<table>
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<tr>
<th><strong>Title</strong></th>
<th>NOVEL PD1 ISOFORMS, AND USES THEREOF FOR POTENTIATING IMMUNE RESPONSES</th>
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<tr>
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<tr>
<td><strong>Citation</strong></td>
<td>US Published patent application US 20140302070. Washington, DC: US Patent and Trademark Office (USPTO), 2014</td>
</tr>
<tr>
<td><strong>Issued Date</strong></td>
<td>2014</td>
</tr>
<tr>
<td><strong>URL</strong></td>
<td><a href="http://hdl.handle.net/10722/206633">http://hdl.handle.net/10722/206633</a></td>
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In one embodiment, the present invention provides a new isoform of human PD1 (Δ42PD1) that contains a 42-nucleotide in-frame deletion located at exon 2 domain. Δ42PD1 does not engage PD-L1/PD-L2, and can induce the production of pro-inflammatory cytokines. In one embodiment, Δ42PD1 can be used as an intramolecular adjuvant to develop a fusion DNA vaccine for enhancing antigen-specific CD8+ T cell immunity and for prevention of pathogenic infection and/or cancer. In one embodiment, soluble Δ42PD1 protein could be a therapeutic target for autoimmune diseases. In other embodiments, proteins or peptides or nucleic acids encoding proteins or peptides containing Δ42PD1 could be used as immunogens for developing antibodies binding specifically to Δ42PD1. In yet another embodiment, neutralizing antibodies could block s842PD1 function and accordingly could be used as treatment for autoimmune disorders.
FIG. 1
FIG. 2C
**FIG. 3A**

**FIG. 3B**
FIG. 4A

FIG. 4B
FIG. 5C
FIG. 6
FIG. 11
FIG. 12
293T cells transiently expressing:

<table>
<thead>
<tr>
<th>Protein treatment</th>
<th>human PD-L1</th>
<th>human PD-L2</th>
<th>murine PD-L1</th>
<th>murine PD-L2</th>
</tr>
</thead>
<tbody>
<tr>
<td>p24&lt;sub&gt;Fc&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>msΔ42PD1-p24&lt;sub&gt;Fc&lt;/sub&gt;</td>
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Cell counts

anti-rabbit Fc

FIG. 13
**FIG. 14**

a) murine splenocytes

b) Relative expression

c) Relative expression

Legend:
- Neg
- LPS
- p24_k
- mΔA42PD1-p24_k
FIG. 16
FIG. 17A
Nucleotides sequences

Soluble PDI: (SEQ ID NO: 19)
ATGCAGATCCCACGCGCGCTTGGCCGCTCGTACAACTGGCTGGCGCCAGGATGGT
TCTTAGACCTCCGAAGGCAACCCCTTGTGGGAACCCCCACACCCCTTCCCGACTGCGTTGAGCACTGCAAGG
GGACACCGCAGCTCTCCACACCTGGCTGTGACAATCGTGTGCTCGTGAAGCACAGCAGCAGCAGCGAGCAAGG
AGCCCAACAACCAGAGCCGAGCAGCTGGCCGCTCTCCCGAGGAAACGCAACCCGCGGCAGAGCAACCTG
GCTCCGTTCGACCAACAGCCAGCGGCTGACTTCCACATGACGCTGGTGACGCCCGGCCGCAAPGA
CAGCCGCACCTACTCTGTTGGCCCATCTCCCGCGCCCGCAAGACCCAGATCAAGAGAGGCGTTGGGCACGA
GNCTACGAGTGACAGAGAAAGGCGAGATGGCCACGCGCCACCCCGCTCACCCACGGCGACGG
GCCAG

Soluble Δ42PDI: (SEQ ID NO: 20)
ATGCAGATCCCACGCGCGCTTGGCCGCTCGTACAACTGGCTGGCGCCAGGATGGT
TCTTAGACCTCCGAAGGCAACCCCTTGTGGGAACCCCCACACCCCTTCCCGACTGCGTTGAGCACTGCAAGG
GGACACCGCAGCTCTCCACACCTGGCTGTGACAATCGTGTGCTCGTGAAGCACAGCAGCAGCAGCGAGCAAGG
AGCCCAACAACCAGAGCCGAGCAGCTGGCCGCTCTCCCGAGGAAACGCAACCCGCGGCAGAGCAACCTG
GCTCCGTTCGACCAACAGCCAGCGGCTGACTTCCACATGACGCTGGTGACGCCCGGCCGCAAPGA
CAGCCGCACCTACTCTGTTGGCCCATCTCCCGCGCCCGCAAGACCCAGATCAAGAGAGGCGTTGGGCACGA
GNCTACGAGTGACAGAGAAAGGCGAGATGGCCACGCGCCACCCCGCTCACCCACGGCGACGG
GCCAG

Amino acid sequences:

Soluble PDI: (SEQ ID NO: 21)
MQ1PQAPFWVWAVQLGLWGFRGFLDQFDREFNPFTPFLALLVELTEGDNATFTCSFSNTSNESSFVLNWYM
SPSNDTDXLAFFDEDSQPGQCDRFCRTVQLPENGRDFHMSVRRARNDGTYLGAIASLAPKPTQIKESLRA
ELRVTERRAVTPAHPSPSRPASQ

Soluble Δ42PDI: (SEQ ID NO: 22)
MQ1PQAPFWVWAVQLGLWGFRGFLDQFDREFNPFTPFLALLVELTEGDNATFTCSFSNTSNESSFVLNWYM
SPSNDTDXLAFFDEDSQPGQCDRFCRTVQLPENGRDFHMSVRRARNDGTYLGAIASLAPKPTQIKESLRA
ELRVTERRAVTPAHPSPSRPASQ

FIG. 18
FIG. 19
FIG. 20
FIG. 21

FIG. 22
FIG. 27
SEQ ID NO: 30-35

FIG. 28
NOVEL PDI ISOFORMS, AND USES THEREOF FOR POTENTIATING IMMUNE RESPONSES

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. provisional application Ser. No. 61/808,993, filed Apr. 5, 2013, which is herein incorporated by reference in its entirety.

BACKGROUND

[0002] Programmed death-1 (PDI, CD279) is a member of the CD28 superfamily that negatively regulates the function of T cells through interaction with its two native ligands PDI-L1 (CD274) and PDI-L2 (CD273). PDI is a type I transmembrane receptor protein composed of a single immunoglobulin (Ig) variable-like domain, a cytoplasmic domain, and two tyrosine-based signaling motifs. The ligands for PDI are PD1-L1 (CD274 or B7-H1) and PD1-L2 (CD273 or B7-DC), which are members of the B7 family.

[0003] PD1-L1 is found expressed on both hematopoietic and non-hematopoietic cells found in immunoprivileged sites including the eye and placenta, and is highly elevated in inflammatory environments. Following activation of an immune response, antigen presenting cells (APCs) and T cells further augment the expression of PD1-L1, while PD1-L2 expression is only found on activated macrophages and DCs. PD1 is constitutively expressed at low levels on resting T cells and is up-regulated on T cells, natural killer T (NKT) cells, B cells and macrophages upon activation.

[0004] The absence of PD1 in mice provides significant resistance against bacterial infection through innate immunity, demonstrating the importance of the regulatory role of PD1 against pathogenic infections. In addition, PD1 plays significant roles in a number of autoimmune diseases, including systemic lupus erythematosus (SLE) and rheumatoid arthritis.

[0005] Recent studies have characterized the inhibitory function of the interaction between PD1 and PD1-L1/2. With PD1 deficient transgenic mice, CD8+ T cells were found to recognize H-2D and proliferate more actively than wildtype cells in response to homologous (H-2D) APCs. In addition, PD1 deficient mice develop spontaneous lupus-like disease and cardiomyopathy, indicating that PD1 has the role to control over-activated T cells. This is more evident from a study that found up-regulated PD1 expression on LCMV-specific CD8+ T cells, which directly contributes to the dysfunction of these T cells and correlated with the failure to control viral replication in mice during chronic infection. It has been known that HIV-1-specific T cells in patients are usually poorly functional due to the loss of CD28 co-stimulation molecule, perforin, and down-regulation of CD87 and IL-7Rα, which are important molecules for maintenance of memory T cells. One of the reasons for the exhausted T cell function during HIV-1 infection is attained by recent studies showing that PD1 is persistently up-regulated on HIV-1 specific CD4+ and CD8+ T cells that have reduced proliferation, cytokine production, and cytotoxicity.

[0006] The role of the PD1/PD-L pathway in chronic infections (Mycobacterium tuberculosis, LCMV, HIV-1, HBV and HCV) has been characterized extensively. The high expression of PD1 on pathogen-specific CD8+ T cells results in these cells being functionally “exhausted,” leading to the failure of clearing persistent infections. In addition, the blockade of the PD1/PD-L1 pathway in vitro and in vivo with antibody or the soluble form (i.e., only containing extracellular domain) of PD1 is able to rescue the function of these exhausted HIV-1 and HCV specific CD8+ and CD4+ T cells by restoring cytokine production, cell proliferation, and cytolyis.

[0007] Progression towards AIDS is markedly correlated with the level of PD1 expression on HIV-1-specific CD8+ T cells and the percentage of cells expressing PD1 with viral load and declining CD4+ counts. In contrast, long-term non-progressors (LTNPs) have significantly lower level of PD1 expression found on HIV-specific memory CD8+ T cells compared to progressors.

[0008] Experiments also demonstrated that blockade of the PD1/PD-L1 interaction can reverse the function of these exhausted viral-specific CD8+ T cells, which was further shown in vivo in LCMV chronically infected mice treated with antibodies against PD1/PD-L1. In LCMV-specific CD8+ T cells with restored proliferation and TNF-α and IFN-γ production that led to reduced viral load. Other studies also found that highly active antiretroviral therapy (HAART) recovered reduced PD1-expressed HIV-1-specific CD8+ T cells, and that blocking of the PD1/PD-L1 pathway could rescue the function of HIV-1-specific T cells. These findings show the importance of the PD1/PD-L1 pathway that results in the exhausted state of T cells during HIV-1 chronic infection, and may act as one of the key host factors in modulating immune response to target HIV-1 infected cells.

[0009] To date, four PD1 isoforms have been reported from alternatively spliced PD1 mRNA. Apart from one of these variants encoding a soluble form of PD1, the other three spliced variants have no function attributed to them. Nevertheless, their highly induced expression following stimulation of human peripheral blood mononuclear cells (PBMCs) likely suggests an immunoregulatory function, which has been shown for variants of the other CD28 family molecules, such as CTLA-4 and CD28. One isoform of CTLA-4 (1/4CTLA-4) could exacerbate experimental autoimmune encephalomyelitis (EAE) diseases in mice, with significantly increased level of CD4+ T cell proliferation and cytokine production compared to wildtype CTLA-4. Interestingly, over-expression of this variant resulted in the down-regulation of wildtype CTLA-4 on CD4+ T cells. For CD28, four spliced variants were identified from human T cells with differential expression. The CD28i isoform was found expressed on the cell surface where it could associate with CD28 to enhance the co-stimulation capacity via CD28, further illustrating that apart from the conventional identified forms, spliced variants of the CD28 receptor family members could have immunoregulatory functions.

SUMMARY OF THE INVENTION

[0010] The present inventors identified and characterized from human healthy PBMC donor a new isoform of PD1 (referred to herein as “Δ42PD1”) that lacks a fragment encoded by 42-nucleotides. In one embodiment, Δ42PD1 comprises the nucleotide sequence of SEQ ID NO:23. In one embodiment, Δ42PD1 comprises the amino acid sequence of SEQ ID NO:1. This isoform is distinct from the wildtype PD1 as it does not bind to PD1-L1 or PD1-L2, and is not recognized by PD1-specific monoclonal antibodies. Like PD1, Δ42PD1 mRNA was found expressed in various immune-related cells.
In one embodiment, the present invention provides PD1 protein isoforms. In one embodiment, the PD1 protein isoform is Δ42PDI, which has an amino acid sequence comprising SEQ ID NO: 1. In one embodiment, the PD1 protein isoforms do not bind to PD-L1 or PD-L2.

In one embodiment, the PD1 protein isoform has a deletion of 14 amino acids at positions corresponding to amino acids 26-39 of the wild-type PD1 protein having an amino acid sequence of SEQ ID NO: 2 and a nucleotide sequence of SEQ ID NO: 24. In one embodiment, the 14 amino acid deletion has a sequence that is DSPDRPWN-PFTFP (SEQ ID NO: 3).

In another embodiment, the PD1 protein isoform has non-conservative substitutions at one or more amino acids at positions corresponding to amino acids 26-39 of the wild-type PD1 protein having an amino acid sequence of SEQ ID NO: 2. In certain embodiments, the PD1 protein isoform has non-conservative substitutions of 1 to 14 amino acids at positions corresponding to amino acids 26-39 of the wild-type PD1 protein having an amino acid sequence of SEQ ID NO: 2. In one embodiment, amino acids 26-39 of the wild-type PD1 protein are DSPDRPWN-PFTFP (SEQ ID NO: 3).

Another aspect of the subject invention provides nucleic acid molecules that encode the PD1 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.

Another aspect of the invention provides PD1 fusion proteins and fusion nucleic acid molecules. In one embodiment, the fusion protein comprises a PD1 protein isoform with an amino acid sequence comprising SEQ ID NO: 1 or a biologically active fragment thereof, and an antigen or peptide. In one embodiment, the fusion nucleic acid molecule comprises a nucleic acid molecule encoding a PD1 protein isoform with an amino acid sequence comprising SEQ ID NO: 1 or a biologically active fragment thereof, and a nucleic acid molecule encoding an antigen or peptide.

In one embodiment, the PD1 nucleic acid molecules of the present invention are formulated into a DNA vaccine formulation.

In one aspect, the soluble form of Δ42PDI (sΔ42PDI) having a nucleic acid sequence of SEQ ID NO: 25, and an amino acid sequence of SEQ ID NO: 26) is fused with the rabbit IgG Fc domain, comprising a nucleic acid sequence of SEQ ID NO: 27, and an amino acid sequence of SEQ ID NO: 28, to induce production of cytokines. In another embodiment, sΔ42PDI is fused with 6×His tag, comprising an amino acid sequence of SEQ ID NO: 29 to induce production of cytokines. In still another embodiment, membrane-bound Δ42PDI is used to induce production of cytokines.

Another aspect of the invention provides uses of Δ42PDI proteins and nucleic acids as immunogens to prepare polyclonal and monoclonal antibodies against human Δ42PDI. In one embodiment, sΔ42PDI is fused with the rabbit IgG Fc domain (sΔ42PDI-Fc), comprising an amino acid sequence of SEQ ID NO: 28 and a nucleotide sequence of SEQ ID NO: 27, and used as an immunogen to inoculate BALB/c mouse for antibody preparation.

In another embodiment, the present invention provides antibodies that bind specifically to Δ42PDI. In some embodiments, the antibody is a monoclonal antibody. In one embodiment, the antibody is CH34. In another embodiment, the antibody is CH101.

In one embodiment, the monoclonal antibody blocks the binding of Δ42PDI to its unknown receptor. In another embodiment, the monoclonal antibody binds a fragment of Δ42PDI, comprising an amino acid sequence of SEQ ID NO: 31.

Another aspect of the present invention provides the uses of the PD1 protein isoforms (e.g., Δ42PDI), nucleic acid molecules, including cDNA molecules, encoding the PD1 protein isoforms, fusion proteins comprising the PD1 protein isoforms, and/or fusion nucleic acid molecules comprising nucleic acid sequences encoding the PD1 protein isoforms, for induction of the production of cytokines (such as, TNF-α, IL-1, and IL-6) in immune cells.

Another aspect of the present invention provides methods for the prevention, treatment, or amelioration of pathogenic infection and/or cancer. The method comprises administering to a subject in need of such prevention and treatment an effective amount of a PD1 protein isoform of the present invention (such as Δ42PDI), a nucleic acid molecule encoding a PD1 protein isoform (e.g., cDNA) of the present invention (such as Δ42PDI protein), and/or a fusion protein and/or a fusion nucleic acid molecule of the present invention.

Another aspect of the present invention provides methods for diagnosis of virus infection diseases and autoimmune disorders.

**BRIEF DESCRIPTION OF THE DRAWINGS**

**FIG. 1** shows the identification of a novel PD1 isoform. (a) Amino acid sequence alignment of Δ42PDI (SEQ ID NO: 1) and PD1 (GenBank accession number: NM_005018) (SEQ ID NO: 2) identified from a representative healthy human PBMC donor. Dashed line represents the 14-amino acid deletion found in Δ42PDI. Signal sequence and the transmembrane region are indicated. IgV domain including the front AGFCCC (SEQ ID NO: 17) β-sheet and the back ABED sheet are highlighted by the arrows. Asterisks show the putative amino acids for ligand interaction. (b) Schematic genomic structure of PD1 with the highlighted location of the exact 42-nucleotide deletion in exon 2. (c) Δ42PDI and PD1 PCR products were amplified from cDNA clones (upper gel) or PD1 alone from the genomic DNA (lower gel) generated from healthy human PBMCs. Lanes 1-7 in both gels represent PCR results from seven human donors. Lanes 8 and 9 are Δ42PDI and PD1 positive controls, respectively. Lane M represents DNA molecular weight marker. (d) Relative mRNA expression of Δ42PDI from subpopulations of PBMCs sorted from five independent healthy blood donors, normalized to housekeeping gene GAPDH and total PBMC samples.

