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(54) Title: DEPDC1 EPITOPE PEPTIDES FOR TH1 CELLS AND VACCINES CONTAINING THE SAME

(57) Abstract: Isolated DEPDC1-derived epitope peptides having Th1 cell inducibility are disclosed herein. Such peptides can be recognized by MHC class II molecules and induce Th1 cells. In preferred embodiments, such a peptide of the present invention can promiscuously bind to MHC class II molecules and induce DEPDC1-specific CTLs in addition to Th1 cells. Such peptides are thus suitable for use in enhancing immune response in a subject, and accordingly find use in cancer immunotherapy, in particular, as cancer vaccines. Also disclosed herein are polynucleotides that encode any of the aforementioned peptides, APCs and Th1 cells induced by such peptides and methods of induction associated therewith. Pharmaceutical compositions that comprise any of the aforementioned components as active ingredients find use in the treatment and/or prevention of cancers or tumors including, for example, bladder cancer and breast cancer.

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Description

Title of Invention: DEPDC1 EPITOPE PEPTIDES FOR TH1 CELLS AND VACCINES CONTAINING THE SAME

Technical Field

[0001] The present invention relates to the field of biological science, more specifically to the field of cancer therapy. In particular, the present invention relates to novel peptides that are extremely effective as cancer vaccines, and drugs for either or both of treating and preventing tumors.

Priority

The present application claims the benefit of Japanese Patent Application No. JP 2016-224624, filed on Novemver 18, 2016, the entire contents of which are incorporated by reference herein.

Background Art

[0002] CD8 positive cytotoxic T lymphocytes (CTLs) have been shown to recognize epitope peptides derived from the tumor-associated antigens (TAAs) found on the major histocompatibility complex (MHC) class I molecule, and then kill the tumor cells. Since the discovery of the melanoma antigen (MAGE) family as the first example of TAAs, many other TAAs have been discovered, primarily through immunological approaches (NPL 1, 2). Some of these TAAs are currently undergoing clinical development as immunotherapeutic targets.

[0003] TAAs which are indispensable for proliferation and survival of cancer cells are valiant as targets for immunotherapy, because the use of such TAAs may minimize the well-described risk of immune escape of cancer cells attributable to deletion, mutation, or down-regulation of TAAs as a consequence of therapeutically driven immune selection. Accordingly, the identification of new TAAs capable of inducing potent and specific anti-tumor immune responses, warrants further development. Currently, the clinical application of peptide vaccination strategies for various types of cancer is ongoing (NPL 3-10). To date, there have been several reports of clinical trials using these TAA derived peptides. Unfortunately, so far these cancer vaccine trials have yielded only a low objective response rate (NPL 11-13). Accordingly, there remains a need in the art for new TAAs suitable for use as immunotherapeutic targets.

[0004] Recently, the present inventors have identified a cancer-testis antigen, DEP domain containing 1 (DEPDC1), that is frequently overexpressed in bladder cancer, breast cancer and several other malignancies using genome-wide cDNA microarray analysis (NPL 14-17). The growth of bladder cancer cells is significantly inhibited by knockdown of DEPDC1 expression using small interfering RNAs; thus, DEPDC1 are

essential for tumor growth and survival, indicating the clinical significance of these antigens as targets for antigen-specific cancer immunotherapy. The present inventors have also identified highly immunogenic DEPDC1-derived short peptides (SPs) that can induce HLA-A24 (A*24:02)-restricted CTLs from peripheral blood mononuclear cells (PBMCs) of bladder cancer patients (PTL 1, NPL 17). In a phase I/ II clinical trial of combination immunotherapy of intravesical BCG inoculation and DEPDC1-derived SPs vaccine demonstrated the promising clinical effect on recurrence prevention for non-muscle invasive bladder cancer as well as safety and good immunogenicity to induce CTLs (NPL 17). Therefore, DEPDC1 is an attractive target molecule for cancer immunotherapy.

