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Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: HIV-1 CORE PROTEIN FRAGMENTS

(57) Abstract

Various peptide fragments of HIV have been identified which interact specifically with particular human leucocyte antigen (HLA) class 1 molecules, to stimulate cytotoxic T lymphocyte immunity. The peptides are known as p24-20, p17-8, p17-3, p24-17, p24-22, p24-23, p24-6 and p24-2. Such fragments can be used in a potential vaccine against AIDS (acquired immune deficiency syndrome), and for diagnostic and therapeutic purposes.

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HIV-1 core protein fragments.

Field of Invention

This invention concerns peptide fragments of HIV (human immunodeficiency virus) and the use thereof in a potential vaccine against AIDS (acquired immune deficiency syndrome), and for diagnostic and therapeutic purposes.

Background to the invention

European Patent Specification No. 0346022 discloses and claims, inter alia, a peptide having the amino acid sequence of a fragment of HIV which interacts specifically with a particular human leucocyte antigen (HLA) class I molecule, to stimulate cytotoxic T lymphocyte immunity.

One such peptide specifically disclosed in the prior application has the sequence NH_2-lysine-arginine - tryptophan-isoleucine-isoleucine-leucine-glycine-leucine-asparagine-lysine-isoleucine-valine-arginine-methionine-tyrosine-cysteine-COOH, which is derived from the gag (group associated antigen) p24 protein of HIV (ie one of the internal core proteins) between residues 263 and 277, and is known as p24-14. The carboxy-terminal cysteine is not part of the gag sequence and is added to facilitate chemical coupling reactions. This peptide interacts specifically with HLA B27, and individuals with HLA B27 (about 7% of the Caucasian population) should respond to the peptide, resulting in production of cytotoxic T lymphocytes (CTL) specific for gag and HLA B27, and

capable of lysing cells infected with HIV.

The present application concerns further such peptides which have now been identified.

Summary of the invention

According to one aspect of the present invention there is provided a peptide having the amino acid sequence of a fragment of HIV which interacts specifically with a particular human leucocyte antigen (HLA) class I molcule, to stimulate cytotoxic T lymphocyte immunity, the peptide having the sequence NH_2-valine-glutamine-asparagine-alanine-asparagine-proline-aspartic acid-cysteine-lysine-threonine-isoleucine-leucine-lysine-alanine-leucine-tyrosine-COOH.

This sequence is derived from the gag p24 protein of HIV. This peptide, which is known as p24-20, interacts specifically with HLA B8 and individuals with HLA B8 (about 15% of the Caucasian population) should respond to the peptide, resulting in production of cytotoxic T lymphocytes (CTL) specific for gag and HLA B8, and capable of lysing cells infected with HIV. Peptide p24-20 has also been recognised by a seropositive donor of the HLA type HLA-A3, 29B44, 14 and therefore can be recognised in association with one of these HLA molecules as well as HLA-B8.

According to another aspect of the present invention there is provided a peptide having the amino acid sequence of a fragment of HIV which interacts specifically with a particular human leucocyte antigen (HLA) class I molecule, to stimulate cytotoxic T lymphocyte immunity, the peptide

having the sequence NH_2-cysteine-glycine-serine-glutamic acid-glutamic acid-leucine-arginine-serine-leucine-tyrosine-asparagine-threonine-valine-alanine-threonine-leucine-COOH.

This sequence is derived from the gag p17 protein of HIV. This peptide, which is known as p17-8, interacts specifically with HLA A2 and individuals with HLA A2 (about 40% of the Caucasian population) should respond to the peptide, resulting in production of cytotoxic T lymphocytes (CTL) specific for gag and HLA A2, and capable of lysing cells infected with HIV.

According to another aspect of the present invention there is provided a peptide having the amino acid sequence of a fragment of HIV which interacts speciifically with a particular human leucocyte antigen (HLA) class I molecule, to stimulate cytotoxic T lymphocyte immunity, the peptide having the sequence NH_2-cysteine-leucine-arginine-proline-glycine-glycine-lysine-lysine-lysine-tyrosine-lysine-leucine-lysine-histidine-isoleucine-valine-COOH.

This sequence is derived from the gag p17 protein of HIV. The amino terminal cysteine is not part of the gag sequence and is added to facilitate chemical coupling reactions. The invention thus also includes within its scope the peptide without the amino terminal cysteine. This peptide, which is known as p17-3, also interacts specifically with HLA B8 and individuals with HLA B8 should respond to the peptide, resulting in production of cytotoxic T lymphocytes (CTL) specific for gag and HLA B8, and capable of lysing cells infected with HIV.

