32	2707 ± 157	9381 ± 235	2115 ± 631	513 ± 103
		+BP1-56	5033 ± 729	11557 ± 1011	5279 ± 1357	570 ± 23
		+BP1-68	4660 ± 823	12202 ± 530	3891 ± 1065	477 ± 88

* Lymph node cells (popliteal and inguinal) taken from female C57 black mice immunized into footpad were cultured with indicated antigens in presence or absence of syngeneic or allogeneic peptides.

Table 202: Blastogenic Responses to STn Hapten and KLH From STn-KLH Immunized Mice

Culture Conditions	STn-KLH	KLH	STn-HSA
Antigen plus Syngeneic Peptide BP1-32 Kb	5.3	18.3	4.12
Antigen plus Allogeneic Peptide BP1-56 Kd(67M*)	8.8	20.3	9.3
Antigen plus Allogeneic Peptide BP1-68 Kd	9.8	25.6	8.2

*BP1-56 Kd peptide with position 67 mutated with valine replacing alanine. This makes this sequence similar to the syngeneic one at this position. This is, however, a conservative substitution.

TABLE 1001A: HUMAN A LOCUS

Human A Locus Allele	61	62	63	64	65	66	67	68	69	70	71	72
Consensus	Asp	Arg (7)	Glu	Thr	Arg	Asn	Val	Lys	Ala	His	Ser	Gln
Frequency	26/ 26	10/ 26	17/ 25	25/ 25	24/ 25	15/ 27	26/ 27	27/ 27	27/ 27	18/ 27	26/ 27	26/ 27
Variability Index	1	13	4.4	1	2.1	3.6	2.1	1	1	3	2.1	2.1
Other A.A.		Gly Gln Leu	Asn Gln		Gly	Lys	Met			Gln	Ala	His

TABLE 1001 A CONTINUED

Human A Locus Allele	73	74	75	76	77	78	79	80	81	82	83
Consensus	Thr	Asp	Arg	Val	Asp	Leu	Gly	Thr	Leu	Arg	Gly
Freguency	25/ 27	15/ 27	27/ 27	21/ 27	21/ 27	27/ 27	24/ 27	24/ 27	24/ 27	24/ 27	24/ 27
Variability Index	2.2	5.4	1	3.9	5.1	1	2.2	2.2	2.2	2.2	2.2
Other A.A.	Ile	His Val	-	Ala Glu	Asn Ser Lys	-	Arg	Ile	Ala	Leu	Ar

TABLE 1001B: HUMAN B LOCUS

Human B Locus	61	62	63	64	65	66	67	68	69	70	71	72
Consensus	Asp	Arg	Glu	Thr	Gln	Ile	Ser	Lys	Thr	Asn	Thr	Gln
Frequency	33/33	31/33	21/33	33/33	31/33	30/33	14/33	33/33	21/33	21/33	21/32	32/32
Variability Index	1	2.1	3.1	1	2.1	3.3	12	1	4.6	6.1	3	1
Other A.A.		Gly	Asn		Arg	Lys Asn	Phe Cys Tyr Met		Arg Ala	Gln Lys Ser	Ala	-

TABLE 1001B CONTINUED

Human B Locus	73	74	75	76	77	78	79	80	81	82	83
Consensus	Thr	Tyr	Arg	Glu	Ser	Leu	Arg	Asn	Leu	Arg	Arg-16
Freguency	32/32	19/32	32/32	31/32	18/32	32/32	32/32	16/33	21/33	16/32	16/32
Variability Index	1	3.4	1	2.1	5.8	1	1	6.2	4.7	6	4
Other A.A.	-	Asp	-	Val	Asp Asn	-	-	Thr Ile	Ala Arg	Leu Ala	Gly-16

TABLE 1001C: HUMAN C LOCUS

Human C Locus	61	62	63	64	65	66	67	68	69	70	71	72
Consensus	Asp	Arg	Glu	Thr	Gln	Lys	Tyr	Lys	Arg	Gln	Ala	Gln
Frequency	17/17	17/17	17/17	17/17	17/17	14/17	17/17	14/17	16/17	17/17	17/17	17/17
Variability Index	1	1	1	1	1	2.4	1	2.4	2.1	1	1	1
Other A.A.						Asn		Asn	Pro		-	-

TABLE 1001 C CONTINUED

Human C Locus	73	74	75	76	77	78	79	80	81	82	83
Consensus	Thr	Asp	Arg	Val	Ser	Leu	Arg	Asn	Leu	Arg	Gly
Freguency	9/17	17/17	17/17	16/16	10/17	17/17	17/17	10/17	17/17	17/17	17/17
Variability Index	3.8	1	1	1	3.4	1	1	3.4	1	1	1
Other A.A.	Ala	-	-	-	Asn	-	-	Lys	-	-	-

TABLE 1001Z: HUMAN CLASS 1
Consensus all Miscellaneous Locus (n=8) 6.[0],
Bewo G7, LA45, BAPCPAP pool, HLAF, HLAJTWIS, HLA-E, HLA CL, HLA12.[4]

	61	62	63	64	65	66	67	68	69	70	71	72
Consensus	Asp	Arg	Glu	Thr	Arg	Asn	Ala	Lys	Ala	His	Ala	Gln
Frequency											7/8	8/8
Variability Index	3.2	4.8	8	1	4.8	11	13	2.7	2.7	11	2.3	2.7
Other A.A.	Glu	Glu Trp	Asn Glu Thr		Gln Gly	Ile Tyr Ser	Thr Val Tyr Cys	Arg	Asp	Gln Asn Thr	Ser	-

TABLE 1001Z CONTINUED

	73	74	75	76	77	78	79	80	81	82	83
Consensus	Thr	Asp	Arg	Val	Asn	Leu	Arg	Thr	Leu	Arg	Gly
Freguency	6/8	5/8	8/8	4/8	5/8	8/8	5/8	5/8	7/8	6/8	5/8
Variability Index	2.7	4.8	1	6	6.4	1	4.8	4.8	2.3	2.7	3.2
Other A.A.	Ile	Phe Glu	-	Met Glu	Asp Ser Ala	-	Gly Gln	Asn Ile	Ala	Leu	Arg

TABLE 1002K: MURINE CLASS I K ALPHA 1 DOMAIN

Murine Class I	61	62	63	64	65	66	67	68	69	70	71	72
Consensus	Glu	Arg	Asn	Thr	Gln	Ile	Ala	Lys	Gly	Asn	Glu	Gln
Frequency	11/11	10/11	5/11	12/12	10/11	5/11	11/12	12/12	7/11	8/11	11/11	11/11
Variability Index	1	2.4	6.6	1	2.2	6.6	2.2	1	4.7	4.1	1	1
Other A.A.		Glu	Glu		Arg	Lys Arg	Val		Asp Ser	Gly Asp	-	-

TABLE 1002K CONTINUED

Murine Class I	73	74	75	76	77	78	79	80	81	82	83
Consensus	Ser	Phe	Arg	Val	Asp	Leu	Arg	Thr	Leu	Leu	Arg
Freguency	8/13	12/14	14/14	14/14	6/13	12/13	14/14	13/14	7/13	12/14	9/14
Variability Index	4.9	2.3	1	1	6.5	2.2	1	2.2	3.7	2.3	3.1
Other A.A.	Ile Trp	Ser	-	-	Asn Ser	Thr	-	Asn	Ala	Gln	Gly

TABLE 1002D: MURINE CLASS I D ALPHA 1 DOMAIN

Murine Class I	61	62	63	64	65	66	67	68	69	70	71	72
Consensus	Glu	Arg	Glu	Thr	Gln	Ile	Ala	Lys	Gly	Gln 6 Asn 6	Glu	Gln
Frequency	17/17	17/20	11/20	18/18	14/17	8/20	17/17	17/18	12/17	6/17	16/17	16/17
Variability Index	1	3.5	5.5	1	3.6	10	1	2.1	4.2	14	2.1	2.1
Other A.A.		Glu Gln	Ile Gln		Arg Trp	Lys Arg Asn		Arg	Asp Ser	His Met	Gly	Arg

TABLE 1002D CONTINUED

Murine Class I	73	74	75	76	77	78	79	80	81	82	83
Consensus	Trp	Phe	Arg	Val	Ser	Leu	Arg	Thr	Leu	Leu	Gly
Freguency	11/18	20/20	20/20	19/19	8/17	19/19	20/20	13/20	12/17	17/19	11/18
Variability Index	4.9	1	1	1	6.4	1	1	2.8	2.8	2.2	4.9
Other A.A.	Ser Asn	-	-	-	Asn Asp	-	-	Asn	Ala	Gln	Ala Arg

TABLE 1002Z: MURINE CLASS MISCELLANEOUS LOCI

Miscellaneous Loci	61	62	63	64	65	66	67	68	69	70	71	72
Consensus	Glu	Arg	Glu	Thr	Gln	Ile	Ala	Lys	Gly	Asn	Glu	Gln
Frequency	18/22	21/24	18/22	24/24	15/24	12/24	18/24	18/24	16/24	12/24	16/24	19/24
Variability Index	3.8	4.6	8.3	1	9.6	10	2.7	5.3	9	12	9	6.3
Other A.A.	Ala Asp	Glu Asp Lys	Ile Glu Asn Thr Arg		Trp Arg Lys Asp Glu	Met Lys Arg Asn	Val	Arg Thr Val	Asp Asn Ala Arg Ser	His Met Ser Gln Ala	Glu Gly Lys Ala His	Glu Arg Lys Tyr

TABLE 1002Z CONTINUED

Miscellaneous Loci	73	74	75	76	77	78	79	80	81	82	83
Consensus	Ser	Phe	Arg	Val	Ser	Leu	Arg	Thr	Leu	Leu	Arg
Freguency	11/24	23/25	19/25	13/25	14/25	24/26	19/25	22/25	12/26	17/26	10/25
Variability Index	15	3.3	5.3	5.8	8.9	3.2	3.9	3.4	8.7	4.6	15
Other A.A.	Ile Trp Asn Arg Gln Phe	Leu Ile	His Gln Lys Arg	Gly Glu	Asn Tyr Ile Asp	Phe Met	Ser Gln	Lys Ser	Ala Met Val	Gln Arg	Arg Gly Asn Asp His

TABLE 1003: MISCELLANEOUS SPECIES CLASS I MOLECULES

Miscellaneous Species	61	62	63	64	65	66	67	68	69	70	71	72
Consensus	Asp	Arg	Glu	Thr	Arg	Asn	Ala	Lys	Ala	Ser	Ala	Gln
Frequency	17/20	17/20	11/20	18/18	14/17	8/20	17/17	17/18	12/17	6/17	21/32	29/33
Variability Index												
Other A.A.	Glu His Val Gln Gly	Glu Gln Leu Gly Thr	Thr Gln Asn Ile Lys Arg	Phe Asp	Gln Glu Ala Trp Gly Asp	Ser Ile Tyr Asp Lys Arg Gly Gln Thr	Val Met Ser Tyr Gly Glu Leu Ile Thr	Leu Glu Phe Asp	Thr Arg Asp Asn Gly Glu Leu	Asn His Gln Asp Thr Trp Val Ile Phe	Ser Glu Trp Lys	Leu Lys Ile Arg

TABLE 1003 CONTINUED

Miscellaneous Species	73	74	75	76	77	78	79	80	81	82	83
Consensus	Thr	Phe	Arg	Val	Asn	Leu	Arg	Thr	Leu	Leu	Gly
Freguency	17/33	14/33	32/33	20/33	17/33	31/33	14/30	20/33	19/32	16/32	20/32
Variability Index											
Other A.A.	Ala Ile Ser Arg Leu	Asp Tyr Ser Arg Leu	Asn	Glu Ser Ala Gly Leu Cys	Asp Ser Ala Gly Thr	Phe Glu	Gly Gln Asn Leu Ser Asp	Ile Asn Val Met Ser	Ala Phe Met Ile Gly	Arg Ile Gln Val	Arg Ala Phe

TABLE 1004: ALL KNOWN SEQUENCES OF MHC CLASS I MOLECULE
ALL SPECIES, RESIDUES #61-83

A

Residue #	61	62	63	64	65	66	67	68	69	70	71	72
	Asp	Arg	Glu	Thr	Gln	Ile	Val	Lys	Ala	Asn	Ala	Gln
	Glu	Gln	Asn	Phe	Arg	Lys	Ala	Arg	Thr	Lys	Ser	His
	Ala	Leu	Gln	Ala	Gly	Asn	Met	Asn	Arg	Ser	Thr	Arg
	His	Glu	Ile		Glu	Ser	Ser	Leu	Asp	Gln	Glu	Lys
	Val	Asp	Thr		Asp	Tyr	Tyr	Gln	Asn	His	Gly	Tyr
	Gln	Lys	Lys		Ala	Asp	Gly	Phe	Gly	Met	Gln	Leu
	Gly	Gly	Arg		Trp	Arg	Glu	Asp	Glu	Asp	Lys	Ile
		Thr			Lys	Gly	Leu		Pro	Gly	Ala	
						Met	Ile		Ser	Thr	His	
							Phe			Val	Trp	
							Cys			Ile		

