

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
5 October 2006 (05.10.2006)

PCT

(10) International Publication Number
WO 2006/103429 A2

(51) International Patent Classification:
C07K 14/725 (2006.01) *A61P 31/18* (2006.01)
CI2N 15/12 (2006.01) *A61P 37/04* (2006.01)
A61K 38/17 (2006.01)

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(21) International Application Number:
PCT/GB2006/001147

(22) International Filing Date: 29 March 2006 (29.03.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0506760.8 1 April 2005 (01.04.2005) GB
0516487.6 10 August 2005 (10.08.2005) GB

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).



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Published:

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2006/103429 A2

(54) Title: HIGH AFFINITY HIV T CELL RECEPTORS

(57) Abstract: The present invention provides TCRs having an affinity (K_D) of less than or equal to $1\mu M$, and/or an off-rate (k_{off}) of $1 \times 10^{-3} S^{-1}$ or slower, for the SLYNTVATL-HLA- A* 0201 complex PROVIDED THAT when the said TCR is presented by cell and comprises SEQ ID NOs: 1 and 2, the cell is not a native T cell. Such TCRs are useful, either alone or associated with a therapeutic agent, for targeting HIV infected cells presenting that complex.

High Affinity HIV T cell receptors

The present invention relates to T-cell receptors (TCRs) having the property of binding to HIV Gag polypeptide-derived SLYNTVATL-HLA-A*0201. The TCRs comprise at least one TCR α chain variable domain and/or at least one TCR β chain variable domain and have a K_D for the said SLYNTVATL-HLA-A*0201 complex of less than or equal to 1 μ M and/or has an off-rate (k_{off}) for the SLYNTVATL-HLA-A*0201 complex of 1×10^{-3} S $^{-1}$ or slower.

Background to the Invention

The Human Immuno-deficiency Virus (HIV) is the causative agent of Acquired Immuno-deficiency Disease Syndrome (AIDS). The virus is an enveloped retrovirus belonging to the lentivirus group. The SLYNTVATL (SEQ ID NO: 16) peptide is derived from the g17 gene product of the Gag gene, one of nine genes which make up the Human Immuno-deficiency Virus-1 (HIV-1). The peptide is loaded by HLA-A*0201 and presented on the surface of HIV infected cells. Therefore, the SLYNTVATL-HLA-A2*0201 complex provides an HIV marker that TCRs can target, for example for the purpose of delivering cytotoxic or immuno-stimulatory agents to the infected cells. However, for that purpose it would be desirable if the TCR had a high affinity and/or a slow off-rate for the peptide-HLA complex.

Brief Description of the Invention

This invention makes available for the first time TCRs having an affinity (K_D) of less than or equal to 1 μ M, and/or an off-rate (k_{off}) of 1×10^{-3} S $^{-1}$ or slower, for the SLYNTVATL-HLA-A*0201 complex PROVIDED THAT when the said TCR is presented by cell and comprises SEQ ID NOs: 1 and 2, the cell is not a native T cell. Such TCRs are useful, either alone or associated with a therapeutic agent, for targeting HIV infected cells presenting that complex.

Detailed Description of the Invention

The present invention provides a T-cell receptor (TCR) having the property of binding to SLYNTVATL-HLA-A*0201 and comprising at least one TCR α chain variable domain and/or at least one TCR β chain variable domain CHARACTERISED IN

5 THAT said TCR has a K_D for the said SLYNTVATL-HLA-A*0201 complex of less than or equal to $1\mu\text{M}$ and/or has an off-rate (k_{off}) for the SLYNTVATL-HLA-A*0201 complex of $1 \times 10^{-3} \text{ S}^{-1}$ or slower, PROVIDED THAT when the said TCR is presented by cell and comprises SEQ ID NOs: 1 and 2, the cell is not a native T cell.

10 The K_D and/or (k_{off}) measurement can be made by any of the known methods. A preferred method is the Surface Plasmon Resonance (Biacore) method of Example 4.

For comparison, the interaction of a disulfide-linked soluble variant of the parental HIV gag TCR (see SEQ ID NO: 9 for TCR α chain and SEQ ID NO: 10 for TCR β chain) and the SLYNTVATL-HLA-A*0201 complex has a K_D of approximately

15 85nM and an off-rate (k_{off}) of $2.21 \times 10^{-2} \text{ S}^{-1}$ as measured by the Biacore-base method of Example 4.

The parental HIV Gag TCR specific for the SLYNTVATL-HLA-A*0201 complex has the following Valpha chain and Vbeta chain gene usage:

20 Alpha chain - TRAV12.2
Beta chain: - TRBV 5.6

The parental HIV Gag TCR can be used as a template from which other TCRs of the invention with high affinity and/or a slow off-rate for the interaction between said

25 TCRs and the SLYNTVATL-HLA-A*0201 complex can be produced. Thus the invention includes TCRs which are mutated relative to the parental HIV Gag TCR α chain variable domain (see Figure 1a and SEQ ID No: 1) and/or β chain variable domain (see Figure 1b and SEQ ID NO: 2) in at least one complementarity determining region (CDR) and/or variable domain framework region thereof. It is also contemplated that other hypervariable regions in the variable domains of the TCRs of

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the invention, such as the hypervariable 4 (HV4) regions, may be mutated within a high affinity mutant TCR.

5 Phage display provides one means by which libraries of TCR variants can be generated. Methods suitable for the phage display and subsequent screening of libraries of TCR variants each containing a non-native disulfide interchain bond are detailed in (Li *et al.*, (2005) *Nature Biotech* **23** (3): 349-354) and WO 2004/04404.

10 Native TCRs exist in heterodimeric $\alpha\beta$ or $\gamma\delta$ forms. However, recombinant TCRs consisting of a single TCR α or TCR β chain have previously been shown to bind to peptide MHC molecules.

In one embodiment the TCR of the invention comprise both an α chain variable domain and an TCR β chain variable domain.

15 As will be obvious to those skilled in the art the mutation(s) in the TCR α chain sequence and/or TCR β chain sequence may be one or more of substitution(s), deletion(s) or insertion(s). These mutations can be carried out using any appropriate method including, but not limited to, those based on polymerase chain reaction (PCR), restriction enzyme-based cloning, or ligation independent cloning (LIC) procedures. 20 These methods are detailed in many of the standard molecular biology texts. For further details regarding polymerase chain reaction (PCR) mutagenesis and restriction enzyme-based cloning see (Sambrook & Russell, (2001) Molecular Cloning – A Laboratory Manual (3rd Ed.) CSHL Press) Further information on LIC procedures can 25 be found in (Rashtchian, (1995) *Curr Opin Biotechnol* **6** (1): 30-6)

It should be noted that any $\alpha\beta$ TCR that comprises similar Valpha and Vbeta gene usage and therefore amino acid sequence to that of the HIV Gag TCR could make a 30 convenient template TCR. It would then be possible to introduce into the DNA encoding one or both of the variable domains of the template $\alpha\beta$ TCR the changes required to produce the mutated high affinity TCRs of the invention. As will be

obvious to those skilled in the art, the necessary mutations could be introduced by a number of methods, for example site-directed mutagenesis.

5 The TCRs of the invention include those in which one or more of the TCR alpha chain variable domain amino acids corresponding to those listed below are mutated relative to the amino acid occurring at these positions in the sequence provided for the parental HIV Gag TCR alpha chain variable domain in Figure 1a and SEQ ID No: 1.

10 Unless stated to the contrary, the TCR amino acid sequences herein are generally provided including an N-terminal methionine (Met or M) residue. As will be known to those skilled in the art this residue may be removed during the production of recombinant proteins. As will also be obvious to those skilled in the art, it may be possible to truncate the sequences provided at the C-terminus and/or N-terminus thereof, by 1, 2, 3, 4, 5 or more residues, without substantially affecting the pMHC binding characteristics of the TCR, all such trivial variants are encompassed by the 15 present invention.

20 As used herein the term “variable region” is understood to encompass all amino acids of a given TCR which are not included within the constant domain as encoded by the TRAC gene for TCR α chains and either the TRBC1 or TRBC2 genes for TCR β chains. (T cell receptor Factsbook, (2001) LeFranc and LeFranc, Academic Press, ISBN 0-12-441352-8)

25 As used herein the term “variable domain” is understood to encompass all amino acids of a given TCR which are encoded by a TRAV gene for TCR α chains and a TRBV gene for TCR β chains. (T cell receptor Factsbook, (2001) LeFranc and LeFranc, Academic Press, ISBN 0-12-441352-8)

30 As is known to those skilled in the art, part of the diversity of the TCR repertoire is due to variations which occur in the amino acid encoded by the codon at the boundary between the variable region, as defined herein, and the constant domain. For example,

the codon that is present at this boundary in the parental HIV Gag TCR sequence results in the presence of the Histidine (H) residue at the C-terminal of the variable region sequences herein. This Histidine replaces the N-terminal Asparagine (N) residue encoded by the TRAC gene shown in Figure 8a.

5

Embodiments of the invention include mutated TCRs which comprise mutation of one or more of alpha chain variable region amino acids corresponding to: 95T, 96N, 97S, 98G, and 100A, for example the amino acids:

10 95S or G

96A

97H

98D

100S

15

The numbering used above is the same as that shown in Figure 1a and SEQ ID No: 1

Embodiments of the invention also include TCRs which comprise mutation of one or more of the TCR beta chain variable region amino acids corresponding to those listed 20 below, are relative to the amino acid occurring at these positions in the sequence provided for the native HIV Gag TCR alpha chain variable region of the native HIV Gag TCR beta chain in Figure 1b and SEQ ID No: 2. The amino acids referred to which may be mutated are: 51Y, 52E, 53E and 54E, for example:

25 51V or A

52R or L

53G

54V

The numbering used above is the same as that shown in Figure 1b and SEQ ID No: 2

30

Further preferred embodiments of the invention are provided by TCRs comprising one of the mutated alpha chain variable region amino acid sequences shown in Fig 6 (SEQ ID Nos: 11 to 13). Phenotypically silent variants of such TCRs also form part of this invention.

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Additional preferred embodiments of the invention are provided by TCRs comprising one of the mutated beta chain variable region amino acid sequences shown in Figure 7. (SEQ ID Nos: 14 and 15). Phenotypically silent variants of such TCRs also form part of this invention.

10

Native TCRs exist in heterodimeric $\alpha\beta$ or $\gamma\delta$ forms. However, recombinant TCRs consisting of $\alpha\alpha$ or $\beta\beta$ homodimers have previously been shown to bind to peptide MHC molecules. Therefore, one embodiment of the invention is provided by TCR $\alpha\alpha$ or TCR $\beta\beta$ homodimers.

15

Further preferred embodiments are provided by TCRs of the invention comprising the alpha chain variable region amino acid sequence and the beta chain variable region amino acid sequence combinations listed below, phenotypically silent variants of such TCRs also form part of this invention:

20

Alpha chain variable region sequence, SEQ ID NO:	Beta chain variable region sequence, SEQ ID NO:
1	2
1	14
1	15
11	2
12	2
13	2
12	15
13	15

12	14
13	14

In another preferred embodiment TCRs of the invention comprising the variable region combinations detailed above further comprise the alpha chain constant domain amino acid sequence shown in Figure 8a (SEQ ID NO: 19) and one of the beta chain amino acid constant domain sequences shown in Figures 8b and 8c (SEQ ID NOs: 20 and 21) or phenotypically silent variants thereof.

As used herein the term “phenotypically silent variants” is understood to refer to those TCRs which have a K_D for the said SLYNTVATL-HLA-A*0201 complex of less than 10 or equal to 1 μM and/or have an off-rate (k_{off}) of $1 \times 10^{-3} \text{ S}^{-1}$ or slower. For example, as is known to those skilled in the art, it may be possible to produce TCRs that incorporate minor changes in the constant domain and/or variable regions thereof compared to those detailed above without altering the affinity and/or off-rate for the interaction with the SLYNTVATL-HLA-A*0201 complex. Such trivial variants are 15 included in the scope of this invention. Those TCRs in which one or more conservative substitutions have been made also form part of this invention.

In one broad aspect, the TCRs of the invention are in the form of either single chain TCRs (scTCRs) or dimeric TCRs (dTcRs) as described in WO 04/033685 and WO 20 03/020763.

A suitable scTCR form comprises a first segment constituted by an amino acid sequence corresponding to a TCR α chain variable region, a second segment 25 constituted by an amino acid sequence corresponding to a TCR β chain variable region sequence fused to the N terminus of an amino acid sequence corresponding to a TCR β chain constant domain extracellular sequence, and a linker sequence linking the C terminus of the first segment to the N terminus of the second segment.

Alternatively the first segment may be constituted by an amino acid sequence corresponding to a TCR β chain variable region, the second segment may be constituted by an amino acid sequence corresponding to a TCR α chain variable region sequence fused to the N terminus of an amino acid sequence corresponding to a TCR α chain constant domain extracellular sequence

The above scTCRs may further comprise a disulfide bond between the first and second chains, said disulfide bond being one which has no equivalent in native $\alpha\beta$ T cell receptors, and wherein the length of the linker sequence and the position of the disulfide bond being such that the variable domain sequences of the first and second segments are mutually orientated substantially as in native $\alpha\beta$ T cell receptors.

More specifically the first segment may be constituted by an amino acid sequence corresponding to a TCR α chain variable region sequence fused to the N terminus of an amino acid sequence corresponding to a TCR α chain constant domain extracellular sequence, the second segment may be constituted by an amino acid sequence corresponding to a TCR β chain variable region fused to the N terminus of an amino acid sequence corresponding to TCR β chain constant domain extracellular sequence, and a disulfide bond may be provided between the first and second chains, said disulfide bond being one which has no equivalent in native $\alpha\beta$ T cell receptors.

In the above scTCR forms, the linker sequence may link the C terminus of the first segment to the N terminus of the second segment, and may have the formula -PGGG-(SGGGG)_n-P- wherein n is 5 or 6 and P is proline, G is glycine and S is serine.

25

-PGGG-SGGGGSGGGGSGGGSGGGSGGGG-P (SEQ ID NO: 17)

-PGGG-SGGGGSGGGGSGGGSGGGSGGGG-P (SEQ ID NO: 18)

A suitable dTCR form of the TCRs of the present invention comprises

a first polypeptide wherein a sequence corresponding to a TCR α chain variable region sequence is fused to the N terminus of a sequence corresponding to a TCR α chain constant domain extracellular sequence, and a second polypeptide wherein a sequence corresponding to a TCR β chain variable region sequence fused to the N terminus a sequence corresponding to a TCR β chain constant domain extracellular sequence, the first and second polypeptides being linked by a disulfide bond which has no equivalent in native $\alpha\beta$ T cell receptors.

