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LYMPHOCYTE INHIBITION BY HLA PEPTIDES

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(57) Novel peptides are provided for modulating the cytotoxic effect of CTL's. Novel peptides cross reactive with the C-terminal or N-terminal portions of the alpha, or alpha2 domains, respectively, of the MHC antigen providing the CTL restriction are employed for CTL modulation. The peptides may be used in vivo or in vitro for controlling cellular lysis.

CLAIM

1. A substantially pure preparation of peptide compound of at least 8 amino acids and having a sequence comprising at least any eight consecutive amino-acid residues of the extended sequence:

aa⁵⁵ G P E Y W D aa⁶² aa⁶³ T aa⁶⁵ aa⁶⁶ aa⁶⁷ K
aa⁶⁹ aa⁷⁰ aa⁷¹ Q T aa⁷⁴ R aa⁷⁶ aa⁷⁷ L aa⁷⁹ aa⁸⁰ aa⁸¹
aa⁸² aa⁸³ Y Y N Q S E A G S H aa⁹⁴ aa⁹⁵ Q aa⁹⁷ M aa⁹⁹ G
C D aa¹⁰³ G aa¹⁰⁵ D aa¹⁰⁷ R aa¹⁰⁹ L R G aa¹¹³ aa¹¹⁴ Q
aa¹¹⁶ A F D G

wherein:

- aa⁵⁵ is E or K;
- aa⁶² is G, Q, E or R;
- aa⁶³ is an acidic amino acid or amide thereof;
- aa⁶⁵ is Q, R or G;
- aa⁶⁶ is I, N or K;
- aa⁶⁷ is an aliphatic neutral amino acid;

aa⁶⁹ is an aliphatic neutral amino acid;
aa⁷⁰ is Q, H, S, N or K;
aa⁷¹ is an aliphatic neutral amino acid;
aa⁷⁴ is D, Y or H;
aa⁷⁶ is E or V;
aa⁷⁷ is D, S or N;
aa⁷⁹ is R or G;
aa⁸⁰ is T, I or N;
aa⁸¹ is an aliphatic non-polar amino acid;
aa⁸² is R or L;
aa⁸³ is G or R;
aa⁹⁴ is T or I;
aa⁹⁵ is a non-polar aliphatic amino acid of
from 5 to 6 carbon atoms;
aa⁹⁷ is an aliphatic amino acid or W;
aa⁹⁹ is an aromatic amino acid;
aa¹⁰³ is a non-polar aliphatic amino acid of
from 5 to 6 carbon atoms;
aa¹⁰⁵ is P or S;
aa¹⁰⁷ is G or W;
aa¹⁰⁹ is L or F;
aa¹¹³ is Y or H;
aa¹¹⁴ is H, Q, D, N or R;
aa¹¹⁶ is Y, D, S, F or H,
which modulates CTL activity.

2. A peptide compound according to Claim 1,
comprising at least eight amino acids within the
sequence:

G S H T (V, I or L) Q R M Y G C D V G S D (W or G)
R F L R G Y H Q Y A Y D G,

or

Q E G P E Y W D (G or R) (E or N) T (R or Q)
(K or N) V K A (H or Q) S Q T (H or D) R (V or
E) (D, S or N) L (G or R) (T or I) (L or A) (R

(11) AU-B-13423/88
(10) 619458

-3-

or L) (G or R) Y Y N Q S E A,

wherein the amino acids in the parentheses indicate that one of the amino acids in the parentheses is present.

5. A method of modulating cytolytic activity of an MHC restricted CTL, said method comprising:

combining a CTL with a peptide compound of at least 8 amino acids and not more than about 30 amino acids having a sequence differing by not more than two mutations from the polymorphic region of the C-terminal half of the α_1 domain and the N-terminal half of the α_2 domain of the MHC protein of the host of said MHC restricted CTL cells, wherein said CTL cells are modulated by said peptide.

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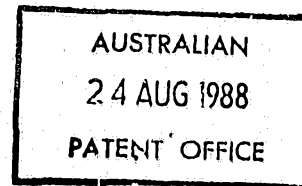
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(54) Title: LYMPHOCYTE INHIBITION BY HLA PEPTIDES

(57) Abstract

Novel peptides are provided for modulating the cytotoxic effect of CTL's. Novel peptides cross reactive with the C-terminal or N-terminal portions of the α , or $\alpha 2$ domains, respectively, of the MHC antigen providing the CTL restriction are employed for CTL modulation. The peptides may be used *in vivo* or *in vitro* for controlling cellular lysis.

LYMPHOCYTE INHIBITION BY HLA PEPTIDES

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FIELD OF THE INVENTION

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HLA peptide compositions affect T-cell activity subject to MHC restriction. Methods and compositions are provided involving the modulation of cytotoxicity toward allogeneic cells.

BACKGROUND OF THE INVENTION

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Cytotoxic T-cells, particularly cytotoxic T-lymphocytes (CTL), are restricted in their activity by recognizing a specific major histocompatibility (MHC) antigen on the surface of the target cell, as well as an antigen exogenous to the host. The foreign antigen may be as a result of transplantation, viral infection, mutation, or the like. The presence of the determinant or restricting site of the MHC protein appears to be essential to the attack by the CTL against the cell carrying the foreign antigen. In this way, the immune system is able to destroy cells in the body, which, if otherwise allowed to proliferate, would result in the proliferation of pathogens or neoplastic cells.

The CTL's are also effective in destroying transplants of various organs or tissue which come from allogeneic hosts. The methods or treatments employed for protecting the transplanted cells frequently result

in the incapacitating of the immune system, subjecting the transplant recipient to opportunistic infection.

It is, therefore, of substantial interest to
5 be able to modulate the cytotoxic system in a host to selectively inhibit a particular population of T-cells from performing their normal physiological function. In this manner, the immune system is substantially maintained intact, while a particular T-cell target may
10 be protected.

DESCRIPTION OF THE RELEVANT LITERATURE

Clayberger et al., J. Exp. Med. (1985) 11:1709-1714 describe HLA-A2 antigen in comparisons with
15 HLA-Aw68 and Aw69. Townsend et al., Cell, (1986) 44:959-968 suggests that CTL recognize segmental epitopes of denatured or degraded proteins in a similar way as helper T-cells. Holmes and Parham, EMBO J. (1985) 4:2849-2854 describe the relationship of HLA-A2.
20 Aw68 and Aw69. CTL target specificity has been taught to be extremely sensitive to changes in structure of human Class I molecules (Durna and Pease, Transplantation, (1986) 41:279-285; Biddison, et al., J. Immunol., (1980) 124:548-552; Spits, et al.,
25 Immunogenetics, (1982) 16:503-512; Gaston, et al., J. Exp. Med., (1983) 158:280-293).

Mutants which affect recognition by CTL have been studied in mice (Natnenson et al., Ann. Rev. Immunol. (1986) 4:471-502; Schulz et al., Proc. Natl. Acad. Sci. USA (1983, 80:207-2011) and humans, (Krangel Biochemistry (1982) 21:6313-6321; Krangel et al., J. Immunol. (1983) 130:1856-1862; Cowan et al., J. Immunol. (1985) 135:2835-2841; Taketani et al., ibid (1984) .

133:816-821; and Vega *et al.*, Proc. Natl. Acad. Sci. USA (1985) 82:7394-7398).

5 These reports have focused considerable
attention on the region between residues 147 and 157,
although other regions can also produce functional
differences (Ezquerro *et al.*, J. Immunol. (1985)
134:2727-2733). Clusters of variability have been
10 reported at the carboxy-terminal end of the first
extracellular domain and at the amino-terminal end of
the second extracellular domain (Ways *et al.*, J. Biol.
Chem. (1985) 26:11924-11933). Sequences between
residues 105-108 of all Class I molecules are related to
15 that of the fibronectin binding tetrapeptide (Auffray
and Novotny, J. Human Immunology (1986) 15:381-390),
which tetrapeptide in either orientation is found to
have cell attachment properties (Pierschbacher and
Ruoslahti, Nature (1984) 309:30-33; Yamada and Kennedy,
J. Cell Biol. (1985) 28:99-104). Substitution at
20 position 107 affecting a single monoclonal antibody
defined epitope of HLA-A2 has been reported by Salter
et al., J. Exp. Med. (1987) 166:283-288.

SUMMARY OF THE INVENTION

25 Methods and compositions are provided for
modulating cytotoxic T-lymphocyte (CTL) activity toward
target cells. The methods employ using peptide
fragments cross-reactive with the region encompassing
the α_1 - and α_2 -domains of major histocompatibility Class
30 I antigens. Particularly, fragments which include at
least a portion of the amino acids between positions 55
and 120 of the Class I antigen are employed.

Accordingly, one aspect of the invention is a
substantially pure preparation of peptide compound of at

least 8 amino acids and having a sequence coming within the extended sequence:

5 aa⁵⁵ G P E Y W D aa⁶² aa⁶³ T aa⁶⁵ aa⁶⁶ aa⁶⁷ K
 aa⁶⁹ aa⁷⁰ aa⁷¹ Q T aa⁷⁴ R aa⁷⁶ aa⁷⁷ L aa⁷⁹ aa⁸⁰ aa⁸¹
 aa⁸² aa⁸³ Y Y N Q S E A G S H aa⁹⁴ aa⁹⁵ Q aa⁹⁷ M aa⁹⁹ G
 C D aa¹⁰³ G aa¹⁰⁵ D aa¹⁰⁷ R aa¹⁰⁹ L G aa¹¹³ aa¹¹⁴ Q
 aa¹¹⁶ A Y D G

10

wherein:

aa⁵⁵ is E or K;
 aa⁶² is G, Q, E or R;
 aa⁶³ is an acidic amino acid or amide thereof;
 15 aa⁶⁵ is Q, R or G;
 aa⁶⁶ is I, N or K;
 aa⁶⁷ is an aliphatic neutral amino acid;
 aa⁶⁹ is an aliphatic neutral amino acid;
 aa⁷⁰ is Q, H, S, N or K;
 20 aa⁷¹ is an aliphatic neutral amino acid;
 aa⁷⁴ is D, Y or H;
 aa⁷⁶ is E or V;
 aa⁷⁷ is D, S or N;
 aa⁷⁹ is R or G;
 25 aa⁸⁰ is T, I or N;
 aa⁸¹ is an aliphatic non-polar amino acid;
 aa⁸² is R or L;
 aa⁸³ is G or R;
 aa⁹⁴ is T or I;
 30 aa⁹⁵ is a non-polar aliphatic amino acid of
 from 5 to 6 carbon atoms;
 aa⁹⁷ is an aliphatic amino acid or W;
 aa⁹⁹ is an aromatic amino acid;

aa¹⁰³ is a non-polar aliphatic amino acid of
 from 5 to 6 carbon atoms;
 aa¹⁰⁵ is P or S;
 5 aa¹⁰⁷ is G or W;
 aa¹⁰⁹ is L or F;
 aa¹¹³ is Y or H;
 aa¹¹⁴ is H, Q, D, N or R;
 aa¹¹⁶ is Y, D, S, F or H,
 10 which modulates CTL activity.

Another aspect of the invention is a method of
 modulating cytolytic activity of a CTL, said method
 comprising:

15 combining CTL with a peptide compound of at
 least 8 amino acids and not more than about 30 amino
 acids having a sequence differing by not more than two
 mutations from the polymorphic region of the C-terminal
 half of the a₁ domain and the N-terminal half of the a₂
 20 domain of the host of said MHC restricted cells,
 wherein said CTL cells are modulated by said
 peptide.

Still another aspect of the invention is a
 method for inhibiting cytolytic activity of a CTL, said
 25 method comprising combining CTL with a peptide compound
 of at least 8 amino acids and having a sequence coming
 within the extended sequence:

30 G S H aa⁹⁴ aa⁹⁵ Q aa⁹⁷ M aa⁹⁹ G C D aa¹⁰³ G
 aa¹⁰⁵ D aa¹⁰⁷ R aa¹⁰⁹ L R G aa¹¹³ aa¹¹⁴ Q aa¹¹⁶ A Y D G

wherein:

aa⁹⁴ is T or I;

aa⁹⁵ is a non-polar aliphatic amino acid of
 from 5 to 6 carbon atoms;
 aa⁹⁷ is an aliphatic amino acid or W;
 5 aa⁹⁹ is an aromatic amino acid;
 aa¹⁰³ is a non-polar aliphatic amino acid of
 from 5 to 6 carbon atoms;
 aa¹⁰⁵ is P or S;
 aa¹⁰⁷ is G or W;
 10 aa¹⁰⁹ is L or F;
 aa¹¹³ is Y or H;
 aa¹¹⁴ is H, Q, D, N or R;
 aa¹¹⁶ is Y, D, S, F or H;

wherein said CTL cells are inhibited by said peptide.

