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(54) **COMPLEXES D'HISTOCOMPATIBILITE ALLOGENES MEDIATEURS DE DESTRUCTION CELLULAIRE**

(54) **ALLOGENEIC HISTOCOMPATIBILITY COMPLEXES AS MEDIATORS OF CELL DESTRUCTION**

(57) Méthodes pour produire et utiliser des protéines du complexe majeur d'histocompatibilité (CMH), dans leurs formes d'origine, tronquées ou fusionnées, formant un complexe avec des peptides de liaison CMH et conjuguées à des molécules de ciblage pour cibler des cellules déterminées dans un sujet en vue de la destruction in vivo de ces dernières via les lymphocytes T. Les protéines CMH sont sélectionnées de manière à être allogènes pour l'hôte et les molécules de ciblage sont sélectionnées pour former une liaison sélective avec les cellules cibles. L'invention porte également sur des préparations pharmaceutiques contenant les complexes peptides-CMH de ciblage.

(57) Methods are disclosed of producing and using Major Histocompatibility Complex (MHC) proteins, in native, truncated of fusion forms, complexed with MHC binding peptides and conjugated to targeting molecules, in order to target selected cells in a subject for T cell mediated destruction in vivo. The MHC proteins are chosen so to be allogeneic with respect to the host and the targeting molecules are chosen to selectively bind to the target cells. Pharmaceutical preparations including the targeted peptide-MHC complexes are also disclosed.

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(57) Abstract

Methods are disclosed of producing and using Major Histocompatibility Complex (MHC) proteins, in native, truncated of fusion forms, complexed with MHC binding peptides and conjugated to targeting molecules, in order to target selected cells in a subject for T cell mediated destruction *in vivo*. The MHC proteins are chosen so to be allogeneic with respect to the host and the targeting molecules are chosen to selectively bind to the target cells. Pharmaceutical preparations including the targeted peptide-MHC complexes are also disclosed.

ALLOGENEIC HISTOCOMPATIBILITY COMPLEXES AS MEDIATORS OF CELL DESTRUCTION

Background of the Invention

Unless the donor and recipient are genetically identical, organs and cells removed from one individual and transplanted to another of the same species are almost invariably destroyed. The transplants, often termed allografts (from the Greek *"allos"* or "different") are killed by the reaction of the recipient's T lymphocytes to a special set of histocompatibility antigens on the transplanted cells. These antigens are so diverse within a species that no two individuals, unless they are identical twins, display identical sets. The T cell response to these antigens, called an alloreaction or alloaggression, is the most powerful cellular immune reaction known and, in fact, is so intense that even transplanted malignant cancer cells fail to survive unless the recipient's immune system is suppressed.

In general, the antigens recognized by antigen-specific receptors on T cells are complexes formed by short peptides with proteins encoded by a group of genes known as the major histocompatibility complex (MHC). Under normal conditions, an individual's T cells encounter peptide-MHC (pep-MHC) complexes in which the MHC component is endogenous or syngeneic ("self") to that individual. In allografts, however, although the MHC components are endogenous to the donor, they are exogenous or allogeneic ("non-self") to the recipient. Thus, the recipient's body's T cells launch an intense attack against the apparently "invading" cells bearing the allogeneic MHC antigens.

In humans, the MHC antigens are divided into two broad classes based, *inter alia*, on their structural characteristics. The MHC Class I proteins (MHC-I) are dimeric molecules in which an approximately 45 kD α chain encoded by a gene within the MHC is non-covalently associated with β_2-microglobulin, an approximately 12 kD molecule which is encoded by a gene outside of the MHC. There are many allelic variants of each α gene but only 1 or 2 for the β2-microglobulin gene. The MHC-I α chain has three globular domains and two of these, α_1 and α_2, differ in sequence amongst allelic variants and are involved in antigen specificity. The third domain (α_3) is far less variable and appears to be involved primarily in β_2-microglobulin-binding and anchoring the MHC-I protein in the cell membrane. The MHC Class II proteins (MHC-II) are also composed of two non-covalently associated chains, α and β, but both of these chains are encoded by genes within the MHC and both are anchored in the cell membrane. In Class II molecules, both the α and β chains have two globular domains at their distal (N-terminal) ends, but it is the

smaller (~28 kD) β chain which is more variable and which appears to be primarily involved in antigen specificity. Despite these apparent differences between MCH Class I and Class II molecules, analysis of the three-dimensional structures of these proteins shows a common structural motif in which two globular domains (α_1 and α_2 in Class I molecules; α_1 and β_1 in Class II molecules) form a cleft between two α-helical stretches with a β-pleated sheet forming a base (see, e.g., Bjorkman et al., 1987; Brown et al., 1988). This cleft is capable of binding small peptides: 8-10 amino acids in length for MHC-I proteins and 10-25 in length for MHC-II proteins. The peptides, which arise from self or non-self proteins that have been partially degraded or "processed," become associated with the MHC-I and MHC-II molecules as these proteins are produced in cells, and the resulting pep-MHC complexes are "presented" on the cell surface, where they may be recognized by specific T cell receptors (TCRs) and may initiate a T cell-mediated immune response.

To be effective, the immune system must be able to react against foreign (non-self) peptides presented in pep-MHC complexes while tolerating self peptides which are also presented in pep-MHC complexes. To produce this tolerance, it is believed that, during development of T cells, all of the TCR molecules on the immature T cells of a given individual are imprinted, as it were, with the capacity to recognize that individual's own self or syngeneic MHC ("synMHC") in association with processed self peptides in pep-synMHC complexes. The basis for this imprinting is emerging from studies of T cell development (reviewed by, e.g., von Boehmer, 1994). In the thymus, immature double positive (CD4+CD8+) thymocytes are stimulated to mature into single positive (CD4+ or CD8+) T cells through "weak" interactions of their TCRs with pep-synMHC complexes on thymic cells (positive selection), whereas double positive cells that interact "strongly" with these complexes on thymic cells undergo programmed cell death or apoptosis (negative selection). Although the processed self peptides involved in these reactions have not been identified, and the kinetics, affinity, and specificity of the reactions in the thymus are all unknown, the MHC proteins involved in both positive and negative selection are necessarily synMHC.

Interestingly, the very same mature T cells that have been selected for relatively weak interaction with pep-synMHC complexes can react specifically and strongly with different (non-self) MHC proteins from other individuals of the same species (allogeneic MHC or "alloMHC"). These reactions, like those restricted by synMHC, also involve pep-MHC complexes (Rötzschke et al., 1991b; Udaka et al., 1992, 1993). There is also some evidence that TCR affinities for pep-alloMHC complexes are generally higher than for pep-synMHC complexes (Sykulev et al., 1994) because there is higher TCR affinity for the alloMHC components than for synMHC components.

Immunologists have long been intrigued by the possibility that immune responses might be engaged to destroy cancer cells. The various strategies proposed include (i) tumor homogenates as vaccines; (ii) polyclonal or monoclonal antibodies to tumor associated antigens (TAA) with or without conjugation to cytotoxic components, and (iii) tumor cells engineered to express cytokines that stimulate enhanced immune responses to tumor cell antigens. For example, antibodies to TAAs have been linked to chemotherapeutic drugs and to plant and bacterial toxins (see, e.g., Vitetta et al., 1987; Pastan et al., 1986). Anti-TAA antibodies have also been linked to other antibodies which bind to T cell receptors and serve as effectors to stimulate T cell cytotoxicity (Liu et al., 1985). In an alternative approach, an analog of the melanocyte-stimulating hormone has been conjugated to an anti-CD3 antibody to target cytotoxic T cells regardless of their specificity to human melanoma cells (Liu et al., 1988).

The use of alloMHC antigens also has been contemplated as a means of stimulating an immunological response against targeted cells. Because pep-MHC complexes are normally formed within cells and are then transported to the plasma membrane where they may be recognized by T cell receptors, attempts to use alloMHC proteins to stimulate an immunological response have focused on the introduction of an alloMHC gene within the target cell. Thus, according to these methods, a recombinant DNA construct encoding at least one alloMHC chain is introduced within the targeted cells. For example, a class I alloMHC gene may cause the production of the corresponding alloMHC chain, which is then complexed within the cell with β2-microglobulin and a self or non-self peptide to form a pep-alloMHC complex. This complex is then transported to the cell surface where it may be recognized as foreign by a host T cell receptor and initiate an alloaggressive immune response.

Thus, Plautz et al. (1993) introduced a foreign MHC gene by direct injection into tumors in mice. These authors used DNA-liposome complexes to deliver the alloMHC gene into mice which had previously been injected with a transplantable CT26 mouse colon adenocarcinoma or MCA 106 fibrosarcoma. It was shown that the alloMHC gene induced a cytotoxic T cell response, attenuated tumor growth and, in some cases, caused complete tumor regression.

In a human clinical study, Nabel et al. (1993) used a similar approach in five patients suffering from melanoma. In these trials, a gene encoding the HLA-B7 MHC Class I protein was introduced by direct injection or by pulmonary catheterization into HLA-B7-negative patients. Again, DNA-liposome complexes were used to mediate the gene transfer. Expression of the alloMHC was shown by PCR or immunohistochemistry in 1-10% of the tumor cells near the site of injection in all five patients. In at least one patient, regression of tumor nodules and metastatic lesions were observed.

In a different approach, Australian Patent AU-B-39005/89 discloses the conjugation of carrier molecules (e.g., an antibody) to the N- or C-terminus of an allogeneic MHC Class I α chain in order to target cytotoxic T cell activity toward particular cells. Although this disclosure broadly uses the phrase "MHC antigen," the teaching is clearly directed to the use only of a conjugated MHC Class I α chain. No results are reported regarding the ability of this construct to initiate an alloaggressive response either in vivo or in vitro.

Summary of the Invention

The present invention relates to the in vivo targeted conversion of selected cells into "pseudoallografts" by exogenously administering ternary pep-MHC complexes in which the MHC protein is allogeneic to the subject. By conjugating a suitable targeting molecule to such a pep-alloMHC complex, the complex can be made to bind selectively to the targeted cells, converting them into targets for alloreactive T cells, including alloreactive cytotoxic T lymphocytes (CTL). This process is referred to herein as "alloconversion." It is believed that the present disclosure reports the first instance in which exogenously supplied pep-alloMHC complexes have been successfully targeted to selected cells and shown to elicit target cell specific cytotoxicity.