[0005] Tumor-specific $CD4^+$ helper T (Th) cells, especially T-helper type 1 (Th1) cells play a critical role in efficient induction of CTL-mediated antitumor immunity (NPL 18). Interferon (IFN)-gamma and tumor necrosis factor (TNF)-alpha primarily produced by Th1 cells is critical for induction and maintenance of long-lived CTL responses, providing help through multiple interactions which are critical in the preservation of immunological memory (NPL19, 20, 21). The IFN-gamma secreted by Th1 cells also mediates direct anti-tumor or anti-angiogenic effect (NPL 22). Furthermore, it has been shown that Th cells must pave the way for entry of CTLs at tumor site (NPL 23). Melief et al. recently reported that human papillomavirus-derived synthetic LPs naturally include CTL epitopes as an attractive vaccine compound for the treatment of vulvar and cervical cancers (NPL 24). Following the injection of LPs, patient's DCs take up LPs, process them, and present possible CTL and Th-cell epitopes in the context of the patient's HLA class I and class II molecules, respectively (NPL 27). In addition, recent clinical studies using a promiscuous telomerase-derived helper-epitope vaccine called GV1001 increased survival of cancer patients when combined with radiotherapy and chemotherapy. (NPL 25, 26). Therefore, identification of TAA-derived Th cell epitopes that can activate specific Th1 cell is important for induction of an effective tumor immunity in tumor-bearing hosts; ideally, the design of effective vaccines should include multiple epitopes to stimulate both CTL and Th1 cells (NPL 24). However, no such epitope derived from DEPDC1 has yet been identified.

Citation List

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Summary of Invention

[0008] Bladder cancer is the most common malignancy involving the genitourinary system. Platinum-based chemotherapy remains the standard of care for first-line treatment of advanced/metastatic urothelial carcinoma (UC) (van der Maase H, et al. J Clin Oncol 1996;156(9):3308-14; Gupta S, et al., Cancers 2017;9(2):pii:E15). There is an estimated overall survival of 9-15 months in metastatic bladder cancer in those who receive the standard treatment with platinum-based chemotherapy; stage IV bladder cancer has a relative 5-year survival rate of about 15% (van der Maase H, et al., supra; De Sanis M, et al., J Clin Oncol 2012;30(2):191-9). Until recently, there were few treatment options for patients who were refractory to chemotherapy or did not tolerate chemotherapy due to serious adverse reactions, and overall outcomes remained very poor (Gupta S, et al., supra). Currently, systemic immunotherapy using programmed cell death protein 1 (PD-1)- and PD-1 ligand (PD-L1)-specific monoclonal antibodies,

i.e., Nivolumab/Pembrolizumab and Atezolizumab, respectively, has been approved for the treatment of urothelial bladder cancer to achieve significant improvement in response rates (Gupta S, et al., supra; Zichi C, et al., Biomed Res Int 2017;2017(561874. Doi:10.1155/2017/5618174)). Another recent clinical trial showed that DEPDC1-derived SP vaccines administered to patients with progressive UC was safe and well tolerated and efficiently activated CTLs (Obara W, et al., Ann Oncol 2017;28(4):798-803).

[0009] In the context of the present invention, the present inventors considered an ideal peptide vaccine for cancer immunotherapy to be one that includes a single polypeptide containing epitopes for both CTL and Th1 cell, both of which are naturally proximal to each other.

[0010] To that end, the present inventors designed a strategy to identify novel DEPDC1-derived Th1 cell epitopes that can be recognized in the context of promiscuous HLA class II molecules and contain CTL epitopes, working on the presumption that epitopes so characterized would induce more efficient T cell-mediated tumor immunity. A computer algorithm predicting HLA class II-binding peptides and known CTL epitope sequences recognized by HLA-A24 (A*24:02) or HLA-A2 (A*02:01)-restricted CTLs was used to select candidate promiscuous HLA-class II-restricted Th1 cell epitopes containing CTL epitopes.

