Description

This invention relates to a peptide composition and method for the detection of antibodies to HTLV-III; more particularly it relates to such a composition and to a highly sensitive, accurate and reliable method for the detection of antibodies to HTLV-III in body fluids, for the diagnosis of AIDS (acquired immune deficiency syndrome), ARC (AIDS related complex) and pre-AIDS conditions. The peptide composition is also useful as a vaccine for AIDS, ARC or pre-AIDS conditions by stimulating the production of antibodies to HTLV-III to provide protection against infection by HTLV-III or LAV in healthy mammals, including humans. The peptide composition comprises a synthetic peptide having about thirty five amino acids and a mixture of peptides. The amino acid sequence of the peptide and each of the peptides useful in the peptide compositions is chemically synthesized to correspond to segments of the envelope protein, p41, and a segment of the gag protein, p24, of HTLV-III. The peptide composition of the present invention has been found to be highly immunoreactive with antibodies in sera of patients with AIDS, ARC and pre-AIDS conditions.

More specifically, the present invention is directed to the use of a peptide composition comprising a peptide or a mixture of chemically synthesized peptides, one peptide containing therein a segment of about thirty five (35) amino acides (hereinafter referred to as the 35mer peptide), one peptide containing therein a segment of about twenty-one (21) amino acids (hereinafter referred to as the 21mer peptide), a third containing therein a segment of nineteen (19) amino acids (hereinafter referred to as the 19mer peptide), and a fourth peptide containing therein a segment of eleven (11) amino acids (hereinafter referred to as the 11mer peptide) in a prescribed sequence. The 35mer peptide or a mixture of the 21mer peptide, the 19mer peptides and optionally the 11mer peptide are useful for the detection of antibodies to the HTLV-III virus in human body fluids of AIDS, ARC or pre-AIDS patients. The amino acids herein mentioned includes substitutions by analogous acids of the prescribed amino acids, as long as the immunogenic structure of the peptides are preserved.

The detection methods include an enzyme-linked immunosorbent assay (ELISA), an immunoradiometric assay (IRMA) and other methods of immuno assay procedures such as enzyme immunoblotting on nitrocellulose paper and hemagglutination using the peptide composition as the antigen. The preferred detection method is by ELISA.

Acquired immune deficiency syndrome (AIDS) has been recently recognized in several countries. Due to its devastating effect on the patients and indications that the disease is spreading, much effort has been devoted to elucidate and identify the cause of the disease. Epidemiologic data suggests that AIDS is caused by an infectious agent that is horizontally transmitted by intimate contact or exposure to blood or certain blood products.

In 1983, F. Barre-Sinoussi et al. of the Institute Pasteur reported the isolation of a T-lymphotropic retrovirus from a patient at risk for AIDS. The retrovirus appeared to be a member of the human T-cell leukemia virus (HTLV) family. However, its immunological response is distinct from known HTLV-I or HTLV-II. F. Barre-Sinoussi et al., Science, 220, pp. 868 (May, 1983).

A similar virus, designated HTLV-III, has also been isolated by R. C. Gallo's group at National Cancer Institute from the blood samples of a large number of AIDS and ARC patients by co-cultivation with a permissive T-cell line H9. See Popovic, M. et al., Science, 224, pp. 497 (1984) and Gallo, R. et al., Science, 224, pp. 500 (1984).

V.S. Kalyanaraman et al. of the Center for Disease Control, Atlanta, Georgia, reported the isolation of a lymphadenopathy associated virus (LAV) in patients with AIDS and the development of a radioimmune-precipitation assay using the major core protein, p25, of LAV. Their test procedure involved the use of the LAV virus propagated in primary cultures of blood lymphocytes and harvested. The p25 core protein was isolated from the harvested virus, labelled with I^{125} and used as a target antigen. The labelled antigen was added to serum and precipitation of at least 15% of the labelled antigen is regarded as a positive result. See V.S. Kalyanaraman et al., Science, 225, pp. 321 (July 1984). However, based on the reported results, the test was positive only for 41% of the AIDS patients and 72% positive for patients with lymphadenopathy syndrome (LAS) otherwise known as ARC. This means that the procedure is not sufficiently sensitive or accurate to be used as a detection or diagnostic tool for screening serum for the presence of antibodies to the AIDS virus.

LAV and HTLV-III, as well as various strains of related retrovirus isolated from AIDS patients share several important characteristics. See Feorino, P. et al., Science, 225, pp. 69 (1984); Levy, J. et al., Science, 225, pp. 840 (1984). These include viral replication in $OKT4^+$ human T-cell leukocytes, in vivo and in vitro; association with impaired T-cell proliferation, the appearance of cytopathic effects; (See Montagnier et al., Human T Cell Leukemia Virus, pp. 363, Cold Spring Harbor Laboratory, 1984; Popovic, M. et al., op. cit.,

and Klatzmann, D. et al. Science, 225, pp. 59 (1984)) and recognition by antibodies in the sera of AIDS and ARC patients. See Montagnier, et al., op. cit.; Levy J. et al., op. cit., Sarngadharan, M. et al., Science, 224, pp. 505 (1984); Safai, B. et al., Lancet, i, pp. 1438 (1984); Brun-Vezinet, F., et al., Lancet, i, pp. 1253 (1984); Brun-Vezinet, F. et al., Science, 226, pp. 453 (1984); Goldbert, J. et al., Lancet, ii, pp. 711 (1984) and Laurence J. et al., New England J. Med., 311, pp. 1269 (Nov. 1984).

In November 1984, L.W. Kitchen et al. reported the use of a HTLV-III infected line, designated H9/HTLV-III, to test the incidents of AIDS in haemophiliac patients. The method involved inactivation of the virus with 2% paraformaldehyde in phosphate buffer and use of the inactivated cells to determine if haemophiliac patients have been inadvertently exposed to AIDS virus through blood transfusion. The data using sera samples from 50 haemophiliacs show that there is an increasing risk for these patients to contract AIDS, because of their need for blood transfusions to sustain life. L.W. Kitchen et al., Nature, 312, pp. 367 (Nov. 1984). This means that there is an urgent need for a safe, reliable and sensitive test to screen each blood sample before its inclusion in blood banks to isolate blood samples which have been contaminated with AIDS virus, and thus avoid the inadvertent spread of AIDS among patients who must receive blood transfusions, e.g. haemophiliac and surgical patients.

In November 1984 and January 1985, R.C. Gallo's group at National Cancer Institute and other collaborators positively concluded that HTLV-III is the causative agent of AIDS and reported the nucleotide sequence of HTLV-III. See Beatrice Hahn et al., Nature, 312, pp. 166 (Nov. 1984), George M. Shaw et al., Science, 226, pp. 1165 (Dec. 1984) and Lee Ratner et al., Nature, 313, pp. 277 (Jan. 1985).

Meanwhile, three other groups also reported the complete nucleotide sequence of the AIDS virus. See Muesing et al., Nature, 313, pp. 450 (Feb. 1985); Sanchez-Pescados, R. et al., Science, 227, pp. 484 (Feb. 1985) and Wain-Hobson et al., Cell, 40, pp. 9 (Jan. 1985). These reports elucidated the structure of the HTLV-III virus at both the DNA level and the projected protein level and permit further serological studies of the different epitopes present on the HTLV-III virus.

Simultaneously, the group at Institute Pasteur reported that LAV has been identified as a causative agent for AIDS, and is considered to be identical to HTLV-III. The assay procedure used by this group also involves propagating LAV in $T4^{+}$ cells of healthy individuals. The viral antigen was then concentrated and deactivated in 0.5 per cent sodium dodecyl sulfate at 37 °C for 15 minutes. Serum samples were then tested against the antigen in an enzyme immunoassay with orthophenylene diamine as substrate. The presence of antibody in serum was found in 68% of AIDS patients, 100% of patients with Kaposi's sarcoma and 100% of pre-AIDS patients. Jeffrey Laurence et al., op. cit.

Recently U.S. Patent 4,520,113 was issued to R.C. Gallo et al.. The Gallo et al. patent describes a method of detecting antibodies in sera of AIDS and pre-AIDS patients by using lysates of a cell line, designated H9/HTLV-III, as the antigen in an enzyme-linked immunosorbent assay (ELISA) or in a strip radioimmunoassay based on the Western Blot technique or anindirect immunofluorescent method. The method is about 85% accurate. The Gallo patent further indicated that several antigens from HTLV-III, p65, (MW 65,000), p60 (MW 60,000), p55 (MW 55,000), p24 (MW 24,000) and p41 (MW 41,000) are recognized by antibodies in sera from AIDS patients, homosexuals and heroin addicts. Of these, major immune reactivity or specificity is directed against p41, a protein constituting the envelope antigen of HTLV-III. This patent further states that it is belived that additional purification and refinement of p41 may lead to an even more sensitive ELISA assay. Based on this statement, the antigen suitable as a test reagent is found to be a p41 segment derived from HTLV-III cultivated in H9 cell line.

It is further reported in Robert C. Gallo et al., Science, 228, pp. 93 (April 1985) that a combined cloning and expression system in E. Coli has been used to identify HTLV-III encoded peptides which react immunologically with antibodies in sera from AIDS patients. Cloned HTLV-III DNA was sheared into fragments and inserted into an expression vector. The inserted DNA was then expressed in E. Coli transformants. Of 300 clones tested, 20 showed specific reactivity with sera from AIDS patients. The 20 clones were analyzed and found to contain segments from the ORF segment of HTLV-III and were identified as clones 175, 191, 13, 31, 162, 113, 121 and 127. Of the eight clones, ORF clones 113, 121 and 127 define the protein encoded by the portion of the env-lor region produced by HTLV-III infected cells and induces antibody production in most if not all AIDS patients.

T.W. Chang et al, Biotechnology, 3, pp. 905 (October 1985) reported the use of a recombinant E. Coli derived viral antigenic peptide as being useful in an immuno assay for the detection of antibodies to HTLV III. The antigenic peptide is described as peptide 121 having a molecular weight of 15,000 daltons.

C.D. Cabradilla et al., Biotechnology, 4, pp. 125 (February 1986) also describes the use of a bacterially synthesized env polypeptide, having 102 amino acides for the serodiagnosis of antibodies to HTLV-III.

Cosand, U.S. Patent 4,629,783 issued December 16, 1986, described the use of chemically synthesized peptides conjugated to carriers as having immunoreactivity with antibodies to HTLV-III and useful as

reagents in the determination of exposure of a human host to the virus. Seven peptides were described, of which two have sequences which are similar to the peptides herein described. One is a peptide having 13 amino acids, conjugated by acetylation to a carrier protein and having the following amino acids sequence:

Y-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-
Gln-Gln-Z-X

wherein X is OH or NH_2, Y is an amino acid to facilitate coupling of the peptide to a carrier, N-terminally acetylated and linked to a peptide or protein of at least 5,000 molecular weight which peptide or protein does not normally bind to antibodies present in a human host. The other is a peptide having 26 amino acids acetylated and conjugated to a carrier protein having the following amino acids sequence:

-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-
Gln-Gln-Leu--Leu-Gly-Ile-Trp-Gly-Cys-Ser-Gly-
Lys-Leu-Ile-Cys-X

wherein X is OH ro NH_2, N-terminally acetylated and linked to a peptide or protein of at least 5,000 molecular weight which peptide or protein does not normally bind to antibodies present in a human host. Cosand also described that a mixture of polypeptide having an amino acids sequence corresponding to:

Y-Asp-Cys-Leu-Thr-Ile-Leu-Lys-Ala-Leu-Gly-
Pro-Ala-Ala-Thr-Leu-Glu-Glu-Met-Met-Thr-
Ala-Cys-X

wherein Y and X has the same meaning defined above and a conjugated polypeptide having 26 amino acids, identified above, as having immunoreactivty that is stronger than the conjugated polypeptide having 26 amino acids alone.

