The subject invention provides novel soluble PD-1 (sPD-1) proteins, nucleic acids, and fusion constructs thereof, for enhancing humoral and cell-mediated immunity of a subject. Also provided are therapeutic compositions comprising the sPD-1 proteins, nucleic acids, and fusion constructs of the subject invention. In a preferred embodiment, the therapeutic composition is formulated as a vaccine composition. Advantageously, the sPD-1 proteins, nucleic acids, and therapeutic compositions provide protective immunity against pathogenic infection including HIV infection. In addition, the subject invention can be used in the prevention and/or treatment of tumor or cancer.
FIG. 1A
1--- Molecular weight marker
2--- negative control
3--- mspd1-p24-fc
4--- mspd1-lgVΔ-p24-fc
5--- p24-fc

FIG. 1B
FIG. 2A

a  mspd1-p24-fc
b  mspd1-p24-lgv  &  fc
  c-p24-fc

- negative control
- samples

Samples
Negative control
FIG. 2B

a

b

Negative control

Samples

c

Negative control

Samples

% of Max

% of Max

% of Max

10^0 10^1 10^2 10^3

10^0 10^1 10^2 10^3

10^0 10^1 10^2 10^3

a-mspd1-p24-fc
b-mspd1-p24-lgv Δ-fc
c-p24-fc

negative control
samples
FIG. 3A
FIG. 3B
a-p24-fc; b-mspd1-p24-fc; c-mspd1-lgv Δ-p24-fc; d-PBS

FIG. 3C
FIG. 3D
FIG. 9A
FIG. 9C

a-p24-fc  b-mspd1-14del-p24-fc  c-mspd1-322mu-p24-fc  d-PBS
FIG. 9D
FIG. 10
FIG. 11A

FIG. 11B

negative
sample
FIG. 12
FIG. 13A

FIG. 13B
1. p24-fc
2. sPD-1-p24-fc
3. sIgVPD-1-p24-fc
4. Ladder
5. Negative control

FIG. 16A

Counts

sPD-1p24-fc  slgVPD-1-p24-fc  p24-fc

PD-L1

PD-L2

A=Cells treated with expression constructs
B=Positive Control

FIG. 16B
FIG. 17C

HIV-1 p24 antibody titer

* *

IgG1

IgG2a

DC-sPD1-p24-fc

DC-sIgV+PD-1-p24-fc

FIG. 17D

IFN-γ ELISpot /10⁶ spleenocytes

P=0.17

P=0.08

DC-sPD-1-p24-fc

DC-sIgV+PD-1-p24-fc

DC only

FIG. 17E

Tetramer+ CD8+ T-cells (%)

P=0.12

DC-sPD-1-p24-fc

DC-sIgV+PD-1-p24-fc

DC only
FIG. 18A

FIG. 18B

FIG. 18C
FIG. 19A

PD-L1

PD-L2

Anti-rabbit Fc

A=Cells treated with hu-sPD-1-p-24-fc protein
B=Positive Control

FIG. 19B

HIV-1 p24 antibody titer

LgG1
LgG2a

p24-fc
hu-sPD-1-p24-fc

FIG. 19C

IFN-γ/10^6 spleenocytes

CD4
CD8

p24-fc
hu-sPD-1-p24-fc
PBS
SOLUBLE PD-1 VARIANTS, FUSION CONSTRUCTS, AND USES THEREOF
CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. provisional application Ser. No. 11/412,557, filed Nov. 11, 2010, which is herein incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

[0002] Programmed death 1 (PD-1), expressed primarily on T cells, is a receptor for B7-H1 molecule (also known as programmed death ligand 1 (PD-L1)) and B7-DC molecule (also known as programmed death ligand 2 (PD-L2)). PD-L1 is expressed on many different cell types, whereas PD-L2 is expressed only on antigen-presenting cells such as B cells, dendritic cells and macrophages.

[0003] The PD-1/PD-L pathway, which transmits negative signals to immune cells, plays a critical role in the modulation of immune responses during infection and cancer. The interaction of PD-1 with PD-L1/L2 inhibits T cell function during HIV infection. A recent study suggested that the blockade of PD-1 during chronic simian immunodeficiency virus (SIV) infection by anti-PD-1 antibody resulted in enhanced B cell responses as well as rapid expansion and restoration of SIV-specific polyfunctional CD8 T cells. Other studies suggested that the blockade of the PD-1/PD-L pathway facilitates the restoration of humoral and cell-mediated immune responses during LCMV and HBV infection.

[0004] Human immunodeficiency virus type 1 (HIV-1) has contributed to an estimated 25 million deaths since it was first recognized in 1981. Currently, over 33 million people worldwide are living with the virus. One of the existing HIV vaccine compositions, obtained by fusing HIV-1 p24 to DSC-205 antibody, enhances CD4 T cell immune responses and cytokine release. In addition, this vaccine composition confers protection against vaccine-gag viral challenge. However, this HIV vaccine composition does not improve Th1 CD8 T cell response. Thus, improved HIV-1 vaccine compositions that enhance host immunity and protect against HIV infection are needed. As will be clear from the disclosure that follows, these and other benefits are provided by the present invention.

BRIEF SUMMARY OF THE INVENTION

[0005] The subject invention provides soluble PD-1 (sPD-1) proteins and nucleic acids, and therapeutic compositions comprising sPD-1 proteins and nucleic acids, for enhancing immunity of a subject. In one embodiment, the sPD-1 proteins, nucleic acids, and compositions are formulated as a vaccine composition.

[0006] One aspect of the subject invention provides sPD-1 protein variants. In an embodiment, the sPD-1 protein variant is mspld-14d1e1, which has an amino acid sequence comprising SEQ ID NO: 11. In an embodiment, the sPD-1 protein variant is mspld-1322mu, which has an amino acid sequence comprising SEQ ID NO: 15. In an embodiment, the sPD-1 protein variant is hspd-14d1e1, which was found in healthy Chinese people. The hspd-14d1e1 variant has an amino acid sequence comprising SEQ ID NO: 25.

[0007] Another aspect of the invention provides nucleic acid molecules that encode the sPD-1 proteins of the subject invention. In an embodiment, the nucleic acid molecule encodes mspld-14d1e1, and has a sequence comprising SEQ ID NO: 12. In an embodiment, the nucleic acid molecule encodes mspl-322mu, and has a sequence comprising SEQ ID NO: 16. In an embodiment, the nucleic acid molecule encodes hspd-14d1e1, and has a sequence comprising SEQ ID NO: 26.

[0008] In addition, the subject invention provides sPD-1 fusion proteins. In specific embodiments, the subject sPD-1 fusion protein comprises SEQ ID NO: 13, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 23, or SEQ ID NO: 27. The subject invention also provides sPD-1 fusion nucleic acid molecules. In specific embodiments, the subject sPD-1 fusion DNA comprises SEQ ID NO: 14, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 24, or SEQ ID NO: 28.

[0009] Another aspect of the subject invention provides methods for the prevention and/or treatment of pathogenic infection, cancer or tumor, and other diseases in which induction of antigen-specific protective immunity would be beneficial. Advantageously, the methods of the subject invention enhance host humoral and cell-mediated immunity. The method comprises administering to a subject in need of such treatment an effective amount of a fusion protein or fusion nucleic acid molecule of the subject invention. In a preferred embodiment, the subject method can be used in the prevention and treatment of HIV or other pathogen infection. In addition, the methods can be used in the prevention and/or treatment of tumor or cancer.

[0010] The subject invention further provides for therapeutic or pharmaceutical compositions. In an embodiment, the composition comprises a therapeutically effective amount of a protein and/or nucleic acid molecule of the subject invention and, optionally, a pharmaceutically acceptable carrier. In a preferred embodiment, the therapeutic composition is a vaccine composition.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] FIG. 1A shows alignment of amino acid sequences of mspld-1-p24-Fc, mspld-1-lgVA-p24-Fc and p24-Fc fusion proteins. FIG. 1B shows Western blot analysis of mspld-1-p24-Fc, mspld-1-lgVA-p24-Fc and p24-Fc. Proteins are detected by anti-rabbit Fc antibody.

[0012] FIG. 2 shows the binding ability of mspld-1-p24-Fc fusion proteins to sPD-1 ligands. (A) shows the binding ability of mspld-1-p24-Fc, mspld-1-lgVA-p24-Fc and p24-Fc to mouse PD-L1, respectively. (B) shows the binding ability of mspld-1-p24-Fc, mspld-1-lgVA-p24-Fc and p24-Fc to mouse PD-L2, respectively.

[0013] FIG. 3 shows that wild-type sPD1 DNA elicits humoral and cell-mediated immune responses against HIV p24. (A) shows serum levels of anti-p24 IgG1 and IgG2a antibodies in mice immunized with p24-Fc, mspld-1-p24-Fc and mspld-1-lgVA-p24-Fc fusion DNA, respectively. Bars represent the average values of three samples (standard deviations). (B) shows the number of IFN-γ-secreting splenocytes specific for p24 epitope gag/Al (AMQMI.LKD1) for CD8 T cells. Bars represent the average values of three samples (standard deviations). (C) shows images of splenocytes isolated from immunized mice. To analyze p24-specific immune response, splenocytes were stained with H122-Kd-AMQMI.LKD1-PE tetramer for CD8 T cell population analysis. (D) shows the number of IFN-γ-secreting splenocytes specific for p24 epitope gag26 (TSNPPPPGDIYKRWWLGL) for CD4 T cells. Bars represent the average values of three samples.
Data represent three experiments on the same batch of immunized mice.

**0014** FIG. 4 shows that immunization with wild-type msPD1 fusion proteins protects mice against viral infection. BALB/c mice immunized with p24-Fc, msPd1-p24-Fc or msPd1-lgGΔA-p24-Fc were challenged with 4x10^5 PFU of vaccinia VTT-HIV-gagpol intranasally three weeks after the last immunization. The mice were sacrificed 3 days after viral challenge.

**0015** Viral titers in lungs were evaluated by plaque-forming assay in Vero cells. Bars represent the average values of five samples (±standard deviations).

**0016** FIG. 5 shows that targeting DCs using sPD-1-p24-fc induces enhanced p24-specific antibody and T cell responses. (A) Expression of PD-L1 and PD-L2 on purified CD11c+ BM-DCs isolated from BALB/c mice were confirmed by flow cytometric analysis using anti-mouse PD-L1 or L2 antibodies (solid line, not shaded). Cells stained with isotype antibody control are shown as shaded histograms. (B) BM-DCs treated with purified msPD1-p24-fc and msPd1-lgGΔA-p24-fc proteins to examine binding. Proteins bound to DCs were detected by flow cytometry using an anti-rabbit Fc-FITC antibody (solid line, not shaded) in parallel to DCs without treatment of proteins as negative control (shaded). 2x10^6 DCs treated with 20 μg of msPD1-p24-fc or msPd1-lgGΔA-p24-fc proteins were introduced to BALB/c mouse by tail vein injection once every three weeks for a total experimental duration of six weeks. Mice that received untreated CD11c+ DCs served as control. (C) Mice sera were collected and analyzed for the presence of IgG1 and IgG2a antibodies specific against HIV-1 p24 by ELISA. (D) IFN-γ producing CD8+ and CD4+ cells were measured by ELISPOT assay in mice splenocytes stimulated using specific peptides gagAl and gag26, respectively. H2-Kd-AMQMLKDTI-PE tetramer staining was performed on isolated splenocytes and analyzed by flow cytometry as a column graph of data from groups of immunized mice. Bars represent the mean values of two replicates with standard error depicted by error bars. Data are representative of two independent immunization experiments. *p<0.05.

**0017** FIG. 6 shows that hdsp1-p24-fc elicits humoral and cell-mediated immune responses against HIV-1 p24. (A) shows that hdsp1-p24-Fc binds to mouse PD-L1. (B) shows that hdsp1-p24-Fc binds to mouse PD-L2. (C) shows high sera levels of anti-p24 IgG1 and IgG2a antibodies in mice immunized with hdsp1-p24-Fc, when compared to mice immunized with p24-Fc. (D) shows the numbers of IFN-γ-secreting splenocytes specific for p24 epitope gagAl (AMQMLKDTI) for CD8 T cells and the numbers of IFN-γ-secreting splenocytes specific for p24 epitope gag26 (TNPPIIPPVGDYKRWILLGL) for CD4 T cells. Bars represent the average values of three samples (±standard deviations).

**0018** FIG. 7A shows alignment of amino acid sequences of msPd1-p24-fc, msPd1-14de1-p24-fc, msPd1-322mu-p24-fc, and p24-fc fusion proteins. FIG. 7B shows Western blot analysis of msPd1-14de1-p24-Fc, msPd1-322mu-p24-Fc, and p24-Fc proteins. Proteins are detected by anti-rabbit Fc antibody.

**0019** FIG. 8 shows the binding ability of msPd1-14de1-p24-Fc, msPd1-322mu-p24-Fc, and p24-fc fusion proteins to sPD-1 ligands, respectively. (A) shows the binding ability of msPd1-14de1-p24-Fc, msPd1-322mu-p24-Fc, and p24-Fc fusion proteins to PD-L1, respectively. (B) shows the binding ability of msPd1-14de1-p24-Fc, msPd1-322mu-p24-Fc, and p24-Fc fusion proteins to PD-L2, respectively.

**0020** FIG. 9 shows that variant sPD1 DNA elicits humoral and cell-mediated immune responses against HIV p24. (A) shows serum levels of anti-p24 IgG1 and IgG2a antibodies in mice immunized with msPd1-14de1-p24-Fc, msPd1-322mu-p24-Fc, and p24-Fc fusion DNA, respectively. Bars represent the average values of three samples (±standard deviations). (B) shows the number of IFN-γ-secreting splenocytes specific for p24 epitope gagAl (AMQMLKDTI) for CD8 T cells. Bars represent the average values of three samples (±standard deviations). (C) shows images of splenocytes isolated from immunized mice. To analyze p24-specific immune response, splenocytes were stained with H2-Kd-AMQMLKDTI-PE tetramer for CD8 T cell population analysis. (D) shows the number of IFN-γ-secreting splenocytes specific for p24 epitope gag26 (TNPPIIPPVGDYKRWILLGL) for CD4 T cells. Bars represent the average values of three samples (±standard deviations). Data represent three experiments on the same batch of immunized mice.

**0021** FIG. 10 shows that immunization with variant sPD1 DNA protects mice against viral infection. BALB/c mice immunized with msPd1-14de1-p24-Fc, msPd1-322mu-p24-Fc, and p24-Fc fusion DNA were challenged with 4x10^5 PFU of vaccinia VTT-HIV-gagpol intranasally three weeks after the last immunization. The mice were sacrificed 3 days after viral challenge. Viral titers in lungs were evaluated by plaque-forming assay in Vero cells. Bars represent the average values of five samples (±standard deviations).

**0022** FIG. 11 shows that hdsp1-14de1-p24-fc elicits humoral and cell-mediated immune responses against HIV-1 p24. (A) shows that hdsp1-14de1-p24-fc does not bind to mouse PD-L1. (B) shows that hdsp1-14de1-p24-fc does not bind to mouse PD-L2. (C) shows high sera levels of anti-p24 IgG1 and IgG2a antibodies in mice immunized with hdsp1-14de1-p24-Fc, when compared to mice immunized with p24-Fc. (D) shows the numbers of IFN-γ-secreting splenocytes specific for p24 epitope gagAl (AMQMLKDTI) for CD8 T cells and the numbers of IFN-γ-secreting splenocytes specific for p24 epitope gag26 (TNPPIIPPVGDYKRWILLGL) for CD4 T cells. Bars represent the average values of three samples (±standard deviations).