[0011] The present invention is based, at least in part, on the discovery of suitable epitope peptides that serve as targets of immunotherapy for inducing Th1 cell response. Recognizing that the DEPDC1 gene is up-regulated in a number of cancer types, including bladder cancer and breast cancer, the present invention targets for further analysis the gene product of DEPDC1 gene, more particularly the polypeptide exemplary set forth in SEQ ID NO: 8 or 10, which is encoded by the gene of GenBank Accession No. NM_001114120.2 (SEQ ID NO: 7) or GenBank Accesssion No. NM_017779.5 (SEQ ID NO: 9). DEPDC1 gene products containing epitope peptides that elicit Th1 cells specific to the corresponding molecule were particularly selected for further study. For example, peripheral blood mononuclear cells (PBMCs) obtained from a healthy donor were stimulated using promiscuous HLA-DRs and/or DPs binding peptide derived from human DEPDC1. Th1 cells that recognize HLA-DRs or DPs positive target cells pulsed with the respective candidate peptides were established, and HLA-DRs and/or DPs restricted epitope peptides that can induce potent and specific immune responses against DEPDC1 were identified. These results demonstrate that DEPDC1 is strongly immunogenic and the epitopes thereof are effective for tumor immunotherapy mediated through Th1 cell response. Additional studies revealed that the promiscuous HLA-DRs and/or DPs binding peptides containing at least one CTL epitope can also stimulate CTL response in the same donor in a DEPDC1 specific manner. These

results confirm that DEPDC1 is strongly immunogenic and that DEPDC1-derived peptides containing both Th1 cell and CTL epitopes are effective for tumor immunotherapy mediated through both Th1 cell and CTL responses.

[0012] It is therefore an object of the present invention to provide peptides having Th1 cell inducibility as well as an amino acid sequence selected from among SEQ ID NOs: 1 to 4. The present invention contemplates modified peptides, i.e., peptides having Th1 cell inducibility that are up to 30 amino acids in length and have a contiguous amino acid sequence selected from the amino acid sequence of SEQ ID NO: 8 or 10 (DEPDC1), as well as functional equivalents thereof. Alternatively, the present invention also provides peptides having both Th1 cell inducibility and CTL inducibility. In some embodiments, the peptides of the present invention correspond to the amino acid sequences of SEQ ID NOs: 1 to 4 or modified versions thereof, in which one, two or several amino acids are substituted, deleted, inserted and/or added, while the ability to induce Th1 cells is maintained.

[0013] When administered to a subject, the present peptides are preferably presented on the surface of one or more antigen-presenting cells (APCs) that in turn induce Th1 cells. When the peptide of the present invention further contains at least one CTL epitope, such APCs also process the peptides to present CTL epitopes generated from the present peptides, and thus induce CTLs targeting the respective peptides. Therefore, it is a further object of the present invention to provide APCs presenting any of the present peptides or fragments thereof, as well as methods for inducing APCs.

[0014] Administration of one or more peptides of the present invention or polynucleotide(s) encoding such peptides, or APCs which present such peptides or fragments thereof results in the induction of a strong anti-tumor immune response. Accordingly, it is yet another object of the present invention to provide pharmaceutical agents or compositions that contain as active ingredient(s) one or more of the following:

(a) one or more peptides of the present invention,

(b) one or more polynucleotides encoding such peptide(s), and

(c) one or more APCs of the present invention.

Such pharmaceutical agents or compositions of the present invention find particular utility as vaccines.

[0015] It is yet a further object of the present invention to provide methods for the treatment and/or prophylaxis (i.e., prevention) of cancers (i.e., tumors), and/or prevention of postoperative recurrence thereof. Methods for inducing Th1 cells or for inducing anti-tumor immunity that include the step of administering one or more peptides, polynucleotides, APCs or pharmaceutical agents or compositions of the present invention are also contemplated. Furthermore, the Th1 cells of the present invention also find use as vaccines against cancer, examples of which include, but are not limited to, bladder

cancer and breast cancer.