**FIG. 2** shows characterization of the function of Δ42PDI isoform. (a) 293T cells transiently transfected to express human PD-L1 or (b) PD-L2, and treated with purified recombinant proteins at series of concentrations –0.5, 2, 5 and 20 μg/ml to investigate binding affinity. The results were analyzed by flow cytometry using a detection antibody against rabbit Fc (shaded) or isotype control (solid line). (c) Plasmids encoding PD1 or Δ42PDI were stably transfected or untransfected 293A cells, and the detection was determined by flow cytometry with a polyclonal anti-PD1 antibody or three monoclonal anti-PD1 antibodies with clone names indicated on the x-axes. Percentage of cells with positive staining (shaded) is shown with corresponding antibodies.
and isotype control (solid line). Data are representative of three independent experiments.

[0026] FIG. 3 shows functional analysis of human sA42PD1 in vitro. (a) Cytokine release profile of human PBMC culture supernatants treated with purified proteins of rabbit Fc, sPD1, or sA42PD1, for 24 h. qRT-PCR analysis of human PBMCs after protein treatment for 6 h, 12 h, and 24 h, for (b) TNFα, (c) IL-6, and (d) IL-1b mRNA expression normalized to GAPDH. LPS served as positive control. Data represents means±SEM of independent experiments. *p<0.05.

[0027] FIG. 4 shows enhanced antigen-specific immunogenicity of msA42PD1-p24c. DNA/EP in mice. (a) purified CD11c BM-DCs from Balb/c mice were treated by purified protein msA42PD1-p24c, p24c, or positive control LPS for 24 h. Supernatants were collected to analyze cytokine releasing of TNFα, IL-6, and IL-1α. Data represent means±SEM of six independent experiments. *p<0.05. Then Balb/c mice were vaccinated using fusion DNA plasmids (20 μg or 100 μg dose), and p24-specific immune responses generated were measured by (b) ELISA for antibody responses, (c) ELISPOT assay for CD4 specific epitope gag26 and (d) CD8 specific epitope gagA1 IFN-γ responses, and H-2Kb p24 tetramer staining for specific CD8 T cell response from splenocytes displayed as scatter plot (n=5) (e). (f) ELISPOT assay was performed on splenocytes using three non-overlapping p24 peptide pools. Data represent means±SEM of at least two independent experiments of three mice per group. *p<0.05, **p<0.01.

[0028] FIG. 5 shows long-term memory responses induced by msA42PD1-p24c vaccine. 30 weeks after immunization, mice were sacrificed to assess long-lived memory response for anti-p24 antibody (a) and CD4 and CD8 IFN-γ ELISpot (b). CFSE proliferation assay was performed on CD4+ T (c) and CD8+ T (d) cells from splenocytes from 30 weeks post-vaccinated mice for five days of stimulation with BM-DCs (ratio 1 DC: 10 T) and p24 peptide pool plus anti-CD28. Anti-CD4/anti-CD28 stimulation served as positive control. Data represent means±SEM of two independent experiments of three mice per group. *p<0.05.

[0029] FIG. 6 shows the efficacy of msA42PD1-p24c vaccine in mice. (a) Effector splenocytes (two weeks post-vaccination) were used for cytotoxicity assay against p24 expressing target AB1-HIV-1-Gag cells at various ratios. Percentage of dead cells was calculated and the dot line showed the background signal of target cells alone. (b) Immunized mice were challenged s.c. by 5x105 AB1-HIV-1-Gag cells three weeks post-vaccination, tumor images were taken twice a week to detect luciferase intensity and representative images at day 17 post-challenge is shown (c). (d) Protection of immunized mice against intranasal virus challenge three weeks after the final immunization with VTTgagpol and (e) virulent WRgagpol. Virus titer was measured from lung homogenates from mice sacrificed 8 days post-challenge on Vero cell plaque formation. (f) Body weight was measured daily overtime and calculated as percentages compared to day 0 of WRgagpol challenge. Functional assay results show the representative data from two independent experiments. Protection studies were performed from at least five mice in each group and data represent the means±SEM. *p<0.05, **p<0.01, ***p<0.001.

[0030] FIG. 7 shows schematic representation of A42 deletion on human PD1 in complex with PD-L2. Protein structure modeling of human PD1 based on the published crystal structure, but including 10 more amino acids upstream of beta-strand A to include the range of the A42 deletion (red). Other beta-standers are labeled for PD1 and PD-L2.

[0031] FIG. 8 shows lack of cross-reactivity of murine immune sera against human PD1 and A42PD1. 293T cells transfected with plasmids encoding PD1 (top) and A42PD1 (bottom) were stained by anti-PD1 and anti-A42PD1 immune sera, respectively, by FACS analysis.

[0032] FIG. 9 shows the specificity of a specific monoclonal antibody (clone CH34) targeting human A42PD1. 293T cells transfected with plasmids encoding human PD1 (right) and A42PD1 (middle) were stained by anti-PD1 and anti-A42PD1 monoclonal antibody CH34 (red) or anti-PD1 monoclonal antibody (blue) or isotype control (black) respectively, by FACS analysis.

[0033] FIG. 10 shows purity of recombinant proteins. Supernatants were collected from 72 h post-transfected 293T cells of plasmids encoding rabbit Fc, sPD1, or sA42PD1, and purified using Protein G agarose. Purified proteins were electrophoresed on SDS-PAGE gel and stained with Coomassie Blue to show a single band corresponding to the encoded protein size.

[0034] FIG. 11 shows that membrane-bound A42PD1 can induce pro-inflammatory cytokines from PBMCs. Stably transfected 293A cells expressing PD1 or A42PD1 were γ-irradiated (50 Gy) then added to freshly isolated PBMCs at 1:1 and real-time PCR was performed on cells harvested at 6 h, 12 h, and 24 h after co-culture to assess the expression of (a) TNFα, (b) IL-6, (c) IL-1B, and (d) IL-10 normalized to GAPDH and untreated control (Neg). *p<0.05.

[0035] FIG. 12 shows that vaccination using human sA42PD1-p24c fusion DNA elicited greater immune response. (a) Schematic representation of fusion DNA plasmid constructs of HIV-1 Gag p24 antigen tagged to rabbit Fc with or without human sA42PD1, lead by a iPα signal sequence, under the CMV promoter. (b) Immunization schedule of Balb/c mice receiving three shots of DNA three weeks apart, and immune response generated were assessed two weeks after the final immunization. (c) Western blot analysis of fusion protein expressed from transiently transfected 293T cells. Numbers represent marker band size (kDa). (d) ELISA measuring anti-p24 antibody response for IgG1 and IgG2a from mice sera, and (e) p24-specific CD4+ and CD8+ T cell response by IFN-γ ELISPOT. Data represents the means±SEM of two independent experiments. *p<0.05, **p<0.001. (f) Western blot analysis of detection of A42PD1-GST protein using a polyclonal anti-PD1 antibody, or serum from mouse immunized with sA42PD1-p24c.

[0036] FIG. 13 shows that murine sA42PD1 does not interact with PD-L1/L2. Binding of murine (m)A42PD1-p24c recombinant protein were examined by treating transiently transfected 293T cells expressing human or murine PD-L1 or PD-L2. p24c was used as a control. Positive staining (solid black line) was achieved by conjugated monoclonal antibodies. Negative staining (shaded) represents isotype control. Red lines show anti-rabbit Fc detection antibody signal if binding of proteins occurred. Data acquired and analyzed by FACS calibur flow cytometer and CellQuest software (BD Biosciences).

[0037] FIG. 14 shows that msA42PD1-p24c recombinant protein can induce pro-inflammatory cytokines from murine splenocytes. qRT-PCR analysis of (a) TNFα, (b) IL-6, and (c) IL-1α expression in freshly isolated murine splenocytes following treatment of recombinant purified proteins (20 μg/mL)
or LPS (0.1 μg/ml) for 6 h, 12 h and 24 h. Data was generated from the means of splenocytes from five individual Balb/c mice of the same age, and normalized to beta-actin and untreated control (Neg). *P<0.05.

[0038] FIG. 15 shows that antibody response against ms42PDI was not found in mice immunized with ms42PDI-p24. Immune mouse serum was used to detect full-length murine Δ42PDI-GST protein by Western blotting to assess if immune response was raised against ms42PDI in ms42PDI-p24 vaccinated mice. A polyclonal anti-murine PDI antibody was used as a positive control. Marker band sizes in kDa are shown.

[0039] FIG. 16 shows that induction of T cell-activating cytokines by s42PDI, in PBMCs. Freshly isolated healthy human PBMCs were treated with s42PDI, sPDI, rabbit Fc recombinant proteins, LPS or left untreated. qRT-PCR was performed on total RNA extracted at 6 h, 12 h and 24 h post-treatment to analyze the expression of (a) IFNb, (b) IL12 and (c) IL15 normalized to GAPDH. Induction was seen with s42PDI for IL12 and IL15 at 6 h post-treatment but did not reach statistical significance compared to rabbit Fc or sPDI. However, s42PDI induced the expression of IFNb significantly at 6 h and 12 h post-treatment with statistical difference. *P<0.05, ***P<0.01.

[0040] FIG. 17 shows comparison of wildtype murine sPDI and s42PDI-based fusion vaccine in mice. (a) To examine binding, recombinant proteins were applied to BMDCs for 30 min at 4°C and stained with anti-rabbit Fc or isotype control. Balb/c mice were immunized with 20 μg of msPDI-p24 or msΔ42PDI-p24 DNA vaccines, and the CD8+ T cell responses measured by Elispot (b) or tetramer staining (c) are shown. Data represented at least two independent experiments with groups of three mice. *P<0.05, ***P<0.001.

[0041] FIG. 18 shows the nucleic acid sequences and the amino acid sequences of soluble PDI and soluble Δ42PDI.

[0042] FIG. 19 shows experimental results for the generation of anti-human Δ42PDI monoclonal antibodies. (A) TimeLine and parameters of the electroporation s42PDI/1C plasmid DNA prime and s42PDI/1Fc protein boost regimen in mice was shown with inoculations and bleed to isolate sera for analysis as indicated. (B) Sera of five immunized mice (MI-MS) were analyzed for anti-s42PDI/1His antibody ELISA titer. Serum of normal mouse (NMS) was used as negative control. (C and D) Sera of immunized mice were analyzed (C) with ELISA for binding potency to sΔ42PDI/1His and sPDI/1His proteins, and (D) with FACS for recognizing 293T, 293T-Δ42PDI and 293T-Δ42PDI cells. Each symbol represents an individual mouse. Data were presented as mean±standard deviation (S.D.). (E) Supernatants of two monoclonal anti-Δ42PDI antibodies (clone CH34 and clone CH101) were tested for recognition of Δ42PDI/1His by ELISA. Plate coated with PBS used as negative control, serum (1:1000) of immunized mouse served as positive control. (C and D) Data were presented as mean±S.D. from three independent experiments.

[0043] FIG. 20 shows the characterization of mouse anti-Human Δ42PDI monoclonal antibodies. (A) 293T-PDI cells and 293T-Δ42PDI cells were stained with anti-human Δ42PDI mAbs (clone CH34 and CH101) or anti-PDI mAb (clone MIIH) and analyzed by flow cytometry. 293T cells served as negative control. The plots are representative of at least three independent experiments. (B) Western blot analysis of cell lysates of 293T and indicated 293T transient transfectants using anti-human Δ42PDI mAbs (clone CH34 and CH101) or anti-mouse PDI pAb as primary antibody respectively. Tubulin is shown as loading control. (C) Supernatants of hybridoma clone CH34 and CH101 were analyzed with mouse mAb rapid ELISA isotyping kit.

[0044] FIG. 21 shows TNFα, IL.6, and IL-1 production from human PBMCs induced by recombinant s42PDI His.

[0045] FIG. 22 shows raised s42PDI level in HIV+ plasma.

[0046] FIG. 23 shows the characterization of anti-Human Δ42PDI monoclonal antibodies. Both CH34 and CH101 are high affinity antibodies by surface plasmon resonance.

[0047] FIG. 24 shows the similar signaling of membrane-bound Δ42PDI and PDI to inhibit the Akt signal pathway in Δ42PDI- and PDI-expressing 293T cell lines.

[0048] FIG. 25 shows CH101 enhanced Δ42PDI signaling triggered by Δ42PDI receptor on PBMCs.

[0049] FIG. 26 shows monoclonal antibodies block the binding of recombinant s42PDI-p24 protein with the unknown Δ42PDI receptor on TH-1 cells.

[0050] FIG. 27 shows development of a double-antibody sandwich-ELISA for specific detection of s42PDI in human body fluid.

[0051] FIG. 28 shows the binding of mAbs to fragments of s42PDI displayed on the surface of yeast cells.

BRIEF DESCRIPTION OF THE SEQUENCES

[0052] SEQ ID NO:1 is the amino acid sequence of a PDI protein isoform (Δ42PDI) of the present invention.

[0053] SEQ ID NO:2 is the amino acid sequence of a wildtype PDI protein.

[0054] SEQ ID NO:3 is the amino acid sequence of the 14 amino acids deleted from the wildtype PDI protein of SEQ ID NO:2.

[0055] SEQ ID NO:4 is the amino acid sequence of a linker sequence.

[0056] SEQ ID NO:5 is the amino acid sequence of a linker sequence.

[0057] SEQ ID NO:6 is the amino acid sequence of gagA1.

[0058] SEQ ID NO:7 is the amino acid sequence of peptide gag26.

[0059] SEQ ID NO:8 is the amino acid sequence of a linker sequence.

[0060] SEQ ID NO:9 is the amino acid sequence of a linker sequence.

[0061] SEQ ID NO:10 is the amino acid sequence of a linker sequence.

[0062] SEQ ID NO:11 is the amino acid sequence of a linker sequence.

[0063] SEQ ID NO:12 is the amino acid sequence of a linker sequence.

[0064] SEQ ID NO:13 is the amino acid sequence of a linker sequence.

[0065] SEQ ID NO:14 is the amino acid sequence of a linker sequence.

[0066] SEQ ID NO:15 is the amino acid sequence of a linker sequence.

[0067] SEQ ID NO:16 is the amino acid sequence of a linker sequence.

[0068] SEQ ID NO:17 is the amino acid sequence of an IgV domain of the PDI protein.

[0069] SEQ ID NO:18 is the nucleic acid sequence encoding the 14 amino acids deleted from the wildtype PDI protein of SEQ ID NO:2.
[0070] SEQ ID NO:19 is a nucleic acid sequence encoding a soluble PD1.
[0071] SEQ ID NO:20 is a nucleic acid sequence encoding a soluble ΔPD1.
[0072] SEQ ID NO:21 is an amino acid sequence of a soluble PD1.
[0073] SEQ ID NO:22 is an amino acid sequence of a soluble ΔPD1.
[0074] SEQ ID NO:23 is a nucleic acid sequence of human Δ42PD1.
[0075] SEQ ID NO:24 is a nucleic acid sequence of human PD1.
[0076] SEQ ID NO:25 is a nucleic acid sequence of human sΔ42PD1.
[0077] SEQ ID NO:26 is an amino acid sequence of human sΔ42PD1.
[0078] SEQ ID NO:27 is a nucleic acid sequence of sΔ42PD1rec.
[0079] SEQ ID NO:28 is an amino acid sequence of sΔ42PD1rec.
[0080] SEQ ID NO:29 is an amino acid sequence of sΔ42PD1frg-1.
[0081] SEQ ID NO:30 is an amino acid sequence of sΔ42PD1frg-2.
[0082] SEQ ID NO:31 is an amino acid sequence of sΔ42PD1frg-3.
[0083] SEQ ID NO:32 is an amino acid sequence of sΔ42PD1frg-4.
[0084] SEQ ID NO:33 is an amino acid sequence of sΔ42PD1frg-5.
[0085] SEQ ID NO:34 is an amino acid sequence of sΔ42PD1frg-6.
[0086] SEQ ID NO:35 is an amino acid sequence of sΔ42PD1frg-6.

DETAILED DESCRIPTION

[0087] The present invention provides fusion proteins comprising peptides derived from the extracellular domain of alternatively spliced isoforms of human PD1 (herein referred to as “Δ42PD1”) (Fig. 1) to regulate innate immunity, as well as uses of Δ42PD1 for potentiating antigen-specific antibody and particularly CD8+ T-cell immune responses.

[0088] In one embodiment, novel PD1 isoform (Δ42PD1) can be used as a potential intramolecular adjuvant for vaccine development to induce high level of functional and long-lived antigen-specific CD8+ T immunity against cancers and infections by pathogens including HIV-1 and Mycobacterium tuberculosis.

