surface of an immune effector cell, wherein the immune effector cell expresses an activating receptor, and

(B) a second epitope-binding domain, the second epitope-binding domain being capable of immunospecifically binding to an epitope of an antigen expressed by a cell infected with a virus; wherein the antigen is detectably present on the cell infected by the virus at a level that is greater than the level at which the antigen is detected on the virus by the bispecific molecule.

[0023] The disclosure further concerns the embodiment of such bispecific molecule, wherein the first epitope-binding domain binds an activating receptor of the effector cell.

[0023a] In a first aspect, there is provided a bispecific molecule comprising:

(A) a first epitope-binding domain, said first epitope-binding domain being capable of immunospecifically binding to an epitope of an activating receptor of an immune effector cell, wherein said immune effector cell is a T-cell, a CD4+ T-cell, a CD8+ T-cell, a natural killer cell, a macrophage, a granulocyte, or a dendritic cell; and

(B) a second epitope-binding domain, said second epitope-binding domain being capable of immunospecifically binding to an epitope of an antigen expressed by a cell infected with a virus, wherein said virus is EBV, HSV, CMV, HIV, HBV, HCV, HPV, or influenza virus, and wherein said antigen is detectably present on said cell infected by said virus at a level that is greater than the level at which said antigen is detected on said virus by said bispecific molecule.

[0024] The disclosure further concerns any of the above-described bispecific molecules, wherein the effector cell is a T-cell, a CD4+ T-cell, a CD8+ T-cell, a natural killer cell, a macrophage, a granulocyte, or a dendritic cell.

[0025] The disclosure further concerns any of the above-described bispecific molecules, wherein the virus is Epstein-Barr virus, herpes simplex virus type 1, herpes simplex virus type 2, cytomegalovirus, human immunodeficiency virus, hepatitis B virus, hepatitis C virus, human papilloma virus or influenza virus.

[0026] The disclosure further concerns any of the above-described bispecific molecules, wherein the cell is latently infected with the virus.

[0027] The disclosure further concerns any of the above-described bispecific molecules, wherein the antigen expressed by the cell infected with the virus is selected from the group consisting of LMP-1, LMP-2, influenza M_2 protein, HIV env protein, HPV E6 and HPV E7.

[0028] The disclosure further concerns any of the above-described bispecific molecules, wherein the antigen expressed by the cell infected with the virus is detectably present on the cell and is not detected on the virus by the bispecific molecule.

[0029] The disclosure further concerns any of the above-described bispecific molecules, wherein the epitope on the effector cell is a CD3 epitope, a CD4 epitope, a CD8 epitope, a CD2 epitope, a CD16 epitope, or an NKG2D epitope.

[0051] The disclosure further concerns methods of killing a cell expressing a viral protein, the method comprises the step of contacting the cell with a bispecific molecule, the bispecific molecule comprising:

(A) a first epitope-binding domain, the first epitope-binding domain being capable of immunospecifically binding to a protein expressed on the surface of an immune effector cell, wherein the immune effector cell expresses an epitope of an activating receptor of an effector cell, and

(B) a second epitope-binding domain, the second epitope-binding domain being capable of immunospecifically binding to an epitope of an antigen expressed by a cell infected with a virus; wherein the antigen is detectably present on the cell infected by the virus at a level that is greater than the level at which the antigen is detected on the virus by the bispecific molecule.

[0052] The disclosure further concerns the embodiment of such method, wherein the first epitope-binding domain of the bispecific molecule binds an activating receptor of the effector cell.

[0052a] In a fourth aspect, there is provided an *in vitro* method of killing a cell containing a viral genome, or a cell expressing a viral protein, said method comprising the step of contacting said cell with any of the above-described bispecific molecules, whereby said cell containing said viral genome, or expressing said viral protein is killed.

[0052b] In a fifth aspect, there is provided use of any of the above-described bispecific molecules, in the manufacture of a medicament for killing a cell containing a viral genome, or a cell expressing a viral protein, whereby said cell containing said viral genome, or expressing said viral protein is killed upon contact with said bispecific molecule.

[0052c] In a sixth aspect, there is provided a bispecific diabody comprising first and second polypeptide chains, wherein:

I. the first polypeptide chain comprises, in the N-terminal to C-terminal direction:

(i) a domain (A) comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for an HIV epitope;

(ii) a domain (B) comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for a CD3 epitope; and

(iii) a domain (C);

II. the second polypeptide chain comprises, in the N-terminal to C-terminal direction:

(i) a domain (D) comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for the CD3 epitope;

(ii) a domain (E) comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) specific for the HIV epitope;

(iii) a domain (F);

wherein:

(1) domains (A) and (B) do not associate with one another to form an epitope-binding domain;

(2) domains (D) and (E) do not associate with one another to form an epitope-binding domain;

(3) domains (A) and (E) associate to form an epitope-binding domain that binds the HIV epitope, and domains (B) and (D) associate to form an epitope-binding domain that binds the CD3 epitope,

(4) domains (C) and (F) are covalently associated together; and

(5) (i) said VL1 comprises CDR1, CDR2 and CDR3 of the VL of anti-HIV antibody A32;

(ii) said VH1 comprises CDR1, CDR2 and CDR3 of the VH of said anti-HIV antibody A32;

(iii) said VL2 comprises CDR1, CDR2 and CDR3 of the VL of humanized anti-CD3 Antibody 2; and

(iv) said VH2 comprises CDR1, CDR2 and CDR3 of the VH of said humanized anti-CD3 Antibody 2.