[0016] Examples of specifically contemplated objects of the present invention include the following:

[1] An isolated peptide having 10-30 amino acids in length and comprising a part of the amino acid sequence of SEQ ID NO: 8 or 10, wherein said peptide comprises an amino acid sequence selected from the group consisting of:

(a) a contiguous amino acid sequence having more than 9 amino acids in length selected from the amino acid sequence of SEQ ID NO: 1, 2, 3 or 4; and

(b) an amino acid sequence in which one, two or several amino acids are substituted, deleted, inserted, and/or added in the amino acid sequence of (a),

wherein said peptide has ability to induceTh1 cells.

[2] The isolated peptide of [1], wherein the peptide or fragment thereof has abilities to bind to at least two kinds of MHC class II molecules.

[3] The isolated peptide of [2], wherein the MHC class II molecules are selected from the group consisting of HLA-DR53, HLA-DR4, HLA-DR15 and HLA-DP5.

[4] The isolated peptide of any one of [1] to [3], wherein said peptide comprises an amino acid sequence of a peptide having DEPDC1-specific CTL inducibility.

[5] The isolated peptide of [4], wherein said peptide comprises the amino acid sequence selected from the group consisting of:

(a) an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 to 4; and

(b) an amino acid sequence in which one, two or several amino acids are substituted, deleted, inserted, and/or added in the amino acid sequence of (a).

[6] An isolated polynucleotide encoding the peptide of any one of [1] to [5].

[7] A composition for inducing at least one of the cells selected from the group consisting of

(i) Th1 cells,

(ii) CTLs,

(iii) APCs having an ability to induce Th1 cells, and

(iv) APCs having an ability to induce CTLs,

wherein the composition comprises one or more peptide(s) of any one of [1] to [5], or one or more polynucleotide(s) encoding them, or a composition for inducing at least one type of cell selected from the group consisting of:

(i) Th1 cells,

(ii) CTLs,

(iii) APCs having an ability to induce Th1 cells, and

(iv) APCs having an ability to induce CTLs,

wherein the composition comprises one or more peptide(s) of any one of [1] to [5], or

one or more polynucleotide(s) encoding them.

[8] A pharmaceutical composition, wherein the composition comprises at least one active ingredient selected from the group consisting of:

(a) one or more peptide(s) of any one of [1] to [5];

(b) one or more polynucleotide(s) of [6];

(c) one or more APC(s) presenting the peptide of any one of [1] to [5] or fragment thereof on their surface;

(d) one or more Th1 cells that recognize(s) an APC presenting the peptide of any one of [1] to [5] or fragment thereof on its surface; and

(e) combination of any two or more of (a) to (d) above; and is formulated for a purpose selected from the group consisting of:

(i) cancer treatment,

(ii) cancer prevention,

(iii) prevention of post-operative recurrence in cancer, and

(iv) combinations of any two or more of (i) to (iii) above.

[9] The pharmaceutical composition of [8], wherein said composition is formulated for administration to a subject that has at least one selected from the group consisting of HLA-DR53, HLA-DR4, HLA-DR15 and HLA-DP5 as an MHC class II molecule, or the pharmaceutical composition of [8], wherein said composition is formulated for administration to a subject that has at least one MHC class II molecule selected from the group consisting of HLA-DR53, HLA-DR4, HLA-DR15 and HLA-DP5.

[10] The pharmaceutical composition of [8] or [9], wherein said composition further comprises one or more peptides having CTL inducibility.

[11] A composition for enhancing an immune response mediated with an MHC class II molecule, wherein the composition comprises at least one active ingredient selected from the group consisting of:

(a) one or more peptide(s) of any one of [1] to [5];

(b) one or more polynucleotide(s) of [6];

(c) one or more APC(s) presenting the peptide of any one of [1] to [5] or fragment thereof on their surface;

(d) one or more Th1 cell(s) that recognize(s) an APC presenting the peptide of any one of [1] to [5] or fragment thereof on its surface; and

(e) combination of any two or more of (a) to (d) above.