15 Yet another aspect of the invention is a
 method of inhibiting cytolytic activity of a CTL, said
 method comprising combining CTL with a peptide compound
 of at least 8 amino acids and having a sequence coming
 within the extended sequence:

20 aa⁵⁵ G P E Y W D aa⁶² aa⁶³ T aa⁶⁵ aa⁶⁶ aa⁶⁷ K
 aa⁶⁹ aa⁷⁰ aa⁷¹ Q T aa⁷⁴ R aa⁷⁶ aa⁷⁷ L aa⁷⁹ aa⁸⁰ aa⁸¹
 aa⁸² aa⁸³ Y Y N Q S E A G S H aa⁹⁴ aa⁹⁵ Q aa⁹⁷ M aa⁹⁹ G
 C D aa¹⁰³ G aa¹⁰⁵ D aa¹⁰⁷ R aa¹⁰⁹ LRG aa¹¹³ aa¹¹⁴ Q
 25 aa¹¹⁶ A Y D G

wherein:

30 aa⁵⁵ is E or K;
 aa⁶² is G, Q, E or R;
 aa⁶³ is an acidic amino acid or amide thereof;
 aa⁶⁵ is Q, R or G;
 aa⁶⁶ is I, N or K;
 aa⁶⁷ is an aliphatic neutral amino acid;

- aa⁶⁹ is an aliphatic neutral amino acid;
 aa⁷⁰ is Q, H, S, N or K;
 aa⁷¹ is an aliphatic neutral amino acid;
 5 aa⁷⁴ is D, Y or H;
 aa⁷⁶ is E or V;
 aa⁷⁷ is D, S or N;
 aa⁷⁹ is R or G;
 aa⁸⁰ is T, I or N, particularly T or I;
 10 aa⁸¹ is an aliphatic no-polar amino acid;
 aa⁸² is R or L, particularly R;
 aa⁸³ is G or R;
 aa⁹⁴ is T or I;
 aa⁹⁵ is a non-polar aliphatic amino acid of
 15 from 5 to 6 carbon atoms;
 aa⁹⁷ is an aliphatic amino acid or W;
 aa⁹⁹ is an aromatic amino acid;
 aa¹⁰³ is a non-polar aliphatic amino acid of
 from 5 to 6 carbon atoms;
 20 aa¹⁰⁵ is P or S;
 aa¹⁰⁷ is G or W;
 aa¹⁰⁹ is L or F;
 aa¹¹³ is Y or H;
 aa¹¹⁴ is H, Q, D, N, or R;
 25 aa¹¹⁶ is Y, D, S, F, or H.

Another aspect of the invention is a method of
 sensitizing MHC restricted cells to CTL, said method
 comprising combining MHC restricted cells with a peptide
 compound of at least 8 amino acids and having a sequence
 30 coming within the extended sequence:

aa⁵⁵ G P E Y W D aa⁶² aa⁶³ T aa⁶⁵
 aa⁶⁶ aa⁶⁷ K aa⁶⁹ aa⁷⁰ aa⁷¹ Q T aa⁷⁴ R aa⁷⁶ aa⁷⁷ L aa⁷⁹
 aa⁸⁰ aa⁸¹ aa⁸² aa⁸³ Y Y N Q S E A

- 5 aa⁵⁵ is E or K, particularly E;
- aa⁶² is G, Q, E or R, particularly R or G;
- aa⁶³ is an acidic amino acid or amide thereof;
- including E and N, particularly E;
- aa⁶⁵ is Q, R or G, particularly Q or R;
- aa⁶⁶ is I, N or K, particularly N or K;
- aa⁶⁷ is an aliphatic neutral amino acid
- 10 including, V, M, S, C and Y, particularly V,
- aa⁶⁹ is an aliphatic neutral amino acid
- including, A, T and P, particularly A;
- aa⁷⁰ is Q, H, S, N or K, particularly Q or H;
- aa⁷¹ is an aliphatic neutral amino acid
- 15 including S, A and T, particularly S;
- aa⁷⁴ is D, Y or H, particularly D or H;
- aa⁷⁶ is E or V;
- aa⁷⁷ is D, S or N, particularly D;
- aa⁷⁹ is R or G, particularly G;
- 20 aa⁸⁰ is T, I or N, particularly T or I;
- aa⁸¹ is an aliphatic non-polar amino acid
- including L or A, particularly L;
- aa⁸² is R or L, particularly R;
- aa⁸³ is G or R, particularly G.

25 Of particular interest is a sequence or sequence fragment of at least 8 amino acids of the sequence:

30 Q E G P E Y W D (G or R) (E or N) T (R or Q)
 (K or N) V K A (H or Q) S Q T (H or D) R (V or E) (D,
 S or N) L (G or R) (T or I) (L or A) (R or L) (G or
 R) Y Y N Q S E A

wherein the CTL cells cause cytolysis of the MHC restricted cells combined with said peptide.

5 Still another aspect of the invention is a method for inhibiting cytolytic activity of a CTL by irreversibly inhibiting the CTL, said method comprising:
combining a CTL with a peptide compound of at least 8 amino acids and having a sequence coming within
10 the extended sequences:

T L Q R M Y G C D V G S D W R F L R G ,

M Y G C D V G S D W R F L R G Y ,

15 M Y G C D V G S D G R F L R G Y ,

G P E Y W D G E T R K V K A , and

G P E Y W D R N T R N V K A ,

20 wherein the peptide compound binds to the CTL.

Yet another aspect of the invention is a method for determining the presence of MHC restricted CTL's, said method comprising:

25 contacting cells with a peptide compound of at least 8 amino acids and having a sequence coming within the extended sequence:

30 aa⁵⁵ G P E Y W D aa⁶² aa⁶³ T aa⁶⁵ aa⁶⁶ aa⁶⁷ K
aa⁶⁹ aa⁷⁰ aa⁷¹ Q T aa⁷⁴ R aa⁷⁶ aa⁷⁷ L aa⁷⁹ aa⁸⁰ aa⁸¹
aa⁸² aa⁸³ Y Y N Q S E A G S H aa⁹⁴ aa⁹⁵ Q aa⁹⁷ M aa⁹⁹ G
C D aa¹⁰³ G aa¹⁰⁵ D aa¹⁰⁷ R aa¹⁰⁹ L R G aa¹¹³ aa¹¹⁴ Q
aa¹¹⁶ A Y D G

wherein:

- 5 aa⁵⁵ is E or K;
 aa⁶² is G, Q, E or R;
 aa⁶³ is an acidic amino acid or amide thereof;
 aa⁶⁵ is Q, R or G;
 aa⁶⁶ is I, N or K;
 aa⁶⁷ is an aliphatic neutral amino acid;
 aa⁶⁹ is an aliphatic neutral amino acid;
 aa⁷⁰ is Q, H, S, N or K;
10 aa⁷¹ is an aliphatic neutral amino acid;
 aa⁷⁴ is D, Y or H;
 aa⁷⁶ is E or V;
 aa⁷⁷ is D, S or N;
 aa⁷⁹ is R or G;
15 aa⁸⁰ is T, I or N, particularly T or I;
 aa⁸¹ is an aliphatic no-polar amino acid;
 aa⁸² is R or L, particularly R;
 aa⁸³ is G or R;
 aa⁹⁴ is T or I;
20 aa⁹⁵ is a non-polar aliphatic amino acid of
 from 5 to 6 carbon atoms;
 aa⁹⁷ is an aliphatic amino acid or W;
 aa⁹⁹ is an aromatic amino acid;
 aa¹⁰³ is a non-polar aliphatic amino acid of
25 from 5 to 6 carbon atoms;
 aa¹⁰⁵ is P or S;
 aa¹⁰⁷ is G or W;
 aa¹⁰⁹ is L or F;
 aa¹¹³ is Y or H;
30 aa¹¹⁴ is H, Q, D, N, or R;
 aa¹¹⁶ is Y, D, S, F, or H.

covalently joined to a compound capable of providing a detectable signal; and

determining the presence of cells to which said detectable signal compound is specifically bound.

5 Still another aspect of the invention is a substantially pure preparation of peptide compound selected from the sequences:

10 T L Q R M Y G C D V G S D W R F L R G ,
 M Y G C D V G S D W R F L R G Y ,
 M Y G C D V G S D G R F L R G Y ,
 G P E Y W D G E T R K V K A , and
 15 G P E Y W D R N T R N V K A .

Yet another aspect of the invention is a pharmaceutical composition comprised of a peptide compound of at least 8 amino acids and having a sequence coming within the extended sequence,

20 aa⁵⁵ G P E Y W D aa⁶² aa⁶³ T aa⁶⁵ aa⁶⁶ aa⁶⁷ K
 aa⁶⁹ aa⁷⁰ aa⁷¹ Q T aa⁷⁴ R aa⁷⁶ aa⁷⁷ L aa⁷⁹ aa⁸⁰ aa⁸¹
 aa⁸² aa⁸³ Y Y N Q S E A G S H aa⁹⁴ aa⁹⁵ Q aa⁹⁷ M aa⁹⁹ G
 25 C D aa¹⁰³ G aa¹⁰⁵ D aa¹⁰⁷ R aa¹⁰⁹ L R G aa¹¹³ aa¹¹⁴ Q
 aa¹¹⁶ A Y D G

wherein:

30 aa⁵⁵ is E or K;
 aa⁶² is G, Q, E or R;
 aa⁶³ is an acidic amino acid or amide thereof;
 aa⁶⁵ is Q, R or G;
 aa⁶⁶ is I, N or K;
 aa⁶⁷ is an aliphatic neutral amino acid;
 aa⁶⁹ is an aliphatic neutral amino acid;

aa⁷⁰ is Q, H, S, N or K;
aa⁷¹ is an aliphatic neutral amino acid;
aa⁷⁴ is D, Y or H;
aa⁷⁶ is E or V;
aa⁷⁷ is D, C or N;
aa⁷⁹ is R or G;
aa⁸⁰ is T, I or N;
aa⁸¹ is an aliphatic non-polar amino acid;
10 aa⁸² is R or L;
aa⁸³ is G or R;
aa⁹⁴ is T or I;
aa⁹⁵ is a non-polar aliphatic amino acid of
from 5 to 6 carbon atoms;
15 aa⁹⁷ is an aliphatic amino acid or W;
aa⁹⁹ is an aromatic amino acid;
aa¹⁰³ is a non-polar aliphatic amino acid of
from 5 to 6 carbon atoms;
20 aa¹⁰⁵ is P or S;
aa¹⁰⁷ is G or W;
aa¹⁰⁹ is L or F;
aa¹¹³ is Y or H;
aa¹¹⁴ is H, Q, D, N or R;
aa¹¹⁶ is Y, D, S, F or H.

25 wherein said peptide compound is present in a
pharmacologically effective dose in a pharmaceutically
acceptable excipient.

30 BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the minimum size of peptide
sequence required for inhibition of cytolysis by HLA-A2
specific CTL.

Fig. 2 shows the effect of pretreatment of CTL and of target cells on the inhibition of cytolysis by HLA-A2 specific CTL.

5 Fig. 3 shows the effect of peptide A2.98-113 on release of granules containing serine esterase during cytolysis of target cells by CTL.

10 Fig. 4 shows the consensus sequence of peptides which constitute the α_1 , α_2 , and α_3 regions of a class I HLA molecule, as well as changes in these sequences in different specific HLA molecules.

Fig. 5 shows the effect of peptides from different HLA-A2 epitopes on cytolysis of target cells by CTL of different specificities.

15 Fig. 6 shows the sensitization of an HLA-Aw69 target cell to cytolysis by clone A2/B17 cells caused by peptide A2.56-69.

20 Fig. 7 shows the effect on sensitization of incubating target cells or clone A2/B17 cells with peptide A2.56-69.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

25 Methods and compositions are provided for modulating the effect of cytotoxic T lymphocytes or diagnosing the presence of CTL's of a predetermined specificity. The compositions are peptide sequences cross reactive with a polymorphic portion of the α_1 or α_2 domains of Class I major histocompatibility antigens, particularly the C-terminal portion of the α_1 domain and
30 the N-terminal portion of the α_2 domain. These major histocompatibility antigens are classified as Groups A, B, and C, having a substantial number of subclassifications within these groups, where the different Class I antigens are able to bind specifically

to complementary CTL's, which are restricted by such antigens. Of particular interest for the α_1 domain is the amino acid sequence from positions 55 to 85, more particularly 55 to 80, usually 55 to 70, desirably including within the sequence a tetrapeptide DGET, GETR, DRET, or YWDG. Of particular interest for the α_2 -domain is the amino acid sequence from positions 90 to 120, more particularly 94 to 116, desirably including within the sequence a tetrapeptide SDWR or SDGR.

The peptides of interest which will serve as the receptor binding peptide will have at least 8 amino acids, usually at least 10 amino acids, and usually not more than about 30 amino acids, more usually not more than about 24 amino acids, desirably having about 12 to 21 amino acids. The amino acid sequence will usually not differ from a naturally occurring sequence by more than 2 amino acids, or mutations, e.g., deletions or insertions, more usually by not more than about 1 amino acid. The sequence employed will usually be from the polymorphic regions of the C-terminal half of the α_1 domain or the N-terminal half of the α_2 domain of the MHC antigen of the host of the MHC restricted T-cells, particularly an HLA-A group antigen.