[0089] As the Δ42 deletion results in the loss of the beta-strand A of human PD1 (Fig. 7), the Δ42PD1 isoform is unable to bind PD-L1/L2 or specific PD1 blocking monoclonal antibodies (Fig. 2), Δ42PD1-mediated enhancement of antigen-specific immunity is unlikely through PD-L1/L2 interaction with dendritic cells but rather through a distinct mechanism.

[0090] The stimulation of pro-inflammatory cytokines by Δ42PD1 contributes to the overall T cell immunity; therefore, Δ42PD1-based fusion DNA vaccine can enhance T cell immunity. In particular, since the enhanced antigen-specific CD8+ T cell immunity confers functional and long-lasting effects in vivo, Δ42PD1-based fusion DNA vaccine offers new opportunities to improve vaccine and immunotherapy efficacy against pathogens and cancers.

[0091] Δ42PD1 is a newly discovered PD1 isoform that could induce pro-inflammatory cytokines for function. This isoform was found among healthy Chinese blood donors whose PBMCs express a PD1 transcript with an identical 42-nucleotide deletion at the beginning of exon 2 (Fig. 1), and differs from other alternatively spliced PD1 variants as reported previously. Δ42PD1 mRNA is preferentially expressed in monocytes, macrophages, NK and NK cells as compared to DCs, B cells and T cells (Fig. 1d). This phenomenon has not been reported for PD1 or spliced variants of other CD28 family members such as CTLA-4 and CD28.

[0092] Soluble forms of PD1, CD28, CD80, CD86 and CTLA-4 can be found in sera of patients suffering from autoimmune diseases such as Sjögren’s syndrome, systemic lupus erythematosus, multiple sclerosis, rheumatoid arthritis, and antibodies detecting naturally occurring sΔ42PD1 can be used in diagnosis (including diagnostic reagents) and/or treatment of autoimmune diseases and infections.

[0093] Δ42PD1, a PD1 spliced variant resulted from a partial exon deletion, is distinct from PD1: firstly, it does not bind to PD-L1/L2, and secondly, recombinant soluble or membrane-bound Δ42PD1 (but not PD1) can induce the expression of TNF-α, IL-6 and IL-113. It is postulated that the Δ42PD1 has distinct immunomodulatory functions that could influence the stimulation of an immune response. Eliciting high levels of functional CD8+ T cell immunity is one of the important determinants of an effective vaccine against intracellular pathogens and cancer. Thus, nucleic acid molecules encoding Δ42PD1 can be used as an intramolecular adjuvant in a fusion DNA vaccine strategy, and can be used to elicit remarkably enhanced functional CD8+ T cell immunity against HIV-1 Gag p24 in vivo (Figs. 4d, e). At a dose of 20 μg of DNA in Balb/c mice, msΔ42PD1-p24/E9P vaccination could achieve robust induction of p24-specific CD8+ (~1000 Ellipsots/10 splenocytes; ~20-fold greater than p24), which are markedly different from those using either three doses of 1 mg of gene-optimized ADVAX DNA vaccine or two doses of 10^7 TCID50 vaccinia-vectorized ADMVA vaccine that only induced 200~250 spot forming units (SFUs)/10^7 splenocytes against the identical GagAl epitope. Meanwhile, ~17% of tetramer+ CD8+ T cells from DNA vaccination was similar to those elicited by rAd5-Gag vaccination with three dosages of 10^10 virus particles, or by a DC-SIGN-targeted lentiviruses-Gag with two doses of 5x10^6 TU (transduction units). The immunogenicity of the fusion DNA/E9P vaccine strategy, therefore, is potent for eliciting anti-HIV CD8+ T cell immunity.

[0094] Furthermore, as long-lasting CD8+ T cell-mediated immunity to a particular intracellular pathogen requires the establishment of a memory cell pool that proliferates rapidly in response to antigen re-encounter, Δ42PD1 fusion DNA induced higher frequencies of not only IFN-γ producing but proliferating p24-specific CD8+ T cells 7.5 months after immunization (Figs. 5b and 5d). Most importantly, msΔ42PD1-p24 vaccine significantly inhibited tumor growth in vivo (Figs. 6b, c) in line with more effective cytotoxic T cells capable of eliminating AB1-HIV-1-Gag tumor cells in vivo (Fig. 6a). In addition, mice vaccinated with msΔ42PD1-p24, were protected against both attenuated (VTGaggpol) and virulent (WRGaggpol) vaccinia viruses from mucosal challenges (Figs. 6d, e) with minimal body weight loss (Fig. 6g). Here, since neither neutralizing antibodies nor T cell immunity against the backbone vaccinia viruses were
generated, the observed protection was also primarily due to the significantly enhanced T cell immunity directed at HIV-1 Gag p24.

[0095] The mechanism of the success of msA42PD1 fusion DNA vaccine in mice can be contributed by the ability of msA42PD1 to induce the expression of TNF-α, IL-6 and IL-10β. These cytokines may play active roles in the generation of antigen-specific adaptive immunity by acting on APCs, such as DCs. TNF-α can induce the maturation of professional antigen presenting DCs and increase the expression of MHC and co-stimulatory molecules, and migration to draining lymph nodes to prime naïve T cells. With the addition of IL-10β, these matured DCs become more potent at promoting the differentiation of IFN-γ-producing T cells in a Th1 manner. While synergistically, TNF-α and IL-6 can provide co-stimulatory cytokine signals to induce the proliferation of T cells, IL-6 has also been found to inhibit the activity of regulatory T cells to ensure the production of IFN-γ by CD4+ T cells.

[0096] As elevated levels of cytokines were not detected systemically in mice seen (Table S3), it is postulated that the high level of functional B and T cell immunity elicited by the sA42PD1-based DNA fusion vaccine can be contributed by the induction of TNF-α, IL-6 and IL-10β at the site of vaccination. Other DNA vaccine studies have also shown that T cell responses were elicited by co-administering plasmids encoding HIV-1 Env and CD86 adjuvant to enable non-bone marrow-derived cells to prime CD8+ T cells at the site of injection assisted by a pro-inflammatory environment that can enhance antigen presentation. As for the weak CD4+ but strong CD8+ T cell responses observed, other cytokine signals such as IL-12 or type 1 IFN may play a role in favoring naïve CD8+ T cell activation. It has also been reported that IL-15 alone can substitute for CD4+ T helper cell in stimulating CD8+ T cell activation and expansion.

[0097] The present invention also shows that TNFα, IL-12 and IL-15 transcripts were increased in PBMCs treated with sA42PD1, after 12 h (Fig. 16). Additionally, the induction of IL-10β and IL-6 by sA42PD1, may also contribute to CD8+ T cell response by inhibiting activation-induced cell death.

Protein Isoforms, Nucleic Acid Molecules, and Fusion Proteins and Fusion Nucleic Acid Constructs

[0098] A first aspect of the subject invention provides PD1 protein isoforms. In one embodiment, the PD1 protein isoform is Δ42PD1, which has an amino acid sequence comprising SEQ ID NO: 1. In one embodiment, the PD1 protein isoforms do not bind to PDLL or PDL2.

[0099] In one embodiment, the PD1 protein isoform has a deletion of 14 amino acids at positions corresponding to amino acids 26-39 of the wild-type PD1 protein having an amino acid sequence of SEQ ID NO:2. In one embodiment, the 14 amino acid deletion has a sequence that is DSPDRPWNPPTFFP (SEQ ID NO:3).

[0100] In another embodiment, the PD1 protein isoform has non-conservative substitutions at one or more amino acids at positions corresponding to amino acids 26-39 of the wild-type PD1 protein having an amino acid sequence of SEQ ID NO:2. In certain embodiments, the PD1 protein isoform has non-conservative substitutions of I to 14 amino acids at positions corresponding to amino acids 26-39 of the wild-type PD1 protein having an amino acid sequence of SEQ ID NO:2. In one embodiment, amino acids 26-39 of the wild-type PD1 protein are DSPDRPWNPPTFFP (SEQ ID NO:3).

[0101] The wild-type PD1 protein is preferably of mammalian origin (such as a wild-type mouse, rabbit, non-human primates, or pig PD1 protein), more preferably, of human origin.

[0102] In certain embodiments, the present invention provides PD1 protein isoforms that are homologous to Δ42PD1 (SEQ ID NO: 1). In an embodiment, the PD1 protein isoform comprises an amino acid sequence that is at least about 80%, 85%, 90%, 93%, 95%, 96%, 97%, 98%, 99%, or 99.5% identical to SEQ ID NO: 1.

[0103] In an embodiment, the present invention provides PD1 protein isoforms that are homologous to Δ42PD1, wherein the PD1 protein isoform has non-conservative substitutions of 1 to 14 amino acids at positions corresponding to amino acids 26-39 of the wild-type PD1 protein having an amino acid sequence of SEQ ID NO:2, and the PD1 protein isoform comprises an amino acid sequence that is at least about 80%, 85%, 90%, 93%, 95%, 96%, 97%, 98%, 99%, or 99.5% identical to amino acids 11-276 of SEQ ID NO: 1.

[0104] In an embodiment, the present invention provides PD1 protein isoforms that are homologous to Δ42PD1, wherein the PD1 protein isoform has a deletion of 14 amino acids at positions corresponding to amino acids 26-39 of the wild-type PD1 protein having an amino acid sequence of SEQ ID NO:2, and the PD1 protein isoform comprises an amino acid sequence that is at least about 80%, 85%, 90%, 93%, 95%, 96%, 97%, 98%, 99%, or 99.5% identical to amino acids 11-276 of SEQ ID NO: 1.

[0105] In certain embodiments, the present invention provides fragments of the PD1 protein isoforms. In certain embodiments, the fragments of the PD1 protein isoforms of the present invention have at least 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 220, 215, 220, 225, 230, 235, 240, 245, 250, 255, 260, 265, 270, 275, or 280 amino acids.

[0106] In a preferred embodiment, the present invention provides soluble fragments of the PD1 protein isoforms of the present invention.

[0107] Another aspect of the subject invention provides nucleic acid molecules that encode the PD1 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. In one embodiment, the PD1 nucleic acid molecules of the present invention are formulated into a DNA vaccine formulation.

[0108] In one embodiment, the nucleic acid molecule encodes a PD1 protein isoform having a deletion of 14 amino acids at positions corresponding to amino acids 26 to 39 of the wild-type PD1 protein having an amino acid sequence of SEQ ID NO:2. In one embodiment, the nucleic acid molecule encodes a PD1 protein isoform having non-conservative substitutions at one or more amino acids at positions corresponding to amino acids 26-39 of the wild-type PD1 protein having an amino acid sequence of SEQ ID NO:2. In certain embodiments, the nucleic acid molecules encode a PD1 protein isoform having non-conservative substitutions at 1 to 14 amino acids at positions corresponding to amino acids 26 to 39 of the wild-type PD1 protein having an amino acid sequence of SEQ ID NO:2. In one embodiment, amino acids 26-39 of the wild-type PD1 protein are DSPDRPWNPPTFFP (SEQ ID NO:3).

[0109] Another aspect of the invention provides PD1 fusion proteins and fusion nucleic acid molecules. In a preferred
embodiment, the PDI fusion nucleic acid molecules of the present invention are formulated into a DNA vaccine formulation. In additional preferred embodiments, the PDI fusion nucleic acid molecules of the present invention are formulated into immunogens for antibody preparation.

[0110] In one embodiment, the PDI fusion protein comprises a Fe domain. In one embodiment, the Fe domain is rabbit IgG1 Fe. In one embodiment, the soluble PDI protein is linked to the antigen via a linker sequence. In an alternative embodiment, the PDI fusion protein comprises a PDI protein fused with a Fe domain, optionally via a linker sequence.

[0111] In one embodiment, the PDI fusion protein comprises a PDI protein of the present invention fused with an antigenic protein fragment.

[0112] In one embodiment, the antigenic protein fragment is a HIV gag p24 antigen fragment. 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-I), influenza, bovine leukemia virus (BLV), Epstein-Barr virus, pertussis, polio, measles, mumps, rubella, smallpox, zoster, anthrax, tetanus, rotavirus, rabies, chickenpox, meningococcus, diphtheria, anaplasmosis, anthrax, plague, encephalitis, pneumococcus, pneumonia, typhus, typhoid fever, streptococcus, staphylococcus, neisseria, lyme disease, cholera, E. coli, shigella, leishmanias, leprosy, cytomegalovirus (CMV), respiratory syncytial virus, parainfluenza, adenovirus, varicella, influenza, dengue, toxoplasmosis, coxsackievirus, schistosomiasis, Mycobacteria tuberculosis, and malaria.

[0113] In certain specific embodiments, the antigenic protein fragment are derived from microbial pathogens including HIV, 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, and Mycobacteria tuberculosis.

[0114] The antigenic protein fragment can also be derived from tumor or cancer cells. In one embodiment, the PDI protein isoforms, and fusion proteins thereof as molecular or protein adjuvants to enhance immune response. Additionally, nucleic acid molecules encoding the PDI protein isoforms, and fusion proteins thereof can also be administered to a subject to enhance immune response.

[0115] 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, gp120, and gp41. In a further embodiment, the PDI fusion protein further comprises a Fe domain. In an embodiment, the PDI fusion protein comprises a rabbit Fe domain for protein purification purpose.

[0116] The term “Fe domain” encompasses the full length and fragments of native human and animal Fe and Fe 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 Fe variants and native Fe’s, the term “Fe domain” includes molecules in monomeric or multimeric form, whether digested from whole antibody or produced by other means.

[0117] The term “Fc variant” refers to a molecule or sequence that is modified from a native Fe but still comprises a binding site for the salvage receptor. Fe domains include molecules having two or more polypeptide chains associated covalently, noncovalently, or by both covalent and non-covalent interactions. IgG molecules typically form dimers; IgM, pentamers; IgD, dimers; and IgA, monomers, dimers, trimers, or tetramers. Multimers may be formed by exploiting the sequence and resulting activity of the native Ig source of the Fe or by derivatizing (as defined below) such a native Fe.

[0118] The Fe domain within the scope of the invention can be of antibodies of any isotype, including IgG, IgA, IgE, IgD, and IgM. IgG 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 Fe domain is IgG1.

[0119] In a further embodiment, the PDI fusion protein of the subject invention comprises a linker sequence that links the soluble PDI domain to the antigen. In addition, the Fe 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. 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. Examples of useful linkers include, but are not limited to, GGGGSGGGG (SEQ ID NO:4), GGTGTTGTGTCAGGAGGAGGA (SEQ ID NO:5), G9Il (SEQ ID NO: 8), G9lku (SEQ ID NO: 9), 9Ser (SEQ ID NO: 10), 5GlyCys2ProCys (SEQ ID NO: 11), 4Gly3Ser (SEQ ID NO: 12), Ser Cys Val Pro Leu Met Arg Cys Gly Gly Cys Asn (SEQ ID NO: 13), Pro Cys Val Pro Leu Met Arg Cys Gly Gly Cys Cys Asn (SEQ ID NO: 14), Gly Asp Leu Ile Tyr Arg Asn Glu Lys (SEQ ID NO: 15), and 9GlyProSerCysValProLeuMetArgCysGlyGlyCysAsn (SEQ ID NO: 16).

[0120] In addition, the subject invention provides PDI fusion nucleic acid constructs, comprising a nucleic acid molecule encoding the subject PDI fusion protein. In one embodiment, the PDI fusion construct comprises a nucleic acid molecule encoding a PDI protein fused with a nucleic acid encoding a protein antigen. In a further embodiment, the PDI fusion construct comprises a Fe DNA. In one embodiment, the soluble PDI DNA is linked to the antigen DNA via a linker sequence. Optionally, the Fe DNA is linked to the PDI-antigen DNA via a linker DNA sequence.

[0121] 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 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-I), influenza, bovine leukemia virus (BLV), Epstein-Barr virus, pertussis, polio, measles, mumps, rubella, smallpox, zoster, anthrax, tetanus, rotavirus, rabies, chickenpox, meningococcus, diphtheria, anaplasmosis, anthrax, plague, encephalitis, pneumococcus, pneumonia, typhus, typhoid fever, streptococcus, staphylococcus, neisseria, lyme disease, cholera, E. coli, shigella, leishmania, leprosy, cytomegalovirus (CMV), respirin-
tory syncytial virus, parainfluenza, adenovirus, varicella, flavivirus, dengue, toxoplasmosis, coecidomyelosis, schistosomiasis, Mycobacteria tuberculosis, and malaria.

[0122] In one embodiment, the present invention provides isolated PD1 isoform and nucleic acid molecules encoding the PD1 isoforms, such as PD1 isoform A42PD1. In certain embodiments, the PD1 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) contaminating factors (such as biological molecules, proteins or peptides, nucleic acids, lipids and carbohydrates and other cellular components).

[0123] 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 having the substituted amino acids retains substantially the same functional activity as the fusion protein in which amino acids have not been substituted.