A - TABLE 1004 CONTINUED

Residue #	73	74	75	76	77	78	79	80	81	82	83
	Thr	Asp	His	Val	Asn	Leu	Arg	Thr	Leu	Arg	Gly
	Ile	His	Gln	Ala	Asp	Thr	Gly	Ile	Ala	Leu	Arg
	Ala	Val	Lys	Glu	Ser	Phe	Gln	Asn	Arg	Ala	Ala
	Ser	Tyr	Arg	Met	Lys	Met	Ser	Lys	Met	Gln	Ser
	Trp	Phe		Gly	Ala	Glu	Asn	Ser	Val	Ile	Asn
	Asn	Glu		Ser	Tyr		Leu	Val	Phe	Val	Asp
	Arg	Ser		Leu	Ile		Ser	Met	Ile		His
	Gln	Leu		Cys	Gly		Asp		Gly		Phe
	Phe				Thr						
	Leu										

B - TABLE 1004 CONTINUED

	61	62	63	64	65	66	67	68	69	70	71	72
	Asp	X	X	Thr	X	X	X	Lys	X	X	X	Gln

B

	73	74	75	76	77	78	79	80	81	82	83
	X	X	Arg	X	X	Leu	Arg	X	Leu	X	X

Table 2001 Human MHC Class 1A

AMINO ACID #	VARIABILITY INDEX	AMINO ACID REGION	MEAN VARIABILITY INDEX FOR AA REGION
1	1.	1 TO 10	1.73
2	1.	2 TO 11	1.73
3	1.	3 TO 12	1.84
4	1.	4 TO 13	1.84
5	1.	5 TO 14	1.84
6	1.	6 TO 15	1.84
7	1.	7 TO 16	1.84
8	1.	8 TO 17	1.95
9	8.3	9 TO 18	1.95
10	1.	10 TO 19	1.33
11	1.	11 TO 20	1.33
12	2.1	12 TO 21	1.33
13	1.	13 TO 22	1.22
14	1.	14 TO 23	1.22
15	1.	15 TO 24	1.22
16	1.	16 TO 25	1.22
17	2.1	17 TO 26	1.22
18	1.	18 TO 27	1.11
19	2.1	19 TO 28	1.11
20	1.	20 TO 29	1.00
21	1.	21 TO 30	1.11
22	1.	22 TO 31	1.22
23	1.	23 TO 32	1.22
24	1.	24 TO 33	1.22
25	1.	25 TO 34	1.22
26	1.	26 TO 35	1.22
27	1.	27 TO 36	1.22
28	1.	28 TO 37	1.22
29	1.	29 TO 38	1.22
30	2.1	30 TO 39	1.22
31	2.1	31 TO 40	1.11
32	1.	32 TO 41	1.00
33	1.	33 TO 42	1.00
34	1.	34 TO 43	1.13
35	1.	35 TO 44	1.24
36	1.	36 TO 45	1.24
37	1.	37 TO 46	1.24
38	1.	38 TO 47	1.24
39	1.	39 TO 48	1.24
40	1.	40 TO 49	1.24
41	1.	41 TO 50	1.24
42	1.	42 TO 51	1.24

Human MHC Class 1A

AMINO ACID #	VARIABILITY INDEX	AMINO ACID REGION	MEAN VARIABILITY INDEX FOR AA REGION
43	2.3	43 TO 52	1.24
44	2.1	44 TO 53	1.11
45	1.	45 TO 54	1.00
46	1.	46 TO 55	1.00
47	1.	47 TO 56	1.12
48	1.	48 TO 57	1.12
49	1.	49 TO 58	1.12
50	1.	50 TO 59	1.12
51	1.	51 TO 60	1.12
52	1.	52 TO 61	1.12
53	1.	53 TO 62	2.32
54	1.	54 TO 63	2.66
55	1.	55 TO 64	2.66
56	2.2	56 TO 65	2.77
57	1.	57 TO 66	2.91
58	1.	58 TO 67	3.02
59	1.	59 TO 68	3.02
60	1.	60 TO 69	3.02
61	1.	61 TO 70	3.22
62	13.	62 TO 71	3.33
63	4.4	63 TO 72	2.24
64	1.	64 TO 73	2.02
65	2.1	65 TO 74	2.46
66	3.6	66 TO 75	2.35
67	2.1	67 TO 76	2.38
68	1.	68 TO 77	2.68
69	1.	69 TO 78	2.68
70	3.	70 TO 79	2.80
71	2.1	71 TO 80	2.72
72	2.1	72 TO 81	2.73
73	2.2	73 TO 82	2.74
74	5.4	74 TO 83	2.74
75	1.	75 TO 84	2.30
76	3.9	76 TO 85	2.30
77	5.1	77 TO 86	2.01
78	1.	78 TO 87	1.60
79	2.2	79 TO 88	1.60
80	2.2	80 TO 89	1.59
81	2.2	81 TO 90	1.60
82	2.2	82 TO 91	1.59
83	2.2	83 TO 92	1.47
84	1.	84 TO 93	1.35

Human MHC Class 1A

AMINO ACID #	VARIABILITY INDEX	AMINO ACID REGION	MEAN VARIABILITY INDEX FOR AA REGION
85	1.	85 TO 94	1.35
86	1.	86 TO 95	1.91
87	1.	87 TO 96	2.02
88	1.	88 TO 97	2.43
89	2.1	89 TO 98	2.43
90	2.3	90 TO 99	2.56
91	2.1	91 TO 100	2.43
92	1.	92 TO 101	2.32
93	1.	93 TO 102	2.43
94	1.	94 TO 103	2.43
95	6.6	95 TO 104	2.43
96	2.1	96 TO 105	2.02
97	5.1	97 TO 106	1.91
98	1.	98 TO 107	1.74
99	3.4	99 TO 108	1.74
100	1.	100 TO 109	1.61
101	1.	101 TO 110	1.61
102	2.1	102 TO 111	1.61
103	1.	103 TO 112	1.50
104	1.	104 TO 113	1.50
105	2.5	105 TO 114	2.37
106	1.	106 TO 115	2.22
107	3.4	107 TO 116	2.85
108	1.	108 TO 117	2.61
109	2.1	109 TO 118	2.61
110	1.	110 TO 119	2.50
111	1.	111 TO 120	2.50
112	1.	112 TO 121	2.50
113	1.	113 TO 122	2.50
114	9.7	114 TO 123	2.50
115	1.	115 TO 124	1.63
116	7.3	116 TO 125	1.63
117	1.	117 TO 126	1.00
118	1.	118 TO 127	1.23
119	1.	119 TO 128	1.23
120	1.	120 TO 129	1.34
121	1.	121 TO 130	1.34
122	1.	122 TO 131	1.34
123	1.	123 TO 132	1.34
124	1.	124 TO 133	1.45
125	1.	125 TO 134	1.45
126	1.	126 TO 135	1.45

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Human MHC Class 1A

AMINO ACID #	VARIABILITY INDEX	AMINO ACID REGION	MEAN VARIABILITY INDEX FOR AA REGION
127	3.3	127 TO 136	1.45
128	1.	128 TO 137	1.22
129	2.1	129 TO 138	1.22
130	1.	130 TO 139	1.11
131	1.	131 TO 140	1.11
132	1.	132 TO 141	1.11
133	2.1	133 TO 142	1.35
134	1.	134 TO 143	1.24
135	1.	135 TO 144	1.40
136	1.	136 TO 145	1.64
137	1.	137 TO 146	1.64
138	1.	138 TO 147	1.64
139	1.	139 TO 148	1.64
140	1.	140 TO 149	1.77
141	1.	141 TO 150	1.99
142	3.4	142 TO 151	2.13
143	1.	143 TO 152	2.34
144	2.6	144 TO 153	2.34
145	3.4	145 TO 154	2.29
146	1.	146 TO 155	2.16
147	1.	147 TO 156	2.78
148	1.	148 TO 157	2.78
149	2.3	149 TO 158	2.89
150	3.2	150 TO 159	2.76
151	2.4	151 TO 160	2.54
152	5.5	152 TO 161	2.63
153	1.	153 TO 162	2.18
154	2.1	154 TO 163	2.31
155	2.1	155 TO 164	2.20
156	7.2	156 TO 165	2.09
157	1.	157 TO 166	1.59
158	2.1	158 TO 167	1.71
159	1.	159 TO 168	1.60
160	1.	160 TO 169	1.60
161	3.3	161 TO 170	1.71
162	1.	162 TO 171	1.59
163	2.3	163 TO 172	1.59
164	1.	164 TO 173	1.46
165	1.	165 TO 174	1.46
166	2.2	166 TO 175	1.46
167	2.2	167 TO 176	1.34
168	1.	168 TO 177	1.22

Human MHC Class 1A

AMINO ACID #	VARIABILITY INDEX	AMINO ACID REGION			MEAN VARIABILITY INDEX FOR AA REGION
169	1.	169	TO	178	1.22
170	2.1	170	TO	179	1.22
171	2.1	171	TO	180	1.11
172	1.	172	TO	181	1.00
173	1.	173	TO	182	1.12
174	1.				
175	1.				
176	1.				
177	1.				
178	1.				
179	1.				
180	1.				
181	1.				
182	2.2				

Table 2002 Human MHC Class 1B

AMINO ACID #	VARIABILITY INDEX	AMINO ACID REGION	MEAN VARIABILITY INDEX FOR AA REGION
1	1.	1 TO 10	1.39
2	1.	2 TO 11	1.63
3	1.	3 TO 12	1.89
4	1.	4 TO 13	1.89
5	1.	5 TO 14	1.89
6	1.	6 TO 15	1.89
7	1.	7 TO 16	1.89
8	1.	8 TO 17	1.89
9	4.9	9 TO 18	1.89
10	1.	10 TO 19	1.50
11	3.4	11 TO 20	1.50
12	3.6	12 TO 21	1.26
13	1.	13 TO 22	1.00
14	1.	14 TO 23	1.00
15	1.	15 TO 24	1.59
16	1.	16 TO 25	1.59
17	1.	17 TO 26	1.59
18	1.	18 TO 27	1.59
19	1.	19 TO 28	1.59
20	1.	20 TO 29	1.59
21	1.	21 TO 30	1.70
22	1.	22 TO 31	1.70
23	1.	23 TO 32	1.92
24	6.9	24 TO 33	1.92
25	1.	25 TO 34	1.33
26	1.	26 TO 35	1.33
27	1.	27 TO 36	1.33
28	1.	28 TO 37	1.33
29	1.	29 TO 38	1.33
30	2.1	30 TO 39	1.33
31	1.	31 TO 40	1.22
32	3.2	32 TO 41	1.41
33	1.	33 TO 42	1.19
34	1.	34 TO 43	1.19
35	1.	35 TO 44	1.19
36	1.	36 TO 45	2.07
37	1.	37 TO 46	2.21
38	1.	38 TO 47	2.21
39	1.	39 TO 48	2.21
40	1.	40 TO 49	2.21
41	2.9	41 TO 50	2.21
42	1.	42 TO 51	2.02

Human MHC Class 1B

AMINO ACID #	VARIABILITY INDEX	AMINO ACID REGION	MEAN VARIABILITY INDEX FOR AA REGION
43	1.	43 TO 52	2.02
44	1.	44 TO 53	2.02
45	9.8	45 TO 54	2.13
46	2.4	46 TO 55	1.25
47	1.	47 TO 56	1.11
48	1.	48 TO 57	1.11
49	1.	49 TO 58	1.11
50	1.	50 TO 59	1.22
51	1.	51 TO 60	1.22
52	1.	52 TO 61	1.22
53	1.	53 TO 62	1.33
54	2.1	54 TO 63	1.54
55	1.	55 TO 64	1.43
56	1.	56 TO 65	1.54
57	1.	57 TO 66	1.77
58	1.	58 TO 67	2.87
59	2.1	59 TO 68	2.87
60	1.	60 TO 69	3.12
61	1.	61 TO 70	3.63
62	2.1	62 TO 71	3.83
63	3.1	63 TO 72	3.72
64	1.	64 TO 73	3.51
65	2.1	65 TO 74	3.75
66	3.3	66 TO 75	3.64
67	12.	67 TO 76	3.52
68	1.	68 TO 77	2.90
69	4.6	69 TO 78	2.90
70	6.1	70 TO 79	2.54
71	3.	71 TO 80	2.55
72	1.	72 TO 81	2.72
73	1.	73 TO 82	3.22
74	3.4	74 TO 83	3.52
75	1.	75 TO 84	3.28
76	2.1	76 TO 85	3.28
77	5.8	77 TO 86	3.17
78	1.	78 TO 87	2.69
79	1.	79 TO 88	2.69
80	6.2	80 TO 89	2.69
81	4.7	81 TO 90	2.17
82	6.	82 TO 91	1.80
83	4.	83 TO 92	1.30
84	1.	84 TO 93	1.00