Most of the reported assay procedures for detecting antibodies to HTLV-III and for diagnosis of AIDS or pre-AIDS conditions involve the use of the HTLV-III or LAV virus. The other assay procedures have not been tested sufficiently up to the present. None of the procedures are 100% accurate. This is undesirable for use in the screening of sera in blood banks. The less than 100% accuracy of the tests may permit contaminated sera from escaping detection and be used in blood transfusions to the severe detriment of blood recipients. Moreover, the use of the HTLV-III virus as the testing agent is dangerous to healthy laboratory workers, requiring extreme precautions to avoid all chances of exposure during the preparative process to make the test reagent. Furthermore, even though the deactivated virus is used in some of the published procedures, exposure to the deactivated virus can cause antibody production in healthy workers, who may then be falsely diagnosed as having AIDS, ARC or pre-AIDS condition. Moreover, presence of cellular materials from H9 cells or E. Coli in the test agent may elicit false positive response in the HTLV-III antibodies screening test from individuals who have antibodies to E. Coli or H9 cells. These false positive reactions can bring severe anxiety to the healthy individuals and their family and may leand to a healthy individual being mistakenly diagnosed as having AIDS and be exiled from normal social activities as a consequence.

The number of tests reporting in Cosand US Patent 4,629,783 is very small. The results indicate that the conjugated polypeptide with 26 amimo acids is not as good as the deactivated virus. Moreover, Cosand does not describe the peptide composition of the present invention.

Furthermore, up to the present, no viable vaccine or method to provide protection against HTLV-III has been reported for AIDS, ARC or pre-AIDS conditions. The use of deactivated virus provokes fears of contracting the disease and would prevent its acceptability and use.

Similarly, the development of monoclonal and polyclonal antibodies to HTLV-III in mammals involves the use of HTLV-III as the immunogen and this presents similar risks in the procedure.

There may also be mentioned: WO 87/06005, which relates to certain synthetic peptides useful for detection of antibodies to HTLV-III virus and to diagnostic and therapeutic compositions and methods of use thereof; EP-A-251612, which discloses human monoclonal antibodies capable of specifically reacting with

an antigenic determinant of LAV/HTLV-III and cell lines producing those monoclonal antibodies; EP-A-201716, which concerns a method for expressing proteins which are immunologically reactive with antibodies to LAV; and WO 86/06414, which provides some peptides having substantially the same sequence as immunolocially significant fragments of AIDS-related viruses.

In Application Serial Nos. 774,644, 837,566 and 847,102, (see European application 86 303 224.9 (214709)), applicants described a 21-mer peptide with twenty-one (21) amino acids as a highly sensitive reagent for the detection of antibodies to HTLV-III, as a vaccine for protection against the AIDS virus, and for the production of monoclonal and polyclonal antibodies to AIDS virus.

Upon extensive clinical testing with the 21mer peptide at vrious blood banks and reserch institutes, it has been found that a small number, 12 out of 449, or about 2.67% of serum samples, mostly at the later stage of AIDS, which tested positive by Western Blot Analysis, gave negative results when tested against the 21mer peptide. Even though the percentage is small, this is considered to be disadvantageous, in that sera of possibly infected individuals may escape detection and be used for transfusion and in surgical procedures.

It is, therefore, an objective of the present invention to further perfect a detection or diagnostic procedure that will ensure detection of antibodies to HTLV-III in all individuals who may have been exposed to or was infected with HTLV-III.

A further objective is to develop a test procedure that is highly sensitive and 100% accurate, i.e. it will not produce false positive or false negative results.

According to the present invention, a peptide composition comprising a peptide of thirty-five amino acids, a mixture of two peptides and optionally a third peptide as the immunogenic reagent for the detection of antibodies to HTLV-III. The peptide composition comprises a peptide of thirty-five amino acids, a mixture of a peptide of about twenty-one amino acids, a peptide of about nineteen amino acids and optionally a third peptide of about eleven amino acids. The peptides were made by solid phase peptide synthesis. The peptide composition has been found to be useful in a highly sensitive and accurate method for the detection of antibodies to HTLV-III in sera and body fluids and diagnosis of AIDS, ARC or pre-AIDS conditions. The peptide composition is useful in stimulating production of antibodies to HTLV-III or LAV in healthy mammals such as Balb/c mice.

According to the present invention, a peptide composition useful for the detection of antibodies to HTLV-III and diagnosis of AIDS, ARC or pre-AIDS conditions in sera or body fluids comprising:

A. a peptide having the following amino acides sequence

-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys-Thr-Thr-Ala-Val-Pro-Trp-Asn-Ala-Ser-,

analogues thereof; or

B. a mixture of:

(i) about 10 to 95 parts by weight of

-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser-,

analogues thereof and segment thereof;

(ii) about 5 to 90 parts by weight of

-Ile-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys-Thr-Thr-Ala-Val-Pro-Trp-Asn-Ala-Ser-,

analogues thereof; and optionally

(iii) about 5 to 50 parts by weight of -Ile-Val-Arg-Met-Tyr-Ser-Pro-Thr-Ser-Ile-Leu- and analogues thereof

It is to be understood that the three letter codes used correspond to the named amino acids:

Ala = alanine,
Arg = arginine,
Asn = asparagine,
Asp = aspartic acid,
Gln = glutamine,
Glu = glutamic acid,
Gly = glycine,
Leu = leucine,
Lys = lysine,
Ile = isoleucine,
Pro = proline,
Met = methionine,
Ser = serine,
Thr = threonine,
Trp = tryptophan,
Tyr = tyrosine,
Val = valine, and
Cys = cysteine.

A highly sensitive and accurate method of detecting antibodies to HTLV-III in body fluids and diagnosis of AIDS, ARC or pre-AIDS condition comprises the following steps:

A. Preparing a peptide composition selected from the comprising:

a. a peptide having the following amino acid; sequence

-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys-Thr-Thr-Ala-Val-Pro-Trp-Asn-Ala-Ser-,

analogues thereof or

b. a mixture of:

(i) about 10 to 95 parts by weight of

-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser-,

analogues thereof

(ii) about 5 to 90 parts by weight of

-Ile-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys-Thr-Thr-Ala-Val-Pro-Trp-Asn-Ala-Ser-,

and analogues thereof; and optionally

(iii) about 5 to 50 parts by weight of -Ile-Val-Arg-Met-Tyr-Ser-Pro-Thr-Ser-Ile-Leu-, analogues thereof.

B. Using about 0.1 ug to about 20 ug per test of the peptide composition as the antigen in an immuno assay procedure.

Further, according to the present invention, the peptide when coupled to a protein or a polymer carrier, or in a polymerized form can be used to stimulate production of antibodies to HTLV-III or LAV in healthy mammals including humans. The method comprises introducing an effective amount of the peptide

composition, wherein each peptide is conjugated to a protein, such as human serum albumin into the body of a healthy mammal by intraperitoneal or subcutaneous injection.

In accordance with the present invention a peptide composition has been chemically synthesized and prepared for the detection of antibodies to HTLV-III in body fluids and diagnosis of AIDS, ARC and pre-AIDS condition, for the vaccination of healthy mammals by stimulating the production of antibodies to HTLV-III or LAV in healthy mammals, and for the development of both monoclonal and polyclonal antibodies to HTLV-III in mammals.

The peptide composition comprises:

A. a peptide having the following amino acids sequence:

-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-
Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-
Cys-Thr-Thr-Ala-Val-Pro-Trp-Asn-Ala-Ser-,

analogues thereof; or

B. a mixture of two peptides:

(i) about 10 to 95 parts by weight of a peptide of about twenty one amino acids, analogues thereof arranged in the following sequence:

-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Leu-Leu-
Gly-Ile-Trp-Gly-Cys-Ser-

(ii) about 5 to 90 parts by weight of a peptide of about nineteen amino acids, analogues thereof arranged in the following sequence:

-Ile-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys-Thr-Thr-Ala-Val-Pro-Trp-
Asn-Ala-Ser-.

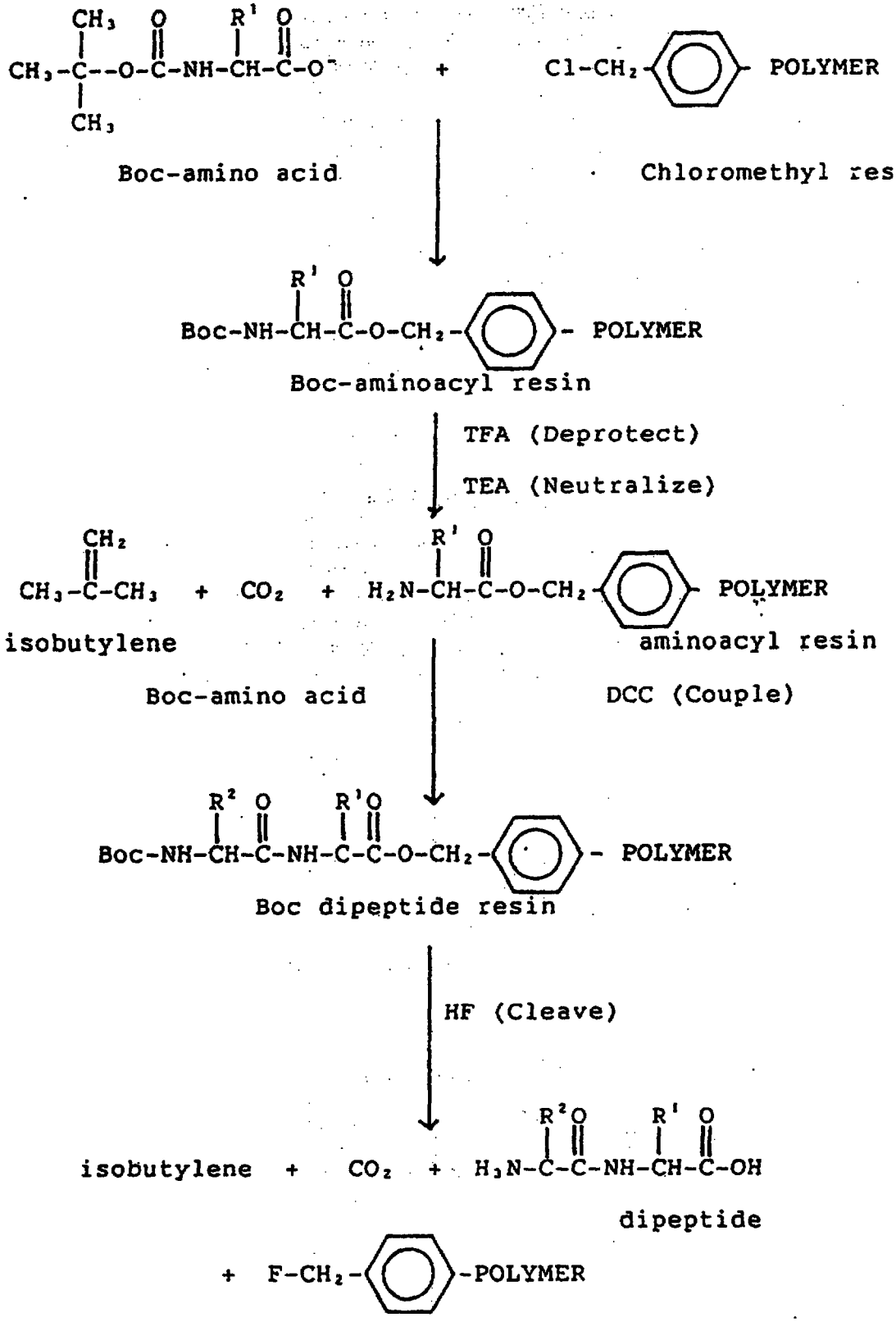
The mixture may further include 5 to 5C parts by weight of a peptide of about eleven amino acids or analogues thereof arranged in the following sequence:
-Ile-Val-Arg-Met-Tyr-Ser-Pro-Thr-Ser-Ile-Leu-.