[0052d] In a seventh aspect, there is provided a bispecific diabody comprising first and second polypeptide chains, wherein:

I. the first polypeptide chain comprises, in the N-terminal to C-terminal direction:

(i) a domain (A) comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for an HIV epitope;

(ii) a domain (B) comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for a CD3

epitope; and

(iii) a domain (C);

II. the second polypeptide chain comprises, in the N-terminal to C-terminal direction:

(i) a domain (D) comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for the CD3 epitope;

(ii) a domain (E) comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) specific for the HIV epitope;

(iii) a domain (F);

wherein:

(1) domains (A) and (B) do not associate with one another to form an epitope-binding domain;

(2) domains (D) and (E) do not associate with one another to form an epitope-binding domain;

(3) domains (A) and (E) associate to form an epitope-binding domain that binds the HIV epitope, and domains (B) and (D) associate to form a epitope-binding domain that binds the CD3 epitope,

(4) domains (C) and (F) are covalently associated together; and

(5) (i) said VL1 comprises CDR1, CDR2 and CDR3 of the VL of anti-HIV antibody 7B2;

(ii) said VH1 comprises CDR1, CDR2 and CDR3 of the VH of said anti-HIV antibody 7B2;

(iii) said VL2 comprises CDR1, CDR2 and CDR3 of the VL of humanized anti-CD3 Antibody 2; and

(iv) said VH2 comprises CDR1, CDR2 and CDR3 of the VH of said humanized anti-CD3 Antibody 2.

[0052e] In an eighth aspect, there is provided a bispecific diabody comprising first, second and third polypeptide chains, wherein:

I. the first polypeptide chain comprises, in the N-terminal to C-terminal direction:

(i) a domain (A) comprising a binding region of a light chain variable

domain of a first immunoglobulin (VL1) specific for an HIV epitope;

(ii) a domain (B) comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for a CD3 epitope; and

(iii) a domain (C) comprising a cysteine residue and an E coil or a K coil;

II. the second polypeptide chain comprises, in the N-terminal to C-terminal direction:

(i) a domain (D) comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for the CD3 epitope;

(ii) a domain (E) comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) specific for the HIV epitope;

(iii) a domain (F) comprising:

(1) a cysteine residue and a K coil if the first polypeptide comprises an E coil; or

(2) a cysteine residue and an E coil if the first polypeptide comprises a K coil;

III. the third polypeptide chain comprises a CH2-CH3 region of an Fc region; and

wherein:

(1) (i) the first polypeptide chain further comprises a CH2-CH3 region of an Fc

region C-terminal to domain (C); or

(ii) the second polypeptide chain further comprises a CH2-CH3 region of an Fc region C-terminal to domain (F);

(2) domains (A) and (B) do not associate with one another to form an epitope-binding domain;

(3) domains (D) and (E) do not associate with one another to form an epitope-binding domain;

(4) domains (A) and (E) associate to form an epitope-binding domain that binds the HIV epitope, and domains (B) and (D) associate to form an epitope-binding domain that binds the CD3 epitope;

(5) domains (C) and (F) are covalently associated together by a disulfide bond;

and

(6) (i) said VL1 comprises CDR1, CDR2 and CDR3 of the VL of anti-HIV antibody A32;

(ii) said VH1 comprises CDR1, CDR2 and CDR3 of the VH of said anti-HIV antibody A32;

(iii) said VL2 comprises CDR1, CDR2 and CDR3 of the VL of humanized anti-CD3 Antibody 2; and

(iv) said VH2 comprises CDR1, CDR2 and CDR3 of the VH of said humanized anti-CD3 Antibody 2.

[0052f] In a ninth aspect, there is provided a bispecific diabody comprising first, second and third polypeptide chains, wherein:

I. the first polypeptide chain comprises, in the N-terminal to C-terminal direction:

(i) a domain (A) comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for an HIV epitope;

(ii) a domain (B) comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for a CD3 epitope; and

(iii) a domain (C) comprising a cysteine residue and an E coil or a K coil;

II. the second polypeptide chain comprises, in the N-terminal to C-terminal direction:

(i) a domain (D) comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for the CD3 epitope;

(ii) a domain (E) comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) specific for the HIV epitope;

(iii) a domain (F) comprising:

(1) a cysteine residue and a K coil if the first polypeptide comprises an E coil; or

(2) a cysteine residue and an E coil if the first polypeptide comprises a K coil;

III. the third polypeptide chain comprises a CH2-CH3 region of an Fc region; and

wherein:

(1) (i) the first polypeptide chain further comprises a CH2-CH3 region of an Fc region C-terminal to domain (C); or

(ii) the second polypeptide chain further comprises a CH2-CH3 region of an Fc region C-terminal to domain (F);

(2) domains (A) and (B) do not associate with one another to form an epitope-binding domain;

(3) domains (D) and (E) do not associate with one another to form an epitope-binding domain;

(4) domains (A) and (E) associate to form an epitope-binding domain that binds the HIV epitope, and domains (B) and (D) associate to form an epitope-binding domain that binds the CD3 epitope;

(5) domains (C) and (F) are covalently associated together by a disulfide bond; and

(6) (i) said VL1 comprises CDR1, CDR2 and CDR3 of the VL of anti-HIV antibody 7B2;

(ii) said VH1 comprises CDR1, CDR2 and CDR3 of the VH of said anti-HIV antibody 7B2;

(iii) said VL2 comprises CDR1, CDR2 and CDR3 of the VL of humanized anti-CD3 Antibody 2; and

(iv) said VH2 comprises CDR1, CDR2 and CDR3 of the VH of said humanized anti-CD3 Antibody 2.