**0023** FIG. 12 shows the structures of various clones useful according to the subject invention.

**0024** FIG. 13 shows the induction of potent p24-specific immune responses by sPD1-14de1-fc vaccination. (A) Schematic representation of constructs encompassing the soluble form of PD-1 or with two amino acid deletions essential for binding with PD-L1/L2 (sIgV-PD-1), p24 and rabbit Fc under the CMV promoter. Rabbit Fc was used as a tag for purification purposes. (B) Mouse immunization schedule is depicted. BALB/c mice were immunized with sPD1-p24-fc, sIgV-PD-1-p24-fc and p24-fc at week 0, 3 and 6 at a low dose of 20 μg or a high dose of 100 μg i.m. with Alum. Mice that received PBS only served as a negative control. Mice sera and splenocytes were collected two weeks after the final immunization for analysis of antibody and T cell responses, respectively. (C) Detection of specific IgG1 and IgG2a antibodies against HIV-1 Gag p24 by ELISA two weeks post immunization. (D) Number of IFN-γ-secreting CD8+ and CD4+ T cells measured by ELISPOT in specific response to HIV-1 Gag p24 epitopes gagAl and gag26, respectively. (F) IFN-γ secreting cells in response to stimulation using three different peptide
pools derived from 59 peptides that spans the whole HIV-1 Gag p24. (G) Representative H2-Kd-AMQMKLKDTI-PE tetramer staining of CD8\(^+\) T cell population is shown in flow cytometric plots or data amalgamated into a column graph (H). Data are representative of three independent immunization experiments. *p<0.05, **p<0.01, ***p<0.001.

[0025] FIG. 14 shows polynucleotyol of sPD-1-p24-fc induced T cells. Balb/c mice were immunized with sPD-1-p24-fc and slgV-PD-1-p24-fc at a dose of 100 \(\mu\)g i.m./EP. Mice that received PBS alone served as control. Splenocytes were collected and analyzed by flow cytometry following intracellular staining using antibodies against IFN-\(\gamma\), TNF-\(\alpha\), and IL-2. (A) Scatter plots indicating CD8\(^+\) or CD4\(^+\) T cells positive for IFN-\(\gamma\) and (B) TNF-\(\alpha\). (C) Column graphs depicting single, double or triple positive CD8\(^+\) or (D) CD4\(^+\) T cells for the cytokines IFN-\(\gamma\), TNF-\(\alpha\), and IL-2. (E) Pie chart analysis representing subpopulations of total cytokine secreting CD8\(^+\) or CD4\(^+\) T cells positive for combinations of IFN-\(\gamma\), TNF-\(\alpha\), and IL-2. Columns represent the mean values of three replicate mice with standard error bars. Data are representative of two independent immunization experiments. *p<0.05, **p<0.01.

[0026] FIG. 15 shows that vaccination with sPD-1-p24-fc induces specific long lasting and protective immunity. Sera and splenocytes derived from mice 50 weeks after immunization were isolated and examined for antibody for CD8\(^+\) and CD4\(^+\) T cell responses. (A) Specific IgG1 and IgG2a antibodies against HIV-1 Gag p24 detected by ELISA. ELISPOT assays using specific HIV-1 Gag p24 epitope for (B) CD8\(^+\) T cells and (C) CD4\(^+\) T cells was performed to test the ability of T cells to produce IFN-\(\gamma\). Mice previously immunized with a dose of 100 \(\mu\)g DNA vaccines were challenged with 2x10\(^5\) PFUs of virulent WRgagpol three weeks post immunization to examine immune protection. Each group contained up to 5 mice. (D) Immunized mice were weighed daily for eight days after vaccinia challenge. (E) Virus titers in the lungs of immunized mice were evaluated by plaque formation on Vero cell monolayers.

[0027] FIG. 16 shows expression and binding characteristics of DNA vaccine constructs. (A) DNA vaccines encoding sPD-1, the mutated form slgV-PD-1, p24 and fc were tested for protein expression by Western blotting. Lower sized band represents p24-fc, while the higher sized band represents sPD-1-p24-fc or slgV-PD-1-p24-fc. (B) 293T cells were transiently transfected with PD-L1 or PD-L2 expression vectors, and the binding profiles of recombinant proteins were examined. Flow cytometric signals were obtained by treating the cells with purified proteins from the constructs followed by detection using anti-rabbit FC-FITC antibody. Controls included transfected 293T cells stained with anti-rabbit FC-FITC antibody (negative, shaded) or anti-mouse PD-L1 or PD-L2 antibodies (positive, solid line, not shaded).

[0028] FIG. 17 shows that targeting dendritic cells (DCs) using sPD-1-p24-fc induces enhanced p24-specific antibody and T cell responses. (A) Expression of PD-L1 and PD-L2 on purified DCs. (B) BM-DCs isolated from Balb/c mice was confirmed by flow cytometric analysis using anti-mouse PD-L1 or PD-L2 antibodies (solid line, not shaded). Cells stained with isotype antibody control are shown as shaded histogram. (B) BM-DCs treated with purified sPD-1-p24-fc and slgV-PD-1-p24-fc proteins to examine binding. Proteins bound to DCs were detected by flow cytometry using an anti-rabbit FC-FITC antibody (solid line, not shaded) in parallel to DCs without treatment of proteins as negative control (shaded). 2x10\(^6\) DCs treated with 20 \(\mu\)g of sPD-1-p24-fc or slgV-PD-1-p24-fc proteins were introduced to Balb/c mouse by tail vein injection once every three weeks for a total experimental duration of six weeks. Mice that received untreated CD11c\(^+\) DCs were served as control. (C) Mice sera were collected and analyzed for the presence of IgG1 and IgG2a antibodies specific against HIV-1 p24 by ELISA. (D) IFN-\(\gamma\) producing CD8\(^+\) and CD4\(^+\) cells were measured by ELISPOT assay in mice splenocytes stimulated using specific peptides gagAl and gag26, respectively. H2-Kd-AMQMKLKDTI-PE tetramer staining was performed on isolated splenocytes and analyzed by flow cytometry as a column graph of data from groups of immunized mice (F). Bars represent the mean values of two replicate mice with standard error depicted by error bars. Data are representative of two independent immunization experiments. *p<0.05.

[0029] FIG. 18 characterizes sPD-1-p24-fc DNA vaccination. CD8\(^+\) T cell ELISPOT assay of immunization strategy by i.m. (A) without electroporation (EP), (B) with purified p24-fc and/or sPD-1-fc with EP, or (C) using DNA vaccines without rabbit Fe tag with EP. All data points represent the mean ± standard error as error bars. *p<0.05.

[0030] FIG. 19 shows that human sPD-1-p24-fc elicits similar p24-specific immunity in mice. (A) Binding profiles of hu-sPD-1-p24-fc protein to murine PD-1 ligands transiently expressed on 293T cells. Flow cytometric signals were obtained by treating the cells with hu-CD11c-p24-fc protein followed by anti-rabbit FC-FITC antibody for detection. Controls included transfected 293T cells stained with anti-rabbit FC-FITC antibody (negative, shaded) or anti-mouse PD-L1 or PD-L2 antibodies (positive, solid line, not shaded). Balb/c mice were immunized with hu-sPD-1-p24-fc and at a dose of 20 \(\mu\)g i.m./EP, or received PBS only serving as a negative control. (B) Detection of specific IgG1 and IgG2a antibodies against HIV-1 Gag p24 by ELISA two weeks post immunization in mice sera. (C) Frequencies of IFN-\(\gamma\)-secreting CD8\(^+\) and CD4\(^+\) T cells in mice splenocytes measured by ELISPOT assay in specific response to HIV-1 Gag p24 epitopes specific for CD3\(^+\) and CD8\(^+\) T cells, respectively. Columns represent the mean values of three replicate mice with standard error as error bars. Data are representative of two independent immunization experiments. *p<0.05, **p<0.01, ***p<0.001.

BRIEF DESCRIPTION OF THE SEQUENCES

[0031] SEQ ID NO: 1 is an amino acid sequence of the wild-type soluble extracellular domain of mouse PD-1 (mouse spl). [0032] SEQ ID NO: 2 is a nucleic acid sequence of the wild-type mouse spl1 DNA. [0033] SEQ ID NO: 3 is an amino acid sequence of HIV p24 useful according to the subject invention. [0034] SEQ ID NO: 4 is a nucleic acid sequence of HIV p24 DNA useful according to the subject invention. [0035] SEQ ID NO: 5 is an amino acid sequence of rabbit Fe domain useful to the subject invention. [0036] SEQ ID NO: 6 is a nucleic acid sequence of rabbit Fe DNA useful to the subject invention. [0037] SEQ ID NO: 7 is an amino acid sequence of mspd1-Igα [0038] SEQ ID NO: 8 is a nucleic acid sequence of mspd1-Igα DNA. [0039] SEQ ID NO: 9 is an amino acid sequence of mspd1-Igα-p24-fc fusion protein.
[0040] SEQ ID NO: 10 is a nucleic acid sequence of mspd1-lgVA-p24-Fc fusion DNA.
[0041] SEQ ID NO: 11 is an amino acid sequence of mspd1-14de1.
[0042] SEQ ID NO: 12 is a nucleic acid sequence of mspd1-14de1 DNA.
[0043] SEQ ID NO: 13 is an amino acid sequence of mspd1-14de1-p24-Fc fusion protein.
[0044] SEQ ID NO: 14 is a nucleic acid sequence of mspd1-14de1-p24-Fc fusion DNA.
[0045] SEQ ID NO: 15 is an amino acid sequence of mspd1-322mu.
[0046] SEQ ID NO: 16 is a nucleic acid sequence of mspd1-322mu DNA.
[0047] SEQ ID NO: 17 is an amino acid sequence of mspd1-322mu-p24-Fc fusion protein.
[0048] SEQ ID NO: 18 is a nucleic acid sequence of mspd1-322mu-p24-Fc fusion DNA.
[0049] SEQ ID NO: 19 is an amino acid sequence of mspd1-p24-Fc fusion protein.
[0050] SEQ ID NO: 20 is a nucleic acid sequence of mspd1-p24-Fc fusion DNA.
[0051] SEQ ID NO: 21 is an amino acid sequence of the wild-type soluble extracellular domain of human PD-1 (human spd1).
[0052] SEQ ID NO: 22 is a nucleic acid sequence of the wild-type human spd1 DNA.
[0053] SEQ ID NO: 23 is an amino acid sequence of Hspd1-p24-Fc fusion protein.
[0054] SEQ ID NO: 24 is a nucleic acid sequence of Hspd1-p24-Fc fusion DNA.
[0055] SEQ ID NO: 25 is an amino acid sequence of Hspd1-14de1.
[0056] SEQ ID NO: 26 is a nucleic acid sequence of Hspd1-14de1 DNA.
[0057] SEQ ID NO: 27 is an amino acid sequence of Hspd1-14de1-p24-Fc fusion protein.
[0058] SEQ ID NO: 28 is a nucleic acid sequence of Hspd1-14de1-p24-Fc fusion DNA.
[0059] SEQ ID NO: 29 is an amino acid sequence of a linker sequence useful according to the subject invention.
[0060] SEQ ID NO: 30 is an amino acid sequence of a linker sequence useful according to the subject invention.
[0061] SEQ ID NO: 31 is an amino acid sequence of a linker sequence useful according to the subject invention.
[0062] SEQ ID NO: 32 is an amino acid sequence of a linker sequence useful according to the subject invention.
[0063] SEQ ID NO: 33 is an amino acid sequence of a linker sequence useful according to the subject invention.
[0064] SEQ ID NO: 34 is an amino acid sequence of a linker sequence useful according to the subject invention.
[0065] SEQ ID NO: 35 is an amino acid sequence of a linker sequence useful according to the subject invention.
[0066] SEQ ID NO: 36 is an amino acid sequence of a linker sequence useful according to the subject invention.
[0067] SEQ ID NO: 37 is an amino acid sequence of a linker sequence useful according to the subject invention.
[0068] SEQ ID NO: 38 is an amino acid sequence of a linker sequence useful according to the subject invention.
[0069] SEQ ID NO: 39 is an amino acid sequence useful according to the subject invention.

[0070] SEQ ID NO: 40 is an amino acid sequence useful according to the subject invention.

DETAILED DISCLOSURE OF THE INVENTION

[0071] The subject invention provides soluble PD-1 (sPD-1) proteins and nucleic acids, and therapeutic compositions comprising soluble PD-1 proteins and nucleic acids, useful for inducing antigen-specific protective immunity against infection and cancer. In one embodiment, the subject sPD-1 proteins, nucleic acids, and compositions are formulated as a vaccine composition. In an embodiment, the subject invention provides novel fusion proteins mspd1-p24Fc, mspd1-14de1-p24Fc, mspd1-322mu-p24Fc, and hspd1-14de1-p24Fc, and nucleic acid molecules encoding these fusion proteins.

[0072] The subject invention is based on the findings that the immune regulatory PD-1/PD-L pathway down-regulates HIV-1-specific CD8+ T cells responses. The present inventors discovered a natural variant of PD-1 present in healthy people that does not interact with either PD-L-1 or PD-L-2 (the ligands of PD-1). In addition, a point mutation, which is essential for PD-1 and its ligands interaction, is discovered.

[0073] In one embodiment, the subject invention provides a novel DNA vaccine design that mimics the binding of programmed death-1 (PD-1) to its ligands expressed on dendritic cells (DCs) for functional activation, by fusible soluble PD-1 with an antigen of interest. Intramuscular immunization via electroporation (EP) of the fusion DNA vaccine elicited robust anti-Gag antibody titers in mice, with both IgG1 (Th2) and IgG2a (Th1) responses detected. High frequencies of Gag-specific, broadly reactive and polyfunctional T cells, especially CD8+ T cells were elicited following immunization. These responses were dose-dependent, long lasting and conferred protection against intranasal challenge with virus-like vaccinia-Gag virus. Specifically, mspd-p24Fc, mspd1-14de1-p24Fc and mspd1-322mu-p24Fc enhance HIV-1 Gag-specific immune responses, as determined by the number of IFN-γ expressed CD4 and CD8 T cells using Elispot assays. Thus, soluble PD-1-based DNA/EP vaccination of the subject invention offers an easy, repeatable and effective way to induce durable and protective CD8+ cell immunity, which has important implications for vaccine development and gene therapy.

[0074] In one embodiment, the mspd1-14de1 protein variant is obtained by deleting amino acids 26-39 of the wild-type mspd1(Amino acids 26-39 are the first 14 amino acids encoded by the second exon of the wild-type mouse PD-1 gene. These 14 amino acids of mspd1 have the same sequence as the first 14 amino acids encoded by the second exon of the human hspd1-14de1 homologue). The mspd1-322mu protein variant is obtained by changing amino acid residue 108 of the wild-type PD-1 protein from Met to Val. The hspd1-14de1 variant, which is derived from a natural isoform of human PD-1, has a deletion of amino acids 26-39 of the wild-type hspd1 (encoded by the first part of the second exon of the wild-type human PD-1 gene).

[0075] The mspd1-p24Fc fusion protein binds to PD-1 ligands PD-L1 and PD-L2, and the binding of PD-1 to PD-L can be blocked by anti-PD-L1/L2 antibodies. It is postulated that the binding of mspd1-p24Fc fusion protein inhibits the PD-1/PD-L pathway, which transmits negative signals to immune cells. In comparison, none of mspd1-14de1-p24Fc, mspd1-322mu-p24Fc, and hspd1-14de1-p24Fc fusion proteins binds to PD-L1 or PD-L2. This indicates that amino acid
residues 26-39 encoded by DNA in exon 2 of spd1 and amino acid residue 108 Met of mspd1 are important for PD-1 binding.