[0124] Examples of non-natural amino acids include, but are not limited to, ornithine, citrulline, hydroxyproline, homoserine, phenylglycine, uracine, isotryptamine, 2,4-diaminobutyric acid, α-amino isobutyric acid, 4-amino-substituted methyl, 2-amino butyric acid, γ-amino butyric acid, ε-amino hexanoic acid, 6-amino hexanoic acid, 2-amino isobutyric acid, 5-amino propionic acid, norleucine, norvaline, sarcosine, homocitrulline, cysteic acid, T-butyglycine, T-butyllalanine, phenylglycine, cyclohexylalanine, C-alanine, fluoroo-amino acids, designer amino acids such as β-methyl amino acids, C-methyl amino acids, N-methyl amino acids, and amino acids 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 (dextrorotatory) form or L (levorotary) form.

[0125] 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.

[0126] 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-flourouracil, 5-bromouracil, hypoxanthine, 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.

[0127] 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 NCBI/NIH website.

[0128] The subject invention also contemplates 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 6xSSPE, 5xDenhardt'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):

$T_m = 81.5 C + 16.6 \log [Na^+] + 0.41(\%GC) - 0.61(\%formamide) - 600/length of duplex in base pairs.$

[0130] Washes are typically carried out as follows:

(1) Twice at room temperature for 15 minutes in 1xSSPE, 0.1% SDS (low stringency wash).
(2) Once at Tm-20°C for 15 minutes in 0.2xSSPE, 0.1% SDS (moderate stringency wash).

[0133] Further, the subject invention provides expression constructs comprising PD1 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.

[0134] 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.

[0135] For expression in animal cells, an expression construct of the invention can comprise suitable promoters that can drive transcription of the polynucleotide sequence. For mammalian cells, suitable promoters include such as, for example, Pcmv. 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, BMPS promoter, and TRP promoter.

Induction of Cytokines

[0136] Another aspect of the present invention provides uses of the PD1 protein isoforms, e.g., A42PD1, nucleic acid molecules encoding the PD1 protein isoforms, fusion pro-

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teins comprising the PD1 protein isoforms, and/or fusion nucleic acid molecules comprising nucleic acid sequences encoding the PD1 protein isoforms for induction of production of cytokines (such as, TNF-α, IL-1, and IL-6) in immune cells.

In one embodiment, the present invention provides a method of inducing the production of TNF-α, IL-1, and/or IL-6, wherein the method comprises administering, to an immune cell (preferably, an immune cell in a subject), a PD1 protein isoform, a nucleic acid molecules encoding the PD1 protein isoform, a fusion protein comprising the PD1 protein isoform, and/or a fusion nucleic acid molecule comprising nucleic acid sequences encoding a PD1 protein isoform of the present invention.

Prevention and/or Treatment of Pathogenic Infection

Another aspect of the present invention provides methods for the prevention, diagnosis, treatment, or amelioration of pathogenic infection. Advantageously, the methods of the subject invention enhance T cell immunity. The method comprises administering to a subject in need of such prevention and treatment an effective amount of a PD1 protein isoform of the present invention (such as Δ42PD1 protein), nucleic acid molecule encoding a PD1 protein isoform of the present invention (such as Δ42PD1 protein), and/or fusion protein and/or fusion nucleic acid molecule of the present invention.

In addition, the methods can be used in the prevention or treatment of diseases where enhanced T cell immunity is beneficial. In a specific embodiment, the subject invention can be used in the prevention, diagnosis, and/or treatment of tumor or cancer.

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.

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.

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.

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.

In one embodiment, the subject invention can be used in the prevention, treatment or amelioration of infection by viral, bacterial, fungal, or other microbial pathogens including, but not limited to, human immunodeficiency virus (HIV), HIV including HIV-1 and HIV-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, bovine leukemia virus (BLV), Epstein-Barr virus, pertussis, polio, measles, mumps, rubella, smallpox, zoster, anthrax, tetanus, rotavirus, rabies, chickenpox, meningococcus, diphtheria, anaplasmosis, anaplasmosis, plague, encephalitis, pneumococcus, pneumonia, typhus, typhoid fever, streptococcus, staphylococcus, neisseria, lyme disease, cholera, E. coli, shigella, leishmania, leprosy, cytomegalovirus (CMV), respiratory syncytial virus, parainfluenza, adenovirus, varicella, flavivirus, dengue, toxoplasmosis, coccidiodomycosis, schistosomiasis, Mycobacteria tuberculosis, and malaria.

In one embodiment, the PD1 protein useful for the treatment or amelioration of tumor comprises an antigenic fragment derived from cancer or tumor cells.

Antibodies

Another aspect of the invention provides antibodies that bind specifically to the PD1 protein isoforms (such as Δ42PD1 protein) of the present invention. In one specific embodiment, the present invention provides CH134—an antibody that binds specifically to the Δ42PD1 protein. Such antibodies are also useful in diagnostic applications, such as but not limited to, tests that utilize FACS, WB, IF, IHC, EILSA, Elispot, and other tests. In another specific embodiment, the present invention provides CH101—an antibody that can both bind specifically to the Δ42PD1 protein and block the binding between the Δ42PD1 and its unknown receptor. Such antibodies, on one hand, are useful in diagnostic applications, such as but not limited to, tests that utilize FACS, WB, IF, IHC, EILSA, Elispot, and other tests. On the other hand, such blocking antibodies are likely to be useful in interfering with Δ42PD1 signaling, as components of therapeutic agents, such as but not limited to therapeutic antibodies, for treating Δ42PD1 related disease conditions.

The term “binding specificity,” “specificity,” “specifically reacts,” or “specifically interacts,” as used herein, refers to the ability of an antibody or other agent to detectably bind an epitope presented on an antigen, such as an epitope of HIV-1 gp120, while having relatively little detectable reactivity with other proteins or structures. Specificity can be relatively determined by binding or competitive assays, using e.g., Biacore instruments. Specificity can be exhibited by, e.g., an about 10:1, about 20:1, about 50:1, about 100:1, about 10,000:1 or greater ratio of affinity/avidity in binding to the specific antigen versus nonspecific binding to other irrelevant molecules.

Antibodies of the present invention can be in any of a variety of forms, including intact immunoglobulin molecules, fragments of immunoglobulin molecules such as Fv, Fab and similar fragments; multimers of immunoglobulin molecules (e.g., diabodies, triabodies, and bi-specific and tri-specific antibodies, as are known in the art; see, e.g., Hulsen and Kortt, J. Immunol. Methods 231:177 189, 1999); fusion constructs containing an antibody or antibody fragment (e.g., a fusion protein containing a fragment of CDR, e.g., 8CD4” (Saltzsweld et al. J. Virol. 74:326 333, 2000); and human or humanized immunoglobulin molecules or fragments thereof.

Antibodies within the scope of the invention can be of any isotype, including IgG, IgA, IgE, IgD, and IgM. IgG isotype antibodies can be further subdivided into IgG1, IgG2, IgG3, and IgG4 subtypes. IgA antibodies can be further subdivided into IgA1 and IgA2 subtypes.
The term “antibody fragment” refers to a portion of a full-length antibody, generally the antigen binding variable region. Examples of antibody fragments include Fab, Fab', F(ab')2, and Fv fragments. Peptide digestion of antibodies produces two identical antigen binding fragments called Fab fragments, each with a single antigen binding site, and a residual “Fc” fragment, so-called for its ability to crystallize readily. Pepsin treatment of an antibody yields an F(ab')2 fragment that has two antigen binding portions which are capable of cross-linking antigen, and a residual other fragment (which is termed pFc'). Additional fragments can include diabodies, linear antibodies, single-chain antibody molecules, and multispecific antibodies formed from antibody fragments.

The subject invention also comprises fusion constructs wherein the antibody, or fragment thereof, may be fused to one or more additional entities. The additional entity (ies) may be for example linkers, toxins, carriers, solid supports, and/or detectable molecules. In this context the binding portion may consist of or consist essentially of the antibody.

Antibodies of the present invention include polyclonal and monoclonal antibodies. The term “monoclonal antibody,” as used herein, refers to an antibody or antibody fragment obtained from a substantially homogeneous population of antibodies or antibody fragments (i.e. the individual antibodies within the population are identical except for possible naturally occurring mutations that may be present in a small subset of the antibody molecules).

Monoclonal antibodies of the invention can be prepared using hybridoma methods, such as those described by Kohler and Milstein, Nature, 256:495 (1975). In a hybridoma method, a mouse or other appropriate host animal is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes may be immunized in vitro, e.g., using the HIV Env-CD4 co-receptor complexes described herein.

The monoclonal antibodies may also be made by recombinant DNA methods, such as those described in U.S. Pat. No. 4,816,567 (Cabilly et al.). DNA encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures (e.g. by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). Libraries of antibodies or active antibody fragments can also be generated and screened using phage display techniques, e.g., as described in U.S. Pat. No. 5,804,440 (Burton et al.) and U.S. Pat. No. 6,096,441 (Barbas et al.). Recombinant antibodies, antibody fragments, and fusions and polymers thereof can be expressed in vitro or in prokaryotic cells (e.g., bacteria) or eukaryotic cells (e.g., yeast, insect, or mammalian cells) and further purified, as using well known methods (see, e.g., Sambrook et al. Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press (1989); and Ausubel et al., Current Protocols in Molecular Biology, John Wiley & Sons, New York, N.Y., 2001, which is updated quarterly).


The humanized antibodies of the present invention may be derived from animal subjects such as mouse, rabbit, and etc. Antibody humanization techniques generally involve the use of recombinant DNA technology to manipulate the DNA sequence encoding one or more polypeptide chains of an antibody molecule. Accordingly, a humanized form of a non-human antibody (or a fragment thereof) is a chimeric antibody or antibody chain (or a fragment thereof, such as an Fv, Fab, Fab', or other antigen-binding portion of an antibody) which contains a portion of an antigen binding site from a non-human (donor) antibody integrated into the framework of a human (recipient) antibody.

Methods for humanizing non-human antibodies are well known in the art. For example, humanized antibodies can be generated according to the methods of Winter and co-workers (Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature, 332:323-327 (1988); Verhoeyen et al., Science, 239:1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Methods that can be used to produce humanized antibodies are also described in U.S. Pat. No. 4,816,567 (Cabilly et al.); U.S. Pat. No. 5,565,352 (Hoogenboom et al.); U.S. Pat. No. 5,721,367 (Kay et al.); U.S. Pat. No. 5,837,243 (Deo et al.); U.S. Pat. No. 5,939,598 (Kucherlapati et al.); U.S. Pat. No. 6,130,364 (Jakobovits et al.); and U.S. Pat. No. 6,180,377 (Morgan et al.).

If desired, the antibodies of the present invention can be modified in any suitable process. For example, the binding affinity of the antibodies can be increased via various methods known in the art. For example, binding characteristics can be improved by direct mutation, methods of affinity maturation, phage display, or chain shuffling within the nucleic acids encoding the antibody molecules. For example, individual residues or combinations of residues can be randomized so that in a population of otherwise identical antigen binding sites, all twenty amino acids are found at particular positions. Binding characteristics can also be improved by methods of affinity maturation. See, e.g., Yang et al. (1995), J. Mol. Bio. 254, 392-403; Hawkins et al. (1992) J. Mol. Bio. 226, 889-896; or Low et al. (1996). J. Mol. Bio. 250, 359-368 (each of which is hereby incorporated by reference in its entirety, particularly with respect to methods of increasing the binding affinity of antibodies). Methods known in the art include for example, Marks et al. BioTechnology, 10:779-783 (1992) describes affinity maturation by VH and VL domain shuffling; random mutagenesis of CDR and/or framework residues is described by Barbas et al. Proc. Natl. Acad. Sci., USA 91:3809-3813 (1994); Schier et al. Gene, 169:147-155 (1995); Yelton et al. J. Immunol., 155:1994-2004 (1995); Jackson et al., J. Immunol., 154(7):3310-3319 (1995); and Hawkins et al. J. Mol. Bio., 226:889-896 (1992).

Strategies for antibody 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 WO 9523813 (which is hereby incorporated by reference in its
entirety) teaches in vitro methods of increasing antibody affinities utilizing alanine scanning mutagenesis. Alanine scanning mutagenesis can also be used, for example, to map the antigen binding residues of an antibody (Kelley et al., 1993, Biochemistry 32:6828-6835; Vajdos et al., 2002, J. Mol. Biol. 320:415-428). Sequence-based methods of affinity maturation (see, U.S. Pat. Application No. 2003/022240 A1 and U.S. Pat. No. 2002/171710 A1, both hereby incorporated by reference in their entireties) may also be used to increase the binding affinities of antibodies.

Therapeutic Compositions and Routes of Administration

[0160] The subject invention further provides for therapeutic or pharmaceutical compositions. In one embodiment, the therapeutic composition is formulated as a vaccine composition.

[0161] 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.

[0162] A vaccine composition is an antigenic preparation that comprises one or more immunogenic antigens used to produce active immunity to a disease. Such compositions may contain suitable pharmaceutically acceptable carriers, such as excipients, adjuvants and/or auxiliaries, and other therapeutically inactive ingredients.

[0163] 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 provides for customized vaccine schedules and compositions to prevent or minimize worsening of the diseases.

[0164] 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.

[0165] In a specific embodiment, a therapeutically effective amount of a protein and/or nucleic acid molecule of the subject invention is typically an amount such that when administered in a pharmaceutically tolerable composition is sufficient to achieve a plasma concentration of from about 0.01 microgram (µg) per milliliter (mL) to about 200 µg/mL.

[0166] 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. Alfonzo R. Gennaro, Mack Publishing Company, Easton, Pa. (1985). Suitable carriers include ethanol, dimethyl sulfoxide, glycerol, silica, 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, lecithin, and equivalent carriers and diluents. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions.

[0167] 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, ethanol, 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.

[0168] The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary, depending such as 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 50% to about 70%, active ingredient (w/w).

[0169] 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.

[0170] The therapeutic composition of the subject invention can include pharmaceutically acceptable solvents of the components involved. Pharmaceutically acceptable solvents 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-ethylamino ethanil, histidine, proline and the like.

[0171] 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.

[0172] 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.

[0173] 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, transdermal, intradermal, transmucosal, intraperitoneal, intramuscular, intracapsular, intraorbital, intracardiac, tracheal, subcutaneous, subcuticular, intrarterial, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection, infusion, and electroproporation, as well as co-administration as a component of any medical device or object to be inserted (temporarily or permanently) into a subject.

[0174] In a preferred embodiment, the microparticles 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.

[0175] 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.

[0176] Sterile injectable solutions are prepared by incorporating the active ingredients in the required amount in the appropriate solvent followed by sterilization filtered. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium as 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 previously sterile-filtered solution thereof.

[0177] In addition, the nucleic acid molecules and compositions 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 invention can be introduced in vivo via a viral vector such as adenovirus (AAV), herpes simplex virus (HSV), retrovirus, papillomavirus, adenovirus, and Epstein-Barr virus (EBV). In addition, the nucleic acid molecules and compositions of the subject invention can also be introduced in vivo via lipofection (DNA transfection via liposomes prepared from synthetic cationic lipids) (Felgenhauer et al., 1987). Synthetic cationic lipids (LIPOFECTIN, Invitrogen Corp., La Jolla, Calif.) can be used to prepare liposomes to encapsulate 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 phosphate precipitation, and by biolistic methods.

Materials and Methods

[0178] Primers and antibodies.

[0179] All primer sequences and antibodies used are listed in Table S1 and S2, respectively.