[0052g] In a tenth aspect, there is provided a pharmaceutical composition comprising the bispecific diabody of any one of the sixth to ninth aspects, and a pharmaceutically acceptable carrier.

[0052h] In an eleventh aspect, there is provided a use of the bispecific diabody of any one of the sixth to ninth aspects, or the pharmaceutical composition of the tenth aspect, in the manufacture of a medicament for treating a latent HIV infection, or a persistent HIV infection, or an inactive HIV infection in an individual in need of such treatment.

[0052i] In a twelfth aspect, there is provided a use of the bispecific diabody of any one of the sixth to ninth aspects, or the pharmaceutical composition of the tenth aspect, in the manufacture of a medicament for killing a cell containing an HIV genome, or a cell expressing an HIV protein.

[0052j] In a thirteenth aspect, there is provided a composition comprising the bispecific diabody of any one of the sixth to ninth aspects, for use in treating a latent HIV infection, or a persistent HIV infection, or an inactive HIV infection.

[0052k] In a fourteenth aspect, there is provided a composition comprising the bispecific diabody of the sixth to ninth aspects, for use in killing a cell containing an HIV genome, or a cell expressing an HIV protein.

Detailed Description of the Invention:

[0053] The present invention relates to bispecific molecules that are capable of localizing an immune effector cell that expresses an activating receptor to a virally infected cell, so as to thereby facilitate the killing of the virally infected cell. In a preferred embodiment, such localization is accomplished using bispecific molecules that are immunoreactive both to an activating receptor of an immune effector cell and to an epitope of an antigen expressed by a cell infected with a virus. The present invention additionally concerns the use of such bispecific molecules in the treatment of latent viral infections, persistent viral infections and inactive viral infections, and the use of such bispecific molecules in methods to kill cells containing a viral genome or cell expressing a viral protein. The invention particularly concerns bispecific molecules that bind to (1) an epitope of an activating receptor of an immune effector cell and (2) an epitope of an antigen expressed by a cell infected with a virus wherein the antigen is detectably present on the cell infected by the virus at a level that is greater than the level at which the antigen is detected on the virus by the bispecific molecules and to such bispecific molecules that are capable of mediating, and more preferably enhancing, the activation and targeting of the immune effector cells to the cell infected by the virus such that the activated immune effector cells kill the cell infected by the virus.

(III) a K coil domain (*i.e.*, $(KVAALKE)_4$;. KVAALKEKVAALKEKVAALKEKVAALKE; **SEQ ID NO:40**) or an E coil domain (*i.e.*, $(EVAALEK)_4$; EVAALEKEVAALEKEVAALEKEVAALEK; **SEQ ID NO:39**).

[00168] In a preferred embodiment, a positively charged, "E-coil" will be appended to one of the polypeptides being used to form the bispecific diabody and a negatively charged "K-coil" will be appended to the second of the diabody's polypeptides.

[00169] Preferably, the first polypeptide chain of such a diabody has the amino acid sequence (**SEQ ID NO:42**) (CDRs are underlined):

DIVMTQSPDS LAVSPGERAT IHC**KSSQTLL YSSNNRHSIA** WYQQRPGQPP
KLLLY**WASMR LS**GVPDRFSG SGSGTDFTLT INNLQAEDVA IYYC**HQYSSH**
PPTFGHGTRV EIKGGGSGGG GQVTLRESGP ALVKPTQTLT LTCTFSGFSL
S**TSGMGVG**WI RQPPGKALEW LA**HIWWDDDK RYNPALKS**RL TISKDTSKNQ
VVLTMTNMDP VDTATYYCAQ **INPAWFAY**WG QGTLVTVSSG GCGGGEVAAL
EKEVAALEKE VAALEKEVAAL EK

wherein residues 1-113 are the light chain variable domain of antibody 7B2 (GenBank Accession No. AFQ31503) (CDR residues are shown in underline), residues 114-121 are the linker GGGSGGGG (**SEQ ID NO:27**), residues 122-239 are the heavy chain variable domain of antibody h3G8 (CDR residues are shown in underline), residues 240-245 are the linker GGCGGG (**SEQ ID NO:38**) and residues 246-277 are the E coil $(EVAALEK)_4$ [*i.e.* (**SEQ ID NO:39)** EVAALEKEVAALEKEVAALEKEVAALEK].

[00170] Preferably, the second polypeptide chain of such a diabody has the amino acid sequence (**SEQ ID NO:43**) (CDRs are underlined):

DIVMTQSPDS LAVSLGERAT INC**KASQSVD FDGDSFMN**WY QQKPGQPPKL
LIY**TTSNLES** GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YC**QQSNEDPY**
TFGQGTKLEI KGGGSGGGGQ VQLVQSGGGV FKPGGSLRLS CEASGFTFT**E**
YYMTWVRQAP GKGLEWLA**YI SKNGEYSKYS PSSNG**RFTIS RDNAKNSVFL
QLDRLSADDT AVYYCAR**ADG LTYFSELLQY IFDL**WGQGAR VTVSSGGCGG
GKVAALKEKV AALKEKVAAL KEKVAALKE

wherein residues 1-111 are the light chain variable domain of antibody h3G8 (CDR residues are shown in underline), residues 112-119 are the linker GGGSGGGG (**SEQ ID NO:27**), residues 120-245 are the heavy chain variable domain of antibody 7B2 (GenBank Accession No. AFQ31502) (CDR residues are shown in underline), residues 246-251 are the linker GGCGGG (**SEQ ID NO:38**) and residues 252-279 are the K coil $(KVAALKE)_4$ [*i.e.*, **SEQ ID NO:40**) KVAALKEKVAALKEKVAALKEKVAALKE].