Each of the peptides may comprise a shorter or longer peptide chain by having more amino acids added to the terminal amino acids of the above sequences or having a few less of the terminal amino acids from either terminal of each of the peptides.

It is expected that as long as the three dimensional conformations of each of the three peptides recognizable by the dominant antibodies to HTLV-III are preserved, the synthetic peptide composition may comprise analogues of the recited amino acids of the above sequences. The term analogues" encompasses each of the entire peptides, portions thereof, peptides that contain substitutions of amino acid residues, insertions and/or deletions of amino acids, or polymeric forms of the peptides. These analogues can also be used to detect and/or elicit production of antibodies to HTLV-III.

The amino acid sequences of the 35mer, 21mer and 19mer peptides in the peptide composition useful as a test reagent for the detection of antibodies to HTLV-III in body fluids and diagnosis of AIDS, ARC and pre-AIDS condition is prepared to correspond to a partial segment of the amino acid sequence of the HTLV-III virus designated as p41, a part of p160, the LOR (long open reading) segment, defining the envelope protein of the HTLV-III virus. The amino acid sequence of the 11mer peptide is prepared to correspond to a partial segment of the amino acid sequence of the HTLV-III virus core protein designated as p24, the gag segment.

The peptides useful as the solid phase immunoadsorbent for the detection of antibodies HTLV-III were synthesized by "classical" Merrifield method of solid phase peptide synthesis according to the following scheme.

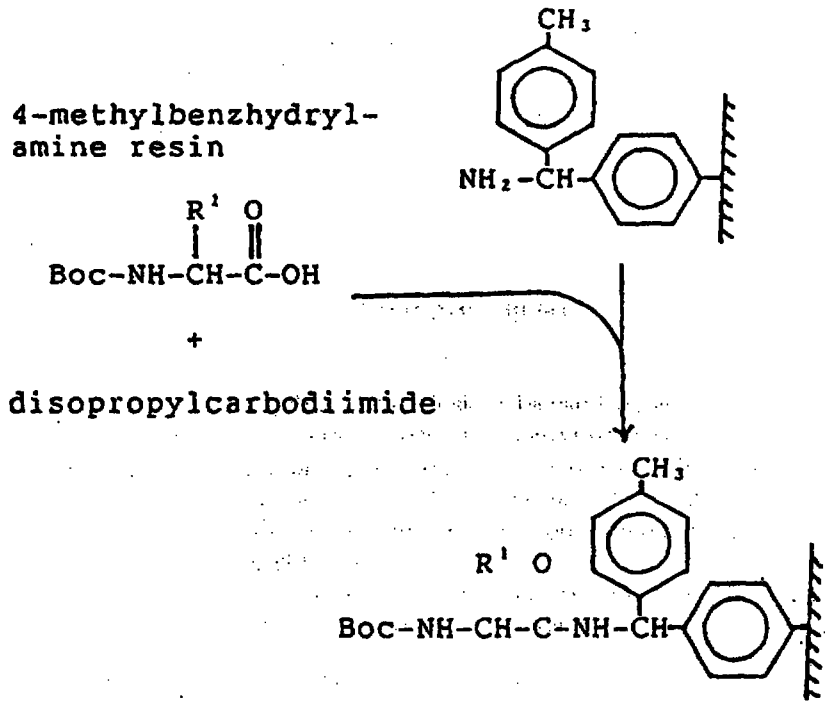


Following the above scheme, Boc-amino acids are added to the resin to prepare the 35mer peptide, the 21mer peptide, the 19mer peptide and the 11mer peptide.

Analogues of each of the peptides can be prepared by varying the above sequence either by adding or subtracting desired Boc-amino acid(s).

Following completion of assembly of the desired blocked peptide on the resin, the peptide-resin is treated with anhydrous hydrofluoric acid to cleave the benzyl ester linking the peptide to the resin in order to liberate the peptide. Side-chain functional groups of amino acids which are blocked during synthesis by benzyl-derived blocking groups are also cleaved from the peptide simultaneously. The free peptide is then analyzed and purified by reverse phase high pressure liquid chromatography (HPLC) and characterized biochemically by amino acid analysis.

Similarly, synthesis of each of the peptides that has an amide group on its C-terminal end can be achieved by using a 4-methylbenzhydrylamine resin according to the following scheme:



Coupling the C-terminal Residue to 4-Methylbenzhydryl amine residue

The peptide composition synthesized according to the above described procedure is highly reactive to antibodies to HTLV-III and can be used as a highly sensitive, accurate, reliable and specific immunoadsorbent for the detection of antibodies against HTLV-III. Results obtained with the peptide composition according to the present invention show that it is more sensitive and specific to antibodies against HTLV-III in body fluids than the Western Blot method, the standard method used for the diagnosis of AIDS. See Tables I, II and III.

It can be observed from Table III that the 35mer peptide has the highest immunoreactivity. However, it is preferable to use a mixture of the 21mer and 19mer peptides, which have amino acids sequences overlapping the 35mer peptide, because it is much more difficult and costly to synthesize the longer chain peptide. This is because the yield of the synthesis process becomes increasingly low for peptides with increasing length.

Based on the high degree of sensitivity and specificity of the peptide composition according to the present invention in the immunoreaction to antibodies to HTLV-III, it is believed that it is also useful as a vaccine for AIDS, ARC or pre-AIDS conditions, and as an immunogen for the development of both monoclonal and polyclonal antibodies to HTLV-III in mammals. The peptide composition as the 35mer peptide or a mixture of each of the peptide itself or when coupled to a protein or a polymer carried, can be introduced to normal subjects to stimulate production of antibodies to HTLV-III, and provide protection against infection by HTLV-III or LAV in healthy individuals. Since the peptide composition according to the present invention is not derived biochemically from the virus, there is no danger of exposing the normal subjects who are to be vaccinated to the disease.

It has been found that when the 21mer peptide conjugated to human serum albumin (HSA), as the carrier protein, was injected intraperitoneally and subcutaneously into healthy Balb/c mice, antibodies to the

21mer peptide cross reactive with HTLV-III were produced by the Balb/c mice. See Application Serial No. 847,102 and Table IV.

The results obtained indicate that the 21mer peptide is also useful as an immunogen for the development of both monoclonal and polyclonal antibodies to HTLV-III in mammals.

Based on the results obtained with the 21mer peptide, it is expected that the peptide composition will also stimulate the production of antibodies to HTLV-III and provide protection against infection by HTLV-III or LAV in healthy individuals. It is also expected that the peptide composition will also be useful as an immunogen for the development of both monoclonal and polyclonal antibodies to HTLV-III in mammals. See Application Serial No. 847,102, Examples 7 and Table IV.

The advantages of using the peptide composition according to the present invention are many.

The peptide composition is chemically synthesized. This means that there is no involvement with the HTLV-III virus at any time during the process of making the test reagent or the vaccine. During the preparation of the vaccine or the vaccination process, there is no risk of exposure of the production workers, individuals in the health profession and those being vaccinated to the HTLV-III virus. Similarly, there is no risk of exposure to HTLV-III in the use of the peptide composition for the development of monoclonal or polyclonal antibodies to HTLV-III in mammals. Further, up to the final step of the test to detect antibodies to HTLV-III, where the test reagent is exposed to the samples of sera or body fluid, there is no risk of exposure of the laboratory worker to the HTLV-III virus. And, risk of exposure in this final step can be avoided by taking the precautionary step of deactivating any virus which may be present by heating at 60 °C for half an hour the samples of sera or body fluids which are to be tested.

Another problem which is avoided by the process of the present invention is the possibility of false positive results caused by the presence of antigenic material from H9 cells co-purified with the HTLV-III viral preparation or E-Coli derived proteins co-purified with expressed viral fragments. Certain normal individuals have antibodies to E. Coli or human leukocyte antigens, e.g. HLA, which are cross reactive with the antigenic materials from H9 cells. Sera samples from these normal individuals may show a positive response in the ELISA or IRMA test even though they have not been exposed to HTLV-III. A diagnosis that a person may be inflicted with AIDS based on this type of false positive response can bring severe anxiety to the person and his/her family, and cause him/her to be excluded from normal social activities. All of these problems can be avoided by using the peptide of the present invention as the test reagent.

A third problem which has plagued this area is the possibility of false negative results. The accuracy of the results using the 21mer peptide alone is about 98% whereas the accuracy of the results using the peptide composition as the antigen is 100% using the following as the cutoff point:

$$\text{Cutoff point} = 0.1 \times A_{492}(RC) + 1 \times A_{492}(NRC)$$

where $A_{492}(NCR)$ is the absorbance reading for the reactive control and A_{492} is the absorbance reading for the non-reactive control.

In fact, the ELISA method using the peptide composition of the present invention in certain cases gave a positive indication confirmed by medical diagnosis where the standard Western Blot test produced a negative result. This indicates that the present method is even more accurate than the Western Blot test.

Extensive clinical trials were conducted on 140 sero-converted specimens collected during the period of late 1979 to 1982 from healthy homosexuals in New York City originally selected for Hepatitis B vaccine studies. These serum samples were also characterized by Western Blot analysis and HTLV-III ELISA tests of the present invention. The results of the tests showed that the sensitivity of the ELISA method using the peptide composition of the present invention is much higher than that of the Western Blot Analysis. Moreover, using the ELISA method with the peptide composition produced positive signals in eleven individuals well before the p24, p55 and p17 bands appeared in the Western Blot Analysis. Similarly, the ELISA method with the peptide composition of the invention is more sensitive and produced earlier diagnostic results than that of ELISA test kits presently available in the market.