[12] A method for inducing an APC having an ability to induce a Th1 cell, said method comprising a step of contacting an APC with the peptide of any one of [1] to [5] in vitro, ex vivo or in vivo.

[13] A method for inducing an APC having an ability to induce a CTL, said method comprising a step selected from the group consisting of:

(a) contacting an APC with the peptide of any one of [1] to [5] in vitro, ex vivo or in vivo; and

(b) introducing a polynucleotide encoding the peptide of any one of [1] to [5] into an APC.

[14] A method for inducing a Th1 cell, said method comprising a step selected from the group consisting of:

(a) co-culturing a CD4-positive T cell with an APC that presents on its surface a complex of an MHC class II molecule and the peptide of any one of [1] to [5] or fragment thereof; and

(b) introducing a polynucleotide encoding both of T cell receptor (TCR) subunits, or polynucleotides encoding each of TCR subunits into a CD4-positive T cell,

wherein the TCR can bind to a complex of an MHC class II molecule and the peptide of any one of [1] to [5] or fragment thereof presented on cell surface, or a method for inducing a Th1 cell, said method comprising a step selected from the group consisting of:

(a) co-culturing a CD4-positive T cell with an APC that presents on its surface a complex of an MHC class II molecule and the peptide of any one of [1] to [5] or fragment thereof; and

(b) introducing a single polynucleotide encoding both of TCR subunits, or multiple polynucleotides each encoding a separate TCR subunit into a CD4-positive T cell,

wherein the TCR can bind to a complex of an MHC class II molecule and the peptide of any one of [1] to [5] or fragment thereof presented on a cell surface if an APC.

[15] A method for inducing a CTL, said method comprising the step selected from the group consisting of:

(a) co-culturing both of a CD4-positive T cell and a CD8-positive T cell with APCs contacted with the peptide of [4] or [5]; and

(b) co-culturing a CD8-positive T cell with an APC contacted with the peptide of [4] or [5].

[16] A method for enhancing an immune response mediated by an MHC class II molecule, wherein the method comprises a step of administering to a subject at least one active ingredient selected from the group consisting of:

(a) one or more peptide(s) of any one of [1] to [5];

(b) one or more polynucleotide(s) of [6];

(c) one or more APC(s) presenting the peptide of any one of [1] to [5] or fragment thereof on their surface;

(d) one or more Th1 cell(s) that recognize(s) an APC presenting the peptide of any one of [1] to [5] or fragment thereof on its surface; and

(e) combination of any two or more of (a) to (d) above.

[17] An isolated APC that presents on its surface a complex of an MHC class II molecule and the peptide of any one of [1] to [5] or fragment thereof.

[18] The APC induced by the method of [12] or [13].

[19] An isolated Th1 cell that recognizes the peptide of any one of [1] to [5] or fragment thereof presented on a surface of an APC.

[20] The Th1 cell induced by the method of [14].

[21] The Th1 cell of [20],wherein the alpha and beta subunits of TCR comprises CDR3 consisting of the amino acid sequence of SEQ ID NOs: 11 and 21, respectively.

[22] A method of inducing an immune response against cancer in a subject in need thereof, said method comprising the step of administering to the subject a composition comprising at least one active ingredient selected from the group consisting of:

(a) one or more peptide(s) of any one of [1] to [5];

(b) one or more polynucleotide(s) of [6];

(c) one or more APC(s) presenting the peptide of any one of [1] to [5] or fragment thereof on their surface;

(d) one or more Th1 cell(s) that recognize(s) an APC presenting the peptide of any one of [1] to [5] or fragment thereof on its surface; and

(e) combination of any two or more of (a) to (d) above.

[23] An antibody or immunologically active fragment thereof against the peptide of any one of [1] to [5].

[24] A vector comprising a nucleotide sequence encoding the peptide of any one of [1] to [5].

[25] A host cell transformed or transfected with the expression vector of [24].

