(12) PATENT
(19) AUSTRALIAN PATENT OFFICE

(11) Application No. AU 199740554 B2
(10) Patent No. 733234

(54) Title

Conjugated peptides, immunological reagent containing same and use thereof for treatment of immunological disorders

(51)[6] International Patent Classification(s)

A61K 038/00 C07K 005/00
A61K 038/04 C07K 007/00
A61K 039/12 C07K 016/00
A61K 039/21 C07K 017/00
A61K 039/385

(21) Application No: 199740554

(22) Application Date: 1997.08.08

(87) WIPO No: WO98/06416

(30) Priority Data

(31) Number	(32) Date	(33) Country
08/695304	1996.08.09	US

(43) Publication Date : 1998.03.06
(43) Publication Journal Date : 1998.05.07
(44) Accepted Journal Date : 2001.05.10

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OPI DATE 06/03/98 APPLN. ID 40554/97
AOJP DATE 07/05/98 PCT NUMBER PCT/US97/13901

AU9740554

CT)

(51) International Patent Classification 6 :
A61K 38/00, 38/04, 39/12, 39/21, 39/385, C07K 5/00, 7/00, 16/00, 17/00

A1

(11) International Publication Number: WO 98/06416

(43) International Publication Date: 19 February 1998 (19.02.98)

(21) International Application Number: PCT/US97/13901

(22) International Filing Date: 8 August 1997 (08.08.97)

(30) Priority Data:
08/695,304 9 August 1996 (09.08.96) US

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: CONJUGATED PEPTIDES, IMMUNOLOGICAL REAGENT CONTAINING SAME AND USE THEREOF FOR TREATMENT OF IMMUNOLOGICAL DISORDERS

(57) Abstract

A heteroconjugate is formed by linking a T cell binding ligand (TCBL) such as Peptide J of β-2 microglobulin to a modified HGP-30 antigenic peptide fragment of p17 gag peptide, such as, for example (I). The heteroconjugate is effective in eliciting a TH1 directed immune response and provides a vaccine composition for treating or preventing AIDS.

ABSTRACT OF THE DISCLOSURE

A heteroconjugate is formed by linking a T cell binding ligand (TCBL) such as Peptide J of β-2 microglobulin to a modified HGP-30 antigentic peptide fragment of p17 gag peptide, such as, for example

A T L Y S V H Q R I D V K D T
K E A L E K I E E E Q N K S (SEQ ID NO: 5)

The heteroconjugate is effective in eliciting a THI directed immune response and provides a vaccine composition for treating or preventing AIDS.

Conjugated Peptides, Immunological Reagent Containing Same and Use Thereof For Treatment of Immunological Disorders

(1) Field of the Invention

This invention relates to peptide conjugates which can be used to form an immunogenic composition useful to activate the immune system of a patient exposed to or at risk of infection by human immunodeficiency virus (HIV) which is the causative organism of the disease known as Acquired Immune Deficiency Syndrome (AIDS).

(2) Discussion of the Prior Art

HIV has several major classes of proteins referred to as the outer envelope structural group (env gp120 and p41), gag (or internal structural proteins p24, p17) and several nonstructural and regulatory genes and encoded proteins. Examination of the immune response and the disease state can be of assistance in designing new agents as vaccines. Generally, the response to HIV gag is polyisotypic (all/most classes or subclasses, IgM, IgG1, IgG3 and IgA) but antibodies to env are usually restricted to IgG1.

In regard to man and HIV it is recognized that the disappearance of a particular subclass of antibodies (IgG3) to a conserved p17 protein molecule is most closely associated with disease progression. Antibodies to HIV of IgG1 and IgG2 subclasses are found in nearly all sera from HIV infected individuals at different stages of the disease. However, the presence of IgG3 is largely associated with gag proteins (p17, p24 and p55). Conversely almost all antibodies to the major viral surface proteins p41 and gp120 are of the IgG1 subclass and are present even in late stage disease.

Several authors have reported that anti-p17 antibodies decline with disease progression and, in part, this was associated with antibodies to certain peptides of p17.

An interesting finding for p17, one of the two major gag proteins, involves a particular 30 amino acid peptide HGP-30, at amino acid residues 86-115, whose sequence contains T and B cell epitopes immunoreactive with p17 of HIV. See U.S. Patent 4,983,387 to Goldstein, et al. HGP-30 has the following sequence:

Y S V H Q R I D V K D T K E A
L E K I E E E Q N K S K K K A (SEQ ID NO:1)

HGP-30 has been conjugated to a large protein, Keyhole Limpet Haemocyanin (KLH), and found to be immunogenic in various animals and man, and the conjugate is well tolerated in both animals and humans. A pilot study of HGP-30 vaccine has shown protection from HIV infection in such SCID hu mice given PBL from HGP-30 immunized donors. More recently it has been shown that the presence of a predominance of IgG3 antibodies in serum of HGP-30 vaccine immunized human donors correlates with protection by PBL in the SCID Hu mouse HIV virus challenge model. This is thought to be quite different than what would be found if SCID hu mice with PBL from hyperimmunized env gp120 donors who had high titers of antibodies to the envelope protein where similarly infected with HIV; in this case it is expected that there would be afforded little or no protection.

There is recent recognition of the need to specifically direct the immune response, such as use of protein carriers, such as antibody, TH1, TH2, TS subclass of antibodies.

Traditionally, small peptides must be attached to carrier proteins in order to elicit immune responses. Often a large protein such as KLH is used. However, it has been observed that heterogenous (impure) KLH yields a better immune response than a more homogenous preparation. It would be desirable to find other methods to direct the response primarily or substantially to murine IgG2a or human IgG3, a TH1 associated pathway. The present inventors recognized that the carrier should

not be directing the response in an undesired direction and since the KLH molecule seems to be predominantly directing the response in the TH2 direction it was concluded that another carrier should be considered. Likewise, other factors such as costs, ease of manufacture, and stability, helped lead to the discovery of this invention.

There is no evidence that changing the nature of a peptide, such as by addition(s) or deletion(s) of one or several amino acids and/or method of attachment to a carrier could or would influence the subclass of antibody generated that recognize the epitope even though it is known that such manipulations can induce different responses such as the stimulation of B and T cells, cytotoxic and lymphoproliferative responses. However, the effect of T cell epitopes on antibody responses that has been reported has been for the presence or absence of antibody production by helper or suppressor immune responses.

One of the present applicants previously reported that addition of a T cell binding ligand to a peptide epitope could alter the nature of the immune response (i.e., TH1 or TH2). It was further shown that the antibodies derived from certain conjugated peptides were better able to recognize the native molecule than were the antibodies prepared by using a conventional peptide-KLH conjugate. It was shown that the antibodies induced by the heteroconjugate had a broader specificity, so that they recognized the peptide epitope not only in the linear form, but also in the native molecule. In some cases the use of the peptide conjugated to KLH was not able to recognize the epitope in the native molecule.

SUMMARY OF THE INVENTION

The present invention relates to certain conjugated peptides comprising at least a first T cell specific binding peptide and a second T cell specific binding peptide covalently linked together, wherein the first

peptide binds to a specific class or subclass of T cells and the second peptide is an antigenic peptide of from about 25 to about 37 amino acids (which may be referred to hereinafter as "modified HGP-30") and which is capable of eliciting TH1 associated antibodies when administered to a human in need thereof, wherein the antigenic peptide has sequence identity with the p17 gag protein of HIV wherein the peptide has a sequence originating with an amino acid residue chosen from residues 75 to 82 and ending with an amino acid residue chosen from residues 106 to 111 of p17 gag protein of HIV.

The second peptide used as T cell specific binding molecule in the conjugated peptides of this invention are peptides which are portions of molecules or analogues of such portions which bind specifically or at least preferentially to specific class or subclass of T cells, such as helper T cells, T_h, suppressor T cells, T_s, cytotoxic T cells, CTC, and the like.

More particularly, the first or antigenic peptides of p17 useful in this invention will generally be between about 25 and 37 amino acids as represented in the following representative cases:

A T L Y S V H Q R I D V K D T
K E A L E K I E E E (SEQ ID NO:2)

S L Y N T V A T L Y S V H Q R
I D V K D T K E A L E K I E E
E Q N K S K (SEQ ID NO:3)

R S L Y N T V A T L Y S V H Q
R I D V K D T K E A L E K I E
E E Q N K S K (SEQ ID NO:4)

A particularly preferred antigenic peptide for use in this invention has the following amino acid sequence

A T L Y S V H Q R I D V K D T
K E A L E K I E E E Q N K S (SEQ ID NO:5)

SEQ ID NO:5 sometimes may be referred to, for convenience, as m-HGP-30, representing a modified version of HGP-30. More generally, as noted above, however, all of the antigenic peptides for use in the present invention may, for convenience, be referred to generically as "modified HGP-30."