For the most part, the amino acid sequences for the α_1 region will come within the following formula:

55 aa⁵⁵ G P E Y W D aa⁶² aa⁶³ T aa⁶⁵ aa⁶⁶ aa⁶⁷ K aa⁶⁹
 30 aa⁷⁰ aa⁷¹ Q T aa⁷⁴ R aa⁷⁶ aa⁷⁷ L aa⁷⁹ aa⁸⁰ aa⁸¹ aa⁸²
 aa⁸³ Y Y N Q S E A

aa⁵⁵ is E or K, particularly E;

aa⁶² is G, Q, E or R, particularly R or G;

aa⁶³ is an acidic amino acid or amide thereof;
including E and N, particularly E;

aa⁶⁵ is Q, R or G, particularly Q or R;

5 aa⁶⁶ is I, N or K, particularly N or K;

aa⁶⁷ is an aliphatic neutral amino acid
including, V, M, S, C and Y, particularly V,

aa⁶⁹ is an aliphatic neutral amino acid
including, A, T and P, particularly A;

10 aa⁷⁰ is Q, H, S, N or K, particularly Q or H;

aa⁷¹ is an aliphatic neutral amino acid
including S, A and T, particularly S;

aa⁷⁴ is D, Y or H, particularly D or H;

aa⁷⁶ is E or V;

15 aa⁷⁷ is D, S or N, particularly D;

aa⁷⁹ is R or G, particularly G;

aa⁸⁰ is T, I or N, particularly T or I;

aa⁸¹ is an aliphatic non-polar amino acid
including L or A, particularly L;

20 aa⁸² is R or L, particularly R;

aa⁸³ is G or R, particularly G.

25 The peptide may be extended by bonding at one
or both termini with other than a wild-type sequence for
the HLA-A2 antigen.

Of particular interest is a sequence or
sequence fragment of at least 8 amino acids of the
sequence:

30

Q E G P E Y W D (G or R) (E or N) T (R or Q) (K or N) V
K A (H or Q) S Q T (H or D) R (V or E) (D, S or N) L (G
or R) (T or I) (L or A) (R or L) (G or R) Y Y N Q S E A

G S H T [V, I, or L] Q R M Y G C D V G S D [W or G] R F
L R G Y H Q Y A Y D G.

5 Where there are two or more amino acids
indicated at the same site, any of the indicated amino
acids may be present.

 The region of particular interest will be the
region from amino acid positions 100 to 116.

10 The classes of amino acids are designated as
follows:

 aliphatic

 non-polar G, A, P, L, I, V

 polar

15 neutral C, S, T, M, N, Q

 acidic D, E

 basic K, R

 aromatic F, H, W, Y

20 The peptides may be prepared in a variety of
ways. Conveniently, they can be synthesized by
conventional techniques employing automatic
synthesizers, such as the Beckman, Applied Biosystem
Inc., or other useful peptide synthesizer apparatus, or
may be synthesized manually. Alternatively, DNA
25 sequences can be prepared which encode the particular
peptide and may be cloned and expressed to provide the
desired peptide. In this instance a methionine may be
the first amino acid.

30 The peptides may also be isolated from natural
sources and purified by known techniques, including, for
example, chromatography on ion exchange materials,
separation by size, immunoaffinity chromatography and
electrophoresis. As used herein, the term "a
substantially pure preparation of peptide compound"

means a preparation of the peptide which is usually greater than about 70% free of materials with which the polypeptide is naturally associated, and preferably greater than about 80% free of these materials; these materials, however, excludes materials with which the peptide may be mixed in the preparation of pharmaceutical compositions.

The sequences may be modified in a variety of ways depending upon their ultimate purpose. Different N- or C- terminal groups may be introduced which allow for linking of the peptide to solid substrates or other molecules. In a synthetic procedure, any molecule may be introduced at the N- or C-terminus which would allow for subsequent reaction, depending upon the purpose for which the peptide is prepared.

For diagnostic purposes, a wide variety of labels may be linked to the terminus, which may provide, directly or indirectly, a detectable signal. For example, fluorescers may be introduced at the terminus or other molecules which provide a linkage to labels such as fluorescers, enzymes, particles, or the like. For example, linkage may be introduced at the terminus, e.g., biotin, which will bind to an avidin conjugate with enzymes or fluorescers. Alternatively, various reactive sites may be introduced at the terminus for linking to particles, solid substrates, macromolecules, or the like. For example, an internal amino moiety of a growing chain bound to a solid substrate with the intermediate side groups protected, may be conjugated with methyldithiobenzoic acid (MDTB). The free mercaptan group may then be used for conjugating with activated olefins. Thus, proteins, such as serum albumin, keyhole limpet hemocyanin, bovine β -globulin,

or the like, may be conjugated to the peptide to provide for an immunogen to produce antibodies to the peptide for use in immunoassays, for affinity chromatography, or the like. Alternatively, the peptide can be bonded to another polypeptide by preparing a DNA sequence which has the peptide at the N-terminus, C-terminus or internal to the protein, so as to provide a fused protein which includes the binding peptide of interest. In this manner, fused proteins may be produced which have enzymatic activity, which enzymatic activity may be modulated by macromolecules, e.g., antibodies, binding to the peptide of interest. Thus, the peptides of the subject invention may be modified in a wide variety of ways for a variety of end purposes while still retaining biological activity.

The subject peptides may also be used in combination with antigenic peptides or proteins of interest to activate CTL's. Thus, the subject peptides may be bound to a protein, either directly or indirectly, so as to be able to present two epitopes to the CTL to which the CTL may bind and be activated. Of particular interest, is where the subject peptides may be bound to a liposome or a bilayer lipid membrane in conjunction with a peptide or protein providing the other determinant site.

Various techniques are available for joining a peptide or protein to a lipid, particularly a phospholipid to provide for the presence of the peptide or protein on the liposome surface. Phosphatidyl choline, phosphatidyl ethanolamine, or other lipid may be used with a bifunctional linking agent, such as MBSE, glutaraldehyde, methyldithiobenzoic acid, or the like. The formation of liposomes with conjugated proteins

finds ample support in the literature, see, for example, U.S. Patent Nos. 3,887,698; 4,261,975; and 4,193,983. The modified peptide or protein is combined with the lipids in an aqueous medium and sonicated to provide the desired liposomes. The liposomes may then be harvested and used in the ways indicated.

The subject peptides, by themselves, or in combination with other peptides or proteins, may be used for diagnosing the presence of CTL's which bind to a subject peptide or the combination of a subject peptide and other peptide or protein. In this manner, conjugates of the subject peptide and the antigenic peptide or protein can be prepared by employing linking agents as described previously. Alternatively, the subject peptide and the antigenic peptide may be bound to a solid surface, such as a particle, container surface, or the like. If desired, the subject peptide and antigenic peptide or protein may be conjugated to a particle or protein which is fluorescent. The binding of the particle or protein will allow for sorting and counting in a fluorescence activated cell sorter.

The subject peptides may also be used for modulating CTL activity in the mammalian host. The modulation may be by inhibiting CTL activity or by sensitizing target cells. This can be achieved by employing apheresis, where the patient's blood is withdrawn from the patient and circulated through a device in which the peptide is present, either bound to the surface, to remove CTL's active with the subject peptide or in a physiologically acceptable medium to bind to the CTL's and inhibit their activity. Alternatively, the subject peptides may be administered

to a host intravascularly, in either an artery or vein, to provide for inhibition or stimulation of the CTL.

5 Examples of inhibitory peptides are presented infra (see Examples 2 and 9), which are derived from both the α_1 and α_2 domain of HLA-A2. In each case the sequence of the inhibitory peptide correlates with the epitope specificity of the CTL. Moreover, as shown in Example 4, inhibition is mediated by an octapeptide, and occurs by peptide binding to the CTL and not the target cell (see Example 5). Since the inhibitory capacity of the individual peptides correlates with CTL specificity, it seems likely that these peptides inhibit by binding to the variable T cell receptor.

15 An example of a peptide which stimulates cytolysis of a HLA-class I bearing target cells by alloreactive CTL is presented in Example 10, infra. The simplest interpretation of the results in Examples 10-12 is that the HLA-A2/B17 specific CTL recognize the A2 56-69 peptide in the context of HLA-Aw69 as a restriction element. This implies that the peptide is binding to the HLA-Aw69 molecule. The data and interpretation are similar to those obtained in the influenza (Bastin et al., J. Exp. Med., 165:1508 (1987));
25 Gotch et al., Nature 326:881 (1987)) and xenogeneic systems (Maryanski et al., Nature 324:578 (1986)), and demonstrate that alloreactive CTL can recognize Class I derived peptides in a Class I restricted fashion. However, the quantities of peptide required to cause sensitization are significantly larger than those
30 reported in other studies. Although the molecular basis for this is as yet unknown, one possible explanation involves the relative handling of exogenous versus endogenous molecules. For example, HLA is an endogenous

molecule, and the exogenous HLA peptides may have to compete with endogenous HLA peptides. Another alternative is that the A2.56-69 peptide may not include all of the residues required for high affinity binding to the target cell.

Two of the CTL epitopes from which the peptides described in the Examples, *infra.* are derived, are situated in very different parts of the HLA-A2 molecule. Residues 62-65 are in an alpha helix which forms part of the peptide binding site (Bjorkman *et al.*, Nature 329:506 (1987)), which is itself thought capable of binding alpha helical peptides. As shown in the Examples, peptides in this region can either inhibit or induce cytolysis. In the induction of cytolysis, it is possible that the peptide may bind to a target cell HLA class I antigen and thereby create a structure which is recognized by the CTL. For example, in the case of A2.56-69 peptide conferring sensitivity to clone A2/B17 cells on HLA-Aw69 cells, the bound peptide presumably substitutes for the α helix of the α_1 domain, since HLA-Aw69 and HLA-A2 have identical α_2 domains.

In contrast, residue 107 is part of a turn between two strands of β structure at some distance from the alpha helices and peptide binding region (Bjorkman *et al.*, supra.) The A2.98-113 peptide may maintain elements of this structure in solution and have little affinity for the peptide binding site of Class I molecules. This interpretation would explain the observation in the Examples that peptides corresponding to this region are inhibitors of HLA-A2 directed cytolysis, but cannot induce cytolysis.

As already indicated, the peptide may be present by itself, or in combination with an antigen

thereby providing a different determinant site of interest. Depending upon whether only the subject peptide is included, or the peptide in combination with other peptides, activation or inhibition can be achieved.

If irreversible inhibition is desired, the conjugate of the subject peptide with the antigen may be joined to a cytotoxic agent, joined to liposomes containing cytotoxic agents, or joined to a specific monoclonal antibody or immunoglobulin, whereby binding of the conjugate to the CTL will result in the complement mediated lysis of the CTL.

The subject peptides, by themselves or as conjugates, may be prepared as formulations in pharmaceutically acceptable media, for example saline, PBS, and glucose, generally at a pharmacologically effective dose, the concentrations of which will be determined empirically in accordance with conventional procedures for the particular purpose. The additives may include bactericidal agents, stabilizers, buffers, or the like. The amount administered to the host will vary depending upon what is being administered, the purpose of the administration, such as prophylaxis or therapy, whether inhibition or activation is desired, the state of the host, the manner of administration, and the like. In order to enhance the half-life of the subject peptide or subject peptide conjugates, the peptides may be encapsulated, introduced into the lumen of liposomes, prepared as a colloid, or other conventional technique may be employed which provides an extended lifetime of the peptides.

The following examples are offered by way of illustration and not by limitation.

EXAMPLES

5

Example 1Preparation of Peptides Derived from
HLA-A2

Four peptides were prepared by conventional synthetic methods using standard solid-phase methods. See Erickson & Merrifield in: The Proteins Vol. 2, 3rd edition (eds. Neurath, H. & Hill, R.L.) p. 255-527 (Academic Press, N.Y. 1970), which is hereby incorporated herein by reference. Three of the peptides had amino acids from the α_2 domain and one of the peptides had amino acids from the α_1 domain of a HLA-A2 antigen. The four peptides had the following compositions and designations:

20

A2.56-69 G P E Y W D G E T R K V K A

A2.94-112 T L Q R M Y G C D V G S D W R F L R G

A2.98-113 M Y G C D V G S D W R F L R G Y

25

Aw.68 98-113 M Y G C D V G S D G R F L R G Y

30

The designations indicate the major histocompatibility antigen from which the peptide is derived, and the position of the amino acids in the antigen.

Example 2Inhibition of HLA-A2 Specific CTL
by Peptides Derived from HLA-A2.98-113
and HLA-A2.94-112

5 Peptides prepared as in Example 1, i.e., those
corresponding to HLA-A2.56-69, HLA-A2.94-112,
HLA-A2.98-113, and HLA-Aw 68.98-113, were preincubated
for 30 min. with $1-3 \times 10^3$ CTLs before addition of 10^3
10 CPM of ^{51}Cr -labeled B-lymphoblastoid target cells. The
cytotoxicity assay was then performed as described by
Clayberger *et al.*, J. Exp. Med. (1984) 162:1709-1714;
and Reiss *et al.*, Proc. Natl. Acad. Sci. USA (1980)
77:5432-5436, which are hereby incorporated herein by
15 reference.

In the first study, the CTL cell line was AJY,
a long term CD8^+ CTL line specific for HLA-A2, and the
target cell was the B-lymphoblastoid cell line JY
(HLA-A2, B7). In the second study the CTL was PWSB, a
20 bulk culture with reactivity against HLA-B17 and the
target was FMB, which expresses HLA-A1, A32, B17. In
each case the percentage of specific release obtained in
the absence of peptide was determined. The lower amount
of specific release in the second study potentially made
25 cytotoxicity more sensitive to inhibition. Stocks of
peptides at 1 mg/ml in PBS were diluted to give final
concentrations in the assay as indicated in Table 1. As
a control inhibitor, the monoclonal antibody PA2.6 which
is directed against the monomorphic determinant of
30 HLA-A, B, C molecules was used (Reiss *et al.*, supra:
McMichael, J. Exp. Med. (1980) 152:195s-203s). The
peptides employed were A2.98-113, A2.94-112, Aw68. 94
112 and A2.56-69. The following table indicates the
results.