[26] A diagnostic kit comprising the peptide of any one of [1] to [5], the polynucleotide of [6] or the antibody of [23].

[27] A method for assessing and/or monitoring Th1 response specific to the peptide comprising the amino acid sequence of SEQ ID NOs: 1, 2, 3 or 4 in a subject, said method comprising:

(a) providing a sample obtained from the subject administered said peptide, wherein said sample comprises T cells;

(b) detecting the presence of T cells expressing the TCR which binds to a complex of an MHC class II molecule and said peptide or a fragment thereof in said sample; and

(c) indicating an induction of Th1 response specific to said peptide when the presence of said T cells is detected in (b).

[28] The method of [27], wherein said TCR comprises a particular pair of alpha and beta subunits each comprising CDR3 consisting of the amino acid sequence of SEQ ID NOs: 11 and 21, respectively.

[0017] In addition to the above, other objects and features of the invention will become

more fully apparent when the following detailed description is read in conjunction with the accompanying figures and examples. However, it is to be understood that both the foregoing summary of the invention and the following detailed description are of exemplified embodiments, and not restrictive of the invention or other alternate embodiments of the invention. In particular, while the invention is described herein with reference to a number of specific embodiments, it will be appreciated that the description is illustrative of the invention and is not constructed as limiting of the invention. Various modifications and applications may occur to those who are skilled in the art, without departing from the spirit and the scope of the invention, as described by the appended claims. Likewise, other objects, features, benefits and advantages of the present invention will be apparent from this summary and certain embodiments described below, and will be readily apparent to those skilled in the art. Such objects, features, benefits and advantages will be apparent from the above in conjunction with the accompanying examples, data, figures and all reasonable inferences to be drawn therefrom, alone or with consideration of the references incorporated herein.

Brief Description of Drawings

[0018] Various aspects and applications of the present invention will become apparent to the skilled artisan upon consideration of the brief description of the figures and the detailed description of the present invention and its preferred embodiments which follows.

[0019] [fig.1-1]Figure 1 presents DEPDC1-derived and promiscuous HLA class II-binding peptides including CTL epitopes predicted by a recently developed computer algorithm. (A) The amino acid sequence of the human DEPDC1 protein was analyzed using an algorithm. Numbers on the horizontal axis indicate amino acid positions at the N-terminus of DEPDC1-derived 15-mer peptides. A lower consensus percentile rank indicates stronger binding affinity to HLA class II molecules. Black bars indicate the position of LPs described in Fig. 1B. (B) The 22-29mer LPs, DEPDC1$_{191\text{-}213}$-LP (LP-1), DEPDC1$_{292\text{-}313}$-LP (LP-2), DEPDC1$_{301\text{-}329}$-LP (LP-3) and DEPDC1$_{613\text{-}634}$-LP (LP-4) with lower consensus percentile ranks for multiple HLA-class II allelic (DRB1*09:01, DRB1*04:05, and DRB1*15:02) products, and 9-mer peptides DEPDC1-A24$_{294\text{-}302}$ and DEPDC1-A2$_{302\text{-}311}$ recognized by HLA-A24 or A2-restricted CTLs respectively were synthesized.

[fig.1-2](C) The amino acid sequence of the human DEPDC1 protein was analyzed using an algorithm. Numbers on the horizontal axis indicate amino acid positions at the N-terminus of DEPDC1-derived 15-mer peptides. A lower consensus percentile rank indicates stronger binding affinity to HLA class II molecules. Black bars indicate the position of LPs described in Fig. 1B.

[0020] [fig.2A]Figure 2 presents induction of DEPDC1-specific Th cells from healthy donors. (A) DEPDC1-specific Th cells were generated from a DR53^{+}healthy donor (HD1) by stimulation with DEPDC1-LP1. The generated Th cells were re-stimulated with autologous PBMCs or L-cells pulsed with DEPDC1-LP1. The number of IFN-gamma-producing Th cells was analyzed by ELISPOT assay. Representative data from at least 3 independent experiments with similar results obtained from HD1 are shown. The HLA class-II genotype of donor HD1 is indicated in a top of the panels. The underlined HLA-class II allele encodes HLA-class II-molecule presenting the peptides to Th cells.