These conjugated peptides offer the advantages previously seen with other conjugated peptides, such as those more generally disclosed in the aforementioned WO 89/12458, of inducing broad spectrum antibodies but, additionally providing a desired TH1 specificity believed to result from the second or antigenic peptide which incorporates a CTL epitope which may modify the response to the desired isotype.

The present invention also relates to pharmaceutically effective compositions containing such antigenic peptide-T-cell binding ligand conjugated peptides (for convenience, may sometimes be referred to as "heteroconjugate") for eliciting immunization to infection against Human Immunodeficiency Virus, HIV, in a human subject. Such compositions, in addition to the heteroconjugate of this invention will, preferably, include suitable immunological adjuvant.

Similarly, the invention relates to the use of such heteroconjugate and the pharmaceutically effective composition containing same for treating or preventing HIV infection and Acquired Immunodeficiency Complex (AIDS) by administering to a human patient in need thereof, a therapeutically or prophylactively effective amount of the heterofunctional conjugate as defined above.

DETAILED DESCRIPTION OF THE INVENTION AND PREFERRED EMBODIMENTS

For the peptides disclosed above and below and as employed in the experimentation described herein, the amino acid sequences thereof, are set forth by the single identification letter symbol as follows:

Amino Acid	Three-letter abbreviation	One-letter symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic Acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic Acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

In addition to recent discoveries leading to the conclusion that HIV p17 is located near the surface of the HIV virion rather than in the internal core as in the case of p24 gag protein, it has been shown that the p17 gag protein is myristylated at its N-terminal; see, e.g., EP 0 245 829. Furthermore, analysis of the peptide (designated HGP-30), which is located nearer to the C-terminal of p17, suggests that this peptide contains an immunodominant B-cell epitope which could induce antibodies that mediate cytotoxicity through ADCC type mechanisms. On the other hand, the peptide designated HGP-34 at amino acid positions 51-84, has an amino acid sequence consistent with a T-cell epitope, therefore capable of eliciting a T-cell immunological response. Analysis of the intermediate region peptide of p17 at

positions 33-50 (also designated HGP-18), near the N-terminal end suggests that this peptide has the appropriate balance of hydrophilicity and hydrophobicity to constitute a transmembrane region peptide if the appropriate charge neutralizing membrane proteins are present.

HGP-30 contains regions with well defined B cell epitopes and other regions with defined T cell epitopes (defined as epitopes stimulating lymphoproliferation and others that stimulate cytotoxic T cells). The B cell epitopes are based upon stimulating the production of antibodies, presumably IgG1 in mice, or of being recognized by antibodies in seropositive individuals. No discrimination as to subclasses in man is made. It was reasoned that if IgG3 in man or IgG2a in mice were the desired type of antibodies and an indicator of the arm being stimulated (TH1 or TH2), then to include several residues from the T cell epitope region at the amino terminus may be of benefit along with deletion of several residues at the carboxyl terminus including all or part of the B cell epitope region. Therefore, the inventors prepared and studied a peptide by modifying HGP-30, and specifically, m-HGP-30 (SEQ ID NO:5) was studied. It was reasoned that T cell epitopes such as Cytotoxic T Cell epitopes might not be suppressive but could be directing the response toward a TH1 response. That being the case the inventors postulated that since TH1 correlates with cellular mechanisms of immune responses, the TH1 effect includes not cytotoxic cells, but antibody dependent cellular cytotoxicity (ADCC) and complement binding, both of which are properties of IgG2a in the mouse and IgG3 in man. Therefore, the antibody response induced is towards IgG2a. In particular, the modified HGP-30 with these goals that was used to prepare the heterofunctional conjugates was m-HGP-30, i.e., SEQ ID NO:5 as follows (here using the 3 letter codes):

Ala Thr Leu Tyr Ser Val His Gln Arg Ile Asp Val Lys Asp Thr Lys Glu Ala Leu Glu Lys Ile Glu Glu Glu Gln Asn Lys Ser

In particular, the antigenic peptides useful in this invention will generally be between about 25 and 37 amino acids as represented in the following representative cases including examples of a longer and shorter antigenic peptide forming one of the peptides of the conjugated peptides of the invention:

A T L Y S V H Q R I D V K D T
K E A L E K I E E E (SEQ ID NO:2)

S L Y N T V A T L Y S V H Q R
I D V K D T K E A L E K I E E
E Q N K S K (SEQ ID NO:3)

R S L Y N T V A T L Y S V H Q
R I D V K D T K E A L E K I E
E E Q N K S K (SEQ ID NO:4)

It should be understood that in any of the above amino acid sequences of the antigenic peptides variations of specific amino acids which do not adversely effect the desired biological activity are contemplated and fall within the scope of the invention. In particular, it is recognized that the foregoing sequences are based upon a specific variant of HIV, namely, HIV-1SF2 (actually, the Ser^{86} analog) and, although this region of interest of HIV is generally fairly highly conserved, other naturally occurring and spontaneously occurring variants, include from one or several (e.g., up to about 10) variations of the amino acids within the sequence of interest. Such natural and spontaneously occurring amino acid variations are specifically contemplated and, in certain cases, it may be advantageous to use mixtures of peptides, the sequences of which, within the guidelines given above, and discussed in more detail below, correspond to two or more natural and spontaneously occurring variants of HIV.

Still further, as well recognized in the art it is often advantageous to make specific amino acid substitutions in order, for example, to provide specific binding sites or for purpose of introducing radioactive or fluorescent tagging of the peptide. Such "designed" amino acid sequences are also within the scope of the antigenic peptides (i.e., modified HGP-30) of this invention.

Examples of different consensus sequences of HIV which are also specifically included within the scope of the modified HGP-30 antigenic peptides for use as the second peptide in the conjugated peptides of this invention include, for instance, the following, wherein the lower case letters represent potential or known cites of amino acid variability resulting from the allelic variations, genetic drift and mutations of the particular consensus sequence; the presence of a "?" symbol reflects that there is no agreed upon consensus for the amino acid at that position of the consensus sequence:

CONSENSUS A:
kSL fNt vat LyC vHq rId
vkD tKe Ald kiE eiq nks k SEQ ID NO: 6

CONSENSUS B:
rSL yNt vat Lyc VHq rIe
VkD tKe Ald kiE eEq nks k SEQ ID NO:7

CONSENSUS C:
rSL ?Nt vat LyC vH? ?Ie
vrD tKe Ald kie eEq nk? Q SEQ ID NO:8

CONSENSUS D:
kSL ?Nt vat LYc VHe rIe
vkd tKe Ale kmE eEq nks k SEQ ID NO:9

CONSENSUS F:
rSL ?Nt v?v Lyf vHq rvE
?kD tke Ale EVE Kaq kQq k SEQ ID NO:10

CONSENSUS G:
kSL ?N? ?a? L?C ?Hq rI?
vkD tke Ale EVE Kaq kns k SEQ ID NO:11

CONSENSUS H:

QSL fNl la? LyC vHq rId

?kD tKe Al? k?? eqn ?Q? ? SEQ ID NO:12

CONSENSUS O:

?SL WNA I?V LWC vHN r??