Thus, it is one object of the present invention to provide pharmaceutical preparations for use in targeting an alloreactive response against selected cells, and comprising a pep-MHC complex, including an MHC heavy chain, an MHC light chain, and an MHC binding peptide; in which at least one of the MHC chains is allogeneic with respect to the diploid MHC genotype of the target cell type, and which is conjugated to at least one targeting molecule capable of selectively binding to the target cell type. In addition, it is preferred that such pharmaceutical preparations include at least one other pep-MHC complex in which at least one MHC chain of the second pep-MHC complex differs from both MHC chains of the first pep-MHC complex. In particular, it is preferred that mixtures of three or more pep-MHC complexes differing in their MHC components be employed, as this will guarantee that at least one MHC protein is allogeneic with respect to any subject. Furthermore, it is preferred that rare MHC components be employed to increase the likelihood of alloantigenicity to any given subject. Similarly, it is preferred that such pharmaceutical preparations include at least one other pep-MHC complex in which the MHC binding peptide of the second pep-MHC complex differs from the MHC binding peptide of the first pep-MHC complex. In particular, it is preferred that mixtures of a number of different, high affinity MHC binding peptides be employed to increase the likely number of T cell clones involved in the alloantigenic response.

In another series of embodiments, the pep-MHC complexes, as described above, may include a flexible molecular linker covalently joining the heavy chain and light chain, the heavy chain and binding peptide, and/or the light chain and binding peptide. Such covalently bound pep-MHC complexes have greater stability, can be produced with only a single MHC binding peptide, and can be conveniently produced recombinantly if the molecular linkers are peptides.

In each of the above-described embodiments, the targeting molecules of the targeted pep-MHC complexes may be antibodies (including single chain antibodies and antibody fragments), hormones, growth factors, or non-hormone, non-growth factor cell receptor ligands. In one preferred embodiment, the targeting molecule is folic acid. In addition, in preferred embodiments, the targeting molecule is covalently bound to the pep-MHC complex and, in one preferred embodiment, the targeting molecule is covalently bound to the pep-MHC complex through a carbodiimide linkage.

The cells to be targeted for destruction by the targeted pep-MHC complexes of the invention may be of any cell type. It is particularly contemplated, however, that the targeted cells are cancer cells and, in one preferred embodiment, that the target cells are ovarian cancer cells.

Thus, it is another object of the invention to provide methods of treating a patient to elicit a cytotoxic T cell response against a population of target cells within the patient by administering to the patient a pharmaceutical preparation of the targeted pep-MHC complexes of the invention. Preferably, such administration is parenteral. In these methods, it is also preferred that the subject is immunized against an alloMHC protein of the pep-MHC complexes prior to administering the targeted pep-MHC complexes of the invention. In particular, it is preferred that this immunization is by administering to the patient whole cells expressing the alloMHC protein. In addition, it is preferred that this immunization is performed between about 1 and about 20 days prior to administering the targeted pep-MHC complex preparation.

In all of the above-described embodiments, the pep-MHC complexes may be MHC Class I complexes including an MHC Class I α chain, a β_2-microglobulin chain, and an MHC Class I binding peptide, or MHC Class II complexes including an MHC Class II α chain, an MHC Class II β chain, and an MHC Class II binding peptide. In addition, in all embodiments described above, the pep-MHC complexes may include MHC protein components which are truncated

proteins lacking transmembrane and/or cytoplasmic domains, or may be fusion proteins including desired antigenic determinants, polypeptide tags, or linkers. Finally, in all embodiments described above, the MHC components may be artificial or man-made MHC components created by site-directed mutagenesis or other techniques so as to differ from all known MHC proteins and, therefore, serve as "universal" alloantigens.

Detailed Description of the Invention

Definitions. In order to more clearly and concisely describe and point out the subject matter of the claimed invention, the following definitions are provided for specific terms which are used in the following description and the claims appended hereto.

As used herein, the term "Major Histocompatibility Complex" and the abbreviation "MHC" mean the complex of genes, found in all vertebrates, which encode the proteins which are primarily responsible for the rapid rejection of allografts and which function in signaling between lymphocytes and antigen presenting cells in normal immune responses. The human MHC region, also referred to as HLA, is found on chromosome six and includes the Class I region (including the Class I α chain genes HLA-A, HLA-B, and HLA-C) and the Class II region (including the subregions for Class II α and β chain genes DP, DQ and DR).

As used herein, the term "MHC protein" means an MHC Class I protein and/or an MHC Class II protein.

As used herein, the term "MHC Class I protein" or "Class I protein" means a covalently or non-covalently joined complex of an MHC Class I α chain and a β_2-microglobulin chain.

As used herein, the term "MHC class I α chain" means a naturally occurring polypeptide, or one encoded by an artificially mutated α gene, corresponding to at least the α_1 and α_2 domains of one of the gene products of an HLA-A, HLA-B or HLA-C gene. As the α_3 domain does not include the primary alloantigenic sites, and as the C-terminal transmembrane and cytoplasmic portions of the α chain are not necessary for membrane binding in the present invention, they may be omitted while maintaining alloantigenicity. Preferably, however, the α_3 domain is included as it aids in stabilizing an MHC Class I protein by interacting with β_2-microglobulin. In addition, the term "MHC Class I α chain" is intended to include variants with and without the usual glycosylation of the α_2 domain. The term is particularly intended to embrace all allelic variants of the Class I α genes, as well as any equivalents, including those which may be produced synthetically or recombinantly by, for example, site-directed mutagenesis of a naturally occurring

variant. An MHC Class I α chain may also be referred to herein as an "MHC Class I heavy chain."

As used herein, the term "β_2-microglobulin" means a naturally occurring polypeptide, or one encoded by an artificially mutated β_2-microglobulin gene, corresponding to the gene product of a β_2-microglobulin gene. The term is particularly intended to embrace all allelic variants of β_2-microglobulin, as well as any equivalents, including those which may be produced synthetically or recombinantly by, for example, site-directed mutagenesis of a naturally occurring variant. When the term "MHC β chain" is used without specifying whether the chain is Class I or Class II, the term is intended to include β_2-microglobulin as well as MHC Class II β chains. A β_2-microglobulin or MHC Class I β chain may also be referred to herein as an "MHC Class I light chain."

As used herein, the term "MHC Class II protein" or "Class II protein" means a covalently or non-covalently joined complex of an MHC Class II α chain and an MHC Class II β chain.

As used herein, the term "MHC Class II α chain" means a naturally occurring polypeptide, or one encoded by an artificially mutated α gene, corresponding to at least the α_1 domain of one of the gene products of an MHC Class II α gene (e.g., a DP, DQ or DR α gene). As the α_2 domain does not include the primary alloantigenic sites, and as the C-terminal transmembrane and cytoplasmic portions of the α chain are not necessary for membrane binding in the present invention, they may be omitted while maintaining alloantigenicity. Preferably, however, the α_2 domain is included as it aids in stabilizing an MHC Class II protein by interacting with the β_2 domain of the β chain. In addition, the term "MHC Class II α chain" is intended to include variants with and without the usual glycosylation of the α_1 and α_2 domains. The term is particularly intended to embrace all allelic variants of the Class II α genes, as well as any equivalents which may be produced synthetically or recombinantly by, for example, site-directed mutagenesis of a naturally occurring variant. An MHC Class II α chain may also be referred to herein as an "MHC Class II heavy chain."

As used herein, the term "MHC Class II β chain" means a naturally occurring polypeptide, or one encoded by an artificially mutated β gene, corresponding to at least the β_1 domain of one of the gene products of an MHC Class II β gene (e.g., DP, DQ or DR β gene). As the β_2 domain does not include the primary alloantigenic sites, and as the C-terminal transmembrane and cytoplasmic portions of the β chain are not necessary for membrane binding in the present invention, they may be omitted while maintaining alloantigenicity. Preferably, however, the β_2 domain is included as it aids in stabilizing an MHC Class II protein by interacting with the α_2 domain of the α chain. In addition, the term "MHC Class II β chain" is intended to include variants with and without the usual glycosylation of the β_1 domain. The term is particularly

intended to embrace all allelic variants of the Class II β genes, as well as any equivalents which may be produced synthetically or recombinantly by, for example, site-directed mutagenesis of a naturally occurring variant. An MHC Class II β chain may also be referred to herein as an "MHC Class II light chain."

As used herein, the term "MHC binding peptide" or "binding peptide" means an MHC Class I binding peptide and/or an MHC Class II binding peptide.

As used herein, the term "MHC Class I binding peptide" means a polypeptide which is capable of selectively binding within the binding cleft formed by a specified MHC Class I protein to form a ternary pep-MHC Class I antigen complex. An MHC Class I binding peptide may be a processed self or non-self peptide or may be a synthetic peptide. For Class I MHC antigens, the peptides are typically 8-10 amino acids in length, although they may be as long 16 or as short as 2 (Udaka et al., 1993; Vturina et al., in preparation)

As used herein, the term "MHC Class II binding peptide" means a polypeptide which is capable of selectively binding within the binding cleft formed by a specified MHC Class II protein to form a ternary pep-MHC Class II antigen complex. An MHC Class II binding peptide may be a processed self or non-self peptide or may be a synthetic peptide. For Class II MHC antigens, the peptides are typically 10-25 amino acids in length, although longer and shorter ones may be effective.

As used herein, the term "pep-MHC complex" means a covalently or non-covalently joined ternary complex of either (a) an MHC Class I protein and an MHC Class I binding peptide which binds to that MHC Class I protein or (b) an MHC Class II protein and an MHC Class II binding peptide which binds to that MHC Class II protein. Similarly, the term "pep-alloMHC complex" means a pep-MHC complex in which the MHC component is allogeneic to a reference genotype (e.g., the genotype of a subject into which it is introduced). A pep-MHC complex may also be referred to as an "MHC antigen."