[fig.2B](B) DEPDC1-LP1-specific and HLA-DR4-restricted Th cells were generated from a healthy donor (HD4) by stimulation with DEPDC1-LP1.

[fig.2C](C) DEPDC1-LP2-specific and HLA-DR4-restricted Th cells were generated from a healthy donor (HD1) by stimulation with DEPDC1-LP2.

[fig.2D](D) DEPDC1-LP3-specific and HLA-DP5-restricted Th cells were generated from a healthy donor (HD3) by stimulation with DEPDC1-LP3.

[fig.2E](E) DEPDC1-LP3-specific and HLA-DR4-restricted Th cells were generated from a healthy donor (HD4) by stimulation with DEPDC1-LP3.

[fig.2F](F) DEPDC1-LP4-specific and HLA-DP5-restricted Th cells were generated from a healthy donor (HD1) by stimulation with DEPDC1-LP4.

[fig.2G](G) DEPDC1-LP4-specific and HLA-DR15-restricted Th cells were generated from a healthy donor (HD5) by stimulation with DEPDC1-LP4.

[fig.2H](H) DEPDC1-LP4-specific and HLA-DP5-restricted Th cells were generated from a healthy donor (HD7) by stimulation with DEPDC1-LP4.

[0021] [fig.3-1]Figure 3 presents natural processing and presentation of DEPDC1-LPs by DCs. (A) HLA-DR4-restricted and DEPDC1-LP2-specific Th cells established from the donor-HD1 recognized autologous DCs loaded with recombinant DEPDC1 protein. Representative data from 2 independent experiments with similar results are shown. (B) HLA-DR4-restricted and DEPDC1-LP3-specific Th cells established from the donor-HD4 recognized autologous DCs loaded with a recombinant DEPDC1 protein.

[fig.3-2](C) Similarly, an HLA-DP5-restricted and DEPDC1-LP4-specific Th cell clone that was derived from HD7 recognized autologous DCs loaded with the recombinant human DEPDC1 protein as well as those pulsed with DEPDC1-LP4 but not those loaded with control protein. The Th cell-responses were substantially reduced in the presence of anti-HLA-DP mAb but not anti-HLA-class I blocking Ab.

[0022] [fig.4]Figure 4 presents DEPDC1-LP2 induces efficient cross-priming of CTLs in vivo. HLA-A2 or HLA-A24 Tgm was immunized with DEPDC1-LP2 emulsified in IFA. After the second vaccination with DEPDC1-LP2, mouse CD8^{+} T-cells in the inguinal lymph nodes were stimulated with BM-DCs pulsed with DEPDC1-$A2_{302\text{-}311}$ SP

or HIV-A2 SP (Fig. 4A) and DEPDC1-$A24_{294-302}$ SP or HIV-A24 SP (Fig 4B). The number of IFN-gamma-producing mouse $CD8^+$ T-cells was analyzed by ex vivo ELISPOT assay. Representative data from at least 3 independent experiments with similar results are shown.

[0023] [fig.5-1]Figure 5 presents various Th1-type cytokines production in DEPDC1-LP-specific Th clones. (A-D) DEPDC1-LP-specific bulk Th cells or Th clone were stimulated with autologous PBMCs pulsed with or without cognate peptides. Concentrations of the indicated cytokines in the culture supernatant were measured as described in the Materials and Methods section. Data are presented as the mean +/- SD of triplicate assays. (A) DEPDC1-LP1-specific bulk Th cells were stimulated with autologous PBMCs pulsed with or without cognate peptides. (B) DEPDC1-LP2-specific Th clone were stimulated with autologous PBMCs pulsed with or without cognate peptides.