Table 1

		<u>% Specific Lysis</u>				
		<u>Concentration</u>	A2.98-113	A2.94-112	Aw68.94-112	A2.56-69
		<u>µg/ml</u>				
5	Trial 1.	160	0	3	52	51
	CTL=AJY	80	4	20	45	38
10	Target=JY	40	18	35	63	61
	Trial 2.	160	27	35	28	20
	CTL=PWSB	80	29	32	30	27
	Target=FMB	40	30	34	35	31

15 In the first case, the percentage specific release obtained in the absence of peptide was about 54, while in the second case it was about 28.

The above results with CTL which are restricted by the HLA-A2 antigen, show inhibition of specific cytotoxicity. With CTL's not restricted by A2, lysis of random target cells occurs with the results approximating the standard specific release obtained in the absence of peptide. These results suggest that the tryptophan at position 107 may be critical. Peptide
 20 A2.98-113 and peptide Aw68.98-113 are homologous except for the substitution of glycine for tryptophan at this position; this substitution resulted in a loss of inhibition of cytolysis by HLA-A2 specific CTL.
 25

The results of treatment of peptide A2.98-113 with different proteases, i.e., trypsin or chymotrypsin, allow the suggestion that arginine 108 is of importance, but that peptides 109-113 are not critical. The major sites of action of trypsin and chymotrypsin are Arg, Lys, and Trp, Phe, Tyr, respectively. Chymotryptic, but
 30

not tryptic, cleavage of the peptide reduced the inhibitory activity (results not shown).

5 Example 3

Effect of Specificity of CTL and Target Cell
on Inhibition of Cytolysis Caused by
HLA-Derived Peptides

10 A number of different CTL cell lines were studied, where the specificity of the cell lines were varied. The results shown in Table 2 indicate that only where the CTL's and the target cells share A2 specificity do the A2-derived peptides provide inhibition.

15

20

25

30

Example 4Minimum Peptide Sequence Required
for Inhibition of HLA-A2 Specific CTL

5 The minimum peptide sequence required for inhibition of cytolysis by HLA-A2 specific CTL was determined by examining the effect of size on the inhibition.

10 A series of peptides which started at positions 98-104 and ended at position 108 of HLA-A2 or HLA-Aw68 were synthesized. The effect of these peptides on cytolysis of JY cells (HLA-A2, B7, DR4,6) by seventeen different HLA-A specific lines or clones were tested. The HLA-A2 specific lines or clones were
15 generated as described in Clayberger et al., supra. Peptides (200 mg/ml) were preincubated with $1-3 \times 10^3$ CTL for 30 minutes prior to addition of 10^3 CPM of ^{51}Cr -labeled target cells. The peptides were present throughout the cytotoxicity assay which was performed as
20 described in Clayberger et al., supra, and in Krensky et al., Proc. Natl. Acad. Sci. USA 79:2365 (1982), which are hereby incorporated herein by reference. Peptides were prepared as stock solutions at 1 mg/ml in phosphate buffered saline and diluted in complete medium (MEM
25 supplemented with 10% calf serum) to give the final concentration used.

30 The results on the inhibition of cytolysis by CTL-A2 is shown in Fig. 1A, where inhibition is expressed as $(1 - [\text{specific cytolysis in the presence of peptide} / \text{specific cytolysis in the absence of peptide}]) \times 100$.

As seen in the figure, peptide 104-108 did not inhibit, peptides 102-108 and 103-108 caused weak inhibition, and the remaining peptides caused good

inhibition of cytolysis. Thus, an octapeptide comprising residues 101-108 was sufficient to cause the inhibitory effect.

5 A major decrease in the inhibitory effect occurs with loss of the cysteine at position 101. This loss may be due to the loss of disulfide cross-linking of two peptide molecules when cysteine 101 is absent.

10 Example 5

Locus of Action of Peptide A2.98-113

The locus at which peptide A2.98-113 interacts to cause an inhibitory effect on HLA-A2 specific CTL mediated cytolysis, i.e., with the CTL and/or with the target cell, was determined as follows.

15 The CTL (1×10^6 CTL-A2) and/or the target cells (^{51}Cr -labeled JY target cells) were incubated with 100 μg of A2.98-113 for 30 min. at 37°C , or alternatively with the control peptide, Aw68.98-113. 20 The sequences of these peptides are presented in Example 1. As an additional control, the cells were incubated with complete medium minus peptide. Following the incubation, the cells were washed three times in complete medium, and tested in a ^{51}Cr -release assay (see 25 Example 2).

The results are presented in Fig. 2, where it may be seen that lysis was inhibited when the CTL, but not the target cells, were pretreated with A2.98-113. Inhibitory effects were not observed when CTL or target 30 cells were pretreated with the control peptide, Aw68.98-113.

Example 6Mechanism of Inhibition of CTL by A2.98-113Effect on CTL Viability

5 To determine whether CTL were inhibited due to their autolysis induced by A2.98-113, either ⁵¹Cr-labeled CTL-A2 cells or unlabeled CTL-A2 cells were incubated with the peptide for 6 hours at 37°C in complete medium. During the 6 hour incubation there was
10 no detectable decrease in cell viability as judged by exclusion of trypan blue or by ⁵¹Cr-release (results not shown).

Example 7Mechanism of Inhibition of CTL by A2.98-113Effect on Release of Granules ContainingSerine Esterase

15 The effect of A2.98-113 on release of granules containing serine esterase during cytolysis of target cells by CTL was determined as follows.

The specificity of release was determined by incubating 3×10^5 HLA-A2 specific CTL with JY cells (HLA-A2; B7; Dr4,6) or IBW4 cells (HLA-A3; B35; DR1) for 2 hours in V bottom microtiter wells. The ratios of
25 CTL:target cells were 1:0.01, 1:0.05, 1:0.10, 1:0.5, and 1:1. After the incubation, the plates were spun at 1000 RPM for 2 minutes, and the supernatant was assayed for serine esterase activity essentially as described in Young et al., Cell 47:183 (1986), which is hereby
30 incorporated herein by reference. The reaction mixtures consisted of 20 μ l of supernatant plus 200 μ l of substrate (2×10^{-4} M N-benzyloxycarbonyl-L-lysine thiobenzyl ester, 2.2×10^{-4} M nitrobenzoic acid, 0.1M Tris-HCl, pH 8.0). After 30 min. at 37°C, the

absorbance was determined at 410 nm. Total serine esterase activity was determined by substituting 0.01% Triton X-100 for stimulator cells.

5 The results, shown in Fig. 3A, indicate that release of the granules occurred when the HLA-A2 specific CTL were incubated with JY cells (closed circles), but not when the HLA-A2 specific CTL were incubated with IBW4 cells (closed squares).

10 The effect of peptide A2.98-113 on release of granules containing serine esterase was determined in a similar fashion, except that the HLA-A2 specific CTL were preincubated with 100 µg of peptide, either A2.98-113 or Aw68.98-113, or with only complete medium, for 30 min. at 37°C prior to the addition of JY target cells at ratios of CTL:target cells of 1:0.01, 1:0.05, 15 1:0.1, 1:0.5 and 1:1.

 As seen in Fig. 3B, complete inhibition of esterase release was seen with 100 µg/ml of A2.98-113 at an effector-to-target ratio of 1:0.1 (closed squares). 20 The control peptide Aw68.98-113 had no effect on esterase release (closed triangles), since release in this case was equal to that obtained with control cells preincubated with complete medium (closed circles).

25 These results, in conjunction with those in Example 5 indicate that the A2.98-113 peptide blocks events which occur early in T cell activation by binding directly to the CTL. This binding may be to the antigen receptor.

30

Example 8Isolation of CTL Specific for the Epitope Shared
by HLA-A2 and HLA-B17, for HLA-B17, and for HLA-2

5 CTL with the various specificities were
derived from the peripheral blood lymphocytes of a
normal donor (HLA-A3; B7; DR6) essentially as described
by Clayberger et al. (1985), supra. For CTL specific
for the epitope shared between HLA-A2 and HLA-B17, the
10 cells were stimulated in primary culture with the
irradiated (10,000R) B-lymphoblastoid cell line Mag
(HLA-A26,33; B17,51) and cloned using the SB cell line
(HLA-A1,2; B17,44; DR2,6) as stimulators. CTL specific
for B17 were derived from the same primary culture, but
15 were cloned using the SH cell line (HLA-A3,w33;
B7,17(w57)) as stimulators. HLA-A2 specific CTL were
derived from cell stimulated in primary culture with the
JY cell line and cloned using the Herluff cell line
(HLA-A2; B12,35; DR4,7) as stimulators. The fine
20 specificity of these CTL clones was assessed using a
panel of 11 targets expressing HLA-B17, 8 targets
expressing HLA-A2 and 15 targets with unrelated HLA
molecules. Multiple clones of the desired specificities
were obtained. An individual clone which caused
25 cytolysis of both HLA-A2 type target cells and HLA-B17
type target cells was designated clone A2/B17. The
cytolysis of target cells of clone A2/B17 was inhibited
by antibody MA2.1. A second clone, which lysed all
HLA-B17 target cells but no others was designated B17.
30 A third clone, which lysed all HLA-A2 target cells but
no others was designated CTL-A2.

The target specificity of clone A2/B17 and the
finding that cytolysis by this clone was blocked by
monoclonal antibody MA2.1 indicates that cells of clone

The epitopes studied and peptides used in the study are shown in Fig. 4, where the protein sequences in the three extracellular domains (α_1 , α_2 and α_3) of eight HLA-A,B molecules are shown using the standard one letter amino acid code. The sequence of HLA.Bw58 subtype of HLA-B17 is from Ways et al., J. Biol. Chem. 260:11924 (1985), that of HLA-A3.1 is from Strachen et al., EMBO J 3:887 (1984), and the remaining sequences of the HLA-A2/28 family are from Holmes et al., J. Immunol. 139:936 (1987). Peptides A2.56-69 and Aw68.56-69, and A2.98-113 and Aw68.98-113, which are derived from α_1 and α_2 , respectively, are indicated by cross-hatching. The two residues found to be critical for the epitopes shared by subtypes of HLA-A2 and HLA-B17 (glycine 62) and subtypes HLA-A2 and HLA-Aw69 (tryptophan 107) are indicated by stippling and the vertical arrows. The consensus sequence is derived from a total of 23 HLA-A,B,C sequences.

The CTL were incubated with peptides at concentrations of 100 $\mu\text{g/ml}$, 200 $\mu\text{g/ml}$, or 300 $\mu\text{g/ml}$. Control samples were incubated in the absence of peptide. The final molar concentrations of peptides used in the assay at 100 $\mu\text{g/ml}$ were $4.9 \times 10^{-5}\text{M}$ for A2.98-113; $5.2 \times 10^{-5}\text{M}$ for Aw68.98-113; $5.9 \times 10^{-5}\text{M}$ for A2.56-69; and $5.9 \times 10^{-5}\text{M}$ for Aw68.56-69. The CTL cells were incubated with the peptides for 20 min. prior to the addition of 10^3 ^{51}Cr -labeled T7529 cells (HLA-Aw33; B17(w58); DR6) or JY cells (HLA-A2; B17; DR4,6). In all cases, the effector-to-target ratios were 1:1.

The results on cytotoxicity, as measured by ^{51}Cr chromium release from the target cells, is shown in Fig. 5. Figures 5A and 5B show the results of the effects of the peptides on cells of clone A2/B17; Fig.

5C shows the effects on cells of clone B17, and Fig. 5D on CTL-A2. The peptides are indicated as follows: (open circles) A2.56-69; (open squares) Aw68.56-69; (open triangles) Aw.98-113; and (closed squares) Aw68.98-113.

Peptide A2.56-69, which encompasses the shared serologic epitope, specifically inhibited the killing of both HLA-A2 and HLA-B17 expressing target cells by clone A2/B17 cells. In contrast, this peptide had no effect upon the lysis of HLA-B17 expressing cells by clone B17 cells. Clone A2/B17 cells were not inhibited by a peptide derived from residues 56-69 of HLA-Aw68.1, or by a series of unrelated peptides. The A2.98-113 peptide did not affect the lysis of HLA-B17 expressing targets by clone A2/B17 cells, but some inhibition was observed at high concentrations with HLA-A2 expressing targets. This difference indicates that the epitopes of HLA-A2 and HLA-B17 recognized by clone A2/B17 cells are not precisely the same.

These results show that the capacity of peptides to inhibit alloreactive CTL is not restricted to the region involving residues 101-108 of the α_2 domain, and that they may be derived from a second epitope of HLA-A2.

The discrepancy of the results achieved with peptide A2.56-69 using clone A2/B17, and those with the PWSB cell line (see Table 2) with respect to the inhibitory effect of this peptide may be explained by the polyclonal nature of the PWSB cells. That is, the PWSB line probably is a mixture of CTL's including individual clones specific for HLA-A2 or HLA-B17.