As used herein, the term "flexible molecular linker" or "molecular linker" means a chemical moiety having a length equal to or greater than that of three carbon-carbon bonds and including at least one freely rotating bond along said length. Preferably, a flexible molecular linker is comprised of one or more amino acid residues but this need not be the case. When comprised of amino acids, a flexible molecular preferably includes at least three or, more preferably, at least five amino acid residues.

As used herein, the term "targeting molecule" means a molecule or chemical moiety which is capable of selectively binding to a portion of the extracellular domain of a cell-surface molecule, moiety or other determinant which is characteristic of, or positively correlated with, a selected cell

type. The cell-surface molecule, moiety or other determinant to which a targeting molecule selectively binds is referred to herein as a "cell-surface target" and, typically, will comprise the extracellular domain of an integral membrane protein or glycoprotein. A targeting molecule may be non-covalently or, preferably, covalently bound to a pep-MHC complex of the invention. Because it may selectively bind to a cell-surface target of a selected or targeted cell type, a targeting molecule may selectively associate or "deliver" the pep-MHC complexes of the invention to the targeted cell type. Targeting molecules include, but are not limited to, antibodies, hormones, growth factors and other cell receptor ligands, as well as fragments of the same which retain selective binding ability.

As used herein, the term "selectively binding" means capable of binding in the electro- and stereospecific manner of an antibody to antigen, or ligand to receptor. With respect to an MHC binding peptide, selective binding entails the non-covalent binding of specific side chains of the peptide within the binding pockets formed in the MHC protein binding cleft in order to form a pep-MHC complex (see, e.g., Brown et al., 1993; Stern et al., 1994). With respect to a targeting molecule, selective binding entails binding to one or more specific cell-surface targets so as to substantially distinguish cell types bearing those targets from cell types without the targets. A targeting molecule should have an intrinsic equilibrium association constant (K_A) for its cell-surface target preferably no lower than about 10^5 M^{-1} and higher values are preferred. More preferred K_A values are greater than 10^6 M^{-1} and, most preferably, greater than 10^8 M^{-1}.

As used herein, the term "substantially pure" means, with respect to the MHC proteins, MHC binding peptides and ternary pep-MHC complexes of the invention, that these proteins or polypeptides are essentially free of other substances to an extent practical and appropriate for their intended use. In particular, the proteins and polypeptides are sufficiently pure and are sufficiently free from other biological constituents of their hosts cells so as to be useful in, for example, generating antibodies or producing pharmaceutical preparations. A substantially pure preparation of the proteins or polypeptides of the invention need not be absolutely free of all other proteins or cell components and, for purposes of administration, may be relatively dilute. One of ordinary skill in the art may produce such substantially pure preparations by application, or serial application, of well-known methods including, but not limited to, HPLC, affinity chromatography and electrophoretic separation.

Alloconversion by Exogenously Administered pep-alloMHC Complexes

The present invention relates to the in vivo targeted conversion of selected cells into "pseudoallografts" by exogenously administering to them ternary pep-MHC complexes in which the MHC protein is allogeneic to the subject. By conjugating a suitable targeting molecule to such a pep-alloMHC complex, the complex can be made to bind selectively to the targeted cells,

converting them into targets for alloreactive T cells, including alloreactive cytotoxic T lymphocytes (CTL). This process is referred to as "alloconversion." It is believed that the present disclosure reports the first instance in which exogenously supplied pep-alloMHC complexes have been successfully targeted to selected cells and shown to elicit target cell specific cytotoxicity.

The success of the present invention is surprising in that the pep-alloMHC complexes are not produced within the target cells and are not presented in the normal manner of pep-MHC complexes. Rather, the pep-alloMHC complexes of the invention are directed to the target cell surfaces by conjugation with targeting molecules which have a high affinity for and selectively bind to the target cells. Thus, the pep-alloMHC complexes of the present invention are not embedded in the cell membrane by the normal transmembrane domains of the MHC proteins but, rather, are non-covalently bound to the cells by the interaction of the targeting molecule with its cell-surface target. Surprisingly, despite the unnatural geometry resulting from the interposition of both the targeting molecule and the cell-surface target between the pep-alloMHC complex and the cell membrane, the pep-alloMHC complexes of the present invention are competent to elicit target cell specific T cell cytotoxicity.

The present invention provides several advantages over prior art methods of selectively destroying targeted cells and, in particular, over prior art methods of cancer therapy. (i) As noted above, T cell responses to alloantigens are the most intense cellular immune responses known. They are, for example, the only ones that can routinely be elicited in vitro with peripheral blood mononuclear cells (PBMC) from an immunologically "naive" individual (i.e. one who has not previously encountered the alloantigen). To elicit a response to other antigens in vitro with PBMC, it is necessary to have just immunized or "primed" the PBMC donor by immunization or infection with the antigen. (ii) Attachment of the alloantigen to a targeting molecule can localize the destructive immune response to the targeted tissue and, therefore, is preferable to techniques such as radiation or chemotherapy. (iii) The enormous polymorphism of the MHC genes makes available many different and rare MHC proteins, and site-directed mutagenesis allows the generation of entirely novel Class I and Class II MHC molecules, "universal alloantigens," which would be allogeneic for any patient. Hence, many different patients can be treated with the same MHC preparation, and the same patient can be treated with different alloMHC antigens in succession. (iv) Because the pep-alloMHC complexes may be made in large quantities and administered exogenously to a great many different patients, there is no need for gene transfer into the cells of individual patients, with the high attendant costs and low rates of successful transfection or transformation. (v) The pep-alloMHC complexes of the invention may be administered locally or systemically and, therefore, may be effective against distant metastases and small, undetected satellite tumors.

Choice of MHC Proteins

The pep-alloMHC complexes of the present invention comprise a ternary complex of an MHC heavy chain, an MHC light chain, and an MHC binding peptide which selectively binds to the MHC binding domain formed by the heavy and light chains. Thus, a Class I pep-alloMHC complex of the present invention comprises a complex of an MHC Class I α (heavy) chain, an MHC Class I light chain (i.e., β_2-microglobulin), and an MHC Class I binding peptide which selectively binds to the MHC Class I binding domain formed by the particular heavy and light chains. Similarly, a Class II pep-alloMHC complex of the present invention comprises a complex of an MHC Class II α (heavy) chain, an MHC Class II β (light) chain, and an MHC Class II binding peptide which selectively binds to the MHC Class II binding domain formed by the particular heavy and light chains.

Because the MHC Class I and Class II genes are amongst the most polymorphic in the human genome, there are many choices available for the particular MHC components which may be used in the present invention. Some MHC alleles, like HLA-A2, are very common (present in around 50% of Caucasians); others, like HLA-X are extremely rare (present in < 1% of Caucasians). To be effective in any given patient, the MHC component of a pep-MHC complex of the present invention must be allogeneic with respect to that patient. That is, it must differ from both of the corresponding MHC chains encoded by the patient's diploid genotype. With respect to MHC Class I antigens, the light chain is a β_2-microglobulin chain which is virtually invariant in the human population and does not appear to be involved in alloantigenicity. Therefore, in preferred embodiments, a Class I alloMHC antigen of the present invention includes a Class I heavy (α) chain which differs from both Class I heavy chains encoded by the patient's diploid genotype. Conversely, with respect to MHC Class II antigens, the light (β) chain is believed to contain the primary alloantigenic sites and, therefore, in preferred embodiments, a Class II alloMHC antigen of the present invention includes a Class II light (β) chain which differs from both Class II β chains encoded by the patient's diploid genotype.

The choice of an appropriate alloMHC protein is well within the discretion and knowledge of one of ordinary skill in the art. In some embodiments, the patient in question may be MHC-genotyped and any MHC chains which are allogeneic relative to that patient may be employed. Alternatively, the patient may be genotyped with respect to other loci which are in linkage disequilibrium with particular MHC alleles, or may be characterized by "race" or other population genetic criteria which will aid in the selection of alloMHC proteins. For commercial purposes, and large scale manufacture of the pep-MHC complexes of the invention, it is preferred that relatively rare MHC allelic variants be produced because such complexes will have utility in a large proportion of patients. Furthermore, it is contemplated that a mixture of pep-MHC complexes, including two or more MHC allelic variants, may be produced in commercial

quantities because such mixtures would have utility in an even larger proportion of the population. Indeed, a mixture of two rare (e.g., < 2%) pep-MHC complexes should be effective in virtually all patients. Furthermore, irrespective of whether the MHC chains represent rare allelic variants, a mixture of three or more pep-MHC complexes including three or more MHC allelic variants would, by definition, include at least one pep-MHC complex including an MHC chain which is allogeneic to any diploid genotype.

Tables 1 and 2 present a partial listing of the many recognized subtypes of the human MHC Class I and Class II genes. The tables show that numerous Class I and Class II allelic variants can be found with allele frequencies less than 5% in the relevant populations. Each such allelic variant will be effective in treating more than 95% of the relevant population. Assuming Hardy-Weinberg equilibrium, a mixture of two such variants, when incorporated into the pep-MHC complexes of the present invention, will be useful in treating more than 99.75% of the relevant population. For a given patient, of course, an appropriate pep-alloMHC can be chosen based upon the patient's genotype. And for any given patient, as noted above, any mixture of three allelic pep-MHC complexes will include at least one pep-alloMHC complex.

In addition, non-naturally occurring pep-MHC complexes may be employed in the present invention. That is, variants of natural MHC Class I or Class II chains may be produced synthetically or recombinantly by, for example, site-directed mutagenesis techniques. Such variants may, in particular, include substituted, inserted or deleted amino acid residues at locations corresponding to the alloantigenic sites of the α_1 and α_2 domains of an MHC Class I α chain or the alloantigenic sites of the corresponding domains of an MHC Class II β chain. Such sites include, for example, the known polymorphic sites of these proteins which distinguish their alleles (see, e.g., Marsh and Bodmer, 1995). Substitutions, insertions or deletions at other sites may, of course, also lead to novel alloantigens and are also contemplated. Production of recombinant genes encoding such non-naturally occurring MHC chains is well within the ability of one of ordinary skill in the art using such standard techniques as site-directed mutagenesis, and the recombinant MHC proteins and pep-MHC complexes may then be produced as described below and elsewhere. The antigenicity of such synthetic pep-alloMHC complexes can easily be ascertained by, for example, testing their ability to elicit a response in vitro from PBMC in a mixed lymphocyte reaction.