The first polypeptide may comprise a TCR α chain variable region sequence is fused to the N terminus of a sequence corresponding to a TCR α chain constant domain extracellular sequence, and a second polypeptide wherein a sequence corresponding to a TCR β chain variable region sequence is fused to the N terminus a sequence corresponding to a TCR β chain constant domain extracellular sequence, the first and second polypeptides being linked by a disulfide bond between cysteine residues substituted for Thr 48 of exon 1 of TRAC*01 and Ser 57 of exon 1 of TRBC1*01 or TRBC2*01 or the non-human equivalent thereof. ("TRAC" etc. nomenclature herein as per T cell receptor Factsbook, (2001) LeFranc and LeFranc, Academic Press, ISBN 0-12-441352-8)

The dTCR or scTCR form of the TCRs of the invention may have amino acid sequences corresponding to human $\alpha\beta$ TCR extracellular constant domain and variable region sequences, and a disulfide bond may link amino acid residues of the said constant domain sequences, which disulfide bond has no equivalent in native TCRs. The disulfide bond is between cysteine residues corresponding to amino acid residues whose β carbon atoms are less than 0.6 nm apart in native TCRs, for example between cysteine residues substituted for Thr 48 of exon 1 of TRAC*01 and Ser 57 of exon 1 of TRBC1*01 or TRBC2*01 or the non-human equivalent thereof. Other sites where cysteines can be introduced to form the disulfide bond are the following residues in exon 1 of TRAC*01 for the TCR α chain and TRBC1*01 or TRBC2*01 for the TCR β chain:

TCR α chain	TCR β chain	Native β carbon separation (nm)
Thr 45	Ser 77	0.533
Tyr 10	Ser 17	0.359
Thr 45	Asp 59	0.560
Ser 15	Glu 15	0.59

5 In addition to the non-native disulfide bond referred to above, the dTCR or scTCR form of the TCRs of the invention may include a disulfide bond between residues corresponding to those linked by a disulfide bond in native TCRs.

The dTCR or scTCR form of the TCRs of the invention preferably does not contain a sequence corresponding to transmembrane or cytoplasmic sequences of native TCRs.

15 TCRs of the invention bind strongly to the SLYNTVATL-HLA-A2*0201. These TCRs also bind to an altered, but still useful, extent to naturally occurring variants of the HIV Gag-derived SLYNTVATL when loaded by HLA-A*0201. Variants of the SLYNTVATL which have been isolated from AIDS patients include the following (Sewell *et al.*, (1997) *Eur J Immunol.* **27**: 2323-2329):

20 SLFNTVATL
SLFNTVAVL
SLSNTVATL
SSFNTVATL
SLLNTVATL
SLYNTIATL
SLYNTIAVL
25 SLFNTIATL
SLFNTIAVL
SLFNFVATL

The mutated amino acids are underlined.

PEGylated TCR Monomers

5 In one particular embodiment a TCR of the invention is associated with at least one polyalkylene glycol chain(s). This association may be cause in a number of ways known to those skilled in the art. In a preferred embodiment the polyalkylene chain(s) is/are covalently linked to the TCR. In a further embodiment the polyethylene glycol chains of the present aspect of the invention comprise at least two polyethylene 10 repeating units.

Multivalent TCR Complexes

15 One aspect of the invention provides a multivalent TCR complex comprising at least two TCRs of the invention. In one embodiment of this aspect, at least two TCR molecules are linked via linker moieties to form multivalent complexes. Preferably the complexes are water soluble, so the linker moiety should be selected accordingly. Furthermore, it is preferable that the linker moiety should be capable of attachment to 20 defined positions on the TCR molecules, so that the structural diversity of the complexes formed is minimised. One embodiment of the present aspect is provided by a TCR complex of the invention wherein the polymer chain or peptidic linker sequence extends between amino acid residues of each TCR which are not located in a variable region sequence of the TCR.

25 Since the complexes of the invention may be for use in medicine, the linker moieties should be chosen with due regard to their pharmaceutical suitability, for example their immunogenicity.

30 Examples of linker moieties which fulfil the above desirable criteria are known in the art, for example the art of linking antibody fragments.

There are two classes of linker that are preferred for use in the production of multivalent TCR molecules of the present invention. A TCR complex of the invention in which the TCRs are linked by a polyalkylene glycol chain provides one 5 embodiment of the present aspect.

The first are hydrophilic polymers such as polyalkylene glycols. The most commonly used of this class are based on polyethylene glycol or PEG, the structure of which is shown below.

10



Wherein n is greater than two. However, others are based on other suitable, optionally substituted, polyalkylene glycols include polypropylene glycol, and copolymers of 15 ethylene glycol and propylene glycol.

Such polymers may be used to treat or conjugate therapeutic agents, particularly 20 polypeptide or protein therapeutics, to achieve beneficial changes to the PK profile of the therapeutic, for example reduced renal clearance, improved plasma half-life, reduced immunogenicity, and improved solubility. Such improvements in the PK profile of the PEG-therapeutic conjugate are believed to result from the PEG molecule or molecules forming a 'shell' around the therapeutic which sterically hinders the reaction with the immune system and reduces proteolytic degradation. (Casey *et al*, 25 (2000) *Tumor Targetting* 4 235-244) The size of the hydrophilic polymer used may in particular be selected on the basis of the intended therapeutic use of the TCR complex. There are numerous review papers and books that detail the use of PEG and similar 30 molecules in pharmaceutical formulations. For example, see Harris (1992) *Polyethylene Glycol Chemistry - Biotechnical and Biomedical Applications*, Plenum, New York, NY. or Harris & Zalipsky (1997) *Chemistry and Biological Applications* of *Polyethylene Glycol ACS Books*, Washington, D.C.

The polymer used can have a linear or branched conformation. Branched PEG molecules, or derivatives thereof, can be induced by the addition of branching moieties including glycerol and glycerol oligomers, pentaerythritol, sorbitol and lysine.

5 Usually, the polymer will have a chemically reactive group or groups in its structure, for example at one or both termini, and/or on branches from the backbone, to enable the polymer to link to target sites in the TCR. This chemically reactive group or groups may be attached directly to the hydrophilic polymer, or there may be a spacer group/moiety between the hydrophilic polymer and the reactive chemistry as shown
10 below:

Reactive chemistry-Hydrophilic polymer-Reactive chemistry

Reactive chemistry-Spacer-Hydrophilic polymer-Spacer-Reactive chemistry

15 The spacer used in the formation of constructs of the type outlined above may be any organic moiety that is a non-reactive, chemically stable, chain, Such spacers include, by are not limited to the following:

-(CH₂)_n- wherein n = 2 to 5

20 -(CH₂)₃NHCO(CH₂)₂

A TCR complex of the invention in which a divalent alkylene spacer radical is located between the polyalkylene glycol chain and its point of attachment to a TCR of the complex provides a further embodiment of the present aspect.

25 A TCR complex of the invention in which the polyalkylene glycol chain comprises at least two polyethylene glycol repeating units provides a further embodiment of the present aspect.

30 There are a number of commercial suppliers of hydrophilic polymers linked, directly or via a spacer, to reactive chemistries that may be of use in the present invention.

These suppliers include Nektar Therapeutics (CA, USA), NOF Corporation (Japan), Sunbio (South Korea) and Enzon Pharmaceuticals (NJ, USA).

Commercially available hydrophilic polymers linked, directly or via a spacer, to reactive chemistries that may be of use in the present invention include, but are not limited to, the following:

PEG linker Description	Source of PEG	Catalogue Number
TCR Monomer attachment		
5K linear (Maleimide)	Nektar	2D2MOHO1
20K linear (Maleimide)	Nektar	2D2MOPO1
20K linear (Maleimide)	NOF Corporation	SUNBRIGHT ME-200MA
20K branched (Maleimide)	NOF Corporation	SUNBRIGHT GL2- 200MA
30K linear (Maleimide)	NOF Corporation	SUNBRIGHT ME- 300MA
40K branched PEG (Maleimide)	Nektar	2D3XOTO1
5K-NP linear (for Lys attachment)	NOF Corporation	SUNBRIGHT MENP-50H
10K-NP linear (for Lys attachment)	NOF Corporation	SUNBRIGHT MENP-10T
20K-NP linear (for Lys attachment)	NOF Corporation	SUNBRIGHT MENP-20T
TCR dimer linkers		
3.4K linear (Maleimide)	Nektar	2D2DOFO2
5K forked (Maleimide)	Nektar	2D2DOHOF
10K linear (with orthopyridyl ds- linkers in place of Maleimide)	Sunbio	
20K forked (Maleimide)	Nektar	2D2DOPOF
20K linear (Maleimide)	NOF Corporation	
40K forked (Maleimide)	Nektar	2D3XOTOF
Higher order TCR multimers		
15K, 3 arms, Mal_3 (for trimer)	Nektar	OJOONO3
20K, 4 arms, Mal_4 (for tetramer)	Nektar	OJOOP04
40 K, 8 arms, Mal_8 (for octamer)	Nektar	OJOOTO8

A wide variety of coupling chemistries can be used to couple polymer molecules to protein and peptide therapeutics. The choice of the most appropriate coupling 5 chemistry is largely dependant on the desired coupling site. For example, the following coupling chemistries have been used attached to one or more of the termini of PEG molecules (Source: Nektar Molecular Engineering Catalogue 2003):

- N-maleimide
- Vinyl sulfone
- 10 Benzotriazole carbonate
- Succinimidyl propionate
- Succinimidyl butanoate
- Thio-ester
- Acetaldehydes
- 15 Acrylates
- Biotin
- Primary amines

As stated above non-PEG based polymers also provide suitable linkers for 20 multimerising the TCRs of the present invention. For example, moieties containing maleimide termini linked by aliphatic chains such as BMH and BMOE (Pierce, products Nos. 22330 and 22323) can be used.

Peptidic linkers are the other class of TCR linkers. These linkers are comprised of 25 chains of amino acids, and function to produce simple linkers or multimerisation domains onto which TCR molecules can be attached. The biotin / streptavidin system has previously been used to produce TCR tetramers (see WO/99/60119) for in-vitro binding studies. However, strepavidin is a microbially-derived polypeptide and as such not ideally suited to use in a therapeutic.

A TCR complex of the invention in which the TCRs are linked by a peptidic linker derived from a human multimerisation domain provides a further embodiment of the present aspect.

5 There are a number of human proteins that contain a multimerisation domain that could be used in the production of multivalent TCR complexes. For example the tetramerisation domain of p53 which has been utilised to produce tetramers of scFv antibody fragments which exhibited increased serum persistence and significantly reduced off-rate compared to the monomeric scFV fragment. (Willuda *et al.* (2001) J. Biol. Chem. 276 (17) 14385-14392) Haemoglobin also has a tetramerisation domain that could potentially be used for this kind of application.

10 A multivalent TCR complex of the invention comprising at least two TCRs provides a final embodiment of this aspect, wherein at least one of said TCRs is associated with a therapeutic agent.

15 In one aspect a TCR (or multivalent complex thereof) of the present invention may alternatively or additionally comprise a reactive cysteine at the C-terminal or N-terminal of the alpha or beta chains thereof.

20 *Diagnostic and therapeutic Use*

25 In one aspect the TCR of the invention may be associated with a therapeutic agent or detectable moiety. For example, said therapeutic agent or detectable moiety may be covalently linked to the TCR.

In one embodiment of the invention said therapeutic agent or detectable moiety is covalently linked to the C-terminus of one or both TCR chains.

30 In one aspect the scTCR or one or both of the dTCR chains of TCRs of the present invention may be labelled with an detectable moiety, for example a label that is

suitable for diagnostic purposes. Such labelled TCRs are useful in a method for detecting a SLYNTVATL-HLA-A*0201 complex which method comprises contacting the TCR ligand with a TCR (or a multimeric high affinity TCR complex) which is specific for the TCR ligand; and detecting binding to the TCR ligand. In tetrameric TCR complexes formed for example, using biotinylated heterodimers, fluorescent streptavidin can be used to provide a detectable label. Such a fluorescently-labelled TCR tetramer is suitable for use in FACS analysis, for example to detect antigen presenting cells carrying the SLYNTVATL-HLA-A*0201 complex for which these high affinity TCRs are specific.

10

Another manner in which the soluble TCRs of the present invention may be detected is by the use of TCR-specific antibodies, in particular monoclonal antibodies. There are many commercially available anti-TCR antibodies, such as α F1 and β F1, which recognise the constant domains of the α and β chains, respectively.

15

In a further aspect a TCR (or multivalent complex thereof) of the present invention may alternatively or additionally be associated with (e.g. covalently or otherwise linked to) a therapeutic agent which may be, for example, a toxic moiety for use in cell killing, or an immune effector molecule such as an interleukin or a cytokine. A multivalent TCR complex of the invention may have enhanced binding capability for a TCR ligand compared to a non-multimeric wild-type or T cell receptor heterodimer of the invention. Thus, the multivalent TCR complexes according to the invention are particularly useful for tracking or targeting cells presenting SLYNTVATL-HLA-A*0201 complexes *in vitro* or *in vivo*, and are also useful as intermediates for the production of further multivalent TCR complexes having such uses. These TCRs or multivalent TCR complexes may therefore be provided in a pharmaceutically acceptable formulation for use *in vivo*.

The invention also provides a method for delivering a therapeutic agent to a target cell, which method comprises contacting potential target cells with a TCR or multivalent TCR complex in accordance with the invention under conditions to allow attachment

of the TCR or multivalent TCR complex to the target cell, said TCR or multivalent TCR complex being specific for the SLYNTVATL-HLA-A*0201 complex and having the therapeutic agent associated therewith.

5 In particular, the soluble TCR or multivalent TCR complex of the present invention can be used to deliver therapeutic agents to the location of cells presenting a particular antigen. This would be useful in many situations and, in particular, against HIV infected cells. A therapeutic agent could be delivered such that it would exercise its effect locally but not only on the cell it binds to. Thus, one particular strategy
10 envisages cytotoxic or immuno-stimulatory molecules linked to TCRs or multivalent TCR complexes according to the invention specific for the SLYNTVATL-HLA-A*0201 complex.

15 Many therapeutic agents could be employed for this use, for instance radioactive compounds, enzymes (perforin for example) or chemotherapeutic agents (cis-platin for example). To ensure that toxic effects are exercised in the desired location the toxin could be inside a liposome linked to streptavidin so that the compound is released slowly. This will prevent damaging effects during the transport in the body and ensure
20 that the toxin has maximum effect after binding of the TCR to the relevant antigen presenting cells.