(II) the heavy chain variable domain of such antibody A32 or such antibody that competes with antibody A32 for binding, or such antibody that binds to the gp120 protein of HIV; and

(III) a K coil domain (*i.e.*, $(KVAALKE)_4$;. KVAALKEKVAALKEKVAALKEKVAALKE; **SEQ ID NO:40**) or an E coil domain (*i.e.*, $(EVAALEK)_4$; EVAALEKEVAALEKEVAALEKEVAALEK; **SEQ ID NO:39**).

[00182] In a preferred embodiment, a positively charged, "E-coil" will be appended to one of the polypeptides being used to form the bispecific diabody and a negatively charged "K-coil" will be appended to the second of the diabody's polypeptides.

[00183] Preferably, the first polypeptide chain of such a diabody has the amino acid sequence (**SEQ ID NO:46**) (CDRs are underlined):

QSALTQPPSA SGSPGQSVTI SC**TGTSSDVG** **GYNYVS**WYQH HPGKAPKLII
S**EVNNRPS**GV PDRFSGSKSG NTASLTVSGL QAEDEAEYYC **SSYTDIHNFV**
FGGGTKLTVL GGGSGGGGEV QLVESGGGLV QPGGSLRLSC AASGFTFS**TY**
AMNWVRQAPG KGLEWVG**RIR** **SKYNNYATYY** **ADSVKG**RFTI SRDDSKNSLY
LQMNSLKTED TAVYYCVR**HG** **NFGNSYVSWF** **AY**WGQGTLVT VSSGGCGGGE
VAALEKEVAA LEKEVAALEK EVAALEK

wherein residues 1-110 are the light chain variable domain of antibody A32 (CDR residues are shown in underline), residues 111-118 are the linker GGGSGGGG (**SEQ ID NO:27**), residues 119-243 are the heavy chain variable domain of humanized anti-CD3 Antibody 2 ("hAntibody 2; CDRs are shown underlined), residues 247-252 are the linker GGCGGG (**SEQ ID NO:38**) and residues 253-280 are the E coil $(EVAALEK)_4$ [*i.e.* (**SEQ ID NO:39)** EVAALEKEVAALEKEVAALEKEVAALEK].

[00184] Preferably, the second polypeptide chain of such a diabody has the amino acid sequence (**SEQ ID NO:47**) (CDRs are underlined):

QAVVTQEPSL TVSPGGTVTL TC**RSSTGAVT** **TSNYAN**WVQQ KPGQAPRGLI
G**GTNKRAP**WT PARFSGSLLG GKAALTITGA QAEDEADYYC **ALWYSNLWV**F
GGGTKLTVLG GGGSGGGGQV QLQESGPGLV KPSQTLSLSC TVSGGSSS**SG**
AHYWSWIRQY PGKGLEWIG**Y** **IHYSGNTYYN** **PSLKS**RITIS QHTSENQFSL
KLNSVTVADT AVYYCAR**GTR** **LRTLRNAFDI** WGQGTLVTVS SGGCGGGKVA
ALKEKVAALK EKVAALKEKV AALKE

wherein residues 1-110 are the light chain variable domain of anti-CD3 Antibody 2 (CDR residues are shown in underline), residues 111-118 are the linker GGGSGGGG (**SEQ ID**

[00187] In a preferred embodiment, a positively charged, "E-coil" will be appended to one of the polypeptides being used to form the bispecific diabody and a negatively charged "K-coil" will be appended to the second of the diabody's polypeptides.

[00188] Preferably, the first polypeptide chain of such a diabody has the amino acid sequence (**SEQ ID NO:48**) (CDRs are underlined):

DIVMTQSPDS LAVSPGERAT IHC**KSSQTLL YSSNNRHSIA** WYQQRPGQPP
KLLLY**WASMR LS**GVPDRFSG SGSGTDFTLT INNLQAEDVA IYYC**HQYSSH**
PPTFGHGTRV EIKGGGSGGG GEVQLVESGG GLVQPGGSLR LSCAASGFTF
S**TYAMN**WVRQ APGKGLEWVG **RIRSKYNNYA TYYADSVKG**R FTISRDDSKN
SLYLQMNSLK TEDTAVYYCV R**HGNFGNSYV SWFAY**WGQGT LVTVSSGGCG
GGEVAALEKE VAALEKEVAA LEKEVAALEK

wherein residues 1-113 are the light chain variable domain of antibody 7B2 (GenBank Accession No. AFQ31503) (CDR residues are shown in underline), residues 114-121 are the linker GGGSGGGG (**SEQ ID NO:27**), residues 122-246 are the heavy chain variable domain of humanized anti-CD3 Antibody 2 ("hAntibody 2;" CDRs are shown underlined), residues 247-252 are the linker GGCGGG (**SEQ ID NO:38**) and residues 253-280 are the E coil $(EVAALEK)_4$ [*i.e.* (**SEQ ID NO:39)** EVAALEKEVAALEKEVAALEKEVAALEK].