Example 10Sensitization of Target Cells to CTL caused by a
HLA-2 Derived Polypeptide

5 Clone A2/B17 was incubated with peptide
A2.56-69 and ⁵¹Cr-labeled target cells at an
effector-to-target ratio of 5:1 for 5 hours, after which
⁵¹chromium released was measured. The concentrations of
peptide were 10, 30, 100, and 300 µg/ml. The results of
10 the effect of peptide on the percent of specific lysis
of the target cells by clone A2/B17 cells are presented
in Fig. 6. The target cells were: (closed square), IBW4
(HLA-A3; B35; DR1); (closed triangle), LB (HLA-Aw68.1;
B40, DR6); (closed circle), Pally (HLA-Aw68.2,26;
15 B14,38; DR1,4), or (open diamond), IDF (HLA-Aw69,26;
B18,38, DR5).

In the absence of peptide, clone A2/B17 cells
do not lyse targets expressing HLA-Aw69, HLA-Aw68.1, and
HLA-Aw68.2 (data not shown). The inability of clone
20 A2/B17 cells to lyse these targets is due to the
differences in the critical residues around position 62
from those found in HLA-A2 and HLA-B17. However, when
peptide A2.56-69 was included in the cytotoxicity assay,
there was significant lysis of HLA-Aw69 expressing
25 targets by A2/B17 cells (Fig. 5). In contrast, targets
expressing HLA-Aw68.1, HLA-Aw68.2, or the unrelated
HLA-A3 molecule were not lysed.

Lysis of HLA-Aw69 cells by clone A2/B17 cells
in the presence of peptide A2.56-69 was blocked by
30 monoclonal antibody DR11-351, which only binds to the
HLA-Aw69 of the target cell. In contrast, the
monoclonal antibody MA2.1 did not inhibit lysis (results
not shown). MA2.1 binds to the epitope of HLA-A2 and
HLA-B17 formed by residues 56-69, but does not bind to

the HLA-Aw69 or peptide A2.56-69. These results demonstrate the involvement of the HLA-Aw69 molecule in the sensitization by peptide A2.56-69.

5 The addition of A2.98-113 peptide to B cell lines which do not express HLA-A2 did not cause sensitization to lysis when target cells expressing a variety of HLA molecules were used. This was true even though a wide range of peptide concentrations (0.1 to
10 300 µg/ml were used.)

 In binding A2.56-69, the HLA-Aw69 molecule is able to present an epitope that mimics the native structure of HLA-A2. That HLA-Aw69 but not other members of the HLA-A2/28 family can be sensitized is of
15 interest. HLA-Aw69 is a recombinant molecule having α_1 derived from HLA-Aw68 and α_2 and α_3 derived from HLA-A2.1 (Holmes and Parham, EMBO J. 4:2849 (1985)). Thus, HLA-2.1 and HLA-Aw69 differ by only 6 amino acids,
20 all residing in the α_1 domain and three of which are present in the A2.56-69 peptide.

Example 11

Locus of Peptide Interaction in Sensitization

 To assess whether sensitization resulted from
25 peptide interaction with the CTL or the target, cells were pretreated with A2.56-69, washed and then tested for cytolysis. More specifically, 1×10^6 clone A2/B17 cells or ^{51}Cr -labeled IDF (HLA-Aw69,26; B18,38; DR5) were incubated with 100 µg of peptide or medium for 30
30 min. at 37°C, washed three times, and cytotoxicity as determined by ^{51}Cr chromium release was measured.

 As seen from the results presented in Fig. 7, target cells expressing HLA-Aw69 were lysed when the targets, but not the CTL, were pretreated with A2.56-69.

Example 12Effect of Peptide A2.56-69 on Release of
Granules Containing Serine Esterase

5 The effect of peptide A2.56-69 on the release
of granules containing serine esterase during co-culture
of A2/B17 cells with HLA-Aw69 expressing cells may be
essentially as described in Example 7, supra, except
10 that the CTL are from clone A2/B17, the target cells are
those expressing HLA-Aw69, and the cells are co-cultured
in the absence or presence of peptide A2.56-69.

 Although the foregoing invention has been
described in some detail by way of illustration and
15 example for purposes of clarity of understanding, it
will be obvious that certain changes and modifications
may be practiced within the scope of the appended
claims.

20

UTILITY

 It is evident from the above results that
cytotoxic cells can be inhibited employing the subject
peptides which are cross-reactive with the polymorphic
regions of a Class I MHC antigen, as demonstrated with
25 the A2 antigen. In this way, CTL's may be specifically
inhibited from lysing target cells that express the same
antigen that CTL's are restricted by. This is of use in
the transplantation of organic tissue to prevent host
rejection of tissue which is restricted by CTL. In
30 addition, it may be of use in prevention/therapy of
autoimmune disease.

 Alternatively, the CTL's may be activated by
employing specific conjugates involving the subject
oligopeptides in conjunction with an antigen of

interest. This is useful in activating CTL to lyse
cells carrying antigens other than those recognized by
the CTL, and thus may induce CTL to lyse cells carrying
5 antigens which are cryptic to the host, for example, in
parasitic diseases and in neoplasia.

The subject compounds may also be used in
viral studies in determining MHC sequences associated
with restriction of T-lymphocytes in the case of viral
10 infection.

The peptides of the invention may also be used
to determine the presence or absence of CTL which are
targeted towards cells bearing the HLA-Class I molecule
from which the peptide is derived. Knowledge of the
15 presence or absence of the targeted CTL is useful in
predicting the success or failure and/or requirement for
CTL modulation in transplantation of organic tissue. It
may also be helpful in determining the mechanisms
whereby cells become cryptic to the immune system. In
20 addition these peptides may also be helpful in the
detection of autoimmune diseases.

25

30

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A substantially pure preparation of
5 peptide compound of at least 8 amino acids and having a
sequence comprising at least any eight consecutive amino-
acid residues of the extended sequence:

aa⁵⁵ G P E Y W D aa⁶² aa⁶³ T aa⁶⁵ aa⁶⁶ aa⁶⁷ K
aa⁶⁹ aa⁷⁰ aa⁷¹ Q T aa⁷⁴ R aa⁷⁶ aa⁷⁷ L aa⁷⁹ aa⁸⁰ aa⁸¹
10 aa⁸² aa⁸³ Y Y N Q S E A G S H aa⁹⁴ aa⁹⁵ Q aa⁹⁷ M aa⁹⁹ G
C D aa¹⁰³ G aa¹⁰⁵ D aa¹⁰⁷ R aa¹⁰⁹ L R G aa¹¹³ aa¹¹⁴ Q
aa¹¹⁶ A Y D G

wherein:

- 15 aa⁵⁵ is E or K;
aa⁶² is G, Q, E or R;
aa⁶³ is an acidic amino acid or amide thereof;
aa⁶⁵ is Q, R or G;
aa⁶⁶ is I, N or K;
20 aa⁶⁷ is an aliphatic neutral amino acid;
aa⁶⁹ is an aliphatic neutral amino acid;
aa⁷⁰ is Q, H, S, N or K;
aa⁷¹ is an aliphatic neutral amino acid;
aa⁷⁴ is D, Y or H;
25 aa⁷⁶ is E or V;
aa⁷⁷ is D, S or N;
aa⁷⁹ is R or G;
aa⁸⁰ is T, I or N;
aa⁸¹ is an aliphatic non-polar amino acid;
30 aa⁸² is R or L;
aa⁸³ is G or R;
aa⁹⁴ is T or I;
aa⁹⁵ is a non-polar aliphatic amino acid of
from 5 to 6 carbon atoms;



aa⁹⁷ is an aliphatic amino acid or W;
aa⁹⁹ is an aromatic amino acid;
aa¹⁰³ is a non-polar aliphatic amino acid of
5 from 5 to 6 carbon atoms;
aa¹⁰⁵ is P or S;
aa¹⁰⁷ is G or W;
aa¹⁰⁹ is L or F;
aa¹¹³ is Y or H;
10 aa¹¹⁴ is H, Q, D, N or R;
aa¹¹⁶ is Y, D, S, F or H,
which modulates CTL activity.

2. A peptide compound according to Claim 1,
15 comprising at least eight amino acids within the
sequence:

G S H T (V, I or L) Q R M Y G C D V G S D (W or G)
R F L R G Y H Q Y A Y D G,

20

or

Q E G P E Y W D (G or R) (E or N) T (R or Q)
(K or N) V K A (H or Q) S Q T (H or D) R (V or
25 E) (D, S or N) L (G or R) (T or I) (L or A) (R
or L) (G or R) Y Y N Q S E A,

25

wherein the amino acids in the parentheses indicate that
one of the amino acids in the parentheses is
30 present.

30



5 3. An extended peptide compound comprising a first peptide according to claim 1 and at least one second peptide bonded to a terminus of said first peptide, whereby the resulting said extended peptide compound has a sequence other than the wild-type sequence of a natural human MHC antigen.

4. A peptide compound according to claim 1, wherein said peptide compound is covalently bonded to a compound capable of providing a detectable signal.

10 5. A method of modulating cytolytic activity of an MHC restricted CTL, said method comprising:

15 combining a CTL with a peptide compound of at least 8 amino acids and not more than about 30 amino acids having a sequence differing by not more than two mutations from the polymorphic region of the C-terminal half of the a₁ domain and the N-terminal half of the a₂ domain of the MHC protein of the host of said MHC restricted CTL cells, wherein said CTL cells are modulated by said peptide.

20 6. A method for inhibiting cytolytic activity of a CTL, said method comprising

combining CTL with a peptide compound of at least 8 amino acids and having a sequence comprising at least any eight consecutive amino acid residues of the extended sequence:

25 G S H aa⁹⁴ aa⁹⁵ Q aa⁹⁷ M aa⁹⁹ G C L
aa¹⁰³ G aa¹⁰⁵ D aa¹⁰⁷ R aa¹⁰⁹ L R G
aa¹¹³ aa¹¹⁴ Q aa¹¹⁶ A Y D G;

30 wherein

aa⁹⁴ is T or I;



aa⁹⁵ is a non-polar aliphatic amino acid of
from 5 to 6 carbon atoms;

5 aa⁹⁷ is an aliphatic amino acid or W;

aa⁹⁹ is an aromatic amino acid;

aa¹⁰³ is a non-polar aliphatic amino acid of
from 5 to 6 carbon atoms;

aa¹⁰⁵ is P or S;

aa¹⁰⁷ is G or W;

10 aa¹⁰⁹ is L or F;

aa¹¹³ is Y or H;

aa¹¹⁴ is H, Q, D, N or R;

aa¹¹⁶ is Y, D, S, F or H;

15 or

aa⁵⁵ G P E Y W D aa⁶² aa⁶³ T aa⁶⁵
aa⁶⁶ aa⁶⁷ K aa⁶⁹ aa⁷⁰ aa⁷¹ Q T aa⁷⁴ R aa⁷⁶
aa⁷⁷ L aa⁷⁹ aa⁸⁰ aa⁸¹ aa⁸² aa⁸³ Y Y N Q S E A

20

wherein

aa⁵⁵ is E or K, particularly E;

aa⁶² is G, Q, E or R, particularly R or G;

25 aa⁶³ is an acidic amino acid or amide thereof;
including E and N, particularly E;

aa⁶⁵ is Q, R or G, particularly Q or R;

aa⁶⁶ is I, N or K, particularly N or K;

aa⁶⁷ is an aliphatic neutral amino acid

30 including, V, M, S, C and Y, particularly V,

aa⁶⁹ is an aliphatic neutral amino acid

including, A, T and P, particularly A;

aa⁷⁰ is Q, H, S, N or K, particularly Q or H;

aa⁷¹ is an aliphatic neutral amino acid including S, A and T, particularly S;
aa⁷⁴ is D, Y or H, particularly D or H;
5 aa⁷⁶ is E or V;
aa⁷⁷ is D, S or N, particularly D;
aa⁷⁹ is R or G, particularly G;
aa⁸⁰ is T, I or N, particularly T or I;
aa⁸¹ is an aliphatic non-polar amino acid
10 including L or A, particularly L;
aa⁸² is R or L, particularly R;
aa⁸³ is G or R, particularly G, wherein said CTL cells are inhibited by said peptide.

15 7. A method of sensitizing MHC restricted cells to CTL, said method comprising:
combining MHC restricted cells with a peptide compound of at least 8 amino acids and having a sequence comprising at least any eight consecutive amino acid
20 residues of the extended sequence:

aa⁵⁵ G P E Y W D aa⁶² aa⁶³ T aa⁶⁵
aa⁶⁶ aa⁶⁷ K aa⁶⁹ aa⁷⁰ aa⁷¹ Q T aa⁷⁴ R aa⁷⁶ aa⁷⁷ L aa⁷⁹
aa⁸⁰ aa⁸¹ aa⁸² aa⁸³ Y Y N Q S E A

25 wherein

aa⁵⁵ is E or K, particularly E;
aa⁶² is G, Q, E or R, particularly R or G;
aa⁶³ is an acidic amino acid or amide thereof;
30 including E and N, particularly E;
aa⁶⁵ is Q, R or G, particularly Q or R;
aa⁶⁶ is I, N or K, particularly N or K;
aa⁶⁷ is an aliphatic neutral amino acid including, V, M, S, C and Y, particularly V,



aa⁶⁹ is an aliphatic neutral amino acid including, A, T and P, particularly A;
 aa⁷⁰ is Q, H, S, N or K, particularly Q or H;
 5 aa⁷¹ is an aliphatic neutral amino acid including S, A and T, particularly S;
 aa⁷⁴ is D, Y or H, particularly D or H;
 aa⁷⁶ is E or V;
 aa⁷⁷ is D, S or N, particularly D;
 10 aa⁷⁹ is R or G, particularly G;
 aa⁸⁰ is T, I or N, particularly T or I;
 aa⁸¹ is an aliphatic non-polar amino acid including L or A, particularly L;
 aa⁸² is R or L, particularly R;
 15 aa⁸³ is G or R, particularly G,
 and wherein the MHC restricted cells become sensitized to the CTL via the binding of said peptide.