[0076] Advantageously, the administration of mspd1-p24Fc, mspd1-14de1-p24Fc, mspd1-322mu-p24Fc, and hspd1-14de1-p24Fc fusion proteins, or fusion DNA thereof, enhanced HIV-1 Gag-specific immune responses. As shown in FIGS. 3-6 and 8-11, administration of mspd1-p24Fc, mspd1-14de1-p24Fc, mspd1-322mu-p24Fc, and hspd1-14de1-p24Fc DNA significantly increased anti-p24 IgG1 (Th2) and IgG2a (Th1) antibody titers. In addition, the administration of mspd1-p24Fc, mspd1-14de1-p24Fc, mspd1-322mu-p24Fc, and hspd1-14de1-p24Fc DNA also significantly increased the number of IFN-γ-expressing CD4 and CD8 T cells in mice. Specifically, mice immunized mspd1-p24Fc, mspd1-14de1-p24Fc, or mspd1-322mu-p24Fc DNA had significantly reduced titers of challenge virus upon vaccinia virus-gagpol (VTV-gagpol) challenges.

[0077] In comparison, mspd1-lgVα-p24Fc, which is obtained by deleting amino acids 89-90 of the mouse PD-1 protein, does not bind to PD-1 ligands PD-L1 and PD-L2. In addition, the administration of mspd1-lgVα-p24Fc DNA does not enhance humoral or cell-mediated immunity in mice. Further, the administration of mspd1-lgVα-p24Fc DNA does not reduce HIV viral titers upon vaccinia viruses-gagpol (MTV-gagpol) challenges.

PD-1 Variants and Fusion Constructs

[0078] A first aspect of the subject invention provides SPD-1 protein variants. In one embodiment, the SPD-1 protein variant is obtained by deleting amino acid residues 26-39 of a wild-type SPD-1 protein. The wild-type SPD-1 protein is preferably of mammalian origin (such as a wild-type mouse, rabbit, non-human primates, or pig PD-1 protein), more preferably, of human origin.

[0079] In an embodiment, the SPD-1 protein variant is mspd1-14de1, which has an amino acid sequence comprising SEQ ID NO: 11. In an embodiment, the SPD-1 protein variant is mspd1-322mu, which has an amino acid sequence comprising SEQ ID NO: 15. In an embodiment, the SPD-1 protein variant is hspd1-14de1, which has an amino acid sequence comprising SEQ ID NO: 25.

[0080] In certain embodiments, the subject invention encompasses PD-1 protein variants that are homologous to mspd1-14de1 (SEQ ID NO: 11), mspd1-322mu (SEQ ID NO: 15), or hspd1-14de1 (SEQ ID NO: 25). In an embodiment, the SPD-1 protein variant has an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99%, or 99.5% identical to SEQ ID NO: 11. In an embodiment, the SPD-1 protein variant has an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99%, or 99.5% identical to SEQ ID NO: 15. In an embodiment, the SPD-1 protein variant has an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99%, or 99.5% identical to SEQ ID NO: 25. In an embodiment, the PD-1 protein variant does not comprise SEQ ID NO: 7.

[0081] A second aspect of the subject invention provides nucleic acid molecules that encode the SPD-1 proteins of the subject invention. The nucleic acid molecules encompass DNA molecules (e.g. genomic DNA and cDNA) and RNA molecules. In addition, the subject nucleic acid molecules may be single-stranded or double-stranded.

[0082] In one embodiment, the nucleic acid molecule encodes a SPD-1 protein, which is obtained by deleting amino acid residues 26-39 of a wild-type SPD-1 protein (such as a wild-type human, mouse, or rabbit SPD-1 protein). In an embodiment, the nucleic acid molecule encodes mspd1-14de1, and has a sequence comprising SEQ ID NO: 12. In an embodiment, the subject nucleic acid molecule encodes mspd1-322mu, and has a sequence comprising SEQ ID NO: 16. In an embodiment, the subject nucleic acid molecule encodes hspd1-14de1, and has a sequence comprising SEQ ID NO: 26.

[0083] In certain embodiments, the subject invention encompasses nucleic acid molecules that are homologous to nucleic acids encoding mspd1-14de1, mspd1-322mu, or hspd1-14de1. In an embodiment, the nucleic acid molecule has a sequence that is at least about 95%, 96%, 97%, 98%, 99%, or 99.5% identical to SEQ ID NO: 12, SEQ ID NO: 16, or SEQ ID NO: 26. In an embodiment, the SPD-1 nucleic acid molecule does not comprise SEQ ID NO: 8.

[0084] A third aspect of the invention provides PD-1 fusion proteins. In one embodiment, the subject invention provides PD-1 fusion proteins, comprising a SPD-1 protein fragment fused with an antigenic protein fragment. In a further embodiment, the SPD-1 fusion protein comprises a Fc domain. In one embodiment, the soluble PD-1 protein is linked to the antigen via a linker sequence. In an alternative embodiment, the PD-1 fusion protein comprises a PD-1 protein fused with a Fc domain, optionally via a linker sequence.

[0085] In an embodiment, the SPD-1 fusion protein comprises the wild-type mouse soluble PD-1 protein (mspd), which has an amino acid sequence comprising SEQ ID NO: 1. In an embodiment, the SPD-1 fusion protein comprises the wild-type human SPD-1 protein (hspd), which has an amino acid sequence comprising SEQ ID NO: 21. In an embodiment, the SPD-1 fusion protein is a variant mouse SPD-1 protein mspd1-14de1, which has an amino acid sequence comprising SEQ ID NO: 11. In an embodiment, the SPD-1 fusion protein is a variant mouse mspd1-14de1-p24Fc, which has an amino acid sequence comprising SEQ ID NO: 15. In an embodiment, the SPD-1 protein is a variant human SPD-1 protein (hspd1-14de1), and has an amino acid sequence comprising SEQ ID NO: 25.

[0086] The antigenic protein fragment can be derived from an immunogenic fragment of viral, bacterial, fungal, or other microbial pathogens including, but not limited to, human immunodeficiency virus (HIV), HSV including HSV-1 and HSV-2, KSHV, HPV including HPV-6, HPV-11, HPV-16, and HPV-18, respiratory syncytial virus, rhinovirus, hepatitis viruses including hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis D virus, hepatitis E virus, hepatitis F virus, and hepatitis G virus, oncoviruses, human T-lymphotropic virus Type I (HTLV-1), influenza virus, bovine leukemia virus (BLV), Epstein-Barr virus, papillomavirus, pneumococcus, streptococcus, staphylococcus, neisseria, E. coli, cytomegalovirus (CMV), respiratory syncytial virus, parainfluenza virus, adenovirus, flavivirus, dengue virus, Mycobacteria tuberculosis, and Plasmodium falciparum; and pathogens causing diseases including, but not limited to, pertussis, polio, measles, mumps, rubella, smallpox, zoster, anthrax, tetanus, rabies, chickenpox, diphtheria, anthrax, plague, encephalitis, pneumonia, typhus, typhoid fever, lyme disease, cholera, shigella, leishmania, leprosy, toxoplasmosis, coccidiomycosis, schistosomiasis, and malaria. The antigenic protein fragment can also be derived from tumor or cancer cells.

[0087] In one embodiment, the soluble PD-1, its variants, and fusion proteins thereof serve as molecular or protein
adjuvants to enhance immune response. Additionally, nucleic acid molecules encoding the soluble PD-1, its variants, and fusion proteins thereof can also be administered to a subject to enhance immune response.

[0088] In an embodiment, the antigenic protein fragment is derived from an immunogenic fragment of an HIV protein domain including, but not limited to, p24, gag, pol, nef, tat, rev, gpl20, and gpr41. In an embodiment, the antigen protein is derived from HIV p24 in a specific embodiment, the antigen protein comprises SEQ ID NO: 3. In a further embodiment, the sPD-1 fusion protein further comprises a Fs domain. In an embodiment, the sPD-1 fusion protein comprises a rabbit Fs domain for protein purification purpose.

[0089] The term “Fs domain” encompasses the full length and fragments of natural human and animal Fs and Fs variant molecules and sequences, including for example, IgG, IgM, IgD, IgE, IgA and subtypes such as for example IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. As with Fs variants and native Fs, the term “Fs domain” includes molecules in monomeric or multimeric form, whether digested from whole antibody or produced by other means.

[0090] In an embodiment, the antigenic protein fragment is derived from a tumor antigen.

[0091] The term “Fs variant” refers to a molecule or sequence that is modified from a native Fs but still comprises a binding site for the salvage receptor. Fs domains include molecules having two or more polypeptide chains associated covalently, noncovalently, or by both covalent and non-covalent interactions. IgG1 molecules typically form dimers; IgM, pentamers; and IgA, monomers, dimers, trimers, or tetramers. Multimers may be formed by exploiting the sequence and resulting activity of the native Fs or by derivatizing (as defined below) such a native Fs.

[0092] The Fs domain within the scope of the invention can be of antibodies of any isotype, including IgG1, IgA, IgE, IgD, and IgM. IgG1 isotype antibodies can be further subdivided into IgG1, IgG2, IgG3, and IgG4 subtypes. IgA antibodies can be further subdivided into IgA1 and IgA2 subtypes. In a specific embodiment, the Fs domain is IgG1.

[0093] In a further embodiment, the sPD-1 fusion protein of the subject invention comprises a linker sequence that links the soluble PD-1 domain to the antigen. In addition, the Fs domain can also be linked to the fusion protein via a linker sequence. Linker sequence is typically a peptide chain. The length of the peptide may be, for example, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 40, 50 or more amino acid residues, but typically is between 5 and 25 residues.

[0094] Depending upon the length and side chain composition, a linker may have, but need not have, greater than average flexibility. Flexibility can be calculated using algorithms known in the art. In an embodiment, the linker sequence is SEQ ID NO: 29. Examples of useful linkers include, but are not limited to, 9Gly (SEQ ID NO: 30), 9Glu (SEQ ID NO: 31), 9Ser (SEQ ID NO: 32), GlyCys2ProCys (SEQ ID NO: 33), 4Gly3Ser (SEQ ID NO: 34), Ser Cys Val Pro Leu Met Arg Cys Gly Gly Cys Cys Asn (SEQ ID NO: 35), Pro Ser Cys Val Pro Leu Met Arg Cys Gly Gly Cys Cys Asn (SEQ ID NO: 36), Gly Asp Leu Ile Tyr Arg Asn Gln Lys (SEQ ID NO: 37), and 9GlyProSerCysValProLeuMetArgCysGlyGlyCysCysAsn (SEQ ID NO: 38).

[0095] In a specific embodiment, the subject sPD-1 fusion protein comprises SEQ ID NO: 13. In another specific embodiment, the subject sPD-1 fusion protein comprises SEQ ID NO: 17. In another specific embodiment, the subject sPD-1 fusion protein comprises SEQ ID NO: 19. In another specific embodiment, the subject sPD-1 fusion protein comprises SEQ ID NO: 23. In another specific embodiment, the subject sPD-1 fusion protein comprises SEQ ID NO: 27.

[0096] In addition, the subject invention provides sPD-1 fusion nucleic acid constructs, comprising a nucleic acid molecule encoding the subject sPD-1 fusion protein. In one embodiment, the sPD-1 fusion construct comprises a nucleic acid molecule encoding a sPD-1 protein fused with a nucleic acid encoding a protein antigen. In a further embodiment, the PD-1 fusion construct comprises a Fs DNA. In one embodiment, the soluble PD-1 DNA is linked to the antigen DNA via a linker sequence. Optionally, the Fs DNA is linked to the sPD-1-antigen DNA via a linker DNA sequence.

[0097] The antigenic nucleic acid molecule of the subject invention encodes immunogenic fragments of viral, bacterial, fungal, or other microbial pathogens including, but not limited to, human immunodeficiency virus (HIV), HSV including HSV-1 and HSV-2, KSHV, HPV including HPV-6, HPV-11, HPV-16, and HPV-18, respiratory syncytial viruses, rhinovirus, hepatitis viruses including hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis D virus, hepatitis E virus, hepatitis F virus, and hepatitis G virus, oncoviruses, human T-lymphotropic virus Type 1 (HTLV-1), influenza virus, bovine leukemia virus (BLV), Epstein-Barr virus, rotavirus, meningococcus, anapllomavirus, streptococcus, staphylococcus, E. coli, cytomegalovirus (CMV), respiratory syncytial virus, parainfluenza virus, adenovirus, flavivirus, dengue virus, Mycobacteria tuberculosis, and Plasmodium falciparum; and pathogens causing diseases including, but not limited to, pertussis, polio, measles, mumps, rubella, smallpox, zoster, anthrax, tetanus, rabies, chickenpox, diphtheria, anthrax, plague, encephalitis, pneumonia, typhus, typhoid fever, lyne disease, cholera, shigella, leishmania, leprosy, toxoplasmosis, coecidiomyosisis, schistosomiasis, and malaria.

[0098] In an embodiment, the antigenic nucleic acid molecule encodes a tumor antigen. In an embodiment, the fusion nucleic acid molecule comprises the wild-type mouse PD-1 (nspd1) DNA (SEQ ID NO: 2). In an embodiment, the fusion nucleic acid molecule comprises the wild-type human PD-1 (hspd1) DNA (SEQ ID NO: 22). In an embodiment, the fusion nucleic acid molecule comprises a variant mouse PD-1 DNA that has a sequence of SEQ ID NO: 12 or SEQ ID NO: 16. In an embodiment, the fusion nucleic acid molecule comprises a variant human PD-1 DNA that has a sequence of SEQ ID NO: 26. In a specific embodiment, the subject PD-1 fusion DNA comprises SEQ ID NO: 14.

[0099] In another specific embodiment, the subject PD-1 fusion DNA comprises SEQ ID NO: 18. In another specific embodiment, the subject PD-1 fusion DNA comprises SEQ ID NO: 20. In another specific embodiment, the subject PD-1 fusion DNA comprises SEQ ID NO: 21. In another specific embodiment, the subject PD-1 fusion DNA comprises SEQ ID NO: 24. In another specific embodiment, the subject PD-1 fusion DNA comprises SEQ ID NO: 28. In certain embodiments, the PD-1 protein or nucleic acid of the subject invention is typically substantially free of other components, such as other biological molecules, proteins or peptides, nucleic acids, lipids and carbohydrates. The term “substantially free of,” as used herein, encompasses preparations of the subject invention having less than about 20%, 10% and preferably less than 5% (by dry weight) con-
taminating factors (such as biological molecules, proteins or peptides, nucleic acids, lipids and carbohydrates and other cellular components).

[0100] If desired, the subject proteins and nucleic acid molecules can be modified by any suitable process. Strategies for protein optimization are sometimes carried out using random mutagenesis. In these cases positions are chosen randomly, or amino acid changes are made using simplistic rules. For example all residues may be mutated to alanine, referred to as alanine scanning. In addition, substitution of amino acids other than those specifically exemplified or naturally present in a fusion protein of the invention are also within the scope of the subject invention. For example, non-natural amino acids can be substituted for the amino acids of the fusion protein, so long as the fusion protein retaining the substituted amino acids retains substantially the same functional activity as the fusion protein in which amino acids have not been substituted.

[0101] Examples of non-natural amino acids include, but are not limited to, ornithine, citrulline, hydroxyproline, homoserine, phenylglycine, taurine, kordotyrosine, 2,4-diaminobutyric acid, α-amino isobutyric acid, 4-aminobutyric acid, 2-amino butyric acid, γ-amino butyric acid, ε-amino hexanoic acid, 6-amino hexanoic acid, 2-amino isobutyric acid, 3-amino propionic acid, norleucine, norvaline, sarcosine, homocitrulline, cysteic acid, t-butylglycine, t-butyllalane, phenylglycine, cyclohexylalanine, β-alanine, fluoro-amino acids, designer amino acids such as β-methyl amion acids, C-methyl amino acids, N-methyl amino acids, and amino analogues in general. Non-natural amino acids also include amino acids having derivatized side groups. Furthermore, any of the amino acids in the protein can be of the D (dextrorotary form) or L (levorotary form). Form.

[0102] The subject invention also concerns variants of nucleic acid molecules that encode functional fusion proteins of the invention. Variant sequences include those sequences wherein one or more nucleotides of the sequence have been substituted, deleted, and/or inserted.