Five other epitopes from the gag p24 protein of HIV have

also been identified as being able to sensitise targets in the CTL assay, but their HLA restrictions have not yet been fully worked out. Details are given below.

Peptide p24-17 is as follows:
NH_2-phenylalanine-arginine-aspartic acid-tyrosine-valine-aspartic acid-arginine-phenylalanine-tyrosine-lysine-threonine-leucine-arginine-alanine-glutamic acid-cysteine-COOH. HLA restriction of this peptide is through one or more of the antigens HLA-A3 or A29 or B44 or B14.

Peptide p24-22 is as follows:
NH_2-leucine-glutamic acid-glutamic acid-methionine-methionine-threonine-alanine-cysteine-glutamine-glycine-valine-glycine-glycine-proline-glycine-tyrosine-COOH. HLA restriction of this peptide is through one or more of the antigens HLA-A3 or A29 or B44 or B14.

Peptide p24-23 is as follows:
NH_2-cysteine-valine-glycine-glycine-proline-glycine-histidine-lysine-alanine-arginine-valine-leucine-COOH. HLA restriction of this peptide is through one or more of the antigens HLA-A1 or B7 or B8.

Peptide p24-6 is as follows:
NH_2-aspartic acid-leucine-asparagine-threonine-methionine-leucine-asparagine-threonine-valine-glycine-glycine-histidine-glutamine-alanine-alanine-cysteine-COOH. HLA restriction of this peptide is through one or more of antigens HLA-A3 or A29 or B44 or B14.

Peptide p24-2 is as follows:
NH_2-valine-histidine-glutamine-alanine-isoleucine-serine-proline-arginine-threonine-leucine-asparagine-alanine-

thryptophan-valine-lysine-cysteine-COOH. HLA restriction of this peptide is through one or more of antigens HLA-A23 or A30 or B8.

By way of explanation, it has been shown that virus proteins such as gag are presented to T cells as degraded peptide fragments (about 15 amino acids) bound to larger HLA class I molecules on the surface of infected cells. Different peptide regions (epitopes) are recognised and interact specifically with different HLA class I molecules. CTL will only recognise target cells that share HLA Class I molecules, ie the T cells recognise a combination of virus antigen plus self HLA. There are probably about 120 different HLA class I molecules, and each individual human has a limited selection, and so will respond only to certain epitopes. Following the example of p24-20, which can be recognised by more than one type of HLA molecule, it is possible the other peptides of the invention may also be restricted by more than one HLA molecule.

The peptides disclosed above all include a region which contains a CTL epitope. It may be possible that one or more individual amino acids in the identified sequences may be altered in naturally occuring variants of the virus, yet still function in the same way, and the invention is intended to cover such and similar variants.

Epitopes recognised with different HLA can be identified and isolated in known manner or made by protein synthesis using known techniques.

Peptides in accordance with the invention can be used as the basis of a vaccine against AIDS, by stimulating

production of CTL responsive to the relevant HLA and so priming the CTL response.

In a further aspect the present invention thus provides a vaccine against AIDS, comprising a peptide of the invention.

The vaccine may comprise more than one peptide, and may additionally comprise one or more other peptides responsive to some of the more common HLA class I molecules to increase effectiveness.

The vaccine may take various different forms. For example, the vaccine may comprise a peptide or mixture of peptides for administration in solution, or absorbtion onto insoluble material or mixing with an adjuvant. The peptide amino acid sequence could alternatively be used to construct synthetic or fusion proteins that contain the relevant peptide epitopes, by known recombinant DNA techniques. These proteins could be used to immunise as soluble protein or absorbed onto insoluble material or mixed wih adjuvant. Alternatively the sequence information could be used to construct recombinant micro-organisms using known techniques which would express the relevant sequences in their own proteins. Examples would be recombinant vaccinia viruses, recombinant polio myelitis viruses, recombinant BCG, recombinant salmonella, recombinant adenovirus.

Another use of the sequence information would be to construct analogs of the peptides, or other chemicals which would interact with eg bind to the HLA molecules involved or the T cell receptors involved and interfere with or stimulate this form of immune response.

Inhibition of this type of immunity might be important if this immune response plays a harmful role in any of the pathology caused by HIV. If so it may be important to regulate the levels of this type of T cell immunity in HIV seropositive individuals so as to achieve a balance between beneficial and harmful effects. Stimulation by such agents may be an alternative way of inducing an immune response in seronegative individuals.