Finally, it should be noted that Class I MHC antigens restrict antigen recognition by CD8+ T cells and, therefore, may be more strongly indicated in cytotoxic T cell immune responses than are Class II MHC antigens, which restrict antigen recognition by CD4+ T cells. Therefore, alloconversion by Class I pep-alloMHC complexes should directly elicit CD8+ T cell mediated cytotoxicity. However, alloresponses to Class II MHC alloantigens are exceptionally intense because of the inflammation they evoke and, therefore, alloconversion by Class II pep-alloMHC

complexes is also contemplated. Naturally, a mixture of Class I and Class II pep-alloMHC complexes may be preferred.

Choice of MHC Binding Peptides

In the past few years an enormous wealth of information has been revealed about the structure of pep-MHC complexes and, in particular, the binding of peptides to MHC antigens. Large numbers of peptides which bind to particular MHC proteins have been identified by various means including, for example, acid elution of the peptides bound to isolated pep-MHC complexes (see, e.g., Buus et al., 1988; Rötzschke et al., 1990a), whole cell binding assays (Christinck et al., 1991), MHC immunoprecipitation from cell lysates (Cerundolo et al., 1991), or in vitro binding to "empty" MHC proteins purified from transfected or transformed cells (Matsumura et al., 1992; Saito et al., 1993; Boyd et al., 1992; Ojcius et al., 1993; Fahnestock et al., 1994), followed by amino acid sequencing of the resulting peptide mix. In addition, a number of amino acid sequence "motifs" have been elucidated which characterize the subset of peptides which can selectively bind to particular MHC proteins. The subset associated with any given MHC protein typically shares the same amino acids at 2 "anchor" positions that bind within "pockets" in the MHC binding site (see, e.g., Rötzschke et al., 1990b; van Bleek and Nathenson, 1990; Falk et al., 1991; Rötzschke et al., 1991a; Pamer et al., 1991; Tsomides et al., 1994, Rammensee et al., 1995). As a result of these studies, many MHC antigen binding peptides appropriate for use with particular MHC proteins in the present invention are already well known to those of ordinary skill in the art. Similarly, many classes of peptides, defined by binding peptide motifs and which, therefore, have a reasonable likelihood of being suitable for use with particular MHC proteins, are already well known in the art. In addition, and most importantly, much has recently been revealed about the ability of many different sequence variants of peptides to elicit an alloreactive T cell response, providing of course that the variant peptides bind to the MHC protein and stabilize it. Thus, while even responses to syngeneic pep-MHC complexes allow much variation in the binding peptide sequence, in alloresponses the cross reactions are much greater. This probably is a consequence of T cell receptors' tending to have higher affinity for alloMHC than for synMHC components. That is, in the ternary pep-MHC complex recognized by TCRs, the peptides appear to contribute relatively less and the alloMHC components relatively more in an allogeneic response, whereas the peptides appear to contribute relatively more and the synMHC components relatively less in a syngeneic response. As a result, one is not greatly restricted by the sequence of the peptide selected and, therefore, relatively small libraries of peptides, restricted only by the few (typically 2) residues that constitute the anchors for any given MHC, can be used to form the ternary pep-alloMHC complexes of the invention.

For Class I or Class II MHC proteins for which binding peptides or binding motifs have not yet been characterized, standard techniques are available which will permit the identification

of appropriate binding peptides without undue experimentation. As a general matter, natural MHC binding peptides may be identified for any particular MHC protein by one of the techniques referenced above, or by any substantially equivalent techniques. Alternatively, a number of candidate peptides (e.g., a "library" of randomly generated peptides, or peptides conforming to an appropriate sequence motif) may be screened by, for example, subjecting a preparation of purified MHC antigen to denaturing conditions, mixing the denatured MHC antigens with a sample of candidate peptides, and subjecting the mixture to renaturing conditions (see, e.g., Garboczi et al., 1992 for appropriate conditions). Pep-MHC complexes that are stable at 37°C may then be isolated from this mixture (e.g., by FPLC or gel filtration), and the peptides binding in the MHC antigens may be identified by HPLC and standard sequence analysis. This method has the particular advantage that peptides which form the strongest or most stable pep-MHC complexes should be disproportionately represented in the renatured pep-MHC complexes.

Again, it should be noted that a given MHC protein can bind and present thousands of different peptides to T cells, and the various MHC proteins bind different (but overlapping) sets of peptides. Accordingly, the alloMHC proteins of the invention are preferably administered in association with a mixture or library of peptides which selectively bind that protein. The diversity of the library should be great enough to engage a large proportion of the alloreactive T cells that can recognize the given alloMHC, but not so large that each individual peptide is present at a concentration too low to be effective. At present, it is contemplated that a library of approximately 100 peptides per alloMHC may be most effective, although larger and smaller libraries (or even single peptides) may be employed.

Targeting Molecules

The pep-alloMHC complexes of the present invention may be conjugated, covalently or non-covalently, to a variety of targeting molecules which will "direct" the alloantigens to selected or targeted cells by selectively binding to an extracellular domain of at least one molecule, moiety or other determinant present on the surface of the target cells. The choice of targeting molecule depends, in part, upon the nature of the target cells and the potential cell-surface targets on those cells. Preferably, the targeting molecules are chosen to selectively bind to a cell-surface target which is characteristic of, or positively correlated with, the targeted cell type. Thus, the cell-surface target may be a molecule, moiety or other determinant which is selectively expressed by the target cells, which selectively binds to the target cells, or which is otherwise positively correlated with the target cells, and which, at least statistically, identifies or substantially distinguishes the target cells from other cells in the area of administration. Preferably, a targeting molecule of the invention should have an intrinsic equilibrium constant K_A for its cell-surface target no lower than $10^5 M^{-1}$ and higher values are more preferred. More preferred K_A values are greater than $10^6 M^{-1}$ and, most preferably, greater than $10^8 M^{-1}$. Significantly, oligomerization of

the targeting molecule or a multiplicity of targeting molecules bound to each pep-alloMHC complex may increase the effective binding constant (or "avidity") per targeting molecule.

Targeting molecules useful in the present invention include, but are not limited to, antibodies, hormones, growth factors and other cell receptor ligands, as well as fragments of the same which retain selective binding ability. Antibodies, including active antibody fragments such as Fab or $F(ab')_2$ fragments, and including single chain antibodies such as scFab antibodies, have already been developed which will detect or selectively bind to antigens characteristic of, or associated with, particular target cells. Such antibodies, or antibody fragments, as well as others yet to be developed, may be used as targeting molecules in accordance with the present invention. Antibody fragments, because of their reduced size, are less likely to impede penetration into tissues, or to create steric hindrance to T cell recognition and, therefore, are preferred. Examples of suitable antibodies, or antibody fragments, include antibodies specific for ganglioside GD2 (characteristic of melanoma cells), the epidermal growth factor receptor (characteristic of neuroblastoma cells) or any other tumor associated antigen (TAA). Hormones (or their analogs) may also be used as targeting molecules of the present invention in order to cause alloconversion of cells bearing the apposite hormone receptors. For example, the melanocyte stimulating peptide hormone (MSH) and MSH-receptor (characteristic of melanoma cells), androgen and the androgen receptor (characteristic of prostatic cancer cells), luteinizing hormone (LH) and the LH receptor (characteristic of ovarian cancer cells), and follicle stimulating hormone (FSH) and the FSH receptor (characteristic of ovarian cancer cells). In addition, other non-hormone, non-growth factor cell receptor ligands (or their analogs) may be employed as targeting molecules (e.g., folate, methotrexate).

As an example, folic acid or folate may be used as a targeting molecule for a variety of cell types which express one or both isoforms (FR-α and FR-β) of the high affinity folate receptor on their surfaces. Specifically, high affinity receptors for folic acid are present on 80% of human ovarian cancers (Miotti et al., 1987; Weitman et al, 1992); uterine carcinomas (Ross et al., 1994); testicular choriocarcinomas (Ross et al., 1994); renal cell carcinomas (Weitman et al., 1992); colon cancers, breast cancers, lymphomas (including Non-Hodgkin's and Non-Burkitt's), fibrous histiocytomas, osteosarcomas, and Wilms' tumors (Ross et al., 1994); and several types of brain tumors including ependymomas, meningiomas, giant cell astrocytomas, juvenile pilocytic astrocytomas, and high grade CNS sarcomas (Weitman et al., 1992; Ross et al., 1994; Coney et al., 1991; Weitman et al., 1994). For such cells, folate (or a folate analog such as methotrexate, CB-3717, or ICI-198,583) is a simple and attractive targeting component.

Preferably, but not necessarily, the targeting molecules of the present invention are relatively small (e.g., < 1 kD) molecules so as to increase penetration into tissue, to reduce the likelihood of steric interference with TCR binding, and to place the pep-alloMHC in close

proximity to the target cell membrane. Larger molecules (e.g., 1-500 kD) such as peptide hormones, growth factors or antibodies (140-190 kD) may, however, also be employed in the present invention. In addition, the targeting molecules of the present invention, as described below, may be attached to the pep-alloMHC complexes using flexible molecular linkers which will allow the pep-alloMHC complex to assume a variety of orientations with respect to the target cell surface and may, thereby, decrease the probability of steric hindrance and increase the probability of effective TCR contact and the elicitation of a cytotoxic response.

Production of pep-MHC Complexes

The pep-MHC complexes of the present invention may be obtained from human cells, or human-hybrid cell lines, which express the desired MHC component using any of a variety of means known in the art. For example, the extracellular domains of MHC molecules may be cleaved off the surface of intact cells with papain, leaving the transmembrane and cytoplasmic domains behind. Alternatively, MHC proteins can be extracted from the membrane fractions of lysed cells using detergents (see, e.g., Nicolle et al., 1994). The soluble MHC proteins may then be isolated (e.g., by immunoprecipitation) along with the diverse set of binding peptide adducts from the cell of origin. Weakly bound peptides may be dissociated from the MHC binding domains (e.g., by incubating the purified MHC at 37°C for 24 hrs.), leaving only MHC protein molecules with strongly bound peptide molecules. Alternatively, essentially all of the bound peptides may be removed by denaturing the MHC proteins and removing the binding peptides (e.g., by acid elution). The MHC proteins may then be refolded with binding peptides of choice.