Human MHC Class 1B

AMINO ACID #	VARIABILITY INDEX	AMINO ACID REGION	MEAN VARIABILITY INDEX FOR AA REGION
85	1.	85 TO 94	1.15
86	1.	86 TO 95	1.53
87	1.	87 TO 96	1.53
88	1.	88 TO 97	2.83
89	1.	89 TO 98	2.83
90	1.	90 TO 99	3.05
91	1.	91 TO 100	3.05
92	1.	92 TO 101	3.05
93	1.	93 TO 102	3.05
94	2.5	94 TO 103	3.19
95	-4.8	95 TO 104	3.04
96	1.	96 TO 105	2.66
97	14.	97 TO 106	2.77
98	1.	98 TO 107	1.58
99	3.2	99 TO 108	1.58
100	1.	100 TO 109	1.36
101	1.	101 TO 110	1.36
102	1.	102 TO 111	1.36
103	2.4	103 TO 112	1.36
104	1.	104 TO 113	1.44
105	1.	105 TO 114	1.98
106	2.1	106 TO 115	1.98
107	2.1	107 TO 116	3.17
108	1.	108 TO 117	3.06
109	1.	109 TO 118	3.06
110	1.	110 TO 119	3.06
111	1.	111 TO 120	3.06
112	1.	112 TO 121	3.06
113	3.2	113 TO 122	3.06
114	6.4	114 TO 123	2.84
115	1.	115 TO 124	2.30
116	14.	116 TO 125	2.30
117	1.	117 TO 126	1.00
118	1.	118 TO 127	1.00
119	1.	119 TO 128	1.00
120	1.	120 TO 129	1.00
121	1.	121 TO 130	1.00
122	1.	122 TO 131	1.17
123	1.	123 TO 132	1.17
124	1.	124 TO 133	1.17
125	1.	125 TO 134	1.17
126	1.	126 TO 135	1.17

Human MHC Class 1B

AMINO ACID #	VARIABILITY INDEX	AMINO ACID REGION	MEAN VARIABILITY INDEX FOR AA REGION
127	1.	127 TO 136	1.17
128	1.	128 TO 137	1.17
129	1.	129 TO 138	1.17
130	1.	130 TO 139	1.17
131	2.7	131 TO 140	1.17
132	1.	132 TO 141	1.00
133	1.	133 TO 142	1.00
134	1.	134 TO 143	1.11
135	1.	135 TO 144	1.11
136	1.	136 TO 145	1.22
137	1.	137 TO 146	1.22
138	1.	138 TO 147	1.33
139	1.	139 TO 148	1.33
140	1.	140 TO 149	1.33
141	1.	141 TO 150	1.33
142	1.	142 TO 151	1.33
143	2.1	143 TO 152	1.54
144	1.	144 TO 153	1.43
145	2.1	145 TO 154	1.43
146	1.	146 TO 155	1.32
147	2.1	147 TO 156	1.83
148	1.	148 TO 157	1.72
149	1.	149 TO 158	1.83
150	1.	150 TO 159	1.83
151	1.	151 TO 160	1.83
152	3.1	152 TO 161	1.83
153	1.	153 TO 162	1.62
154	1.	154 TO 163	2.26
155	1.	155 TO 164	2.26
156	6.1	156 TO 165	2.26
157	1.	157 TO 166	1.75
158	2.1	158 TO 167	1.99
159	1.	159 TO 168	1.99
160	1.	160 TO 169	2.10
161	1.	161 TO 170	2.10
162	1.	162 TO 171	2.24
163	7.4	163 TO 172	2.24
164	1.	164 TO 173	1.60
165	1.	165 TO 174	1.60
166	1.	166 TO 175	1.60
167	3.4	167 TO 176	1.60
168	2.1	168 TO 177	1.53

Human MHC Class 1B

AMINO ACID #	VARIABILITY INDEX	AMINO ACID REGION			MEAN VARIABILITY INDEX FOR AA REGION
169	2.1	169	TO	178	1.56
170	1.	170	TO	179	1.45
171	2.4	171	TO	180	1.61
172	1.	172	TO	181	1.47
173	1.	173	TO	182	1.47
174	1.				
175	1.				
176	1.				
177	2.7				
178	2.4				
179	- 1.				
180	2.6				
181	1.				
182	1.				

Table 2003 Mouse MHC Class 1K

AMINO ACID #	VARIABILITY INDEX	AMINO ACID REGION	MEAN VARIABILITY INDEX FOR AA REGION
1	1.	1 TO 10	1.51
2	2.2	2 TO 11	1.51
3	1.	3 TO 12	1.39
4	1.	4 TO 13	1.39
5	1.	5 TO 14	1.39
6	1.	6 TO 15	1.39
7	1.	7 TO 16	1.39
8	1.	8 TO 17	1.39
9	4.9	9 TO 18	1.39
10	1.	10 TO 19	1.22
11	1.	11 TO 20	1.22
12	1.	12 TO 21	1.22
13	1.	13 TO 22	1.38
14	1.	14 TO 23	1.52
15	1.	15 TO 24	1.92
16	1.	16 TO 25	1.92
17	1.	17 TO 26	1.92
18	1.	18 TO 27	1.92
19	3.2	19 TO 28	1.92
20	1.	20 TO 29	1.82
21	1.	21 TO 30	1.94
22	2.6	22 TO 31	1.94
23	2.4	23 TO 32	2.08
24	5.	24 TO 33	1.94
25	1.	25 TO 34	1.54
26	1.	26 TO 35	1.54
27	1.	27 TO 36	1.54
28	1.	28 TO 37	1.54
29	2.2	29 TO 38	1.54
30	2.2	30 TO 39	1.42
31	1.	31 TO 40	1.30
32	4.	32 TO 41	1.45
33	1.	33 TO 42	1.15
34	1.	34 TO 43	1.27
35	1.	35 TO 44	1.27
36	1.	36 TO 45	1.44
37	1.	37 TO 46	1.44
38	1.	38 TO 47	1.44
39	1.	39 TO 48	1.44
40	1.	40 TO 49	1.63
41	2.5	41 TO 50	1.77
42	1.	42 TO 51	1.62

Mouse MHC Class 1K

AMINO ACID #	VARIABILITY INDEX	AMINO ACID REGION	MEAN VARIABILITY INDEX FOR AA REGION
43	2.2	43 TO 52	1.74
44	1.	44 TO 53	1.62
45	2.7	45 TO 54	1.62
46	1.	46 TO 55	1.75
47	1.	47 TO 56	2.02
48	1.	48 TO 57	2.02
49	2.9	49 TO 58	2.02
50	2.4	50 TO 59	1.83
51	1.	51 TO 60	1.69
52	2.2	52 TO 61	1.69
53	1.	53 TO 62	1.71
54	1.	54 TO 63	2.27
55	4.	55 TO 64	2.27
56	3.7	56 TO 65	2.09
57	1.	57 TO 66	2.38
58	1.	58 TO 67	2.50
59	1.	59 TO 68	2.50
60	1.	60 TO 69	2.87
61	1.	61 TO 70	3.18
62	2.4	62 TO 71	3.18
63	6.6	63 TO 72	3.04
64	1.	64 TO 73	2.87
65	2.2	65 TO 74	3.00
66	6.6	66 TO 75	2.38
67	2.2	67 TO 76	2.32
68	1.	68 TO 77	2.75
69	4.7	69 TO 78	2.87
70	4.1	70 TO 79	2.50
71	1.	71 TO 80	2.31
72	1.	72 TO 81	2.58
73	4.9	73 TO 82	2.71
74	2.3	74 TO 83	2.53
75	1.	75 TO 84	2.40
76	1.	76 TO 85	2.40
77	6.5	77 TO 86	2.40
78	2.2	78 TO 87	1.85
79	1.	79 TO 88	1.73
80	2.2	80 TO 89	2.19
81	3.7	81 TO 90	2.19
82	2.3	82 TO 91	1.92
83	3.1	83 TO 92	1.79
84	1.	84 TO 93	1.58

Mouse MHC Class 1K

AMINO ACID #	VARIABILITY INDEX	AMINO ACID REGION	MEAN VARIABILITY INDEX FOR AA REGION
85	1.	85 TO 94	1.58
86	1.	86 TO 95	2.04
87	1.	87 TO 96	2.04
88	1.	88 TO 97	2.50
89	5.6	89 TO 98	2.88
90	2.2	90 TO 99	3.23
91	1.	91 TO 100	3.22
92	1.	92 TO 101	3.22
93	1.	93 TO 102	3.43
94	1.	94 TO 103	3.43
95	5.6	95 TO 104	3.55
96	1.	96 TO 105	3.09
97	5.6	97 TO 106	3.09
98	4.8	98 TO 107	2.85
99	9.1	99 TO 108	2.59
100	2.1	100 TO 109	1.78
101	1.	101 TO 110	1.67
102	3.1	102 TO 111	1.67
103	1.	103 TO 112	1.46
104	2.2	104 TO 113	1.46
105	1.	105 TO 114	1.75
106	1.	106 TO 115	1.75
107	3.2	107 TO 116	2.45
108	2.2	108 TO 117	2.23
109	1.	109 TO 118	2.11
110	1.	110 TO 119	2.11
111	1.	111 TO 120	2.11
112	1.	112 TO 121	2.31
113	1.	113 TO 122	2.31
114	5.1	114 TO 123	2.31
115	1.	115 TO 124	1.90
116	8.	116 TO 125	1.90
117	1.	117 TO 126	1.20
118	1.	118 TO 127	1.20
119	1.	119 TO 128	1.20
120	1.	120 TO 129	1.20
121	3.	121 TO 130	1.20
122	1.	122 TO 131	1.00
123	1.	123 TO 132	1.00
124	1.	124 TO 133	1.00
125	1.	125 TO 134	1.00
126	1.	126 TO 135	1.00

Mouse MHC Class 1K

AMINO ACID #	VARIABILITY INDEX	AMINO ACID REGION	MEAN VARIABILITY INDEX FOR AA REGION
127	1.	127 TO 136	1.00
128	1.	128 TO 137	1.00
129	1.	129 TO 138	1.12
130	1.	130 TO 139	1.12
131	1.	131 TO 140	1.12
132	1.	132 TO 141	1.24
133	1.	133 TO 142	1.24
134	1.	134 TO 143	1.24
135	1.	135 TO 144	1.37
136	1.	136 TO 145	1.65
137	1.	137 TO 146	1.65
138	2.2	138 TO 147	1.65
139	1.	139 TO 148	1.53
140	1.	140 TO 149	1.65
141	2.2	141 TO 150	1.65
142	1.	142 TO 151	1.53
143	1.	143 TO 152	2.15
144	2.3	144 TO 153	2.15
145	3.8	145 TO 154	2.02
146	1.	146 TO 155	1.87
147	1.	147 TO 156	2.57
148	1.	148 TO 157	2.57
149	2.2	149 TO 158	2.57
150	1.	150 TO 159	2.45
151	1.	151 TO 160	2.45
152	7.2	152 TO 161	2.45
153	1.	153 TO 162	1.94
154	1.	154 TO 163	2.59
155	2.3	155 TO 164	2.59
156	8.	156 TO 165	2.71
157	1.	157 TO 166	2.01
158	1.	158 TO 167	2.13
159	1.	159 TO 168	2.13
160	1.	160 TO 169	2.26
161	1.	161 TO 170	2.26
162	2.1	162 TO 171	2.39
163	7.5	163 TO 172	2.28
164	1.	164 TO 173	2.09
165	3.5	165 TO 174	2.51
166	1.	166 TO 175	2.26
167	2.2	167 TO 176	2.26
168	1.	168 TO 177	2.26

Mouse MHC Class 1K

AMINO ACID #	VARIABILITY INDEX	AMINO ACID REGION			MEAN VARIABILITY INDEX FOR AA REGION
169	2.3	169	TO	178	2.26
170	1.	170	TO	179	2.13
171	2.3	171	TO	180	2.27
172	1.	172	TO	181	2.14
173	5.6	173	TO	182	2.14
174	5.2				
175	1.				
176	1.				
177	2.2				
178	1.				
179	1.				
180	-2.4				
181	1.				
182	1.				