[0103] The nucleotides that can be substituted for natural nucleotides of DNA have a base moiety that can include, but is not limited to, inosine, 5-fluorouracil, 5-bromouracil, 5-haloalkyluracil, 1-methylguanine, 5-methylcytosine, and tritylated bases. The sugar moiety of the nucleotide in a sequence can also be modified and includes, but is not limited to, arabinose, xylose, and hexose. In addition, the adenine, cytosine, guanine, thymine, and uracil bases of the nucleotides can be modified with acetyl, methyl, and/or thio groups. Sequences containing nucleotide substitutions, deletions, and/or insertions can be prepared and tested using standard techniques known in the art.

[0104] Unless otherwise specified, as used herein percent sequence identity and/or similarity of two sequences can be determined using the algorithm of Karlin and Altschul (1990), modified as in Karlin and Altschul (1993). Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul et al. (1990). BLAST searches can be performed with the NBLAST program, score=100, wordlength=12, to obtain sequences with the desired percent sequence identity. To obtain gapped alignments for comparison purposes, Gapped BLAST can be used as described in Altschul et al. (1997). When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (NBLAST and XBLAST) can be used. See NCB/NIH website.

[0105] The subject invention also contemplating those nucleic acid molecules having sequences which are sufficiently homologous with the nucleic acid sequences exemplified herein so as to permit hybridization with that sequence under standard stringent conditions and standard methods (Maniatis et al., 1982). As used herein, “stringent” conditions for hybridization refers to conditions wherein hybridization is typically carried out overnight at 20-25°C below the melting temperature (Tm) of the DNA hybrid in 6x SSPE, 5x Denhardt’s solution, 0.1% SDS, 0.1 mg/ml denatured DNA. The melting temperature, Tm, is described by the following formula (Beltz et al., 1983):

\[ Tm = 81.5 + 16.6 \log [Nar] + 0.41(\% GC - 0.11)(\% formamide) - 600/\text{length of duplex in base pairs}. \]

[0106] Washes are typically carried out as follows:

[0107] (1) Twice at room temperature for 15 minutes in 1x SSPE, 0.1% SDS (low stringency wash).

[0108] (2) Once at Tm-20°C for 15 minutes in 0.2x SSPE, 0.1% SDS (moderate stringency wash).

[0109] Further, the subject invention provides expression constructs comprising PD-1 nucleic acid molecules or fusion constructs thereof. Expression constructs of the invention generally include regulatory elements that are functional in the intended host cell in which the expression construct is to be expressed. Regulatory elements include promoters, transcription termination sequences, translation termination sequences, enhancers, and polyadenylation elements.

[0110] An expression construct of the invention can comprise a promoter sequence operably linked to a nucleic acid sequence encoding a peptide of the invention. Multiple copies of promoters or multiple promoters can be used in an expression construct of the invention. In a preferred embodiment, a promoter can be positioned about the same distance from the transcription start site as it is from the transcription start site in its natural genetic environment. Some variation in this distance is permitted without substantial decrease in promoter activity. A transcription start site is typically included in the expression construct.

[0111] For expression in animal cells, an expression construct of the invention can comprise suitable promoters that can drive transcription of the nucleotide sequence. For mammalian cells, suitable promoters include for example, Penv, actin promoter, metallothionein promoter, NF-kappaB promoter, EGR promoter, SRE promoter, IL-2 promoter, NFAT promoter, osteocalcin promoter, SV40 early promoter and SV40 late promoter, Lck promoter, BMP5 promoter, and TRP-1 promoter.

Protection against Pathogenic Infection and Cancer

[0112] Another aspect of the subject invention provides methods for the prevention and/or treatment of pathogenic infection and/or cancer. Advantageously, the methods of the subject invention induce antigen-specific humoral and cell-mediated immunity. In one embodiment, the method comprises administering, to a subject in need of such treatment, an effective amount of a fusion protein or fusion nucleic acid molecule of the subject invention.

[0113] In an embodiment, the subject invention provides a method of inducing protective immunity against pathogenic infection and/or cancer. In a specific embodiment, the method comprises administering a composition comprising a fusion nucleic acid molecule, wherein the fusion nucleic acid molecule comprises a nucleic acid encoding an antigen of interest; a PD-1 nucleic acid encoding a wild-type soluble PD1 protein, a nucleic acid encoding a spdl-14 del protein of the
invention, or a nucleic acid encoding a spδ-322 del1 protein of the invention; and, optionally, a nucleic acid encoding Fc domain and a linker nucleic acid sequence that links the spδ-1 nucleic acid and the antigen nucleic acid. In one embodiment, the composition is administered by intramuscular injection via electroporation (EP).

[0114] In another specific embodiment, the method comprises administering a composition comprising a fusion protein, wherein the fusion protein comprises an antigen of interest; a soluble PD-1 protein selected from a wild-type soluble PD1 protein, a spδ-14de1 protein of the invention, or a spδ-322 del1 protein of the invention; and, optionally, a Fc domain and a linker sequence that links the spδ-1 PD-1 protein and the antigen protein.

[0115] The methods can be used for prevention and/or treatment of infection and other diseases where induction of antigen-specific humoral and cell-mediated immunity is beneficial. In a specific embodiment, the subject invention can be used in the prevention and/or treatment of tumor or cancer.

[0116] The term “treatment” or any grammatical variation thereof (e.g., treat, treating, and treatment etc.), as used herein, includes but is not limited to, ameliorating or alleviating a symptom of a disease or condition, reducing, suppressing, inhibiting, lessening, or affecting the progression, severity, and/or scope of a condition.

[0117] The term “prevention” or any grammatical variation thereof (e.g., prevent, preventing, and prevention etc.), as used herein, includes but is not limited to, delaying the onset of symptoms, preventing relapse to a disease, decreasing the number or frequency of relapse episodes, increasing latency between symptomatic episodes, or a combination thereof. Prevention, as used herein, does not require complete inhibition or elimination of symptoms.

[0118] The term “effective amount,” as used herein, refers to an amount that is capable of treating or ameliorating a disease or condition or otherwise capable of producing an intended therapeutic effect.

[0119] The term “subject,” as used herein, describes an organism, including mammals such as primates, to which treatment with the compositions according to the subject invention can be provided. Mammalian species that can benefit from the disclosed methods of treatment include, but are not limited to, apes, chimpanzees, orangutans, humans, monkeys; and other animals such as dogs, cats, horses, cattle, pigs, sheep, goats, chickens, mice, rats, guinea pigs, and hamsters.

[0120] In certain embodiments, in case of prevention of pathogenic infection or cancer, the spδ-1-based composition of the invention is administered to a subject that does not suffer from the pathogenic infection or cancer type to be prevented, or a subject that does not exhibit symptoms of the pathogenic infection or cancer type to be prevented.

[0121] In one embodiment, the subject invention can be used in the prevention and/or treatment of infection by viral, bacterial, fungal, or other microbial pathogens including, but not limited to, human immunodeficiency virus (HIV), HSV including HSV-1 and HSV-2, KSHV, HPV including HPV-6, HPV-11, HPV-16, and HPV-18, respiratory syncytial virus, rhinovirus, hepatitis viruses including hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis D virus, hepatitis E virus, hepatitis F virus, and hepatitis G virus, oncoviruses, human T-lymphotropic virus Type 1 (HTLV-1), influenza virus, bovine leukemia virus (BLV), Epstein-Barr virus, rotavirus, meningococcus, anaplasma, pneumococcus, streptococcus, staphylococcus, E. coli, cytomegalovirus (CMV), respiratory syncytial virus, parainfluenza virus, adenovirus, dengue virus, Mycobacteria tuberculosis, and Plasmodium falciparum; and pathogens causing diseases including, but not limited to, pertussis, polio, measles, mumps, rubella, smallpox, zoster, anthrax, tetanus, rabies, chickenpox, diphtheria, anthrax, plague, encephalitis, pneumonia, typhus, typhoid fever, lyme disease, cholera, shigella, leishmania, leprosy, toxoplasmosis, coxsackievirus, schistosomiasis, and malaria.

[0122] In a specific embodiment, the subject invention can be used to prevent and/or treat HIV infection. In certain embodiments, the method comprises administering to a subject in need of such treatment an effective amount of a fusion protein, comprising an amino acid sequence selected from SEQ ID NOs: 13, 17, 19, 23, and 27. In specific embodiments, the subject method comprises administering to a subject in need of such treatment an effective amount of a fusion DNA, comprising a nucleic acid sequence selected from SEQ ID NOs: 14, 18, 20, 24, and 28.

[0123] In addition, the methods can be used in the prevention and/or treatment of diseases where enhanced humoral and cell-mediated immunity is beneficial. In an embodiment, the subject invention can be used in the prevention and/or treatment tumor or cancer.

[0124] In one embodiment, the spδ-1 protein useful for the prevention and/or treatment of tumor comprises an antigenic fragment derived from cancer or tumor cells. Soluble PD-1 proteins useful for the prevention and/or treatment of tumor or cancer also include, for example, the wild-type mspd-1 (SEQ ID NO:1), the wild-type hsdp1 (SEQ ID NO: 21), msdp1-14de1 (SEQ ID NO: 11), msdp1-322mu (SEQ ID NO: 15), hsdp1-14de1 (SEQ ID NO: 25), or fusion proteins thereof. Additionally or alternatively, the PD-1 protein useful for the prevention and/or treatment of tumor or cancer comprises an amino acid sequence that is at least 95%, 96%, 97%, 98%, 99%, or 99.5% identical to the wild-type mspd-1 (SEQ ID NO:1), the wild-type hsdp1 (SEQ ID NO: 21), msdp1-14de1 (SEQ ID NO: 11), msdp1-322mu (SEQ ID NO: 15), hsdp1-14de1 (SEQ ID NO: 25), or fusion proteins thereof.

[0125] In specific embodiments, spδ-1 nucleic acid molecules useful for the prevention and/or treatment of tumor or cancer include, for example, the wild-type mspd-1 DNA (SEQ ID NO:2), the wild-type hsdp1 DNA (SEQ ID NO: 22), msdp1-14de1 DNA (SEQ ID NO: 12), msdp1-322mu DNA (SEQ ID NO: 16), hsdp1-14de1 DNA (SEQ ID NO: 26), or fusion DNA thereof.

[0126] Additionally or alternatively, the spδ-1 nucleic acid molecule useful for the prevention and/or treatment of tumor or cancer comprises a sequence that is at least 90%, 95%, 96%, 97%, 98%, 99%, or 99.5% identical to the wild-type mspd-1 DNA (SEQ ID NO:2), the wild-type hsdp1 DNA (SEQ ID NO: 22), msdp1-14de1 DNA (SEQ ID NO: 12), msdp1-322mu DNA (SEQ ID NO: 16), hsdp1-14de1 DNA (SEQ ID NO: 26), or fusion DNA thereof.

Therapeutic Compositions and Routes of Administration

[0128] The subject invention further provides for therapeutic or pharmaceutical compositions. In an embodiment, the composition comprises a therapeutically effective amount of a protein and/or nucleic acid molecule of the subject invention and, optionally, a pharmaceutically acceptable carrier.

[0129] In one embodiment, the proteins and/or nucleic acid molecules are formulated into a vaccine composition for
administration to subjects having certain risks of pathogenic infection. A vaccine composition is an antigenic preparation that comprises one or more immunogenic antigens used to produce active immunity to a disease. In addition, the compositions of the subject invention can be administered to a subject with existing infection, and provide for customized vaccine schedules and compositions to prevent or minimize worsening of the diseases.

[0130] The subject invention contemplates therapeutic compositions useful for practicing the therapeutic methods described herein. The therapeutic composition can be any form of pharmaceutical format, including injectable formulations such as liquid and lyophilized injections.

[0131] In a specific embodiment, a therapeutically effective amount of a protein and/or a nucleic acid molecule of the subject invention is typically an amount such that when administered in a physiologically tolerable composition is sufficient to achieve a plasma concentration of from about 0.01 microgram (ug) per milliliter (ml) to about 200 ug/ml. Stated differently, the dosage can vary from about 0.1 mg/kg to about 300 mg/kg, preferably from about 0.2 mg/kg to about 200 mg/kg, most preferably from about 0.5 mg/kg to about 20 mg/kg, in one or more dose administrations daily, for one or several days.

[0132] Suitable non-toxic pharmaceutically acceptable carriers for use with the agent will be apparent to those skilled in the art of pharmaceutical formulation. See, for example, Remington's Pharmaceutical Sciences, seventeenth edition, ed. Alfonso R. Gennaro, Mack Publishing Company, Easton, Pa. (1985). Suitable carriers include ethanol, dimethyl sulfoxide, glycerol, silicone, alumina, starch, sorbitol, inositol, xylitol, D-xylene, mannitol, powdered cellulose, microcrystalline cellulose, talc, colloidal silicon dioxide, calcium carbonate, magnesium carbonate, calcium phosphate, calcium aluminum silicate, aluminum hydroxide, sodium starch phosphate, lactitins, and equivalent carriers and diluents. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions.

[0133] Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethan and the like. The therapeutic composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like which enhance the effectiveness of the active ingredient.

[0134] The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary, depending on the type of the condition and the subject to be treated. In general, a therapeutic composition contains from about 5% to about 95% active ingredient (w/w). More specifically, a therapeutic composition contains from about 20% (w/w) to about 80%, or about 30% to about 70%, active ingredient (w/w).

[0135] The preparation of a pharmacological composition that contains active ingredients dissolved or dispersed therein is well understood in the art and need not be limited based on formulation. Typically such compositions are prepared as injectables either as liquid solutions or suspensions; however, solid forms suitable for solution, or suspensions, in liquid prior to use also can be prepared. The preparation also can be emulsified.

[0136] The therapeutic composition of the subject invention can include pharmaceutically acceptable salts of the components therein. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of a polypeptide) that are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, tartaric, mandelic and the like. Salts formed with the free carboxyl groups also can be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylaminoethanol, histidine, procaine and the like.

[0137] As used herein, the terms “pharmaceutically acceptable”, “physiologically tolerable” and grammatical variations thereof, as they refer to compositions, carriers, diluents and reagents, are used interchangeably and represent that the materials are capable of administration to or upon a mammal.

[0138] The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients, e.g., compound, carrier suitable for administration.

[0139] The compositions of the subject invention can be administered to the subject being treated by standard routes, including oral, inhalation, or parenteral administration including intravenous, subcutaneous, topical, intradermal, transmucosal, intraperitoneal, intramuscular, intracapsular, intraorbital, intracardiac, transtracheal, subcutaneous, subcuticular, intrarricular, subcapsular, subarachnoid, intraspinal, epidural and intrathecal injection, infusion, and electroporation, as well as co-administration as a component of any medical device or object to be inserted (temporarily or permanently) into a subject.

[0140] In a preferred embodiment, the microcrystals of the subject invention can be formulated for parenteral administration. The preparation of an aqueous composition that contains one or more agents, such as a protein or nucleic acid molecule of the subject invention, will be known to those of skill in the art in light of the present disclosure. Typically, such compositions can be prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for using to prepare solutions or suspensions upon the addition of a liquid prior to injection can also be prepared; and the preparations can also be emulsified.

[0141] The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi.

[0142] Sterile injectable solutions are prepared by incorporating the active ingredients in the required amount in the appropriate solvent followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions,
the preferred methods of preparation are vacuum drying and
freeze-drying techniques, which yield a powder of the active
ingredient, plus any additional desired ingredient from a pre-
viously sterile-filtered solution thereof

[0143] In addition, the nucleic acid molecules and com-
positions of the subject invention can be delivered in vivo into a
host cell by methods known in the art. In one embodiment, the
nucleic acid molecules and compositions of the subject inven-
tion can be introduced in vivo via a viral vector such as aden-
associated virus (AAV), herpes simplex virus (HSV),
retrovirus, papillomavirus, adeno virus, and Epstein-Barr virus
(EBV). In addition, the nucleic acid molecules and com-
positions of the subject invention can also be introduced in
vivo via lipofection (DNA transfection via liposomes pre-
pared from synthetic cationic lipids) (Felgner et al., 1987).
Synthetic cationic lipids (LIPOFECTIN, Invitrogen Corp.,
La Jolla, Calif.) can be used to prepare liposomes to encap-
sulate the nucleic acid molecules of the invention. The nucleic
acid molecules of the subject invention can also be introduced in
vivo as naked DNA using methods known in the art, such as
transfection, microinjection, electroporation, calcium phos-
phate precipitation, and by biolistic methods.