Other suitable therapeutic agents include:

- small molecule cytotoxic agents, i.e. compounds with the ability to kill mammalian cells having a molecular weight of less than 700 daltons. Such compounds could also contain toxic metals capable of having a cytotoxic effect. Furthermore, it is to be understood that these small molecule cytotoxic agents also include pro-drugs, i.e. compounds that decay or are converted under physiological conditions to release cytotoxic agents. Examples of such agents include cis-platin, maytansine derivatives, rachelmycin, calicheamicin,
25 docetaxel, etoposide, gemcitabine, ifosfamide, irinotecan, melphalan,
30

mitoxantrone, sorfimer sodiumphotofrin II, temozolmide, topotecan, trimetrate glucuronate, auristatin E vincristine and doxorubicin;

- peptide cytotoxins, i.e. proteins or fragments thereof with the ability to kill mammalian cells. Including but not limited to, ricin, diphtheria toxin, pseudomonas bacterial exotoxin A, DNAase and RNAase;
- radio-nuclides, i.e. unstable isotopes of elements which decay with the concurrent emission of one or more of α or β particles, or γ rays. including but not limited to, iodine 131, rhenium 186, indium 111, yttrium 90, bismuth 210 and 213, actinium 225 and astatine 213; chelating agents may be used to facilitate the association of these radio-nuclides to the high affinity TCRs, or multimers thereof;
- prodrugs, including but not limited to, antibody directed enzyme pro-drugs;
- immuno-stimulants, i.e. moieties which stimulate immune response. Including but not limited to, cytokines such as IL-2 and IFN, Superantigens and mutants thereof, TCR-HLA fusions and chemokines such as IL-8, platelet factor 4, melanoma growth stimulatory protein, etc, antibodies or fragments thereof, complement activators, xenogeneic protein domains, allogeneic protein domains, viral/bacterial protein domains, viral/bacterial peptides and anti-T cell determinant antibodies (e.g. anti-CD3 or anti-CD28) or antibody analogues such as NanobodiesTM and AffybodiesTM.

Soluble TCRs or multivalent TCR complexes of the invention may be linked to an enzyme capable of converting a prodrug to a drug. This allows the prodrug to be converted to the drug only at the site where it is required (i.e. targeted by the sTCR).

25

It is expected that the high affinity SLYNTVATL (SEQ ID NO: 16)-HLA-A*0201 specific TCRs disclosed herein may be used in methods for the diagnosis and treatment of AIDS.

For treatment, therapeutic agent localisation in the vicinity of HIV infected (CD4⁺) 30 cells would enhance the effect of toxins or immunostimulants. For vaccine delivery,

the vaccine antigen could be localised in the vicinity of antigen presenting cells, thus enhancing the efficacy of the antigen. The method can also be applied for imaging purposes.

5 One embodiment is provided by a membrane preparation comprising a TCR of the invention. Said membrane preparation may be prepared from cells or may comprise a synthetic membrane.

10 Another embodiment is provided by a cell harbouring an expression vector comprising nucleic acid encoding a TCR of the invention. For example, said cell may be a T cell.

Further embodiments of the invention are provided by a pharmaceutical composition comprising:

15 a TCR or a multivalent TCR complex of the invention (optionally associated with a therapeutic agent), or a membrane preparation comprising a TCR of the invention, or a plurality of cells harbouring an expression vector comprising nucleic acid encoding a TCR of the invention, together with a pharmaceutically acceptable carrier;

20 The invention also provides a method of treatment of AIDS comprising administering to a subject suffering such AIDS an effective amount of a TCR or a multivalent TCR complex of the invention, or a membrane preparation comprising a TCR of the invention, or a plurality of cells harbouring an expression vector comprising nucleic acid encoding a TCR of the invention. In a related embodiment the invention provides for the use of a TCR or a multivalent TCR complex of the invention, or a membrane preparation comprising a TCR of the invention, or a plurality of cells harbouring an expression vector comprising nucleic acid encoding a TCR of the invention, in the preparation of a composition for the treatment of AIDS. Further specific embodiments of these uses and methods of the invention are provided wherein the TCR, or
25 multivalent TCR complex of the invention, or a membrane preparation comprising a TCR of the invention is administered in a form which is associated with a therapeutic
30

agent. In other preferred embodiments the cells harbouring an expression vector comprising nucleic acid encoding a TCR of the invention are CD8⁺ T cells.

Therapeutic or imaging TCRs in accordance with the invention will usually be supplied
5 as part of a sterile, pharmaceutical composition which will normally include a pharmaceutically acceptable carrier. This pharmaceutical composition may be in any suitable form, (depending upon the desired method of administering it to a patient). It may be provided in unit dosage form, will generally be provided in a sealed container and may be provided as part of a kit. Such a kit would normally (although not necessarily)
10 include instructions for use. It may include a plurality of said unit dosage forms.

Without wishing to be limited by theory, it is expected that the TCRs of the invention will provide effective targeting agents capable of delivering therapeutic agents such as immunostimulants and/or cytotoxic agents to HIV infected (CD4⁺) cells. In particular, it
15 is expected that the administration of the TCRs of the present invention when associated with immunostimulants and/or cytotoxic agents in combination with conventional anti-retrovirus drug therapies and/or IL-2 treatment will be able to target HIV infected cells.

The following is a list of anti-retroviral drugs currently approved for use in the US:
20

Agenerase (amprenavir) - protease inhibitor
Combivir -combination of Retrovir (300mg) and Epivir (150mg)
Crixivan (indinavir) - protease inhibitor
Epivir (3tc / lamivudine) - nucleoside analog reverse transcriptase inhibitor
25 Epzicom (a combination of 2 nucleoside reverse transcriptase inhibitors (NRTIs in the same pill; 600mg of Ziagen (abacavir) and 300mg of Epivir (3TC).
Emtriva [emtricitabine (FTC)]
Fortovase (saquinavir) - protease inhibitor
Fuzeon (enfuvirtide) - Fusion inhibitor
30 Hivid (ddc / zalcitabine) - nucleoside analog reverse transcriptase inhibitor
Invirase (saquinavir) - protease inhibitor

Kaletra (lopinavir) - protease inhibitor

Lexiva (Fosamprenavir) - Protease Inhibitor approved 10/20/03

Norvir (ritonavir) - protease inhibitor

Rescriptor (delavirdine) - non nucleoside analog reverse transcriptase inhibitor

5 Retrovir, AZT (zidovudine) - nucleoside analog reverse transcriptase inhibitor

Reyataz (atazanavir; BMS-232632) - protease inhibitor

Sustiva (efavirenz) - non nucleoside analog reverse transcriptase inhibitor

Trizivir (3 non nucleosides in one tablet; abacavir + zidovudine + lamivudine

Truvada (Emtricitabine + Tenofovir DF)

10 Videx (ddl / didanosine) nucleoside analog reverse transcriptase inhibitor

Videx EC; (ddl / didanosine) nucleoside analog reverse transcriptase inhibitor;

Viracept (nelfinavir) - protease inhibitor

Viramune (nevirapine) - non nucleoside analog Reverse transcriptase inhibitor

Viread (tenofovir disoproxil fumarate) Nucleotide Reverse transcriptase inhibitor

15 (Adenosine Class)

Zerit (d4t / stavudine) - nucleoside analog reverse transcriptase inhibitor

Ziagen (abacavir) - nucleoside analog reverse transcriptase inhibitor

The pharmaceutical composition may be adapted for administration by any appropriate
20 route, for example parenteral, transdermal or via inhalation, preferably a parenteral
(including subcutaneous, intramuscular, or, most preferably intravenous) route. Such
compositions may be prepared by any method known in the art of pharmacy, for example
by mixing the active ingredient with the carrier(s) or excipient(s) under sterile conditions.

25 Dosages of the substances of the present invention can vary between wide limits,
depending upon the disease or disorder to be treated, the age and condition of the
individual to be treated, etc. and a physician will ultimately determine appropriate
dosages to be used.

Additional Aspects

A scTCR or dTCR (which preferably is constituted by constant and variable sequences corresponding to human sequences) of the present invention may be provided in 5 substantially pure form, or as a purified or isolated preparation. For example, it may be provided in a form which is substantially free of other proteins.

The sequence(s) of the nucleic acid or nucleic acids encoding the TCRs of the 10 invention may be altered so as to optimise the level of expression obtained in the host cell. The host cell may be any appropriate prokaryotic or eukaryotic cell. For example, the host cell may be an *E.coli* cell or a human T cell. The alterations made to these genetic sequences are silent, that is they do not alter the amino acid sequence encoded. There are a number of companies which offer such expression optimisation services, including, GeneArt, Germany.

15

The invention also provides a method of producing a high affinity TCR having the property of binding to SLYNTVATL-HLA-A*0201. CHARACTERISED IN THAT the TCR (i) comprises at least one TCR α chain variable domain and/or at least one TCR β chain variable domain and (ii) has a K_D for the said SLYNTVATL-HLA- 20 A*0201 complex of less than or equal to 1 μ M and/or an off-rate (k_{off}) for the SLYNTVATL-HLA-A*0201 complex of 1×10^{-3} S $^{-1}$ or slower, wherein the method comprises:

(a) the production of a TCR comprising the α and β chain variable domains 25 of the parental HIV Gag TCR wherein one or both of the α and β chain variable domains comprise a mutation(s) in one or more of the amino acids identified in claims 7 and 8;

(b) contacting said mutated TCR with SLYNTVATL-HLA-A*0201 under 30 conditions suitable to allow the binding of the TCR to SLYNTVATL-HLA- A*0201; and measuring the K_D and/or k_{off} of the interaction.

Preferred features of each aspect of the invention are as for each of the other aspects *mutatis mutandis*. The prior art documents mentioned herein are incorporated to the fullest extent permitted by law.

5

Examples

The invention is further described in the following examples, which do not limit the scope of the invention in any way.

10

Reference is made in the following to the accompanying drawings in which:

Figure 1a and 1b details the alpha chain variable domain amino acid and beta chain variable domain amino acid sequences of the parental HIV Gag TCR respectively.

15

Figures 2a and 2b show respectively the DNA sequence of soluble versions of the parental HIV Gag TCR α and β chains.

20

Figures 3a and 3b show respectively the HIV Gag TCR α and β chain extracellular amino acid sequences produced from the DNA sequences of Figures 2a and 2b.

Figures 4a and 4b show respectively the DNA sequence of soluble versions of the HIV Gag TCR α and β chains mutated to encode additional cysteine residues to form a non-native disulfide bond. The mutated codon is indicated by shading and the introduced restriction enzyme recognition sites are underlined..

Figures 5a and 5b show respectively the HIV Gag TCR α and β chain extracellular amino acid sequences produced from the DNA sequences of Figures 4a and 4b. The introduced cysteine in each chain is indicated by shading.

30

Figure 6 details the alpha chain variable domain amino acid sequences of the high affinity HIV Gag TCR variants.

5 Figure 7 details the beta chain variable domain amino acid sequences of the high affinity HIV Gag TCR variants.

Figure 8a details the amino acid sequence of a soluble portion of TRAC.

Figure 8b details the amino acid sequence of a soluble portion of TRBC1.

10 .

Figure 8c details the amino acid sequence of a soluble portion of TRBC2.

Figure 9 details the DNA sequence of the pEX954 plasmid.

15 Figure 10 details the DNA sequence of the pEX821 plasmid.

Figure 11 details the beta chain amino acid sequences of the parental soluble HIV Gag TCR variant fused via a peptide linker to wild-type human IL-2. The amino acids of the linker and IL-2 are indicated in italics.

20

Figure 12 provides the Biacore response curves generated for the interaction of the soluble disulfide-linked parental HIV Gag TCR and the SLYNTVATL-HLA-A*0201 complex.

25 Figure 13 provides a plasmid map of the pEX954 plasmid.

Figure 14 provides a plasmid map of the pEX821 plasmid.

30 Figure 15a provides the full-length DNA sequence of the parental HIV Gag TCR α chain optimised for expression in human T cells.

Figure 15b provides the full-length DNA sequence of the parental HIV Gag TCR β chain optimised for expression in human T cells.

5 Figure 16a provides the full-length amino acid sequence of the parental HIV Gag TCR α chain.

Figure 16b provides the full-length amino acid sequence of the parental HIV Gag TCR β chain optimised for expression in human T cells.

10 Figures 17a provides FACS analysis data for untransduced control CD8 $^{+}$ T cells.

Figure 17b provides FACS analysis data demonstrating expression of the parental HIV Gag TCR on the surface of transduced CD8 $^{+}$ T cells.

15 Figures 18a and 18b provide the amino acids sequences of the alpha and beta chains of a soluble disulfide-linked high affinity c11c6 HIV Gag TCR respectively.

20 Figure 19 demonstrates the ability of soluble disulfide-linked high affinity c11c6 HIV Gag TCRs to inhibit the activation of the SLYNTVATL-HLA-A*0201 reactive OX84 polyclonal T cell line in the presence of To cells infected with HIV as measured by IFN- γ and TNF- α production.

25 Figure 20 demonstrates the ability of soluble disulfide-linked high affinity c11c6 HIV Gag TCRs to inhibit the activation of the SLYNTVATL-HLA-A*0201 reactive OX84 polyclonal T cell line in the presence of SLYNTVATL peptide-pulsed uninfected To cells as measured by IFN- γ and TNF- α production.

Figure 21 demonstrates the ability of soluble disulfide-linked high affinity c11c6 HIV Gag TCRs to stain SLYNTVATL peptide-pulsed T2 cells.

Example 1 – Production of soluble disulfide-linked TCRs comprising the parental HIV Gag TCR variable regions

5 Figures 4a and 4b provide the DNA sequences of soluble disulfide-linked alpha beta chains from a parental TCR which is specific for the SLYNTVATL-HLA-A*0201 complex. These DNA sequences can be synthesis de-novo by a number of contract research companies, for example GeneArt (Germany). Restriction enzyme recognition sites are also added to these DNA sequences in order to facilitate ligation of these
10 DNA sequences into the pGMT7-based expression plasmids, which contain the T7 promoter for high level expression in E.coli strain BL21-DE3(pLysS) (Pan *et al.*, *Biotechniques* (2000) **29** (6): 1234-8)

15 The TCR alpha chain sequences contain introduced *ClaI* and *SalII* restriction enzyme recognition sites and this sequence was ligated into pEX954 (see Figures 9 and 13) cut with *ClaI* and *XhoI*.

20 The TCR beta chain sequences contain introduced *AseI* and *AgeI* restriction enzyme recognition sites and were ligated into pEX821 (see Figures 10 and 14) cut with *NdeI/AgeI*.

Restriction enzyme recognition sites as introduced into DNA encoding the TCR chains

25 *ClaI* - ATCGAT
SalII - GTCGAC
AseI - ATTAAT
AgeI - ACCGGT

Ligation

30 The cut TCR alpha and beta chain DNA and cut vector were ligated using a rapid DNA ligation kit (Roche) following the manufacturers instructions.

Ligated plasmids were transformed into competent E.coli strain XL1-blue cells and plated out on LB/agar plates containing 100mg/ml ampicillin. Following incubation overnight at 37°C, single colonies were picked and grown in 10 ml LB containing 5 100mg/ml ampicillin overnight at 37°C with shaking. Cloned plasmids were purified using a Miniprep kit (Qiagen) and the insert was sequenced using an automated DNA sequencer (Lark Technologies).

Figures 5a and 5b show respectively the soluble disulfide linked parental HIV gag 10 TCR α and β chain extracellular amino acid sequences produced from the DNA sequences of Figures 4a and 4b

Example 2- Production of high affinity variants of the soluble disulfide linked HIV Gag TCR

15 The soluble disulfide-linked native HIV Gag TCR produced as described in Example 1 can be used a template from which to produce the TCRs of the invention which have an increased affinity for the SLYNTVATL (SEQ ID NO: 16) -HLA-A*0201 complex.