Table 2004 Mouse MHC Class 1D

AMINO ACID #	VARIABILITY INDEX	AMINO ACID REGION	MEAN VARIABILITY INDEX FOR AA REGION
1	2.2	1 TO 10	1.83
2	2.3	2 TO 11	1.82
3	1.	3 TO 12	1.69
4	1.	4 TO 13	1.80
5	3.4	5 TO 14	1.80
6	1.	6 TO 15	1.67
7	1.	7 TO 16	1.78
8	1.	8 TO 17	2.04
9	4.4	9 TO 18	2.40
10	1.	10 TO 19	2.32
11	2.1	11 TO 20	2.32
12	1.	12 TO 21	2.32
13	2.1	13 TO 22	2.62
14	1.	14 TO 23	2.78
15	2.1	15 TO 24	3.26
16	2.1	16 TO 25	3.26
17	3.6	17 TO 26	3.26
18	4.6	18 TO 27	3.11
19	3.6	19 TO 28	2.86
20	1.	20 TO 29	2.71
21	2.1	21 TO 30	3.00
22	4.	22 TO 31	3.30
23	3.7	23 TO 32	3.27
24	5.8	24 TO 33	3.11
25	2.1	25 TO 34	2.74
26	2.1	26 TO 35	2.74
27	2.1	27 TO 36	2.74
28	2.1	28 TO 37	2.74
29	2.1	29 TO 38	2.63
30	3.9	30 TO 39	2.52
31	5.1	31 TO 40	2.23
32	3.7	32 TO 41	1.95
33	2.1	33 TO 42	1.68
34	2.1	34 TO 43	1.57
35	2.1	35 TO 44	1.46
36	2.1	36 TO 45	1.82
37	2.1	37 TO 46	1.82
38	1.	38 TO 47	1.71
39	1.	39 TO 48	1.88
40	1.	40 TO 49	2.01
41	2.3	41 TO 50	2.22
42	1.	42 TO 51	2.09

Mouse MHC Class 1D

AMINO ACID #	VARIABILITY INDEX	AMINO ACID REGION	MEAN VARIABILITY INDEX FOR AA REGION
43	1.	43 TO 52	2.23
44	1.	44 TO 53	2.23
45	5.7	45 TO 54	2.23
46	2.1	46 TO 55	1.76
47	1.	47 TO 56	1.65
48	2.7	48 TO 57	1.65
49	2.3	49 TO 58	1.48
50	3.1	50 TO 59	1.46
51	1.	51 TO 60	1.25
52	2.4	52 TO 61	1.25
53	1.	53 TO 62	1.36
54	1.	54 TO 63	1.81
55	1.	55 TO 64	1.31
56	1.	56 TO 65	2.07
57	1.	57 TO 66	2.97
58	1.	58 TO 67	2.97
59	2.1	59 TO 68	3.08
60	1.	60 TO 69	3.29
61	1.	61 TO 70	4.59
62	3.5	62 TO 71	4.70
63	5.5	63 TO 72	4.56
64	1.	64 TO 73	4.50
65	3.6	65 TO 74	4.50
66	10.	66 TO 75	4.24
67	1.	67 TO 76	3.34
68	2.1	68 TO 77	3.88
69	4.2	69 TO 78	3.77
70	14.	70 TO 79	3.45
71	2.1	71 TO 80	2.33
72	2.1	72 TO 81	2.40
73	4.9	73 TO 82	2.41
74	1.	74 TO 83	2.41
75	1.	75 TO 84	2.41
76	1.	76 TO 85	2.41
77	6.4	77 TO 86	2.41
78	1.	78 TO 87	1.87
79	1.	79 TO 88	1.87
80	2.8	80 TO 89	2.39
81	2.8	81 TO 90	2.32
82	2.2	82 TO 91	2.25
83	4.9	83 TO 92	2.42
84	1.	84 TO 93	2.03

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Mouse MHC Class 1D

AMINO ACID #	VARIABILITY INDEX	AMINO ACID REGION	MEAN VARIABILITY INDEX FOR AA REGION
85	1.	85 TO 94	2.03
86	1.	86 TO 95	2.41
87	1.	87 TO 96	2.41
88	1.	88 TO 97	3.61
89	6.2	89 TO 98	3.72
90	2.1	90 TO 99	4.20
91	2.1	91 TO 100	4.09
92	3.9	92 TO 101	3.98
93	1.	93 TO 102	3.69
94	1.	94 TO 103	3.86
95	4.8	95 TO 104	3.98
96	1.	96 TO 105	3.89
97	13.	97 TO 106	3.89
98	2.1	98 TO 107	2.91
99	11.	99 TO 108	2.80
100	1.	100 TO 109	1.80
101	1.	101 TO 110	1.80
102	1.	102 TO 111	1.80
103	2.7	103 TO 112	1.80
104	2.2	104 TO 113	1.63
105	3.9	105 TO 114	2.91
106	1.	106 TO 115	2.62
107	3.2	107 TO 116	2.89
108	1.	108 TO 117	2.67
109	1.	109 TO 118	2.67
110	1.	110 TO 119	2.82
111	1.	111 TO 120	2.82
112	1.	112 TO 121	3.82
113	1.	113 TO 122	3.82
114	15.	114 TO 123	3.82
115	1.	115 TO 124	2.42
116	3.7	116 TO 125	2.53
117	1.	117 TO 126	2.26
118	1.	118 TO 127	2.26
119	2.5	119 TO 128	2.26
120	1.	120 TO 129	2.11
121	11.	121 TO 130	2.11
122	1.	122 TO 131	1.23
123	1.	123 TO 132	1.34
124	1.	124 TO 133	1.34
125	2.1	125 TO 134	1.34
126	1.	126 TO 135	1.23

Mouse MHC Class 1D

AMINO ACID #	VARIABILITY INDEX	AMINO ACID REGION	MEAN VARIABILITY INDEX FOR AA REGION
127	1.	127 TO 136	1.34
128	1.	128 TO 137	1.34
129	1.	129 TO 138	1.60
130	1.	130 TO 139	1.60
131	2.2	131 TO 140	1.71
132	2.1	132 TO 141	1.74
133	1.	133 TO 142	1.63
134	1.	134 TO 143	1.74
135	1.	135 TO 144	1.86
136	2.1	136 TO 145	1.98
137	1.	137 TO 146	1.97
138	3.6	138 TO 147	1.98
139	1.	139 TO 148	1.72
140	2.1	140 TO 149	1.83
141	2.5	141 TO 150	2.25
142	1.	142 TO 151	2.22
143	2.1	143 TO 152	2.49
144	2.2	144 TO 153	2.38
145	2.2	145 TO 154	2.37
146	1.	146 TO 155	3.35
147	2.1	147 TO 156	3.80
148	1.	148 TO 157	3.90
149	2.1	149 TO 158	3.90
150	6.4	150 TO 159	3.79
151	2.1	151 TO 160	3.25
152	3.7	152 TO 161	3.25
153	1.	153 TO 162	2.98
154	2.1	154 TO 163	3.38
155	12.	155 TO 164	3.27
156	5.5	156 TO 165	2.17
157	3.1	157 TO 166	1.72
158	1.	158 TO 167	1.51
159	1.	159 TO 168	1.51
160	1.	160 TO 169	1.73
161	2.1	161 TO 170	1.73
162	1.	162 TO 171	1.62
163	5.	163 TO 172	1.62
164	1.	164 TO 173	1.57
165	1.	165 TO 174	1.77
166	1.	166 TO 175	1.77
167	1.	167 TO 176	1.77
168	1.	168 TO 177	1.92

Mouse MHC Class 1D

AMINO ACID #	VARIABILITY INDEX	AMINO ACID REGION			MEAN VARIABILITY INDEX FOR AA REGION
169	3.2	169	TO	178	1.92
170	1.	170	TO	179	1.70
171	1.	171	TO	180	1.81
172	1.	172	TO	181	2.05
173	4.5	173	TO	182	2.16
174	3.				
175	1.				
176	1.				
177	2.5				
178	1.				
179	1.				
180	2.1				
181	3.4				
182	2.1				

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CLAIMS

1. A pharmaceutically acceptable CMI-specific immunogenic composition, said composition comprising

(a) a non-naturally occurring conjugate of a primary epitope which is substantially the same as a T cell epitope of a cancer-, microbial-, parasitic-, or virally infected cell-associated antigen, and of an immunomodulatory peptide, or

(b) a mixture of (1) primary antigen bearing a primary epitope which is substantially the same as a T-cell epitope of a cancer-, microbial-, parasitic-, or virally infected cell-associated antigen, (2) an immunomodulatory peptide,

where the conjugate of (a) and the immunomodulatory peptide of (b) have a molecular weight of less than 5,000 daltons,

where said immunomodulatory peptide of (a) or (b) comprises an allopeptide moiety of at least five amino acids whose amino acid sequence corresponds essentially to that of a polymorphic region of an MHC-encoded polymorphic Class I or Class II antigen,

and where, when said composition is administered to a subject from whom said immunomodulatory peptide is allogeneic or xenogeneic, an immune response is elicited wherein the cellular immune response is stronger, and the humoral immune response weaker, than if said composition, with the immunomodulatory peptide omitted, were administered to said subject.

2. The composition of claim 1 wherein the MHC encoded antigen is an MHC Class I antigen.

3. The composition of claim 2 wherein the MHC Class I antigen is a mouse antigen.

4. The composition of claim 3 wherein the antigen is selected from the group consisting of H2K, H2D and H2L.

5. The composition of claim 2 wherein the MHC Class I antigen is a human antigen.

6. The composition of claim 5 wherein the antigen is selected from the group consisting of HLA-A, HLA-B and HLA-C.

7. The composition of claim 2 wherein the polymorphic region is a region of the α_1 or α_2 domains of said MHC Class I antigen.

8. The composition of claim 2 wherein the polymorphic region is a segment corresponding to residues 62-83 of said MHC Class I antigen, or a segments which is a polymorphic subsequence of the 62-83 segment.

9. The composition of claim 8 wherein the polymorphic region is selected from the group consisting of segments 62-69, 75-83 and 62-83.

10. The composition of claim 2 wherein the polymorphic region is the segment corresponding to residues 92-102 of said MHC Class I antigen, or a segment which is a polymorphic subsequence of the 92-102 segment.

11. The composition of claim 1 wherein said allopeptide moiety is at least nine amino acids in length.

12. The composition of claim 11 in which said allopeptide moiety is not more than 24 amino acids in length.

13. The composition of claim 1 wherein the immunomodulatory peptide comprises a plurality of allopeptide moieties, which may be the same or different.

14. The composition of claim 1 wherein the immunomodulatory peptide comprises a promiscuous T-cell epitope which is not substantially the same as a T-cell epitope of an MHC-encoded polypeptide.

15. The composition of claim 1 wherein the composition comprises the conjugate (a).

16. The composition of claim 15 wherein the conjugate is a chimeric peptide.

17. The composition of claim 1 wherein the composition

comprises the mixture (b).

18. The composition of claim 1 wherein the mixture comprises a plurality of different such immunomodulatory peptides.

19. The composition of claim 1 wherein each molecule of said conjugate of (a) or said primary antigen of (b) bears a plurality of primary epitopes, which may be same or different.

20. The composition of claim 1 wherein the primary epitope is cancer-associated.

21. The composition of claim 20 wherein the primary epitope is an epitope associated with an adenocarcinana.

22. The composition of claim 1 wherein the primary epitope is microbe-associated.

23. The composition of claim 22 wherein the primary epitope is selected from the group consisting of epitopes associated with tuberculosis, staphylococcus, E. coli, Shigella and Candida.

24. The composition of claim 1 wherein the primary epitope is parasite-associated.

25. The composition of claim 24 wherein the primary epitope

is selected from the group consisting of epitopes associated with Plasmodia, Leishmania, Toxoplasma or Schistosoma.