[00189] Preferably, the second polypeptide chain of such a diabody has the amino acid sequence (**SEQ ID NO:49**) (CDRs are underlined):

QAVVTQEPSL TVSPGGTVTL TC**RSSTGAVT TSNYAN**WVQQ KPGQAPRGLI
G**GTNKRAP**WT PARFSGSLLG GKAALTITGA QAEDEADYYC **ALWYSNLWV**F
GGGTKLTVLG GGGSGGGGQV QLVQSGGGVF KPGGSLRLSC EASGFTFT**EY**
YMTWVRQAPG KGLEWLA**YIS KNGEYSKYSP SSNG**RFTISR DNAKNSVFLQ
LDRLSADDTA VYYCAR**ADGL TYFSELLQYI FDL**WGQGARV TVSSGGCGGG
KVAALKEKVA ALKEKVAALK EKVAALKE

wherein residues 1-110 are the light chain variable domain of antibody Anti-CD3 Antibody 2 (CDR residues are shown in underline), residues 111-118 are the linker GGGSGGGG (**SEQ ID NO:27**), residues 119-244 are the heavy chain variable domain of antibody 7B2 (GenBank Accession No. AFQ31502) (CDR residues are shown in underline), residues 245-250 are the linker GGCGGG (**SEQ ID NO:38**) and residues 251-278 are the K coil $(KVAALKE)_4$ [*i.e.*, **SEQ ID NO:40**) KVAALKEKVAALKEKVAALKEKVAALKE].

Anti-Fluorescein x anti-HIV env Bispecific Molecule

[00195] The first polypeptide chain of the bispecific diabody preferably has:

(I) the variable light domain of antibody 7B2 or an antibody that competes with antibody 7B2 for binding, or an antibody that binds to the env protein of HIV;

(II) the heavy chain variable domain of anti-fluorescein antibody 4-4-20 or an antibody that competes with anti-fluorescein antibody 4-4-20 for binding, or an antibody that binds to fluorescein; and

(III) an E coil domain (*i.e.*, $(EVAALEK)_4$; EVAALEKEVAALEKEVAALEKEVAALEK; **SEQ ID NO:39**) or a K coil domain (*i.e.*, $(KVAALKE)_4$;. KVAALKEKVAALKEKVAALKEKVAALKE; **SEQ ID NO:40**).

[00196] The second polypeptide chain of such bispecific diabody preferably has:

(I) the light chain variable domain of anti-fluorescein antibody 4-4-20 or such antibody that competes with antibody 4-4-20 for binding, or such antibody that binds to fluorescein;

(II) the variable heavy domain of such antibody 7B2 or such antibody that competes with antibody 7B2 for binding, or such antibody that binds to the env protein of HIV; and

(III) a K coil domain (*i.e.*, $(KVAALKE)_4$;. KVAALKEKVAALKEKVAALKEKVAALKE; **SEQ ID NO:40**) or an E coil domain (*i.e.*, $(EVAALEK)_4$; EVAALEKEVAALEKEVAALEKEVAALEK; **SEQ ID NO:39**).

[00197] In a preferred embodiment, a positively charged, "E-coil" will be appended to one of the polypeptides being used to form the bispecific diabody and a negatively charged "K-coil" will be appended to the second of the diabody's polypeptides.

[00198] Preferably, the first polypeptide chain of such a diabody has the amino acid sequence (**SEQ ID NO:52**) (CDRs are underlined):

DIVMTQSPDS LAVSPGERAT IHC**KSSQTLL YSSNNRHSIA** WYQQRPGQPP
KLLLY**WASMR LS**GVPDRFSG SGSGTDFTLT INNLQAEDVA IYYC**HQYSSH**
PPTFGHGTRV EIKGGGSGGG GEVKLDETGG GLVQPGRPMK LSCVASGFTF
S**DYWM**NWVRQ SPEKGLEWVA **QIRNKPYNYE TYYSDSVKG**R FTISRDDSKS
SVYLQMNNLR VEDMGIYYCT G**SYYGMDY**WG QGTSVTVSSG GCGGGEVAAL
EKEVAALEKE VAALEKEVAA LEK

wherein residues 1-113 are the light chain variable domain of antibody 7B2 (GenBank Accession No. AFQ31503) (CDR residues are shown in underline), residues 114-121 are the linker GGGSGGGG (**SEQ ID NO:27**), residues 122-239 are the heavy chain variable domain of antibody 4-4-20, residues 240-245 are the linker GGCGGG (**SEQ ID NO:38**) and residues 246-277 are the E coil $(EVAALEK)_4$ [*i.e.* (**SEQ ID NO:39)** EVAALEKEVAALEKEVAALEKEVAALEK].

[00199] Preferably, the second polypeptide chain of such a diabody has the amino acid sequence (**SEQ ID NO:53**) (CDRs are underlined):

DVVMTQTPFS LPVSLGDQAS ISC**RSSQSLV HSNGNTYLR**W YLQKPGQSPK
VLIY**KVSNRF S**GVPDRFSGS GSGTDFTLKI SRVEAEDLGV YFC**SQSTHVP**
WTFGGGTKLE IKGGGSGGGG QVQLVQSGGG VFKPGGSLRL SCEASGFTFT
EYYMTWVRQA PGKGLEWLA**Y ISKNGEYSKY SPSSNG**RFTI SRDNAKNSVF
LQLDRLSADD TAVYYCAR**AD GLTYFSELLQ YIFDL**WGQGA RVTVSSGGCG
GGKVAALKEK VAALKEKVAA LKEKVAALKE

wherein residues 1-112 are the light chain variable domain of antibody 4-4-20, residues 113-120 are the linker GGGSGGGG (**SEQ ID NO:27**), residues 121-246 are the heavy chain variable domain of antibody 7B2 (CDR residues are shown in underline), residues 247-252 are the linker GGCGGG (**SEQ ID NO:38**) and residues 253-280 are the K coil $(KVAALKE)_4$ [*i.e.*, **SEQ ID NO:40**) KVAALKEKVAALKEKVAALKEKVAALKE].