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Sequences</th>
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<tr>
<td>PO1 forward</td>
<td>5'-GGAGT GAGAA AGGCG GCGCT CT-3'</td>
</tr>
<tr>
<td>PO1 reverse</td>
<td>5'-CTCTG CTTGG GAAATG CCGTG ACC-3'</td>
</tr>
<tr>
<td>PO-L1 forward</td>
<td>5'-AGGCG ATGCC AGHAA GHTGA GGAA-3'</td>
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</table>

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Sequences</th>
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</thead>
<tbody>
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<td>hTNFa-f</td>
<td>5'--CGG AAG CAG TCA QAT CAT CTT-3'</td>
</tr>
<tr>
<td>hTNFa-r</td>
<td>5'--ACC TCC TCA QCT TGA-3'</td>
</tr>
<tr>
<td>hIL4-f</td>
<td>5'--GCT ACA TCC TCG ACG GCA TCT-3'</td>
</tr>
<tr>
<td>hIL4-r</td>
<td>5'--GTT CCT CCT TCC TCC TTT CAT-3'</td>
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<tr>
<td>hIL6-f</td>
<td>5'--AAG CTT ATG GCC CTA ACC AG-3'</td>
</tr>
<tr>
<td>hIL6-r</td>
<td>5'--AGC TCT ATC GTG CAC ATA AG-3'</td>
</tr>
<tr>
<td>hu-IFN-b-f</td>
<td>5'--ACC TCA AGT TCC ACA AG-3'</td>
</tr>
<tr>
<td>hu-IFN-b-r</td>
<td>5'--AGT CTC ATT CCA GCC AGT GC-3'</td>
</tr>
<tr>
<td>hu-IL-12-f</td>
<td>5'--AGA CAT CAT CAA ACC TGA CC-3'</td>
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</table>

Real-time PCR for cytokine expression

Human
TABLE S1-continued

<table>
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<tr>
<th>Primer name</th>
<th>Sequences</th>
</tr>
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<tr>
<td>hu-IL-12-r</td>
<td>5'-AAC GAG AAG TAG GAA TAC CCA CCT-3'</td>
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<tr>
<td>hIL-15F2</td>
<td>5'-TCT CTT CTA CTA AAG ACA TCC-3'</td>
</tr>
<tr>
<td>hIL-15R2</td>
<td>5'-GCT GAG TAC AGA GCA ACA-3'</td>
</tr>
<tr>
<td>hGRPMF f</td>
<td>5'-CAA GTC CAT GCC ACT ACT GCC-3'</td>
</tr>
<tr>
<td>hGRPMR r</td>
<td>5'-ACC TTC ACC ACC TTC TGG-3'</td>
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**Murine**

<table>
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<th>Sequences</th>
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<tr>
<td>THP-a-PW</td>
<td>5'-CAT CTT CTA AAA ATT CAT GTA GGT AGA-3'</td>
</tr>
<tr>
<td>THP-a-RV</td>
<td>5'-TGG GAG TAG ACA AGG TAC ACC CC-3'</td>
</tr>
<tr>
<td>mIL6-f</td>
<td>5'-GTA GCT ATG GTA CTC AGA AGA C-3'</td>
</tr>
<tr>
<td>mIL6-r</td>
<td>5'-ACG AGU ATG CA CTG GCA GAA-3'</td>
</tr>
<tr>
<td>mIL8a-f</td>
<td>5'-TCC CAG GAT GGG GGC ATG AGG-3'</td>
</tr>
<tr>
<td>mIL8a-r</td>
<td>5'-TGG TTC TTC TTC ATC TCG GAG CCA-3'</td>
</tr>
<tr>
<td>b-actin-f</td>
<td>5'-GTT GGC CCG CCT AGC CAC CA-3'</td>
</tr>
<tr>
<td>b-actin-r</td>
<td>5'-CCG TGT GGC TTA GGG TTC AGG GGG-3'</td>
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</table>

**TABLE S2-continued**

<table>
<thead>
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<th>Antibody name</th>
<th>Company source</th>
</tr>
</thead>
<tbody>
<tr>
<td>donkey anti-goat IgG (H + L)</td>
<td>Invitrogen</td>
</tr>
<tr>
<td>Alexa Fluor 647</td>
<td>Invitrogen</td>
</tr>
</tbody>
</table>

Cell Isolation and Gene Cloning.

**[0180]** Peripheral blood mononuclear cells (PBMCs) were freshly isolated fromuffy coats of anonymous healthy human blood donors using Ficoll-Hypaque (GE Healthcare). Human full-length PD1, PD-L1 and PD-L2 genes were amplified from PBMCs with respective primer pairs: PD1 forward/PD1 reverse, PD-L1 forward/PD-L1 reverse, and PD-L2 forward/PD-L2 reverse.

PCR analysis of PD1 and D42PD1.

**[0181]** Cellular genomic DNA was extracted from human PBMCs using the QIAamp DNA Blood Kit (Qiagen). PD1 amplification from genomic DNA amplification used primer pair PD1 forward/PD1 reverse. Another primer pair (nPD1 forward/nPD1 reverse) flanks the deletion region to detect both PD1 and D42PD1 cDNA samples by PCR. All PCR products were electrophoresed in 2% agarose gel.

Quantitative Real-Time (qRT)PCR of D42PD1 Transcript Expression.

**[0182]** cDNA templates were generated using Superscript VILO Master Mix (Invitrogen) from total RNA extracted using RNAiso (Takara Bio Inc), followed by real-time PCR reactions performed with SYBR Premix Ex Taq II (Takara Bio Inc) with specific primer pairs (listed in Table S1) in the Viia 7 instrument (Applied Biosystems) and analyzed with Viia7 RUO software (Applied Biosystems) normalized to GAPDH (for human) or beta-actin (for murine) and untreated negative control.

DNA Plasmids and Fusion Proteins.

**[0183]** The extracellular domains of PD-L1 and PD-L2 were amplified from cDNA of human PBMC using primer pairs EU forward/EL1 reverse and EL2 forward/EL2 reverse, respectively. The extracellular domains (i.e. soluble forms) of PD1 and D42PD1 were amplified from the PD1 and D42PD1 genes using primer pair ED1 forward/ED1 reverse. The amplified ectodomains of PD1 and D42PD1, and PD-L1 and PD-L2 were inserted into the expression vector pVAX fused with the CH2-CH3 domain of rabbit IgG (Fc) in one open reading frame to generate sPD1Fc, sD42PD1Fc, PD-L1Fc, and PD-L2Fc, respectively.

**[0184]** The 14APD1 mutant was generated by an overlapping PCR-based technique to introduce a run of fourteen alamines into the deletion region using the primer pair 14aPD1 forward/14aPD1 reverse. Fusion DNA vaccine plasmids with HIV-1 Gag p24 insert alone or linked to human or murine sD42PD1 contain the CMV promoter and transcription led by the tPA signal sequence, which improves the adaptive immunogenicity of encoded antigen by DNA vaccines likely due to increased protein expression.
[0185] PD-1 signal sequence is still intact in the construct, thus cleavage for protein translation does affect the overall fusion protein composition.

[0186] To increase the flexibility of the fusion protein, a linker (GGGGSOGG) (SEQ ID NO:4) (at sequence: GGGGTGTTGACGGAGGAGGA) (SEQ ID NO:5) was applied between the sPD-1 and HIV-1 p24 gene. Recombinant fusion proteins were produced by transient transfection of 293T cells using polyethyleneamine (PEI) for 72 h and purified with protein-G agarose (Invitrogen), and quantified using a Micro BCA protein kit (Thermo Scientific). Endotoxin contamination was not detected in all protein preparations as tested by the LPS-TOXATE kit (sensitivity 0.03 EU/ml; Sigma-Aldrich). Recombinant proteins were detected by Western blotting with specific antibodies and analyzed with Odyssey Infrared Imaging System (LI-COR Biosciences).

Molecular Modeling.

[0187] The model of human α42PD-1 complex was built from the original PD-1 crystal structure (PDB: 3H1K) using the INSIGHTII (Molecular Simulations, Inc., San Diego, Calif.), with the Δ42 deletion and beta-strands being highlighted.


[0191] 293T cells transiently expressing human or murine PD-1 (and PD-1.2) were incubated with 20 μg/ml of purified sPD1Δm, sΔ42PD1Δm, rabbit Fc, mPD1-p24Δ, msΔ42PD1-p24Δ, or p24Δ proteins, and detected with anti-rabbit Fc conjugated antibody by flow cytometry.

Vaccination of Mice.

[0191] All animal experiments received approval from the Committee on the Use of Live Animals in Teaching and Research, Laboratory Animal Unit, The University of Hong Kong. Female Balb/c mice at 5-8 weeks old were used for DNA immunization (or placebo PBS) by intramuscular (i.m.) injection with electroporation (EP) given every three weeks at a dose of 20 or 100 μg in 100 μl volume PBS per mouse for three times (FIG. 12c). Injection of 100 μl PBS alone served as the placebo group. Two weeks after the final immunization, mice were sacrificed, and sera and splenocytes were collected for immune response analysis. Each group contained 3-5 individual mice with independent immunization studies performed at least three times.

Cytokine Detection in Immunized Mice Serum

**TABLE S3**

<table>
<thead>
<tr>
<th>Immunized mouse</th>
<th>IL-2</th>
<th>IFN-γ</th>
<th>TNF-α</th>
<th>IL-4</th>
<th>IL-5</th>
<th>IL-6</th>
<th>IL-10</th>
<th>IL-17</th>
<th>GM-CSF</th>
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<td>0</td>
<td>0</td>
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<tr>
<td>mouse 3</td>
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<tr>
<td>msPD1-p24Δ mouse 1</td>
<td>0</td>
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Numbers = concentration in pg/ml.

Quantification of Cytokines.

[0188] 1x10⁶ PBMCs were treated with purified proteins of sPD1Δm, sΔ42PD1Δm, or rabbit Fc (20 μg/ml) or 1x10⁶ mouse splenocytes treated with msΔ42PD1-p24Δ, msPD1-p24Δ, or p24Δ (20 μg/ml) or LPS (100 ng/ml). The concentration of 20 μg/ml is close to 6.7 μg/ml of sPD-1 and 25 μg/ml of polyclonal anti-PD-1 antibody to achieve their required in vivo effects.

[0189] Supernatants were then harvested for analysis of cytokine release using the Human or Mouse Th1/Th2 FlowCytomix multiplex kit (Bender MedSystems). Data were generated using FACSCalibur instrument (BD Biosciences) and analyzed by FlowCytomixPro software (Bender MedSystems).

Antibody Responses.

[0193] Specific antibody responses were assessed by ELISA. Briefly, high affinity protein-binding ELISA plates (BD Biosciences) were coated with HIV-1 p24 protein (Abcam), and serially diluted mouse sera were added, and antibodies were quantified by goat-anti-rabbit peroxidase (HRP)-labeled anti-mouse IgG1 or IgG2a antibody (Sigma). Data acquired using VICTOR 1420 Multilabel Counter (PerkenElmer)" optical density over control was used for analysis.

Evaluation of HIV-1 Gag p24-Specific T Cell Responses.

[0194] ELISPOT (Millipore) were used to assess IFN-γ-producing T cells. Briefly, peptide gagAl (AMQMLKDTI
(SEQ ID NO:6); specific for CD8+ T cells and peptide gag26 (TSNPIIPVGDYKRWILELG (SEQ ID NO:7); specific for CD4+ T cells) were used to stimulate cells for 20 h and added to IFN-γ ELISPOT plates, with PMA (500 ng/ml) and calcium ionomycin (1 μg/ml) as positive control, or media only as negative control.

[0195] Peptide pool consisting of 59-members of Gag p24 libraries (each peptide contains 15aa or 10aa overlap) 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) and used to assess epitopic breadth of T cell response.

[0196] Elispots were identified by an immunospot reader and image analyzer (Thermo Scientific). Helicobacter pylori (H:K-2 AMQMLKDTI) (SEQ ID NO:6) (Beckman Coulter) tetramer was used to identify p24-specific CD8+ T cell population. Flow cytometry data was acquired and analyzed on a BD Aria III flow cytometer (BD Biosciences).

T Cell Proliferation.

[0197] Splenocytes were isolated from immunized mice 30 weeks post-immunization, labeled with CFSE (5 μM, Invitrogen), and stimulated with p24 peptide pool (2 μg/ml; donated by NIH, catalog: 8117), anti-CD28 antibody (2 μg/ml; eBioscience), in the presence of bone marrow-derived (BM)-DCs at a ratio of 1 DC: 10 splenocytes for 5 days. Positive control included anti-CD3 (2 μg/ml) and anti-CD28 antibodies (2 μg/ml). Surface staining occurred for CD3/CD4/CD8+ T cell markers, and flow cytometry with FACSCalibur (BD Bioscience) was used to analyse CFSE proliferation signals on T cells.

Cytotoxicity Assay.

[0198] Splenocytes isolated from mice two weeks after the last vaccination were served as effector cells. Effector cells were stimulated with p24 peptide pool (2 μg/ml) and anti-CD28 antibody (2 μg/ml; eBioscience) for 16 h before used. A1B1 cell line (Cell Bank Australia) transduced to express HIV-1 gag served as target cells. A luciferase reporter was also introduced to the A1B1-HIV-1-Gag cells. Assay was performed according to manufacturer’s instructions using the Live/Dead® Cell-Mediated Cytotoxicity Kit (Invitrogen).

[0199] Briefly, target cells were pre-stained with DIOC and co-cultured with effector cells at varying ratios for 2 h before all cells were stained with propidium iodide (PI), and analyzed by flow cytometry. Percentage of dead cells was calculated by subtracting the percentage of PI+ target only cells for each test sample.

Tumor Challenge.

[0200] Mice were subcutaneously challenged with 5x10^5 A1B1-HIV-1-Gag cells. Briefly, a transfer vector pBABE-HIVgag/Luc was inserted with a CMV promoter and co-transfected with pCL packaging vector into 293T cells to produce virus particles. Retrovirus-containing supernatants were used to infect A1B1 mesothelioma cells with puromycin selection and single clones were expanded. Following tumor challenge, in vivo images were taken twice a week to detect the intensity of luciferase on the flank of mice by Xenogen IVIS 100 in vivo imaging system.

Virus Challenge and Plaque Assay.

[0201] Mice three weeks post-vaccination were intramuscularly challenged using modified vaccinia virus that expresses HIV-1 gag and pol genes from attenuated strain TianTan (VTIgagpol) (for 20 μg dose mice group) or virulent strain Western Reserve (WRgagpol) (for 100 μg dose mice group) at 4x10^7 and 2x10^7 PFU/L, respectively.

[0202] Mice were sacrificed eight days post-challenge to determine virus titers in the lung homogenates, prepared by physical disruption, and cultured on Vero cell monolayer to monitor cytotoxic effect over time. Body weight of WRgagpol infected mice were monitored daily for eight days prior to sacrifice.

Statistical Analysis.

[0203] All statistical analyses were performed using the paired two-tailed Student’s t test. P values less than 0.05 were considered statistically significant. Data were presented as mean values the standard error of the mean (SEM) of at least three independent experiments (n ≥ 3 mice per group per experiment) unless indicated.

Generation of Mouse Derived Monoclonal Antibodies Against Human A42PD1

[0204] Cell Culture.

[0205] SP2/0-Ag14 myeloma cells (ATCC, Ca. No. CRL-1581), 293T cells, and Human PDI or A42PD1 stably expressing 293T cell lines (293T-PDI, 293T-A42PD1) were maintained in Dulbecco’s modified Eagle’s medium (DMEM) (Cat. No. 11995, Life Technologies) supplemented with 10% fetal bovine serum (FBS) plus 1/100 pen/strep (Cat. No. 15140, Life Technologies). DG-75 B cell line and Jurkat T cell line were maintained in RPMI supplemented with 10% heat-inactivated FBS, 2 mM L-glutamine and 1/100 pen/strep (1640 complete medium). All above-mentioned cells were maintained in a 37°C humidified 5% CO2 incubator. Suspension-adapted HEK293 cells (FreeStyle™ 293-F) (Cat No. R79007, Life Technologies) were cultured in the serum-free FreeStyle™ 293-F Expression Medium (Cat. No. 12338-018, Life Technologies) in 37°C incubator with a humidified atmosphere of 8% CO2 on an orbital shaker platform rotating at 135 rpm.

[0206] Expression and Purification of Recombinant Protein.

[0207] Recombinant proteins sA42PD1Fc, sPDIHIs and sA42PD1HIs were expressed using FreeStyle™ 293 Expression System. Briefly, fusion expressing plasmid pVAX-sA42PD1-Fc, pVAX-sA42PD1-His were used to transfect 293-F. Dilute 200 μg plasmid and 200 μg Polyethylenimine (PEI) in 8 ml Opti-MEM and mix gently, followed by incubating for 15 min at room temperature. Then the mixture was added into 200 ml 293-F cells (106 cells/ml). After 6 days culture, the fusion protein containing supernatant was collected and then purified using Recombinant Protein G (rProtein G) Agarose (Cat. No. 15920-010, Life Technologies) (for sA42PD1Fc) and Dynabeads™ His-Tag Isolation & Purification Kit (Cat. No. 10103D, Life Technologies) (for sA42PD1HIs and sPDIHIs) respectively, following the manufacturers’ instructions. Plasmids used for protein preparation were previously constructed[25]. Concentrations and purity of proteins were determined by BCA Protein Assay Kit (Cat. No. 23227, Thermo Scientific) and Coomassie Brilliant Blue-stained SDS-PAGE respectively.

[0208] Immunization and Cell Fusion.

[0209] All animal experiments received approval from the Committee on the Use of Live Animals in Teaching and
Research, Laboratory Animal Unit, The University of Hong Kong, Hong Kong SAR, China. For immunization, 100 μg s442PD1c plasmid in 50 μl PBS was injected intramuscularly (i.m.) in the quadriceps of female BABL/c mouse (8-10 weeks of age) on week 0 and 1. Immediately following injection, electroporation (EP) was performed at the injection site using a 2-needle array with a 0.5 cm gap. Electroporation parameters were: 120 V/cm between the electrodes; 50-ms pulse length; 6 pulses, given by a TERESA (Shanghai Teresa Healthcare) generator. After DNA plus EP priming, 20 μg s442PD1Fc proteins emulsified in Freund’s complete adjuvant was immunized subcutaneously on week 6, followed by 20 μg immunogen in Freund’s incomplete adjuvant subcutaneously on week 9. Mouse serum were collected seven days after the forth immunization for ELISA and Flow Cytometry. Hybridoma producing monoclonal antibodies (mAbs) against human A42PD1 were generated as described by Kohler and Milstein. At day 7 following the last boosting, 1.5x10^8 spleen cells of the immunized mice were collected and fused with Sp 2/0 myeloma cells at a ratio of 10:1 using Polyethylene glycol solution (Cat. No. P7181, Sigma). Hybridoma cells were selected in HAT medium (DMEM supplemented with 20% FBS and 2% HAT) for 10 days and then switched to FIT medium (DMEM supplemented with 20% FBS and 1% HAT).