26. The composition of claim 1 wherein the primary epitope is virally infected cell-associated.

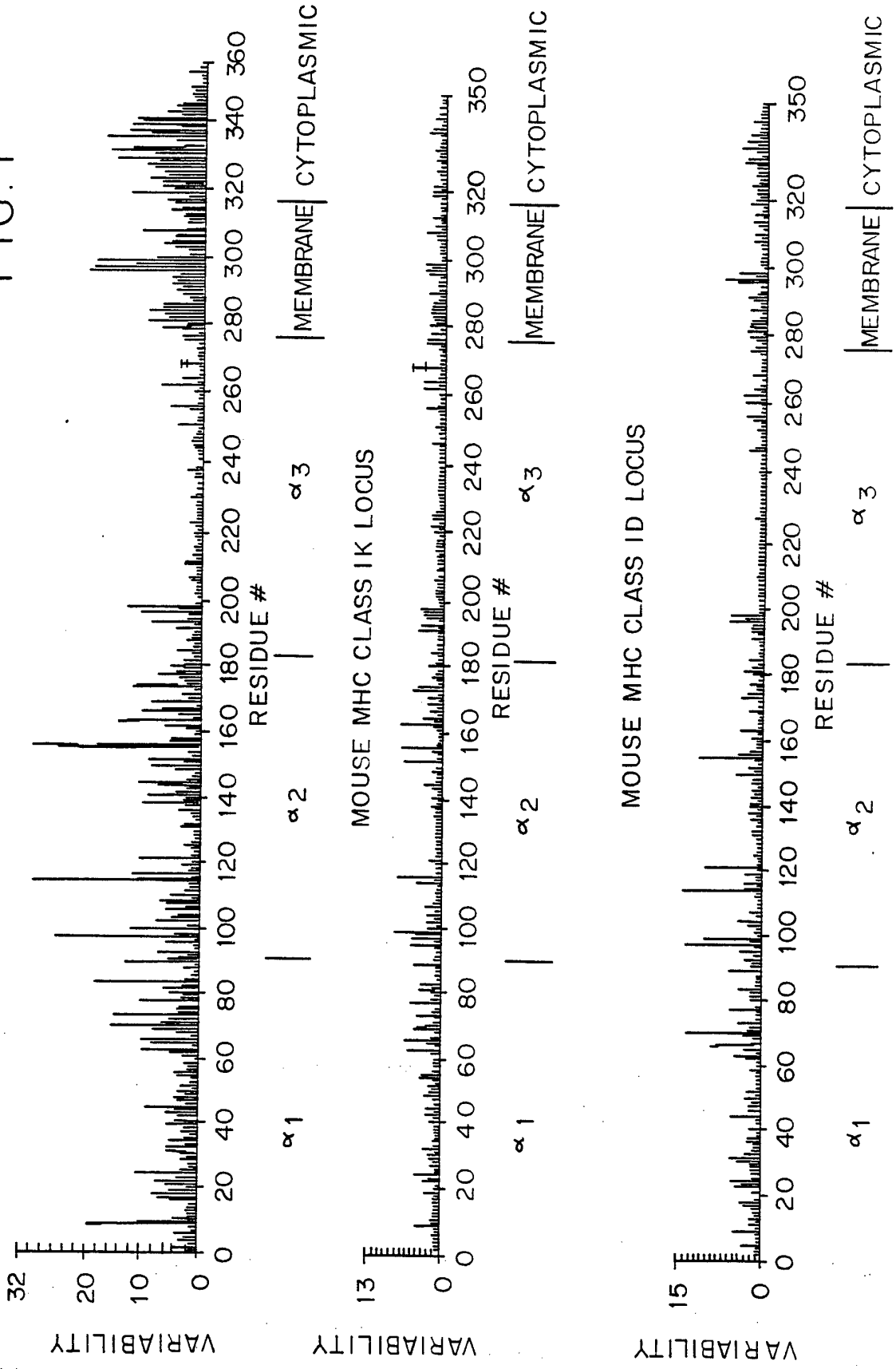
27. The composition of claim 1 wherein the virus is selected from the group consisting of HIV, influenza, EBV, HBV, HSV and rabies.

28. A method of eliciting a CMI-specific response which is prophylactic or therapeutic for a disease selected from the group consisting of cancer, microbial infections, viral infections, and parasitic infestations, which comprises administering to a subject to be protected a composition according to claim 1, where the primary epitope is at least substantially the same as a T-cell epitope of an antigen associated with that disease, such that a CMI-specific response which is protective for said subject against that disease is elicited.

29. The method of claim 28 wherein the humoral response to said composition is no stronger than that represented by an antibody titer of 1/80.

30. The method of claim 28 wherein the T cell stimulation index is at least 2.

FIG. 1


MOUSE MHC CLASS I
VARIABILITY
32
20
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0 20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360
RESIDUE #
α1
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MEMBRANE
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RESIDUE #
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MEMBRANE
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0
0 20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 350
RESIDUE #
α1
α2
α3
MEMBRANE
CYTOPLASMIC

FIG. 2

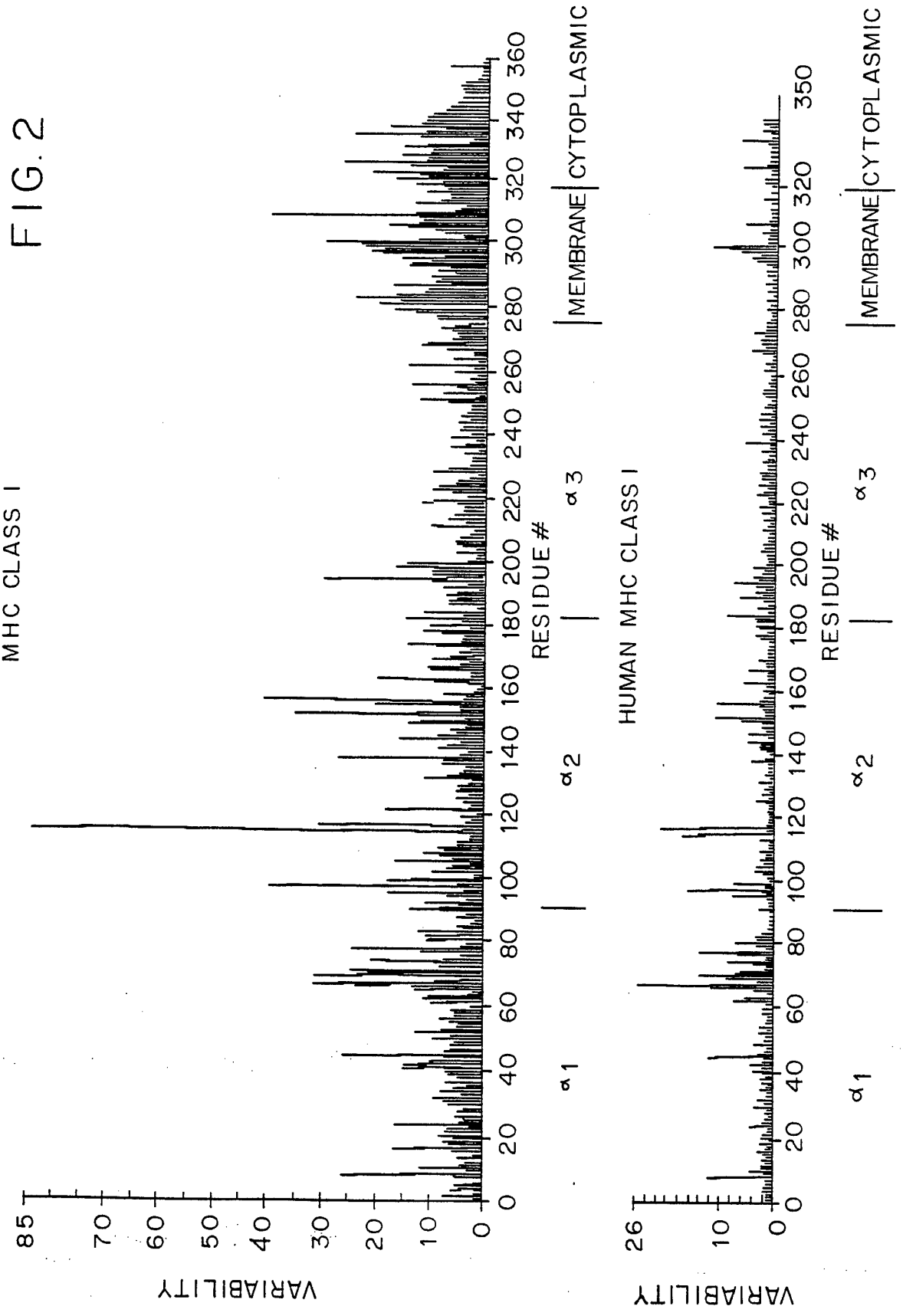

MHC CLASS I
VARIABILITY
85
70
60
50
40
30
20
10
0
0 20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360
RESIDUE #
α1
α2
α3
MEMBRANE
CYTOPLASMIC
HUMAN MHC CLASS I
VARIABILITY
26
10
0
0 20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 350
RESIDUE #
α1
α2
α3
MEMBRANE
CYTOPLASMIC

FIG. 3

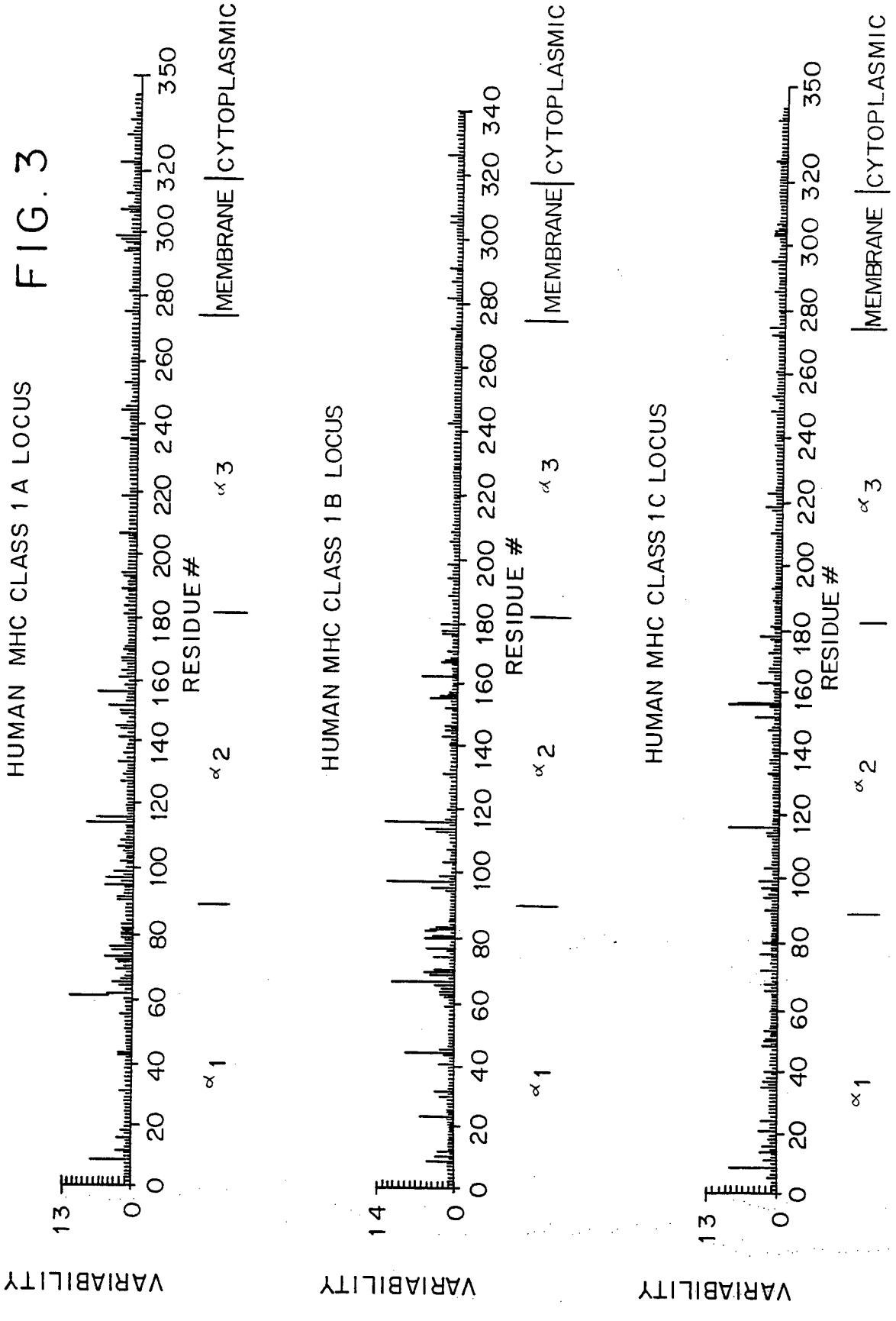

HUMAN MHC CLASS 1A LOCUS
VARIABILITY
13
0
0 20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 350
RESIDUE #
α1
α2
α3
MEMBRANE
CYTOPLASMIC
HUMAN MHC CLASS 1B LOCUS
VARIABILITY
14
0
0 20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340
RESIDUE #
α1
α2
α3
MEMBRANE
CYTOPLASMIC
HUMAN MHC CLASS 1C LOCUS
VARIABILITY
13
0
0 20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 350
RESIDUE #
α1
α2
α3
MEMBRANE
CYTOPLASMIC