Further, with appropriate amino acid analogue substitutions, it is expected that various peptide analogues based on the prescribed amino acid sequences can be synthesized with properties giving rise to lower background readings or better adsorption capacity to solid phases useful for HTLV-III antibodies screening assays.

Various peptide analogs based on the above described amino acid sequence have been prepared. These analogs and their reactivities with the anti HTLV-III antibodies present in sera of AIDS and ARC patients are described in Example 8 and Table III. The relativity in an ELISA test for the same amount of each peptide by weight per volume was compared with the reactivity of the 21mer peptide, which was assigned a reactivity of 100.

Our results indicate that the 21mer peptide of the present invention is unique in it size and sequence in that mere deletion of -Arg-, or -Ile- frm the N- terminal or -Trp-Gly-Cys-Ser- from the C-terminal, or cleaving this peptide in between the -Lys- and -Asp- to form two peptides of 10 and 11 amino acids, all resulted in dramatic loss of antigenicity conferred by the 21mer peptide. In addition, -Lys- at position 12 from the C-terminal also appear to be important, in that addition of -Lys- to the above said peptide with 11 amino acids restores significantly the antigenicity. Similarly C-terminal amino acids such as -Ile-Trp-Gly-Cys-Ser- also play an important role in the antigenicity to the antibody-antigen interaction. See Table III, peptides 4 and 6.

Analogues of the 19mer peptide have also been prepared to produce similar results. See Table III. The 19mer peptide also shares at its N-terminal the amino acid sequence of Ile-Trp-Gly-Cys-Ser with the 21 mer peptide. This again clearly demonstrates the importance of this region. Deletion of the segment from the 19mer resulted in the complete loss of antiqenicity.

The 11mer peptide has an amino acid sequence corresponding to a segment of the gag protein. A comparison of ELISA results using the 11mer peptide as the solid phase immuno adsorbent with Western Blot shows that the 11mer peptide is highly immunoreactive. Using a total of 266 samples of which 159 had been shown to be p24 positive and 107 were shown be p24 negative by the Western Blot method, and 11mer peptide showed corresponding results for 114 out of the 159 and 105 out of the 107. See Exmple 10 and Table V.

Moreover, because the peptides of the present invention are synthetically prepared, the quality can be controlled, and as a result, reproducibility of the test results can be assured. Also, since very small amounts of the peptides are required for each test procedure, and the expense of preparing the peptide is relatively low, the cost of screening body fluids for antibodies to HTLV-III and diagnosis of AIDS, ARC and pre-AIDS and the preparation of a vaccine is relatively low.

The peptide composition prepared in accordance with the present invention can be used to detect HTLV-III infection anddiagnose AIDS, ARC and pre-AIDS condition by using it as the test reagent in an enzyme-linked immunosorbent assay (ELISA), an enzyme immunodot assay, a hemagglutination assay or a radioimmunoradiometric assay (IRMA), preferably ELISA. The ELISA technique using a mixture of the 21mer and 19mer peptides is exemplified in Example 1, the ELISA method with a mixture of the 21mer peptide, 19mer peptide and 11mer peptide is exemplified in Example 2.

It is to be noted that in the following methods, 0.25% by weight of glutaldehyde may be added in the coating buffer to facilitate better peptide binding onto the plates or beads. Further, horseradish peroxidase conjugated mouse monoclonal anti human IgG antibody may be used in place of horseradish peroxidase conjugated goat anti human IgG as a second antibody tracer. The gelatins useful in these processes can include calf skin gelatin, pig skin gelatin, fish gelatin or any known available gelatin proteins or be replaced with albumin proteins.

The following examples illustrate the invention.

EXAMPLE 1

Detection of Antibodies to HTLV-III by an Enzyme-Linked Immunoadsorbent Assay

A mixture in a ratio by weight of 10:1 of the 21mer and 19mer peptides, synthesized as described was prepared.

Wells of 96-well plates were coated at 37°C for 1 hour with the mixture of the 21mer peptide and the 19mer peptide, at 1.0 ug per well in 100 ul 0.1 M $NaHCO_3$ buffer, pH 9.5. The wells were washed three times with phosphate buffered saline (PBS) and then incubated with 250 ul of 3% by weight gelatin in PBS at 37°C for 1 hour to block non-specific protein binding sites, followed by three more washes with PBS containing 0.05% by volume Tween 20. The test sera (blood taken from a human patient or normal individual) were diluted with PBS containing 20% by volume normal goat serum, 1% by weight gelatin and 0.05% by volume Tween 20 at a dilution of 1:20, volume to volume, respectively. 200 ul of the diluted sera were added to each well and allowed to react for 15 minutes at 37°C. The wells were then washed six times with 0.05% by volume Tween 20 in PBS in order to remove unbound antibodies. Horseradish peroxidase conjugated goat anti human IgG was used as a second antibody tracer to bind with the AIDS antibody-antigen complex formed in positive wells. 100 ul of peroxidase labeled goat anti human IgG at a dilution of 1:3000 in 1% by volume normal goat serum, 0.05% by volume Tween 20 in PBS was added to each well and incubated at 37°C for another 15 minutes.

The wells were washed six times with 0.05% by volume Tween 20 in PBS to remove unbound antibody and reacted with 100 ul of the substrate mixture containing 0.04% by weight orthophenylenediamine (OPD) and 0.012% by volume hydrogen peroxide in sodium citrate buffer, pH 5.0. The mixture was incubated at

37° for 15 min. This substrate mixture was used to detect the peroxidase label by forming a color product. Reactions were stopped by the addition of 100 ul of 2.N H_2SO_4 and the color yield measured using an ELISA reader which quantifies the color reading at 492nm (i.e. OD was measured at 492nm). Assays were performed with 1:20 dilution. Normal serum samples were used as non-reactive controls. Sera samples obtained from an individual diagnosed as having AIDS is included as reactive controls. Absorbence readings greater than a cut off absorbance reading determined according to the following formula were taken as positive.

Cutoff point = $0.1 \times A_{492}(RC) + 1 \times A_{492}(NCR)$

wherein $A_{492}(RC)$ is the absorbance reading for the reactive control and A_{492} (NCR) is the absorbance reading for the non-reactive control. The results are shown in Table I.

TABLE I

Comparison of ELISA
using a 10:1 mixture of 21mer and
19mer peptides with Western Blot Analysis

Patient Diagnosis	No. Positive/No. Tested ELISA	Western Blot
Acquired Immunodeficiency Syndrome	187/187	186/187
Pediatric AIDS	11/11	11/11
AIDS related complex	116/116	116/116
Persistent Generalized Lymphoadnopathy	29/29	29/29
Lymphadenophathy	6/7	6/7
Healthy Homosexuals	48/57	48/57
Transexual	1/1	1/1
Bisexual	0/3	0/3
Intravenous Drug Abuser	20/21	20/21
Sexual Partner of AIDS patient	12/18	12/18
Hemophiliac	6/6	6/6
Transfusion	3/3	3/3
Idiopathic Thrombocytopenic Purpura	1/1	1/1
Non-AIDS Kaposi's Sarcoma	0/3	0/3
Health Care Worker(mostly with needle sticks)	0/14	0/14
Acute Myelogenous Leukemia	0/12	0/12
Chronic Myelogenous Leukemia	0/2	0/2
Acute Lymphocytic Leukemia	0/6	0/6
Chronic Lymphocytic Leukemia of the B type	0/32	0/32
Chronic Lymphocytic Leukemia of the T type	0/2	0/2
Non-Hodgkin's Lymphoma	0/4	0/4
Hodgkin's Disease	0/1	0/1

Hairy Cell Leukemia	1/2	1/2
Macroglobulinemia	0/1	0/1
Mycosis Fungoide	0/1	0/1
Primary Immunodeficiency including Common variable immunodeficiencies, Hypogammaglobulinemia and Thymoma	0/25	0/25
Gamma Globulin Preparation	0/1	0/1
Other Diseases Unrelated to HTLV-III infection		
Viral infections	1/6	1/6
Sarcoidosis	0/2	0/2
Patient with papilloma	0/1	0/1
Non-AIDS	0/3	0/3
Normal Control	0/38	0/38
Brother of AIDS patient	0/1	0/1
Daughter of AIDS patient	0/1	0/1
Wife of AIDS patient	1/2	1/2
Infant of AIDS patient	0/1	0/1
Repeat reactive by Abbott test	7/54	7/54
	450/674	449/674

The results in Table I of the ELISA test procedure according to the present invention with 674 sera samples show that the method is extremely accurate and highly specific. No immunoreactivity was found in normal subjects or patients who were identified as not being inflicted with AIDS or ARC. In fact, the ELISA results confirmed medical diagnosis of AIDS which was missed by Western Blot Analysis.

It is to be noted that the cut off absorbance reading is very stringent. It was determined statistically based upon a large number of clinical trial data (larger than 10,000 tests).

EXAMPLE 2

The procedure of Example 1 was repeated using the same sera samples as in Example 1 except that the well plates were precoated with a mixture of the 21mer peptide, 19mer peptide and 11mer peptide in a weight ratio of 10:1:1. The results are presented in Table II.

TABLE II

Comparison of ELISA using a 10:1:1 mixture of 21mer, 19mer and 11mer peptides with Western Blot Analysis

Patient Diagnosis	No. Positive/No. Tested ELISA	Western Blot
Acquired Immunodeficiency Syndrome (AIDS/KS,AIDS/IVDA,AIDS/HD,AIDS/OI etc.)	187/187	186/187
Pediatric AIDS	11/11	11/11
AIDS related complex	116/116	116/116
Persistent Generalized Lymphoadnopathy	29/29	29/29
Lymphadenophathy	6/7	6/7
Healthy Homosexuals	48/57	48/57
Transexual	1/1	1/1
Bisexual	0/3	0/3
Intravenous Drug Abuser	20/21	20/21
Sexual Partner of AIDS patient	12/18	12/18
Hemophiliac	6/6	6/6
Transfusion	3/3	3/3
Idiopathic Thrombocytopenic Purpura	1/1	1/1
Non-AIDS Kaposi's Sarcoma	0/3	0/3
Health Care Worker(mostly with needle sticks)	0/14	0/14

Acute Myelogenous Leukemia	0/12	0/12
Chronic Myelogenous Leukemia	0/2	0/2
Acute Lymphocytic Leukemia	0/6	0/6
Chronic Lymphocytic Leukemia of the B type	0/32	0/32
Chronic Lymphocytic Leukemia of the T type	0/2	0/2
Non-Hodgkin's Lymphoma	0/4	0/4
Hodgkin's Disease	0/1	0/1
Hairy Cell Leukemia	1/2	1/2
Macroglobulinemia	0/1	0/1
Mycosis Fungoide	0/1	0/1
Primary Immunodeficiency including Common variable immunodeficiencies, Hypogammaglobulinemia and Thymoma	0/25	0/25
Gamma Globulin Preparation	0/1	0/1
Other Diseases Unrelated to HTLV-III infection		
Viral infections	1/6	1/6
Sarcoidosis	0/2	0/2
Patient with papilloma	0/1	0/1
Non-AIDS	0/3	0/3
Normal Control	0/38	0/38
Brother of AIDS patient	0/1	0/1
Daughter of AIDS patient	0/1	0/1
Wife of AIDS patient	1/2	1/2
Infant of AIDS patient	0/1	0/1
Repeat reactive by Abbott test	7/54	7/54
	450/674	449/674

The above results indicate that the 11mer peptide may also be included in the immuno assay procedure to produce similar results as shown for the mixture of 21mer and 19mer peptides.