Example 8
Redirected CD8-Mediated Killing of HIV env-Expressing Cells

[00200] As a further example of the utility of the bispecific molecules of the present invention, the above-described anti-CD3 x anti-HIV gp120 and anti-CD3 x anti-HIV env bispecific molecules were evaluated for their ability to facilitate redirected CD8-mediated killing of HIV env-expressing cells in the presence of T cells.

[00201] The above-described anti-fluorescein x anti-HIV env and anti-CD3 x anti-RSV F protein bispecific molecules were used as controls.

[00202] In one such investigation, Jurkat 522 FY (HxB2 gp160) cells were incubated in the presence of tetracycline in order to induce their expression of HIV gp160. The cells were incubated for 24 hours in the presence of pan T cells (D54670) (at an Effector : Target ratio of 10:1) and one of the above-described bispecific molecules. The cytotoxicity of the

What Is Claimed Is:

1. A bispecific molecule comprising:

   (A) a first epitope-binding domain, said first epitope-binding domain being capable of immunospecifically binding to an an epitope of an activating receptor of an immune effector cell, wherein said immune effector cell is a T-cell, a CD4+ T-cell, a CD8+ T-cell, a natural killer cell, a macrophage, a granulocyte, or a dendritic cell; and

   (B) a second epitope-binding domain, said second epitope-binding domain being capable of immunospecifically binding to an epitope of an antigen expressed by a cell infected with a virus, wherein said virus is EBV, HSV, CMV, HIV, HBV, HCV, HPV, or influenza virus, and wherein said antigen is detectably present on said cell infected by said virus at a level that is greater than the level at which said antigen is detected on said virus by said bispecific molecule.

2. The bispecific molecule of claim 1, wherein said cell is latently infected with said virus.

3. The bispecific molecule of claim 1 or 2, wherein said antigen expressed by said cell infected with said virus is selected from the group consisting of LMP-1, LMP-2, influenza virus M_2 protein, HIV env protein, HPV E6 and HPV E7.

4. The bispecific molecule of any one of claims 1-3, wherein said antigen expressed by said cell infected with said virus is detectably present on said infected cell by said bispecific molecule and is not detected on said virus by said bispecific molecule.

5. The bispecific molecule of any one of claims 1-4, wherein said epitope on said effector cell is a CD3 epitope, a CD4 epitope, a CD8 epitope, a CD2 epitope, a CD16 epitope, a TCR epitope, or an NKG2D epitope.

6. The bispecific molecule of any one of claims 1-5, wherein said first epitope-binding domain is an epitope-binding domain from a CD3 antibody, a CD4 antibody, a CD8 antibody, a CD2 antibody, a CD16 antibody, a TCR antibody, or an NKG2D antibody.

7. The bispecific molecule of any one of claims 1-3, wherein:

(1) said antigen expressed by said cell infected with said virus is detectably present on said cell at a level that is at least 2 times greater than the level at which said antigen is detected on said virus by said bispecific molecule; or

(2) said antigen expressed by said cell infected with said virus is detectably present on said cell at a level that is at least 5 times greater than the level at which said antigen is detected on said virus by said bispecific molecule; or

(3) said antigen expressed by said cell infected with said virus is detectably present on said cell at a level that is at least 10 times greater than the level at which said antigen is detected on said virus by said bispecific molecule; or

(4) said antigen expressed by said cell infected with said virus is detectably present on said cell at a level that is at least 100 times greater than the level at which said antigen is detected on said virus by said bispecific molecule.

8. The bispecific molecule of any one of claims 1-7, wherein said antigen expressed by said cell infected with said virus is detectably present on said infected cell by said bispecific molecule and is not detected by said bispecific molecule on a cell not infected with said virus.

9. The bispecific molecule of any one of claims 1-8, wherein said molecule is a diabody, said diabody being a covalently linked complex comprising at least two polypeptide chains.

10. The bispecific molecule of any one of claims 1-9, wherein said molecule comprises an Fc domain or portion thereof.

11. The bispecific molecule of claim 9, wherein said molecule is composed of three polypeptide chains, wherein:

(A) the first polypeptide chain comprises, in the N-terminal to C-terminal direction:

(i) a domain (A) comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for an epitope (1);

(ii) a domain (B) comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for an epitope (2); and

(iii) a domain (C) comprising a cysteine residue, and an E coil or a K coil;

(B) the second polypeptide chain comprises, in the N-Terminal to C-terminal direction:

(i) a domain (D) comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for epitope (2);

(ii) a domain (E) comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) specific for epitope (1);

(iii) a domain (F) comprising:

(1) a cysteine residue and a K coil if the first polypeptide has an E coil; or

(2) a cysteine residue and an E coil if the first polypeptide has a K coil; and

(iv) a CH2-CH3 region of an Fc region;

(C) the third polypeptide chain comprises a CH2-CH3 region of an Fc region; and

(D) (i) domains (A) and (B) do not associate with one another to form an epitope-binding domain;

(ii) domains (D) and (E) do not associate with one another to form an epitope-binding domain;

(iii) domains (A) and (E) associate to form an epitope-binding domain that binds epitope (1), and domains (B) and (D) associate to form a epitope-binding domain that binds said epitope (2), wherein:

(a) epitope (1) is said epitope of an activating receptor of an immune effector cell, and epitope (2) is said epitope of an antigen expressed by a cell infected with a virus; or

(b) epitope (1) is said epitope of an antigen expressed by a cell infected with a virus, and epitope (2) is said epitope of an activating receptor of an immune effector cell; and

(iv) domains (C) and (F) are covalently associated together by a disulfide bond.