FIG. 4

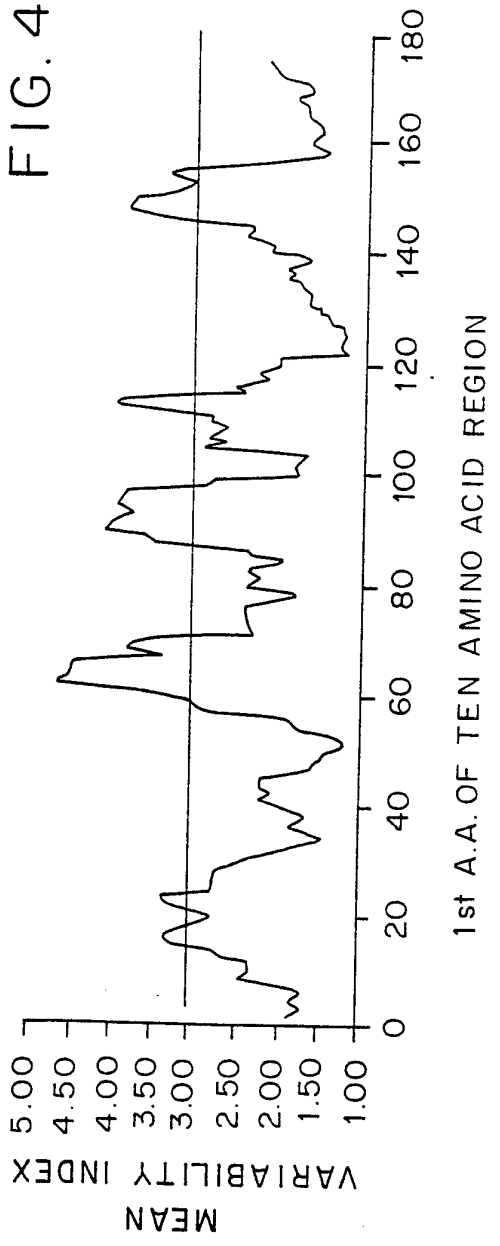

MEAN VARIABILITY INDEX
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0
20
40
60
80
100
120
140
160
180
1st A.A. OF TEN AMINO ACID REGION

FIG. 5

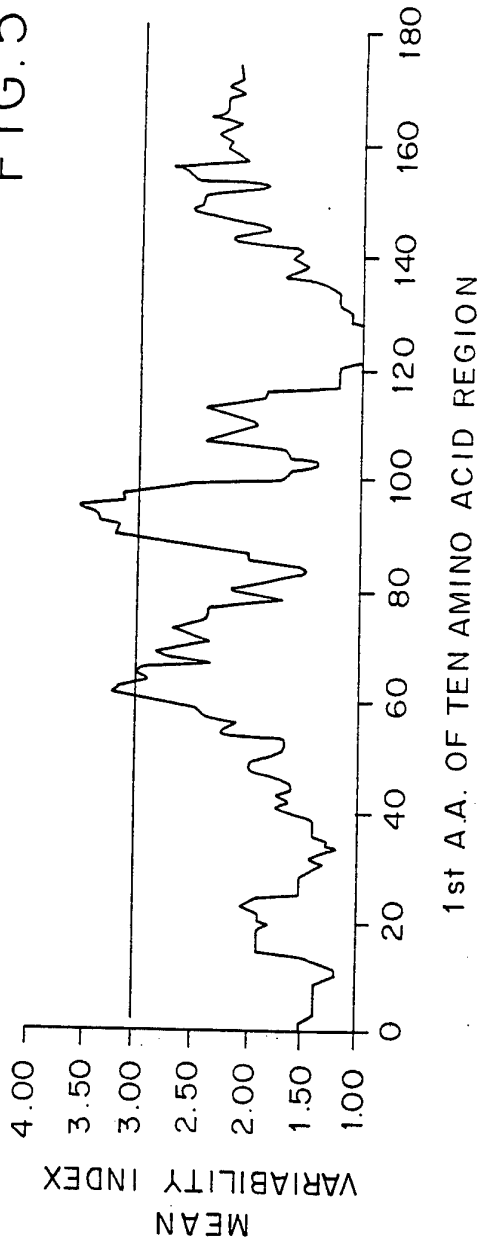

MEAN VARIABILITY INDEX
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0
20
40
60
80
100
120
140
160
180
1st A.A. OF TEN AMINO ACID REGION

FIG. 6

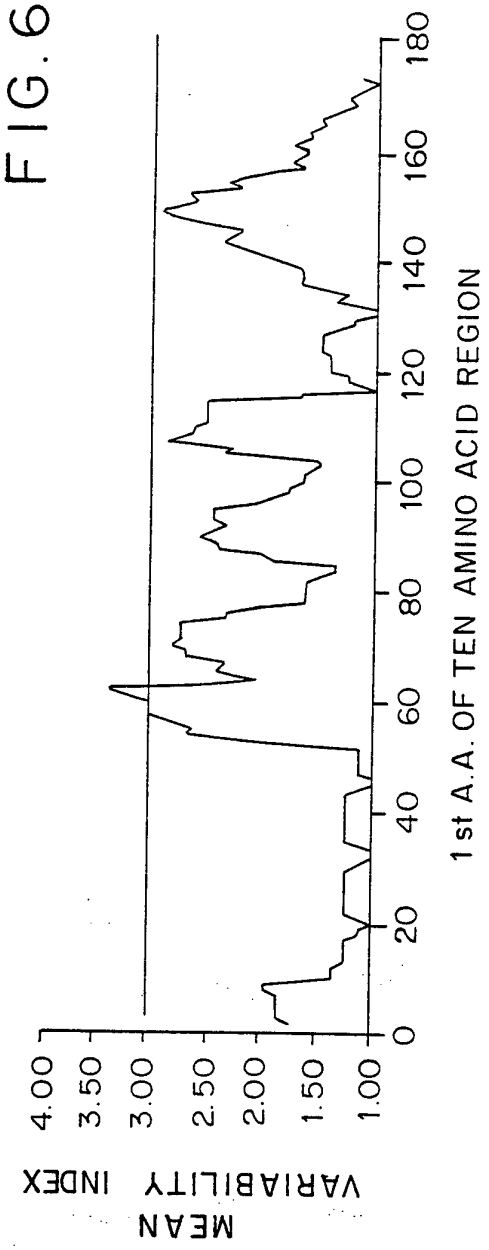

MEAN VARIABILITY INDEX
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0
20
40
60
80
100
120
140
160
180
1st A.A. OF TEN AMINO ACID REGION

FIG. 7

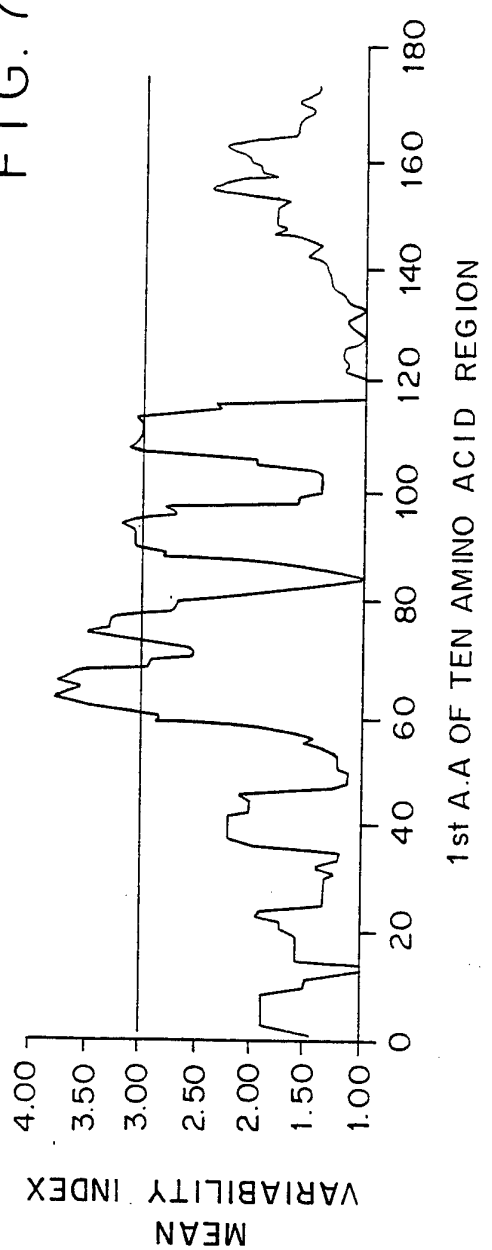

MEAN VARIABILITY INDEX
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0
20
40
60
80
100
120
140
160
180
1st A.A OF TEN AMINO ACID REGION

FIG. 8

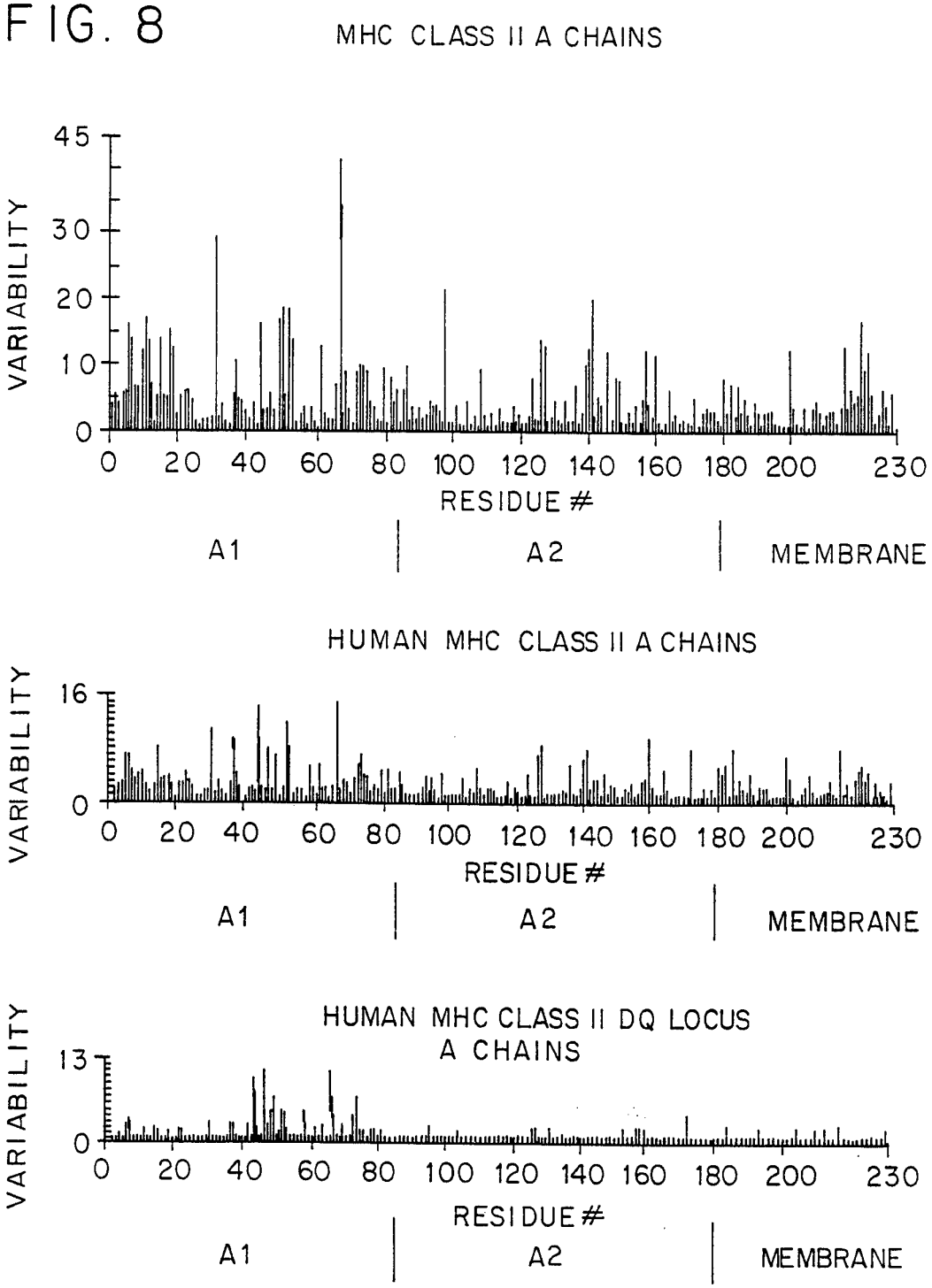

MHC CLASS II A CHAINS
VARIABILITY
45
30
20
10
0
0 20 40 60 80 100 120 140 160 180 200 230
RESIDUE #
A1
A2
MEMBRANE
HUMAN MHC CLASS II A CHAINS
VARIABILITY
16
0
0 20 40 60 80 100 120 140 160 180 200 230
RESIDUE #
A1
A2
MEMBRANE
HUMAN MHC CLASS II DQ LOCUS
A CHAINS
VARIABILITY
13
0
0 20 40 60 80 100 120 140 160 180 200 230
RESIDUE #
A1
A2
MEMBRANE