20 Phage display is one means by which libraries of HIV Gag TCR variants can be generated in order to identify high affinity mutants. For example, the TCR phage display and screening methods described in (Li *et al.*, (2005) *Nature Biotech* **23** (3): 349-354) can be adapted and applied to HIV Gag TCRs.

25 The amino sequences of the mutated TCR alpha and beta chain variable domains which, when combined with an appropriate TCR chain, demonstrate high affinity for the SLYNTVATL-HLA-A*0201 complex, are listed in Figures 6 and 7 respectively. (SEQ ID Nos: 11-13 and 14-15 respectively) As is known to those skilled in the art the necessary codon changes required to produce these mutated chains can be introduced 30 into the DNA encoding these chains by site-directed mutagenesis. (QuickChangeTM Site-Directed Mutagenesis Kit from Stratagene)

Briefly, this is achieved by using primers that incorporate the desired codon change(s) and the plasmids containing the relevant TCR chain DNA as a template for the mutagenesis:

5

Mutagenesis was carried out using the following conditions : 50ng plasmid template, 1 μ l of 10mM dNTP, 5 μ l of 10x Pfu DNA polymerase buffer as supplied by the manufacturer, 25 pmol of fwd primer, 25 pmol of rev primer, 1 μ l pfu DNA polymerase in total volume 50 μ l. After an initial denaturation step of 2 mins at 95C, 10 the reaction was subjected to 25 cycles of denaturation (95C, 10 secs), annealing (55C 10 secs), and elongation (72C, 8 mins). The resulting product was digested with DpnI restriction enzyme to remove the template plasmid and transformed into E. coli strain XL1-blue. Mutagenesis was verified by sequencing.

15

Example 3 – Expression, refolding and purification of soluble TCR

The expression plasmids containing the mutated α -chain and β -chain respectively as prepared in Examples 1 or 2 were transformed separately into *E.coli* strain BL21pLysS, and single ampicillin-resistant colonies were grown at 37°C in TYP (ampicillin 100 μ g/ml) medium to OD₆₀₀ of 0.4 before inducing protein expression with 0.5mM IPTG. Cells were harvested three hours post-induction by centrifugation for 30 minutes at 4000rpm in a Beckman J-6B. Cell pellets were re-suspended in a buffer containing 50mM Tris-HCl, 25% (w/v) sucrose, 1mM NaEDTA, 0.1% (w/v) NaAzide, 10mM DTT, pH 8.0. After an overnight freeze-thaw step, re-suspended cells were sonicated in 1 minute bursts for a total of around 10 minutes in a Milsonix XL2020 sonicator using a standard 12mm diameter probe. Inclusion body pellets were recovered by centrifugation for 30 minutes at 13000rpm in a Beckman J2-21 centrifuge. Three detergent washes were then carried out to remove cell debris and membrane components. Each time the inclusion body pellet was homogenised in a Triton buffer (50mM Tris-HCl, 0.5% Triton-X100, 200mM NaCl, 10mM NaEDTA,

0.1% (w/v) NaAzide, 2mM DTT, pH 8.0) before being pelleted by centrifugation for 15 minutes at 13000rpm in a Beckman J2-21. Detergent and salt was then removed by a similar wash in the following buffer: 50mM Tris-HCl, 1mM NaEDTA, 0.1% (w/v) NaAzide, 2mM DTT, pH 8.0. Finally, the inclusion bodies were divided into 30 mg aliquots and frozen at -70°C. Inclusion body protein yield was quantitated by solubilising with 6M guanidine-HCl and measurement with a Bradford dye-binding assay (PerBio).

Approximately 30mg of TCR β chain and 60mg of TCR α chain solubilised inclusion bodies were thawed from frozen stocks, samples were then mixed and the mixture diluted into 15ml of a guanidine solution (6 M Guanidine-hydrochloride, 10mM Sodium Acetate, 10mM EDTA), to ensure complete chain de-naturation. The guanidine solution containing fully reduced and denatured TCR chains was then injected into 1 litre of the following refolding buffer: 100mM Tris pH 8.5, 400mM L-Arginine, 2mM EDTA, 5mM reduced Glutathione, 0.5mM oxidised Glutathione, 5M urea, 0.2mM PMSF. The redox couple (2-mercaptoethylamine and cystamine (to final concentrations of 6.6mM and 3.7mM, respectively) were added approximately 5 minutes before addition of the denatured TCR chains. The solution was left for 5 hrs \pm 15minutes. The refolded TCR was dialysed in Spectrapor 1 membrane (Spectrum; Product No. 132670) against 10 L 10 mM Tris pH 8.1 at 5°C \pm 3°C for 18-20 hours. After this time, the dialysis buffer was changed to fresh 10 mM Tris pH 8.1 (10 L) and dialysis was continued at 5°C \pm 3°C for another 20-22 hours.

sTCR was separated from degradation products and impurities by loading the dialysed refold onto a POROS 50HQ anion exchange column and eluting bound protein with a gradient of 0-500mM NaCl over 50 column volumes using an Akta purifier (Pharmacia). Peak fractions were stored at 4°C and analysed by Coomassie-stained SDS-PAGE before being pooled and concentrated. Finally, the sTCR was purified and characterised using a Superdex 200HR gel filtration column pre-equilibrated in HBS-EP buffer (10 mM HEPES pH 7.4, 150 mM NaCl, 3.5 mM EDTA, 0.05% nonidet p40). The peak eluting at a relative molecular weight of approximately 50 kDa was

pooled and concentrated prior to characterisation by BIACore surface plasmon resonance analysis.

5 *Example 4 – Biacore surface plasmon resonance characterisation of sTCR binding to specific pMHC*

A surface plasmon resonance biosensor (Biacore 3000TM) was used to analyse the binding of a sTCR to its peptide-MHC ligand. This was facilitated by producing single pMHC complexes (described below) which were immobilised to a streptavidin-coated binding surface in a semi-oriented fashion, allowing efficient testing of the binding of a soluble T-cell receptor to up to four different pMHC (immobilised on separate flow cells) simultaneously. Manual injection of HLA complex allows the precise level of immobilised class I molecules to be manipulated easily.

15 Biotinylated class I HLA-A*0201 molecules were refolded *in vitro* from bacterially-expressed inclusion bodies containing the constituent subunit proteins and synthetic peptide, followed by purification and *in vitro* enzymatic biotinylation (O'Callaghan *et al.* (1999) *Anal. Biochem.* **266**: 9-15). HLA-A*0201-heavy chain was expressed with a C-terminal biotinylation tag which replaces the transmembrane and cytoplasmic domains of the protein in an appropriate construct. Inclusion body expression levels of ~75 mg/litre bacterial culture were obtained. The MHC light-chain or β 2-microglobulin was also expressed as inclusion bodies in *E. coli* from an appropriate construct, at a level of ~500 mg/litre bacterial culture.

20 25 *E. coli* cells were lysed and inclusion bodies are purified to approximately 80% purity. Protein from inclusion bodies was denatured in 6 M guanidine-HCl, 50 mM Tris pH 8.1, 100 mM NaCl, 10 mM DTT, 10 mM EDTA, and was refolded at a concentration of 30 mg/litre heavy chain, 30 mg/litre β 2m into 0.4 M L-Arginine-HCl, 100 mM Tris pH 8.1, 3.7 mM cystamine, 6.6 mM β -cysteamine, 4 mg/ml of the SLYNTVATL peptide required to be loaded by the HLA-A*0201 molecule, by addition of a single

pulse of denatured protein into refold buffer at < 5°C. Refolding was allowed to reach completion at 4°C for at least 1 hour.

5 Buffer was exchanged by dialysis in 10 volumes of 10 mM Tris pH 8.1. Two changes of buffer were necessary to reduce the ionic strength of the solution sufficiently. The protein solution was then filtered through a 1.5 μ m cellulose acetate filter and loaded onto a POROS 50HQ anion exchange column (8 ml bed volume). Protein was eluted with a linear 0-500 mM NaCl gradient. HLA-A*0201-peptide complex eluted at approximately 250 mM NaCl, and peak fractions were collected, a cocktail of protease 10 inhibitors (Calbiochem) was added and the fractions were chilled on ice.

15 Biotinylation tagged pMHC molecules were buffer exchanged into 10 mM Tris pH 8.1, 5 mM NaCl using a Pharmacia fast desalting column equilibrated in the same buffer. Immediately upon elution, the protein-containing fractions were chilled on ice and protease inhibitor cocktail (Calbiochem) was added. Biotinylation reagents were then added: 1 mM biotin, 5 mM ATP (buffered to pH 8), 7.5 mM MgCl₂, and 5 μ g/ml BirA enzyme (purified according to O'Callaghan *et al.* (1999) *Anal. Biochem.* **266**: 9-15). The mixture was then allowed to incubate at room temperature overnight.

20 The biotinylated pHLA-A*0201 molecules were purified using gel filtration chromatography. A Pharmacia Superdex 75 HR 10/30 column was pre-equilibrated with filtered PBS and 1 ml of the biotinylation reaction mixture was loaded and the column was developed with PBS at 0.5 ml/min. Biotinylated pHLA-A*0201 molecules eluted as a single peak at approximately 15 ml. Fractions containing 25 protein were pooled, chilled on ice, and protease inhibitor cocktail was added. Protein concentration was determined using a Coomassie-binding assay (PerBio) and aliquots of biotinylated pHLA-A*0201 molecules were stored frozen at -20°C. Streptavidin was immobilised by standard amine coupling methods.

30 Such immobilised complexes are capable of binding both T-cell receptors and the coreceptor CD8 $\alpha\alpha$, both of which may be injected in the soluble phase. Specific

binding of TCR is obtained even at low concentrations (at least 40 μ g/ml), implying the TCR is relatively stable. The pMHC binding properties of sTCR are observed to be qualitatively and quantitatively similar if sTCR is used either in the soluble or immobilised phase. This is an important control for partial activity of soluble species and also suggests that biotinylated pMHC complexes are biologically as active as non-biotinylated complexes.

The interactions between HIV Gag sTCR containing a novel inter-chain bond and its ligand/ MHC complex or an irrelevant HLA-peptide combination, the production of which is described above, were analysed on a Biacore 3000TM surface plasmon resonance (SPR) biosensor. SPR measures changes in refractive index expressed in response units (RU) near a sensor surface within a small flow cell, a principle that can be used to detect receptor ligand interactions and to analyse their affinity and kinetic parameters. The probe flow cells were prepared by immobilising the individual HLA-peptide complexes in separate flow cells via binding between the biotin cross linked onto β 2m and streptavidin which have been chemically cross linked to the activated surface of the flow cells. The assay was then performed by passing sTCR over the surfaces of the different flow cells at a constant flow rate, measuring the SPR response in doing so.

20

To measure Equilibrium binding constant

Serial dilutions of the parental or mutated HIV Gag sTCR were prepared and injected at constant flow rate of 5 μ l min⁻¹ over two different flow cells; one coated with ~1000 RU of specific SLYNTVATL-HLA-A*0201 complex, the second coated with ~1000 RU of non-specific HLA-A2 -peptide complex. Response was normalised for each concentration using the measurement from the control cell. Normalised data response was plotted versus concentration of TCR sample and fitted to a hyperbola in order to calculate the equilibrium binding constant, K_D . (Price & Dwek, Principles and Problems in Physical Chemistry for Biochemists (2nd Edition) 1979, Clarendon Press, Oxford).

To measure Kinetic Parameters

5

For high affinity TCRs K_D was determined by experimentally measuring the dissociation rate constant, k_d , and the association rate constant, k_a . The equilibrium constant K_D was calculated as k_d/ka .

10 TCR was injected over two different cells one coated with ~300 RU of specific HLA-A2-nyeso peptide complex, the second coated with ~300 RU of non-specific HLA-A2-peptide complex. Flow rate was set at 50 μ l/min. Typically 250 μ l of TCR at ~3 μ M concentration was injected. Buffer was then flowed over until the response had returned to baseline. Kinetic parameters were calculated using Biaevaluation software. The dissociation phase was also fitted to a single exponential decay equation enabling 15 calculation of half-life.

Results

20 The interaction between a soluble disulfide-linked native HIV Gag TCR (consisting of the α and β TCR chains detailed in SEQ ID NOs 9 and 10 respectively) and the SLYNTVATL-HLA-A*0201 complex was analysed using the above methods and demonstrated a K_D of 85 nM and an off-rate (k_{off}) of $2.21 \times 10^{-2} \text{ S}^{-1}$. (See Figure 12 for Biacore response curves)

25

The TCRs specified in the following table have a K_D of less than or equal to 1 μ M and/or a k_{off} of $1 \times 10^{-3} \text{ S}^{-1}$ or slower.

Alpha chain variable domain sequence, SEQ ID NO:	Beta chain variable domain sequence, SEQ ID NO:
1	2
1	14

1	15
11	2
12	2
13	2
12	15
13	15
12	14
13	14

Example 5 – Production of a soluble high affinity HIV Gag TCR –WT human IL-2

5 *fusion protein*

The methods substantially as described in Examples 1 to 3 can be used to produce a soluble high affinity HIV Gag TCR–WT human IL-2 fusion protein. Briefly, the DNA encoding the desired linker and WT human IL-2 are added into the 3' end of the DNA sequence of the soluble disulfide-linked parental HIV Gag TCR beta chain
10 immediately prior to the TAA ("Stop") codon. Figure 11 provides the amino acid sequence of a fusion protein comprising a disulfide-linked parental HIV Gag TCR beta chain fused to WT human IL-2 via linker sequence.(SEQ ID NO: 24) The linker and IL-2 portion of this fusion protein are indicated in italics. The DNA encoding this
15 construct can then be ligated into pEX821. The soluble parental HIV Gag TCR- IL-2 fusion protein can then be expressed by combining this beta chain fusion protein with the soluble disulfide-linked parental HIV Gag alpha chain TCR chain detailed in Figure 5a (SEQ ID NO: 9) using the methods substantially as described in Example 3.

20 *Example 6 – Recombinant expression of the parental HIV Gag TCR on the surface of T cells.*

DNA constructs encoding the signal sequence, extracellular, transmembrane and intracellular domains of the parental HIV Gag TCR chains were synthesised (GeneArt, Germany). These TCR α chain and TCR β chain DNA sequences, provided in Figures 15a and 15b respectively, are altered from the parental HIV Gag TCR DNA sequences so as to enhance expression levels of the encoded TCR chains in human T cells whilst maintaining the native amino acid sequence. Figures 16a and 16b provide the full-length amino acid sequences encoded by the DNA sequences of Figures 15a and 15b respectively.

10 TCR α chain and TCR β chain DNA sequences were then inserted together into a Lentiviral expression vector. This vector contains DNA encoding both the parental HIV Gag TCR α chain and β chain as a single open reading frame with the in-frame Foot and Mouth Disease Virus (FMDV) 2A cleavage factor amino acid sequence (LLNFDLLKLAGDVESNPG (SEQ ID NO: 31)) separating the TCR chains. (de
15 Felipe *et al.*, *Genet Vaccines Ther* (2004) 2 (1): 13) On mRNA translation the TCR α chain is produced with the 2A peptide sequence at its C-terminus and the TCR β chain is produced as a separate polypeptide.