12. The bispecific molecule of any one of claims 1-11, wherein:

(A) said activating receptor is CD3, and said antigen expressed by a cell infected with a virus is EBV LMP-1; or

(B) said activating receptor is TCR, and said antigen expressed by a cell infected with a virus is EBV LMP-2; or

(C) said activating receptor is CD3, and said antigen expressed by a cell infected with a virus is HPV E6 in the context of MCH class I; or

(D) said activating receptor is CD16, and said antigen expressed by a cell infected with a virus is HIV env; or

(E) said activating receptor is CD3, and said antigen expressed by a cell infected with a virus is HIV gp120; or

(F) said activating receptor is CD3, and said antigen expressed by a cell infected with a virus is HIV env.

13. A pharmaceutical composition comprising the bispecific molecule of any one of claims 1-12, and a pharmaceutically acceptable carrier.

14. Use of the bispecific molecule of any one of claims 1-12, or the pharmaceutical composition of claim 13, in the manufacture of a medicament for treating a latent virus infection, or a persistent virus infection, or an inactive virus infection in an individual in need of such treatment.

15. An *in vitro* method of killing a cell containing a viral genome, or a cell expressing a viral protein, said method comprising the step of contacting said cell with the bispecific molecule of any one of claims 1-12, whereby said cell containing said viral genome, or expressing said viral protein is killed.

16. Use of the bispecific molecule of any one of claims 1-12, in the manufacture of a medicament for killing a cell containing a viral genome, or a cell expressing a viral protein, whereby said cell containing said viral genome, or expressing said viral protein is killed upon contact with said bispecific molecule.

17. A bispecific diabody comprising first and second polypeptide chains, wherein:

I. the first polypeptide chain comprises, in the N-terminal to C-terminal direction:

(i) a domain (A) comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for an HIV epitope;

(ii) a domain (B) comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for a CD3

epitope; and

(iii) a domain (C);

II. the second polypeptide chain comprises, in the N-terminal to C-terminal direction:

(i) a domain (D) comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for the CD3 epitope;

(ii) a domain (E) comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) specific for the HIV epitope;

(iii) a domain (F);

wherein:

(1) domains (A) and (B) do not associate with one another to form an epitope-binding domain;

(2) domains (D) and (E) do not associate with one another to form an epitope-binding domain;

(3) domains (A) and (E) associate to form an epitope-binding domain that binds the HIV epitope, and domains (B) and (D) associate to form an epitope-binding domain that binds the CD3 epitope,

(4) domains (C) and (F) are covalently associated together; and

(5) (i) said VL1 comprises CDR1, CDR2 and CDR3 of the VL of anti-HIV antibody A32;

(ii) said VH1 comprises CDR1, CDR2 and CDR3 of the VH of said anti-HIV antibody A32;

(iii) said VL2 comprises CDR1, CDR2 and CDR3 of the VL of humanized anti-CD3 Antibody 2; and

(iv) said VH2 comprises CDR1, CDR2 and CDR3 of the VH of said humanized anti-CD3 Antibody 2.

18. The bispecific diabody of claim 17, wherein:

(i) said domain (C) comprises a cysteine residue and an E coil or a K coil; and

(ii) said domain (F) comprises a K coil if said domain (C) comprises an E coil, or an E coil if said domain (C) comprises a K coil.

19. A bispecific diabody comprising first and second polypeptide chains, wherein:

I. the first polypeptide chain comprises, in the N-terminal to C-terminal direction:

(i) a domain (A) comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for an HIV epitope;

(ii) a domain (B) comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for a CD3 epitope; and

(iii) a domain (C);

II. the second polypeptide chain comprises, in the N-terminal to C-terminal direction:

(i) a domain (D) comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for the CD3 epitope;

(ii) a domain (E) comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) specific for the HIV epitope;

(iii) a domain (F);

wherein:

(1) domains (A) and (B) do not associate with one another to form an epitope-binding domain;

(2) domains (D) and (E) do not associate with one another to form an epitope-binding domain;

(3) domains (A) and (E) associate to form an epitope-binding domain that binds the HIV epitope, and domains (B) and (D) associate to form a epitope-binding domain that binds the CD3 epitope,

(4) domains (C) and (F) are covalently associated together; and

(5) (i) said VL1 comprises CDR1, CDR2 and CDR3 of the VL of anti-HIV antibody 7B2;

(ii) said VH1 comprises CDR1, CDR2 and CDR3 of the VH of said anti-HIV antibody 7B2;

(iii) said VL2 comprises CDR1, CDR2 and CDR3 of the VL of humanized anti-CD3 Antibody 2; and

(iv) said VH2 comprises CDR1, CDR2 and CDR3 of the VH of said

humanized anti-CD3 Antibody 2.