I?D tQQ AIQ kLK eVM ?kS A SEQ ID NO:13

For example, for Consensus sequence A, above, the following species have been identified; the dashes represent identity of amino acid with the consensus sequence [it is noted, however, that only amino acids at positions 74 to 93 are identified; the amino acids at positions 94 to 111 or higher at the C-terminal end or at positions 73 and below at the N-terminal end may be readily determined from the published sequences; the same applies to the exemplary species for Consensus sequences B, C, D]:

CONSENSUS.A	rSL fNt vat LyC VHq rId vk	SEQ ID NO:6
HIV-1U455	R-- Y-T VAV -Y- --Q R-D VK	SEQ ID NO:14
HIV-1MAL	K-- Y-T VAG -Y- --Q R-D VK	SEQ ID NO:15
HIV-1TN243	K-- F-T VAT -W- --Q R-E VK	SEQ ID NO:16

The following are examples of Consensus Sequence B above:

CONSENSUS.B	rSL yNt vAt LYC vHQ rIe vk	SEQ ID NO:7
HIV-1SF2	R-- Y-T V-T --- V-- R-D VK	SEQ ID NO:17
HIV-1TB132	R-- Y-T I-V --- V-- K-E VK	SEQ ID NO:18
HIV-1LAI	R-- Y-T V-T --- V-- R-E IK	SEQ ID NO:19
HIV-1HXB2R	R-- Y-T V-T --- V-- R-E IK	SEQ ID NO:20
HIV-1MN	K-- Y-T V-T --- V-- K-E IK	SEQ ID NO:21
HIV-1JH3	K-- F-T V-T --- V-- R-E VK	SEQ ID NO:22
HIV-1JRCSF	T-- Y-T V-T --- V-- R-E IK	SEQ ID NO:23
HIV-1OYI	R-- Y-T V-T --- V-- K-E VK	SEQ ID NO:24
HIV-1NY5CG	R-- F-T V-V --- V-- R-D VK	SEQ ID NO:25
HIV-1NL43	R-- Y-T I-V --- V-- R-D VK	SEQ ID NO:26
HIV-1CDC4	R-- Y-T V-T --- V-- R-E VR	SEQ ID NO:27
HIV-1HAN	R-- Y-T V-T --- V-- K-E VK	SEQ ID NO:28
HIV-1CAM1	R-- Y-T V-T --- V-- K-D KV	SEQ ID NO:29
HIV-1RF	K-- Y-A V-T --- V-- N-E VR	SEQ ID NO:30

HIV-1D31 R-- F-T V-T --- V-- R-E IK SEQ ID NO:31
HIV-1BH102 R-- Y-T V-T --- V-- R-E IK SEQ ID NO:32
HIV-1PV22 R-- Y-T V-T --- V-- R-E IK SEQ ID NO:33
HIV-1JRFL R-- Y-T V-T --- V-- R-E VK SEQ ID NO:34

The following are exemplary of the sequences for species of Consensus Sequence C:

CONSENSUS.C rSL ?Nt vat Lyc VH? ?ie vr SEQ ID NO:8
HIV-1ZAM18 K-- F-T VVT -WC --E DIT VR SEQ ID NO:35
HIV-1ZAM19 K-- H-A VAV -YC --K XIT VR SEQ ID NO:36
HIV-1ZAM20 R-- Y-T VAT -YC --A GIE VR SEQ ID NO:37

Consensus Sequence C includes the following exemplary species:

CONSENSUS.D kSL yNT VAT LYC VH? RiE VK SEQ ID NO:9
HIV-1ELI R-- Y-- --- --- --K G-D -K SEQ ID NO:38
HIV-1ZUZ6 R-- F-- --- --- --E R-E -K SEQ ID NO:39
HIV-1NDK R-- Y-- --- --- --E R-E -K SEQ ID NO:40

Similarly, other naturally occuring species within Consensus A, Consensus B, Consensus C, Consensus D, as well as Consensus F, Consensus G, Consensus H, Consensus O, whether presently known or existing, or subsequently discovered or subsequently arising, can be used as the modified HGP-30 antigenic peptide in the conjugated peptides of this invention. It is well known in the art that these various consensus sequences are generally derived from, and are prevalent in different geographical regions of the world and are often referred to as "clades" (also known as "subtypes") of the HIV virus. Representative of these clades of modified HGP-30 include the following consensus sequences (wherein the letter designations generally correspond to the consensus sequences as given above) and any allelic variations thereof:

Thailand-B:
YCV HQK IEV KDT KEA LEK IEE EQN KSK KKA SEQ ID NO:41
Thailand-A/E:
WCV HQR IEV KDT KEA LDK IEE VQN KSQ QKT SEQ ID NO:42

Uganda-A:
YCV HQR IDV KDT KEA LNK IEE MQN KNK QRT SEQ ID NO:43
Kenya-A:
YCV HQR IDV KDT KEA LDK IEE IQN KSK QKT SEQ ID NO:44
Brazil-A/E:
YFV HQR VEV KDT KEA LDK LEE EQN KSQ QKT SEQ ID NO:45
Brazil-B:
YCV HQK IDV RDT KEA LEK VEE EQN KSK EKA SEQ ID NO:46
Uganda-B:
YCV HQR IDV KDT KEA LDK IEE EQN KSK KKE SEQ ID NO:47
Uganda-C:
YCV HKG IEV RDT KEA LDK IEE EQN KIQ QKT SEQ ID NO:48
India-C:
YCV H?? IEV RDT KEA LDK IEE EQN K?Q QKT SEQ ID NO:49
Uganda-D:
YCV HER IKV ADT KEA LDK IEE EQT KSK KKA SEQ ID NO:50

As can be seen from the above aligned consensus sequences and species for the various consensus sequences, there is some variation amongst HIV subtypes in the gag protein sequence. Moreover, there is considerable variation in the specific numbering of amino acids among different HIV strains. In the present invention, the numbering of sequences is based on the sequence of HIV strain 1SF2 or MN; however, it is the amino acid sequence itself, allowing for variations observed amongst HIV subtypes, that is important. The sequences listed above are illustrative of the types of amino acid changes that can be made in the antigenic modified HGP-30 peptides of the invention and the conjugated peptides based thereon. In addition to the variations in the amino acids among the various HIV strains, it is also recognized that the amino acids at the N-terminal and C-terminal may be present as the free acid (amino or carboxyl groups) or as the salts, esters, ethers, or amides thereof. In particular amide end groups at the C-terminal and acetylation, e.g., myristyl,

etc. at the N- or C-terminal, are often useful without effecting the immunological properties of the peptide.

The first and second peptides of the conjugated peptides of the present invention can be prepared by conventional processes for synthesizing proteins, such as, for example, solid phase peptide synthesis, as described by Merrifield, R. B., 1963, J. of Am. Chem. Soc., 85:2149-2154. It is also within the scope of the invention and within the skill in the art to produce the novel conjugated peptides of this invention or the peptide components thereof by genetic engineering technology.

In the present invention, the above modified HGP-30 antigenic peptides are conjugated to a T cell binding peptide. Various T cell binding peptides can be used in this invention and examples include those shown in Table 1, below. These include, for example, peptide J from β-2-microglobulin 35-50 (Parham et al, 1983, J Biol Chem. 258:6179; Zimmerman et al, WO 89/12458); TCBLs from MHC class 1 α3 domain positions 223-229 (Salter et al, 1990, Nature 345:41), the MHC class II β2 domain 135-149 (Konig et al, 1992, Nature 356: 796; Cammarota et al, 1992, Nat 356:799) or Interleukin Iβ 163-171 (Nencioni et al, 1987 J. Immunol. 139:800). As examples of other peptides which may be used as the first peptide of the conjugated peptides of the invention, mention may be made of the aforementioned WO 89/12458. Guidelines for selection of these or other suitable T cell binding peptides are discussed therein as well as in the Zimmerman et al articles. Mention may be made of, for example, the molecules known as B7 (Freeman et al, Science 262:909); B70 (Azuma et al, 1993, Nature 366:76); GL1 (Hathcock et al, 1993, Science 262:905); CD58 (Arulanandam et al, 1993, Proc. Nat. Acad. Sci. 90:11613), CD40 (van Essen et al, 1995, Nature 378:620); and ICAM-1 (Becker et al, 1993, J. Immunol. 151:7224). The reader is referred to these literature articles for further details.

TABLE 1

TCBL Peptides used in Heteroconjugate Construction

Name/Amino Acid Sequence	Molecule/a.a. positions	
MHC Class I	MHC-$I_{\alpha 3}$	
DQT QDT E	223-229	(SEQ ID NO:51)
Lymphokine	IL-1_{β}	
VQG EES NDK	163-171	(SEQ ID NO:52)
MHC Class II	MHC-$II_{\beta 2}$	
NGQ EEK AGV VST GLI	135-149	(SEQ ID NO:53)
β-2-Microglobulin	β-2-M	
DLL KNG ERI EKV E	35-47	(SEQ ID NO:54)

Conjugated peptides prepared by conjugating the antigenic peptides based on the modified HGP-30 epitopes to any of these T cell binding peptides have been shown by the inventors to elicit an immune response to HIV that can be directed toward the desired TH1 as evidenced by the numerous examples of the TH1 characteristic antibody IgG2a (mouse) or IgG3 (man). The order of the first T cell binding peptide and second modified HGP-30 peptide is not usually critical and may be reversed. For example, if first peptide=A and modified HGP-30=B then the conjugated peptide may have the sequence A-B or B-A. Also, while the first peptide and modified HGP-30 may be directly coupled to each other, in some cases a small linker sequence or a larger heterolinker molecule may be used to couple the two peptides. For example, as the spacer, one or a few, up to about 5, preferably, up to about 3, neutral amino acids, such as glycine, may be used to link the peptides. A preferred spacer peptide is GGG, however, the spacer may be made larger or smaller and altered to include other molecules besides the amino acid glycine. As examples of heterolinkers mention may be made of, for example, N-succinimidyl-3-(2-pyridylthio)propinate (SPDP), m-maleimidobenzoyl-N-hydroxy-succimide (MBS) as well as any of the other reagents employed to link peptides,

including without limitation those disclosed in the aforemention WO 89/12458.