In preferred embodiments, recombinant DNA technology is used to introduce nucleic acids encoding MHC chains (including truncated MHC chains or MHC fusion proteins) into cells of any one of the several well established protein expression systems. In these approaches, the recombinant sequences are preferably, but not necessarily, engineered to eliminate the transmembrane and cytoplasmic domains of the MHC chains. To purify the recombinantly produced MHC molecules and introduce MHC binding peptides, a variety of known procedures may be employed.

For example, to produce a Class I protein in E. coli, the heavy chain may be expressed in one culture and the light chain (i.e., β_2-microglobulin) in another. Both proteins may be isolated as inclusion bodies, washed, solubilized (e.g., in high concentration urea), mixed with appropriate MHC binding peptides, and subjected to renaturing conditions (e.g., dialysis to remove urea) to allow folding of the MHC chains and formation of the pep-MHC complexes. (See, e.g., Garboczi et al., 1992).

As another example, MHC heavy chain and light chain constructs (preferably lacking the transmembrane and cytoplasmic domains) may be co-expressed in Drosophila cells. These heavy and light chain constructs may be properly folded within these insect cells, and secreted as "empty" MHC molecules because the Drosophila cells lack the cellular machinery used by vertebrate cells to process and bind peptides to nascent MHC molecules in the endoplasmic reticulum. These empty MHC molecules may then be harvested and loaded with one or more peptides of choice. (See, e.g., Stern and Wiley, 1992; Kozono et al., 1994; Wallny et al., 1995; U.S. Pat. No. 5,314,813).

Recombinant techniques also allow for the production of mutated MHC proteins for use in the present invention. As noted above, for example, site-directed mutagenesis may be employed to create new MHC proteins, different from all known MHC alleles and, therefore, useful as allogeneic MHC components in all patients. In addition, particular amino acid residues may be substituted or added (e.g., to the C-terminus or a truncated C-terminus) to aid in the covalent attachment of a targeting moiety. Thus, for example, residues with side chains that are subject to specific chemical reactions (e.g., cysteine) may be added and used as attachment points for targeting molecules.

In order to enhance the stability of the pep-MHC complexes of the invention, it may be desirable to covalently join the MHC proteins and peptides by flexible molecular linkers. Such linkers include, but are not limited to, short polypeptide chains and may include any relatively small (e.g., < 5 kD) organic chemical moieties which are flexible in that they include at least one single bond between the termini and about which there is free rotation. Thus, for example, a short polypeptide of 5-15 amino acids, preferably including many small residues (e.g., alanine, glycine, or serine), may be employed. Alternatively, bifunctional molecules (e.g., an α,ω-dicarboxylic acid or an α,ω-diamine) of a lower alkyl chain may be employed and such flexible molecular linkers may be reacted with the N- or C-termini of the proteins/peptides or with reactive groups of the amino acid side chains. Many other cross-linking agents, of course, are well known in the art and may be employed as substantial equivalents.

Finally, using recombinant techniques, it is now possible to produce an MHC protein, or even a pep-MHC complex, as a single polypeptide chain. Thus, for example, a recombinant DNA construct may be produced as a single artificial gene which encodes both the heavy and light chains of an MHC protein joined in tandem, which encodes an MHC heavy chain joined to a desired MHC binding peptide, which encodes an MHC light chain joined to a desired MHC binding peptide, or which encodes both the heavy and light chains of an MHC antigen as well as a desired MHC binding peptide all joined together. Such recombinant proteins may include sequences encoding a flexible molecular linker between the structural domains (i.e., heavy chain, light chain, and/or peptide) to allow for proper folding of the pep-MHC complex. The

recombinant construct may also include a "sequence tag" (e.g., a poly-His tail or antigenic determinant) which will aid in purification of the protein. (See, e.g., Kozono et al., 1994; Mottez et al., 1995).

Production of Targeted pep-MHC Complexes

The targeting molecules of the present invention may be attached to the MHC antigens of the present invention in any of a number of ways. For targeting molecules which are polypeptides, a recombinant construct may be produced which encodes the targeting molecule joined to one of the termini of the MHC proteins or the MHC binding peptide. For embodiments employing a non-polypeptide linker, a variety of well known chemical reactions may be employed to covalently join the targeting molecule to an MHC chain.

For embodiments in which the targeting molecule is not a polypeptide, a variety of standard techniques may be used to covalently join the targeting molecule to a pep-MHC complex of the invention. These techniques may be used to join the targeting molecule to the N- or C-termini of the MHC chains, taking advantage of the reactivity of the terminal amine and carboxyl groups, or taking advantage of the reactivity of some of the side chains (e.g., cysteine, lysine) of the residues comprising the MHC chains. As will be obvious to one of skill in the art, either the targeting molecule or the MHC chains may be modified by one or more chemical reactions to introduce or block reactive groups before reacting them together. A disulfide linkage may, for example, be created according to standard methods such as those employed by Liu et al. (1988) in creating an MSH-antiCD3 conjugate. Alternatively, carbodiimide bonds may be formed between carboxyl and amine groups by standards methods such as those employed by Kranz et al. (1995) in creating a folate-antiTCR conjugate. These and other methods may be used according to the present invention without further instruction to one of skill in the art.

As a general matter, it is preferred that a multiplicity of targeting molecules be attached to each pep-MHC complex. This is particularly true for relatively small targeting molecules which are unlikely to create steric hindrance to TCR recognition. Indeed, for relatively small targeting molecules, attachment may be relatively non-selective (e.g., attachment of folate to the primary amine groups of lysine residues) and result in an average of 5-10 targeting molecules per pep-MHC complex. For larger targeting molecules, a lower molar ratio of targeting molecules to pep-MHC complexes may be preferred and, in addition, more selective attachment of the targeting molecule may be desired. Thus, for relatively large targeting molecules, it may be desired to selectively join only one or two targeting molecules to positions at or near the C-termini of the MHC chains. As noted above, this may be facilitated by the use of recombinant MHC chains in which particular reactive residues (e.g., cysteine, lysine) have been introduced at or near the C-terminus of a (complete or truncated) heavy or light chain.

Finally, it should also be noted that it is preferred that the targeting molecules are not covalently joined to the peptide portion of the pep-MHC complexes in order to avoid steric hindrance of TCR contact (although N-terminal or C-terminal binding of targeting molecules to the longer and exposed ends of MHC Class II binding peptides may not interfere with T cell recognition). Thus, in some preferred embodiments, the targeting molecules are covalently attached to the MHC chains in the absence of the MHC binding peptides and prior to formation of the pep-MHC complexes. Alternatively, when the amino acid sequences of the peptides and MHC proteins permit, reactions may be chosen which exploit a reactive group which is present on the side chains of the MHC protein chains but which is absent from the peptide's side chains.

Methods of Treatment

The methods of the present invention may be employed to cause the selective destruction of targeted cells in mammalian hosts by using pep-MHC complexes which are allogeneic to the host and which selectively bind to the targeted cells by means of a targeting molecule which is covalently bound to the pep-alloMHC complex. It is particularly contemplated that the hosts will be human patients and that the targeted cells will be cancerous or other neoplastic cells. Nonetheless, it should be emphasized that the present invention may be used, without substantial or undue modification, in the treatment of non-human mammals by merely substituting that species' equivalent of the human MHC proteins for the human MHC proteins discussed herein. It should also be emphasized that the target cells of the invention need not be cancerous or other neoplastic cells but, rather, may be any cells which are, for whatever reason, undesirable and for which a sufficiently selective molecular target is available. Thus, for example, the present invention may be directed at healthy as well as malignant cells whenever the overall therapeutic benefits are considered positive or when it is desired to eradicate an entire type of cell (e.g., during bone marrow explantation/reimplantation techniques).

The pep-MHC complexes of the present invention may be formulated as a pharmaceutical preparation in a pharmaceutically acceptable carrier (e.g., saline solution, phosphate buffered saline solution, Hank's solution, Ringer's solution, lactated Ringer's solution, dextrose/saline, glucose solution, etc.) which is suitable for parenteral administration of proteins. The pharmaceutical preparation should be sterile and non-pyrogenic and may, as determined by the pharmacist or attending physician, include tonicity adjusting agents, wetting agents, bactericidal agents, preservatives, stabilizers, and the like. See, e.g., Remington's Pharmaceutical Sciences, Mack Publishing Company, Philadelphia, PA, 17th edition (1985). Alternatively, for purposes of transport and storage, the pep-MHC complexes may be formulated in lyophilized form with instructions for preparing a pharmaceutically acceptable carrier solution. In order to assist the practitioner in choosing a pep-MHC preparation which is likely to be allogeneic for a given patient, the preparations may be identified with respect to the allelotypes of the MHC chains

contained in the pep-MHC complexes or, when the preparations contain sufficiently rare allelotypes for a given population, may be identified with respect to the populations for which they are expected to be most effective. The practitioner may perform an MHC genotyping of the patient and choose a preparation containing pep-MHC complexes which are allogeneic to that patient, or may simply employ a preparation containing one or more pep-MHC complexes with rare MHC allelic variants. In particular, if the preparation contains a recombinantly produced MHC chain with a novel antigenicity not known to exist in nature, or if it contains at least three allelotypes, it may be labeled to indicate that it is expected to be useful in all members of the species and may be used without genotyping the patient. The pep-MHC complexes may be conjugated with a great variety of different targeting molecules and, for each, the packaged preparations may be identified as to the tissues and/or cells against which they are directed.