20 T cells were transduced with the above Lentiviral vector. Briefly, primary T cells were stimulated for 24 hours using anti-CD3/anti-CD28 beads. A concentrated Lentivirus supernatant, expressing the TCR genes, was then incubated with the stimulated T cells to allow viral transduction. The anti-CD3/anti-CD28 beads were then removed and the transduced T cells were cultured until they attained a “resting volume” of 200-300 fL.

25 Presentation of parental HIV Gag TCRs on the surface of the transduced cells was confirmed by FACS analysis using HLA-A*0201-SLYNTVALT PE tetramer and anti-CD8 monoclonal antibody FITC co-staining.

Results

Figure 17b provides the FACS analysis data which demonstrates the successful expression of the parental HIV Gag TCR on the surface of transduced CD8⁺ T cells.

5 Figure 17a provides FACS analysis data generated using control untransduced T cells.

Example 7 – Inhibition of CTL activation by soluble high affinity HIV Gag TCRs

10 The following assays were carried out to demonstrate that the soluble high affinity c11c6 HIV Gag TCR was capable of inhibiting activation of a SLYNTVATL-HLA-A*0201 reactive polyclonal T cell line.

*Inhibition of activation of the OX84 SLYNTVATL-HLA-A*0201 reactive polyclonal T cell line in the presence of HIV infected cells*

15 The soluble c11c6 high affinity HIV Gag TCR utilised in this experiment contained the TCR alpha chain variable domain and TCR beta chain variable regions shown in Figure 6c (SEQ ID NO: 13) and Figure 7b (SEQ ID NO: 15) respectively. The full amino acid sequences of the TCR alpha and beta chains of this soluble TCR are 20 provided by Figure 18a (SEQ ID NO: 29) and Figure 18b (SEQ ID NO: 30) respectively.

IFN- γ and TNF- α production was used as the read-outs for CTL activation.

25 *Reagents*

R10 Assay media: 10% FCS (heat-inactivated, Gibco, cat# 10108-165), 88% RPMI 1640 (Gibco, cat# 42401-018), 1% glutamine (Gibco, cat# 25030-024) and 1% penicillin/streptomycin (Gibco, cat# 15070-063).

Peptide: (obtained from various sources) initially dissolved in DMSO (Sigma, cat# D2650) at 4mg/ml and frozen.

5 The BD™ Cytometric Bead Array Kit, Human Th1/Th2 cytokine Kit II (BD Biosciences, San Diego, US) contains all the reagents required for the assay.

T cell activation Assay

10 Chronically HIV infected To target cells (HXB2 and HIV3B HIV Lab strains) were washed and re-suspended in R10 media. As a control uninfected To target cells were pulsed with 1nM of SLYNTVATL peptide, for 30 minutes at 37°C, 5%CO₂.

Test Samples:

15 25,000 HIV infected To target cells in R10 media per well of a 96 well U-bottom plate.

2 x 10⁻⁷ M high affinity c11c6 HIV Gag TCR or parental HIV Gag TCR in R10 media per well.

20 5000 OX84 polyclonal effector T cell line in R10 media per well.

Controls:

As above substituting irrelevant soluble TCRs (HLA-A*0201-Tax specific and HLA-A*0201-NY-ESO specific TCRs) or the high affinity HIV Gag TCRs.

25 The plate was then incubated for 4 hours at 37°C, 5% CO₂. The culture supernatant was removed to measure the levels of IFN- γ and TNF- α present using the following method.

30 *IFN- γ and TNF- α assay*

BD™ Cytometric Beads coated with (a) anti-IFN γ capture antibodies and (b) anti-TNF α capture antibodies were prepared according to the manufacturers instructions

A number of assay tubes were then prepared containing the following additions:

5

50 μ l of mixed anti-IFN γ and anti-TNF α BD™ Cytometric Beads in BD Assay

Diluent

50 μ l of PE Dectection Reagent

10

Followed by either:

50 μ l of the culture supernatant taken from the T cell activation assay wells. (Test Samples)

Or

15

50 μ l of mixed IFN γ and TNF α standards prepared at a range of concentrations by serial dilution of stock standards. (Calibration Standards)

20

The tube were then incubated in the dark for 3 hours prior to being washed with 1ml of BD Wash Buffer and centrifuged. Finally, the beads were re-suspended in 300 μ l of the Wash Buffer and the level of IFN γ and TNF α present was determined by Flow Cytometry according to manufacturer's instructions.

25

*Inhibition of the SLYNTVATL-HLA-A*0201 specific OX84 polyclonal T line in the presence of uninfected SLYNTVATL peptide pulsed To cells*

The same regents and methods as used for the above CTL activation assay were used except that:

30

2000 OX84 polyclonal effector T cells were used in each T cell activation assay.

Uninfected To lymphoblastoid cells, pulsed with 10^{-10} - 10^{-8} M SLYNTVATL peptide were used as the target cells

Results

5

The soluble high affinity c11c6 HIV Gag TCR strongly inhibited activation of the SLYNTVATL-HLA-A*0201 reactive OX84 polyclonal T cell line in the presence of To cells infected by HIV as measured by IFN- γ and TNF- α production. (See Figure 19)

10

The soluble high affinity c11c6 HIV Gag TCR strongly inhibited activation of the SLYNTVATL-HLA-A*0201 reactive OX84 polyclonal T cell line in the presence of SLYNTVATL-pulsed uninfected To cells as measured by IFN- γ and TNF- α production. (See Figure 20)

15

*Example 8 - Quantification of cell surface SLYNTVATL-HLA-A*0201 antigens on peptide-pulsed T2 cells by fluorescence microscopy using high affinity c11c6 HIV Gag TCR*

20

The number of SLYNTVATL-HLA-A*0201 antigens on peptide-pulsed T2 lymphoblastoid cell was determined (on the assumption that one fluorescence signal relates to a single labelled TCR bound to its cognate pMHC ligand on the surface of the target cell) by single molecule fluorescence microscopy using a soluble high-affinity c11c6 HIV Gag TCR. This was facilitated by using biotinylated TCR to target the antigen-expressing cancer cells and subsequent labelling of cell-bound TCR by streptavidin-R phycoerythrin (PE) conjugates. Individual PE molecules were then imaged by 3-dimensional fluorescence microscopy.

25

T2 lymphoblastoid cells were pulsed with the HIV Gag-derived SLYNTVATL peptide, or an irrelevant peptide (SLLMWITQC) at a range of concentrations (10^{-5} - 10^{-10} M) for 90 minutes at 37°C. After pulsing the cells were washed twice with 500 μ l of PBS. Cells were incubated in 200 μ l of TCR solution (100 nM high-affinity c11c6

HIV Gag TCR), in PBS, 0.5% BSA albumin) for 30 min at room temperature. TCR solution was removed, and cells were washed three times with 500 μ l of PBS. Cells were incubated in 200 μ l of streptavidin-PE solution (5 μ g ml⁻¹ streptavidin-PE in PBS containing 0.5% BSA) at room temperature in the dark for 20 min. Streptavidin-PE solution was removed and cells were washed three times with 500 μ l of PBS. Wash media was removed, and cells kept in 400 μ l of R10, without Phenol Red before 5 imaging by fluorescence microscopy.

Fluorescence microscopy. Fluorescent microscopy was carried out using an Axiovert 10 200M (Zeiss) microscope with a 63x Oil objective (Zeiss). A Lambda LS light source containing a 300W Xenon Arc lamp (Sutter) was used for illumination, and light intensity was reduced to optimal levels by placing a 0.3 and a 0.6 neutral density filter into the light path. Excitation and emission spectra were separated using a TRITC/DiI 15 filter set (Chroma). Cells were imaged in three dimensions by z-stack acquisition (21 planes, 1 μ m apart). Image acquisition and analysis was performed using Metamorph software (Universal Imaging) as described (Irvine *et al.*, *Nature* **419**: p845-9, and Purbhoo *et al.*, *Nature Immunology* **5**: p524-30).

Results

20

As shown by Figure 21 the above method was used successfully to image high affinity 25 c11c6 HIV Gag TCR bound to SLYNTVATL -HLA-A*0201 antigens on the surface of peptide-pulsed T2 cells. These results show the threshold for counting epitopes on SLYNTVATL peptide-pulsed cells using the high affinity c6c11 HIV Gag TCR is approximately 10⁻⁹ M peptide.

Claims

1. A T-cell receptor (TCR) having the property of binding to SLYNTVATL-HLA-A*0201 and comprising at least one TCR α chain variable domain and/or at least one TCR β chain variable domain CHARACTERISED IN THAT said TCR has a K_D for the said SLYNTVATL-HLA-A*0201 complex of less than or equal to $1\mu M$ and/or has an off-rate (k_{off}) for the SLYNTVATL-HLA-A*0201 complex of $1 \times 10^{-3} S^{-1}$ or slower, PROVIDED THAT when the said TCR is presented by cell and comprises SEQ ID NOS: 1 and 2, the cell is not a native T cell.
10
2. A TCR as claimed in claim 1 comprising both an α chain variable domain and an TCR β chain variable domain.
15
3. A TCR as claimed in claim 1 which is an $\alpha\alpha$ or $\beta\beta$ homodimer.
4. A T-cell receptor (TCR) as claimed in any of the preceding claims wherein the said K_D and/or k_{off} is/are as measured by Surface Plasmon Resonance.
20
5. A TCR as claimed in any of the preceding claims which is mutated relative to the parental HIV Gag TCR α chain variable domain (SEQ ID No: 1) and/or β chain variable domain (SEQ ID NO: 2) in at least one complementarity determining region.
25
6. A TCR as claimed in any of the preceding claims which is mutated relative to the parental HIV Gag TCR α chain variable region (SEQ ID No: 1) and/or β chain variable region (SEQ ID NO: 2) in at least one variable domain framework region thereof.
30
7. A TCR as claimed in any of the preceding claims wherein one or more of alpha chain variable region amino acids 95T, 96N, 97S, 98G, and 100A using the numbering shown in SEQ ID NO: 1 is/are mutated.

8. A TCR as claimed in any of the preceding claims wherein one or more of beta chain variable region amino acids 51Y, 52E, 53E and 54E using the numbering shown in SEQ ID NO: 2 is/are mutated.

5 9. A TCR as claimed in any of claims 1 to 6 comprising one or more of alpha chain variable region amino acids 95S, 95G, 96A, 97H, 98D or 100S using the numbering shown in SEQ ID NO: 1.

10. 10. A TCR as claimed in any of claims 1 to 6 or 9 comprising one or more of beta chain variable region amino acids 51V, 51A, 52R, 52L, 53G or 54V using the numbering shown in SEQ ID NO: 2.

15. 11. A TCR as claimed in any of claims 1 to 6 comprising one of the alpha chain variable region amino acid sequences shown in (SEQ ID Nos: 11 to 13), optionally comprising one or more phenotypically silent substitutions.

12. 12. A TCR as claimed in any of claims 1 to 6 or 11 comprising one of the beta chain variable region amino acid sequences shown in (SEQ ID Nos: 14 to 15), optionally comprising one or more phenotypically silent substitutions.

20. 13. A TCR as claimed in claim 2 comprising the alpha and beta chain variable region pairings shown in the following table, optionally comprising one or more phenotypically silent substitutions:

25

Alpha chain variable region sequence, SEQ ID NO:	Beta chain variable region sequence, SEQ ID NO:
1	2
1	14
1	15

11	2
12	2
13	2
12	15
13	15
12	14
13	14

14. A TCR as claimed in any preceding claim further comprising the alpha chain constant domain amino acid sequence shown in SEQ ID NO: 19, and/or one of the beta chain amino acid constant domain sequences shown in SEQ ID NOs: 20 and 21, 5 optionally comprising one or more phenotypically silent substitutions.

15. A TCR as claimed in any preceding claim which is a dimeric T cell receptor (dTCR) or a single chain T cell receptor (scTCR).

10 16. A TCR as claimed in of claims 4 to 15 which is an scTCR comprising a first segment constituted by an amino acid sequence corresponding to a TCR α chain variable region

15 a second segment constituted by an amino acid sequence corresponding to a TCR β chain variable region sequence fused to the N terminus of an amino acid sequence corresponding to a TCR β chain constant domain extracellular sequence, and

20 a linker sequence linking the C terminus of the first segment to the N terminus of the second segment.

17. A TCR as claimed in any of claims 4 to 15 which is an scTCR comprising a first segment constituted by an amino acid sequence corresponding to a TCR β chain

variable region

5 a second segment constituted by an amino acid sequence corresponding to a TCR α chain variable region sequence fused to the N terminus of an amino acid sequence corresponding to a TCR α chain constant domain extracellular sequence, and

10 a linker sequence linking the C terminus of the first segment to the N terminus of the second segment.

10

18. A TCR as claimed in claim 16 or 17 further comprising a disulfide bond between bond between the first and second chains, said disulfide bond being one which has no equivalent in native $\alpha\beta$ T cell receptors, and wherein the length of the linker sequence and the position of the disulfide bond being such that the variable 15 domain sequences of the first and second segments are mutually orientated substantially as in native $\alpha\beta$ T cell receptors.

19. An scTCR as claimed in any of claims 16 to 18 wherein in the binding part the linker sequence links the C terminus of the first segment to the N terminus of the 20 second segment.

20. A scTCR as claimed in any of claims 16 to 19 wherein in the binding part the linker sequence has the formula -PGGG-(SGGGG)₅-P- (SEQ ID NO: 17) or -PGGG-(SGGGG)₆-P- (SEQ ID NO: 18) wherein P is proline, G is glycine and S is serine.

25

21. A TCR as claimed in any of claims 1, 2 or 4 to 15 which is a dTCR comprising a first polypeptide wherein a sequence corresponding to a TCR α chain variable region sequence is fused to the N terminus of a sequence corresponding to a TCR α chain constant domain extracellular sequence, and

30

a second polypeptide wherein a sequence corresponding to a TCR β chain variable region sequence fused to the N terminus a sequence corresponding to a TCR β chain constant domain extracellular sequence,

5 the first and second polypeptides being linked by a disulfide bond which has no equivalent in native $\alpha\beta$ T cell receptors.

22. A TCR as claimed in claim 21 wherein the disulfide bond links amino acid residues of the said constant domain sequences, which disulfide bond has no 10 equivalent in native TCRs.

23. A TCR as claimed in claim 22 wherein the said disulfide bond is between cysteine residues corresponding to amino acid residues whose β carbon atoms are less than 0.6 nm apart in native TCRs.

15 24. A TCR as claimed in claim 22 wherein the said disulfide bond is between cysteine residues substituted for Thr 48 of exon 1 of TRAC*01 and Ser 57 of exon 1 of TRBC1*01 or TRBC2*01 or the non-human equivalent thereof.

20 25. A TCR as claimed in any of claims 15 to 24 wherein the dTCR or scTCR binding part includes a disulfide bond between residues corresponding to those linked by a disulfide bond in native TCRs.