The following are exemplary of applications for various embodiments of the conjugated peptides of the invention but, it is understood that the invention is not restricted to the following described examples.

Embodiment 1. Use of the conjugate of e.g., Peptide J and the modified HGP-30 sequence to direct the immune response as a prophylactic vaccine for a TH1 directed immune response to prevent the infection by HIV.

Embodiment 2. Use of the conjugate to direct the immune response as a therapeutic vaccine for a TH1 directed immune response in HIV infected persons perhaps in conjunction with other therapies to reduce viral load and to control or cure the infection by HIV. Embodiment 3. Use of the first peptide as a carrier for the modified HGP-30 sequence to direct the immune response as a prophylactic vaccine to induce a TH1, TH2 or mixed TH1/TH2 directed immune response to prevent the infection by HIV.

Embodiment 4. Use of a first peptide as a carrier for the modified HGP-30 sequence to direct the immune response as a therapeutic vaccine to induce a TH1, TH2 or mixed TH1/TH2 directed immune response against the HIV virus and virus infected cells in HIV infected persons perhaps in conjunction with other therapies to reduce the viral load and to control or cure the infection by HIV.

Examples of other therapies which may be used in conjunction with the conjugated peptidess of this invention include, for example, protease inhibitors, reverse transcriptase inhibitors and the like.

Examples

I. Peptides

The T cell binding peptide of the conjugated peptide used in these studies includes a region of β-2 microglobulin, Peptide J shown underlined for a MHC Class I-like action

PEPTIDE J DLL KNG ERI EKV EGG C-amide SEQ ID NO:55

The conjugate with the modified HGP-30 sequence and the Peptide J contained a spacer of one additional glycine substituted for the C-terminal cysteine for a total of three glycine residues. Accordingly, the conjugated peptide had the following formula

JHD LLK NEG ERI EKV EGG
GAT LYS VHQ RID VKD TKE
ALE KIE FEQ NKS SEQ ID NO:56

wherein the underlined portion represents m-HGP-30.

The peptides were all synthesized by Quality Controlled Biochemicals, Inc. (QCB) (Hopkinton, MA) using the FMOC procedure and a double coupling protocol for the first 8 residues. Usually the peptide is prepared with the carboxyl terminus as an amide form. All of the peptides were purified at QCB using preparative HPLC, and analyzed by an analytical HPLC, amino acid analysis and mass spectrophotometer. The peptides were greater than 95%, usually greater than 98%, pure by HPLC criteria. The dry peptides obtained from QCB were stored in glass vials with desiccant at -20°C.

II. Preparation of Conjugates

KLH Conjugations

Keyhole Limpet Haemocyanin (KLH) (Pierce) may be conjugated to the HGP-30 or modified HGP-30 peptide by a glutaraldehyde conjugation method. KLH may also be conjugated to HGP-30 via the EDC method as described above. The alternative conjugation techniques are useful to evaluate if the method of conjugation was important as far as the nature of the immune response evoked. In both cases a 1:1 mg weight ratio of peptide to KLH is used. Conjugation of the antigenic peptide to KLH may also be carried out by formation of a thioether using a halogenated N-terminal acetyl derivative. To reduce any oxidized sulfhydryls or disulfides that may form during storage, the resulting dissolved T cell binding ligands (TCBL) are added to an equal molar quantity of

tris-(2-carboxylethyl)phosphine (TCEP) which is dissolved to a concentration of 3.5 mg/ml in 0.2 M sodium phosphate buffer (pH 6.8). Next, 50 µl of 0.1 M ethylenediaminetetraacetic acid disodium (EDTA) is added to give a final concentration of 0.005 M. The mixture is then gassed with nitrogen, and allowed to incubate with stirring using a "V" shaped stir bar in a sealed screw cap plastic conical reaction vessel (total container volume 1.5 ml) for at least 45 minutes, but usually less than 120 minutes, at room temperature. In the Thioether protocol, the KLH is treated with TCEP, and separated using a P6DG column (Bio-Rad). The HGP-30 peptide and KLH are allowed to incubate for 18 hours to allow conjugation to occur. Then the reaction mixture is exhaustively dialyzed against 3-4 changes of 1 L each change of PBS over 3-5 days and the product is sterile filtered (0.2µ low protein binding filter).

The conjugated peptide may be synthesized as a single peptide without any conjugation step or by conjugation of the Peptide J and the modified HGP-30 by using the thioether method or by any other conjugation method known to the skilled practitioner.

The final products, the peptide, conjugated peptide, peptide-KLH control, are analyzed for protein or peptide using the BCA protein assay, and adjusted to contain between 200-400 µg/ml of total protein or peptide, and stored frozen (-20°C) in 1.5 ml aliquots ready for thawing and administered in combination with an adjuvant (e.g., alum, ICFA, SAF-1) or carrier (e.g., liposomes or Novasomes).

III. Immunization, Anti-sera Collection and Processing

In a series of experiments, groups (5-10 per group) of 10-16 week old BALB/c female mice (Taconic Farms, Germantown, NY) are immunized and test bled according to the following schedule. Schedule A immunizations on day 0, day 7, test bleeding on days 14, 28 and 42.

The antigens are prepared with adjuvants and carriers as follows. The antigens are emulsified for Incomplete Freund's adjuvant (Life Technology, Gaithersburg MD) supplemented with Muramyl Dipetide (Pierce). Other adjuvants, which may be used include, for example, alum, Ribi (Immunochem Research Inc. Hamilton, Montana) and a proprietary adjuvant "Novasomes" (Novavax, Rockville MD). These adjuvants and carrier systems are used according to the manufactures' direction. The Novasome system is evaluated with or without a Lipid A supplement.

Unanesthetized mice are placed in the palm of one hand with the nape held between the thumb and forefinger, and the little finger wrapped around the lower abdomen. The mice are inoculated with 0.2-0.4 ml of the emulsion equally divided between a subcutaneous site in the nape of the neck and intraperitonealy in the lower abdomen. Other routes which could be used include subcutaneously, intramuscularly, etc. The inoculum contains 250 μg/ml of KLH conjugate, conjugated peptide or peptide alone, unless otherwise stated.

The mice are anesthetized by Metofane™ (Pitman-Moore Mundelein, IL) for retrorbital bleeding and ear tagging. Blood from individual mice on the specified days is collected from the retrorbital vein using a 5 3/4" glass pasteur pipette, transferred to 1.5 ml centrifuge tube and allowed to clot. The clots are separated from the walls of the tube by use of a flexible thin wire extending to the bottom of the tube and encircling the inner circumference of the tube, and the cells/and clot are separated by centrifugation from the sera. The sera from individual animals are collected and placed in labeled storage vials and stored frozen until ready for testing. At the first time of blood collection, each mouse is also ear tagged for identification purposes with an aluminum band imprinted with a unique sequential number (National Band and Tag, Lexington, Ky).

IV. ELISA Assays

The sera is tested for the presence of antibody by an indirect ELISA. In this procedure, high binding plates (Maxi Sorb; Nunc, Naperville, IL) are used. The plates are coated at 4°C with the HGP-30 (SEQ ID NO:1) or modified HGP-30 (SEQ ID NO:5) from a different preparation or a control peptide at a concentration of 1.0 μg/ml in 0.15 M bicarbonate coating buffer (pH 9.6) using 118 μl/well, and stored at 4°C for 1-7 days. Prior to use, the wells are washed at least 2 times with PBS containing 0.05% Tween™ 20 (PBSTw), blocked with 150 μl of 0.2% bovine serum albumin (BSA) (Sigma Chemicals St Louis, MO) in PBSTw for 15-30 minutes, and washed at least two more times with PBSTw. Antibodies to the coating HGP-30, modified HGP-30 or a control peptide in the sample are assayed as follows. The control peptide used in this case is derived from an env V-3 peptide about 20 amino acids in length, however, as is well known in the art, other controls can be used to measure non-specific antibody response as measure of background.