EXAMPLE 3

Detection of Antibodies to HTLV-III by an Immunoradiometric Assay (IRMA)

Wells of 96-well flexible-polyvinylchloride (PVC) plates are coated at 37°C for 1 hour with a mixture of 10:1:1 by weight of the 21mer peptide, the 19mer peptide and 11mer peptide, prepared as described, at 1.0 ug per well in 100 ul 0.1M $NaHCO_3$ buffer, pH 9.5. The wells are washed three times with phosphate buffered saline (PBS) and then incubated with 250 ul of 3% by weight gelatin in PBS at 37°C for 1 hour to

block non-specific protein binding sites, followed by three more washes with PBS containing 0.05% by volume Tween 20. The test sera (blood taken from a human patient or normal individual) are diluted with PBS containing 20% by volume normal goat serum, 1% by weight gelatin and 0.05% by volume Tween 20 at a dilution of 1:20 (volume to volume) respectively. 200 ul of the diluted sera are added to each well and allowed to react for 15 minutes at 37°C. The wells are then washed six times with 0.05% by volume Tween 20 in PBS in order to remove unbound antibodies. I^{125} labeled affinity purified goat anti human IgG (Fc) is used as a second antibody tracer that binds with the antibody-antigen complex formed in positive wells. 100 ul of I^{125} labeled goat anti human IgG of 50,000-200,000 cpm in 1% by volume normal goat serum, 0.05% by volume Tween 20 in PBS was added to each well and incubated at 37°C for 15 minutes.

The wells six washed five times with 0.05% by volume Tween 20 in PBS to remove unbound second antibody and dried. The wells are cut and counted by a gamma-scintillation counter. Assays are performed inwith 1:20 dilution, volume to volume, respectively. Normal sera sample as negative controls were also tested simultaneously. Cpm readings greater than the average readings of normal sera samples +4 SD (standard deviation) are taken as positive.

EXAMPLE 4

Detection of Antibodies to HTLV-III by a Hemagglutination Assay using 10:1 Mixture of the Peptide Composition Coated Gelatin Particles, Sheep Red Blood Cells or Latex Beads as Solid Phase Immunoadsorbent

One ml thoroughly washed human blood cells, Ox red blood cells, gelatin particles, or polystyrene latex beads are coated with a 10:1 by weight mixture of the 21mer peptide and the 19mer peptide at a concentration in the range of 1 ug/ml to 1mg/ml. The coated cells, particles or beads are then incubated with serially diluted serum samples in the wells of a multi-well U-shaped or lattice shaped microplate. After being left at room temperature for about an hour, the agglutination patterns on the bottom are read, and the largest dilution showing a positive reaction is recorded.

This is a one-step assay which could be used for both qualitative and quantative analysis of the presence of anti-HTLV-III antibodies in specimens including sera or biofluids.

EXAMPLE 5

A diagnostic AIDS, ARC and pre-AIDS specific test kit for HTLV-III antibodies detection can be constructed. The test kit comprises a compartmented enclosure containing multiple 96-well plates coated prior to use with 1 ug of the peptide composition of the present invention in 100 ul pH 9.5 0.1M $NaHCO_3$ buffer per well. The kit further comprises materials for enzyme detection in separate sealed containers consisting of: 1) normal human serum (as non reactive control); 2) heat inactivated, NP40 solubilized AIDS serum (as reactive control); 3) normal goat serum; 4) peroxidase labelled-goat anti human IgG; and 5) a color change indicator consisting of orthophenylenediamine (OPD) and hydrogen peroxide in phosphate citrate buffer. The procedure described in Example 1 is to be followed.

In this test kit, 96-well plates, precoated with the peptide of the present invention, can be replaced by polystyrene beads, latex particles, or multiple mini-columns filled with controlled pore size glass beads, or nitrocellulose paper strip precoated with the peptide of the present invention for use as the solid phase immunoadsorbent.

EXAMPLE 6

A second test kit for detecting antibodies using the immunoradiometric assay (IRMA) comprises a compartmented enclosure containing multiple 96-well bendable polyvinylchloride (PVC) plates precoated with the peptide composition according to the present invention at a concentration of 1 ug of peptide composition in 100 ul of pH 9.5 0.1M $NaHCO_3$ buffer per well and materials for radioimmunoassay including: 1) normal human serum (as non-reactive control); 2) heat inactivated, NP40 solubilized AIDS serum (as reactive control); 3) normal goat serum; and, 4) I^{125} labeled coated anti human IgG. The procedure described in Example 3 is to be followed.

In this test kit, 96-well PVC plates precoated with the peptide of the present invention can be replaced by polystyrene beads precoated with the peptide of the present invention for use as the solid phase immunoadsorbent.

EXAMPLE 7

A third test kit for detecting HTLV-III antibodies using the hemagglutination assay comprised a compartmented enclosure containing multiple -well U-shaped or lattiged shaped microplates and materials for hemagglutination assay including (1) a bottle of human O red blood cells, Ox red blood cells, gelatin particles or latex polystyrene beads precoated with a mixture of 10:1:1 by weight of the peptide composition (2) normal human serum (as a non-reactive control); and, (3) heat inactivated, NP40 solubilized AIDS positive serum (as reactive control). The procedure described in Example 4 is to be followed.

EXAMPLE 8

The 35mer peptide, 19mer peptide, 11mer peptide and eight analogues of the 21mer peptide, and overlapping 19mer peptide were prepared in their amide form according to the classical Merrifield method described herein above. The amino acid sequence of each of the analogues are shown in Table III.

The immunoreactivity of the various peptides at 1 ug per well level was tested using a pooled AIDS serum sample. The reactivity of each peptide was compared with the reactivity of the 21mer peptide which was set as 100%.

TABLE III

Peptide	Amino Acid Sequence	% Reactivity
1	-Val-Trp-Gly-Ile-Lys-Gln-Leu-Gln-Ala-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-	5
2	-Gln-Leu-Gln-Ala-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-	13.5
3	-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-	5
4	-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Leu-Leu-Gly-Ile-	28.3
5	-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Leu-Leu-Gly-Ile-	30.3
6	-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser-	100
7	-Arg-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser-	66.8
8	-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser-	53.9
9	-Lys-Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser-	38.3
10	-Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser-	8
11	-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser-	20.0
12	-Gly-Lys-Leu-Ile-Cys-Thr-Thr-Ala-Val-Pro-Trp-Asn-Ala-Ser-	2.42
13	-Cys-Ser-Gly-Lys-Leu-Ile-Cys-Thr-Thr-Ala-Val-Pro-Trp-Asn-Ala-Ser-	57.5
14	-Ile-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys-Thr-Thr-Ala-Val-Pro-Trp-Asn-Ala-Ser-	99.0
15	-Leu-Lys-Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys-Thr-Thr-Ala-Val-Pro-Trp-Asn-Ala-Ser-	73.4
16	-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys-Thr-Thr-Ala-Val-Pro-Trp-Asn-Ala-Ser-	107.1
17	-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Lys-Leu-Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys-Thr-Thr-Ala-Val-Pro-Trp-Asn-Ala-Ser-	132.0

EXAMPLE 9

Varying Amounts of a mixture of 21mer Peptide and 19 mer Peptide

Following the procedure described in Example 1, mixtures of the 21mer peptide with the 19mer peptide with various weight ratios, from 10:1.25 to 4:1, were used as the immunoadsorbent. The wells were coated with 1ug of the mixture. The serum samples used were two samples from patients which had been diagnosed as positive for AIDS and two samples from patients with autoimmune diseases. The two AIDS positive samples were screened out from over 500 HTLV III positive AIDS serum specimens and had been showned to be negative in ELISA using only the 21mer peptide as the immunoadsorbent. The results are shown in Table IV. The results indicate that the various mixtures of the 21mer and 19mer peptides gave more accurate results than the 21mer peptide alone.

TABLE IV

Weight Ratio of Peptide Composition			Absorbance				
				AIDS Positive Samples		Autoimmune Diseases	
21mer ug/ml	+ 19mer ug/ml	Positive Control	Normal Serum	1	2	1	2
5	0	1.01	0.03	0.04	0.05	0.09	0.04
5	0.625	1.17	0.02	0.62	0.46	0.07	0.04
5	1.25	1.30	0.02	1.16	1.08	0.07	0.03
5	2.50	1.50	0.02	1.57	1.76	0.07	0.04
7.5	0	0.92	0.03	0.04	0.06	0.09	0.05
7.5	1.25	1.28	0.02	1.12	0.94	0.08	0.07
7.5	2.5	1.54	0.03	1.58	1.59	0.07	0.04
10.0	0	0.98	0.03	0.04	0.06	0.09	0.05
10.0	0.625	1.11	0.02	0.51	0.33	0.09	0.07
10.0	1.25	1.31	0.02	1.02	0.86	0.09	0.06
10.0	2.50	1.72	0.03	1.69	1.67	0.09	0.07

EXAMPLE 10

1 ug of the 11mer peptide was used to coat each well of a 96-well plate. The procedure of Example 1 was followed. 266 serum samples were scanned on the 11mer coated plate. The results are shown in Table V. Out of the 159 samples indicated as sero-positive by Western Blot Analysis, the 11mer peptide showed reactivity with 114 samples. Out of the 107 samples indicated as sero-negative by Western Blot Assay, the 11mer peptide showed non-reactivity with 105 samples.

The results indicates that the 11mer peptide is immunoreactive with antibodies to HTLV-III.

TABLE V

Comparison of ELISA using 11mer Peptide with Western Blot Method

ELISA		WESTERN BLOT	
$p24^{+(a)}$	$p24^{-(b)}$	$p24^{+}$	$p24^{-}$
114/159	105/107	159	107

(a) = p24 positive
(b) = p24 negative

EXAMPLE 11

Analogue peptides having the following amino acid sequences were synthesized based on the known amino sequences of the trans-membrane region of various strains of HTLV-III.

1.

Arg-Val-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Arg-Asp-
Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser

is based on an ARV-2 isolate,

2.

Arg-Val-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Gln-Asp-
Gln-Arg-Leu-Leu-Gly-Met-Trp-Gly-Cys-Ser

is based on a MAL isolate from Zaire;

3.

Ala-Arg-Val-Thr-Ala-Ile-Glu-Lys-Tyr-Leu-Gln-
Asp-Gln-Ala-Arg-Leu-Asn-Ser-Trp-Gly-Cys-Ala-
Phe-Arg-Gln-Val-Cys-His-Thr-Thr-Val-Pro-Trp

is based on a West African isolate of HTLV-III.

The synthesized peptides (1) and (2) were tested against sera which were known to be positive for antibodies to HTLV-III. The results showed that peptides (1) and (2) were immunoreactive to antibodies to HTLV-III.