**0210** Indirect ELISA.

**0211** For hybridoma screening, two weeks after fusion, supernatants were tested for specific antibody production by indirect ELISA. Briefly, 100 μl s442PD1His (0.2 μg/ml) was coated in 96-well plates overnight at 4°C. The wells were then washed three times with phosphate buffer solution containing 0.1% Tween-20 (PBS-T), and blocked with 200 μl of PBS containing 4% nonfat milk at 37°C for 1 h. After washing, supernatants (100 μl/well) were added to the plates and incubated for 1 h at 37°C. After three times washing, 100 μl per well of Goat anti-Mouse IgG H&L (HRP) secondary antibody (Cat. No. ab97040, Abcam) diluted 1:50,000 was added to plates. Then plates were incubated at 37°C for 1 h. After extensive washes, the enzymatic reaction was developed with 3,3',5,5'-tetramethylbenzidine (TMB) liquid substrate (Cat. No. T4444, Sigma) and stopped by adding 0.2 M H2SO4. The optical density was measured at 450 nm (O.D. 450 nm) with a VICTOR3 1420 Multilabel Counter (Perkin Elmer).

**0212** Flow Cytometry and Antibodies.

**0213** For indirect staining, cells were initially incubated with mouse serum, hybridoma supernatant or purified monoclonal antibodies followed by staining with Alexa Fluor® 647 Goat anti-Mouse IgG (H+L) (Cat. No. A-21235, Life Technologies) after washing with FACS buffer (PBS with 2% FBS and 0.1% NaN3). For direct staining, cells were incubated with fluorescein-labeled mAbs or isotype-matched negative control Abs, or for intracellular staining, cells were fixed and permeabilized using Fixation/Permeabilization Solution Kit (Cat. No. 554714, BD Biosciences) according to the manufacturer’s instructions. All the stained tubes were incubated for 15 min at room temperature. Cells were resuspended in 0.4 ml PBS and then subjected to FACSCalibur or FACS Aria III Flow Cytometer (BD Biosciences), and data were analyzed with FlowJo software.

**0214** Labeled anti-human antibodies used in current research include Pacific Blue-CD3 (clone UCHT1, 558117, BD Pharmingen), FITC-CD11c (clone 3.9, 301603, Biologend), PE-Cy7-CD14 (clone 61D3, 25-0149-42, eBioSciences), PerCP-CD19 (clone 340421, BD Bioscience), Alexa Fluor 488-CD56 (clone HCD56, 318312, Biologend), PerCP-Cy5.5-HLA-DR (clone I2.243, 307630, Biologend), PE-PE-D1 (clone EH12.1, 560795, BD Pharmingen), Alexa Fluor 647-A42PD1 (clone CH101, clone CH34), Alexa Fluor 647-IgG1 (clone MG121, Invitrogen), Alexa Fluor 647-IgG2b (clone MOPC-11, 400330, Biologend), PE-IgG1 (clone MOPC-21, 400112, Biologend).

**0215** Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation using Ficoll-paque premium (Cat. No. 17-5442-02, GE Healthcare) from fresh healthy blood donors. Briefly, whole blood was diluted 1:4 with sterile PBS and centrifuged at 400g for 30 min without brake. The isolated PBMCs were washed twice with PBS at 200g for 5 min. After washing, the cells were counted and resuspended in pre-warmed 1640 complete medium at a concentration of 2x10^6 cells/ml.

**Surface Plasmon Resonance**

**0216** Binding avidity analyses were performed with a Biacore 1000 optical biosensor (GE Healthcare). Immobilization of recombinant s442PD1Fc to CMS sensor chip was performed following the standard amine coupling procedure. Concretely, carboxyl groups on the sensor chip surface was activated by injection of N-Hydroxysuccinimide (NHS) and 1-Ethyl-3-(3-dimethylaminopropyl) carbodimide hydrochloride (EDC) in Amine Coupling Kit (Cat No. BR-1000-50, GE Healthcare). Then recombinant s442PD1Fc at a concentration of 30 μg/ml in 10 mM sodium acetate buffer (pH 5.0) was allowed to flow over the chip surface at a rate of 5 μl/min for 7 min, and the final response bound turned out to be 7579 RU. After unreacted protein was washed out, excess active ester groups on the sensor surface were capped by injection of 1 M ethanolamine (pH 8.5) at a flow rate of 5 μl/min for 7 min. As background to correct instrument and buffer artifacts, a reference was generated under the same conditions without immobilization the recombinant protein. Binding experiments were performed at 25°C in HBS-EP buffer (Cat No. BR-1006-69, GE Healthcare). Binding kinetics were analyzed by passing various concentrations of anti-human A42PD1 mAbs CH34 and CH101 over the chip surface for 3 min. Dissociation of bound analytes was monitored while the surface was washed with buffer for 4 min at a flow rate of 30 μl/min. Remaining analytes were removed in the surface regeneration step with injection of 10 mM glycine-HCl (pH 2.0) for 2x30 sec at a flow rate of 30 μl/min. The kinetic parameters were determined after subtraction of the blank cell from each response value, by collectively fitting the overlaid sensograms locally using Biacore x100 Evaluation software (version 2.0.1) to the 1:1 Langmuir binding model.

**Cell Surface A42PD1 Signaling Assay**

**0217** To determine antagonist activity of A42PD1 specific monoclonal antibodies, 1x10^5 cells were centrifuged at 200xg for 5 min and resuspended with 100 μl PBS containing 1 μg purified A42PD1 specific monoclonal antibodies or isotype matched control antibodies, cells were incubated at room temperature for 10 min, then PBMCs were added with a ratio of 1:50 followed by centrifugation at 200xg for 5 min and resuspension with 100 μl DMEM complete media.
Then cells were incubated at room temperature for 15 min, followed by intracellular staining of p-Akt and flow cytometrical analysis.

Double Antibody Sandwich-ELISA

[0218] Microtiter plates (Cat. No. 3690, Corning) were coated with antibody CH54 (10 μg/ml) at 37°C. For 2 h followed by incubating with PBS containing 4% skim milk to block nonspecific binding. Plasma or serum specimens were diluted at 1/2 and added to wells in duplicate, along with reconstituent 42PD1/FC proteins as standards. The plates were then incubated for 2 h at 37°C. After washing, biotin labeled antibody CH110 (5 μg/ml) (labeled using Biotin Protein Labeling Kit, Cat. No. D-20655, Life Technologies) were added and incubated for an additional 2 h at 37°C. Following the addition of horseradish peroxidase (HRP) conjugated Streptavidin (Cat. No. SA10001, Life Technologies) (1:2000), color reactions were developed using 3,3',5,5'-tetramethylbenzidine (TMB) liquid substrate (Cat. No. T4444, Sigma) and subsequently stopped with 0.2 M H2SO4. The optical density was measured at 450 nm (O.D. 450 nm) with a VICTOR3 1420 Multilabel Counter (Perkin-Elmer).

EXAMPLES

[0219] Following are examples that illustrate procedures and embodiments for practicing the invention. The examples should not be construed as limiting.

Example 1

Novel PD1 Isoform

[0220] To investigate the polymorphism of PD1 gene, mRNA transcripts from PBMCs from 25 human healthy donors were examined. In one representative donor with seven clones, RT-PCR and sequence analysis showed that seven clones harbored an identical isoform of PD1, which consists of a 42-base pairs deletion from the start of exon 2 which is equivalent to a 14 amino acid in-frame deletion (DSPRDRWNPPT-FFP) (SEQ ID NO.3) (Figs. I a-b). The PD1 isoform was designated as Δ42PD1.

[0221] To verify that this deletion is not due to intrinsic genomic defect from multiple donors, PCR was performed using primers that flank the deleted region. As a control, genomic DNA only detected wildtype PD1 (FIG. 1c, lanes 1-7, lower gel), while both wildtype PD1 and Δ42PD1 transcripts were readily detected from cDNA generated from five of seven donor PBMCs (FIG. 1c, lanes 1-7, upper gel), which are confirmed by sequence analysis. Hence, this transcript is likely due to alternative splicing, and not mutation on the chromosomal level. Alternative splicing of pre-mRNA is usually found in mammalian cells under two conditions: mutation of the junction site between introns and exons, or alternative selection of splicing sites.

[0222] For the latter, an AG dinucleotide splicing donor is often required, and indeed, there exists an alternative AG splicing donor at the 3' terminus of the deletion region of exon 2 that probably leads to the formation of the Δ42PD1 mRNA (FIG. 1b). In total, 24 out of 25 donors harbored the Δ42PD1 isoform.

[0223] To determine the expression profile of Δ42PD1 among immune cells found in PBMCs, quantitative real-time RT-PCR with the use of specific primers was performed to measure the mRNA expression of Δ42PD1 in different cell types. [0224] For this purpose, cell sub-populations were sorted from PBMCs from five independent healthy donors according to various cell markers: NK cells (CD3-CD56+), T cells (CD3+, CD3+CD4+ T and CD3+CD8+ T cells), B cells (CD3-CD19+), NKT cells (CD3+CD56+), monocytes (CD3-CD11c+CD14+), macrophages (CD3-CD11c+CD68+), and dendritic cells (DCs; CD3-CD11c+). As shown in FIG. 1d, the relative expression of Δ42PD1 was found highest among monocytes, macrophages, NK and NK T cells, and to a lesser extent on B cells, T cells (CD4 or CD8) and DCs. Δ42PD1 is distinct from PD1 and does not interact with PD-L1/L2.

[0225] To gain a better understanding of the possible function of Δ42PD1, DNA plasmid vectors were generated to express soluble forms of PD1 or Δ42PD1 protein tagged to rabbit Fc, denoted as sΔPD1Fc, and sΔ42PD1Fc respectively. Soluble forms of PD1 or Δ42PD1 protein only encode the extracellular regions and the former has been used to characterize the function of PD1 previously.

[0226] In addition, to account for tertiary structural disruptions with the deleted 14 amino acids, alanines are substituted back to generate s14APD1Fc. Purified proteins of sPD1Fc, s42PD1Fc, and s14APD1Fc, were generated by transient transfection of 293T cells with subsequent purification from culture supernatants. The purity of these proteins was checked by Coomassie blue-stained SDS-PAGE gel electrophoresis (FIG. 10).

[0227] To determine if these proteins could bind to PD1 ligands, they were used to treat 293T cells transiently transfected with human PD-L.1 or PD-L.2 at different concentrations, and signals from binding were detected by anti-rabbit Fc antibody using flow cytometry (FIG. 2a,b). As expected, sPD1Fc was bound to both PD-L.1 and PD-L.2, but neither to s42PD1Fc nor to s14APD1Fc.

[0228] The results show that the protein encoded by the Δ42PD1 isoform is unlikely to interact with PD1 ligands and the 14 alanines were insufficient to restore the binding.

[0229] To demonstrate that Δ42PD1 and PD1 are distinct molecules, the full-length membrane-bound form of Δ42PD1 and PD1 are expressed by stable transfection of 293A cell line (293A-PD1 and 293A-Δ42PD1) and commercial antibodies were used for detection by flow cytometry. PD1-specific monoclonal antibodies (clones EH21.2.1, MH4, and EH12. 217) detected PD1 but were unable to detect Δ42PD1 (FIG. 2c). As these commercial antibodies bind to the PD1/PD-L interacting moieties, these results further reinforce that Δ42PD1 differs from PD1 structurally at the PD-L binding interface. Commercial polyclonal anti-PD1 antibody could detect both PD1 and Δ42PD1 (FIG. 2c), suggesting that Δ42PD1 could still be recognized, likely through a region conserved between PD1 and the Δ42PD1 isoform outside the PD-L binding interface.

[0230] These results indicate that the confirmation of this Δ42PD1 isoform differs from PD1 primarily at the domain of PD-L1/L2 interaction.

[0231] This Example also examines the structure of Δ42PD1 in silico. As the 14-amino acid deletion partially exists in the published PD1 crystal structure, the inventors re-modeled human PD1 and included the initial 14-amino acids in the structure in the beta-strand A of human PD1 (FIG. 7; highlighted red). Based on the model, the deletion of the N-terminal beta-strand A, which extensively interacts with
the core structure, could result in a conformation that is distinct from the correct folding of wildtype PD1, and thus readers Δ42PD1 unable to bind to PD-L1/L2.

**Example 2**

Δ42PD1 Induces the Production of Pro-Inflammatory Cytokines in Human PBMCs

[0232] This Example investigates the function of Δ42PD1 using the purified sΔ42PD1Fc proteins to treat human PBMCs and measured the production of cytokines.

[0233] Briefly, PBMCs were treated with purified sPD1Fc, sΔ42PD1Fc, or rabbit Fe recombinant proteins for 24 h, and supernatants were collected to determine the cytokine release profile by a multiplex assay. Untreated cells or LPS served as negative and positive controls, respectively.

[0234] As shown in FIG. 3a, PBMCs treated with sΔ42PD1Fc had significantly higher levels of TNF-α, IL-6 and IL-1β cytokine production, when compared to sPD1Fc or rabbit Fe. Other cytokines IFN-γ, IL-2, IL-4, IL-5, IL-8, IL-10, IL-12p70 and TNF-β were not detected following treatment by these recombinant proteins (data not shown).

[0235] For verification, quantitative real-time PCR was performed at 6, 12 h and 24 h post-treatment of PBMCs, and relative mRNA expression of TNFα, IL-6, and IL-1β was also found significantly increased with sΔ42PD1Fc protein treatment compared with sPD1Fc that remained comparable to rabbit Fe (FIGS. 3b-d).

[0236] Moreover, another version of recombinant protein in which soluble Δ42PD1 was fused with a 6×His tag named sΔ42PD1His was also used to treat PBMCs, and successfully induced production of pro-inflammatory cytokines in a dose dependent manner (FIG. 28).

[0237] In addition, to confirm that not only the soluble form can induce such effects, cytokine induction is also examined using γ-irradiated 293A cells stably expressing surface PD1 or Δ42PD1 and co-cultured with PBMCs, and the same trend as with using the soluble form of proteins at least for the 6 h time point after treatment was observed (FIG. 11).

[0238] The results show that both soluble and membrane-bound Δ42PD1 could induce the production of pro-inflammatory cytokines.

[0239] Subsequently, human and mouse sΔ42PD1 nucleic acid molecules were used as an intramolecular adjuvant to develop a fusion DNA vaccine with HIV-1 Gag p24 antigen (sΔ42PD1-p24) to immunize mice, and the fusion DNA vaccine elicited a significantly enhanced level of anti-p24 IgG1/ IgG2a antibody titers, and important p24-specific CD8+ T cell responses that lasted for more than 7.5 months. Furthermore, p24-specific CD8+ T cells possess functionally improved proliferative and cytotoxic capacities resulting in the protection of immunized mice against pathogenic viral challenge.

[0240] The results show that Δ42PD1 has an immune regulatory function distinct from PD1.

**Example 3**

Δ42PD1 Fused to Antigen Promotes Specific Adaptive Immunity In Vivo

[0241] As TNF-α, IL-6 and IL-1β have cooperative and key roles in the generation of adaptive immunity, this Example investigates whether Δ42PD1 can perform this function in vivo.

[0242] Briefly, a fusion DNA vaccine construct comprised of HIV-1 Gag p24 is generated for use as the target immunogen with human sΔ42PD1 tagged to rabbit Fe (sΔ42PD1-p24Fc; FIG. 12a); DNA encoding p24, is used as control. The rabbit Fe used only contains the CH2-CH3 domain and thus does not bind to rabbit Fc receptor. The γA-leader was fused with the leader sequence of PD1 to increase protein release, while the signal peptide cleavage of Δ42PD1 remains the same as wildtype PD1. Expression of their encoded protein was confirmed by Western blotting (FIG. 12b).

[0243] The DNA vaccine constructs were delivered at a dose of 20 μg/shot to Balb/c mice intramuscularly (i.m.) with electroporation (EP) according to our previously used immunization regimen (FIG. 12c).