First, an appropriate dilution, usually from 1:100 to 1:10,000 of the test antisera is made in 0.2% BSA in PBSTw, and 100 ml thereof is added per well. After all of the wells are loaded, the plates are sealed with an adhesive plate sealer (ICN, Costa Mesa, CA), and incubated for 2 hours at 37°C. The plates are then washed at least three times with PBSTw (>250 μl/well per wash) and drained. The wells are loaded with 100 μl of a dilution, usually 1:5000 in 5.0% BSA in PBSTw, of the enzyme-antibody conjugate, HRP-goat anti-murine immunoglobulins (Kirkegaard and Perry Laboratories (KPL), Gaithersburg, MD). The plates are incubated for 1.5 hours with the enzyme-antibody conjugate before a final series of three washing steps and color development using as the substrate, 100 μl/well of o-phenylenediamine dihydrochloride (OPD (Sigma)). The substrate is prepared by dissolving a 5.0 mg tablet in 12.5 ml of urea hydrogen

peroxide phosphate citrate buffer (pH 5.0). The color reaction is stopped after about 60 minutes with 100 µl of 4.0 N H2SO4, and the color is read as optical density (OD) or absorption (A_{490}) at 490 nanometers on an ELISA plate reader. Data is printed out and also saved on the hard drive of the computer attached to the plate reader for use in further analysis. Data points are collected in duplicates, and the values reported as the average of both readings.

As discussed below, in some cases, the second incubation (1.5 hours) is carried out with isotyping antisera of Goat anti-murine heavy chain specific class or subclass (µ, α, γ1, 2a, 2b and 3) (Sigma or ICN), and then an enzyme conjugate, HRP-rabbit-anti-goat immunoglobulins (KPL), is used before the substrate color development step.

EXAMPLE 1

Previously it was observed that a TB related heterofunctional conjugate can stimulate a TH1 or TH2 antigen specific immune response (Zimmerman, Vaccine Res. 5:91-102, 1996a, 5:103-118, 1996b). Table 2 demonstrates that such heteroconjugate of a modified HGP-30 shows responses as an ELISA signal observable at several different dilutions starting at 1:200 to 1:6400 by testing the antisera reactivity against wells coated with either the immunizing, but unconjugated modified HGP-30 peptide or control peptide, measuring the OD, and calculating the differences between the two OD values which is indicative of specific antibody. These mice are immunized using Incomplete Freund's adjuvant at 40 µg per dose per animal. A large number of the animals produced specific antisera with a substantial titer. Whereas with the TB heterofunctional conjugate no discernable specific immune response is observed until a sensitive challenge protocol is used with the immunogenic modified HGP-30

heterofunctional conjugate, 2/3 of the animals at the dose used even as early as day 35 showed an immune response at a 1:800 dilution.

TABLE 2

Titer Analysis of Anti-HGP30 in heteroconjugate Immunized mice

Mouse Number	Dilution	1/200 Modified HGP-30	Control Peptide	Net HGP-30	1/400 Modified HGP-30	Control Peptide	Net HGP-30	1/800 Modified HGP-30	Control Peptide	Net HGP-30
Prebled		0.090	0.090	0.000	0.070	0.070	0.010	0.050	0.060	0.000
S12		0.820	0.110	0.700	0.490	0.060	0.430	0.290	0.060	0.230
S13		0.610	0.100	0.610	0.390	0.080	0.310	0.230	0.070	0.160
S14		0.230	0.110	0.110	0.140	0.060	0.080	0.100	0.060	0.030
S15		1.330	0.090	1.230	1.220	0.070	1.160	1.080	0.060	1.020
S16		1.210	0.110	1.100	1.030	0.080	0.960	0.810	0.070	0.740

Mouse Number	Dilution	1/1600 Modified HGP-30	Control Peptide	Net HGP-30	1/3200 Modified HGP-30	Control Peptide	Net HGP-30	1/6400 Modified HGP-30	Control Peptide	Net HGP-30
Prebled		0.060	0.060	0.000	0.050	0.050	0.010	0.060	0.050	0.000
S12		0.150	0.050	0.100	0.100	0.050	0.050	0.070	0.050	0.020
S13		0.120	0.070	0.050	0.100	0.070	0.030	0.080	0.070	0.010
S14		0.090	0.070	0.020	0.080	0.070	0.010	0.070	0.060	0.000
S15		0.910	0.060	0.850	0.690	0.060	0.630	0.470	0.060	0.410
S16		0.590	0.070	0.520	0.420	0.060	0.360	0.250	0.070	0.180

EXAMPLE 2

Three groups of mice are set up for evaluation using immunization doses of 40, 8 and 1.6 μg on day 0 and day 14 with heteroconjugates using HGP-30 preparation with the β-2-microglobulin TCBL, Peptide J. These mice are then test bled on day 35 and the sera are analyzed for the presence of an antibody immune response. Table 3 shows the results with the initial day 35 test bleed and results with antisera collected 14 days after a booster inoculation on day 42. As can be seen from Table 3, following the booster all of the animals with the higher dose responded and even several of those with the next lowest dose also responded.

TABLE 3

Dose Response with varying dose for immunization with JH heteroconjugate

	Results of Initial Test Bleed (day 35)		Results 14 days after Test bleed after booster	
Group#	Number of animals*	Number of Responders	No. of Animals	No. of Responders
40 μg	12	5	12	9
8μg	13	0	13	2
1.6 μg	13	0	13	0

\# = antigen (as specified above) in emulsion (0.2mL) of equal parts of Incomplete Freund's adjuvant and sterile saline per animal per inoculation

* = Number of BALB/c females immunized

@ = Number of animals with specific antibody signal at 1:200 dilution of over 0.2 (> 3 times the value of the control peptide)

EXAMPLE 3

In order to evaluate and identify advantageous adjuvants and carriers for use with the heteroconjugates, other than Incomplete Freund's adjuvant which is not

approved by the FDA for use in man the following test is performed. A single conjugate peptide preparation JH is used at a 25 microgram dose per animal. This is used to immunize groups of mice on day 0 and 14 and then the mice are test bled on day 35. The resultant antisera are evaluated in a standard ELISA for HGP-30 specific antibody. As seen in Table 4 none of the Alum adjuvant group showed a response whereas significant numbers of animals immunized using either Incomplete Freund's Adjuvant or the Commercial formulation of TDM/MPL preparation of RIBI available from Sigma Chemicals induced an immune response. As before (Table 3) after the booster on day 42 all animals tested on day 56 for the Ribi and Freund's adjuvants demonstrated antibody response (data not shown). However, the alum heteroconjugate immunized animals were still non-responsive.

TABLE 4

Summary of day 35 response using JH heterofunctional conjugate (Peptide J as and m-HGP-30) to generate an immune response with different Adjuvants

Group#	Number of animals*	Number of responders@
ALUM	14	0
ICFA	12	9
RIBI	10	8
No antigen or Adjuvant	14	0

\# = 25 μg of antigen in emulsion (0.2mL) of adjuvant and sterile saline per animal per inoculation

* = Number of BALB/c females immunized

@ = Number of animals with specific antibody signal at 1:200 dilution of over 0.2 (>3 times the value of the control peptide)

EXAMPLE 4

Since the adjuvant has been shown to be of some importance in eliciting an immune response another adjuvant, Novasomes, is tested. In addition, since the inclusion of Lipid A is thought to be beneficial this Novasome adjuvant is evaluated with or without Lipid A addition. The Novasomes are supposed to possess many of the properities of liposomes but with the added properities of ease of manufacturing and long term stability. As seen in Table 5 no advantage is seen using Novasomes with or without Lipid A for most antigens. Indeed, one group of animals immunized with Novasomes and the heteroconjugate had the Lipid A subset seemingly having a lower signal than does the group without Lipid A. It should be noted that often Alum is used in conjunction with the Liposomes but not enough material was available to make such a comparison.

TABLE 5

Summary for various antigens and conjugates of TCBL and KLH with HGP-30 or m-HGP-30 to generate an immune response using Novosome or Alum as adjuvant.

Group	Number of animals	Number of responders
Novosomes + m-HGP-30	7	0
Novosomes + m-HGP-30 with Lipid A	7	0
Novosomes + HGP-30	7	0
Novosomes + HGP-30 with Lipid A	7	0
Novosomes + HGP-30-KLH	6	6
Novosomes + HGP-30-KLH with Lipid A	5	5
Novosomes + m-HGP-30 Heteroconjugate	6	6

Alum + m-HGP-30 Heteroconjugate with Lipid A	6	0
Alum + m-HGP-30	6	0
Alum + HGP-30	6	0

In this example the animals are immunized on days 0 and 7 with the antigen and the test bleedings are taken on day 28. See legend to Table 2 for other details.