Peptide (3) was tested against sera which have been found to be positive for antibodies to the West African isolate of HTLV-III. The results showed that peptide (3) was immunoreactive to antibodies to the West African isolate of HTLV-III.

Based on peptide (3), it is expected that a synthetic peptide with the following amino acid sequence:

Arg-Val-Thr-Ala-Ile-Glu-Lys-Tyr-Leu-Gln-Asp-Gln-

Ala-Arg-Leu-Asn-Ser-Trp-Gly-Cys-Ala-

will be immunoreactive to the West African isolate of HTLV-III.

This example shows that analogue peptides can be synthesized, using information based on the known amino acid sequence of various strains of HTLV-III, as immunoreactive agents to test for the presence of antibodies to various strains of HTLV-III in body fluids.

Claims

Claims for the following Contracting States : BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. A peptide for the detection of antibodies to HTLV-III in body fluids and diagnosis of AIDS, ARC or pre-AIDS conditions characterised in that it comprises about thirty-five amino acids and has an amino acid sequence:

-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys-Thr-Thr-Ala-Val-Pro-Trp-Asn-Ala-Ser-

or an analogue thereof, such "analogue" being a peptide containing a substitution, insertion and/or deletion of an amino acid or a polymeric form of such a peptide, which can detect and/or elicit production of antibodies to HTLV-III.

2. A peptide characterised in that it comprises about eleven amino acids and has the amino acid sequence:
   -Ile-Val-Arg-Met-Tyr-Ser-Pro-Thr-Ser-Ile-Leu-
   or an analogue thereof (as defined in claim 1).

3. A peptide composition for the detection of antibodies to HTLV-III in body fluids and diagnosis of AIDS, ARC or pre-AIDS conditions characterised in that it comprises a mixture of :
   (i) from about 10 to about 95 parts, by weight, of a peptide of about twenty-one amino acids having an amino acid sequence:

-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser-

   or an analogue thereof (as defined in claim 1);
   (ii) from about 5 to about 90 parts, by weight, of a peptide of about nineteen amino acids having an amino acid sequence :

-Ile-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys-Thr-Thr-Ala-Val-Pro-Trp-Asn-Ala-Ser-

   or an analogue thereof (as defined in claim 1);

Peptide (3) was tested against sera which have been found to be positive for antibodies to the West African isolate of HTLV-III. The results showed that peptide (3) was immunoreactive to antibodies to the West African isolate of HTLV-III.

Based on peptide (3), it is expected that a synthetic peptide with the following amino acid sequence:

Arg-Val-Thr-Ala-Ile-Glu-Lys-Tyr-Leu-Gln-Asp-Gln-Ala-Arg-Leu-Asn-Ser-Trp-Gly-Cys-Ala-

will be immunoreactive to the West African isolate of HTLV-III.

This example shows that analogue peptides can be synthesized, using information based on the known amino acid sequence of various strains of HTLV-III, as immunoreactive agents to test for the presence of antibodies to various strains of HTLV-III in body fluids.

Claims

Claims for the following Contracting States : BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. A peptide for the detection of antibodies to HTLV-III in body fluids and diagnosis of AIDS, ARC or pre-AIDS conditions characterised in that it comprises about thirty-five amino acids and has an amino acid sequence:

-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys-Thr-Thr-Ala-Val-Pro-Trp-Asn-Ala-Ser-

or an analogue thereof, such "analogue" being a peptide containing a substitution, insertion and/or deletion of an amino acid or a polymeric form of such a peptide, which can detect and/or elicit production of antibodies to HTLV-III.

2. A peptide characterised in that it comprises about eleven amino acids and has the amino acid sequence:
   -Ile-Val-Arg-Met-Tyr-Ser-Pro-Thr-Ser-Ile-Leu-
   or an analogue thereof (as defined in claim 1).

3. A peptide composition for the detection of antibodies to HTLV-III in body fluids and diagnosis of AIDS, ARC or pre-AIDS conditions characterised in that it comprises a mixture of :
   (i) from about 10 to about 95 parts, by weight, of a peptide of about twenty-one amino acids having an amino acid sequence:

-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser-

   or an analogue thereof (as defined in claim 1);
   (ii) from about 5 to about 90 parts, by weight, of a peptide of about nineteen amino acids having an amino acid sequence :

-Ile-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys-Thr-Thr-Ala-Val-Pro-Trp-Asn-Ala-Ser-

   or an analogue thereof (as defined in claim 1);

* C L M S *

and, optionally,
(iii) from about 5 to about 50 parts, by weight, of a peptide of about eleven amino acids having an amino acid sequence :
-Ile-Val-Arg-Met-Tyr-Ser-Pro-Thr-Ser-Ile-Leu- or an analogue thereof (as defined in claim 1).

4. An immunoassay method for the detection of antibodies to HTLV-III and diagnosis of AIDS, ARC or pre-AIDS conditions characterised in that it comprises :
(A) preparing a peptide as claimed in claim 1 or claim 2 or a peptide composition as claimed in claim 3; and
(B) using an effective amount thereof as a coating antigen to detect antibodies to HTLV-III in an immunoassay.

5. A method as claimed in claim 4 wherein the immunoassay is ELISA or IRMA.

6. A method as claimed in claim 5 wherein, for each immunoassay test, from about 0.1 to about 20µg of the peptide composition per test is used as the antigen, or about 1µg of the peptide composition per test in a buffer is used as the antigen.

7. A method as claimed in claim 6 wherein the pH is from about 7 to about 10.

8. A test kit for the detection of antibodies to HTLV-III and the diagnosis of AIDS, ARC or pre-AIDS conditions characterised in that the immunoadsorbent is a peptide per test as claimed in claim 1 or claim 2 or a peptide composition as claimed in claim 3.

9. A vaccine for eliciting the production of antibodies to HTLV-III in mammals or an immunogen for the development of monoclonal or polyclonal antibodies to HTLV-III in mammals characterised in that it comprises a peptide as claimed in claim 1 or claim 2 or an analogue thereof (as defined in claim 1), or it comprises a mixture of:
(i) a peptide of about twenty-one amino acids having an amino acid sequence as defined in claim 3 or an analogue thereof (as defined in claim 1);
(ii) a peptide of about nineteen amino acids having an amino acid sequence as defined in claim 3 or an analogue thereof (as defined in claim 1); and, optionally,
(iii) a peptide of about eleven amino acids having an amino acid sequence as claimed in claim 2 or as defined in claim 3 or an analogue of either thereof (as defined in claim 1).

10. A vaccine or an immunogen as claimed in claim 9 wherein the peptide is coupled to a protein or to a polymer, or is polymeric.

11. A vaccine or an immunogen as claimed in claim 10 wherein the protein is human serum albumin.

12. A method for the production of monoclonal or polyclonal antibodies to HTLV-III in mammals characterized in that it comprises using an immunogen produced by a process as claimed in any of claims 9 to 11.

Claims for the following Contracting States : AT, ES

1. A process for the production of a peptide for the detection of antibodies to HTLV-III in body fluids and diagnosis of AIDS, ARC or pre-AIDS conditions characterized in that it comprises chemically synthesizing a peptide of about thirty-five amino acids having an amino acid sequence:

-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys-Thr-Thr-Ala-Val-Pro-Trp-Asn-Ala-Ser-

or an analogue thereof, such "analogue" being a peptide containing a substitution, insertion and/or deletion of an amino acid or a polymeric form of such a peptide, which can detect and/or elicit

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production of antibodies to HTLV-III.

2. A process for the production of a peptide characterized in that it comprises chemically synthesizing a peptide of about eleven amino acids having the amino acid sequence:
-Ile-Val-Arg-Met-Tyr-Ser-Pro-Thr-Ser-Ile-Leu-
or an analogue thereof (as defined in claim 1).

3. A peptide composition for the detection of antibodies to HTLV-III in body fluids and diagnosis of AIDS, ARC or pre-AIDS conditions characterized in that it comprises a mixture of :
(i) from about 10 to about 95 parts, by weight, of a peptide of about twenty-one amino acids having an amino acid sequence:

-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser-

or an analogue thereof;
(ii) from about 5 to about 90 parts, by weight, of a peptide of about nineteen amino acids having an amino acid sequence :

-Ile-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys-Thr-Thr-Ala-Val-Pro-Trp-Asn-Ala-Ser-

or an analogue thereof (as defined in claim 1);
and, optionally,
(iii) from about 5 to about 50 parts, by weight, of a peptide of about eleven amino acids having an amino acid sequence :
-Ile-Val-Arg-Met-Tyr-Ser-Pro-Thr-Ser-Ile-Leu- or an analogue thereof (as defined in claim 1).

4. A process for the production of a peptide composition as claimed in claim 3 characterised in that it comprises mixing the components thereof.

5. An immunoassay method for the detection of antibodies to HTLV-III and diagnosis of AIDS, ARC or pre-AIDS conditions characterised in that it comprises :
(A) producing a peptide (composition) by a process as claimed in claims 1, 2 or 4 or preparing a peptide composition as claimed in claim 3; and
(B) using an effective amount thereof as a coating antigen to detect antibodies to HTLV-III in an immunoassay.

6. A method as claimed in claim 5 wherein the immunoassay is ELISA or IRMA.

7. A method as claimed in claim 6 wherein, for each immunoassay test, from about 0.1 to about 20µg of the peptide (composition) per test is used as the antigen, or about 1µg of the peptide (composition) per test in a buffer is used as the antigen.

8. A method as claimed in claim 7 wherein the pH is from about 7 to about 10.

9. A test kit for the detection of antibodies to HTLV-III and the diagnosis of AIDS, ARC or pre-AIDS conditions characterised in that the immunoadsorbent is a peptide (composition) produced by a process as claimed in claims 1, 2 or 4 or a peptide composition as claimed in claim 3.

10. A process for the production of a vaccine for eliciting the production of antibodies to HTLV-III in mammals or of an immunogen for the development of monoclonal or polyclonal antibodies to HTLV-III in mammals characterised in that it comprises the use of at least one peptide having an amino acid sequence as defined in claims 1 or 2 or at least one analogue thereof (as defined in claim 1) or produced by a process as claimed in claims 1, 2 or 4, or it comprises the use of a mixture of:

(i) a peptide of about twenty-one amino acids having an amino acid sequence as defined in claim 3 or an analogue thereof (as defined in claim 1);
(ii) a peptide of about nineteen amino acids having an amino acid sequence as defined in claim 3 or an analogue thereof (as defined in claim 1); and, optionally,
(iii) a peptide of about eleven amino acids having an amino acid sequence as defined in claim 2 or an analogue thereof (as defined in claim 1) or produced by a process as claimed in claim 2.

11. A process as claimed in claim 10 wherein the peptide used is coupled to a protein or to a polymer, or is polymeric.

12. A process as claimed in claim 11 wherein the protein is human serum albumin.

13. A method for the production of monoclonal or polyclonal antibodies to HTLV-III in mammals characterised in that it comprises using an immunogen produced by a process as claimed in any of claims 10 to 12.