8. A method for inhibiting cytolytic activity of a CTL by irreversibly inhibiting the CTL, said method comprising:

combining a CTL with a peptide compound of at least 8 amino acids and having a sequence comprising at least any eight consecutive amino acid residues of the extended sequence:

aa⁵⁵ G P E Y W D aa⁶² aa⁶³ T aa⁶⁵
 aa⁶⁶ aa⁶⁷ K aa⁶⁹ aa⁷⁰ aa⁷¹ Q T aa⁷⁴
 R aa⁷⁶ aa⁷⁷ L aa⁷⁹ aa⁸⁰ aa⁸¹ aa⁸² aa⁸³ Y Y N Q S E A G S
 H aa⁹⁴ aa⁹⁵ Q aa⁹⁷ M
 30 aa⁹⁹ G C D aa¹⁰³ G aa¹⁰⁵ D aa¹⁰⁷ R aa¹⁰⁹ L R G
 aa¹¹³ aa¹¹⁴ Q aa¹¹⁶ A Y D G

wherein:

aa⁵⁵ is E or K;



- 5 aa⁶² is G, Q, E or R;
 aa⁶³ is an acidic amino acid or amide thereof;
 aa⁶⁵ is Q, R or G;
 aa⁶⁶ is I, N or K;
 aa⁶⁷ is an aliphatic neutral amino acid;
 aa⁶⁹ is an aliphatic neutral amino acid;
 aa⁷⁰ is Q, H, S, N or K;
 aa⁷¹ is an aliphatic neutral amino acid;
 10 aa⁷⁴ is D, Y or H;
 aa⁷⁶ is E or V;
 aa⁷⁷ is D, S or N;
 aa⁷⁹ is R or G;
 aa⁸⁰ is T, I or N;
 15 aa⁸¹ is an aliphatic no-polar amino acid;
 aa⁸² is R or L;
 aa⁸³ is G or R;
 aa⁹⁴ is T or I;
 aa⁹⁵ is a non-polar aliphatic amino acid of
 20 from 5 to 6 carbon atoms;
 aa⁹⁷ is an aliphatic amino acid or W;
 aa⁹⁹ is an aromatic amino acid;
 aa¹⁰³ is a non-polar aliphatic amino acid of
 from 5 to 6 carbon atoms;
 25 aa¹⁰⁵ is P or S;
 aa¹⁰⁷ is G or W;
 aa¹⁰⁹ is L or F;
 aa¹¹³ is Y or H;
 aa¹¹⁴ is H, Q, D, N, or R;
 30 aa¹¹⁶ is Y, D, S, F, or H

and wherein said peptide is ^{joined} ~~linked~~ with an agent which causes cytotoxicity, and wherein said peptide binds to said CTL cell.



9. A method for determining the presence of MHC restricted CTL's, said method comprising

5

contacting cells with a peptide compound of at least 8 amino acids and having a sequence comprising at least any eight consecutive amino acid residues of the extended sequence:

10 aa⁵⁵ G P E Y W D aa⁶² aa⁶³ T aa⁶⁵ aa⁶⁶ aa⁶⁷ K
aa⁶⁹ aa⁷⁰ aa⁷¹ Q T aa⁷⁴ R aa⁷⁶ aa⁷⁷ L aa⁷⁹ aa⁸⁰ aa⁸¹
aa⁸² aa⁸³ Y Y N Q S E A G S H aa⁹⁴ aa⁹⁵ Q aa⁹⁷ M aa⁹⁹ G
C D aa¹⁰³ G aa¹⁰⁵ D aa¹⁰⁷ R aa¹⁰⁹ LRG aa¹¹³ aa¹¹⁴ Q
aa¹¹⁶ A Y D G

15

wherein:

20

25

30

- aa⁵⁵ is E or K;
- aa⁶² is G, Q, E or R;
- aa⁶³ is an acidic amino acid or amide thereof;
- aa⁶⁵ is Q, R or G;
- aa⁶⁶ is I, N or K;
- aa⁶⁷ is an aliphatic neutral amino acid;
- aa⁶⁹ is an aliphatic neutral amino acid;
- aa⁷⁰ is Q H, S, N or K;
- aa⁷¹ is an aliphatic neutral amino acid;
- aa⁷⁴ is D, Y or H;
- aa⁷⁶ is E or V;
- aa⁷⁷ is D, S or N;
- aa⁷⁹ is R or G;
- aa⁸⁰ is T, I or N, particularly T or I;
- aa⁸¹ is an aliphatic no-polar amino acid;
- aa⁸² is R or L, particularly R;
- aa⁸³ is G or R;



aa⁹⁴ is T or I;
aa⁹⁵ is a non-polar aliphatic amino acid of
from 5 to 6 carbon atoms;
5 aa⁹⁷ is an aliphatic amino acid or W;
aa⁹⁹ is an aromatic amino acid;
aa¹⁰³ is a non-polar aliphatic amino acid of
from 5 to 6 carbon atoms;
aa¹⁰⁵ is P or S;
10 aa¹⁰⁷ is G or W;
aa¹⁰⁹ is L or F;
aa¹¹³ is Y or H;
aa¹¹⁴ is H, Q, D, N, or R;
aa¹¹⁶ is Y, D, S, F, or H.

15 covalently joined to a compound capable of providing a
detectable signal; and
determining the presence of cells to which said
detectable signal compound is specifically bound.

20 10. A method according to Claim 9 wherein
said extended sequence is of the sequence:

25 G S H T (V, I or L) Q R M Y G C D V G S D (W or G) R
F L R G Y H Q Y A Y D G

or

30 Q E G P E Y W D (G or R) (E or N) T (R or Q)
(K or N) V K A (H or Q) S Q T (H or D) R (V or E) (D, S
or N) L (G or R) (T or I) (L or A) (R or L) (G or R) Y Y
N Q S E A,



wherein the amino acids in the parentheses indicate that one of the amino acids in the parentheses is present.

11. A substantially pure preparation of peptide compound selected from the sequences:

5 T L Q R M Y G C D V G S D W R F L R G,
M Y G C D V G S D W R F L R G Y,
M Y G C D V G S D G R F L R G Y,
G P E Y W D G E T R K V K A, and
G P E Y W D R N T R N V K A.

10 12. A pharmaceutical composition comprised of a peptide compound of at least 8 amino acids and having a sequence comprising at least any 8 ^{consecutive} amino acid residues of:-

aa⁵⁵ G P E Y W D aa⁶² aa⁶³ T aa⁶⁵ aa⁶⁶ aa⁶⁷ K
aa⁶⁹ aa⁷⁰ aa⁷¹ Q T aa⁷⁴ R aa⁷⁶ aa⁷⁷ L aa⁷⁹
15 aa⁸⁰ aa⁸¹ aa⁸² aa⁸³ Y Y N Q S E A G S H aa⁹⁴
aa⁹⁵ Q aa⁹⁷ M aa⁹⁹ G C D aa¹⁰³ G aa¹⁰⁵ D aa¹⁰⁷
R aa¹⁰⁹ L R G aa¹¹³ aa¹¹⁴ Q aa¹¹⁶ A Y D G

wherein:

aa⁵⁵ is E or K;
20 aa⁶² is G, Q, E or R;
aa⁶³ is an acidic amino acid or amide thereof;
aa⁶⁵ is Q, R or G;
aa⁶⁶ is I, N or K;
aa⁶⁷ is an aliphatic neutral amino acid;
aa⁶⁹ is an aliphatic neutral amino acid;



- 5 aa⁷⁰ is Q, H, S, N or K;
aa⁷¹ is an aliphatic neutral amino acid;
aa⁷⁴ is D, Y or H;
aa⁷⁶ is E or V;
aa⁷⁷ is D, S or N;
aa⁷⁹ is R or G;
aa⁸⁰ is T, I or N;
10 aa⁸¹ is an aliphatic non-polar amino acid;
aa⁸² is R or L;
aa⁸³ is G or R;
aa⁹⁴ is T or I;
aa⁹⁵ is a non-polar aliphatic amino acid of
from 5 to 6 carbon atoms;
15 aa⁹⁷ is an aliphatic amino acid or W;
aa⁹⁹ is an aromatic amino acid;
aa¹⁰³ is a non-polar aliphatic amino acid of
from 5 to 6 carbon atoms;
20 aa¹⁰⁵ is P or S;
aa¹⁰⁷ is G or W;
aa¹⁰⁹ is L or F;
aa¹¹³ is Y or H;
aa¹¹⁴ is H, Q, D, N or R;
aa¹¹⁶ is Y, D, S, F or H.

25

wherein said peptide compound is present in a pharmacologically effective dose in a pharmaceutically acceptable medium.

30

13. A peptide compound according to Claim 1 wherein said peptide compound begins with Q aa⁹⁷ M and terminates with A aa¹¹⁶, wherein aa⁹⁷ is R.

5 14. a peptide compound according to Claim 1 wherein said peptide compound begins with PEY and terminates with QSE.

15. A method according to Claim 8, wherein said peptide compound and said agent which causes cytotoxicity are joined with liposome.

10 DATED this 11th day of June 1991

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UNIVERSITY
Patent Attorneys for the
Applicant:

F.B. RICE & CO.