EXAMPLE 5

This example investigates the specificity of the antibodies induced by the heteroconjugate and compares the HGP-30 and modified HGP-30 KLH derived antibodies. For this purpose the conjugated peptide antibodies from several different adjuvant or dose groups are analyzed. The results are shown in Table 6. The antibodies are analyzed for reactivity to not only the immunizing antigen sequence which is shown but also for other modified HGP-30's as shown in italics and for a control peptide A shown in the first column. The last column is an indicator of the ratio of reactivity of the antibodies induced by m-HGP-30 (SEQ ID NO:5) and HGP-30 (SEQ ID NO:1). Interestingly, the m-HGP-30 KLH immunized antisera show a strong preference for the modified HGP-30 and yet this same sequence in the heterofunctional conjugate is able to induce antibodies which often have the more desirable broader specificity as seen by the original HGP-30-KLH conjugate as previously reported for a TB heterofunctional conjugate (Zimmerman, et al., 119, ibid).

TABLE 6

Evaluation of the antigen specificity of the HGP-30 conjugated peptide derived antibodies

		Control	m-HGP	Net m-HGP	HGP	Net HGP	Ratio m-HGP/HGP
779	NOVA	0.074	0.684	0.610	0.771	0.697	0.875
780		0.104	0.808	0.705	0.896	0.792	0.890
781		0.072	0.396	0.325	0.537	0.466	0.697
782		0.075	0.633	0.559	0.785	0.711	0.786
783		0.093	0.833	0.741	0.887	0.794	0.933
784		0.083	0.856	0.773	0.951	0.868	0.891
429	JH LO	0.078	0.356	0.279	0.070	0.000	0.000
432		0.071	0.604	0.533	0.065	0.000	0.000
435	JH	0.099	0.302	0.204	0.093	0.000	0.000
437	MED	0.078	1.010	0.932	0.860	0.782	1.192
438		0.095	1.235	1.140	0.940	0.845	1.349
439		0.085	0.427	0.342	0.076	0.000	0.000
441		0.073	0.477	0.405	0.075	0.002	0.000
442		0.086	0.633	0.547	0.083	0.000	0.000
443		0.084	1.380	1.296	1.287	1.203	1.077
444		0.092	1.230	1.138	1.168	1.076	1.058
446		0.075	1.040	0.965	1.230	1.155	0.835
447		0.054	1.180	1.126	1.039	0.985	1.143
448		0.115	0.635	0.520	0.648	0.533	0.976
511	JHICFA	0.075	1.338	1.264	0.074	0.000	0.000
512		0.140	1.491	1.351	0.606	0.466	2.899
513		0.082	1.099	1.018	1.100	1.019	0.999
514		0.080	0.770	0.690	0.126	0.046	15.000
515		0.083	1.169	1.087	1.088	1.005	1.082
516		0.065	0.982	0.917	0.991	0.926	0.990
517		0.093	0.907	0.814	0.994	0.901	0.903
518		0.093	1.281	1.188	1.451	1.358	0.875
519		0.124	1.410	1.286	0.110	0.000	NA
520		0.099	1.402	1.303	1.279	1.181	1.103
523	JH	0.151	1.457	1.306	0.213	0.063	20.730
525	RIBI	0.168	1.506	1.338	0.418	0.251	5.331
526		0.240	1.246	1.006	0.119	0.000	NA
528		0.106	1.474	1.368	0.667	0.561	2.439
530		0.093	1.064	0.971	0.068	0.000	NA
531		0.133	1.402	1.269	1.361	1.228	1.033

EXAMPLE 6

It is known that the amino acid sequence of β-2 microglobulin, is highly conserved between species, including man and mouse. The T cell binding sequence from human β-2-microglobulin does contain an epitope as indicated by its ability to generate, albeit probably not frequently, antibodies including monoclonal antibody in mice. However, since the ultimate use of the heteroconjugate is in man and not the mouse some amount of immunogenicity of the T cell binding peptide in the mouse is allowable. Indeed, as can be seen in a fraction of the cases where specific anti-HGP-30 were generated, the generation of anti-J antibodies is observed, but with a low frequency(1/10 the rate for m-HGP-30). Even with a second booster at day 42 the number of responders is still only a fraction of the m-HGP-30.

TABLE 7

Evaluation of the T cell binding reactivity of the HGP-30 conjugate derived antibodies

Group Responders	No. of Animals*	No. of m-HGP30 responders	No. of TCBL (peptide J)
EXPERIMENT 1			
ALUM	14	0	0
ICFA	12	9	1
RIBI	10	8	0
No antigen or Adjuvant	14	0	0
EXPERIMENT 2			
ICFA	12	6	2
EXPERIMENT 3			
Novasomes	6	6	0

= 25 μg of antigen in emulsion (0.2mL) of adjuvant and sterile saline/animal per inoculation

* = Number of animals with specific antibody signal at 1:200

@ = Number of animals with specific antibody signal at 1:200 dilution of over 0.2 (>2 times the value of the control peptide)

EXAMPLE 7

Examination of the isotype profile of these broad specificity antibodies shows that, as desired, the antibodies show an isotype distribution expected for a TH1 response (Table 8). These are the animals shown in Table 4. These data are more difficult to evaluate for clear demonstrable improvement, compared to the specificity in Table 7 or that reported by Zimmerman et al (1996b, *ibid*). This is apparently because adjuvants and carriers strongly influence the type of response; i.e., Alum being a very strongly TH2 and indicate high levels of IgG1, low IgG2a and poor DTH; Complete Freund's being an improvement toward a weak TH1 (some IgG2a and weak DTH) but still strongly TH2 (IgG1). These effects depend upon which Ribi formulation is used and, furthermore, in this study Incomplete Freund's was used and not Complete Freunds. It can be see that the isotypes characteristic of a TH1 (IgG2a and IgG2b) are found. As can be seen the alum is not effective with this JH heteroantigen while Incomplete Freund's, Ribi and Novasomes were able to work with JH. No reversal is seen and examination of the ratio of IgG2a/IgG1 suggests that the heteroconjugate of the modified HGP-30 favors a TH1 IgG2a above that seen with the peptide conjugated to KLH as previously reported.

With regard to dose of antigen no difference is seen as has been reported. It has also been reported that low doses of antigen also favour TH1 IgG2a with Leishmania antigens in BALB/c mice, the strain used here. Not shown is data collected at separate times for the antibodies from the dose studies (Table 3) and the Novasome JH heteroconjugate groups found in Table 5. The modified HGP-30 heteroconjugate induces more of those immunoglobulins TH1 subtypes than the modified HGP-30

conjugated to KLH. This is demonstrated in the last column showing ratios of IgG2/IgG1 where a larger number shows more of a TH1 response.

TABLE 8

Isotype analysis of JH heteroconjugate derived anti HGP-30 antibodies

	IgA	IgM	Net Signal as Anti m-HGP 30 as IgG1	IgG2a	IgG2b	IgG3	Ratio Ig2a/ IgG1
511 JH	0.041	0.178	0.790	0.201	0.317	0.085	0.25
512 ICFA	0.015	0.082	0.255	0.222	0.102	0.254	0.87
513	0.023	0.057	0.347	0.057	0.048	0.243	0.16
515	0.022	0.000	1.227	0.327	0.132	0.040	0.27
516	0.009	0.263	0.985	0.005	0.009	0.000	0.01
517	0.017	0.000	0.769	0.000	0.016	0.000	0.00
518	0.006	0.060	1.000	0.271	0.011	0.025	0.27
519	0.000	0.105	0.911	0.202	0.068	0.038	0.22
520	0.060	0.605	1.046	1.013	1.295	0.415	0.97
521	0.023	0.013	0.092	0.010	0.152	0.000	0.11
523 JH	0.005	0.000	0.218	0.270	0.580	0.000	1.24
524 RIBI	0.000	0.000	0.726	0.821	0.217	0.581	1.13
525	0.019	0.000	0.361	0.563	0.898	0.073	1.56
526	0.000	0.000	0.592	0.227	0.032	0.000	0.38
527	0.000	0.000	0.053	0.886	1.235	0.000	16.72
528	0.000	0.101	1.142	0.347	1.154	0.970	0.30
529	0.000	0.000	0.093	0.119	0.000	0.236	1.28
530	0.000	0.000	0.342	0.791	0.234	0.094	2.31
531	0.000	0.000	0.206	0.862	0.289	0.102	4.18
532	0.014	0.000	0.519	0.077	0.118	0.020	0.15

Claims

1. A conjugated peptide capable of eliciting a TH1 response when administered to a human, said conjugated peptide comprising a first T cell specific binding peptide and a second T cell specific binding peptide, said first and second peptides being derived from different molecules and covalently linked together, wherein said first T cell specific binding peptide binds to a specific class or subclass of T cells and said second T cell specific binding peptide is an antigenic peptide capable of eliciting TH1 associated antibodies and having sequence identity with the p17 gag protein of HIV wherein the peptide has a sequence originating with an amino acid residue chosen from residues 75 to 82 and ending with an amino acid residue chosen from residues 106 to 111 of p17 gag protein of HIV.