EXAMPLES

[0144] Following are examples that illustrate embodiments for
practicing the invention. These examples should not be
construed as limiting. All percentages are by weight and all
solvent mixture proportions are by volume unless otherwise
noted.

Example 1

Construction of Mouse sPD-1 Vaccine Candidates

[0145] This Example illustrates the construction of mouse
sPD-1-p24 fusion constructs. To construct mouse sPD-1-p24-
Fc construct, PVAX vector that carries the wild-type msPD-1-
gen and p24 gene was fused with rabbit Fc DNA. The vector and
the p24-Fc DNA were linked by a linker encoding
GGGGSGGSGG (SEQ ID NO: 29). The transcript is under the
control of promoter Pmcm.

[0146] Mouse sPD1 protein variant msdp1-igVΔ was
obtained by deleting amino acids 89-99 of the mouse
PD-1 protein, which forms the C'β loop of the IgG domain and is
essential for PD-1 and PD-L1/L2 interaction. PVAX vector
encoding msdp1-igVΔ was linked with p24-rabbit Fc DNA via
a linker encoding GGGSGGSGG (SEQ ID NO: 29) linker
sequence. In addition, p24-Fc fusion construct was obtained
by linking the PVAX vector carrying p24 with rabbit Fc DNA.
The transcript is under the control of promoter Pmcm.

[0147] FIG. 1A shows alignment of amino acid sequences
of msPd1-p24-Fc, msdp1-igVΔ-p24-Fc, and p24-Fc fusion
proteins. FIG. 1B shows Western blot results of various fusion
constructs useful according to the subject invention. Briefly,
293T cells were transfected with various fusion constructs
using polyethyleneimine (PEI) and the supernatants were col-
clected 72 hours post transfection. Proteins were detected by
anti-rabbit Fc antibody. FIG. 1B shows that msPd1-p24-Fc
and msdp1-igVΔ-p24-Fc are about 72KD in size, while p24-
Fc is about 58KD in size. The results also show that msPd1-
p24-Fc, msdp1-igVΔ-p24-Fc, and p24-Fc fusion proteins are
soluble.

Example 2

Binding Ability of Mouse sPD-1 Fusion Protein to
sPD-1 Ligands

[0148] This Example shows the binding ability of msPD-1
fusion proteins to mouse sPD-1 ligands. Briefly, 293T cells
were transfected with PD-L1, PD-L2, and PD-L1/2. The binding
of sPD-1 proteins to PD-1 ligands was detected by FITC-anti
rabbit Fc antibody using flow cytometer, and the results were
analyzed by flowJo.

[0149] The results, as shown in FIGS. 2A-B, reveal that
msPd1-p24-Fc binds to mouse PD-1 ligands PD-L1 and PD-
L2. In contrast, the variant msdp1-igVΔ fusion protein does
not bind to mouse PD-1 ligands. In addition, p24-Fc does not
affect the interaction between PD-1 and PD-L1/L2.

Example 3

Induction of Humoral and Cell-Mediated Immune
Responses by Wild-Type Mouse sPD1 Vaccine

[0150] This Example shows that the wild-type msPD1-
p24-Fc potently induces humoral and cell-mediated immune
responses. Briefly, Balb/c mice were primed at week 0 and
boosted at week 3 and week 6 with 20μg mouse DNA vectors
encoding msPd1-p24-Fc, msdp1-igVΔ-p24-Fc, or p24-Fc via
intramuscular electroporation. Mice that received PBS
served as controls.

[0151] Two weeks after the last immunization, mice sera
were collected and contacted with HIV-1 p24 viral proteins.
The levels of anti-p24 IgG and IgG2a antibodies were mea-
sured by ELISA. The level of anti-p24 antibody in control
samples is not shown because the absorbance readings of
these samples fell below the cutoff values for determining
antibody titers. The anti-p24 antibody endpoint titer is
defined as the reciprocal of the highest dilution of a test
sample that produces a reading of at least two-fold greater
than that of the control sample with the same dilution. The
results show that mouse immunized with msPd1-p24-Fc had
high IgG1 and IgG2a titers, when compared to mice
immunized with p24-Fc or msdp1-igVΔ-p24-Fc.

[0152] To examine p24-specific immune responses, the
number of IFN-γ-secreting splenocytes specific for p24
epitope gag/Δ (AMQMLKDTI) (SEQ ID NO: 39) for CD8 T
cells and the number of splenocytes specific for p24 epitope
gag26 (PSVPPGDIYKRWILGI) (SEQ ID NO: 40) for CD4 T
cells was determined using ELISPOT assay. In addition,
splenocytes isolated from immunized vaccinated mice were
subjected to H2d-Kd-AMQMLKDTI-PE tetramer staining, and
CD8 T cell and CD4 T cell population was analyzed.

[0153] The results show that mice immunized with msPd1-
p24-Fc had high anti-p24 antibody titers (FIG. 3A) and high
number of IFN-γ-secreting splenocytes (FIGS. 3B and 3D),
when compared to mice immunized with p24-Fc or msdp1-
igVΔ-p24-Fc. Splenocytes isolated from mice immunized
with msPd1-p24-Fc contained about five-fold higher H2d-
Kd-AMQMLKDTI tetramer-positive cells (FIGS. 3C), when
compared to mice immunized with p24-Fc or msdp1-igVΔ-
p24-Fc.

Example 4

Reduction of VTF-HIV-Gagpol Titers in Mice
Immunized with Wild-Type Mouse sPD1 Fusion
Protein

[0154] This Example shows that immunization with the
wide-type msPD1 fusion protein protects against viral infec-
tion. Briefly, Balb/c mice were primed at week 0 and boosted
at week 3 and week 6 with 20μg mouse DNA encoding
msPd1-p24-Fc, msdp1-igVΔ-p24-Fc, or p24-Fc via intramuscular
electroporation. Mice that received PBS served as
controls. Three weeks after immunization, mice were challenged with 4 × 10^10 PFU vaccinia VTT-HIV-gagpol intranasally. The mice were sacrificed 3 days after viral challenge and viral titers in the lungs were evaluated by plaque assay. The results show that mice immunized with mspd1-p24-Fc exhibited significantly reduced VTT-HIV-gagpol titers upon viral challenge (FIG. 4).

**[0155]** FIG. 5 shows that targeting dendritic cells using sPD-1-p24-fc induces enhanced p24-specific antibody and T cell responses.

**Example 5**

**Induction of Humoral and Cell-Mediated Immune Responses by Wild-Type Human sPD1 Vaccine**

**[0156]** Human sPD-1-p24-Fc was constructed by fusing PVAX vector carrying hspD1-p24 with rabbit Fc DNA. The vector and the p24-Fc DNA were linked by a linker encoding GGGS(GG(SEQ ID NO: 29). The transcription is under the control of promoter Penv.

**[0157]** To analyze the binding ability of hspD1-1-p24-Fc to sPD-1 ligands, 293T cells were transfected with mouse PD-L1 and PD-L2, respectively. The binding of sPD-1 proteins to PD-1 ligands was detected by mouse sPD-1-Fc proteins and FITC-anti rabbit Fc antibody using flow cytometry, and the results were analyzed by FlowJo. The results, as shown in FIGS. 6A and 6B, reveal that hspd1-p24-fc fusion protein binds to PD-1 ligands.

**[0158]** To examine the induction of immune responses by hspD1-1-p24-Fc, Balb/c mice were primed at week 0 and boosted at week 3 and week 6 with 20 μg mouse DNA encoding hspD1-p24-Fc or p24-Fc via intramuscular electroporation. Mice that received PBS served as controls.

**[0159]** Two weeks after the last immunization, sera were collected. The levels of anti-p24 IgG1 and IgG2a antibodies were measured by ELISA. The levels of anti-p24 antibody in control samples is not shown because the absorbance readouts of these samples fell below the cutoff values for determining antibody titers. The anti-p24 antibody endpoint titers are defined as the reciprocal of the highest dilution of a test sample that produces a reading of at least two-fold greater than that of the control sample with the same dilution. The results, as shown in FIG. 6C, reveal that mice immunized with hspd1-p24-fc had high IgG1 and IgG2a titers, when compared to mice immunized with p24-Fc.

**[0160]** To examine p24-specific immune response, the number of IFN-γ-secreting splenocytes specific for p24 epitope gagAI (AMQMLKDI) (SEQ ID NO: 39) for CD8 T cells and the number of IFN-γ-secreting splenocytes specific for p24 epitope gag26 (TSNPPVPGYIYKRHWILGL) (SEQ ID NO: 40) for CD4 T cells was determined by ELispot assay. Bars represent the average values of three samples (±standard deviations). The results, as shown in FIG. 6D, reveal that wild-type hspD1 1-p24-Fc binds to mouse PD-L1 and PD-L2, and potently elicits humoral and cell-mediated immune responses.

**Example 6**

**Construction of Mouse sPD-1 Variant Vaccine Candidates**

**[0161]** This Example illustrates the construction of variant mspD1 vaccine candidates. Mouse sPD1 variants, mspd1-14dc1 and mspd1-322mu, were constructed (FIG. 7A). The mspd1-14dc1 variant is obtained by deleting amino acids 26-39 of the wild-type mspd1 (encoded by the first part of the second exon of the wild-type mouse PD-1 gene). The mspd1-322mu variant is obtained by changing amino acid residue 108 of the wild-type mouse PD-1 protein from Met to Val.

**[0162]** Mouse sPD-1 fusion constructs were obtained by fusing PVAX vector carrying mspd1 variant-p24 with rabbit Fc DNA. The PVAX vector and the p24-Fc DNA were linked by a linker encoding GGGS(GG(SEQ ID NO: 29). The transcription is under the control of promoter Penv.

**[0163]** FIG. 7A shows alignment of amino acid sequences of mspd1-p24-Fc, mspd1-14dc1-p24-Fc, mspd1-322mu-p24-Fc, and p24-Fc fusion proteins. FIG. 7B shows Western blot results of various fusion proteins useful according to the subject invention. Briefly, 293T cells were transfected with various fusion constructs using polyethyleneimine (PEI) and the supernatants were collected 72 hours post transfection. Proteins were detected by anti-rabbit Fc antibody. FIG. 7B shows that mspd1-14dc1-p24-Fc, and mspd1-322mu-p24-Fc are about 72KD in size, while p24-Fc is about 50KD in size. The results also show that mspd1-14dc1-p24-Fc, and mspd1-322mu-p24-Fc fusion proteins are soluble.

**Example 7**

**Binding Ability of Mouse sPD-1 Variant Fusion Proteins to sPD-1 Ligands**

**[0164]** This Example shows that mspD1-1 variant fusion proteins, mspd1-14dc1-p24-Fc and mspd1-322mu-p24-Fc, do not bind to mouse sPD-1 ligands PD-L1 and PD-L2 (FIG. 8). Briefly, 293T cells were transfected with PD-L1 (PD-L1 and PD-L2). The binding of sPD-1 proteins to PD-1 ligands was detected by mouse sPD-1-p24-Fc proteins and FITC-anti rabbit Fc antibody using flow cytometry, and the results were analyzed by FlowJo.

**Example 8**

**Induction of Humoral and Cell-Mediated Immune Responses by Variant sPD1 Vaccines**

**[0165]** This Example shows that mspD1 variants potently elicit humoral and cell-mediated immune responses. Briefly, Balb/c mice were primed at week 0 and boosted at week 3 and week 6 with 20 μg mouse DNA vectors encoding mspd1-14dc1-p24-Fc, mspd1-322mu-p24-Fc, or p24-Fc via intramuscular electroporation. Mice that received PBS served as controls.

**[0166]** Two weeks after the last immunization, sera were collected. The levels of anti-p24 IgG1 and IgG2a antibodies were measured by ELISA. The level of anti-p24 antibody in control samples is not shown because the absorbance readouts of these samples fell below the cutoff values for determining antibody titers. The anti-p24 antibody endpoint titers are defined as the reciprocal of the highest dilution of a test sample that produces a reading of at least two-fold greater than that of the control sample with the same dilution. The results show that mice immunized with msp1-14dc1-p24-Fc or msp1-322mu-p24-Fc had high IgG1 and IgG2a titers, when compared to mice immunized with p24-Fc.

**[0167]** To examine p24-specific immune response, the number of IFN-γ-secreting splenocytes specific for p24 epitope gagAI (AMQMLKDI) (SEQ ID NO: 39) for CD8 T cells and the number of IFN-γ-secreting splenocytes specific
for p24 epitope gag26 (TNNPPIPVGDIYKRVWIIILG1) (SEQ ID NO: 40) for CD4 T cells was determined by EL1spot assay. [0168] The results show that mice immunized with mspld1-14de1-p24-Fc or mspld1-32mmu-p24-Fc had high anti-p24 antibody titers (FIG. 9A) and a high number of IFN-γ-secreting splenocytes (FIGS. 9B and 9D). Splenocytes isolated from mice immunized with mspld1-14de1-p24-Fc or mspld1-32mmu-p24-Fc contained higher H2dKd-AMQMLKDTTI tetramer-positive cells (FIG. 9C), when compared to mice immunized with p24-Fc.

Example 9
Reduction of VTT-HIV-Gagp1 Fusions in Mice Immunized with Variant Mouse sPD1 Fusion Proteins

[0169] This Example shows that immunization with variant mspld1-14de1-p24-Fc or mspld1-32mmu-p24-Fc protects against HIV infection. Briefly, Balb/c mice were primed at week 0 and boosted at week 3 and week 6 with 20 μg mouse DNA encoding mspld1-14de1-p24-Fc or mspld1-32mmu-p24-Fc via intramuscular electroporation. Three weeks after immunization, mice were challenged with 4x10^6 PFU of VTT-HIV-gagp1 intranasally. Mice that received PBS served as controls.

[0170] The mice were sacrificed 3 days after viral challenge and viral titers in lungs were evaluated by plaque assay. The results show that mice immunized with mspld1-14de1-p24-Fc or mspld1-32mmu-p24-Fc exhibited significantly reduced VTT-HIV-gagp1 titers upon viral challenge (FIG. 10).

Example 10
Induction of Humoral and Cell-Mediated Immune Responses by Variant Human sPD1 Vaccine

[0171] In this Example, variant hsPD1 construct, hsPD1-14de1-p24-Fc, was constructed. To analyze the binding ability of hsPD1-14de1-p24-Fc protein to PD-L1 and PD-L2, 293T cells were transfected with PD-L1 and PD-L2, respectively. The binding of hsPD1-14de1-p24-Fc protein to PD-L1 were detected by FITC-anti rabbit Fc antibody using flow cytometry, and the results were analyzed by flowJo. The results, as shown in FIGS. 11A and 11B, reveal that hsPD1-14de1-p24-Fc fusion protein binds to PD-L1 ligands.

[0172] To examine the induction of immune responses by hsPD1-14de1-p24-Fc, Balb/c mice were primed at week 0 and boosted at week 3 and week 6 with 20 μm/mouse DNA encoding hsPD1-14de1-p24-Fc or p24-Fc via intramuscular electroporation. Mice that received PBS served as controls.

[0173] Two weeks after the last immunization, mice sera were collected. The levels of anti-p24 IgG1 and IgG2a antibodies were determined by ELISA. The levels of anti-p24 antibody in control samples are not shown because the absorbance readouts of these samples fell below the cutoff values for determining antibody titers. The anti-p24 antibody endpoint titer is defined as the reciprocal of the highest dilution of a test sample that produces a reading of at least two-fold greater than that of the control sample with the same dilution.