FIG. 9

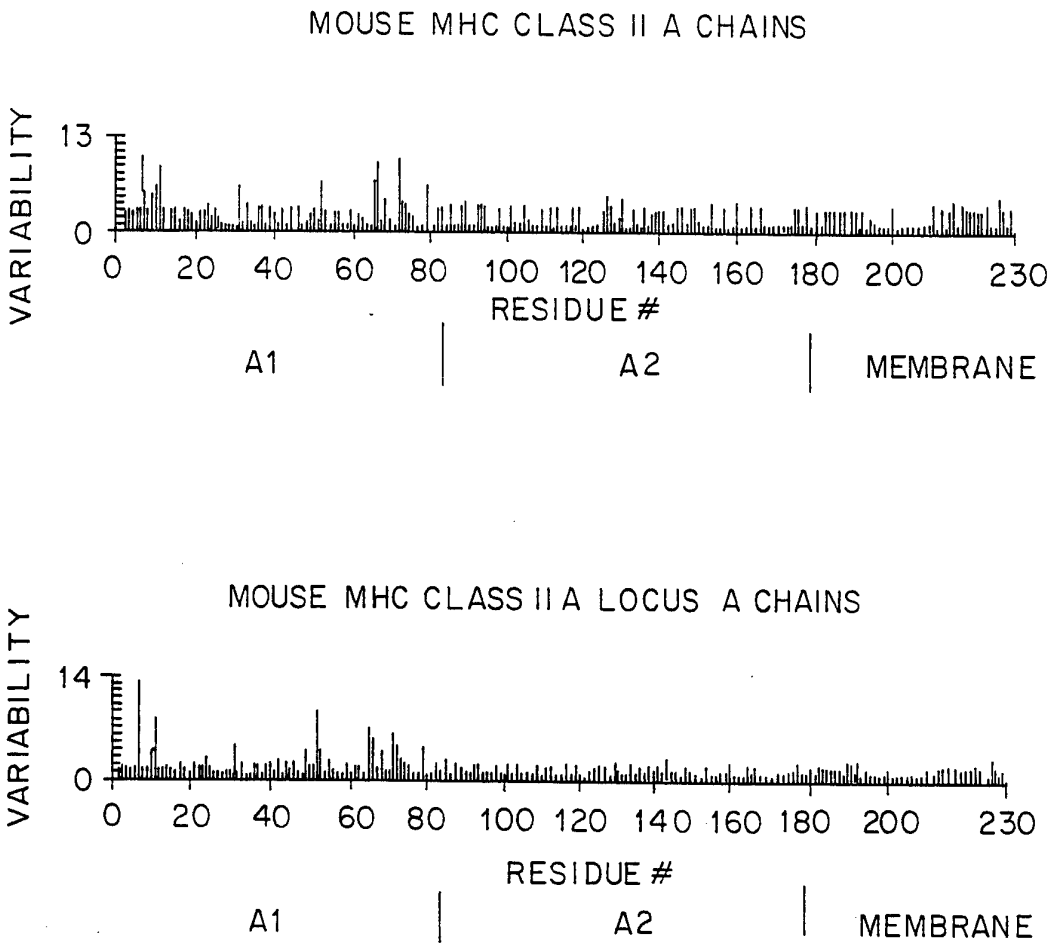
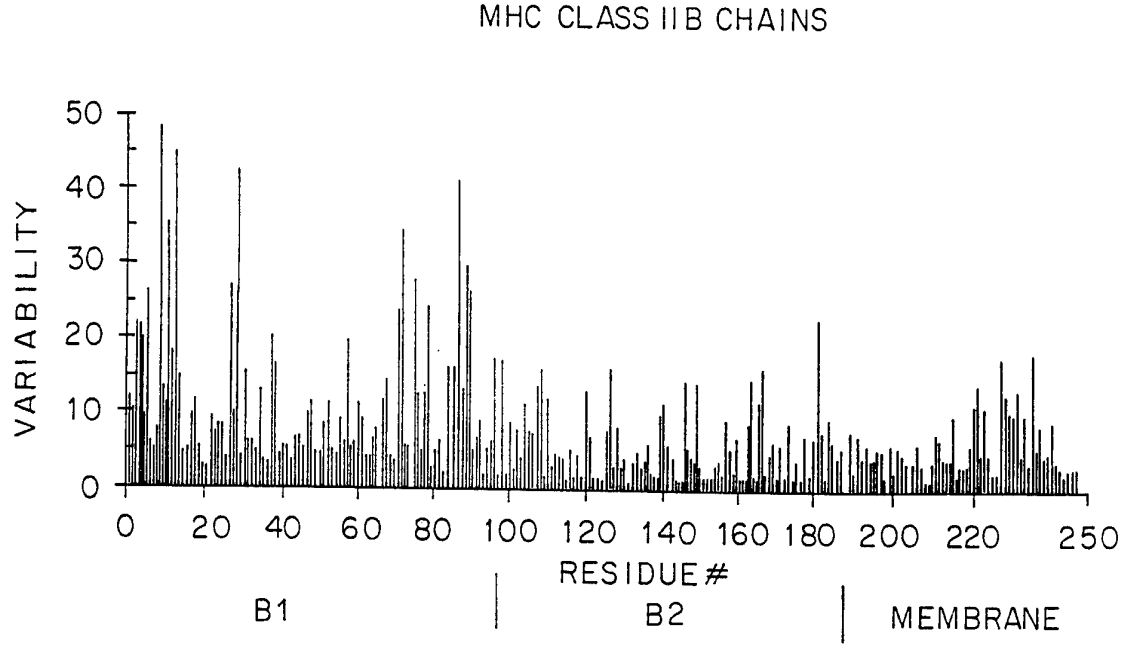

MOUSE MHC CLASS II A CHAINS
VARIABILITY
13
0
0 20 40 60 80 100 120 140 160 180 200 230
RESIDUE #
A1
A2
MEMBRANE
MOUSE MHC CLASS II A LOCUS A CHAINS
VARIABILITY
14
0
0 20 40 60 80 100 120 140 160 180 200 230
RESIDUE #
A1
A2
MEMBRANE

FIG. 10


MHC CLASS II B CHAINS
VARIABILITY
50
40
30
20
10
0
0 20 40 60 80 100 120 140 160 180 200 220 250
RESIDUE #
B1
B2
MEMBRANE

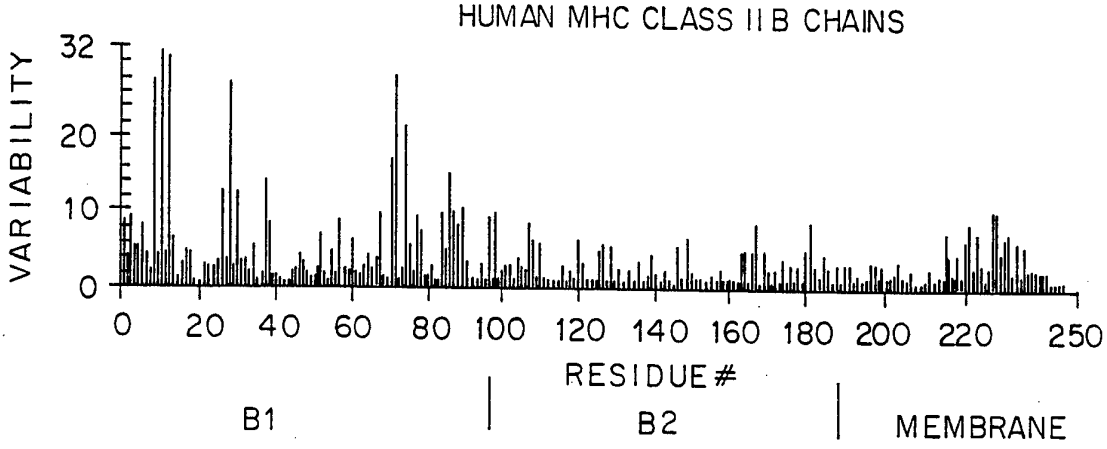

HUMAN MHC CLASS II B CHAINS
VARIABILITY
32
20
10
0
0 20 40 60 80 100 120 140 160 180 200 220 250
RESIDUE #
B1
B2
MEMBRANE

FIG. 11

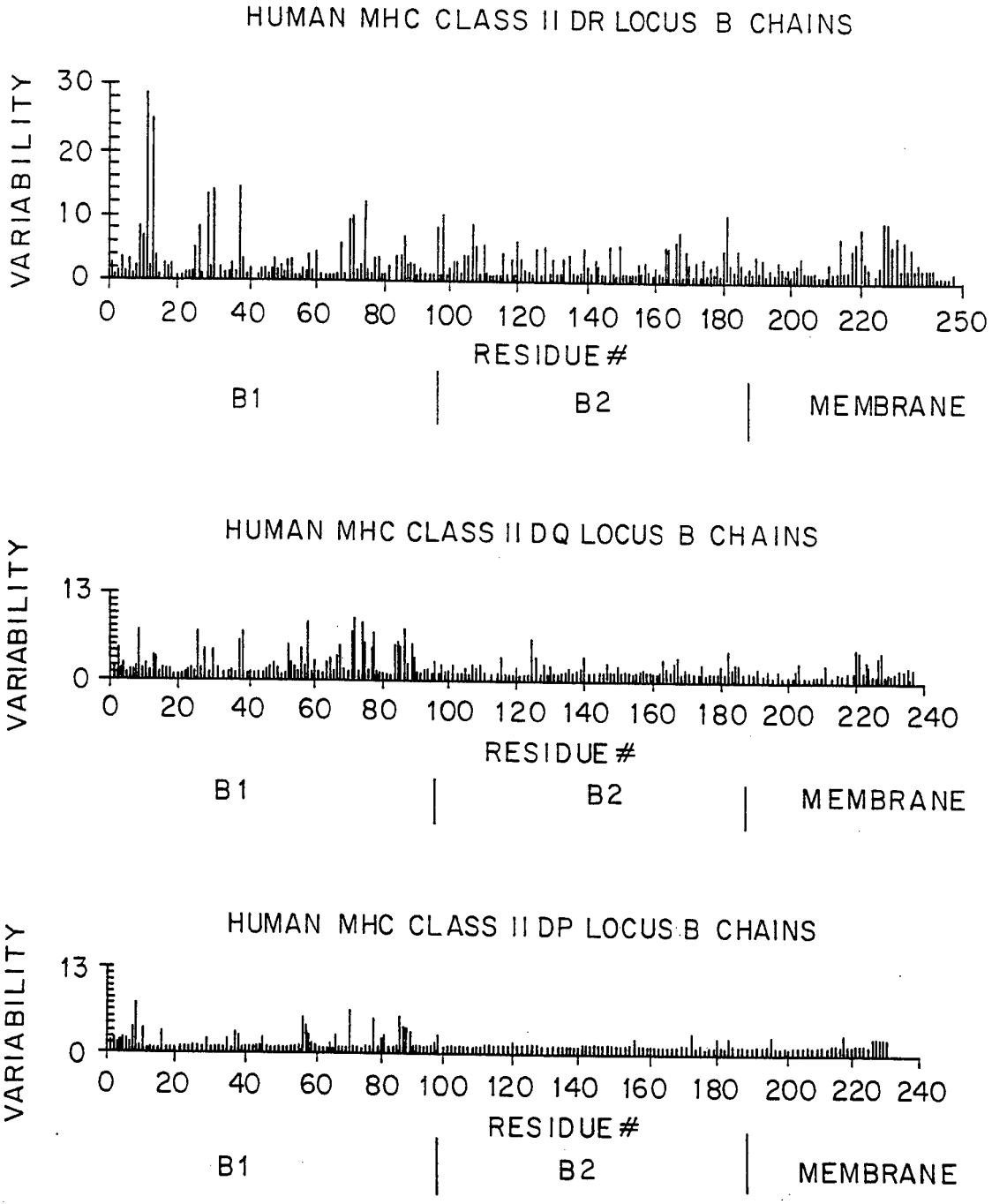

HUMAN MHC CLASS II DR LOCUS B CHAINS
VARIABILITY
30
20
10
0
0 20 40 60 80 100 120 140 160 180 200 220 250
RESIDUE#
B1
B2
MEMBRANE
HUMAN MHC CLASS II DQ LOCUS B CHAINS
VARIABILITY
13
0
0 20 40 60 80 100 120 140 160 180 200 220 240
RESIDUE#
B1
B2
MEMBRANE
HUMAN MHC CLASS II DP LOCUS B CHAINS
VARIABILITY
13
0
0 20 40 60 80 100 120 140 160 180 200 220 240
RESIDUE#
B1
B2
MEMBRANE

