binding domains of antibody 7B2 and the anti-fluorescein epitope binding domains of antibody 4-4-20 was used as a control.

[0020] **Figure 7** shows the ability of bispecific diabodies comprising the anti-CD3 epitope binding domains of antibody hAntibody 2 and either the anti-HIV gp120 epitope binding domains of antibody A32 or the anti-HIV env epitope binding domains of antibody 7B2 to facilitate redirected CD8-mediated killing of HIV gp140-expressing HEK 293 D371 cells (CM244, subtype AE, R5-tropic) in the presence of pan T cells (D47239). The Effector : Target ratio was 10:1. A bispecific diabody comprising the anti-HIV env epitope binding domains of antibody 7B2 and the anti-fluorescein epitope binding domains of antibody 4-4-20 was used as a control.

Summary:

[0021] The present disclosure 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 disclosure 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 disclosure 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.

[0022] In detail, the disclosure concerns a bispecific molecule comprising:

(A) a first epitope-binding domain, the first epitope-binding domain being capable of immunospecifically binding to an epitope of a protein expressed on the

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 disclsure 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.

[0030] The disclosure further concerns any of the above-described bispecific molecules, wherein the first antigen binding domain is an antigen binding domain from a CD3 antibody, a CD4 antibody, a CD8 antibody, a CD2 antibody, a CD16 antibody, or an NKG2D antibody.

[0031] 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 at a level that is at least 2 times greater than the level, if any, at which the antigen is detected on the virus by the bispecific molecule.

[0032] 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 at a level that is at least 5 times greater than the level, if any, at which the antigen is detected on the virus by the bispecific molecule.

[0033] 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 at a level that is at least 10 times greater than the level, if any, at which the antigen is detected on the virus by the bispecific molecule.

[0034] 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 at a level that is at least 100 times greater than the level, if any, at which the antigen is detected on the virus by the bispecific molecule.

[0035] The disclosure further concerns any of the above-described bispecific molecules, 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 by the bispecific molecule on a cell that is not infected by the virus.

[0036] The disclosure further concerns any of the above-described bispecific molecules, wherein the antigen is detectably present on the cell infected by the virus at a level that is at least 2 times greater than the level at which the antigen is detected by the bispecific molecule on a cell that is not infected by the virus.

[0037] The disclosure further concerns any of the above-described bispecific molecules, wherein the antigen is detectably present on the cell infected by the virus at a level that is at

least 5 times greater than the level at which the antigen is detected by the bispecific molecule on a cell that is not infected by the virus.

[0038] The disclosure further concerns any of the above-described bispecific molecules, wherein the antigen is detectably present on the cell infected by the virus at a level that is at least 10 times greater than the level at which the antigen is detected by the bispecific molecule on a cell that is not infected by the virus.

[0039] The disclosure further concerns any of the above-described bispecific molecules, wherein the antigen is detectably present on the cell infected by the virus at a level that is at least 100 times greater than the level at which the antigen is detected by the bispecific molecule on a cell that is not infected by the virus.

[0040] 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 a cell not infected with the bispecific molecule.

[0040a] In a second aspect, there is provided a pharmaceutical composition comprising any of the above-described bispecific molecules, and a pharmaceutically acceptable carrier.

[0041] The disclosure further concerns a pharmaceutical composition comprising a bispecific molecule and a pharmaceutically acceptable carrier; the bispecific molecule comprising:

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

[0042] The disclosure further concerns the embodiment of such pharmaceutical composition, wherein the first epitope-binding domain binds an activating receptor of the effector cell.

[0042a] In a third aspect, there is provided use of any of the above-described bispecific molecules, or the pharmaceutical composition of the second aspect, 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.

[0043] The disclosure further concerns a method of treating a latent virus infection in an individual in need of such treatment, the method comprising the step of administering a

therapeutically effective amount of a bispecific molecule to the individual, 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 said 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.

[0044] 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.

[0045] The disclosure further concerns a method of treating a persistent virus infection in an individual in need of such treatment, the method comprising the step of administering a therapeutically effective amount of a bispecific molecule to the individual, 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.

[0046] 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.

[0047] The disclosure further concerns methods of treating an inactive virus infection in an individual in need of such treatment, the method comprising the step of administering a therapeutically effective amount of a bispecific molecule to the individual, 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.

[0048] 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.

[0049] The disclosure further concerns methods of killing a cell containing a viral genome, 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.

[0050] 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.

[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.

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.

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 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 cell 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 cell and is not detected on a cell not infected by said bispecific molecule.

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.