Preferably, the pep-alloMHC complexes of the invention are administered by injection, perfusion or catheterization in the area in which the target cells are known or expected to be densest. Thus, for ovarian cancer, in which spread of malignant cells into the peritoneum is common, intraperitoneal injections would be preferred. Alternatively, solid tumor sites may be directly injected with the pep-alloMHC complexes of the invention and lymph nodes may be injected or perfused to treat lymphatic cancers. The present invention, however, is particularly useful in that the targeting molecules allow for the selective binding of the pep-alloMHC complexes to the targeted cells. Thus, the present invention has particular utility in the alloconversion and consequent destruction of metastatic cells, small undetected satellite tumors, or cancerous cells which are diffusely spread through a tissue (e.g., brain tissue). For such treatments, more systemic administration of the pep-alloMHC complexes, including intravenous or intra(cranio)ventricular administration, may be preferred.

In order to elicit the most effective cytotoxic T cell response against alloconverted target cells, it is desirable that the patient be first immunized with the pep-alloMHC antigen to prime the immune system of the patient against the alloMHC. In preferred treatments, any such immunization should be performed approximately 1-20 and, more preferably, about 5-15 days prior to the initiation of the targeted pep-alloMHC treatments. To accomplish this immunization, the pep-alloMHC complex may be introduced into the patient without the targeting molecules. Preferably, whole cells expressing the pep-alloMHC antigens are introduced into the patient to immunize against the alloMHC antigen. In preferred embodiments, the introduced cells are antigen presenting cells (APCs) such as B cells, macrophages or dendritic cells. For example, EBV transformed B cells expressing the alloMHC antigen may be introduced within the patient. If the whole cells are not APCs, they preferably are also transformed or transfected to express B7, a co-stimulatory factor involved in antigen presentation. As a matter of convenience, if the MHC antigens of the invention are obtained from mammalian cells which express the antigens on their

cell surfaces, the same cells or line of cells that are used to produce the pep-alloMHC complexes also may be used to immunize the patient and prime the cytotoxic response. This is particularly preferred if these cells are APCs. In order to be effective, immunizing doses of the alloMHC antigen or pep-alloMHC complex should include an immunogenically effective amount of the alloMHC antigen. Preferably, such immunogenically effective doses are provided by a modest number of APC, as contained in a blood transfusion (e.g., at least 10^5 and, more preferably, about 10^7 - 10^9 cells).

The pep-alloMHC complexes of the invention may be administered in a single dose, or in several doses, separated by an interval of one or several weeks. In addition to monitoring the response of the target cells (e.g., tumors) by standard techniques such as biopsies or CAT scans, the ability of the treatment to activate cytotoxic T cells in vivo can be assessed by (a) measuring the patient's T cell reactivity with the pep-alloMHC presented by a different APC than was used for immunization and/or (b) by drawing peripheral blood mononuclear cells (PMBCs) from the patient and testing for their in vitro cytotoxic activity against, or proliferation in response to, incubation with cells expressing the alloMHC antigens, and preferably the pep-alloMHC complexes, which had been administered to the patient.

It should be noted that following treatment, the patient may develop a humoral response against the administered pep-alloMHCs. This response may decrease the effectiveness of the treatment by removing some of the pep-alloMHCs from the lymph or plasma before they reach and bind to their target cells. Thus, after the development of a humoral response, or as a matter of course after a few weeks of treatment, it is recommended that further treatments, if necessary, employ either a different peptide or set of peptides associated with the same alloMHC antigen or, more preferably, employ a different alloMHC antigen in targeted pep-alloMHC complexes. As before, it is preferred, that this second round of treatment be preceded by immunization of the patient to enhance the expected cytotoxic response. As necessary, additional rounds of treatment may be performed, always using different pep-alloMHC complexes, until the desired level of destruction of the target cells is achieved.

Examples

In order to illustrate some of the applications and methods which may be employed in the practice of the present invention, and to demonstrate the expected utility of the invention, the following non-limiting experimental examples are provided. As will be obvious to one of ordinary skill in the art, the particular techniques, reagents and/or cells employed in the examples below may be freely substituted with other, substantially equivalent techniques, reagents and/or cells.

Expression of Recombinant MHC Proteins in Bacteria and In Vitro pep-MHC Complex Formation

E. coli bacteria were transformed with expression vectors encoding either a murine K^b heavy chain (without transmembrane and cytoplasmic domains) or β_2-microglobulin ("β_2m"). To isolate the recombinant proteins (produced as inclusion bodies), the following general protocol was employed:

One colony of the K^b or β_2m bacteria was chosen and grown in 200 ml LB + 0.1 ml Carbenicillin (100 mg/ml stock) at 37°C in a shaker overnight. 200 ml of the culture was transferred to approximately 4.0 L of LB + Carbenicillin. O.D. 600 nm at this point was < 0.2. The culture was then incubated, checking O.D. every hour initially, and every 15-30 min once O.D. 600 nm reached approximately 0.7. IPTG (0.5 M stock) was added at 1ml/L when the O.D. reached approximately 0.8 for the K^b culture or 1.2 for the β_2m culture. The bacterial cultures were then incubated an additional 3-3.5 hrs, with the O.D. remaining between 1.8 and 2.5.

Bacteria were harvested by centrifugation at 2-4000 rpm for 30 min at 4°C and pellets were weighed. The pellets were resuspended in bacterial lysis buffer (1 g/ml), lysozyme (100 μg/ml) and PMSF (50 μg/ml). Samples were then passed three times through a French Press at 100-1200 psi. RNAse T1 (130 units/ml) and DNAse I (40 μg/ml) were added and the sample was incubated at room temperature for 15 min. The sample was then centrifuged at 1200 rpm for 20 min at 4°C. The pellets were washed with lysis buffer and 0.5% Triton X100 until gray and sandy in appearance. Pellets were resuspended in a denaturing buffer comprising 8.0 M urea, 50 mM 2-(N-morpholino)ethanesulfonic acid (pH 6.5), 0.1 mM EDTA, and 0.1 mM DTT in a total volume of approximately 10.5 ml. This suspension was then centrifuged at 1200 rpm for 20 min.

The isolated and purified K^b and β_2m proteins were refolded in the presence of the SV9 peptide (see below) in a refolding buffer at 10°C for 36 hrs. Specifically, approximately 18.6 mg of the K^b protein, 14.4 mg of the β_2m protein, and 6 mg of the SV9 peptide, were allowed to refold in 100 mM Tris HCl (pH 8), 400 mM L-arginine HCl, 2 mM EDTA, 5 mM reduced glutathione, 0.5 mM oxidized glutathione, 0.5 mM PMSF (100 mM stock in isopropanol), and dH_20 to a total volume of 600 ml. The samples were then concentrated to 200 μl using an Amicron concentrator.

Targeted Destruction of Cells Mediated by Exogenously Supplied pep-MHC Complexes

To determine if cytotoxic T lymphocytes (CTLs) can lyse target cells to which are attached, via a targeting molecule and cell-surface target, an exogenous pep-MHC complex, an in vitro assay of CTL-mediated specific lysis of target cells was employed.

A murine leukemia cell line, designated F2-MTX^{r}A ("F2-MTX"), was selected as the target cells. F2-MTX is a methotrexate resistant subline of the L1210 murine leukemia cell line selected for increased expression of the FRβ folate receptor by growth in low folic acid medium (Brigle et al., 1994). Thus, in this experiment, the folate receptor was used as the cell-surface target.

For the pep-MHC complexes, a recombinant human HLA-A2 Class I protein was produced in E. coli essentially as described in Garboczi et al. (1992). The recombinant HLA-A2 was isolated and refolded in the presence of the peptide ILKEPVHGV ("IV9," SEQ ID NO: 1), derived from the reverse transcriptase enzyme of the human immunodeficiency virus-type 1 (HIV-1), to form the soluble pep-MHC complex "A2-IV9." The complex was isolated by HPLC and was highly reactive with anti-A2 antibodies that are specific for native, properly folded A2-peptide complexes.

Folic acid was employed as the targeting molecule to selectively bind to the FRβ proteins expressed on the surface of the F2-MTX cells. Thus, folate was conjugated to the A2-IV9 pep-MHC complex as follows: An approximately 6 mM solution of folic acid was prepared in DMSO (~ 2.6 mg/ml) and was activated with an approximately five-fold molar excess of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC, ~ 6.4 mg/ml) for 30 min in the dark at room temperature. A roughly sixty-fold molar excess of the activated folate (~ 5.0 μl at 6.0 x 10^{-3} M) was reacted with the recombinant A2-IVP pep-MHC complex (~ 100 μl at 0.5 x 10^{-5} M) in an amine free buffer (MOPS) for 1.25 hrs in the dark at room temperature and neutral pH. To separate the folate-targeted pep-MHC complexes ("Folate-A2-IV9") from free folate, the mixture was either passed through a Sephadex G25 column in PBS or subjected to ultrafiltration through a Centricon membrane with a 10 kDa cut-off. It is estimated that this procedure resulted in the attachment of roughly 4-5 folate targeting molecules to each pep-MHC complex.

Finally, a cytotoxic T lymphocyte (CTL) clone, #2175, that recognizes the A2-IV9 pep-MHC complex was employed as the effector cells to investigate selective killing of Folate-A2-IV9 targeted F2-MTX cells. The #2175 clone was derived from an H-2^b mouse expressing a human HLA-A2 transgene. The mouse had previously been immunized with the IV9 peptide injected at the base of the tail with Freund's incomplete adjuvant.

A standard cytolytic assay was carried out in 96 well plates using ^{51}Cr labeled F2-MTX cells as target cells. The release of ^{51}Cr in the presence or absence of CTL, the Folate-A2-IV9 targeted pep-MHC complex, and/or molar excesses (~ 10^{-4} to 10^{-6} M) of free folate, were then used to assess the "percent specific lysis" defined as

$$\% \text{ spec. lysis} = (a-b)/(t-b),$$

where a = ^{51}Cr released in wells containing Folate-A2-IV9-targeted F2-MTX cells and #2175 CTLs, b = ^{51}Cr released spontaneously from Folate-A2-IV9-targeted F2-MTX in the absence of the CTLs, and t = total ^{51}Cr released from F2-MTX cells lysed with a detergent (0.1% NP40). In all cases, each well contained the same number of MTX tumor cells (approximately 5000) in a total volume of 0.2 ml. When present, a 10-fold excess of CTLs (approximately 50,000) were contained in each well. After 4 hr at 37°C (in a 5% CO_2 atmosphere), the culture supernatants were removed from each well and 0.05 ml aliquots were counted in a γ-spectrometer to measure released ^{51}Cr. Controls were typically performed in triplicate or quadruplicate and experimentals in duplicate or triplicate. This same cytolytic assay was also used in the examples which follow.