Peptides in accordance with the invention can also be used for diagnostic purposes. In particular, it has been found that it is possible to use such a peptide in some patients to identify T lymphocyte response in a relatively simple assay. Briefly, fresh peripheral blood mononuclear cells (or lymphocytes obtained from biopsy material) are prepared and added at ratios of 50:1, 25:1 and 10:1 to 10^4 ^{51}-chromium labelled B lymphoblastoid cells matched for the relevant HLA molecule. The HLA type of patient is determined in known manner by tissue typing, and B lymphoblastoid cells obtained from a donor of known HLA type (again determined in known manner by tissue typing) amd transformed with Epstein Barr using known techniques. A peptide in accordance with the invention, which interacts with the relevant HLA molecule, is also added to the cells in a concentration of 10 to 100 u molar. After 4 hours incubation the supernatant is removed and the released ^{51}-chromium measured. The ^{51}-chromium released is compared to that released by incubation of labelled cells in detergent, which gives a maximum value, and released by labelled cells incubated in medium alone, which gives a minimum value. If lysis (defined by ^{51}-chromium relase of at least two times the minimum value) is observed it means that there are cytotoxic T lymphocytes in the patients' mononuclear cells and these

are likely to be indicative of infection with HIV.

Such an assay, together with antibody measurements, may also be useful for measurement of the patient's general immune response to HIV, and may have prognostic implications. This approach may represent a very simple method which can be used for measuring cell mediated immunity in HIV seropositive patients. It may also be possible to automate the method.

Hence, in a further aspect the present invention provides a method of assaying cells for the presence of cytotoxic T lymphocytes, comprising incubating cells with labelled B lymphoblastoid cells matched for HLA type in the presence of a peptide of the invention which interacts with the relevant HLA type, and determining the amount of label released.

By comparing the amount of released label with known standards an indication of the degree of lysis can be obtained and hence of likely infection with HIV.

Peptides of the invention may also have potential use in therapy. These and similar peptides (and other peptide epitodes that have been identified previously in studies on influenza virus) have been used to stimulate cytotixic T lymphocytes to grow in vitro. The method involves exposing cultured peripheral blood mononuclear cells or B lymphoblastoid cell lines, which have been treated for one hour with an appropriate peptide at approximately 10-100 ug/ml then washed in tissue culture medium and irradiated to 3000 rads. The cells are then cultured in the presence of interleukin-2 at 10 units/ml. Using this method cytotoxic T lymphocyte cell lines specific for the peptide

have been grown, and these lines have been expanded up to 10^8 cells. These expanded cytotoxic T cell lines could be used to treat patients by reinfusion.

Preliminary data indicates that patients with AIDS or the AIDS related complex show low levels of cytotoxic T cell activity, whereas those who are infected with HIV but are healthy show high levels. Part of the immune deficiency syndrome therefore may be a result of impaired cytotoxic T cell activity. The proposal therefore would be to reinfuse autologous cytotoxic T cells grown in vitro on synthetic peptide pulsed cells. Initially patients who had previously had a high cytotoxic T cell activity would be treated at a stage when their levels of these cells was declining. The cytotoxic T cell lines could be prepared from frozen lymphocytes taken earlier in the patient's infection.

Thus in another aspect the invention provides a method of treating a patient for AIDS or related conditions, comprising administering cytotoxic T cells treated with a peptide in accordance with the invention which interacts with a HLA molecule present in the patient.

The cytotoxic T cells are preferably derived from lymphocytes taken from the patient at an earlier stage. The lymphocytes may be stored in frozen condition until used for preparation of the cytotoxic T cell line.

In addition it may be useful to treat patients for AIDS or related conditions by vaccination with a peptide in accordance with the invention.

The peptide epitope p24-12 is found to be quite well

conserved between different strains of HIV 1 and HIV 2, and it was found that cytotoxic T lymphocytes from a patient infected with HIV 1 cross-reacted on the HIV 2 peptide sequence. Similar properties may apply to the peptides of the invention. In this case, the peptide of the invention may be useful in vaccines to stimulate protection against both HIV 1 and HIV 2, and also for diagnostic and therapeutic purposes with patients infected with HIV 1 and/or HIV 2.

CLAIMS

1. A peptide having the amino acid sequence of a fragment of HIV which interacts specifically with a particular human lymphocyte antigen (HLA) class I molecule, to stimulate cytotoxic T lymphocyte immunity, the peptide having the sequence NH_2-valine-glutamine-asparagine-alanine-asparagine-proline-aspartic acid-cysteine-lysine-threonine-isoleucine-leucine-lysine-alanine-leucine-tyrosine-COOH.