2. An immunogenic composition comprising the conjugated peptide of claim 1 and an immunogenic carrier.

3. A method of eliciting a TH1 response in a human patient in need thereof, comprising administering to said patient an immunologically effective amount of the conjugated peptide of claim 1.

4. The method of claim 3 wherein the heterofunctional conjugate is administered in combination with an immune response adjuvant.

EDITORIAL NOTE - 40554/97

Page 33 of this specification is an abstract which follow after the sequence listings pages 34-59.

Raw Sequence Listing

Sequence Listing

(1) GENERAL INFORMATION

(i) APPLICANTS:DANIEL H. ZIMMERMAN, PREM S. SARIN

(ii) TITLE OF INVENTION:Modified HGP-30 Heteroconjugates, Compositions and Methods of Use

(iii) NUMBER OF SEQUENCES:50

(iv) CORRESPONDENCE ADDRESS:

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(B) STREET:413 N. Washington Street

(C) CITY:Alexandria

(D) STATE:Virginia

(E) COUNTRY:USA

(F) ZIP:22314

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE:Diskette, 5.25 inch, 1.2 mb storage
3.5 inch, 1.44 mb storage

(B) COMPUTER:Dell System 210; Intel 80 286 Microprocessor

(C) OPERATING SYSTEM:MS DOS 6.22

(D) SOFTWARE:Word Perfect, Version 5.1

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:08/695,304

(B) FILING DATE:August 9, 1996

(vii) ATTORNEY/AGENT INFORMATION:

(A) NAME:Richard A. Steinberg

(B) REGISTRATION NUMBER:26,588

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(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE:(703) 549-2282

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(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:30 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:HGP-30

(B) LOCATION:85 to 114

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV

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

Tyr Ser Val His Gln Arg Ile Asp Val Lys Asp Thr Lys Glu Ala Leu
5 10 15
Glu Lys Ile Glu Glu Glu Gln Asn Lys Ser Lys Lys Lys Ala
20 25 30

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:25 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:82 to 106

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV

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

Ala Thr Leu Tyr Ser Val His Gln Arg Ile Asp Val Lys Asp Thr Lys
5 10 15
Glu Ala Leu Glu Lys Ile Glu Glu Glu
20 25

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:36 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:76 to 111

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV

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

```
Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Ser Val His Gln Arg Ile
                5                  10                  15
Asp Val Lys Asp Thr Lys Glu Ala Leu Glu Lys Ile Gln Glu Glu Gln
            20                  25                  30
Asn Lys Ser Lys
        35
```

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:37 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:75 to 111

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV

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

```
Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Ser Val His Gln Arg
                5                  10                  15
Ile Asp Val Lys Asp Thr Lys Glu Ala Leu Gln Lys Ile Glu Glu Glu
            20                  25                  30
Gln Asn Lys Ser Lys
        35
```

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:29 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:82 to 110

(B) LOCATION:

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV

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

Ala Thr Leu Tyr Ser Val His Gln Arg Ile Asp Val Lys Asp Thr Lys
5 10 15
Glu Ala Leu Glu Lys Ile Glu Glu Glu Gln Asn Lys Ser
20 25

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:37 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:75 to 111

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV, CONSENSUS A

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

Lys Ser Leu Phe Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln Arg
5 10 15
Ile Asp Val Lys Asp Thr Lys Glu Ala Leu Asp Lys Ile Glu Glu Ile
20 25 30
Gln Asn Lys Ser Lys
35

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:37 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:75 to 111

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV, CONSENSUS B

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

```
Arg Ser Lys Tyr Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln Arg
                5                  10                  15
Ile Glu Val Lys Asp Thr Lys Glu Ala Leu Asp Lys Ile Glu Glu Glu
            20                  25                  30
Gln Asn Lys Ser Lys
        35
```

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:37 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:75 to 111

(D) OTHER INFORMATION:fragment of p-17 protein of HIV, CONSENSUS C

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

```
Arg Ser Leu Xaa Asn Thr Val Ala Thr Leu Lys Cys Val His Xaa Xaa
                5                  10                  15
Ile Glu Val Arg Asp Thr Lys Glu Ala Leu Asp Lys Ile Glu Glu Glu
            20                  25                  30
Gln Asn Lys Xaa Gln
        35
```

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:37 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:75 to 111

(D) OTHER INFORMATION:fragment of p-17 protein of HIV, CONSENSUS D

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

Lys Ser Leu Xaa Asn Thr Val Ala Thr Leu Tyr Cys Val His Glu Arg
5 10 15
Ile Glu Val Lys Asp Thr Lys Glu Ala Leu Glu Lys Met Glu Glu Glu
20 25 30
Gln Asn Lys Ser Lys
35

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:37 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:75 to 111

(D) OTHER INFORMATION:fragment of p-17 protein of HIV,

CONSENSUS F

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

Arg Ser Leu Xaa Asn Thr Val Xaa Val Leu Tyr Phe Val His Gln Arg
5 10 15
Val Glu Xaa Lys Asp Thr Lys Glu Ala Leu Glu Glu Val Glu Lys Ala
20 25 30
Gln Lys Gln Gln Lys
35

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:37 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:75 to 111

(D) OTHER INFORMATION:fragment of p-17 protein of HIV,

CONSENSUS G

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

Lys Ser Leu Xaa Asn Xaa Xaa Ala Xaa Leu Xaa Cys Xaa His Gln Arg
1 5 10 15
Ile Xaa Val Lys Asp Thr Lys Glu Ala Leu Glu Glu Val Glu Lys Ala
20 25 30
Gln Lys Asn Ser Lys
35

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:37 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:75 to 111

(D) OTHER INFORMATION:fragment of p-17 protein of HIV,

CONSENSUS H

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

Gln Ser Leu Phe Asn Leu Leu Ala Xaa Leu Tyr Cys Val His Gln Arg
1 5 10 15
Ile Asp Xaa Lys Asp Thr Lys Glu Ala Leu Xaa Lys Xaa Xaa Glu Gln
20 25 30
Asn Xaa Gln Xaa Xaa
35

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:37 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:75 to 111

(D) OTHER INFORMATION:fragment of p-17 protein of HIV,

CONSENSUS O

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

Xaa Ser Leu Trp Asn Ala Ile Xaa Val Leu Trp Cys Val His Asn Arg
5 10 15
Xaa Xaa Ile Xaa Asp Thr Gln Gln Ala Ile Gln Lys Leu Lys Glu Val
20 25 30
Met Xaa Lys Ser Ala
35

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:20 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:75 to 94

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV-1U455

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

Arg Ser Leu Tyr Asn Thr Val Ala Val Leu Tyr Cys Val His Gln Arg
5 10 15
Ile Asp Val Lys
20

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:20 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:75 to 94

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV-1MAL

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

Lys Ser Leu Tyr Asn Thr Val Ala Gly Leu Tyr Cys Val His Gln Arg
5 10 15
Ile Asp Val Lys
20

(3) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:20 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:75 to 94

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV-1TN243

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

Lys Ser Leu Phe Asn Thr Val Ala Thr Leu Trp Cys Val His Gln Arg
5 10 15
Ile Glu Val Lys
20

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:20 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:75 to 94

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV-1SF2

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

Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln Arg
5 10 15
Ile Asp Val Lys
20

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:20 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:75 to 94

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV-1TB132

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

Arg Ser Leu Tyr Asn Thr Ile Ala Val Leu Tyr Cys Val His Gln Lys
5 10 15
Ile Glu Val Lys
20

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:20 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:75 to 94

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV-1LAI

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

Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln Arg
5 10 15
Ile Glu Ile Lys
20

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:20 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:75 to 94