The results, summarized in the following table, show that F2-MTX target cells, which are murine cells of MHC type H-2^d and lack the human HLA-A2 class I MHC, are specifically lysed by CTL that recognize the A2-IV9 peptide complex only if Folate-A2-IV9 is present. The presence of free folic acid at a concentration of about 1 x 10^{-5} M completely inhibited cytolysis by blocking the binding of Folate-A2-IV9 to the target cells' high affinity receptors for folate.

^{51}Cr-F2-MTX + CTL #2175 +	% Specific Lysis
Folate-A2-IV9, undil.	57
Folate-A2-IV9, dil. 1:10	27
Folate-A2-IV9, dil. 1:100	11
Folate-A2-IV9, undil. + Free Folate	0
Folate-A2-IV9, dil. 1:10 + Free Folate	0
Folate-A2-IV9, dil. 1:100 + Free Folate	0

As another test of the targeted pep-MHC complexes of the present invention, CTLs from an H-2^d mouse were used to mediate the destruction of F2-MTX cells (also H-2^d) targeted with the murine allogeneic MHC Class I component K^b. In these experiments, the CTL effectors were derived from an H-2^d mouse ("dm2") which had been immunized with live cells ("T2-K^b") expressing the murine K^b alloantigen. Briefly, an alloreactive CD8+ T cell line was produced by injecting dm2 mice with live cells expressing K^b loaded with the K^b-binding Sendai virus nonapeptide SV9 ("T2-K^b-SV9 cells"). These immunizing T2-K^b cells were from a line of human TAP$^-$ TXB cells transfected with the mouse K^b gene. One week after immunization, the spleens were removed from the mice and spleen cells were put into culture with irradiated T2-K^b-SV9 cells and then maintained by weekly exposure to these cells and growth factors (i.e., lymphokines, including IL-2). The resulting "anti-K^b" T cells were used as the effector CTLs in the following experiments. In these experiments, as described above, a standard cytolytic assay was carried out

to measure specific lysis using internal ^{51}Cr-labeling of target cells, with the same numbers of target cells (approximately 5,000) and CTLs (approximately 50,000) per well.

The murine K^b heavy chain (without transmembrane and cytoplasmic domains) and β_2m were produced and isolated as described above, and refolded in the presence of the peptide FAPGNYPAL ("SV9," SEQ ID NO: 2), derived from the Sendai virus, to form the soluble pep-MHC complex "K^b-SV9." This pep-MHC complex was then conjugated with folate (approximately 5 folate groups per K^b), essentially as described above, to form targeted pep-MHC complexes, and these were used to target F2-MTX cells at a variety of concentrations. As a control, murine erythroleukemia cells ("MEL"), which lack the high affinity folate receptor, were tested as target cells. The results, shown below, demonstrate that the Folate-K^b-SV9 targeted pep-alloMHC complexes effectively and specifically targeted the F2-MTX cells, but not the MEL cells, for CTL-mediated lysis in a dose-dependent manner.

Concentration (M/L) of Folate-K^b-SV9	% Specific Lysis	
	F2-MTX cells	MEL cells
0	10	1
10^{-14}	9	0
10^{-13}	11	0
10^{-12}	15	2
10^{-11}	26	1
10^{-10}	39	1

A repeat of this experiment yielded a higher rate of specific lysis but no dose-dependence:

Concentration (M/L) of Folate-K^b-SV9	% Specific Lysis	
	F2-MTX cells	MEL cells
0	5	-
10^{-13}	32	4
10^{-12}	32	2
10^{-11}	35	2
10^{-10}	31	2
10^{-9}	30	2

Next, to demonstrate that the selective lysis of the F2-MTX cells was dependent upon binding between the folate (targeting molecule) and the high affinity folate receptor (cell-surface

target) on these cells, the ability of free folate to inhibit Folate-K^b-SV9 targeted lysis was measured. The results, shown below, demonstrate that the lysis is highly specific and that free folate inhibits the specific lysis in a dose-dependent manner.

Free Folate (M/L)	% Specific Lysis
0	26
10^{-11}	24
10^{-10}	23
10^{-9}	23
10^{-8}	20
10^{-7}	15
10^{-6}	10
10^{-5}	8
10^{-4}	1

T Cells Recognize Allogeneic MHC Proteins Regardless of Peptide Adduct

As a test of the specificity of T cell responses to particular peptides in pep-alloMHC complexes, the anti-K^b T cells raised against T2-K^b-SV9 cells were tested for their ability to lyse T2-K^b cells loaded with one of three peptides: (1) the peptide FAPGNYPAL ("SV9," SEQ ID NO: 2), derived from the Sendai virus, (2) the peptide RGYVYQGL ("VSV," SEQ ID NO: 3), derived from the vesicular stomatitis virus, or (3) the peptide SIINFEKL ("OVA," SEQ ID NO: 4), derived from ovalbumin.

Thus, using the anti-K^b CTLs, T2-K^b cells were employed as the target cells, with or without the addition of one of the K^b-binding peptides SV9, VSV, or OVA. The results, shown below, demonstrate that it is the K^b component of the pep-alloMHC complexes presented by these target cells, and not the specific peptide presented in the pep-alloMHC complex, which evokes the T cell response. Thus, despite the fact that the anti-K^b CTLs were raised against T2-K^b-SV9 cells, the specific lysis of the target cells is not increased by addition of the SV9 peptide. As further controls, ^{51}Cr-labeled target cells which were either T2 (but not transfected with K^b) or T2-L^d (T2 transfected with L^d rather than K^b) were tested. Neither the T2 nor the T2-L^d target cells were substantially lysed (~5%), indicating that lysis is dependent upon the presence of the K^b component of the pep-alloMHC complex.

^{51}Cr-T2-K^b + anti-K^b CTL +	% Specific Lysis
no peptide added	71
SV9 peptide 5 μg/ml	71
SV9 peptide 0.5 μg/ml	72
VSV peptide 5 μg/ml	73
VSV peptide 0.5 μg/ml	75
OVA peptide 5 μg/ml	73
OVA peptide 0.5 μg/ml	71

Targeted Destruction of Cells Mediated by Exogenously Supplied pep-alloMHC Complexes In Vivo

As a further test of the methods of the present invention, a non-human animal model may be employed in which the target cells are transplantable tumor cells. Thus, for example, F2-MTX^{r}A ("F2-MTX") murine leukemia cells (Brigle et al., 1994) may be transplanted into DBA/2 mice. The F2-MTX cell line originated in DBA/2 mice (MHC subtype H-2^d) and, therefore, the tumor cells will not be rejected. To establish the model and control values, the animals are injected intraperitoneally with varying numbers of tumor cells and survival curves for each dose are determined and/or the approximate number of cells required to reliably cause death in a desired experimental period (e.g., 3-6 weeks) is determined.

Since the expected period of time between injection of the tumor cells and death is sufficiently long, the animals may be injected with the tumor cells and then immunized against an alloMHC protein. As described above, this immunization is intended to "prime" the subject's immune system for the targeted pep-alloMHC complexes of the invention. The immunogen in this step will preferably consist of intact cells expresssing the alloMHC protein (e.g., murine RMA-S cells (H-2^b), human T2 cells transfected with a K^b gene, Drosophila cells transfected with both K^b and β_2m genes) and may be administered by any of several standard routes (e.g., intraperitoneal, subcutaneous). As described above, because of the relative contributions to TCR binding of the peptide and MHC components in an allogeneic response, it is not necessary that the immunizing alloMHC protein be loaded with the same peptides that will be employed in the targeted pep-MHC complexes, but this may be desirable. 1-2 weeks after immunization, the subjects are injected intraperitoneally with the targeted pep-alloMHC complexes of the invention. For example, if the subjects are DBA/2 mice (H-2^d) and the target cells are derived from the F2-MTX tumor cell line (H-2^d), MHC Class I subtype K^b protein will be allogeneic. Thus, as described above, soluble K^b and β_2m may be produced recombinantly, allowed to refold in the

presence of appropriate MHC binding peptides (e.g., a single immunodominant peptide or, preferably, a library of tightly binding peptides), and be conjugated to a targeting molecule which selectively binds to the target cells (e.g., folate which binds the FRβ folate receptor on F2-MTX cells). The subjects are then followed, with or without booster injections with the same or different targeted pep-alloMHC complexes, and the effect of the treatment on survival is determined.

Alternatively, if the expected period of time between injection of the tumor cells and death is too short (e.g., 3-4 weeks), the subjects may be immunized against the desired alloMHC protein 1-2 weeks prior to injection with tumor cells. Such a protocol avoids premature death of the subjects before an immune response can be raised against the alloantigens, but does not otherwise adversely affect the interpretation of the results.

Finally, as additional controls, the subjects may be immunized against the alloMHC protein before or after injection with tumor cells but without subsequent treatment with the targeted pep-alloMHC complexes of the invention. In such experiments, immunization with untargeted alloMHC should have no effect on survival because the allogeneic response will not be selectively directed against the tumor cells.