[0174] To examine p24-specific immune response, the number of IFN-γ-secreting splenocytes specific for p24 epitope gag26 (TSNPPIPVGDIYKRVWIIILG1) (SEQ ID NO: 39) for CD8 T cells and the number of IFN-γ-secreting splenocytes specific for p24 epitope gag26 (TSNPPIPVGDIYKRVWIIILG1) (SEQ ID NO: 40) for CD4 T cells was determined by EL1spot assay.

Although hsPD1-14de1-p24-Fc protein does not bind to PD-1, the results show that hsPD1-14de1-p24-Fc significantly enhanced humoral and cell-mediated immune responses upon HIV viral challenge (FIGS. 11C and 11D).

Example 11
Antigen Targeting to Dendritic Cells by sPD1-Based Vaccine Amplifies CD8⁺ T Cell Immunity

[0175] This Example shows that sPD1-based vaccine improves CD8⁺ T cell immunity by targeting vaccine antigens to dendritic cells (DCs), while blocking the negative effects of the PD-1/PD-L pathway on T cell function simultaneously.

[0176] HIV-1 Gag p24 was chosen as a test antigen because it has been commonly used in other DC-targeting strategies as a model immunogen. Three DNA vaccines, p24-Fc, sPD1-1-p24-Fc, and slvG-PD1-1-p24-Fc, were designed (FIGS. 13a and 16a). slvG-PD1-1-p24-Fc differs from sPD1-1-p24-Fc by two essential amino acids in the functional IgV domain of sPD1, rendering it unable to bind PD-1 ligands.

[0177] The results show that PD-L 1 and PD-L 2 interact with recombinant sPD1-1-p24-Fc protein, but do not interact with slvG-PD1-1-p24-Fc or p24-Fc proteins (FIG. 16b).

[0178] In addition, Balb/c mice bone marrow (BM) derived CD1 c4DCs that expresses PD-L1 and PD-L2 (FIG. 17a) binds to sPD1-1-p24-Fc, but does not bind to slvG-PD1-1-p24-Fc (FIG. 17b).

[0179] 2x10⁷ BM-DCs were pulsed with 20 g sPD1-1-p24-Fc or control proteins, and infused back into Balb/c mice via the tail vein in accordance to a standard immunization schedule. Compared to slvG-PD1-1-p24-Fc, sPD1-1-p24-Fc pulsed BM-DCs elicited higher levels of anti-p24 IgG1 (Th2) and IgG2a (Th1) antibody responses (p<0.05; FIG. 17c). Increased levels of p24-specific CD8⁺ T cell immunity was also evident as determined by IFN-γ ELISPOT (FIG. 17d) and H2-Kd-AMQMLKDTTI (Gag-A1) tetramer assays (p<0.05; FIG. 17e). The results show that the sPD1-based protein vaccine induced p24 specific CD8⁺ T cell immunity by targeting dendritic cells.

[0180] The results also show that sPD1-1-p24-Fc can be used as a DNA vaccine against infection. The present inventions have previously shown that intramuscular (i.m.) EP enhances the immunogenicity of DNA vaccines consistently.

[0181] In this Example, i.m. sPD1-1-p24-Fc/EP vaccination was conducted, using a vaccine dose of 20 μg or 100 μg (FIG. 13b). The results show that sPD1-1-p24-Fc/EP elicited significantly higher levels of IgG1 (4-fold; p<0.01) and IgG2a (8-fold; p<0.01) antibody responses, when compared to the slvG-PD1-1-p24-Fc/EP control (FIG. 13c), in addition to potent and dose-dependent anti-Gag CD8⁺ (p<0.001) and CD4⁺ (p<0.05) T cell responses as determined by IFN-γ ELISPOTs (FIGS. 13d and 13e). Specifically, approximatley 700 and 1600 ELISPOTs/10⁶ splenocytes were found against the CD8⁺ specific Gag-A1 epitope at the doses 20 μg and 100 μg, respectively. This greatly contrasts with the 200-300 ELISPOTs/10⁶ splenocytes against the same epitope elicited by 1 mg/i.m. ADVAX (a codon-optimized HIV DNA vaccine) or 10⁹ TCID50/î.m. ADMAV (a vaccinia MVA-vectorized HIV-1 vaccine) as previously described by the present inventors.

[0182] The p24-specific T cell immunity was not confined to the single Gag-A1 epitope. Approximately 800-1000 ELISPOTs/10⁶ splenocytes was reactive to each of the three
non-overlapping peptide pools spanning the entire p24 protein, indicating a broad breadth in anti-Gag Gag T cell responses following vaccination with sPD-1-p24-fc/EP (FIG. 13f). Additionally, over 12.7% and 22% of CD8+ T cells were positive for H2-Kd-Gag: Al tetramer binding in the 20 μg and 100 μg sPD-1-p24-fc/EP dose groups respectively, which is significantly higher than that of the slgV-EP-1-p24-fc/EP group (p<0.05, FIGS. 3g and 13b), and is comparable to those observed in Balb/c mice using a heterologous prime-boost protocol with two live vectors, L. monocytogenes and Ad5.18

[0183] In this example, this investigation determines the ability of p24-specific T cell populations to secrete IFN-γ, TNF-α and IL-2 in response to antigen stimulation. Compared to sPD-1-p24-fc/EP, sPD-1-p24-fc/EP elicited substantially higher frequencies of p24-specific CD8+ T cells producing IFN-γ (47.1%) and TNF-α (23.6%), and elevated frequencies of p24-specific CD4+ T cells producing IFN-γ (6.8%) and TNF-α (3.6%) (FIGS. 14a and 14b). The results show that the proportion of effector-producing CD8+ and CD4+ T cell populations was similar in the order of IFN-γ/TNF-α > IFN-γ/TNF-α/IL-2 (FIGS. 14a and 14b). Upon analyzing total cytokine-producing p24-specific CD8+ T cells, high frequency of cells secreting IFN-γ (42.2%), IFN-γ/TNF-α (44.8%) and IFN-γ/TNF-α/IL-2 (9.4%) are indicative of enhanced vaccine potency (FIG. 14c).

[0184] To characterize sPD-1-p24-fc/EP vaccination and investigate its underlying mechanism(s) of immune induction, additional experiments were performed. Specifically, this example compared sPD-1-p24-fc DNA vaccination with or without EP at the 20 μg dose. Without EP, sPD-1-p24-fc induced 10-fold less IFN-γ-secreting CD8+ T cells than sPD-1-p24-fc/EP (FIGS. 18a and 13b), likely due to the omission of EP’s effective recruitment of DCs to the site. In addition, the lack of statistical difference between sPD-1-p24-fc and sLV-EP-p24-fc induced CD8+ T cells when delivered without EP (FIG. 18a) indicates that sPD-1 alone does not have a strong adjuvant effect.

[0185] In another experiment, mice were co-immunized with a mixture of 20 μg of sPD-1-fc and p24-fc by i.m./EP, and no statistical difference between these two groups in their IFN-γ/CD8+ T cell response was found (FIG. 18b), indicating that de novo synthesis of sPD-1-fc alone was insufficient to potentiate immunogenicity. This shows the importance of DC-targeting via fusion of the antigen to sPD-1.

[0186] To exclude a role of rabbit-Fc in enhancing p24-specific immunity, the rabbit Fc fragment was removed from sPD-1-p24-fc and p24-fc to generate sPD-1-p24 and p24 DNA vaccines for immunization. In corroboration to sPD-1-p24-fc/EP, sPD-1-p24/EP induced significantly higher levels of IFN-γ/CD8+ T cell response than p24/EP (FIG. 18c). Also, there was no statistical difference between sPD-1-p24-fc/EP and sPD-1-p24/EP in their ability to induce p24-specific IFN-γ+CD8+ T cell responses (FIGS. 18a and 18c).

[0187] In another experiment, a human (hu)-hSPD-1-p24-fc vaccine was used for comparative study, as it is known that hu-hSPD-1 cross-reacts with murine PD-1.1 and PD-1.223 (FIG. 19a). The results show that hu-hSPD-1-p24-fc/EP induced significantly greater levels of p24-specific IFN-γ+ CD8+T cell and antibody responses, when compared to p24-fc/EP in Balb/c mice (FIGS. 19b and 19c). Anti-human PD-1 responses were also induced due to the sequence divergence from murine PD-1, which may account for the difference between murine sPD-1-p24-fc/EP and hu-sPD-1-p24-fc/EP in the observed immunogenicity profile (i.e. p24-specific CD4+ T cell response was weak in mice immunized with hu-sPD-1-p24-fc/EP (FIG. 13e and FIG. 19c).

[0188] To determine whether sPD-1-p24-fc/EP elicited long-lived p24-specific memory T cell responses, groups of mice 7.5 months were sacrificed after the third immunization with 20 μg DNA vaccine. Besides persistent anti-p24 IgG1 and IgG2a antibody responses (FIG. 15a), p24-specific CD8+ (p<0.05) and CD4+ (p<0.05) memory T cell responses were sustained in mice immunized with sPD-1-p24-fc/EP compared with controls (FIGS. 15b and 15c).

[0189] To investigate if cellular immunity elicited by sPD-1-p24-fc/EP leads to protection, Balb/c mice immunized with DNA vaccines at a dose of 100 μg (FIG. 13b) were challenged intranasally with 2x106 PFU of a virulent strain of vaccinia modified to express HIV-1 gag and pol (WRgagpol). Eight days post-challenge, a significant reduction in virus titers in the lungs was observed in mice vaccinated with sPD-1-p24-fc/EP compared to controls (p<0.01; FIG. 15d). Mice immunized with the placebo or slgV-EP-1-p24-fc/EP showed >25% body weight loss within eight days after virus inoculation in contrast to mice immunized with sPD-1-p24-fc/EP that survived the challenge with <5% body weight loss (FIG. 15e). Since there were no anti-vaccinia neutralizing antibodies involved, the results indicated that p24-specific T cell immunity induced by sPD-1-p24-fc/EP provided significant protection against mucosal challenge by a virulent virus.

[0190] To summarize, this Example demonstrates that targeting of HIV-1 p24 to DCs by sPD-1 as a DNA vaccine enhanced the magnitude, breadth, and polyfunctionalit of specific CD8+ T cell immunity. The sPD-1-based DNA vaccine can be used for inducing protective and long-lasting CD8+ T cell immunity against pathogenic infections including HIV-1, tuberculosis, and malaria.

Material and Methods

[0191] Construction of sPD-1-Based Vaccine and Controls

[0192] Three DNA vaccines, sPD-1-p24-fc, slgV-EP-1-p24-fc, and p24-fc, were constructed in the background of pVAX1 (FIG. 13a). The coding region for the extracellular domain of murine PD-1 (sPD-1) was obtained by nested PCR from mouse cDNA 10,26, and the HIV-1 p24 fragment was amplified from an aborted ease isolate HIV-1021Fh44 of a Chinese patient without codon-optimization.27 To increase the flexibility of the fusion protein, a linker was applied between the sPD-1 and HIV-1 p24 gene.

[0193] A mutant form of sPD-1 (slgV-EP-1) was also cloned following the same strategy as wild type PD-1. slgV-EP-1 does not react with PD-1 ligands 42,43 due to a two essential amino acid (position 89-90) in-frame deletion in the IgV domain. Plasmid expressing HIV-1 p24 alone served as a control.

[0194] All of the plasmids contained a rabbit Fc tag to facilitate protein purification and characterization. DNA transfection into (HEK-293T) cells was performed using Polyethylenimine (PEI), and protein expression was detected by Western blotting assay using anti-rabbit Fc antibody.

[0195] Recombinant proteins were purified from the transfected cell supernatants by affinity chromatography using Protein G Sepharose (Invitrogen), and protein concentration was measured by Micro BCA Protein Assay Kit (Thermo Scientific).

[0196] Binding Characteristics of sPD-1 Fusion Proteins

[0197] 106 293T cells transiently expressing PD-1.1 and PD-1.2 were incubated with 2 μg of purified sPD-1-p24-fc,
slgV-PD-1-p24-fc or p24-fc fusion protein. Goat anti-rabbit IgG (H+L-)–FITC (Invitrogen) was used to capture the positive cells. Transfected 293T cells stained by FITC-rat anti-mouse PD-L1 or PD-L2 antibodies (eBioscience) and FITC-rat IgG1 isotype served as positive and negative controls, respectively. Data was acquired on FACSCalibur instrument (BD Biosciences) and analyzed using BD CellQuest software.

Mouse Immunization

All animal experiments were approved by the Committee on the Use of Live Animals in Teaching and Research of the University of Hong Kong. 5–8 weeks old female Balb/c mice were bred under standard pathogen-free conditions in the Laboratory Animal Unit, University of Hong Kong. Mice were housed in cages under standard conditions with regulated temperature and humidity, fed with pelleted food and tap water, and cared for according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals.

The immunization procedure was similar to the previous protocols described in 1,5,15 (FIG. 136). Mice received three DNA immunizations by intramuscular (i.m.) injection with or without EP given every three weeks at a dose of 20 μg or 100 μg per mouse. Two weeks after the final immunization, mice were sacrificed, and sera and spleen cells (splenocytes) were collected for immune response analysis.

Enzyme-Linked Immunosorbent Assay (ELISA)

Specific antibody responses were assessed by ELISA as previously described15,16. Briefly, high affinity, protein-binding ELISA plates (BD Biosciences) were coated with HIV-1 p24 protein (Abcam). Serial diluted sera were then added and antibodies detected by goat-radish peroxidase (HRP)-labeled anti-mouse IgG1 or IgG2a antibody (Sigma). Relative antibody titer was expressed as the reciprocal highest dilution of samples producing at least two-fold greater optical density readout over that of the control serum sample at the same dilution.

Evaluation of HIV-I Gag p24-Specific T Cell Responses

IFN-γ-producing T cells were evaluated by an ELISPOT assay (Millipore) as previously described 15,16. 2 μg/ml of HIV-1 p24 peptide or peptide pools (at a final concentration of 2 μg/ml for each peptide, donated by NIH) were used to stimulate splenocytes in vitro. Peptide pool consisting of 59-members of Gag p24 libraries were divided into 3 pools of 19-20 peptides that span from amino acids 1-87 (pool 1), 77-167 (pool 2) and 157-231 (pool 3). Peptide gagAl (AMQMLKDIT) is specific for CD8+ T cells, whereas peptide gag26 (TSPHPVMDIDKRWITLGL) is specific for CD4+ T cells15,16.

Cells stimulated by 500 ng/ml PMA plus 1 μg/ml calcium ionophore or left in media only served as positive and negative controls, respectively. Cells were stimulated at 37°C, 5% CO2, and 100% humidity for 20 h. Spots were identified by an immunospot reader and image analyzer (Thermo Scientific).

For intracellular cytokine staining (ICS), splenocytes were stimulated with HIV-1 p24 peptide pool (2 μg/ml for each peptide) in the presence of co-stimulatory anti-CD28 antibody (2 μg/ml, eBioscience) for 20-24 h at 37°C. 10 μg/ml Brefeldin A (BFA; Sigma) was added for the last 5 h to accumulate intracellular cytokines. Cells were washed and incubated with 2,4G2 mAb for 15 min at 4°C to block Fcγ.

After surface staining with anti-mouse CD3-APC/cy7, CD4-PE/cy5, CD8-Perp/cy5.5 antibodies (eBioscience), cells were permeabilized in 100 μl Fixation/Permeabilization solution (BD Biosciences) for 20 min at 4°C, washed with Perm/Wash™ buffer (BD), and then stained intracellularly with anti-IFN-γ-PE, anti-IL-2-PE/cy7, anti-TNF-α-FITC (eBioscience). Tetramer positive CD8+ T cell population was evaluated using phycoerythrin (PE)-conjugated major histocompatibility complex (MHC) class I tetramer H2d-Kd-AM-QMLKDIT (Beckman Coulter). Flow cytometric data were acquired and analyzed on a BD Aria III flow cytometer (BD Biosciences).