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV-1HXB2R

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

Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln Arg
5 10 15
Ile Glu Ile Lys
20

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:20 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:75 to 94

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV-1MN

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

Lys Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln Lys
5 10 15
Ile Glu Ile Lys
20

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:20 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:75 to 94

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV-1JH3

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

Lys Ser Leu Phe Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln Arg
5 10 15
Ile Glu Val Lys
20

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:20 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:75 to 94

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV-1JRCSF

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

Thr Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln Arg
5 10 15
Ile Glu Ile Lys
20

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:20 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:75 to 94

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV-1DYI

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

Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln Lys
5 10 15
Ile Glu Val Lys
20

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:20 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:75 to 94

(D) OTHER INFORMATION: fragment of p-17 gag protein of HIV-1NY5CG

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:25:

Arg Ser Leu Phe Asn Thr Val Ala Val Leu Tyr Cys Val His Gln Arg
5 10 15
Ile Asp Val Lys
20

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:20 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:75 to 94

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV-1NL43

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

Arg Ser Leu Tyr Asn Thr Ile Ala Val Leu Tyr Cys Val His Gln Arg
5 10 15
Ile Glu Val Lys
20

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:20 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:75 to 94

(B) LOCATION:

(D) OTHER INFORMATION:fragment of p-17 protein of HIV-1CDC4

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

Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln Arg
5 10 15
Ile Glu Val Arg
20

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:20 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:75 to 94

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV-1HAN

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

Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln Lys
5 10 15
Ile Glu Val Lys
20

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:20 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:75 to 94

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV-1CAM1

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

Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln Lys
5 10 15
Ile Asp Lys Val
20

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:20 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:75 to 94

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV-1RF

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

Lys Ser Leu Tyr Asn Ala Val Ala Thr Leu Tyr Cys Val His Gln Asn
5 10 15
Ile Glu Val Arg
20

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:20 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:75 to 94

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV-1D31

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

Arg Ser Leu Phe Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln Arg
5 10 15
Ile Glu Ile Lys
20

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:20 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:75 to 94

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV-1BH102

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

Arg Ser Leu Thr Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln Arg
5 10 15
Ile Glu Ile Lys
20

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:20 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:75 to 94

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV-1PV22

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

Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln Arg
5 10 15
Ile Glu Ile Lys
20

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:20 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:75 to 94

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV-1JRFL

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

Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln Arg
5 10 15
Ile Glu Val Lys
20

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:20 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:75 to 94

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV-1ZAM18

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

Lys Ser Leu Phe Asn Thr Val Val Thr Leu Trp Cys Val His Glu Asp
5 10 15
Ile Thr Val Arg
20

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:20 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:75 to 94

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV-1ZAM19

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

Lys Ser Leu His Asn Ala Val Ala Val Leu Tyr Cys Val His Lys Xaa
5 10 15
Ile Thr Val Arg
20

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:20 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:75 to 94

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV-1ZAM20

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

```
Arg Ser Leu Tyr Asn Thr Val Ala Thr Lys Tyr Cys Val His Ala Gly
                5                   10                  15
Ile Glu Val Arg
            20
```

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:20 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:75 to 94

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV-1ELI

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

```
Arg Ser Leu Tyr Asn Thr Val Ala Thr Lys Tyr Cys Val His Lys Gly
                5                   10                  15
Ile Asp Val Lys
            20
```

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:20 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:75 to 94

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV-1ZUZ6

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

Arg Ser Leu Phe Asn Thr Val Ala Thr Lys Tyr Cys Val His Glu Arg
5 10 15
Ile Glu Val Lys
20

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:20 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:75 to 94

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV-1NDK

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

Arg Ser Leu Tyr Asn Thr Val Ala Thr Lys Tyr Cys Val His Glu Arg
5 10 15
Ile Glu Val Lys
20

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:30 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:85 to 114

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV; Thailand-B

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

```
Tyr Cys Val His Gln Lys Ile Glu Val Lys Asp Thr Lys Glu Ala Leu
                5                  10                  15
Glu Lys Ile Glu Glu Glu Gln Asn Lys Ser Lys Lys Lys Ala
            20                  25                  30
```

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:30 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:85 to 114

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV; Thailand-NE

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

```
Trp Cys Val His Gln Arg Ile Glu Val Lys Asp Thr Lys Glu Ala Leu
                5                  10                  15
Asp Lys Ile Glu Glu Val Gln Asn Lys Ser Gln Gln Lys Thr
            20                  25                  30
```

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:30 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:85 to 114

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV; Uganda-A

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

```
Tyr Cys Val His Gln Arg Ile Asp Val Lys Asp Thr Lys Glu Ala Leu
                5                  10                  15
Asn Lys Ile Glu Glu Met Gln Asn Lys Asn Lys Gln Arg Thr
            20                  25                  30
```

(3) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:30 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:85 to 114

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV; Kenya-A

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

```
Tyr Cys Val His Gln Arg Ile Asp Val Lys Asp Thr Lys Glu Ala Leu
                5                  10                  15
Asp Lys Ile Glu Glu Ile Gln Asn Lys Ser Lys Gln Lys Thr
            20                  25                  30
```

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:30 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:85 to 114

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV; Brazil-A/E

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

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

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:30 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:85 to 114

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV; Brazil-B

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

```
Tyr Cys Val His Gln Lys Ile Asp Val Arg Asp Thr Lys Glu Ala Leu
                5                   10                  15
Glu Lys Val Glu Glu Glu Gln Asn Lys Ser Lys Glu Lys Ala
            20                  25                  30
```

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:30 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:85 to 114

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV; Uganda-B

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

```
Tyr Cys Val His Gln Arg Ile Asp Val Lys Asp Thr Lys Glu Ala Leu
                5                   10                  15
Asp Lys Ile Glu Glu Glu Gln Asn Lys Ser Lys Lys Lys Glu
            20                  25                  30
```

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:30 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:85 to 114

(D) OTHER INFORMATION:fragment of p-17 protein of HIV

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

Tyr Cys Val His Lys Gly Ile Glu Val Arg Asp Thr Lys Glu Ala Leu
5 10 15
Asp Lys Ile Glu Glu Glu Gln Asn Lys Ile Gln Gln Lys Thr
20 25 30

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:30 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:85 to 114

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV; India-C

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

Tyr Cys Val His Xaa Xaa Ile Glu Val Arg Asp Thr Lys Glu Ala Leu
5 10 15
Asp Lys Ile Glu Glu Glu Gln Asn Lys Xaa Gln Gln Lys Thr
20 25 30

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:30 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:85 to 114

(D) OTHER INFORMATION:fragment of p-17 gag protein of HIV; Uganda-D

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

Tyr Cys Val His Glu Arg Ile Lys Val Ala Asp Thr Lys Glu Ala Leu
5 10 15
Asp Lys Ile Glu Glu Glu Gln Thr Lys Ser Lys Lys Lys Ala
20 25 30

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:7 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:223 to 229

(D) OTHER INFORMATION:fragment of MHC-$I_{\alpha 3}$

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

Asp Gln Thr Gln Asp Thr Glu
5

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:9 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:163 to 171

(D) OTHER INFORMATION:fragment of IL-1_{β}

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

Val Gln Gly Glu Glu Ser Asn Asp Lys
5

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:15 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:135 to 149

(D) OTHER INFORMATION:fragment of MHC-$II_{\beta 2}$

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

Asn Gly Gln Glu Glu Lys Ala Gly Val Val Ser Thr Gly Leu Ile
5 10 15

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:13 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:35 to 47

(D) OTHER INFORMATION:fragment of β-2 microglobulin

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

Asp Leu Leu Lys Asn Gly Glu Arg Ile Glu Lys Val Glu
5 10

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:15 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(D) OTHER INFORMATION:C-terminal amide of Peptide J from β-2 microglobulin with glycine spacers

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

Asp Leu Leu Lys Asn Gly Glu Arg Ile Glu Lys Val Glu Gly Gly
5 10 15

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:45 amino acids

(B) TYPE:amino acid

(C) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(D) OTHER INFORMATION:conjugate of Peptide J (SEQ ID NO:55) with peptide fragment of HIV-1 p17 (SEQ ID NO:5)

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

Asp Leu Leu Lys Asn Gly Glu Arg Ile Glu Lys Val Glu Gly Gly Gly
5 10 15

Ala Thr Leu Tyr Ser Val His Gln Arg Ile Asp Val Lys Asp Thr Lys
20 25 30

Glu Ala Leu Glu Lys Ile Glu Glu Glu Gln Asn Lys Ser
35 40 45