26. A TCR as claimed in any of claims 14 to 23 wherein the dTCR or scTCR 25 binding part does not contain a sequence corresponding to transmembrane or cytoplasmic sequences of native TCRs.

27. A TCR as claimed in any preceding claim wherein the TCR is associated with at least one polyalkylene glycol chain(s).

28. A TCR as claimed in claim 27 wherein the polyalkylene glycol chain(s) is/are covalently linked to the TCR.

29. A TCR as claimed in claim 27 or claim 28 wherein the polyalkylene glycol chain(s) comprise(s) at least two polyethylene glycol repeating units.

30. A TCR as claimed in any preceding claim further comprising a reactive cysteine at the C terminal or N-terminal of the alpha or beta chains thereof.

10 31 A TCR as claimed in any preceding claim associated with a therapeutic agent or detectable moiety.

32 A TCR as claimed in claim 31 wherein the TCR is covalently linked to a therapeutic agent or detectable moiety.

15 33. A TCR as claimed in claim 31 wherein the therapeutic agent or detectable moiety is covalently linked to the C terminus of one or both TCR chains.

20 34 A TCR as claimed in any of claims 31 to 33 associated with a therapeutic agent which is an immune effector molecule.

35. A TCR as claimed in claim 34 wherein the immune effector molecule is a cytokine.

25 36. A TCR as claimed in claim 34 wherein the immune effector molecule is IL-2, or a functional variant or fragment thereof.

37. A TCR as claimed in any of claims 31 to 33 wherein the therapeutic agent is a cytotoxic agent.

38. A TCR as claimed in any of claims 31 to 33 wherein the therapeutic agent is a radionuclide.

5 39. A multivalent TCR complex comprising at least two TCRs as claimed in any of the preceding claims.

40. A multivalent TCR complex comprising at least two TCRs as claimed in any of the preceding claims linked by a non-peptidic polymer chain or a peptidic linker sequence.

10

41. A TCR complex as claimed in claim 40 wherein the polymer chain or peptidic linker sequence extends between amino acid residues of each TCR which are not located in a variable region sequence of the TCR.

15

42. A TCR complex as claimed in either of claims 40 or 41 in which the TCRs are linked by a polyalkylene glycol chain or a peptidic linker derived from a human multimerisation domain.

20

43. A TCR complex as claimed in claim 42 wherein a divalent alkylene spacer radical is located between the polyalkylene glycol chain and its point of attachment to a TCR of the complex.

44. A TCR complex as claimed in claim 40 or claim 41 wherein the polyalkylene glycol chain comprises at least two polyethylene glycol repeating units.

25

45. A multivalent TCR complex comprising at least two TCRs as claimed in any of claims 1 to 30 wherein (i) at least one of said TCRs is associated with a therapeutic agent as claimed in any of claims 31 to 38.

30

46. A membrane preparation comprising a TCR as defined in any of claims 1 to 26.

47. A cell harbouring an expression vector comprising nucleic acid encoding a TCR as defined in any of claims 1 to 26.

48. A pharmaceutical composition comprising a TCR or a multivalent TCR complex as claimed in any of claims 1 to 45, or a membrane preparation as claimed in claim 46, or a plurality of cells as claimed in claim 47, together with a pharmaceutically acceptable carrier.

49 A method of treatment of AIDS comprising administering to a subject 10 suffering AIDS an effective amount of a TCR or a multivalent TCR complex as claimed in any of claims 1 to 45, or a membrane preparation as claimed in claim 46, or a plurality of T cells presenting a plurality of TCRs as claimed in any of claims 1 to 30.

50 A method as claimed in claim 49 wherein a TCR or multivalent TCR complex, or membrane preparation comprising a TCR is administered, in a form which is 15 associated with a therapeutic agent.

51 A method as claimed in claim 49 wherein a plurality of T cells presenting a 20 plurality of TCRs are administered and the T cells are CD8+ T cells.

52 The use of a TCR or a multivalent TCR complex as claimed in any of claims 1 to 45, or a membrane preparation as claimed in claim 46, or a plurality of cells as 25 claimed in claim 47, in the preparation of a composition for the treatment of AIDS.

53. The use as claimed in claim 52 wherein a TCR or multivalent TCR complex, or membrane preparation comprising a TCR is administered, in a form which is 30 associated with a therapeutic agent.

54 The use as claimed in claim 52 wherein a plurality of T cells presenting a plurality of TCRs are administered and the T cells are CD8+ T cells.

55. A method of producing a high affinity TCR having the property of binding to SLYNTVATL-HLA-A*0201 CHARACTERISED IN THAT the TCR (i) comprises at least one TCR α chain variable region and/or at least one TCR β chain variable region and (ii) has a K_D for the said SLYNTVATL-HLA-A*0201 complex of less than $1\mu\text{M}$ and/or an off-rate (k_{off}) for the SLYNTVATL-HLA-A*0201 complex of less than 1×10^{-3} said method comprising:

10 (a) the production of a TCR comprising the α and β chain variable regions of the HIV Gag TCR wherein one or both of the α and β chain variable regions comprise a mutation(s) in one or more of the amino acids identified in claims 7 and 8;

(b) contacting said mutated TCR with SLYNTVATL-HLA-A*0201 under conditions suitable to allow the binding of the TCR to SLYNTVATL-HLA-A*0201;

15 And measuring the K_D and/or k_{off} of the interaction.

1/24

Figure 1a

10 20
* *
M A Q K E V E Q N S G P L S V P E G A I A S L N C T Y S D

30 40 50
* * *
R G S Q S F F W Y R Q Y S G K S P E L I M F I Y S N G D K

60 70 80
* * *
E D G R F T A Q L N K A S Q Y I S L L I R D S K L S D S A

90 100 110
* * *
T Y L C A V R T N S G Y A L N F G K G T S L L V T P H
(SEQ ID No: 1)

Figure 1b

	10	20	
	*	*	
M E A G V T Q S P T H L I K T R G Q Q V T L R C S P K S G			
30	40	50	
*	*	*	
H D T V S W Y Q Q A L G Q G P Q F I F Q Y Y E E E E R Q R			
60	70	80	
*	*	*	
G N F P D R F S G H Q F P N Y S S E L N V N A L L L G D S			
90	100	110	
*	*	*	
A L Y L C A S S D T V S Y E Q Y F G P G T R L T V T			
(SEQ ID NO: 2)			

Figure 2a

atggcccagaaggaggaggcagaattctggaccctcagtgccatgtggagggaggccattgcctctcaattgcacttaca
tgaccgagggtcccagtccttctgttacagacaatattctggaaaagccctgagttgataatgttcatatactccaaatgg
tgacaaaagaagatggaaggttacagcacagctcaataaagccagccagttatattccctgtcatcagagactccaagctc
agtgattcagccacacctacctctgtcggtgcgcacaaattccggatgcactcaacttcggcaaaaggcacctcgctgttgc
cacacccatatccagaaccctgaccctgccgttaccagctgagagactctaaatccagtgacaagtctgtgcctattca
ccgattttgattctcaaacaaatgttcacaaatggattctgttatcacagacaactgtgttagacatgaggatc
tatggacttcaagagcaacagtgtggccctggagcaacaaatctgactttgcattgtgcacaaacgcctcaacaacagcatta
ttccagaagacacccatcccccagcccagaaatgttctaa
(SEQ ID No: 3)

Figure 2b

atggaggctggagtcacacaaaactccacacacccgtatcaaaaacgagaggacagcaagtgactctgagatgtctctctaa
gtctggcatgacactgtgtcctgttaccaacaggccctgggtcaggggccccagtttatcttcagtattatgaggaggaag
agagacagagaggcaactccctgtatcgattctcaggtcaccagttccctaactatagctctgactgtgaacgccttg
ttgctggggactcggccctctatctgtgccagcaggcaccgttcctacgaggacttccggccggccaccagg
ctcacggtcacagaggacctgaaaaacgtgtccacccgaggctgtgttgcattcagaaggcacttccac
acccaaaaggccacactgggtgtgccctggccacaggcttctaccccgaccacgtggagctgagctgtgggtgaatggaa
ggaggtgcacagtgggtcagcacagacccgcagccctcaaggagcagccgcctcaatgactccagatacgctctg
agcagccgcctgagggtctggccacccctgtcaggacccttccgctgtcaagtccagttctacggc
tcggagaatgacgagtggacccaggataggccaaaccctcaccatgtcagcgccgaggcctgggttagagca
gactaa

(SEQ ID No: 4)

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Figure 3a

M A Q K E V E Q N S G P L S V P E G A I A S L N C T Y S D R G S Q S F
F W Y R Q Y S G K S P E L I M F I Y S N G D K E D G R F T A Q L N K A
S Q Y I S L L I R D S K L S D S A T Y L C A V R T N S G Y A L N F G K
G T S L L V T P H I Q N P D P A V Y Q L R D S K S S D K S V C L F T D
F D S Q T N V S Q S K D S D V Y I T D K T V L D M R S M D F K S N S A
V A W S N K S D F A C A N A F N N S I I P E D T F F P S P E S S
(SEQ ID No: 5)

Figure 3b

M E A G V T Q S P T H L I K T R G Q Q V T L R C S P K S G H D T V S W
Y Q Q A L G Q G P Q F I F Q Y Y E E E E R Q R G N F P D R F S G H Q F
P N Y S S E L N V N A L L L G D S A L Y L C A S S D T V S Y E Q Y F G
P G T R L T V T E D L K N V F P P E V A V F E P S E A E I S H T Q K A
T L V C L A T G F Y P D H V E L S W W V N G K E V H S G V S T D P Q P
L K E Q P A L N D S R Y A L S S R L R V S A T F W Q D P R N H F R C Q
V Q F Y G L S E N D E W T Q D R A K P V T Q I V S A E A W G R A D
(SEQ ID No: 6)

Figure 4a

ccatcgatggcccagaaggaggtggagcagaattctggacccctcagtgtccagagggagccatgcctctcaattgc
acttacagtgaccgagggtcccagtccttctgttacagacaatattctggaaaagccctgagttgataatgttcatatactc
caatggtgacaaaagaagatggaaggttacagcacagctcaataaagccagccagtatattccctgctcatcagagactcc
aagctcagtgattcagccacccatctgtcgccgtgcgcacaaattccgggtatgcactcaacttggcaaaggcacctgc
tgttggtcacacccatccagaaccctgaccctgcgttaccagctgagagactctaagtgtgacaagtctgtctgc
ctattcaccgattttgattctcaaaacaaatgttgcacaaagaaggattctgtatcacagacaaaatgtgtctgtagacat
gaggtctatggacttcaagagaacagtgtggcctggagcaacaaatctgactttgcatgtgcaaacgcctcaacaac
agcattattccagaagacacccatcccccagccagaaagtccctaa
(SEQ ID No: 7)

Figure 4b

tctctattaatggaggctggagtcacacaaaagtcccacacacacgtcaaaacgagaggacagcaagtgactctgagatg
ctctcctaagtctggcatgacactgtgtctgttaccaacaggccctgggtcagggggcccaagtttatcttcagtattatga
ggaggaagagagacagagaggcaacitccctgatcgattctcaggtcaccagttccctaactatacgctgagctgaatgtg
aacgccttgtgctggggactcgcccttatctgtgccagcagcgcacaccgtctcctacgaggcgtacttcggccgg
gcaccagg
ctcacggtcacagaggacactgaaaaacgtgttcccacccgaggtcgctgtgttgcattcagaaggcagatctccac
acccaaaaggccacactgggtgtgcctggccaccgggttttaccccgaccacgtggagctgagctgggtgggtgaatgggaa
ggaggtgcacagtgggtctgcacagacccgcagccctcaaggaggcagccgcctcaatgactccagatacgctctg
agcagccgcctgagggtctggccacccctgtggcaggaccccgcaaccacttccgctgtcaagtccagttctacggc
tcggagaatgacgagtggacccaggataggccaaacccgtcacccagatcgtcagcgcggaggcctgggttagagca
gactaa
(SEQ ID No: 8)

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Figure 5a

M A Q K E V E Q N S G P L S V P E G A I A S L N C T Y S D R G S Q S F
F W Y R Q Y S G K S P E L I M F I Y S N G D K E D G R F T A Q L N K A
S Q Y I S L L I R D S K L S D S A T Y L C A V R T N S G Y A L N F G K
G T S L L V T P H I Q N P D P A V Y Q L R D S K S S D K S V C L F T D
F D S Q T N V S Q S K D S D V Y I T D K C V L D M R S M D F K S N S A
V A W S N K S D F A C A N A F N N S I I P E D T F F P S P E S S
(SEQ ID No: 9)

Figure 5b

M E A G V T Q S P T H L I K T R G Q Q V T L R C S P K S G H D T V S W
Y Q Q A L G Q G P Q F I F Q Y Y E E E E R Q R G N F P D R F S G H Q F
P N Y S S E L N V N A L L L G D S A L Y L C A S S D T V S Y E Q Y F G
P G T R L T V T E D L K N V F P P E V A V F E P S E A E I S H T Q K A
T L V C L A T G F Y P D H V E L S W W V N G K E V H S G V C T D P Q P
L K E Q P A L N D S R Y A L S S R L R V S A T F W Q D P R N H F R C Q
V Q F Y G L S E N D E W T Q D R A K P V T Q I V S A E A W G R A D
(SEQ ID No: 10)

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Figure 6a

M A Q K E V E Q N S G P L S V P E G A I A S L N C T Y S D R
G S Q S F F W Y R Q Y S G K S P E L I M F I Y S N G D K E D
G R F T A Q L N K A S Q Y I S L L I R D S K L S D S A T Y L
C A V R S A H G Y S L N F G K G T S L L V T P H
(SEQ ID NO: 11)

Figure 6b

M A Q K E V E Q N S G P L S V P E G A I A S L N C T Y S D R
G S Q S F F W Y R Q Y S G K S P E L I M F I Y S N G D K E D
G R F T A Q L N K A S Q Y I S L L I R D S K L S D S A T Y L
C A V R S A H G Y A L N F G K G T S L L V T P H
(SEQ ID NO: 12)

Figure 6c

M A Q K E V E Q N S G P L S V P E G A I A S L N C T Y S D R
G S Q S F F W Y R Q Y S G K S P E L I M F I Y S N G D K E D
G R F T A Q L N K A S Q Y I S L L I R D S K L S D S A T Y L
C A V R G A H D Y A L N F G K G T S L L V T P H
(SEQ ID NO: 13)

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Figure 7a

M E A G V T Q S P T H L I K T R G Q Q V T L R C S P K S G H
D T V S W Y Q Q A L G Q G P Q F I F Q Y V R G V E R Q R G N
F P D R F S G H Q F P N Y S S E L N V N A L L L G D S A L Y
L C A S S D T V S Y E Q Y F G P G T R L T V T
(SEQ ID NO: 14)

Figure 7b

M E A G V T Q S P T H L I K T R G Q Q V T L R C S P K S G H
D T V S W Y Q Q A L G Q G P Q F I F Q Y A L G E E R Q R G N
F P D R F S G H Q F P N Y S S E L N V N A L L L G D S A L Y
L C A S S D T V S Y E Q Y F G P G T R L T V T
(SEQ ID NO: 15)

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Figure 8a

I Q N P D P A V Y Q L R D S K S S D K S V C L F T
D F D S Q T N V S Q S K D S D V Y I T D K
(SEQ ID NO: 19)

Figure 8b

E D L N K V F P P E V A V F E P S E A E I S H T Q K A T
L V C L A T G F F P D H V E L S W W V N G K E V H S G V
(SEQ ID NO: 20)

Figure 8c

E D L K N V F P P E V A V F E P S E A E I S H T Q K A T
L V C L A T G F Y P D H V E L S W W V N G K E V H S G V
(SEQ ID NO: 21)

Figure 9**PEX954**

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Figure 9 Cont.