Mouse Immunization of Antigen Pulsed-Dendritic Cells

Bone marrow DCs (BM-DCs) from Balb/c mice were enriched by Dynabeads Mouse DC Enrichment Kit (Invitrogen). Two million CD11c+ BM-DCs were co-cultured with 20 μg of purified sPD-1-p24-fc or slgV-PD-1-p24-fc proteins for 1 h at 4°C. Cells were then washed extensively with PBS and transduced into mice via tail vein injection. Untreated DCs alone served as control. Immunization procedure and immune responses analysis were the same as described above.

Vaccinia Viral Challenges

Immunized mice were challenged intranasally with 2×10^6 PFUs vaccinia strain Western Reserve (WR) virus modified to express HIV-1 gag and pol genes. Animal body weight was monitored daily. Groups of animals were also sacrificed 8 days post challenge to measure viral titers in their lungs. Lung homogenates were prepared by physical disruption, and virus titers in the lungs were determined by a plaque-forming assay on monolayer Vero cells and monitored for cytopathic effect.

Statistical Analysis

All statistical analyses were performed using the paired one-tailed Student’s t-test. P values less than 0.05 were considered statistically significant. Data were presented as mean values±standard error of at least three independent experiments.

All references, including publications, patent applications and patents, cited herein are hereby incorporated by reference to the same extent as if each reference was individually and specifically indicated to be incorporated by reference and was set forth in its entirety herein.

The terms “a” and “an” and “the” and similar referents as used in the context of describing the invention are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context.

Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. Unless otherwise stated, all exact values provided herein are representative of corresponding approximate values (e.g., all exact exemplary values provided with respect to a particular factor or measurement can be considered to also provide a corresponding approximate measurement, modified by “about,” where appropriate).

The use of any and all examples, or exemplary language (e.g., “such as”) provided herein, is intended merely to
better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise indicated. No language in the specification should be construed as indicating any element is essential to the practice of the invention unless as much is explicitly stated.

[0210] The description herein of any aspect or embodiment of the invention using terms such as "comprising", "having", "including" or "containing" with reference to an element or elements is intended to provide support for a similar aspect or embodiment of the invention that "consists of", "consists essentially of", or "substantially comprises" that particular element or elements, unless otherwise stated or clearly contradicted by context (e.g., a composition described herein as comprising a particular element should be understood as also describing a composition consisting of that element, unless otherwise stated or clearly contradicted by context).

[0211] It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application.

REFERENCES


[0223] 12. Ideyama, J. et al. Comparable T helper 1 (Th1) and CD8 T-cell immunity by targeting.


SEQUENCE LISTING

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Cys Ser Lys Pro Met Cys Pro Pro Pro Gly Leu Leu Gly Gly Pro Ser 65 70 75 80
Val Phe Ile Phe Pro Pro Lys Asp Thr Leu Met Ile Ser Arg 85 90 95
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Arg Pro Pro Leu Arg Glu Gin Phe Ser Ser Thr Ile Arg Val 130 135 140
Ser Thr Leu Pro Ile Ala His Glu Asp Trp Leu Arg Gly Lys Glu Phe 145 150 155 160
Lys Cys Lys Val His Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr 165 170 175
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<220> FEATURE:
<223> OTHER INFORMATION: mapd1-14del

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Gln Ala Ala Phe Cys Asn Gly Leu Ser Gln Pro Val Gln Asp Ala Arg
65  70  75  80
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85  90  95
Leu Aep Thr Arg Arg Aen Ser Gly Ile Tyr Leu Cys Gly Ala Aen
100 105 110
Ser Leu His Pro Lys Ala Lys Ile Glu Glu Ser Pro Gly Ala Glu Leu
115 120 125
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| Gly Ala Aam Ala Thr Phe Thr Cys Ser Leu Ser Aam Trp Ser Glu Asp |
| 35  | 40              | 45              |                 |
| Leu Met Leu Aam Trp Aam Arg Leu Ser Pro Ser Aam Gin Thr Glu Lys |
| 50  | 55              | 60              |                 |
| Gin Ala Ala Phe Cys Aam Gin Leu Ser Gin Pro Val Gin Aam Gin Asp |
| 65  | 75              | 80              |                 |
| Phe Gin Ile Ile Gin Leu Pro Aam Arg His Asp Phe His Met Aam Ile |
| 85  | 90              | 95              |                 |
| Leu Asp Thr Arg Arg Aam Asp Ser Gly Ile Tyr Leu Cys Gin Aam Ala |
| 100 | 105             | 110             |                 |
| Ser Leu His Pro Lys Ala Lys Ile Gin Ser Pro Gin Aam Glu Leu     |
| 115 | 120             | 125             |                 |
| Val Val Thr Glu Arg Ile Leu GluThr Ser Thr Arg Tyr Pro Ser Pro  |
| 130 | 135             | 140             |                 |
| Ser Pro Lys Pro Glu Gly Arg Phe Gin Pro Glu Phe Arg Gly Gly Gly |
| 145 | 150             | 155             | 160             |
| Ser Gly Gly Pro Ile Val Gin Aam Leu Gin Gly Gin Met Val His     |
| 165 | 170             | 175             |                 |
| Gin Pro Ile Ser Pro Arg Thr Leu Aam Ala Trp Val Lys Val Ile Gin |
| 180 | 185             | 190             |                 |
| Glu Lys Ala Phe Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser |
| 195 | 200             | 205             |                 |

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<210> SEQ ID NO 15
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Arg Ser Leu Thr Phe Tyr Pro Ala Trp Leu Thr Val Ser Glu Gly Ala
  35   40   45
Asn Ala Thr Phe Thr Cys Ser Leu Ser Asn Trp Ser Glu Asp Leu Met
  50   55   60
Leu Asn Trp Asn Arg Leu Ser Pro Ser Asn Glu Thr Glu Lys Glu Ala
  65   70   75   80
Asa Phe Cys Asn Gly Leu Ser Glu Pro Val Glu Asp Ala Arg Phe Glu
  85   90   95
Ile Ile Gln Leu Pro Asn Arg Asp Arg Phe His Val Asn Ile Leu Asp
 100  105  110
Thr Arg Arg Asn Ser Gly Ile Tyr Leu Cys Gly Ala Ile Ser Leu
 115  120  125
His Pro Lys Ala Lys Ile Glu Glu Ser Pro Gly Ala Glu Leu Val
 130  135  140
Thr Glu Arg Ile Leu Glu Thr Ser Thr Arg Tyr Pro Ser Pro Ser Pro
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Lys Pro Glu Gly Arg Phe Glu Pro Phe Arg Gly Gly Gly Ser Gly
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Asn Ala Thr Phe Thr Cys Ser Leu Ser Asn Trp Ser Glu Asp Leu Met 50 55 60
Leu Asn Trp Asn Arg Leu Ser Pro Ser Asn Gin Thr Glu Lys Gin Ala 65 70 75 80
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Ile Ile Gin Leu Pro Asn Arg His Asp Phe His Val Asn Ile Leu Arg 100 105 110
Thr Arg Arg Asn Asp Ser Gly Ile Tyr Leu Cys Gin Ala Ile Ser Leu 115 120 125
His Pro Lys Ala Lys Ile Glu Glu Ser Pro Gly Ala Glu Leu Val Val 130 135 140
Thr Glu Arg Ile Leu Glu Thr Ser Thr Arg Tyr Pro Ser Pro Ser Pro 145 150 155 160
Lys Pro Glu Gly Arg Phe Gin Pro Glu Phe Arg Gly Gly Gly Ser Gly 165 170
Gly Gly Pro Ile Val Gin Asn Leu Gin Gly Gin Met Val His Gin Pro 180 185 190
Ile Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Ile Glu Glu Lye 195 200 205
Ala Phe Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly 210 215 220
Ala Thr Pro Gin Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly His 225 230 235 240
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atagctgcaaa accccgaggg gcacaattgta ctaacgccc ctaaacttctg aacttttaa 600
gcattggtaaa aagtaattagc agggaggttc ttattgctcag aaagaatcccc cattttttca 660
gcatattcag aaggggacagc ccccaagatg ttaaaccacc tgcataacatt agtttgggaa 720
cactaacgag ccaagccat gttataagaa aaccaaatag agagacgtag gcagctgaat 780
agatgcatc ccggcggctc agggttgctc gcaaacgagc agtggagaga acacggggga 840
agttcatacag caggaatacct tagtaattctt cagggccaa taggtgggat gacaaataaa 900
cccatcttcc cggcggctcc atctatattt aagatgtaaa tttctggttct aaataaaata 960
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agacagagct tagacggttt ctaaataact ctaaggggct cagcggotc caacgagggta 1080
aaaaattttg tgaagggactg agggagcagc ggaagggctcc aatctctgga aactttttct 1140
ttaaagactt gggagacagc acatttcaag aagcttttga tggagcagtac tggagttgtg 1200
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caccgagcac ctaatagga aaccaatct cc aaggtgacag gaggctccct gcagctggaag 1800
gctctacacca tggggcggttcc cgggagagc gattggctgag cctctggagc 1860
acgatcaacg gctttaccc ttcgacatc tcggatgagtc ggggaggaag ccggagagca

1920
gagggacact agacagacac gcggaacgtg ccggcacagc acggctctca tttctcttac

1980
tagcagctct cagtgcttac gaggagagtc aagttcttaca cttgcttctg

2040
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2100
taatcttagg

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<211> LENGTH: 2110
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: mspd1-p24-Pc

SEQUENCE: 20

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tcggggcgc ttcctagaggt ccccaatgag cccctgaggt cccctcactt ctccccagcc  120
tgctccacag ttgctcgggg acgaaatgcc accctcaacct gcagcttgcg ccaagtgcgc  180
gagcatctta gtcggcactg gaaccgctcg aggccccagca accagaactga aaaaaccggc  240
gctttctgta atgctttagc ccaacccgct caggtagcct gctttcagat catcacgtg  300
cccacccgg atgaccttcca caatgacata cttgacacac ggcgaatga caggggatct  360
taacccttg gggccatcct ctcgacccg aagccaaaaa tegagagag ctcggagaca  420
gagctcgggc taacagagag aatctcgagag acctcaacca gataacccag ccccctccggc  480
aagccagaag ggcgttttca accgaaactc cgggggctgg gttgttcagc aggagacgt  540
atgctaacca accctggaag gcacagtgta catcagccca tttcgccagt aacgttatat  600
gcattggtta aagtaatagc agaagagcct ttgtacccag aagtaatacc accgttttca  660
gcattcag aaggaagcc ccccaagat tttacaccag aaggaagtgc aagtaagggat  720
cacacctg cccgtcaaat gtttaacagac acctcaactg aggacgctgc aagtaagggat  780
agatcctcc caggtgggac aggccgagtt gcaccgagcc agatgagaga acaaggggga  840
agtgacatag cggagaactac tgaattcttt cggagccagc tagatggagc gacaacata  900
cacacctcc caggtggaat aacttctaaa agatggtatat tccgggtgtt aataaaaaa  960
gtaagaagrt atagccctac cagcattgagc gacaataagc aagccaaa aagccagttt 1020
agacatag taagcagctgt cttaaacact ctatggagcgc cagaagcttc acaacaggtta 1080
aaaaatggag cgaccagaca ctttgtgact ccaaaaattc accaagattg ttaagcatatt 1140
ttaaaagct tggccagcag acgtagaca gaaasatga tgcagcagct tggggtggct 1200
ggggacggt gcataaacat aagattttgt atcccttgtcg agcatacaca ggcacagcag 1260
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aaggacaccc ctcctgctctt cagcaacccc gcagtaagct ggtctggtgt gaggctgagc 1560
caggatgacc cggcgctgca gttctacagc tcataacata aacgacaggt gcggccgacc 1620
cggcgcgagc tcagggcagc gcagttccacag acgacagct ggttggctag cacatcctcc 1680
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cctccgaga cccacatcc aacgcagcag cgcagccccg cggcgacagc ggcagccag 1800
gtcacacca cggcgcccc gcgggagagt cgtcgccgag ccggccgctg aagttctgct 1860
atgtcacag cttttcttccc tccgacacat cttggagagt gcggagacag cggagagcaac 1920
gagcgaacct aacaagacac gcgcacgctg ctcggacgcg aaggttcota ctctctctac 1980
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agcaagctt caagacacac gacgttaggg cagcgagggcg aagtcctcaac ctgctcctgg 2040
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taatctccag 2110

<210> SEQ ID NO: 21
<211> LENGTH: 174
<212> TYPE: PRT
<213> ORGANISM: human
<220> FEATURE:
<223> OTHER INFORMATION: human spdl

<400> SEQUENCE: 21

Met Gln Ile Pro Gin Ala Pro Trp Pro Val Val Trp Ala Val Leu Gin 1 5 10 15
Leu Gly Trp Arg Pro Gly Trp Phe Leu Asp Ser Pro Asp Arg Pro Trp 20 25 30 35
Arg Pro Thr Phe Ser Pro Ala Leu Leu Val Val Thr Gln Gys Asp 40 45
Arg Ala Thr Thr Cys Ser Phe Ser Asn Thr Ser Glu Ser Phe Val 50 55 60
Leu Asn Thr Tyr Arg Met Ser Pro Ser Aen Gln Thr Asp Lys Leu Ala 65 70 75 80
Asp Ala Phe Glu Asp Asp Arg Ser Gin Pro Gly Gin Asp Cys Arg Phe Arg 85 90 95
Val Thr Gin Leu Pro Gin Gly Arg Asp Phe His Met Ser Val Val Arg 100 105 110
Ala Arg Arg Gin Asp Ser Gly Thr Tyr Leu Cys Gin Ala Ile Ser Leu 115 120 125
Ala Pro Lys Ala Gin Ile Lys Gin Ser Leu Arg Ala Glu Leu Arg Val 130 135 140
Thr Gin Arg Arg Ala Gin Val Pro Thr Ala His Pro Ser Pro Ser Pro 145 150 155 160
Arg Pro Ala Gin Pro Glu Phe Arg Gly Gly Gln Ser Gly Gly Gly Gly 165 170 175

<210> SEQ ID NO: 22
<211> LENGTH: 531
<212> TYPE: DNA
<213> ORGANISM: human
<220> FEATURE:
<223> OTHER INFORMATION: human spdl

<400> SEQUENCE: 22

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cagggagtt tgtatcagc cccagacagg cctggaacc ccccaacott ctccccagggc 120
cgctgcctgg gacacgacc acctctcaact gcagctctctt cacaactgc 180
gagacgcttg tgtactaaactgt gtaacgctag agccccagca accagccgga caagctggcc 240
gcgctccag aggacgacag cagcgaggg ccagctgctgt ccaacactg 300
cccacggg ggtccattcag ctaggactgt gtcgagggc ggcgcaatag cagggcacc 360
tactacctgg ggcctagcttc ctgccccg ccagacgcg tcaagagagctccggggg 420
gagctcagag tcacagagag aagggccgaa gttcccacag cccacccag gcctctaccc 480
agggcagccg gcgcagcggga attcgggggt ggtcggtggt cagggaggg a

<210> SEQ ID NO 23
<211> LENGTH: 695
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: hepd1-p24-Fc