[0244] As shown in FIG. 12d, antibody responses detected in mice were ELISA for both IgG2a (Th1: 1.5-fold) and IgG1 (Th2; 7-fold) raised against p24 were significantly higher (P<0.05) in mice immunized with sΔ42PD1-p24Fc than p24Fc. For T cell responses, IFN-γ-producing cells were measured using ELISPOT assay against Gag peptides specific for CD4+ (gag26) and CD8+ (gagAI) T cells. Almost 10-fold greater number of IFN-γ+ ELISpot for gagAI-specific CD8+ T cells were detected in splenocytes of sΔ42PD1-p24Fc-immunized mice compared to p24Fc-immunized group (P<0.001) or placebo (PBS). However, gag26-specific CD8+ ELISpot remained low and there were no differences between the two immunized groups or placebo (FIG. 12e).

[0245] Immunization with human sΔ42PD1 fused to p24Fc elicited a substantial level of CD8+ T cell response and modest antibody responses against p24, indicating a functional role of human sΔ42PD1 in DNA vaccination in mice.

[0246] To determine whether human sΔ42PD1 could be immunogenic in mice due to sequence diversity, the inventors examined whether immune recognition and response have been directed against human sΔ42PD1. Indeed, mouse serum from sΔ42PD1-p24Fc-immunized mice recognized Δ42PD1-GST purified protein by Western blotting (FIG. 12f), indicating that anti-human Δ42PD1 immunity may have interfered with the generation of anti-p24 immune response.

**Example 4**

Murine SAΔ42PD1 Fusion DNA Vaccine Elicits an Enhanced Level of Antigen-Specific CD8+ T Cell Immunity in Mice

[0247] The murine version of fusion DNA construct was generated by substituting human sΔ42PD1 with murine (m)Δ42PD1 with deletions at the same nucleotide positions to generate msΔ42PD1-p24Fc. While the native Δ42PD1 isoform was not detected in splenocytes of Balb/c or C57BL/6N mice by RT-PCR and sequencing (data not shown), the equivalent (m)Δ42PD1 isoform was used to study the efficacy of our DNA fusion vaccine strategy in mice.

[0248] To verify the function of murine counterparts, recombinant msΔ42PD1-p24Fc proteins were generated and tested for binding to PD-L1/L2 expressed on transiently transfected 293T cells (FIG. 13). msΔ42PD1-p24Fc, or p24Fc did not bind to either human or murine PD1 ligands.

[0249] To investigate whether the recombinant msΔ42PD1-p24Fc protein could induce pro-inflammatory cytokines, splenocytes from Balb/c mice were treated with purified proteins msΔ42PD1-p24Fc, or p24Fc. The results show that an increased level (~2-fold) of mRNA expression of Ifnα from 12 h and 24 h post-treatment was significantly induced
by mSA42PD1-p24e protein compared to p24e (P=0.05; FIG. 14a). For IL-6 and II-1α, a modest but statistically significant elevated level of gene expression was detected at 6 h (~1.3-fold; P<0.05) and 24 h (~1.6-fold; P=0.05) (FIGS. 14a,c).

[0250] However, the release of these cytokines 24 h post-treatment did not reach any significant differences compared to control (data not shown). Given the heterogeneity of splenocytes, bone marrow-derived dendritic cells (BM-DCs) were isolated and cultured to perform the same experiment. As shown in FIG. 4c, higher levels of pro-inflammatory cytokines TNF-α (~3-fold), II-6 (~1.5-fold) and II-1α (~5-fold) were produced by mSA42PD1-p24e-treated BM-DCs compared to p24e. Same as human sA42PD1-p24e, mSA42PD1-p24e can also stimulate the expression of pro-inflammatory cytokines and the p24 antigen was not a contributing factor for this induction.

[0251] In vivo vaccination experiments were performed to determine if a higher level of antigen-specific immunity could be achieved compared to the human sA42PD1 counterpart using the same immunization regimen (FIG. 12c), but with two different doses (20 µg and 100 µg DNA shot). Antibody responses show significantly higher level of IgG1 (Th2) and IgG2a (Th1) in sera of mice vaccinated with 20 µg of mSA42PD1-p24e compared to p24e (3- and 4-fold, respectively; P<0.05; FIG. 4b), which was further amplified at the 100 µg dose. Unlike human sA42PD1-p24e, no immune response was raised against the mSA42PD1 portion of the fusion molecule mSA42PD1-p24e, as immunized mouse serum did not detect mSA42PD1 protein by Western blotting (FIG. 15).

[0252] Meanwhile, IFN-γ ELISPOT assay detected a significantly increased level of p24-specific CD4+ T cell responses (~10000 ELispots/106 splenocytes; ~3.5-fold) and CD8+ (~10000 ELispots/106 splenocytes; ~15-fold) from mice vaccinated with 20 µg dose mSA42PD1-p24e compared to p24e or placebo (FIGS. 4c,d). However, no significant improvement was found in mice vaccinated at 100 µg dosage, which suggests that a low dose of mSA42PD1-p24e was sufficient to achieve this level of IFN-γ+ T cell response.

[0253] The antigen specificity of CD8+ T cells from mice vaccinated (20 µg dose) with mSA42PD1-p24e was examined, and the results show a greater frequency of p24-specific tetramer+ CD8+ T cells at an average of 17% at 3 weeks compared to those in p24e group (~11-fold; P=0.05; FIG. 4e). Additionally, epitope breadth was enhanced in splenocytes detected using three non-overlapping p24 peptide pools (FIG. 4f).

Example 5

Long-Term Memory CD8+ T Cells Immune Responses is Sustained in MSA42PD1-P24e Vaccinated Mice

[0254] To determine if long-term memory responses can be achieved with mSA42PD1-p24e, p24-specific cell-mediated immunity was examined 30 weeks (7.5 months) post-vaccination. Anti-p24 antibody titers were retained at 100 µg groups, with IgG1 and IgG2a responses being higher for mSA42PD1-p24e compared to p24e; however, at 20 µg dose, antibody responses of both groups remained relatively low (FIG. 5a). Although memory CD4+ IFN-γ+ ELispots was not apparent unless a higher dose of 100 µg DNA vaccine was used (~2-fold; P<0.05; FIG. 5b), CD8+ T cell immunity is long-lived, as a significant level of CD8+ IFN-γ+ ELispots could still be detected 30 weeks after mSA42PD1-p24e DNA vaccination in two doses (FIG. 5b).

[0255] Also, proliferative memory T cells were evaluated by CFSE assay for both CD4+ and CD8+ T cells in splenocytes isolated from 30 weeks post-vaccinated mice. The data showed that CD4+ T cells from p24e- or mSA42PD1-p24e-vaccinated mice (at 100 µg dose) were minimally proliferative upon stimulation with BM-DCs plus p24 peptide pool (FIG. 5c). However, ~16% of CD8+ T cells of the mSA42PD1-p24e group proliferated following stimulation, while p24e group remained at levels similar to the placebo group (FIG. 5d). Overall, the use of mSA42PD1 as an intramolecular adjuvant in the DNA vaccine vastly improved the elucidation of the levels of antigen-specific long-lived B and T cell immunity, especially CD8+ T cell immune responses compared to antigen alone.

Example 6

The efficacy of MSA42PD1-P24e DNA Vaccine in Mice

[0256] To assess the efficacy of our fusion DNA vaccine, this Example determines whether these CD8+ T cells are cytolytic and provide protection. C11, assay was performed using a modified mesothelioma cell line (AB1) to express HIV-1 Gag with luciferase as target cells (AB1-HIV-1-Gag). Splenocytes isolated from vaccinated mice (two weeks post-vaccination) were co-cultured at various ratios with AB1-HIV-1-Gag target cells and the frequency of dead target cells was measured.

[0257] Compared to p24e, or placebo groups, splenocytes isolated from mSA42PD1-p24e-immunized mice were able to kill efficiently even at a ratio of one effector T cell to two target cells (FIG. 6a).

[0258] To evaluate whether mSA42PD1-p24e protects vaccinated mice from tumor challenge, mice were immunized with 100 µg mSA42PD1-p24e, and p24e i.m./EP (FIG. 12c). Three weeks after the last boost, mice were challenged subcutaneously (s.c.) using 5x105 AB1-HIV-1-Gag tumor cells, and in vivo imaging was performed twice a week up to 3 weeks.

[0259] As shown in FIGS. 6b and 6c, the results showed that the tumor growth in mSA42PD1-p24e-vaccinated mice was inhibited up to 17 days compared to p24e, and PBS control, showing that mSA42PD1-p24e vaccination conferred protective immunity against tumor growth systematically.

[0260] Furthermore, the protection of vaccinated mice against virus infection was assessed. Briefly, mSA42PD1-p24e, p24e, and PBS vaccinated mice were challenged (at three weeks post-vaccination) by either vaccinia virus strain TianTan (WRgagpol) (for 20 µg dose vaccinated mice) or virulent strain Western Reserve (WRgagpol) (for 100 µg dose vaccinated mice).

[0261] Significantly less virus titer was found in lung homogenates of mSA42PD1-p24e group compared to p24e, or placebo groups (FIGS. 6d, 6e), and significantly reduced body weight loss (FIG. 6f). The results show the immunogenic advantage of mSA42PD1-p24e, in eliciting p24-specific protective immunity.
Example 7

Mouse Immunization and Cell Fusion to Generate Anti-Human Δ42PD1 mAbs

[0262] When human sΔ42PD1-p24 was used to immunize mice, a strong antibody response was induced against the Δ42PD1 protein. This response does not significantly cross-react with human PD1 (FIG. 8), which is similar to that PDI-specific monoclonal antibody does not cross-react with Δ42PD1 (FIG. 2c). It is demonstrated that it is feasible to generate a monoclonal antibody specific to Δ42PD1 in animals. The key point is that anti-Δ42PD1 specific antibody can only be elicited using Δ42PD1 or soluble Δ42PD1 as immunogen, which is one of the key inventions of this patent application.

[0263] To elicit human Δ42PD1 specific antibody response, a DNA prime/protein boost immunization regimen was utilized. Briefly, mice were immunized with sΔ42pd1g plasmids, which fused expression the extracellular domain of human Δ42PD1 (soluble Δ42PD1 (sΔ42PD1)) and rabbit IgG1 Fc region, at weeks 0 and 3 by intramuscular injection plus electroporation, followed by two additional subcutaneous injections of purified recombinant sΔ42PD1Fc protein in three-week intervals (FIG. 19A). One week after the second protein boost (week 10), serum samples were collected for analysis for the presence of antibodies recognizing soluble Δ42PD1 protein. Serial three-fold dilution starting from 1/100 of sera were assessed in an indirect ELISA using immobilized sΔ42PD1His protein purified from the supernatants of the 293T cells, and IgG anti-sΔ42PD1 titers were measured. Similar levels of antibody titers were observed in serum samples from mice #1-4, and serum antibody titer of mouse #5 was approximately 2-fold higher (FIG. 19B).

[0264] Before performing cell fusion assay for hybridoma generation, the recognition bias of serum samples from inoculated mice to human PD1 or Δ42PD1 was assessed. Firstly, the binding activity of serum samples to sΔ42PD1His and sPD1His proteins was determined by ELISA. Both proteins were bound with all five serum samples. However, the optical density at 450 nm (O.D.450 nm) value was at least 2-fold higher for sΔ42PD1His than PD1His (FIG. 19C). Secondly, it was found that the cell count was significantly increased in the presence of Δ42PD1 and PD1 on cell surface using 293T, 293T-Δ42PD1, and 293T-PD1 cell lines. The mean fluorescence intensities (MFI) of serum samples interacting with the three cell lines were used to generate the scatter plot shown in FIG. 19D. As expected, antibodies in serum samples did not engage with 293T cells, however bound with high affinity to Δ42PD1 expressed on 293T cell surface, with relatively lower affinity to cell surface PD1, and this difference is statistically significant (p=0.0043). Together, these data demonstrated that human Δ42PD1 is immunogenically different from PD1 and that antibodies generated in Δ42PD1 immunized mice have a strong bias toward Δ42PD1 recognition compared to PD1 recognition.

[0265] Since serum from the #4 mouse possesses the strongest bias toward soluble Δ42PD1 and cell surface-expressed natural Δ42PD1 compared with soluble PD1 and cell surface PD1. Therefore, the #4 mouse was immunized a final time at week 12 and sacrificed at week 13 to harvest spleen cells for fusion with SP2/0-Ag14 myeloma cells. Since high affinity mAbs were desired, hybridoma culture supernatants were screened for Δ42PD1-specific IgG but not IgM or IgA based on their ability to bind to sΔ42PD1His immobilized in micro-
titer plates in indirect ELISA. Subsequently, two hybridoma cell lines (clone CH34 and CH100) secreting Δ42PD1 highly reactive mAbs were identified (FIG. 19E).

Example 8

Anti-Human Δ42PD1 Mabs do not Cross-React with PD1

[0266] To evaluate cross-reactivity of mAbs induced by Δ42PD1 to PD1, binding of anti-Δ42PD1 mAbs to cell surface expressed human Δ42PD1 and human PD1 was flow cytometrically analyzed using 293T, 293T-Δ42PD1 and 293T-PD1 cell lines. Both anti-Δ42PD1 mAbs (clone CH34 and CH100) specifically recognized human Δ42PD1 without cross-reacting to human PD1 (FIG. 20A) (FIG. 8). Second, the applicability and specificity of anti-Δ42PD1 mAbs in Western blot was explored. Besides human PD1 and Δ42PD1, mouse Δ42PD1 was also considered which exhibits approximately 64% amino acid sequence homology with human sΔ42PD1, notwithstanding that mouse Δ42PD1 isoform have not been discovered yet. 293T cells were transiently transfected with human Δ42pd1, human pd1 and mouse sΔ42pd1g, respectively. Two days post-transfection, cells were lyzed and whole cell lysates were prepared for Western blot. As shown in FIG. 20B, the two anti-Δ42PD1 mAbs (clone CH34 and CH100) recognized denatured human PD1 and Δ42PD1 but not mouse Δ42PD1. Both anti-Δ42PD1 mAbs could flow cytometrically distinguish human Δ42PD1 from PD1, auguring a crucial role in future functional research on human Δ42PD1. Therefore, isotypes and avidity were identified of the anti-Δ42PD1 mAbs to facilitate future utilization using rapid ELISA mouse mAb isotyping kit (37503, Pierce Biotechnology) and surface plasmon resonance, respectively. As shown in FIG. 20C and FIG. 23, isotype of clone CH34 is IgG2b/Kappa and clone CH101 turn out to be IgG1/Kappa. Collectively, these data demonstrated that both anti-human Δ42PD1 mAbs specifically engage with cell surface human Δ42PD1 but not human PD1 in flow cytometry, interestingly recognize both human Δ42PD1 and PD1 but not mouse Δ42PD1 in Western blot.

Example 9

Raised Plasma SA42PD1 in HIV Infection

[0267] Chronic immune activation is a characteristic feature of progressive HIV disease. Indeed, polyclonal B-cell activation was one of the first described immunological abnormalities in HIV-infected individuals. Subsequently, increased T-cell turnover, increased frequencies of T cells with an activated phenotype, and increased serum levels of proinflammatory cytokines and chemokines were observed. Notably, the degree of immune activation is a better predictor of disease progression than plasma viral load. However, the underlying causes of immune activation have remained elusive.

[0268] SA42PD1 could induce production of proinflammatory cytokines in vivo, which could lead to immune activation. To explore whether SA42PD1 plays a role in HIV progression, we determined SA42PD1 level in HIV+ (n=11) and HIV− (n=21) plasma using DAS-ELISA. As shown in FIG. 22, SA42PD1 level in HIV+ plasma is significantly higher than HIV− plasma.

[0269] This result indicated that SA42PD1 plays a role in HIV infection and progression. Besides HIV infection, some
other viral infection and autoimmune diseases also featured as immune activation. So it is very important to determine the plasma level of sΔ42PDI in these patients.

Example 10

Augmentation of Membrane-Bound Δ42PDI
Signaling by Specific MAb

[0270] Engagement of PDI by its ligands triggers transduction of inhibitory signal which could inhibit PI3K-Akt signal pathway. PBMCs express ligands of PDI and also unknown ligand(s) of Δ42PDI, which is confirmed by a proinflammatory cytokine release response to Δ42PDI treatment. So we attempted to trigger PDI and Δ42PDI signaling by mixing human PBMCs and human PDI or Δ42PDI expressing 293T cells with different ratio to determine whether Δ42PDI which possesses exactly the same intracellular region with PDI could also transduce inhibitory signal while bound by its unknown ligand(s). As expected, treatment with PBMCs significantly decreased the phosphorylation of Akt in 293T-PDI cells but not in 293T cells, compared with untreated cells. Similarly, the level of phosphorylated Akt in 293T-Δ42PDI cells was significantly lower upon PBMC stimulation (FIG. 24). These results strongly suggest a negatively immune regulatory function of membrane-bound Δ42PDI, although no direct evidence of the inhibitory effect has been obtained yet.