FIG. 12

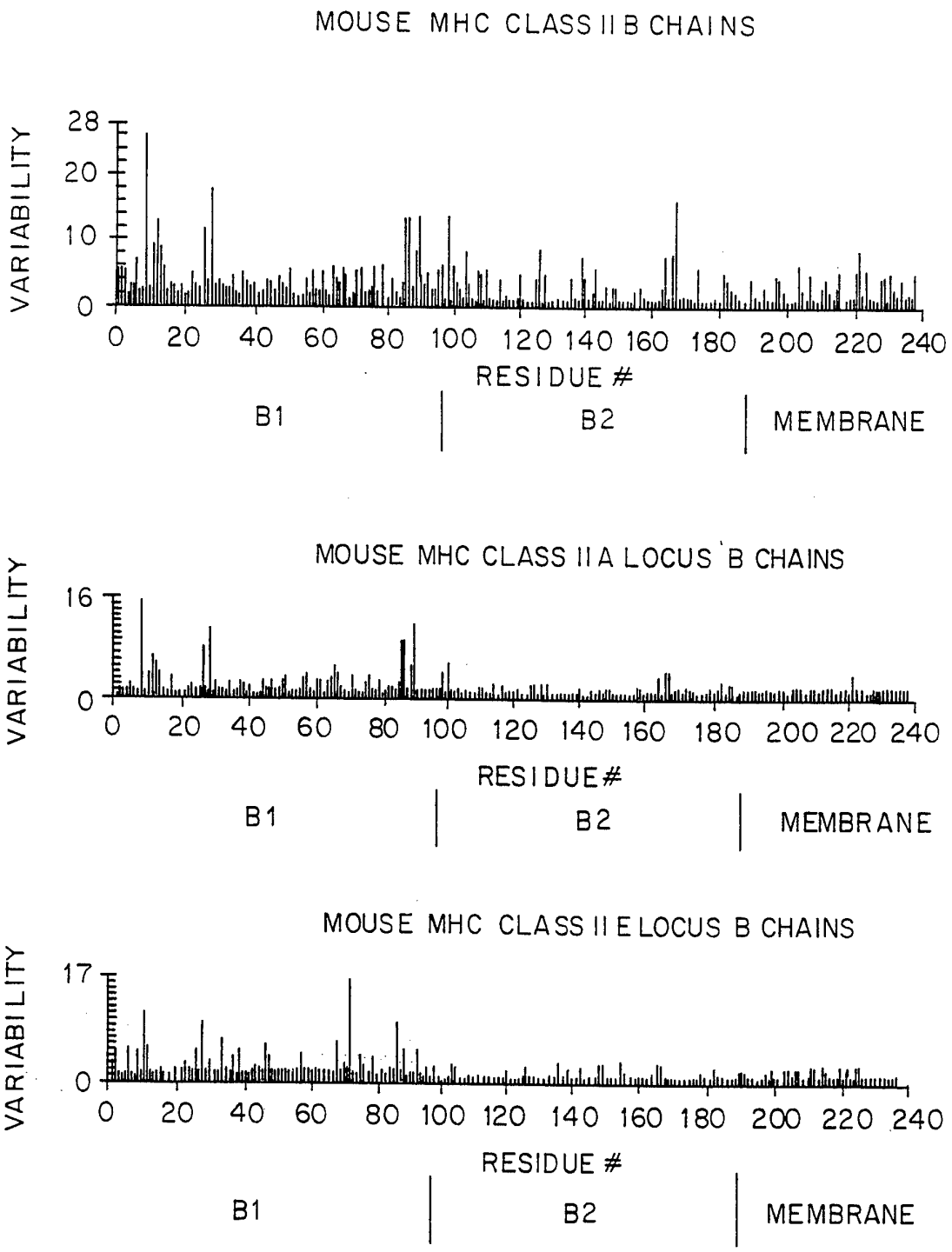

MOUSE MHC CLASS II B CHAINS
VARIABILITY
28
20
10
0
0 20 40 60 80 100 120 140 160 180 200 220 240
RESIDUE #
B1
B2
MEMBRANE
MOUSE MHC CLASS II A LOCUS B CHAINS
VARIABILITY
16
0
0 20 40 60 80 100 120 140 160 180 200 220 240
RESIDUE #
B1
B2
MEMBRANE
MOUSE MHC CLASS II E LOCUS B CHAINS
VARIABILITY
17
0
0 20 40 60 80 100 120 140 160 180 200 220 240
RESIDUE #
B1
B2
MEMBRANE

FIG. 13

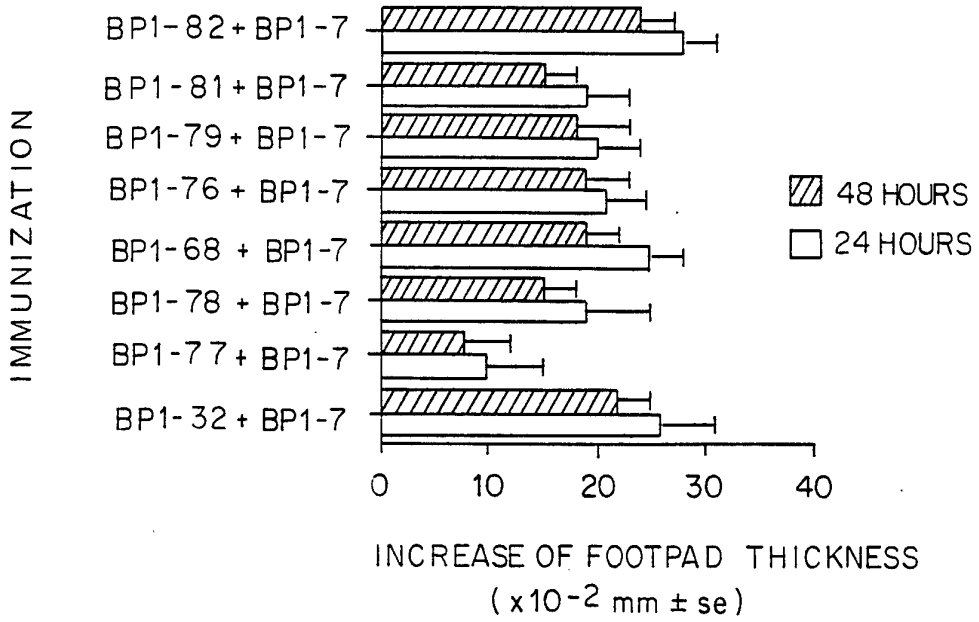
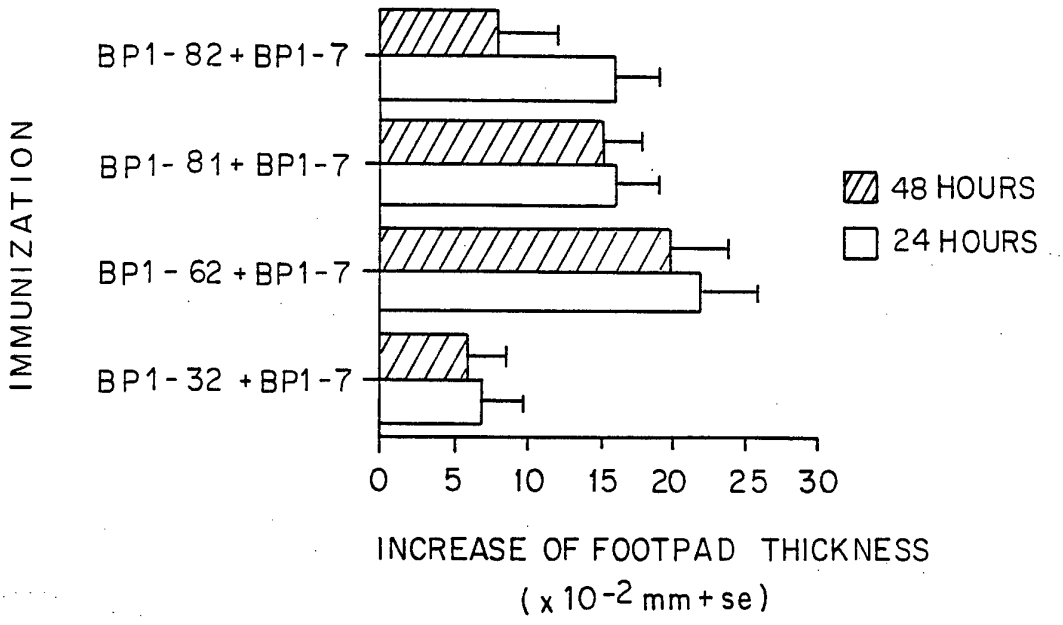

BP1-82+BP1-7
BP1-81+BP1-7
BP1-79+BP1-7
BP1-76+BP1-7
BP1-68+BP1-7
BP1-78+BP1-7
BP1-77+BP1-7
BP1-32+BP1-7
IMMUNIZATION
48 HOURS
24 HOURS
0
10
20
30
40
INCREASE OF FOOTPAD THICKNESS
(x10-2 mm ± se)

FIG. 14


BP1-82+BP1-7
BP1-81+BP1-7
BP1-62+BP1-7
BP1-32+BP1-7
IMMUNIZATION
48 HOURS
24 HOURS
0
5
10
15
20
25
30
INCREASE OF FOOTPAD THICKNESS
(x10-2 mm+se)

INTERNATIONAL SEARCH REPORT

Inter ıal Application No
PCT/US 95/04540

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K39/00 C07K14/74

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)
IPC 6 A61K C07K

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,93 09810 (ANERGEN) 27 May 1993 see the whole document ---	1-30
A	WO,A,90 05142 (IMPERIAL CANCER RESEARCH TECHNOLOGY) 17 May 1990 see the whole document ---	1-30
A	WO,A,94 04557 (PRESIDENT AND FELLOWS OF HARVARD COLLEGE) 3 March 1994 see the whole document ---	1-30
	-/--	

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

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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

"E" earlier 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 when the document is taken alone

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 June 1995	04 -07- 1995

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Moreau, J

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

INTERNATIONAL SEARCH REPORT

Interna .l Application No
PCT/US 95/04540

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CANCER LETTERS, vol. 70, no. 3, 16 July 1993 pages 143-150, DENTON G. ET AL. 'Induction of antibody responses to breast carcinoma associated mucins using synthetic peptide constructs as immunogens' see the whole document ---	1-30
A	CANCER IMMUNOLOGY IMMUNOTHERAPY , vol. 36, no. 1, 1993 pages 9-17, DING L. ET AL. 'Immunogenicity of synthetic peptides related to the core peptide sequence encoded by the human MUC1 mucin gene ...' cited in the application see the whole document ---	1-30
A	JOURNAL OF IMMUNOLOGY, vol. 137, no. 7, 1 October 1986 BALTIMORE US, pages 2311-2318, SINGH B. ET AL. 'Alloantigenic sites on class I major histocompatibility complex antigens ...' cited in the application see the whole document -----	1-30

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/04540

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

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

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

Remark : Although claims 28-30 are directed to a method of treatment of the human/animal body, the search has beem carried out and based on the alleged effects of the compound/composition.

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

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

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

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

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

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

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

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

Remark on Protest

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

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

International application No.
PCT/US95/04540

INTERNATIONAL SEARCH REPORT

Box III TEXT OF THE ABSTRACT (Continuation of item 5 of the first sheet)

A CMI-specific immunogenic composition, comprising

(a) a non-naturally occurring conjugate of a primary epitope which is substantially the same as a T cell epitope of a cancer-, microbial-, parasitic-, or virally infected cell- associated antigen, and of an immunomodulatory peptide, or

(b) a mixture of (1) primary antigen bearing a primary epitope which is substantially the same as a T-cell epitope of a cancer-, microbial-, parasitic-, or virally infected cell- associated antigen, (2) an immunomodulatory peptide,

where the conjugate of (a) and the immunomodulatory peptide of (b) have a molecular weight of less than 5,000 daltons,

where said immunomodulatory peptide of (a) or (b) comprises an allopeptide moiety of at least five amino acids whose amino acid sequence correpsonds essentially to that of a polymorphic region of an MHC-encoded polymorphic Class I or Class II antigen.

The composition modulates a stronger cellular immune response thar the humoral immune response and is useful for treatment of tumors.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interna .l Application No
PCT/US 95/04540

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9309810	27-05-93	AU-A-	3220693	15-06-93
		CA-A-	2123228	27-05-93
		JP-T-	7501526	16-02-95
WO-A-9005142	17-05-90	EP-A-	0442926	28-08-91
		JP-T-	4501719	26-03-92
WO-A-9404557	03-03-94	NONE		