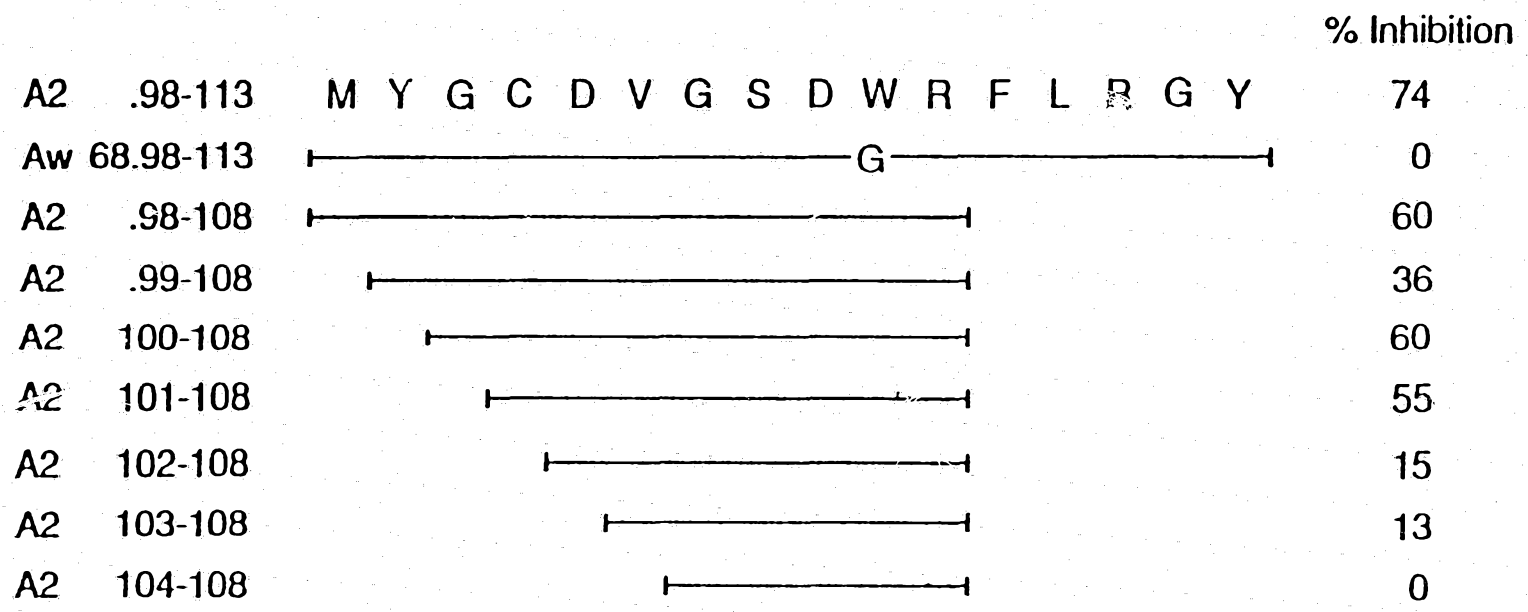


FIG. 1

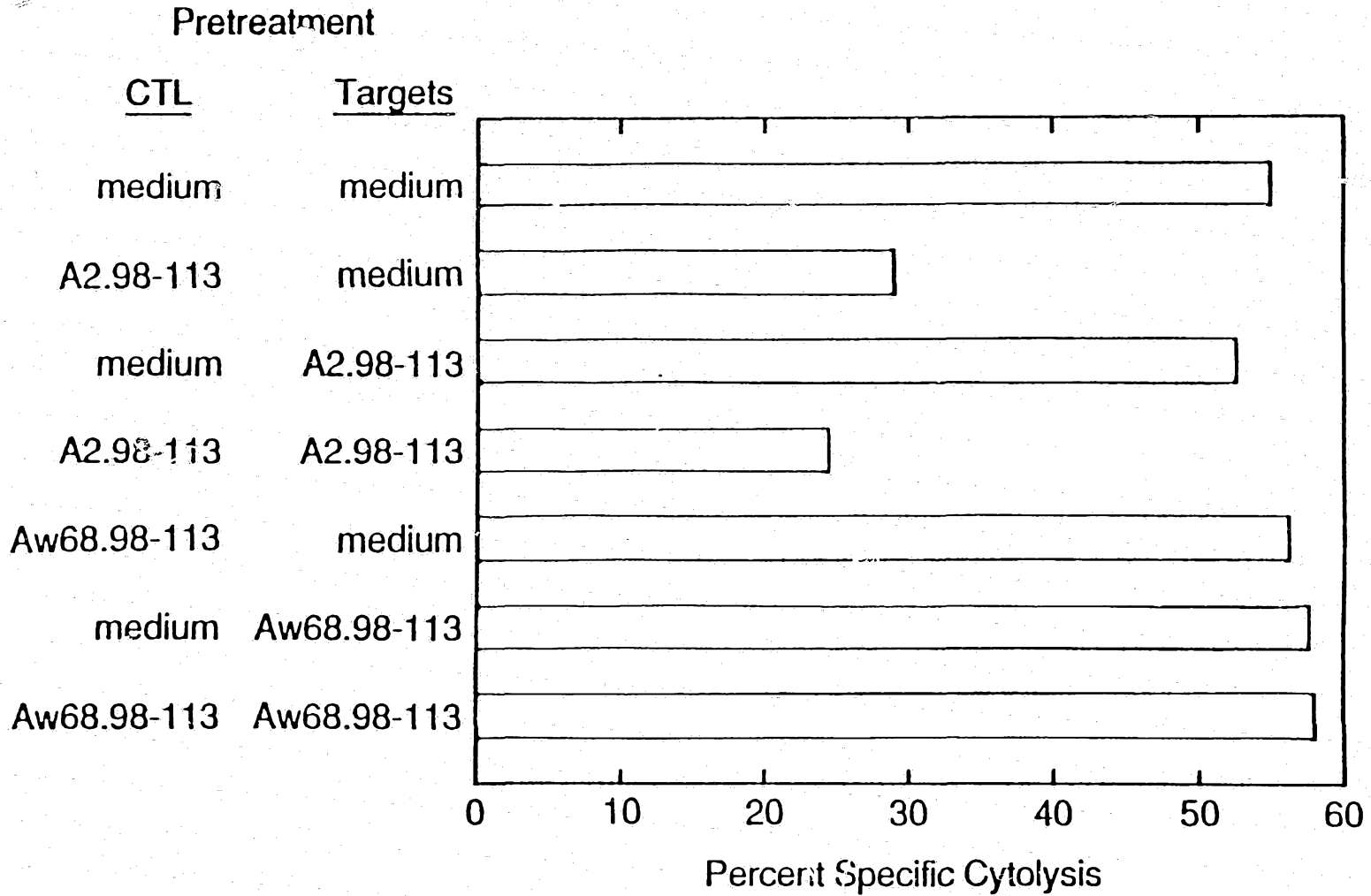


FIG. 2

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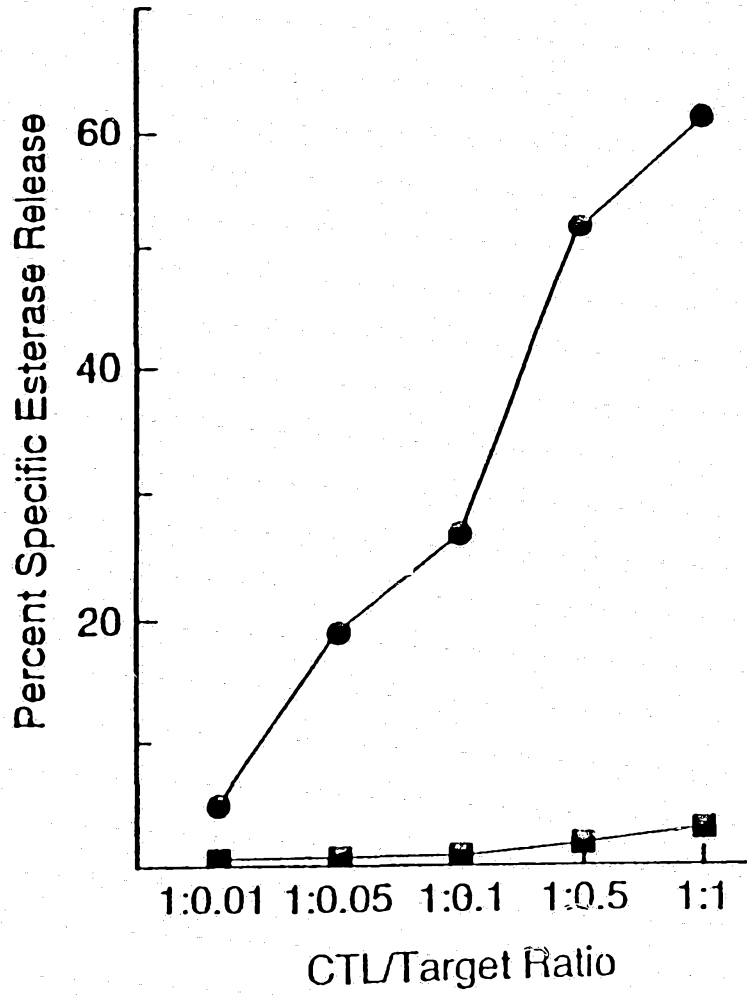


FIG. 3A

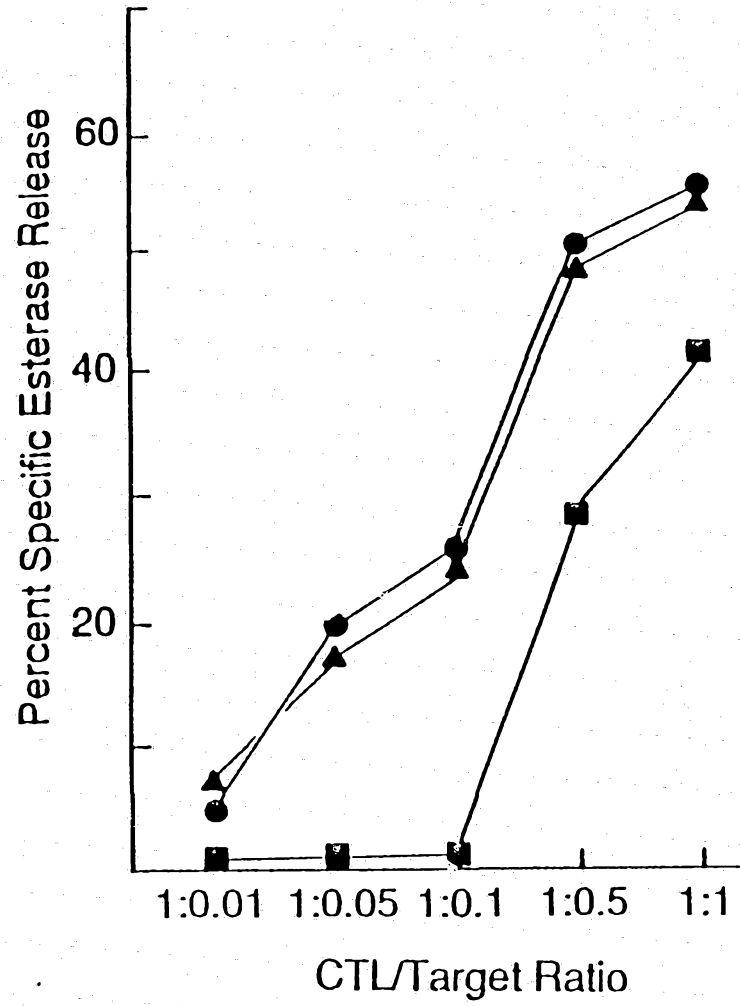


FIG. 3B

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FIG. 4

A2/B17

	1	10	20	30	40	50	60	70	80	90	
consensus	GSHSMEFSYTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMEPRAPWIEQEGPEYWDRETQIVKAQSQTDRDLRTRLCGYNQSEA										
B17(Bw58)	-----AM-----T-----G-RNM-SA-Y-N-IALR-----										
A2.1	-----F-----Q-----G-RK-II-II-V-G-----										
A2.3	-----F-----Q-----G-RK-II-II-V-G-----										
A2.2F	-----F-----R-----G-RK-II-II-V-G-----										
Aw69	-----Q-----N-RN-----V-G-----										
Aw68.2	-----M-----Q-----N-RN-----V-G-----										
Aw68.1	-----Q-----N-RN-----V-G-----										
A3.1	-----F-----Q-----Q-RN-----V-G-----										
consensus	GSHSRYFYTSVSBPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMEPRAPWIEQEGPEYWDRETQIVKAQSQTDRDLRTRLCGYNQSEA										

A2/Aw69

	100	110	120	130	140	150	160	170	180	
consensus	GSHTIQRMYGCDVGPDRLLPGYHQYAYDGKDYIALNEDLRSWTAADTAAQITQRKWEAARVAEQLRAYLECTCWEWLRRYLENGKETLQRA									
B17(Bw58)	-----I-----L-----HD-S-----S-----L-----									
A2.1	-----V-----S-W-F-----K-----M-----T-KII-----II-----T									
A2.3	-----V-----S-W-F-----K-----M-----T-KII-----T-II-E-----W-----T									
A2.2F	-----L-----S-W-F-----K-----M-----T-KII-----II-----W-----T									
Aw69	-----V-----S-W-F-----K-----M-----T-KII-----II-----T									
Aw68.2	-----F-----K-----M-----T-KII-----II-----W-----T									
Aw68.1	-----M-----S-W-F-----R-D-----K-----M-----T-KII-----II-----W-----T									
A3.1	-----I-----S-----F-----R-D-----M-----K-----HE-----D-----T									
consensus	GSHTIQRMYGCDVGPDRLLRGYHQYAYDGKDYIALNEDLRSWTAADTAAQITQRKWEAARVAEQLRAYLECTCWEWLRRYLENGKETLQRA									

A3

	190	200	210	220	230	240	250	260	270	
consensus	DPPKTHVTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDELVETRPAGDGTFFQKWAAVVVPSEEGRYTCHVQHEGLPKPLTLRW									
B17(Bw58)	-----V-----R-----									
A2.1	-----A-----M-----AV-----S-----Q-----									
A2.3	-----A-----M-----AV-----S-----Q-----									
A2.2F	-----A-----M-----AV-----S-----Q-----									
Aw69	-----A-----M-----AV-----S-----Q-----									
Aw68.2	-----A-----M-----AV-----S-----V-----Q-----									
Aw68.1	-----A-----M-----AV-----S-----V-----Q-----									
A3.1	-----M-----									
consensus	DPPKTHVTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDELVETRPAGDGTFFQKWAAVVVPSEEGRYTCHVQHEGLPKPLTLRW									