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(SEQ ID NO: 22)

Figure 10

PEX821

Figure 10 Cont.

ggtaagcggcagggtcggAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGAAACGCCTGGTATCTTATAGCCTGTC
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(SEQ ID NO: 23)

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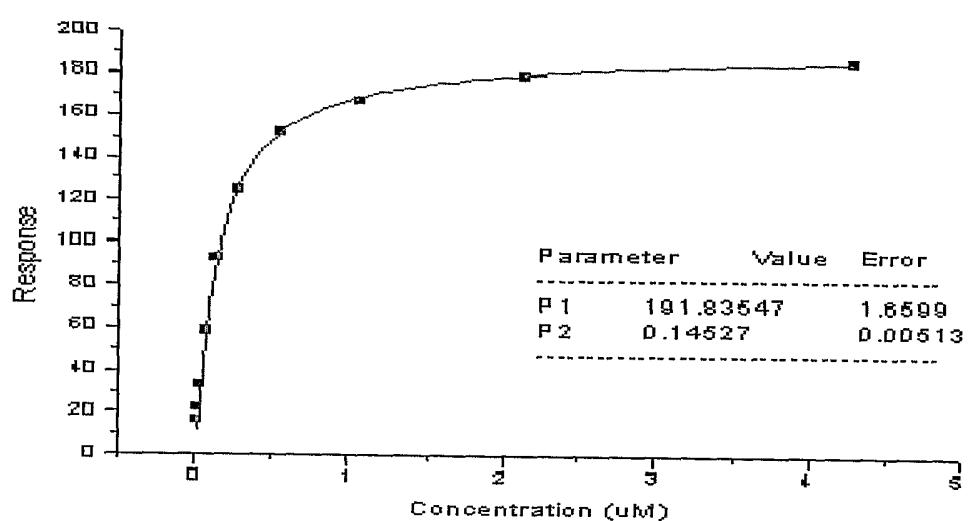
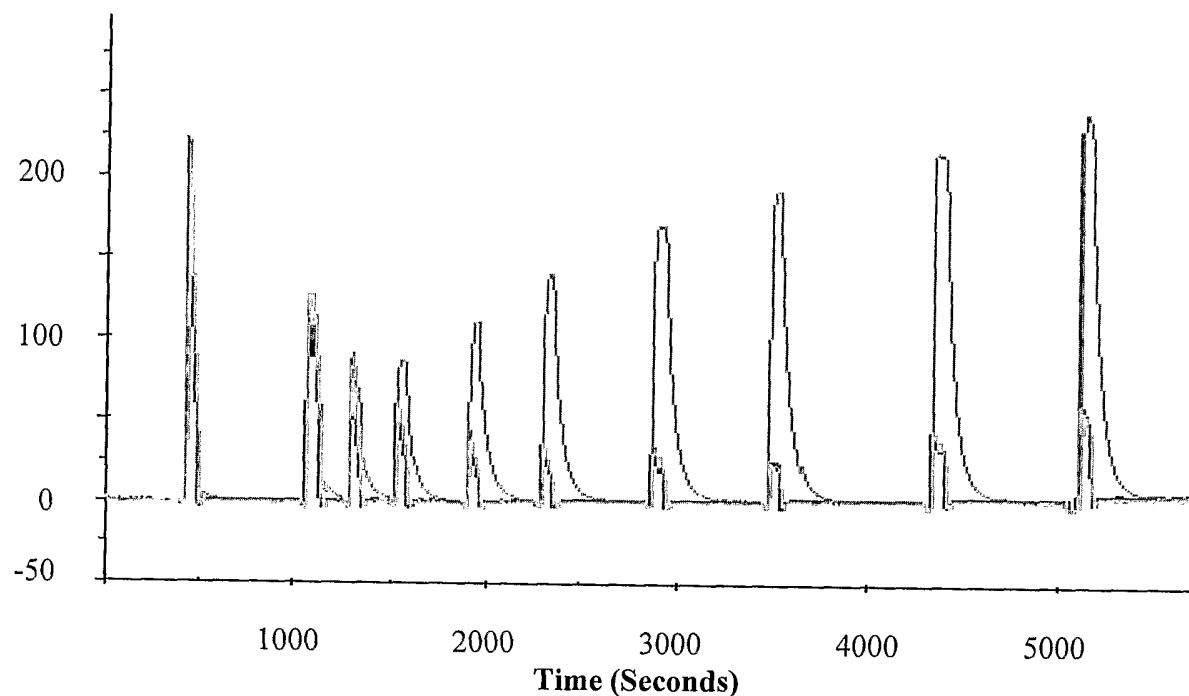
Figure 11

M E A G V T Q S P T H L I K T R G Q Q V T L R C S P K S G H D T V S W
Y Q Q A L G Q G P Q F I F Q Y Y E E E E R Q R G N F P D R F S G H Q F
P N Y S S E L N V N A L L L G D S A L Y L C A S S D T V S Y E Q Y F G
P G T R L T V T E D L K N V F P P E V A V F E P S E A E I S H T Q K A
T L V C L A T G F Y P D H V E L S W W V N G K E V H S G V C T D P Q P
L K E Q P A L N D S R Y A L S S R L R V S A T F W Q D P R N H F R C Q
V Q F Y G L S E N D E W T Q D R A K P V T Q I V S A E A W G R A D P G
A P T S S S T K K T Q L Q L E H L L D L Q M I L N G I N N Y K N P K
L T R M L T F K F Y M P K K A T E L K H L Q C L E E E L K P L E E V L
N L A Q S K N F H L R P R D L I S N I N V I V L E L K G S E T T F M C
E Y A D E T A T I V E F L N R W I T F C Q S I I S T L T
(SEQ ID NO: 24)

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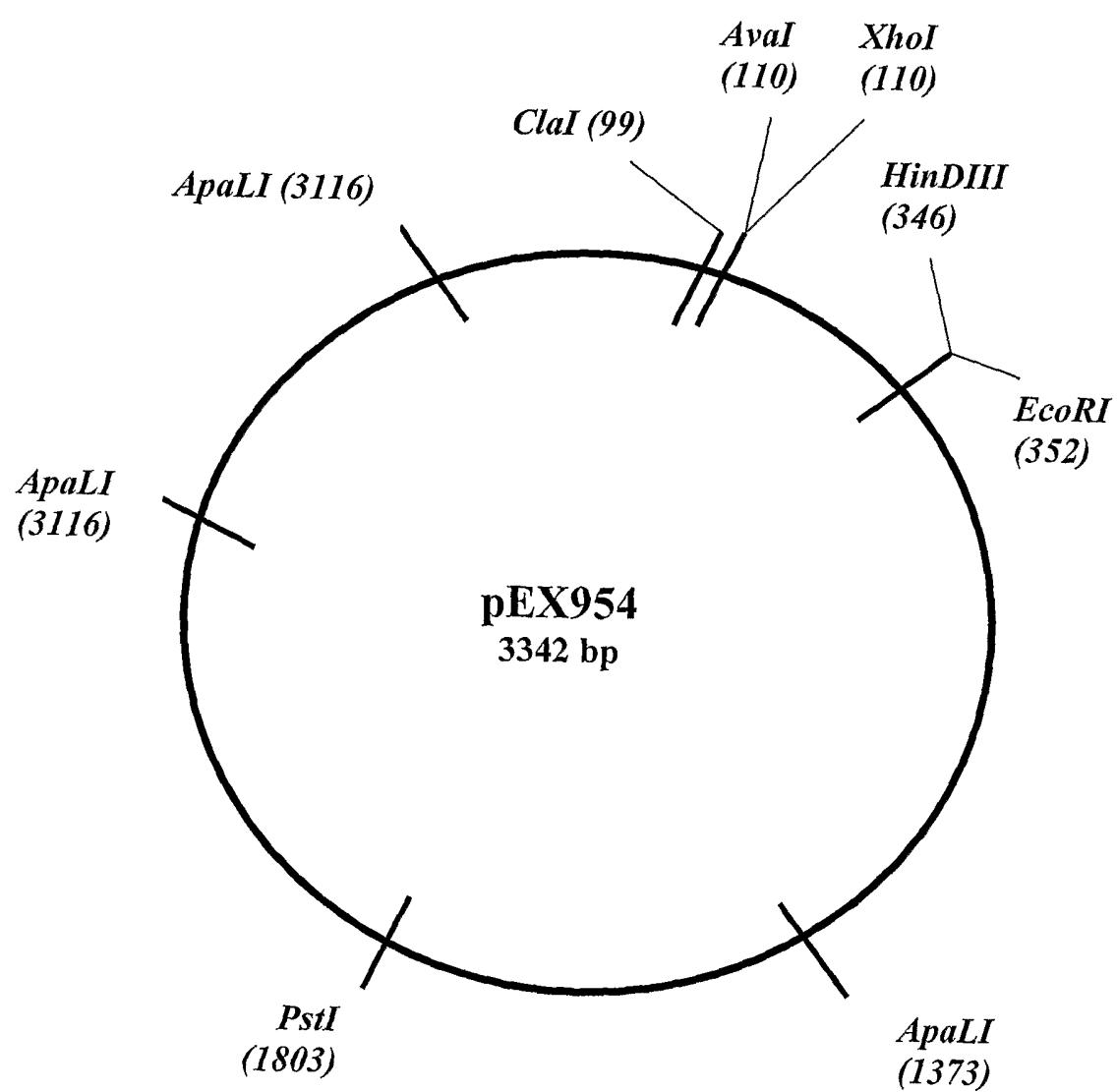
Figure 12

Response (RU)



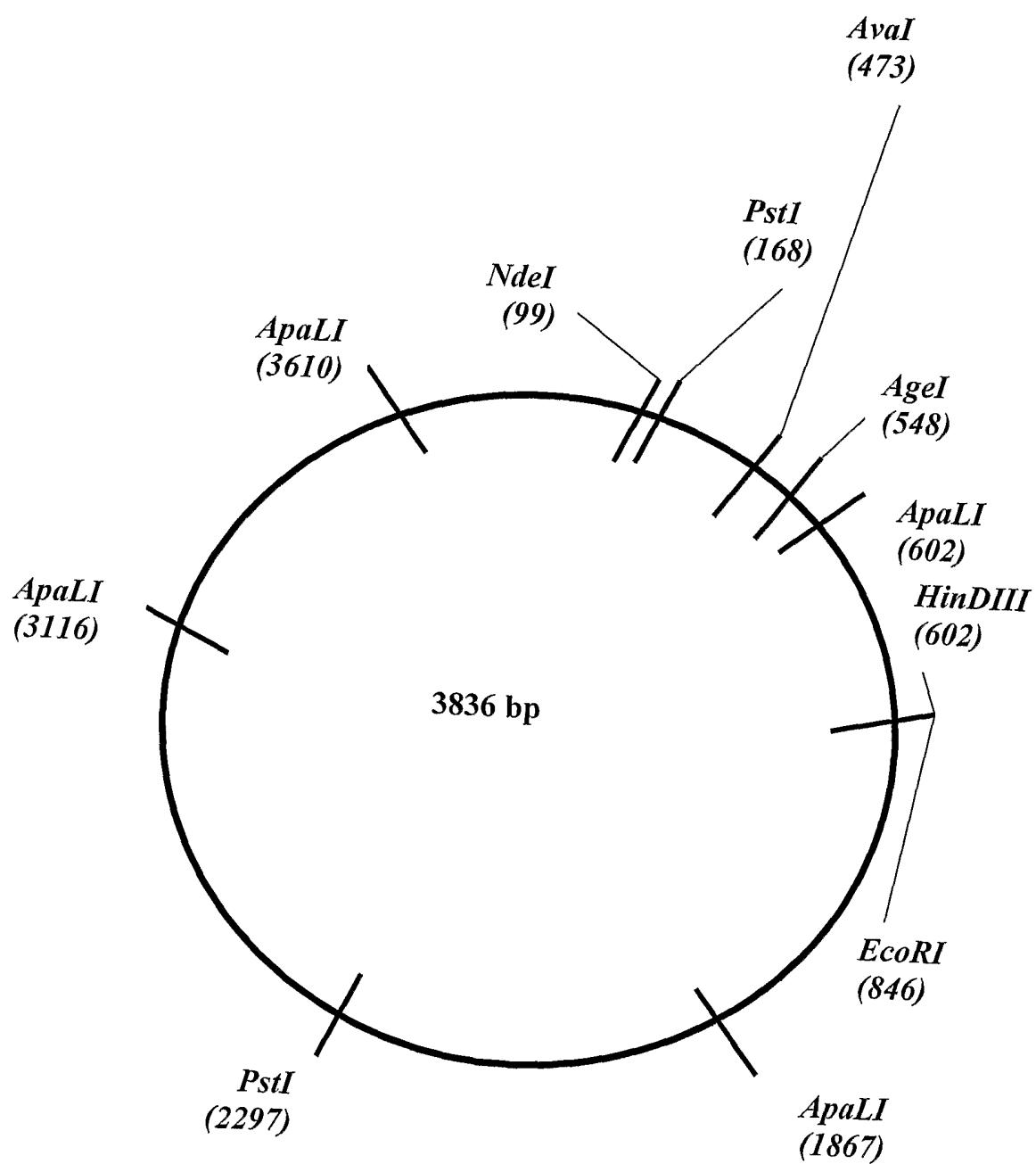
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Figure 13



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Figure 14



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Figure 15a

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(SEQ ID NO: 25)

Figure 15b

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(SEQ ID NO: 26)

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Figure 16a

M M K S L R V L L V I L W L Q L S W V W S Q Q K E V E Q N S G P L S V
P E G A I A S L N C T Y S D R G S Q S F F W Y R Q Y S G K S P E L I M
F I Y S N G D K E D G R F T A Q L N K A S Q Y I S L L I R D S K L S D
S A T Y L C A V R T N S G Y A L N F G K G T S I L L V T P H I Q N P D P
A V Y Q L R D S K S S D K S V C L F T D F D S Q T N V S Q S K D S D V
Y I T D K T V L D M R S M D F K S N S A V A W S N K S D F A C A N A F
N N S I I P E D T F F P S P E S S C D V K L V E K S F E T D T N L N F
Q N L S V I G F R I L L L K V A G F N L L M T L R L W S S
(SEQ ID NO: 27)