Patentansprüche
Patentansprüche für folgende Vertragsstaaten : BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Peptid zum Nachweis von Antikörpern gegen HTLV-III in Körperflüssigkeiten und zur Diagnose von AIDS, ARC oder pre-AIDS-Bedingungen, dadurch gekennzeichnet, daß es etwa 35 Aminosäuren aufweist und die Aminosäuresequenz

-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys-Thr-Thr-Ala-Val-Pro-Trp-Asn-Ala-Ser-

oder eines Analogons davon hat, wobei ein solches "Analogon" ein Peptid ist, das eine Substitution, Insertion und/oder Deletion einer Aminosäure oder eine polymere Form eines solchen Peptids enthält, das die Produktion von Antikörpern gegen HTLV-III nachweisen und/oder entdecken kann.

2. Peptid, dadurch gekennzeichnet, daß es aus etwa 11 Aminosäuren besteht und die Aminosäuresequenz -Ile-Val-Arg-Met-Tyr-Ser-Pro-Thr-Ser-Ile-Leu- oder eines Analogons davon (wie es in Anspruch 1 definiert ist) hat.

3. Peptidzusammensetzung zum Nachweis von Antikörern gegen HTLV-III in Körperflüssigkeiten und zur Diagnose von AIDS, ARC oder pre-AIDS-Bedingungen, dadurch gekennzeichnet, daß sie eine Mischung aus
(i) von etwa 10 bis etwa 95 Gew.-Teilen eines Peptids von etwa 21 Aminosäuren mit der folgenden Aminosäuresequenz

-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser-

oder eines Analogons davon (wie es in Anspruch 1 definiert ist),
(ii) von etwa 5 bis etwa 90 Gew.-Teilen eines Peptids von etwa 19 Aminosäuren mit der folgenden Aminosäuresequenz

-Ile-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys-Thr-Thr-Ala-Val-Pro-Trp-Asn-Ala-Ser-

oder eines Analogons davon (wie es in Anspruch 1 definiert ist), und gegebenenfalls
(iii) von etwa 5 bis etwa 50 Gew.-Teilen eines Peptids von etwa 11 Aminosäuren mit der folgenden Aminosäuresequenz
-Ile-Val-Arg-Met-Tyr-Ser-Pro-Thr-Ser-Ile-Leu- oder eines Analogons davon (wie es in Anspruch 1 definiert ist) enthält.

4. Immunoassey-Verfahren zum Nachweis von Antikörpern gegen HTLV-III und zur Diagnose von AIDS, ARC oder pre-AIDS-Bedingungen, dadurch gekennzeichnet, daß es
(a) die Herstellung eines Peptids nach Anspruch 1 oder Anspruch 2 oder einer Peptidzusammensetzung nach Anspruch 3 und
(b) die Verwendung eine wirksame Menge davon als ein Beschichtungsantigen, um Antikörper gegen HTLV-III in einem Immunoassey nachzuweisen, umfaßt.

5. Verfahren nach Anspruch 4, dadurch gekennzeichnet, daß der Immunoassey ELISA oder IRMA ist.

6. Verfahren nach Anspruch 5, dadurch gekennzeichnet, daß für jeden Immunoassey-Test von etwa 0,1 bis etwa 20µg der Peptidzusammensetzung pro Test als das Antigen oder etwa 1µg der Peptidzusammensetzung pro Test in einem Puffer als das Antigen verwendet werden.

7. Verfahren nach Anspruch 6, dadurch gekennzeichnet, daß der pH von etwa 7 bis etwa 10 beträgt.

8. Testkit zum Nachweis von Antikörpern gegen HTLV-III und zur Diagnose von AIDS, ARC oder pre-AIDS-Bedingungen, dadurch gekennzeichnet, daß das Immunoabsorbens ein Peptid pro Test nach Anspruch 1 oder Anspruch 2 oder einer Peptidzusammensetzung nach Anspruch 3 ist.

9. Vakzin zum Bestimmen der Produktion von Antikörpern gegen HTLV-III in Säugetieren oder eines Immunogens zur Entwicklung eines monoclonalen oder polyclonalen Antikörpers gegen HTLV-III in Säugetieren, dadurch gekennzeichnet, daß es ein Peptid nach Anspruch 1 oder Anspruch 2 oder eines Analogons davon (wie in Anspruch definiert) oder eine Mischung aus
(i) einem Peptid von 21 Aminosäuren mit einer Aminosäuresequenz nach Anspruch 3 oder eines Analogons davon (wie es in Anspruch 1 definiert ist),
(ii) einem Peptid von etwa 19 Aminosäuren mit einer Aminosäresequenz wie sie gemäß Anspruch 3 oder eines Analogons davon (wie es in Anspruch 1 definiert ist) und gegebenenfalls
(iii) einem Peptid von etwa 11 Aminosäuren mit einer Aminosäuresequenz nach Anspruch 2 oder nach Anspruch 3 oder eines Analogons davon (wie es in Anspruch 1 definiert ist) enthält.

10. Vakzin oder Immunogen nach Anspruch 9, dadurch gekennzeichnet, daß das Peptid an ein Protein oder an ein Polymer gekoppelt ist oder das Peptid polymer ist.

11. Vakzin oder Immunogen nach Anspruch 10, dadurch gekennzeichnet, daß das Protein Humanserumalbumin ist.

12. Verfahren zur Herstellung monoclonaler oder polyclonaler Antikörper gegen HTLV-III in Säugetieren, dadurch gekennzeichnet, daß es die Verwendung eines nach einem Verfahren nach einem der Ansprüche 9 bis 11 hergestellten Immunogens umfaßt.

Patentansprüche für folgende Vertragsstaaten : AT, ES

1. Verfahren zur Herstellung eines Peptids zum Nachweis von Antikörpern gegen HTLV-III in Körperflüssigkeiten und zur Diagnose von AIDS, ARC oder pre-AIDS-Bedingungen, dadurch gekennzeichnet, daß es die chemische Synthese eines Peptides von etwa 35 Aminosäuren der nachfolgenden Aminosequenz

Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys-Thr-Thr-Ala-Val-Pro-Trp-Asn-Ala-Ser-

oder eines Analogons davon umfaßt, wobei ein solches "Analogon" ein Peptid ist, das eine Substitution, Insertion und/oder Deletion einer Aminosäure oder eine polymere Form eines solchen Peptids enthält, die die Produktion von Antikörpern gegen HTLV-III nachweisen und/oder entdecken kann.

2. Verfahren zur Herstellung eines Peptids, dadurch gekennzeichnet, daß es die chemische Synthese eines Peptids von etwa 11 Aminosäuren mit der folgenden Aminosäuresequenz -Ile-Val-Arg-Met-Tyr-Ser-Pro-Thr-Ser-Ile-Leu- oder eines Analogons davon (wie es in Anspruch 1 definiert ist) umfaßt.

3. Peptidzusammensetzung zum Nachweis von Antikörern gegen HTLV-III in Körperflüssigkeiten und zur Diagnose von AIDS, ARC oder pre-AIDS-Bedingungen, dadurch gekennzeichnet, daß sie eine Mischung von

(i) von etwa 10 bis etwa 95 Gew.-Teilen eines Peptids von etwa 21 Aminosäuren mit der folgenden Aminosäuresequenz

Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser-

oder eines Analogons davon

(ii) von etwa 5 bis etwa 90 Gew.-Teilen eines Peptids von etwa 19 Aminosäuren mit der folgenden Aminosäuresequenz

-Ile-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys-Thr-Thr-Ala-Val-Pro-Trp-Asn-Ala-Ser-

oder eines Analogons davon (wie es in Anspruch 1 definiert ist), und gegebenenfalls

(iii) von etwa 5 bis etwa 50 Gew.-Teilen eines Peptids von etwa 11 Aminosäuren mit der folgenden Aminosäuresequenz -Ile-Val-Arg-Met-Tyr-Ser-Pro-Thr-Ser-Ile-Leu- oder eines Analogons davon (wie es in Anspruch 1 definiert ist) enthält.

4. Verfahren zur Herstellung einer Peptidzusammensetzung nach Anspruch 3, dadurch gekennzeichnet, daß es das Mischen der Komponenten umfaßt.

5. Immunoassey-Verfahren zum Nachweis von Antikörpern gegen HTLV-III und zur Diagnose von AIDS, ARC oder pre-AIDS-Bedingungen, dadurch gekennzeichnet, daß es

(A) die Herstellung eines Peptids (eine zusammensetzung) durch ein Verfahren nach den Ansprüchen 1, 2 oder 4 oder die Herstellung einer Peptidzusammensetzung nach Anspruch 3 und

(B) die Verwendung einer wirksamen Menge davon als ein Beschichtungsantigen umfaßt, um Antikörper gegen HTLV-III in einem Immunoassey nachzuweisen.

6. Verfahren nach Anspruch 5, dadurch gekennzeichnet, daß der Immunoassey ELISA oder IRMA ist.

7. Verfahren nach Anspruch 6, dadurch gekennzeichnet, daß für jeden Immunoassey-Test von etwa 0,1 bis etwa 20µg des Peptides (Zusammensetzung) pro Test als das Antigen oder etwa 1 µg des Peptids (der Zusammensetzung) pro Test in einem Puffer als das Antigen verwendet werden.

8. Verfahren nach Anspruch 7, dadurch gekennzeichnet, das der pH von etwa 7 bis etwa 10 beträgt.

9. Testkit zum Nachweis von Antikörpern gegen HTLV-III und zur Diagnose von AIDS, ARC oder pre-AIDS-Bedingungen, dadurch gekennzeichnet, daß das Immunoabsorbens ein(e) nach einem Verfahren gemäß den Ansprüchen 1, 2 oder 4 hergestelltes Peptid (hergestellte Zusammensetzung) oder eine Peptidzusammensetzung nach Anspruch 3 ist.

10. Verfahren zur Herstellung eines Vakzins zum Entdecken der Produktion von Antikörpern gegen HTLV-III in Säugetieren oder eines Immunogens zur Entwicklung von monoclonalen oder polyclonalen Antikörpers gegen HTLV-III in Säugetieren, dadurch gekennzeichnet, daß es die Verwendung von zumindest einem Peptid mit einer Aminosäuresequenz nach den Ansprüchen 1 oder 2 oder zumindest eines Analogons davon (wie in Anspruch 1 definiert ist) umfaßt oder durch ein Verfahren nach den Ansprüchen 1, 2 oder 4 hergestellten Peptids oder die Verwendung einer Mischung von
(i) eines Peptids von etwa 21 Aminosäuren mit einer Aminosäuresequenz wie sie gemäß Anspruch 3 oder eines Analogons davon (wie es in Anspruch 1 definiert ist),
(ii) eines Peptids von etwa 19 Aminosäuren mit einer Aminosäresequenz nach Anspruch 3 oder eines Analogons davon (wie es in Anspruch 1 definiert ist), und gegebenenfalls
(iii) eines Peptids von etwa 11 Aminosäuren mit einer Aminosäuresequenz nach Anspruch 2 oder eines Analogons davon (wie es in Anspruch 1 definiert ist) oder durch ein Verfahren gemäß Anspruch 2 hergestellt worden ist, umfaßt.

11. Verfahren nach Anspruch 10, dadurch gekennzeichnet, daß das verwendete Peptid an ein Protein oder ein Polymer gekoppelt ist oder das Peptid polymer ist.