2. A peptide having the amino acid sequence of a fragment of HIV which interacts specifically with a particular human leucocyte antigen (HLA) class I molecule, to stimulate cytotoxic T lymphocyte immunity, the peptide having the sequence NH_2-cysteine-glycine-serine-glutamic acid-glutamic acid-leucine-arginine-serine-leucine-tryosine-asparagine-threonine-valine-alanine-threonine-COOH.

3. A peptide having the amino acid sequence of a fragment of HIV which interacts specifically with a particular human leucocyte antigen (HLA) class I molecule, to stimulate cytotoxic T lymphocyte immunity, the peptide having the sequence NH_2-leucine-arginine-proline-glycine-glycine-lysine-lysine-lysine-tyrosine-lysine-leucine-lysine-histidine-isoleucine-valine-COOH.

4. A peptide according to claim 3, further comprising an amino terminal cysteine.

5. A peptide having the amino acid sequence of a fragment

of HIV which interacts specifically with a particular human leucocyte antigen (HLA) class I molecule, to stimulate cytotoxic T lymphocyte immunity, the peptide having the sequence NH_2-phenylalanine-arginine-aspartic acid-tyrosine-valine-aspartic acid-arginine-phenylalanine-tyrosine-lysine-threonine-leucine-arginine-alanine-glutamic acid-cysteine-COOH.

6. A peptide having the amino acid sequence of a fragment of HIV which interacts specifically with a particular human leucocyte antigen (HLA) class I molecule, to stimulate cytotoxic T lymphocyte immunity, the peptide having the sequence NH_2-leucine-glutamic acid-glutamic acid-methionine-methionine-threonine-alanine-cysteine-glutamine-glycine-valine-glycine-glycine-proline-glycine-tyrosine-COOH.

7. A peptide having the amino acid sequence of a fragment of HIV which interacts specifically with a particular human leucocyte antigen (HLA) class I molecule to stimulate cytotoxic T lymphocyte immunity, the peptide having the sequence NH_2-cysteine-valine-glycine-glycine-proline-glycine-histidine-lysine-alanine-arginine-valine-leucine-COOH.

8. A peptide having the amino acid sequence of a fragment of HIV which interacts specifically with a particular human leucocyte antigen (HLA) class I molecule to stimulate cytotoxic T lymphocyte immunity, the peptide having the sequence NH_2-aspartic acid-leucine-asparagine-threonine-methionine-leucine-asparagine-threonine-valine-glycine-glycine-histidine-glutamine-alanine-alanine-cysteine-COOH.

9. A peptide having the amino acid sequence of a fragment of HIV which interacts specifically with a particular human leucocyte antigen (HLA) class I molecule to stimulate cytotic T lymphocyte immunity, the peptide having the sequence NH_2-valine-histidine-glutamine-alanine-isoleucine-serine-proline-arginine-threonine-leucine-asparagine-alanine-tryptophan-valine-lysine-cysteine-COOH.

10. A vaccine against AIDS, comprising a peptide in accordance with any one of claim 1 to 9.

11. A vaccine according to claim 10, comprising more than one peptide, each peptide being responsive to a different HLA class I molecule.

12. A vaccine according to claim 10 or 11, comprising a peptide or mixture of peptides for administration in solution, or absorbtion onto insoluble material or mixing with an adjuvant.

13. A vaccine according to claim 10 or 11, comprising a synthetic or fusion protein that contains the relevant peptide epitope(s), for administration in solution, or absorbed onto insoluble material or mixed with adjuvant.

14. A vaccine according to claim 10 or 11, comprising a recombinant micro-organism constructed to express the relevant peptide sequence(s).

15. A method of assaying cells for the pressure of cytotoxic T lymphocytes, comprising incubating cells with labelled B lymphoblastoid cells matched for HLA type in the presence of a peptide in accordance with any one of

claims 1 to 9, which interacts with the relevant HLA type, and determining the amount of label released.

16. A method of treating a patient for AIDS or related conditions, comprising administering to the patient cytotoxic T cells treated with peptide in accordance with any one of claims 1 to 9 which interacts with a HLA molecule present in the patient.