Figure 16b

M G P G L L C W A L L C L L G A G L V D A G V T Q S P T H L I K T R G
Q Q V T L R C S P K S G H D T V S W Y Q Q A L G Q G P Q F I F Q Y Y E
E E E R Q R G N F P D R F S G H Q F P N Y S S E L N V N A L L L G D S
A L Y L C A S S D T V S Y E Q Y F G P G T R L T V T E D L K N V F P P
E V A V F E P S E A E I S H T Q K A T L V C L A T G F Y P D H V E L S
W W V N G K E V H S G V S T D P Q P L K E Q P A L N D S R Y C L S S R
L R V S A T F W Q N P R N H F R C Q V Q F Y G L S E N D E W T Q D R A
K P V T Q I V S A E A W G R A D C G F T S E S Y Q Q G V L S A T I L Y
E I L L G K A T L Y A V L V S A L V L M A M V K R K D S R G
(SEQ ID NO: 28)

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Figure 17a

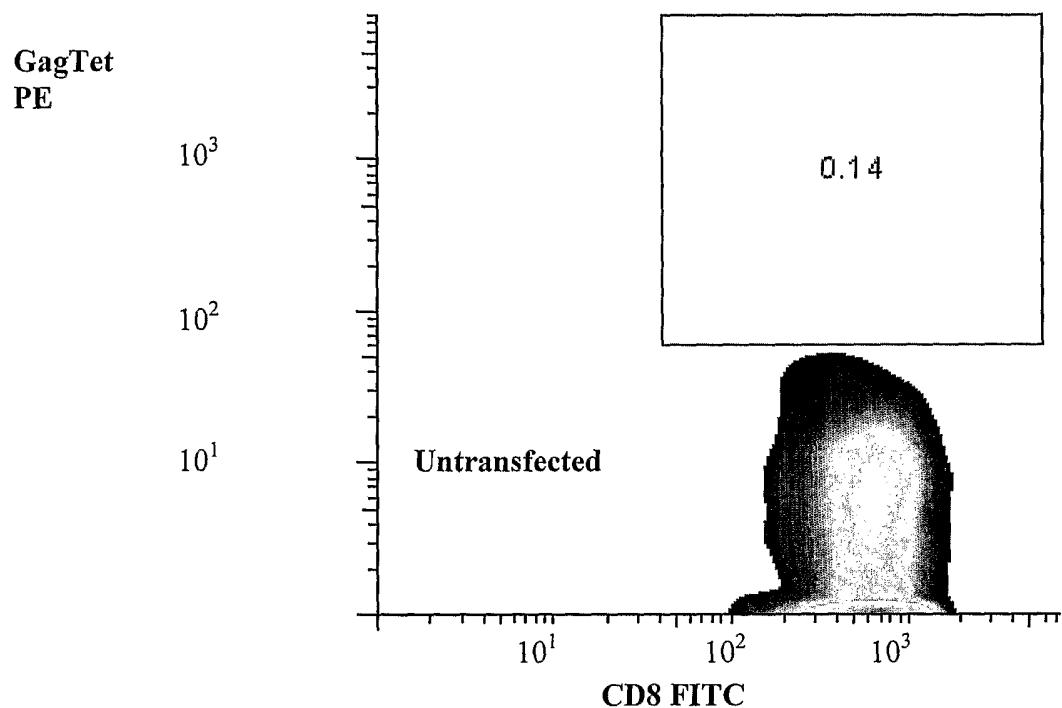


Figure 17b

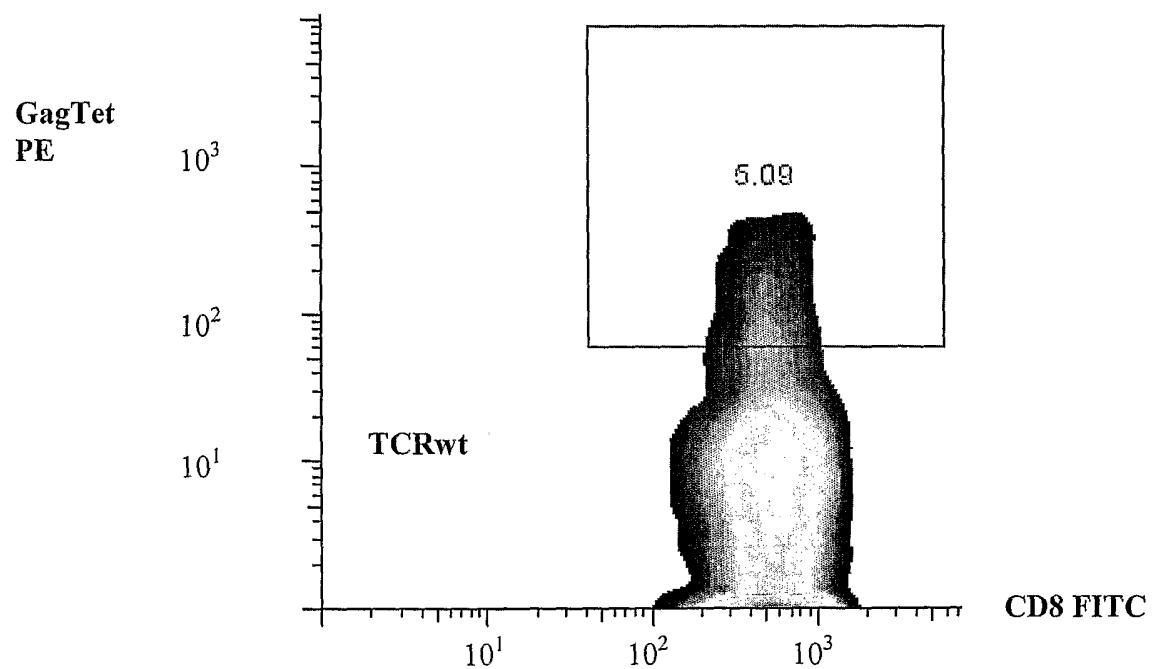


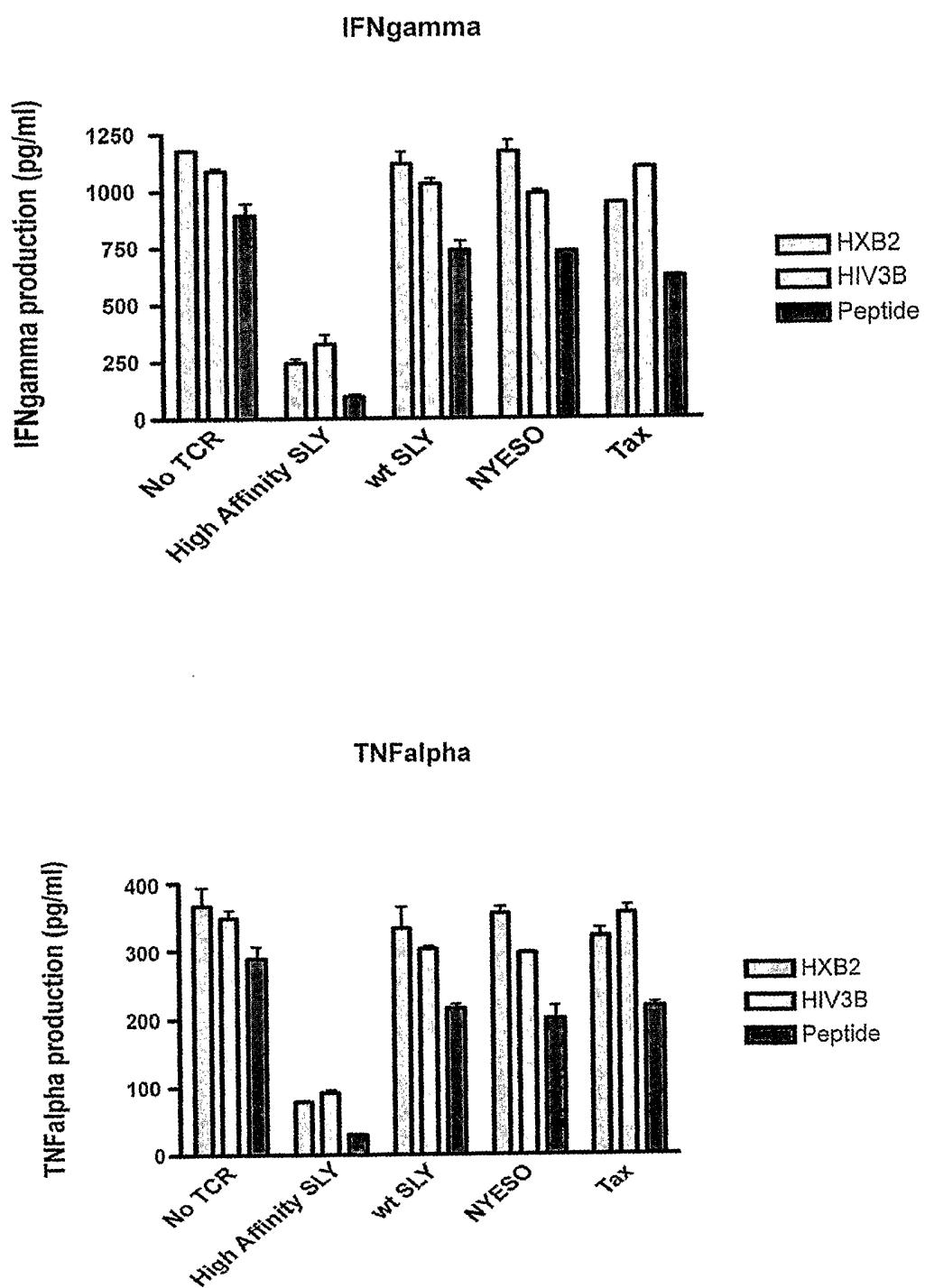
Figure 18a

M A Q K E V E Q N S G P L S V P E G A I A S L N C T Y S D R
G S Q S F F W Y R Q Y S G K S P E L I M F I Y S N G D K E D
G R F T A Q L N K A S Q Y I S L L I R D S K L S D S A T Y L
C A V R G A H D Y A L N F G K G T S L L V T P H I Q N P D P
A V Y Q L R D S K S S D K S V C L F T D F D S Q T N V S Q S
K D S D V Y I T D K C V L D M R S M D F K S N S A V A W S N
K S D F A C A N A F N N S I I P E D T F F P S P E S S
(SEQ ID NO: 29)

Figure 18b

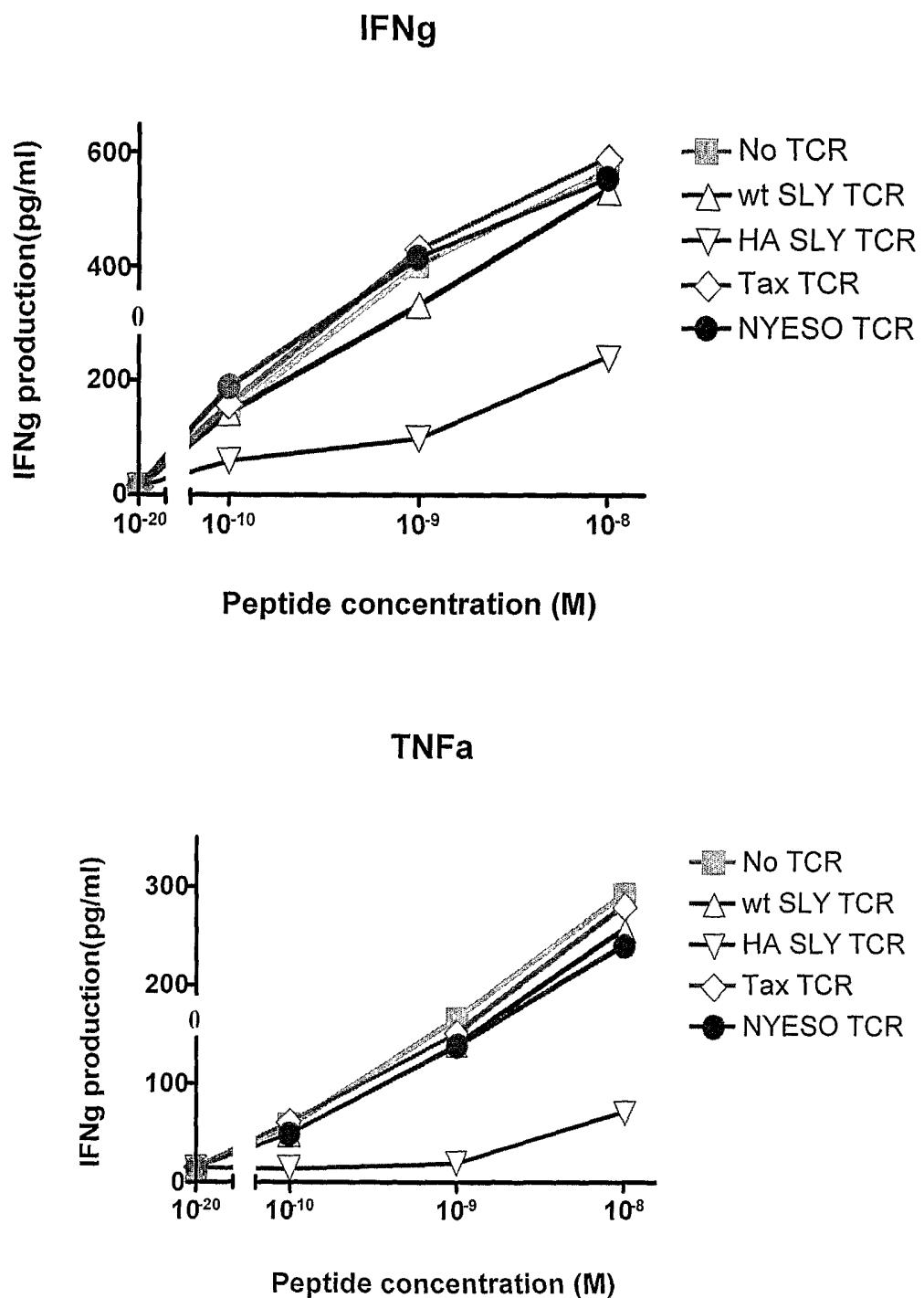
M E A G V T Q S P T H L I K T R G Q Q V T L R C S P K S G H
D T V S W Y Q Q A L G Q G P Q F I F Q Y A L G E E R Q R G N
F P D R F S G H Q F P N Y S S E L N V N A L L L G D S A L Y
L C A S S D T V S Y E Q Y F G P G T R L T V T E D L K N V F
P P E V A V F E P S E A E I S H T Q K A T L V C L A T G F Y
P D H V E L S W W V N G K E V H S G V C T D P Q P L K E Q P
A L N D S R Y A L S S R L R V S A T F W Q D P R N H F R C Q
V Q F Y G L S E N D E W T Q D R A K P V T Q I V S A E A W G
R A D
(SEQ ID NO: 30)

Figure 19



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Figure 20



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Figure 21