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PCT/US88/00245

FIG. 5A

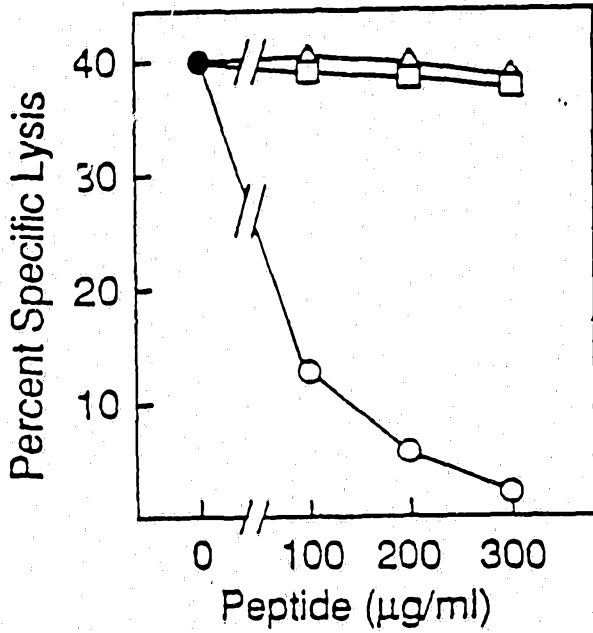


FIG. 5B

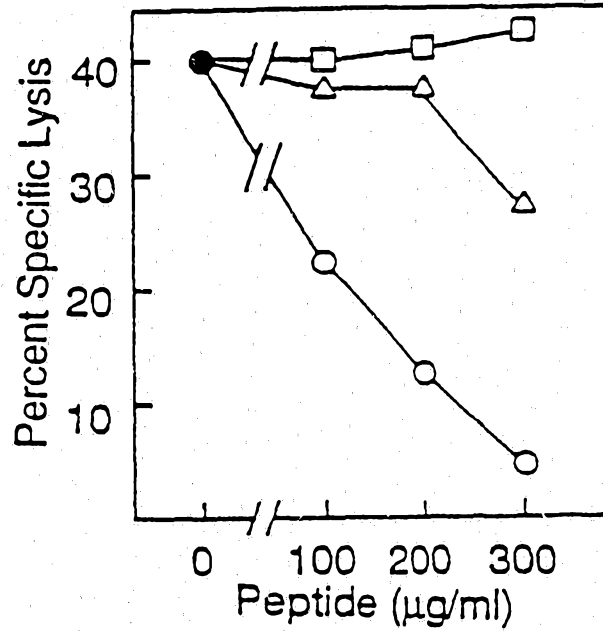


FIG. 5C

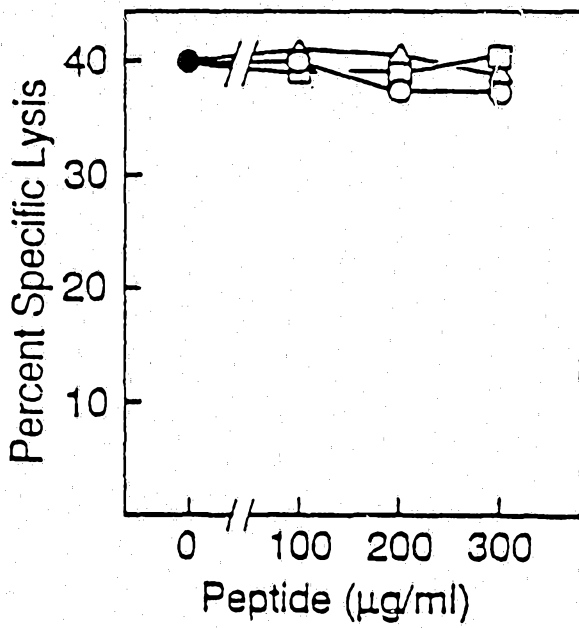
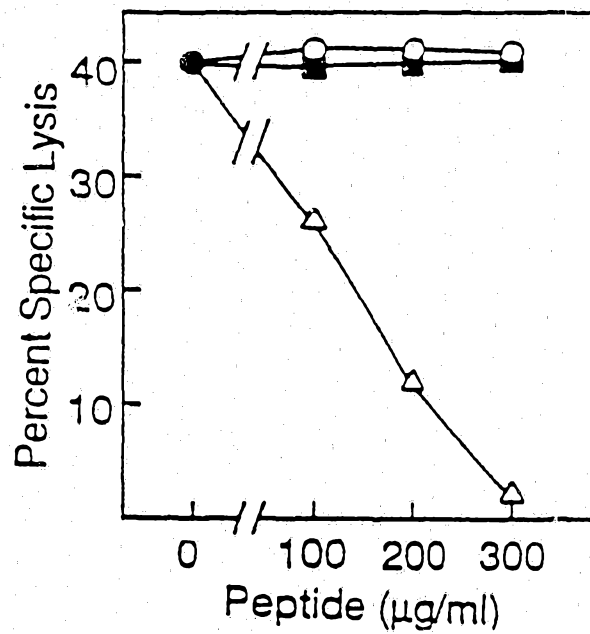
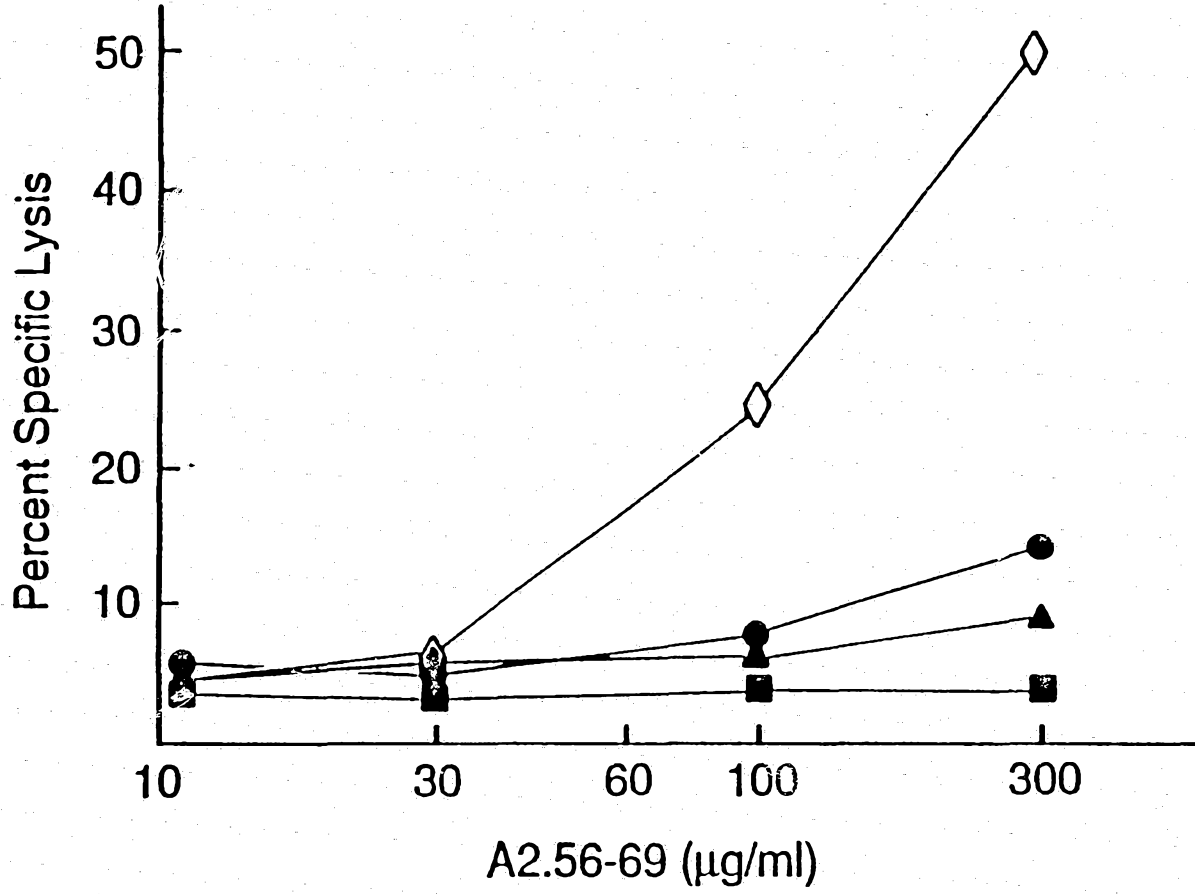


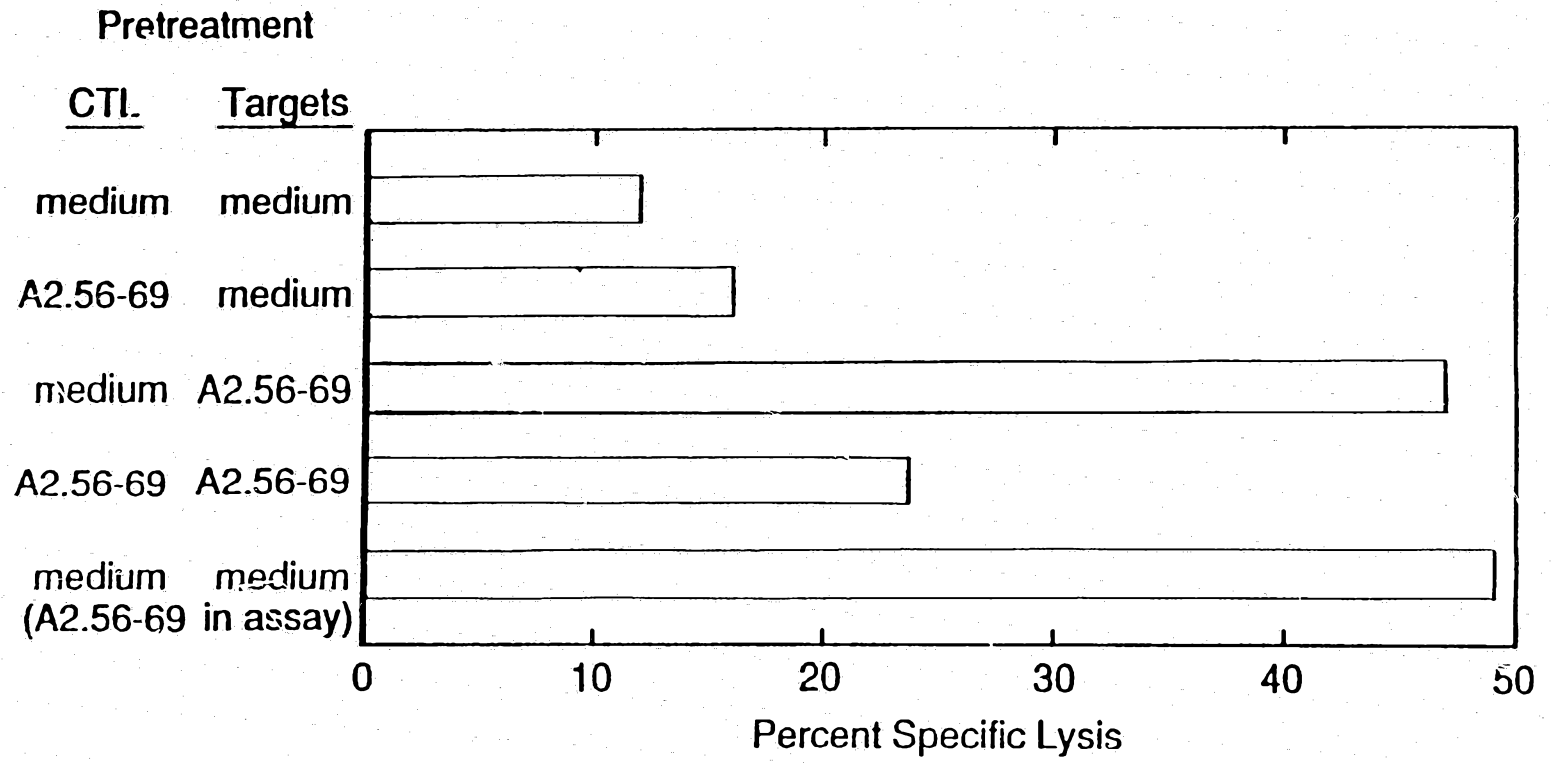
FIG. 5D





A2.56-69 ($\mu\text{g/ml}$)

FIG. 6



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FIG. 7

INTERNATIONAL SEARCH REPORT

International Application No PCT/US88/00245

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ¹		
According to International Patent Classification (IPC) or to both National Classification and IPC IPC(4) C07K 7/06 7/08, 7/10; A61K 37/02; C12Q 1/04; US. CL. 530/324, 325, 326, 327, 328, 345, 802; 514/12, 13, 14, 15, 16; 435/29		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁴		
Classification System	Classification Symbols	
US.	530/324, 325, 326, 327, 328, 345, 802; 514/12, 13, 14, 15, 16; 435/29	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁴		
CHEMICAL ABSTRACT AND BIOLOGICAL ABSTRACT ON LINE COMPUTER SEARCH		
III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹⁴		
Category ⁶	Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁸
X	THE JOURNAL OF IMMUNOLOGY, VOLUME 134, ISSUED APRIL, 1985 (KOLLER ET AL), "CLONING AND COMPLETE SEQUENCE OF AN HLA-A ₂ GENE: ANALYSIS OF TWO HLA-A ALLELES AT THE NUCLEOTIDE LEVEL" PAGES 2727-2733. SEE PAGE 2731 IN PARTICULAR.	1-2, 12
X	PROC. NATL. ACAD. SCI. USA, VOLUME 82, ISSUED NOVEMBER, 1985 (VEGA ET AL), "STRUCTURAL ANALYSIS OF AN HLA-B27 FUNCTIONAL VARIANT: IDENTIFICATION OF RESIDUES THAT CONTRIBUTE TO THE SPECIFICITY OF RECOGNITION BY CYTOLYTIC T LYMPHOCYTES", PAGES 7394-7398. SEE P. 7396, 7397, 7398 IN PARTICULAR.	1-2, 5-7, 12
P, X	CHEMICAL ABSTRACTS, (COLUMBUS, OHIO, USA), VOLUME 106, ISSUED 1987, (PARHAM ET AL), "INHIBITION OF ALLOREACTIVE CYTOTOXIC T LYMPHOCYTES BY PEPTIDES FROM THE α_2 DOMAIN OF HLA-A ₂ , SEE PAGE 516, COLUMN 1, THE ABSTRACT NO. 154384x, NATURE, 1987 325 (6105), 625-8, (ENG.).	1-2, 5-6 11-12
<p>⁶ Special categories of cited documents: ¹³</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"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)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"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</p> <p>"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"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.</p> <p>"Z" document member of the same patent family</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search ¹		Date of Mailing of this International Search Report ¹
07 MARCH 1988		04 APR 1988
International Searching Authority ¹		Signature of Authorized Officer ¹⁹
ISA/US		CHRISTINA CHAN <i>Christina Chan</i>