17. A method of treating a patient for AIDS or related conditions, comprising vaccinating the patient with a peptide in accordance with any one of claims 1 to 9.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 91/00013

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: C 07 K 7/08, A 61 K 39/21, C 12 Q 1/00, C 12 P 21/00

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	C 07 K; A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	IMMUNOLOGY, vol. 67, 1989, T. Mathiesen et al: "Mapping of IgG subclass and T-cell epitopes on HIV proteins by synthetic peptides ", see page 453 - page 459 see Table 2, peptides 9-11 and 34 and pages 457-8	3,9
Y		1,3,7,9-14
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X	JOURNAL OF ACQUIRED IMMUNE DEFICIENCY SYNDROMES, vol. 4, 1989, B. Wahren et al: "HIV-1 Peptides Induce a Proliferative Response in Lymphocytes from Infected Persons ", see page 448 - page 456 especially Table 2, peptides 10 and 11	3
Y		1,3,7,9-14
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* Special categories of cited documents:[10]

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

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

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

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
25th April 1991	24. 05. 91
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	M. SOTELO

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	EP, A1, 0346022 (MEDICAL RESEARCH COUNCIL) 13 December 1989, see the whole document	1,3,7,9-14
X		15
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Y	Dialog Information Services, File 157, AIDSLINE, Accession no. 01344489, Parekh BS et al: "Antigenic analysis of HIV-1 major capsid protein, p 24", Int Conf AIDS Jun 4-9 1989, 5 p 651 (abstract no. C.555)	1
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Y	EP, A1, 0290893 (GENETIC SYSTEMS CORPORATION) 17 November 1988, see page 5, line 29 - line 34; claims 1-19	1
	--	
A	WO, A1, 8606414 (GENETIC SYSTEMS CORPORATION) 6 November 1986, see page 9; claims 24,33	1
	--	
A	WO, A2, 8902277 (BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM) 23 March 1989, see page 28 - page 30; claim 8	1
	--	
P,X	EP, A2, 0356007 (MEDICAL RESEARCH COUNCIL) 28 February 1990, see claims 1-16	1,7
	--	
Y	WO, A1, 8602383 (INSTITUT PASTEUR) 24 April 1986, see page 21 - page 24; claim 9	3,7,9-14
	--	
X	EP, A2, 0284587 (VIROVAHL S.A.) 28 September 1988, see page 5; claims 7,9	7
Y		7,9-14
	--	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	AIDS, vol. 3, No. 12, 1989, R. Bridget Ferns et al: "Epitope location of 13 anti-gag HIV-1 monoclonal antibodies using oligopeptides and their cross reactivity with HIV-2 ", see page 829 - page 834 especially Table 1, peptide 2	9
Y		9-14

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FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

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

1. [ ] Claim numbers 16-17, because they relate to subject matter not required to be searched by this Authority, namely:

See PCT Rule 39.1(iv)

Method for treatment of the human or animal body by means of surgery or therapy, as well as diagnostic methods.

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

3. [ ] Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [X] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

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

See next sheet.

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

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

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the the claims. It is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additonal fee.

Remark on Protest

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

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

FURTHER INFORMATION CONTINUED PCT/ISA/210(supplemental sheet(2))

The general problem underlying the invention - finding HIV peptides which interact specifically with HLA class I molecules - is not novel and a solution to it has already been found or does not involve an inventive step having regard to the state of the art as illustrated by EP, A1, 346 022 (MEDICAL RESEARCH COUNCIL), cited in the application. Therefore the original single general inventive concept is not acceptabel anymore, making it necessary to reconsider the technical relationship between the different solutions mentioned. This leads to their regrouping under distincts subjects as listed below, each now falling under its own inventive concept.

1. Peptides according to claims 1,3,4,9 (and use thereof in vaccines according to claims 10-14) as well as a method for asaying cells according to claims 15 which peptides interact with HLA B8 molecules.

2. Peptides according to claim 2 (and corresponding use in vaccines) interacting with HLA A2.

3. Peptides according to claims 5,6 and 8 (and use thereof in vaccines) interacting with HLA A3.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.PCT/GB 91/00013

SA 43503

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 23/03/91
The European Patent office is in no way liable for theseparticulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A1- 0346022	13/12/89	GB-A-	2220939	24/01/90
		JP-A-	2036198	06/02/90
EP-A1- 0290893	17/11/88	AU-D-	1508088	03/11/88
		AU-D-	7720187	19/05/88
		BE-A-	1000811	11/04/89
		CH-A-	675728	31/10/90
		DE-A-	3727703	05/05/88
		FR-A-	2603107	26/02/88
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		JP-A-	1063392	09/03/89
		JP-A-	1085928	30/03/89
		LU-A-	86972	02/03/88
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		EP-A-	0220273	06/05/87
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