TABLE 1

MHC CLASS I ANTIGEN ALLELE FREQUENCIES					
ANTIGEN	FREQUENCY				
	CAUC	AF-AM	JPN	CHN	MEX
A1	28.6	10.1	1.4	9.2	10.1
A2	45.8	30.3	42.4	54.0	43.0
A3	20.6	16.3	1.2	7.1	14.8
A11	9.9	3.8	19.7	33.1	7.3
A23	3.2	14.3	0.0	1.6	5.5
A24	16.8	8.8	58.1	32.9	26.7
A25	6.1	1.6	0.0	0.8	4.2
A26	7.3	3.2	20.4	3.8	6.7
A28	8.8	20.8	0.0	0.8	20.8
A29	4.7	6.7	0.0	2.0	8.4
A30	4.7	18.8	0.8	7.3	8.4
A31	4.4	3.8	14.8	9.6	10.1
A32	9.6	1.6	0.2	1.2	6.7
A33	2.0	16.1	14.8	8.8	11.3
A34	1.2	9.8	0.2	0.0	2.4
A36	0.8	5.3	0.0	0.4	0.6
A43	0.0	0.2	0.2	0.0	0.0
AX	0.4	3.8	0.0	0.0	1.2
B7	17.7	15.5	9.6	6.9	11.8
B8	18.1	6.3	0.0	3.6	9.0
B13	5.9	1.6	3.4	15.7	3.0
B14	7.6	6.3	0.4	0.8	12.4
B18	9.2	6.9	0.0	2.2	7.6
B27	7.5	2.6	0.8	3.4	4.9
B35	15.4	14.8	15.4	9.8	28.1
B37	4.4	2.2	1.6	3.8	1.2
B38	7.6	0.2	0.8	3.4	3.6
B39	3.6	1.6	8.4	5.1	11.8
B41	2.0	4.2	0.0	0.0	6.7
B42	0.0	10.5	0.0	0.0	0.6
B44	19.7	10.5	14.3	6.7	17.4
B45	1.2	7.1	0.0	0.8	3.6
B46	0.0	0.0	8.8	16.1	0.0
B47	1.2	0.0	0.2	0.0	1.2
CAUC = North American Caucasian, AF-AM = African-American, JPN = Japanese, CHN = Chinese, and MEX = Mexican					

TABLE 1 (cont'd)

MHC CLASS I ANTIGEN ALLELE FREQUENCIES					
ANTIGEN	FREQUENCY				
	CAUC	AF-AM	JPN	CHN	MEX
B48	0.4	0.6	5.5	5.5	4.5
B49	4.4	3.6	0.0	0.4	4.9
B50	4.4	2.2	0.0	3.0	5.5
B51	6.9	6.7	17.2	13.0	7.6
B5102	0.0	1.8	0.6	1.2	0.0
B52	2.4	1.4	20.3	4.9	5.9
B53	1.6	22.6	0.2	0.0	4.2
B54	0.0	0.0	12.4	8.6	0.0
B55	4.4	1.4	5.7	6.7	1.8
B56	1.6	0.0	3.0	1.2	0.6
B57	7.3	7.6	0.0	2.0	2.4
B58	2.0	13.7	1.4	6.7	3.0
B59	0.0	0.2	3.8	0.4	0.0
B60	8.2	2.2	10.7	20.1	6.1
B61	5.9	0.2	20.3	9.6	10.1
B62	10.3	2.6	16.3	14.3	3.0
B63	0.8	4.9	0.2	0.4	0.6
B67	0.0	1.6	3.2	1.6	0.6
B70	3.2	15.9	3.2	1.6	4.9
B73	0.8	0.6	0.0	0.0	0.6
B75	0.0	0.8	2.2	7.3	2.4
B76	0.4	0.0	0.0	0.0	0.0
B7801	0.0	0.6	0.0	0.0	0.0
BX	0.8	1.6	0.2	0.0	0.6
Cw1	8.2	1.6	22.9	21.5	7.3
Cw2	10.1	18.6	0.4	2.0	12.2
Cw4	17.9	37.3	8.4	11.5	32.9
Cw5	7.1	4.0	1.0	2.4	9.2
Cw6	17.6	13.7	2.2	19.0	12.2
Cw7	37.1	27.2	28.1	29.3	49.2
Cw9	7.5	4.0	25.9	29.6	8.0
Cw10	7.5	6.9	17.0	12.4	11.6
CwX	6.3	2.6	2.8	1.2	3.6
CAUC = North American Caucasian, AF-AM = African-American, JPN = Japanese, CHN = Chinese, and MEX = Mexican					

TABLE 2

MHC CLASS II ANTIGEN ALLELE FREQUENCIES					
ANTIGEN	FREQUENCY				
	CAUC	AFR-AM	JPN	CHN	MEX
DR1	18.5	8.4	10.7	4.5	10.1
DR3	17.7	19.5	0.4	7.3	14.4
DR4	23.6	6.1	40.4	21.9	29.8
DR7	26.2	11.1	1.0	15.0	16.6
DR8	5.5	10.9	25.0	10.7	23.3
DR9	3.6	4.7	24.5	19.9	6.7
DR10	3.6	1.4	1.2	3.4	3.0
DR11	17.0	18.1	4.9	19.4	18.1
DR12	2.8	5.5	13.1	17.6	5.7
DR13	21.7	16.5	14.6	12.2	10.5
DR14	2.4	3.8	10.3	4.2	15.2
DR15	19.9	14.8	30.9	22.0	15.0
DR16	2.4	1.8	1.6	2.2	7.5
DRX	12.8	37.0	1.2	1.6	0.0
DQ1	67.4	50.2	69.0	50.7	51.3
DQ2	37.3	22.7	1.2	15.5	24.3
DQ3	21.7	7.1	32.9	19.4	21.3
DQ4	4.4	13.0	27.6	8.2	18.8
DQ7	28.1	23.1	27.1	38.8	43.0
DQX	0.0	32.6	0.2	0.0	0.0
CAUC = North American Caucasian, AF-AM = African-American, JPN = Japanese, CHN = Chinese, and MEX = Mexican					

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: EISEN, HERMAN N
KRANZ, DAVID M

(ii) TITLE OF INVENTION: ALLOGENEIC HISTOCOMPATIBILITY COMPLEXES AS MEDIATORS OF CELL DESTRUCTION

(iii) NUMBER OF SEQUENCES: 4

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

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(viii) ATTORNEY/AGENT INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..9
(D) OTHER INFORMATION: /note= "IV9 peptide"

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

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1 5

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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(D) OTHER INFORMATION: /note= "SV9 peptide"

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(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
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(B) LOCATION: 1..8
(D) OTHER INFORMATION: /note= "VSV"

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

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1 5

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..8
(D) OTHER INFORMATION: /note= "OVA"

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

Ser Ile Ile Asn Phe Glu Lys Leu
1 5

CLAIMS

What is claimed is:

1. A pharmaceutical preparation for use in targeting an alloreactive response against a target cell type comprising

a first pep-MHC complex including an MHC heavy chain, an MHC light chain, and an MHC binding peptide; wherein at least one of said MHC chains is allogeneic with respect to a diploid MHC genotype of said target cell type; and

at least one targeting molecule capable of selectively binding to said target cell type;

wherein said targeting molecule is bound to said pep-MHC complex.

2. A pharmaceutical preparation as in claim 1 further comprising

a second pep-MHC complex including an MHC heavy chain, an MHC light chain, and an MHC binding peptide; wherein at least one of said MHC chains is allogeneic with respect to a diploid MHC genotype of said target cell type; and

at least a second targeting molecule capable of selectively binding to said target cell type;

wherein said second targeting molecule is bound to said second pep-MHC complex; and

wherein at least one MHC chain of said second pep-MHC complex differs from both MHC chains of said first pep-MHC complex.

3. A pharmaceutical preparation as in claim 1 further comprising

a second pep-MHC complex including an MHC heavy chain, an MHC light chain, and an MHC binding peptide; wherein at least one of said MHC chains is allogeneic with respect to a diploid MHC genotype of said target cell type; and

at least a second targeting molecule capable of selectively binding to said target cell type;

wherein said second targeting molecule is bound to said second pep-MHC complex; and

wherein said MHC binding peptide of said second pep-MHC complex differs from said MHC binding peptide of said first pep-MHC complex.

4. A pharmaceutical preparation as in claim 1 wherein said pep-MHC complex further comprises

a flexible molecular linker covalently joining a pair selected from the group consisting of (1) said heavy chain and said light chain, (2) said heavy chain and said binding peptide, and (3) said light chain and said binding peptide.

5. A pharmaceutical preparation as in claim 1 wherein said heavy chain is an MHC Class I α chain; said light chain is a β_2-microglobulin chain; and said binding peptide is an MHC Class I binding peptide.

6. A pharmaceutical preparation as in claim 1 wherein said heavy chain is an MHC Class II α chain; said light chain is an MHC Class II β chain; and said binding peptide is an MHC Class II binding peptide.

7. A pharmaceutical preparation as in claim 1 wherein said targeting molecule is selected from the group consisting of antibodies, hormones, growth factors, and non-hormone, non-growth factor cell receptor ligands.

8. A pharmaceutical preparation as in claim 7 wherein said targeting molecule is folic acid.

9. A pharmaceutical preparation as in claim 1 wherein said targeting molecule is covalently bound to said pep-MHC complex.

10. A pharmaceutical preparation as in claim 1 wherein said targeting molecule is covalently bound to said pep-MHC complex through a carbodiimide linkage.

11. A method of treating a patient to target a cytotoxic T cell response against a population of target cells within said patient comprising administering to said patient a pharmaceutical preparation of claim 1 in an amount sufficient to cause T cell mediated destruction of cells within said population.

12. A method as in claim 11 wherein said administration is parenteral.

13. A method as in claim 11 further comprising the step of

immunizing said patient to an alloMHC protein of said pep-MHC complex prior to administering said preparation,

wherein said immunization comprises administering to said patient an immunogenically effective amount of said alloMHC protein.

14. A method as in claim 13 wherein said immunization comprises

administering to said patient whole cells expressing said alloMHC protein.

15. A method as in claim 13 wherein said immunization is performed between about 1 and about 20 days prior to administering said preparation.

16. A method as in claim 11 wherein said target cells are cancer cells.

17. A method as in claim 16 wherein said target cells are ovarian cancer cells.

18. A method as in claim 17 wherein said targeting molecule is selected from the group consisting of folate, folate analogs, follicle stimulating hormone (FSH), follicle stimulating hormone analogs, luteinizing hormone and luteinizing hormone analogs.

19. A method as in claim 16 wherein said target cells are brain tumor cells and said targeting molecule is selected from the group consisting of folate and folate analogs.