<400> SEQUENCE: 23

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20  25  30
Asn Pro Pro Thr Phe Ser Pro Ala Leu Leu Val Val Thr Glu Gly Asp
35  40  45
Asn Ala Thr Phe Thr Cys Ser Phe Ser Asn Thr Ser Glu Ser Phe Val
50  55  60
Leu Asn Trp Tyr Arg Met Ser Pro Ser Asn Gin Thr Asp Lys Leu Ala
65  70  75  80
Ala Phe Pro Glu Asp Arg Ser Gin Pro Gly Gin Asp Cys Arg Phe Arg
85  90  95
Val Thr Gin Leu Pro Asn Gly Arg Arg Phe His Met Ser Val Val Arg
100 105 110
Ala Arg Arg Asn Asp Ser Gly Thr Tyr Leu Cys Gly Ala Ile Ser Leu
115 120 125
Ala Pro Lys Ala Gin Ile Lys Glu Ser Leu Arg Ala Glu Leu Arg Val
130 135 140
Thr Gin Arg Arg Ala Gin Val Pro Thr Ala His Pro Ser Pro Ser Pro
145 150 155 160
Arg Pro Ala Gin Gin Pro Gin Phe Arg Gly Gly Gin Ser Gly Gly Gin
165 170 175
Pro Ile Val Gin Asn Leu Gin Gin Gin Met Val His Gin Pro Ile Ser
180 185 190
Pro Arg Thr Leu Asn Ala Trp Val Lys Val Ile Gin Gly Ala Phe
195 200 205
Ser Pro Gin Val Ile Pro Met Phe Ser Ala Leu Ser Gin Ala Gly Ala Thr
210 215 220
Pro Gin Asp Gin Gin Thr Met Leu Gin Thr Val Gin Gin Gin Gin Gin Alpa
225 230 235 240
Asn Met Gin Met Leu Lys Gin Thr Ile Gin Gin Gin Gin Gin Gin Gin
245 250 255
Asp Arg Gin Gin Pro Gin Val Gin Gin Gin Gin Gin Gin Gin Gin Gin
260 265 270
Arg Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin
275 280 285
Glu Gin Ile Gin Gin Gin Met Thr Asn Gin Pro Pro Ile Pro Val Gin Gin
290 295 300
Ile Tyr Lys Gin Thr Ile Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin
305 310 315 320
Tyr Ser Pro Thr Ser Ile Gin Leu Gin Leu Gin Gin Gin Gin Gin Gin Gin
325 330 335
Phe Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin
340 345 350
Tyr Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin
355 360 365
Asp Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin
370 375 380
Ala Ser Gin Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gin
340  345  350
355  360  365

Asn Ser Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala
370  375  380

Ala Thr Leu Glu Glu Met Met Thr Ala Cys Gin Gly Val Gly Gly Pro
385  390  395  400

Gly His Lys Ala Arg Val Leu Met Gin Tyr Ile Lys Ala Asn Ser Lys
405  410  415

Phe Ile Gly Ile Thr Glu Leu Lys Leu Gly Gly Ser Asn Asp Ile
420  425  430

Phe Asn Asn Phe Thr Val Ser Phe Trp Leu Arg Val Pro Lys Val Ser
435  440  445

Ala Ser His Leu Gin Gin Tyr Leu Glu Ala Thr Asn Thr Lys Val Asp
450  455  460

Lys Thr Val Ala Pro Ser Thr Cys Ser Lys Pro Met Cys Pro Pro Pro
465  470  475  480

Glu Leu Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys Pro Lys
485  490  495

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
500  505  510

Asp Val Ser Gin Asp Asp Pro Glu Val Gin Phe Thr Trp Tyr Ile Asn
515  520  525

Asn Gin Gin Val Gin Gin Arg Gin Thr Ala Arg Pro Pro Leu Gin Gin Gin Phe
530  535  540

Asn Ser Thr Ile Arg Val Val Ser Thr Leu Pro Ile Ala His Gin Asp
545  550  555  560

Trp Leu Arg Gly Lys Glu Phe Lys Cys Lys Val His Gin Lys Ala Leu
565  570  575

Pro Ala Pro Ile Gin Thr Ile Ser Lys Ala Gin Arg Gin Pro Leu
580  585  590

Glu Pro Lys Val Tyr Thr Met Gin Gly Pro Pro Arg Glu Leu Gin Gin Ser
595  600  605

Arg Ser Val Ser Leu Thr Cys Met Ile Asn Gin Phe Tyr Pro Ser Gin
610  615  620

Ile Ser Gin Val Trp Gin Lys Asn Gin Gly Lys Ala Gin Gin Gin Thr Lys
625  630  635  640

Thr Thr Pro Thr Val Leu Gin Asp Ser Gin Ser Tyr Phe Leu Tyr Ser
645  650  655

Lys Leu Ser Val Pro Thr Ser Gin Trp Gin Arg Gin Gin Gin Gin Val Phe Thr
660  665  670

Cys Ser Val Met His Gin Glu Ala Leu Gin Gin Gin Gin Tyr Thr Gin Lys Ser
675  680  685

Ile Ser His Ser Gin Gin Gin Lys
690  695

<210> SEQ ID NO 24
<211> LENGTH: 2104
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE: 
<223> OTHER INFORMATION: hepdl-p24-Fc
<260> SEQUENCE: 24

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tgctcaagag  
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gtxtcagac  

<210> SEQ ID NO 25
Met Gln Ile Pro Gln Ala Pro Trp Pro Val Val Trp Ala Val Leu Gln
1  5  10  15
Leu Gly Trp Arg Pro Gly Trp Phe Leu Ala Leu Leu Val Val Thr Glu
20 25 30
Gly Asp Asn Ala Thr Phe Thr Cys Ser Phe Ser Asn Thr Ser Glu Ser
35 40 45
Phe Val Leu Asn Trp Tyr Arg Met Ser Pro Ser Asn Glu Thr Asp Lys
50 55 60
Leu Ala Ala Phe Pro Glu Asp Arg Ser Gln Pro Gly Gln Asp Cys Arg
65 70 75 80
Phe Arg Val Thr Glu Leu Pro Asn Gly Arg Arg Asp Phe His Met Ser Val
85 90 95
Val Arg Ala Arg Arg Asn Ser Gly Thr Tyr Leu Cys Gly Ala Ile
100 105 110
Ser Leu Ala Pro Lys Ala Gln Ile Lys Glu Ser Leu Arg Ala Glu Leu
115 120 125
Arg Val Thr Glu Arg Ala Glu Val Pro Thr Ala His Pro Ser Pro
130 135 140
Ser Pro Arg Pro Ala Gly Gln Pro Glu Phe Arg Gly Gly Gly Ser Gly
145 150 155 160
Gly Gly

<210> SEQ ID NO 26
<211> LENGTH: 489
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: hspd1-14del

<400> SEQUENCE: 26

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1  5  10  15
Cys Gly Cys Cys Thr Gly Gly Cys Ala Gly Thr Cys Gly Thr
20 25 30
Cys Thr Gly Gly Cys Gly Thr Gly Cys Thr Ala Cys Ala
35 40 45
Cys Thr Gly Gly Cys Thr Gly Gly Cys Gly Cys Ala Cys Ala
50 55 60
Gly Ala Thr Gly Thr Cys Thr Thr Ala Cys Ala Gly Cys Cys Thr
65 70 75 80
Gly Cys Thr Cys Gly Thr Gly Ala Cys Gly Ala Cys Gly Ala
85 90 95
Gly Gly Gly Ala Cys Ala Cys Glu Cys Ala Cys Ala Cys Cys Thr
100 105 110
Thr Cys Ala Cys Thr Gly Cys Ala Gly Cys Thr Thr Cys Thr Cys
115 120 125
Cys Ala Ala Cys Ala Thr Cys Gly Gly Ala Gly Ala Gly Cys
130 135 140
Thr Thr Cys Gly Thr Gly Thr Cys Thr Ala Ala Ala Cys Thr Gly Gly Thr
145                     150        155        160

Ala Cys Cys Gly Cys Ala Thr Gly Ala Gly Cys Cys Cys Ala Gly
165                     170        175

Cys Ala Ala Cys Cys Ala Gly Ala Cys Gly Gly Ala Cys Ala Ala Ala
180                    185        190

Cys Thr Gly Gly Cys Cys Gly Cys Thr Thr Cys Cys Cys Cys Gly
195                     200        205

Ala Gly Gly Ala Cys Gly Cys Cys Cys Ala Gly Cys Ala Gly Cys Cys
210                    215        220

Cys Gly Gly Cys Cys Ala Gly Ala Cys Thr Gly Cys Cys Gly Cys
225                     230        235        240

Thr Thr Cys Cys Gly Thr Gly Thr Cys Ala Cys Ala Ala Ala Cys
245                    250        255

Thr Gly Cys Cys Cys Ala Ala Cys Gly Gly Gly Gly Thr Gly Ala
260                    265        270

Cys Thr Thr Cys Ala Cys Ala Cys Ala Gly Ala Gly Cys Gly Thr Gly
275                    280        285

Gly Thr Cys Ala Gly Gly Cys Cys Cys Gly Cys Cys Cys Cys Ala
290                    295        300

Ala Thr Gly Ala Cys Ala Gly Cys Gly Cys Ala Cys Ala Thr Ala
305                    310        315        320

Cys Cys Thr Cys Thr Gly Thr Gly Gly Gly Cys Ala Cys Ala Cys
325                    330        335

Thr Cys Cys Cys Thr Gly Cys Cys Cys Cys Cys Cys Ala Ala Ala
340                    345        350

Cys Gly Cys Ala Gly Ala Thr Cys Ala Ala Ala Ala Ala Gly Ala
355                    360        365

Cys Cys Thr Cys Gly Gly Cys Ala Gly Ala Ala Gly Cys Thr Cys
370                    375        380

Ala Gly Gly Thr Ala Cys Ala Ala Gly Ala Ala Gly Ala Ala
385                    390        395        400

Gly Gly Cys Ala Ala Ala Gly Thr Gly Cys Cys Cys Cys Ala Cys
405                    410        415

Ala Gly Cys Cys Cys Ala Ala Cys Cys Cys Cys Cys Cys Cys Cys
420                    425        430

Thr Cys Ala Cys Cys Cys Ala Gly Gly Cys Cys Cys Cys Cys Gly
435                    440        445

Gly Cys Cys Ala Gly Cys Cys Gly Ala Ala Thr Thr Cys Cys Gly
450                    455        460

Gly Gly Cys Ala Gly Cys Gly Ala Ala Thr Cys Gly Cys Ala
465                    470        475        480

Gly Gly Ala Gly Gly Ala Gly Gly Ala
485

<210> SEQ ID NO 27
<211> LENGTH: 681
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: hspd1-16del-p24-Pc
<400> SEQUENCE: 27
Met  Gln  Ile  Pro  Gln  Ala  Pro  Trp  Pro  Val  Val  Trp  Ala  Val  Leu  Gln
1      5            10            15
Leu  Gly  Trp  Arg  Pro  Gly  Trp  Phe  Leu  Ala  Leu  Leu  Val  Val  Thr  Glu
20  25                              30
Gly  Asp  Asn  Ala  Thr  Phe  Thr  Cys  Ser  Ser  Ser  Asn  Thr  Ser  Glu  Ser
35  40  45
Phe  Val  Leu  Asn  Trp  Tyr  Arg  Met  Ser  Pro  Ser  Asn  Glu  Thr  Asp  Lys
50  55                                    60
Leu  Ala  Ala  Phe  Pro  Glu  Asp  Arg  Ser  Glu  Pro  Gly  Glu  Asp  Cys  Arg
65           70                      75                          80
Phe  Arg  Val  Thr  Glu  Leu  Pro  Asn  Gly  Arg  Asp  Phe  His  Met  Ser  Val
85                                    90                          95
Val  Arg  Ala  Arg  Arg  Asn  Ser  Gly  Thr  Tyr  Leu  Cys  Gly  Ala  Ile
100                                           105             110
Ser  Leu  Ala  Pro  Lys  Ala  Gln  Ile  Lys  Gly  Ser  Leu  Arg  Ala  Glu  Leu
115                                           120            125
Arg  Val  Thr  Glu  Arg  Ala  Glu  Val  Pro  Thr  Ala  His  Pro  Ser  Pro
130                                           135            140
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<400> SEQUENCE: 37
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<400> SEQUENCE: 39
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We claim:
1. A soluble PD-1 protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 15, and SEQ ID NO: 25.
2. A PD-1 nucleic acid molecule encoding a soluble PD-1 protein of claim 1.
3. A soluble PD-1 fusion protein, comprising a soluble PD-1 protein fragment and an antigenic protein fragment, wherein the soluble PD-1 protein fragment comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 21, SEQ ID NO: 11, SEQ ID NO: 15, and SEQ ID NO: 25.
4. The soluble PD-1 fusion protein of claim 3, wherein the antigenic protein fragment is derived from an immunogenic protein fragment of a viral, bacterial, or fungal pathogen, or cancer or tumor cells.
5. The PD-1 fusion protein of claim 4, wherein the pathogen is selected from the group consisting of HIV, HSV, respiratory syncytial virus, rhinovirus, hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis D virus, hepatitis E virus, hepatitis F virus, hepatitis G virus, oncoviruses, human T-lymphotropic virus Type I (HTLV-I), influenza virus, bovine leukemia virus (BLV), Epstein-Barr virus, rotavirus, anapapillomavirus, pneumococci, streptococci, staphylococci, E. coli, cytomegalovirus (CMV), respiratory syncytial virus, parainfluenza virus, adenovirus, flavivirus, dengue virus, Mycobacteria tuberculosis, or Plasmodium falciaparum.
6. The PD-1 fusion protein of claim 4, wherein the antigenic protein fragment is derived from HIV p24.
7. The PD-1 fusion protein of claim 3, further comprising a Fc domain.
8. The PD-1 fusion protein of claim 7, further comprising a linker sequence, wherein the linker sequence links the soluble PD-1 domain and the antigen.
9. The PD-1 fusion protein of claim 3, comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 13, 17, 19, 23, and 27.
10. A sPD-1 fusion nucleic acid molecule encoding a PD-1 fusion protein of claim 3.
11. The sPD-1 fusion nucleic acid molecule of claim 10, wherein the nucleic acid molecule comprises a sequence selected from the group consisting of SEQ ID NOs: 14, 18, 20, 24, and 28.
12. A vaccine composition comprising the sPD-1 fusion nucleic acid molecule of claim 10.
13. A method for preventing or treating pathogenic infection and/or tumor or cancer, comprising administering, to a subject in need of such prevention or treatment, an effective amount of a fusion nucleic acid of claim 10.
14. The method of claim 13, wherein the pathogenic infection is caused by a pathogen selected from the group consisting of human immunodeficiency virus (HIV), HSV, respiratory syncytial virus, rhinovirus, hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis D virus, hepatitis E virus, hepatitis F virus, hepatitis G virus, oncoviruses, human T-lymphotropic virus Type I (HTLV-I), influenza virus, bovine leukemia virus (BLV), Epstein-Barr virus, rotavirus, anapapillomavirus, pneumococci, streptococci, staphylococci, E. coli, cytomegalovirus (CMV), respiratory syncytial virus, parainfluenza virus, adenovirus, flavivirus, dengue virus, Mycobacteria tuberculosis, or Plasmodium falciaparum.
15. The method of claim 14, wherein the fusion nucleic acid comprises an antigenic nucleic acid fragment encoding HIV p24 and the pathogenic infection is HIV infection.
16. The method of claim 15, wherein the fusion nucleic acid comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 18, 20, 24, and 28.
17. The method of claim 13, wherein the fusion nucleic acid is delivered by injection.
18. The method of claim 17, wherein the fusion nucleic acid is delivered via electroporation.
19. A method for preventing or treating pathogenic infection and/or tumor or cancer, comprising administering, to a subject in need of such prevention or treatment, an effective amount of a fusion protein of claim 3.
20. The method of claim 19, wherein the pathogenic infection is caused by a pathogen selected from the group consisting of human immunodeficiency virus (HIV), HSV, respiratory syncytial virus, rhinovirus, hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis D virus, hepatitis E virus, hepatitis F virus, hepatitis G virus, oncoviruses, human T-lymphotropic virus Type I (HTLV-I), influenza virus, bovine leukemia virus (BLV), Epstein-Barr virus, rotavirus, anapapillomavirus, streptococci, staphylococci, E. coli, shigella, cytomegalovirus (CMV), respiratory syncytial virus, adenovirus, flavivirus, Mycobacteria tuberculosis, or Plasmodium falciaparum.

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