20. The bispecific diabody of claim 19, wherein:

(i) said domain (C) comprises a cysteine residue and an E coil or a K coil; and

(ii) said domain (F) comprises a K coil if said domain (C) comprises an E coil, or an E coil if said domain (C) comprises a K coil.

21. A bispecific diabody comprising first, second and third polypeptide chains, wherein:

I. the first polypeptide chain comprises, in the N-terminal to C-terminal direction:

(i) a domain (A) comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for an HIV epitope;

(ii) a domain (B) comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for a CD3 epitope; and

(iii) a domain (C) comprising a cysteine residue and an E coil or a K coil;

II. the second polypeptide chain comprises, in the N-terminal to C-terminal direction:

(i) a domain (D) comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for the CD3 epitope;

(ii) a domain (E) comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) specific for the HIV epitope;

(iii) a domain (F) comprising:

(1) a cysteine residue and a K coil if the first polypeptide comprises an E coil; or

(2) a cysteine residue and an E coil if the first polypeptide comprises a K coil;

III. the third polypeptide chain comprises a CH2-CH3 region of an Fc region; and wherein:

(1) (i) the first polypeptide chain further comprises a CH2-CH3 region of an Fc

region C-terminal to domain (C); or

(ii) the second polypeptide chain further comprises a CH2-CH3 region of

an Fc region C-terminal to domain (F);

(2) domains (A) and (B) do not associate with one another to form an epitope-binding domain;

(3) domains (D) and (E) do not associate with one another to form an epitope-binding domain;

(4) domains (A) and (E) associate to form an epitope-binding domain that binds the HIV epitope, and domains (B) and (D) associate to form an epitope-binding domain that binds the CD3 epitope;

(5) domains (C) and (F) are covalently associated together by a disulfide bond; and

(6) (i) said VL1 comprises CDR1, CDR2 and CDR3 of the VL of anti-HIV antibody A32;

(ii) said VH1 comprises CDR1, CDR2 and CDR3 of the VH of said anti-HIV antibody A32;

(iii) said VL2 comprises CDR1, CDR2 and CDR3 of the VL of humanized anti-CD3 Antibody 2; and

(iv) said VH2 comprises CDR1, CDR2 and CDR3 of the VH of said humanized anti-CD3 Antibody 2.

22. A bispecific diabody comprising first, second and third polypeptide chains, wherein:

I. the first polypeptide chain comprises, in the N-terminal to C-terminal direction:

(i) a domain (A) comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for an HIV epitope;

(ii) a domain (B) comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for a CD3 epitope; and

(iii) a domain (C) comprising a cysteine residue and an E coil or a K coil;

II. the second polypeptide chain comprises, in the N-terminal to C-terminal direction:

(i) a domain (D) comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for the CD3 epitope;

(ii) a domain (E) comprising a binding region of a heavy chain variable

domain of the first immunoglobulin (VH1) specific for the HIV epitope;

(iii) a domain (F) comprising:

(1) a cysteine residue and a K coil if the first polypeptide comprises an E coil; or

(2) a cysteine residue and an E coil if the first polypeptide comprises a K coil;

III. the third polypeptide chain comprises a CH2-CH3 region of an Fc region; and

wherein:

(1) (i) the first polypeptide chain further comprises a CH2-CH3 region of an Fc

region C-terminal to domain (C); or

(ii) the second polypeptide chain further comprises a CH2-CH3 region of an Fc region C-terminal to domain (F);

(2) domains (A) and (B) do not associate with one another to form an epitope-binding domain;

(3) domains (D) and (E) do not associate with one another to form an epitope-binding domain;

(4) domains (A) and (E) associate to form an epitope-binding domain that binds the HIV epitope, and domains (B) and (D) associate to form an epitope-binding domain that binds the CD3 epitope;

(5) domains (C) and (F) are covalently associated together by a disulfide bond; and

(6) (i) said VL1 comprises CDR1, CDR2 and CDR3 of the VL of anti-HIV antibody 7B2;

(ii) said VH1 comprises CDR1, CDR2 and CDR3 of the VH of said anti-HIV antibody 7B2;

(iii) said VL2 comprises CDR1, CDR2 and CDR3 of the VL of humanized anti-CD3 Antibody 2; and

(iv) said VH2 comprises CDR1, CDR2 and CDR3 of the VH of said humanized anti-CD3 Antibody 2.

23. A pharmaceutical composition comprising the bispecific diabody of any one of claims 17-22, and a pharmaceutically acceptable carrier.

24. Use of the bispecific diabody of any one of claims 17-22, or the pharmaceutical composition of claim 23, in the manufacture of a medicament for treating a latent HIV infection, or a persistent HIV infection, or an inactive HIV infection in an individual in need of such treatment.

25. Use of the bispecific diabody of any one of claims 17-22, or the pharmaceutical composition of claim 23, in the manufacture of a medicament for killing a cell containing an HIV genome, or a cell expressing an HIV protein.

26. A composition comprising the bispecific diabody of any one of claims 17-22, for use in treating a latent HIV infection, or a persistent HIV infection, or an inactive HIV infection.

27. A composition comprising the bispecific diabody of any one of claims 17-22, for use in killing a cell containing an HIV genome, or a cell expressing an HIV protein.

28. The use of claim 24 or 25, or the composition for use according to claim 26 or 27, wherein said cell infected with said HIV is latently infected with said HIV.