To determine whether monoclonal antibody CH34 and CH101 have agonist or antagonist activities, 293T-Δ42PDI cells were treated with Δ42PDI specific mAbs or isotype matched controls. Levels of phosphorylated Akt in 293T-Δ42PDI cells were detected subsequently or followed by mixing with PBMCs. As shown in FIG. 25, no blocking effect of Δ42PDI specific mAbs on attenuation of Akt phosphorylation in 293T-Δ42PDI cells triggered by PBMC were observed, indicating non antagonist activities of CH34 and CH101 on Δ42PDI signaling. Unexpectedly, anti-Δ42PDI mAb clone CH101 synergistically decrease p-AKT intensity induced by unknown Δ42PDI ligand(s) expressed on PBMCs. These results suggested that membrane-bound Δ42PDI could functionally transduce inhibitory signal through cytoplasmic region, and play a role in immune system.

[0271] Given that both Δ42PDI and its unknown ligand(s) expressed among PBMCs, mAb CH101 probably behaves as a Δ42PDI agonist in vivo and potentially contribute to autoimmune disease treatment.

Example 11

Blockage of Δ42PDI Binding to its Receptor by Specific mAb

[0272] To determine if Δ42PDI specific monoclonal antibody could block the engagement of Δ42PDI with its unknown receptor(s), we mixed sΔ42PDI15 recombinant protein with various doses of CH34, CH101 and isotype matched mouse derived control monoclonal antibodies, and then used the mixture to incubate THP-1 cells, followed by staining with fluorescent labeled antibody to detect the binding of sΔ42PDI15 recombinant proteins to THP-1 cells. mAb CH101 blocked the binding of Δ42PDI to its unknown receptor on THP-1 with a dose dependent pattern. On the contrary, mAb CH34 did not block the binding of Δ42PDI to its receptor (FIG. 26).

[0273] sΔ42PDI could induce production of proinflammatory cytokines in vivo, which play a key role in autoimmune disorders. The blockage of the binding of sΔ42PDI to its receptor by specific monoclonal antibody is one potential way to treat these autoimmune disease.

Example 12

Double-Monoclonal Antibody Sandwich Indirect ELISA for sΔ42PDI Detection

[0274] PDI has a soluble form which interferes with physiological functions of PDI: PDI-Ls axis and leads to autoimmune disease. It is highly possible that soluble form of Δ42PDI also exists and plays a role in a particular ailment. Therefore we wanted to develop a double-monoclonal antibody sandwich indirect enzyme-linked immunosorbent assay (DAS-ELISA) based on Δ42PDI specific mAbs (clone CH34 and CH101) for the assessment of s42PDI concentrations in human body fluid. The top concern for the development of sΔ42PDI-detecting DAS-ELISA system is whether sPDI could also be detected, considering the fact that both CH34 and CH101 recognize human PDI by Western blot. So we tested the DAS-ELISA system using commercially available recombinant human sPDI15 and home-made recombinant sΔ42PDI15. As shown in FIG. 27, antibody CH34 and CH101 based DAS-ELISA system could detect ultra-trace level of human sΔ42PDI but not sPDI.

Example 13

Binding of mAb to Δ42PDI Fragments

[0275] For mapping the epitope of Δ42PDI specific mAbs, 6 fragments (Δ42PDI F 1–6) of Δ42PDI (FIG. 28A) were displayed on the surface yeast cells (Y-F1 to Y-F6). The binding of CH34 and CH101 to the Δ42PDI fragments were analyzed by flow cytometry. As shown in FIG. 28C, mAb could bind the Δ42PDI F2 but not other fragments. However, CH34 failed to bind any of the 6 fragments of Δ42PDI.

[0276] All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

[0277] 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. In addition, any elements or limitations of any invention or embodiment thereof disclosed herein can be combined with any and/or all other elements or limitations (individually or in any combination) or any other invention or embodiment thereof disclosed herein, and all such combinations are contemplated with the scope of the invention without limitation thereto.

REFERENCES


[0364] Ueta, C., Kawasumi, H., Fujitawa, H., Miyagawa, T., Kida, H., Ohmoto, Y., Kishimoto,


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Phe Val Leu Asn Trp Tyr Arg Met Ser Pro Ser Asn Gln Thr Asp Lys 50    55    60
    
Leu Ala Ala Phe Pro Gln Asp Arg Ser Gln Pro Gly Gln 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 Gln Ser Gln Thr Cys Leu 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 Phe Glu Thr Leu Val Val Gly Val 145    150    155    160
Gly Gly Leu Leu Gly Ser Leu Val Leu Val Trp Leu Ala Val 165    170    175
    
Ile Cys Ser Arg Ala Arg Arg Gly Thr Ile Gly Ala Arg Arg Thr Gly 180    185    190
Gln Pro Leu Lys Glu Asp Pro Ser Ala Val Pro Val Phe Ser Val Asp 195    200    205
    
Tyr Gly Leu Asp Phe Glu Trp Arg Glu Lys Thr Pro Glu Pro Pro 210    215    220
Val Pro Cys Val Pro Gln Thr Glu Tyr Ala Thr Ile Val Phe Pro 225    230    235    240
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Am Ala Thr Phe Thr Cys Ser Phe Ser Asn Thr Ser Glu Ser Phe Val
50 55 60
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Ala Phe Pro Glu Asp Arg Ser Gln Pro Gly Gln Asp Cys Arg Phe Arg
85 90 95
Val Thr Gln Leu Pro Asn Gly Arg Asp 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
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165 170 175
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180 185 190
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195 200 205
Leu Lys Glu Asp Pro Ser Ala Val Pro Val Phe Ser Val Asp Tyr Gly
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Glu Leu Asp Phe Gln Trp Arg Glu Lys Thr Pro Glu Pro Pro Val Pro
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SEQ ID NO 24
LENGTH: 867
TYPE: PRO
ORIGIN: Homo sapiens

SEQUENCE: 24

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<211> LENGTH: 868
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

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Cys Thr Gly Gly Cys Thr Gly Gly Cys Gly Cys Cys Cys Ala 50 55 60
Gly Ala Thr Gly Thr Thr Thr Thr Cys Thr Gls Cys Cys Cys 65 70 75 80
Gly Cys Thr Cys Gly Thr Gly Gly Ala Cys Cys Cys Gla Ala 85 90 95
Gly Gly Gly Ala Ala Cys Ala Cys Cys Cys Ala Cys Cys Thr 100 105 110
Thr Cys Ala Cys Cys Thr Gly Cys Ala Gly Thr Cys Thr Cys 115 120 125
Cys Ala Ala Cys Ala Cys Thr Cys Gly Gly Ala Gly Ala Gly Cys 130 135 140
Thr Thr Cys Gly Thr Gly Cys Thr Ala Ala Ala Cys Thr Gly Gly 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 Ala Gly Ala Cys Gly Gly Ala Cys Ala Gly 180 185 190
Cys Thr Gly Gly Cys Cys Gly Cys Cys Thr Cys Cys Cys Cys Cys Gly  
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355  360  365  
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370  375  380  
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385  390  395  400  
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405  410  415  
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420  425  430  
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<212> TYPE: PRT  
<213> ORGANISM: Homo sapiens  
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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 Gly 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
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Phe Arg Val Thr Gln Leu Pro Asn Gly Arg Asp Phe His Met Ser Val
85 90 95
Val Arg Ala Arg Arg Arg Asp Ser 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 Arg Ala Glu Val Pro Thr Ala His Pro Ser Pro
130 135 140
Ser Pro Arg Pro Ala Gly Gln Phe Gln Thr Leu Val
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<210> SEQ ID NO: 27
<211> LENGTH: 1176
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 27

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35 40 45
Cys Thr Gly Gly Cys Thr Gly Gly Cys Gly Cys Ala Gly Cys
50 55 60
Gly Ala Thr Gly Gly Thr Cys Thr Thr Ala Gly Cys Cys Thr
65 70 75 80
Gly Cys Thr Cys Gly Thr Gly Thr Gly Ala Cys Cys Gly Ala
85 90
Gly Gly Gly Ala Cys Ala Ala Cys Gly 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 Ala Gly Ala Gly Cys
130 135 140
Thr Thr Cys Gly Thr Gly Cys Thr Ala Ala Ala Cys Thr Gly Gly
145 150 155 160
 Ala Cys Cys Gly Ala Thr Gly Ala Gly Cys Cys Cys Ala Gly
165 170 175
Cys Ala Ala Cys Ala Gly Ala Cys Gly Cys Ala Gly Ala Gly
180 185 190
Cys Thr Gly Gly Cys Gly Cys Thr Cys Thr Cys Cys Cys Gly
195 200 205
Ala Gly Gly Ala Cys Gly Cys Ala Gly Cys Cys Ala Gly Cys Cys
210 215 220
Cys Gly Gly Cys Ala Gly Gly Ala Cys Thr Cys Gly Cys Cys
225 230 235 240
Thr Thr Cys Gly Thr Gly Thr Ala Cys Ala Cys Ala Ala Cys
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260 265 270
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Cys Cys Ala Cys Ala Ala Cys Ala Gly Gly Cys Ala Cys Thr Cys 805 810 815
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Thr Ala Ala 1175

<210> SEQ ID NO 28
<211> LENGTH: 391
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<400> SEQUENCE: 28

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Gly Asp Asn Ala Thr Phe Thr Cys Ser Ser Asn Thr Ser Glu Ser 35 40 45

Phe Val Leu Asn Trp Tyr Arg Met Ser Pro Ser Asn Gln Thr Aep 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 Gln Leu Pro Asn Gly Arg Asp Phe His Met Ser Val 85 90 95

Val Arg Ala Arg Arg Asp 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 Arg Ala Glu Val Pro Thr Ala His Pro Ser Pro 130 135 140

Ser Pro Arg Pro Ala Gln Glu Leu Glu Ala Thr Asn Thr Lys Val Asp 145 150 155 160

Lys Thr Val Ala Pro Ser Thr Cys Ser Lys Pro Met Cys Pro Pro Pro 165 170 175

Glu Leu Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys Lys Pro Lys 180 185 190

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val 195 200 205

Asp Val Ser Glu Asp Arg Pro Glu Val Glu Phe Thr Trp Tyr Ile Ser 210 215 220

Asn Glu Gln Val Arg Thr Ala Arg Pro Leu Arg Glu Gln Gln Phe 225 230 235 240

Asn Ser Thr Ile Arg Val Val Ser Thr Leu Pro Ile Ala His Gln Asp 245 250 255
Trp Leu Arg Gly Lys Glu Glu Lys Cys Lys Val His Asn Lys Ala Leu 260 265 270

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Arg Gly Gin Pro Leu 275 280 285

Glu Pro Lys Val Tyr Thr Met Gly Pro Pro Arg Glu Glu Ser Ser Leu 290 295 300

Arg Ser Val Ser Leu Thr Cys Met Ile Asn Gly Phe Tyr Pro Ser Asp 305 310 315 320

Ile Ser Val Glu Trp Glu Lys Asn Gly Lys Ala Glu Asp Asn Tyr Lys 325 330 335

Thr Thr Pro Thr Val Leu Asp Ser Asp Gly Ser Tyr Phe Leu Tyr Ser 340 345 350

Lys Leu Ser Val Pro Thr Glu Trp Gin Arg Gly Asp Val Phe Thr 355 360 365

Cys Ser Val Met His Gin Ala Leu His Asn His Tyr Thr Gin Lys Ser 370 375 380

Ile Ser His Ser Pro Gly Lys 385 390

<210> SEQ ID NO 29
<211> LENGTH: 317
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 29

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 Ala 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 Met Ser Pro Ser Asn Gin Thr Asp Lys 50 55 60

Leu Ala Ala Phe Pro Gin Asp Arg Ser Gin Pro Gly Gin Asp Cys Arg 65 70 75 80

Phe Arg Val Thr Gin Leu Pro Asn Gly Arg Asp Phe His Met Ser Val 85 90 95

Val Arg Ala Arg Arg Asp Ser Gly Thr Tyr Leu Cys Gly Ala Ile 100 105 110

Ser Leu Ala Pro Lys Ala Gin Ile Lys Gin 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 Gin His His His His His 145 150 155

<210> SEQ ID NO 30
<211> LENGTH: 30
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 30

Leu Leu Val Val Thr Glu Gly Asp Asn Ala Thr Phe Thr Cys Ser Phe 1 5 10 15
Ser Asn Thr Ser Glu Ser Phe Val Leu Asn Trp Tyr Arg Met
     20   25   30

<210> SEQ ID NO 31
<211> LENGTH: 30
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 31

Glu Ser Phe Val Leu Asn Trp Tyr Arg Met Ser Pro Ser Asn Gln Thr
     1    5    10   15
Amp Lys Leu Ala Ala Phe Pro Glu Asp Arg Ser Gln Pro Gly
     20   25   30

<210> SEQ ID NO 32
<211> LENGTH: 30
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 32

Ala Phe Pro Glu Asp Arg Ser Gln Pro Gly Gln Asp Cys Arg Phe Arg
     1    5   10   15
Val Thr Gln Leu Pro Asn Gly Arg Asp Phe His Met Ser Val
     20   25   30

<210> SEQ ID NO 33
<211> LENGTH: 30
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 33

Pro Asn Gly Arg Asp Phe His Met Ser Val Val Arg Ala Arg Arg Asn
     1    5   10   15
Asp Ser Gly Thr Tyr Leu Cys Gly Ala Ile Ser Leu Ala Pro
     20   25   30

<210> SEQ ID NO 34
<211> LENGTH: 30
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 34

Tyr Leu Cys Gly Ala Ile Ser Leu Ala Pro Lys Ala Gln Ile Lys Glu
     1    5   10   15
Ser Leu Arg Ala Glu Leu Arg Val Thr Glu Arg Arg Ala Glu
     20   25   30

<210> SEQ ID NO 35
<211> LENGTH: 30
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 35

Glu Leu Arg Val Thr Glu Arg Arg Ala Glu Val Pro Thr Ala His Pro
     1    5    10   15
Ser Pro Ser Pro Arg Pro Ala Gly Gin Phe Gin Thr Leu Val
     20   25   30
We claim:
1. An isolated PD1 protein isoform having a deletion of 14 amino acids at positions corresponding to amino acids 26-39 of the wild-type PD1 protein having an amino acid sequence of SEQ ID NO:2, or a fragment of the PD1 protein isoform wherein the fragment has at least 30 amino acids and wherein the fragment has said deletion of 14 amino acids at positions corresponding to amino acids 26-39 of the wild-type PD1 protein having an amino acid sequence of SEQ ID NO:2.
2. The PD1 protein isoform of claim 1, which does not bind to PD-L1 or PD-L2.
3. The PD1 protein isoform of claim 1, wherein the 14 amino acids deleted from the wild-type PD1 protein are DSP-DRPWNPTFFIP (SEQ ID NO:3).
4. The PD1 protein isoform of claim 1, comprising SEQ ID NO:1 or a fragment thereof comprising at least 30 consecutive amino acids of SEQ ID NO:1.
5. A nucleic acid molecule encoding:
a PD1 protein isoform having a deletion of 14 amino acids at positions corresponding to amino acids 26-39 of the wild-type PD1 protein having an amino acid sequence of SEQ ID NO:2; or
a fragment of the PD1 protein isoform wherein the fragment has at least 30 amino acids, wherein the fragment has said deletion of 14 amino acids at positions corresponding to amino acids 26-39 of the wild-type PD1 protein having an amino acid sequence of SEQ ID NO:2.
6. The nucleic acid molecule of claim 5, wherein the nucleic acid molecule is a cDNA molecule.
7. An antibody that binds specifically to the PD1 protein isoform of claim 1.
8. The antibody of claim 7, where the antibody cannot bind to wild type PD1.
9. A fusion protein comprising:
a PD1 protein isoform having a deletion of 14 amino acids at positions corresponding to amino acids 26-39 of the wild-type PD1 protein having an amino acid sequence of SEQ ID NO:2; or
a fragment of the PD1 protein isoform wherein the fragment has at least 30 amino acids, wherein the fragment has said deletion of 14 amino acids at positions corresponding to amino acids 26-39 of the wild-type PD1 protein having an amino acid sequence of SEQ ID NO:2.
10. The fusion protein of claim 9, further comprising an antigen protein or peptide.
11. A fusion nucleic acid molecule comprising a nucleic acid molecule of claim 5 and a nucleic acid molecule encoding an antigen or peptide.
14. A method of inducing the production of TNF-α, IL-1, or IL-6, comprising administering to an immune cell a nucleic acid molecule of claim 5.
15. A method of inducing the production of TNF-α, IL-1, or IL-6, comprising administering to an immune cell a fusion nucleic acid molecule of claim 11.
16. A method of inducing the production of TNF-α, IL-1, or IL-6, comprising administering to an immune cell a fusion protein of claim 9.
17. A method of preventing pathogenic infection or cancer, comprising administering to a subject in need of such prevention a nucleic acid molecule of claim 5.
18. A method of preventing pathogenic infection or cancer, comprising administering to a subject in need of such prevention a fusion nucleic acid molecule of claim 11.
19. A method of preventing pathogenic infection or cancer, comprising administering to a subject in need of such prevention a fusion protein of claim 9.
20. An immunogen that generates an antibody of claim 7.

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