12. Verfahren nach Anspruch 11, dadurch gekennzeichnet, daß das Protein Humanserumalbumin ist.

13. Verfahren zur Herstellung monoclonaler oder polyclonaler Antikörper gegen HTLV-III in Säugetieren, dadurch gekennzeichnet, daß es die Verwendung eines durch ein Verfahren nach einem der Ansprüche 10 bis 12 hergestellten Immunogens umfaßt.

Revendications

Revendications pour les Etats contractants suivants : BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Un peptide pour la détection d'anticorps à HTLV-III dans des fluides corporels et le diagnostic des conditions du SIDA, de l'ARC ou du pré-SIDA, caractérisé en ce qu'il comprend environ trente cinq acide aminés et présente une séquence d'acides aminés :

-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Leu-Leu-
Gly-Ile-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys-Thr-Thr-Ala-Val-
Pro-Trp-Asn-Ala-Ser-

ou son analogue, un tel "analogue" étant un peptide contenant une substitution, une insertion et/ou une suppression d'un acide aminé ou d'une forme polymère d'un tel peptide, qui peut détecter et/ou déterminer la production d'anticorps à HTLV-III.

2. Un peptide caractérisé en ce qu'il comprend environ onze acides aminés et présente la séquence d'acides aminés : -Ile-Val-Arg-Met-Tyr-Ser-Pro-Thr-Ser-Ile-Leu- ou son analogue (tel que défini à la revendication 1).

3. Une composition à base de peptide pour la détection d'anticorps à HTLV-III dans des fluides corporels et le diagnostic des conditions du SIDA, de l'ARC ou du pré-SIDA, caractérisée en ce qu'elle comprend un mélange :
(i) d'environ 10 à environ 95 parties, en poids, d'un peptide d'environ vingt et un acides aminés présentant une séquence d'acides aminés :

-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Leu-Leu-
-Gly-Ile-Trp-Gly-Cys-Ser-

ou son analogue (tel que défini à la revendication 1) ;
(ii) d'environ 5 à environ 90 parties, en poids, d'un peptide d'environ dix neuf acides aminés présentant une séquence d'acides aminés :

-Ile-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys-Thr-Thr-Ala-Val-Pro-
Trp-Asn-Ala-Ser-

ou son analogue (tel que défini à la revendication 1) ;
et, facultativement,
(iii) d'environ 5 à environ 50 parties, en poids, d'un peptide d'environ onze acides aminés présentant une séquence d'acides aminés :
-Ile-Val-Arg-Met-Tyr-Ser-Pro-Thr-Ser-Ile-Leu- ou son analogue (tel que défini à la revendication 1).

4. Un procédé d'immunodosage pour la détection d'anticorps à HTLV-III et le diagnostic des conditions du SIDA, de l'ARC ou du pré-SIDA, caractérisé en ce qu'il comprend :
(A) la préparation d'un peptide tel que revendiqué à la revendication 1 ou à la revendication 2, ou une composition à base de peptide telle que revendiquée à la revendication 3 ; et
(B) l'utilisation d'une quantité efficace de ces derniers en tant qu'antigène de revêtement pour détecter des anticorps à HTLV-III dans un immunodosage.

5. Un procédé tel que revendiqué à la revendication 4, dans lequel l'immunodosage est ELISA ou IRMA.

6. Un procédé tel que revendiqué à la revendication 5 dans lequel, pour chaque test d'immunodosage, d'environ 0,1 à environ 20 µg de la composition à base de peptide par test est utilisé en tant qu'antigène, ou environ 1 µg de la composition à base de peptide par test dans un tampon est utilisé en tant qu'antigène.

7. Un procédé tel que revendiqué à la revendication 6 dans lequel le pH est d'environ 7 à environ 10.

8. Une trousse à test pour la détection d'anticorps à HTLV-III et le diagnostic des conditions du SIDA, de l'ARC ou du pré-SIDA, caractérisée en ce que l'immuno-adsorbant est un peptide par test tel que revendiqué à la revendication 1 ou à la revendication 2, ou une composition à base de peptide telle que revendiquée à la revendication 3.

9. Un vaccin pour déterminer la production d'anticorps à HTLV-III chez des mammifères ou d'un immunogène pour le développement d'anticorps monoclonaux ou polyclonaux à HTLV-III chez les mammifères, caractérisé en ce qu'il comprend un peptide tel que revendiqué à la revendication 1 ou à la revendication 2 ou son analogue (tel que défini à la revendication 1), ou qu'il comprend un mélange de :
(i) un peptide d'environ vingt et un acides aminés présentant une séquence d'acides aminés telle que définie à la revendication 3 ou son analogue (tel que défini à la revendication 1) ;
(ii) un peptide d'environ dix neuf acides aminés présentant une séquence d'acides aminés telle que définie à la revendication 3 ou son analogue (tel que défini à la revendication 1) : et, facultativement,
(iii) un peptide d'environ onze acides aminés présentant une séquence d'acides aminés telle que revendiquée à la revendication 2 ou telle que définie à la revendication 3 ou leur analogue (tel que défini à la revendication 1).

10. Un vaccin ou un immunogène tel que revendiqué à la revendication 9, dans lequel le peptide est couplé à une protéine ou à un polymère, ou est un corps polymère.

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11. Un vaccin ou un immunogène tel que revendiqué à la revendication 10, dans lequel la protéine est de l'albumine de sérum humain.

12. Un procédé pour la production d'anticorps monoclonaux ou polyclonaux à HTLV-III chez les mammifères, caractérisé en ce qu'il comprend l'utilisation d'un immunogène produit par un procédé tel que revendiqué dans l'une quelconque des revendications 9 à 11.

Revendications pour les Etats contractants suivants : AT, ES

1. Un procédé pour la réalisation d'un peptide pour la détection d'anticorps à HTLV-III dans des fluides corporels et le diagnostic des conditions du SIDA, de l'ARC ou du pré-SIDA, caractérisé en ce qu'il comprend la synthétisation chimique d'un peptide d'environ trente cinq acides aminés présentant une séquence d'acides aminés :

-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys-Thr-Thr-Ala-Val-Pro-Trp-Asn-Ala-Ser-

ou son analogue, un tel "analogue" étant un peptide contenant une substitution, une insertion et/ou une suppression d'un acide aminé ou d'une forme polymère d'un tel peptide, qui peut détecter et/ou déterminer la production d'anticorps à HTLV-III.

2. Un procédé pour la réalisation d'un peptide caractérisé en ce qu'il comprend la synthétisation chimique d'un peptide d'environ onze acides aminés ayant la séquence d'acides aminés :
-Ile-Val-Arg-Met-Tyr-Ser-Pro-Ihr-Ser-Ile-Leu- ou son analogue (tel que défini à la revendication 1).

3. Une composition à base de peptide pour la détection d'anticorps à HTLV-III dans des fluides corporels et le diagnostic des conditions du SIDA, de l'ARC ou du pré-SIDA, caractérisée en ce qu'elle comprend un mélange :
(i) d'environ 10 à environ 95 parties, en poids, d'un peptide d'environ vingt et un acides aminés présentant une séquence d'acides aminés :

-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser-

ou son analogue ;
(ii) d'environ 5 à environ 90 parties, en poids, d'un peptide d'environ dix neuf acides aminés présentant une séquence d'acides aminés :

-Ile-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys-Thr-Thr-Ala-Val-Pro-Trp-Asn-Ala-Ser-

ou son analogue (tel que défini à la revendication 1) ;
et, facultativement,
(iii) d'environ 5 à environ 50 parties, en poids, d'un peptide d'environ onze acides aminés présentant une séquence d'acides aminés :
-Ile-Val-Arg-Met-Tyr-Ser-Pro-Thr-Ser-Ile-Leu- ou son analogue (tel que défini à la revendication 1).

4. Un procédé pour la réalisation d'une composition à base de peptide telle que revendiquée à la revendication 3, caractérisé en ce qu'il comprend le mélange de ses constituants.

5. Un procédé d'immunodosage pour la détection d'anticorps à HTLV-III et le diagnostic des conditions du SIDA, de l'ARC ou du pré-SIDA, caractérisé en ce qu'il comprend :
(A) la réalisation d'un peptide (composition) par un procédé tel que revendiqué aux revendications 1, 2 ou 4, ou la préparation d'une composition à base de peptide telle que revendiquée à la revendication 3 ; et
(B) l'utilisation d'une quantité efficace de ces derniers en tant qu'antigène de revêtement pour détecte des anticorps à HTLV-III dans un immunodosage.

6. Un procédé tel que revendiqué à la revendication 5, dans lequel l'immunodosage est ELISA ou IRMA.

7. Un procédé tel que revendiqué à la revendication 6 dans lequel, pour chaque test d'immunodosage, d'environ 0,1 à environ 20 µg de peptide (composition) par test est utilisé en tant qu'antigène, ou environ 1 µg de peptide (composition) par test dans un tampon est utilisé en tant qu'antigène.

8. Un procédé tel que revendiqué à la revendication 7, dans lequel le pH est d'environ 7 à environ 10.

9. Une trousse à test pour la détection d'anticorps à HTLV-III et le diagnostic des conditions du SIDA, de l'ARC ou du pré-SIDA, caractérisée en ce que l'immuno-adsorbant est un peptide (composition) produit par un procédé tel que revendiqué à la revendication 1, 2 ou 4, ou une composition à base de peptide telle que revendiquée à la revendication 3.

10. Un procédé pour la réalisation d'un vaccin pour déterminer la production d'anticorps à HTLV-III chez des mammifères ou d'un immunogène pour le développement d'anticorps monoclonaux ou polyclonaux à HTLV-III chez les mammifères, caractérisé en ce qu'il comprend l'utilisation d'au moins un peptide présentant une séquence d'acides aminés telle que définie aux revendications 1 ou 2 ou au moins son analogue (tel que défini à la revendication 1) ou produite par un procédé tel que revendiqué aux revendications 1, 2 ou 4, ou qu'il comprend l'utilisation d'un mélange de :
(i) un peptide d'environ vingt et un acides aminés présentant une séquence d'acides aminés telle que définie à la revendication 3 ou son analogue (tel que défini à la revendication 1) :
(ii) un peptide d'environ dix neuf acides aminés présentant une séquence d'acides aminés telle que définie à la revendication 3 ou son analogue (tel que défini à la revendication 1) ; et, facultativement,
(iii) un peptide d'environ onze acides aminés présentant une séquence d'acides aminés telle que définie à la revendication 2 ou son analogue (tel que défini à la revendication 1), ou réalisée par un procédé tel que revendiqué à la revendication 2.

11. Un procédé tel que revendiqué à la revendication 10, dans lequel le peptide utilisé est couplé à une protéine ou à un polymère, ou est un corps polymère.

12. Un procédé tel que revendiqué à la revendication 11, dans lequel la protéine est de l'albumine de sérum humain.

13. Un procédé pour la production d'anticorps monoclonaux ou polyclonaux à HTLV-III chez les mammifères, caractérisé en ce qu'il comprend l'utilisation d'un immunogène produit par un procédé tel que revendiqué dans l'une quelconque des revendications 10 à 12.