[fig.5-2](C) DEPDC1-LP3-specific bulk Th cells were stimulated with autologous PBMCs pulsed with or without cognate peptides. (D) DEPDC1-LP3-specific bulk Th cells were stimulated with autologous PBMCs pulsed with or without cognate peptides.

[0024] [fig.6-1]Figure 6 presents the results of TCR gene analysis of DEPDC1-LP2-specific Th cells. (A-B) DEPDC1-LP2 specific T cell repertoire was analyzed by deep cDNA sequencing of TCR-alpha and -beta genes using a next-generation sequencer. The TCR-alpha is indicated at the top panels, and TCR-beta is indicated at the lower panels. Y axis indicates the number of times for Th cell stimulation with DCs pulsed with the cognate peptide. Cloning of DEPDC1-LP2-specific bulk Th cells was done after five times stimulation of bulk Th cells with irradiated PBMC pulsed with the cognate peptide. The total stimulation frequency was 9 times. X axis indicates top 10 frequent V-J-CDR3 sequences (details are shown in the right boxes), Z axis indicates frequency of individual V-J-CDR3 sequence.

[fig.6-2](C) The TCR-negative murine T cell line TG40 expressing full-length TCR-alpha and -beta genes isolated from DEPDC1-LP2-specific T cell clones specifically responded to the HLA-DR expressing L-cells that were pulsed with cognate peptides, as revealed by the cell-surface expression of CD69 and CD137.

[0025] [fig.7]Figure 7 presents the presence of DEPDC1-LP-specific Th cells in the PBMCs of patients. PBMCs isolated from urothelial carcinoma (UC) patients were stimulated with a mixture of DEPDC1-LP1, -2, -3, -4, plus IL-2 and IL-7 in vitro and the frequency of individual DEPDC1-LP-specific T cells was assessed using ELISPOT. Th cell responses specific to DEPDC1-LP4 were observed in 2 UC patients (A, B). HLA-class II restriction of the DEPDC1-LP-specific Th cells was determined by means of a blocking assay using monoclonal antibodies specific to HLA-DR or -DP.

Description of Embodiments

[0026] Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the present invention, the preferred methods, devices, and materials are now described. However, before the present materials and methods are described, it is to be understood that the present invention is not limited to the particular sizes, shapes, dimensions, materials, methodologies, protocols, etc. described herein, as these may vary in accordance with routine experimentation and optimization. It is also to be understood that the terminology used in the description is for the purpose of describing the particular versions or embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

[0027] The disclosure of each publication, patent or patent application mentioned in this specification is specifically incorporated by reference herein in its entirety. However, nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

[0028] I. Definitions

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the present invention belongs. However, in case of conflict, the present specification, including definitions, will control.

[0029] The words "a", "an", and "the" as used herein mean "at least one" unless otherwise specifically indicated.

[0030] The terms "isolated" and "purified" used in relation with a substance (e.g., peptide, antibody, polynucleotide, etc.) indicates that the substance is substantially free from at least one substance that may else be included in the natural source. Thus, an isolated or purified peptide refers to peptide that are substantially free of cellular material such as carbohydrate, lipid, or other contaminating proteins from the cell or tissue source from which the peptide is derived, or substantially free of chemical precursors or other chemicals when chemically synthesized.

[0031] The term "substantially free of cellular material" includes preparations of a peptide in which the peptide is separated from cellular components of the cells from which it is isolated or recombinantly produced. Thus, a peptide that is substantially free of cellular material includes preparations of polypeptide having less than about 30%, 20%, 10%, or 5% (by dry weight) of heterologous protein (also referred to herein as a "contaminating protein"). When the peptide is recombinantly produced, it is also preferably substantially free of culture medium, which includes preparations of peptide with culture medium less than about 20%, 10%, or 5% of the volume of the peptide

preparation. When the peptide is produced by chemical synthesis, it is preferably substantially free of chemical precursors or other chemicals, which includes preparations of peptide with chemical precursors or other chemicals involved in the synthesis of the peptide less than about 30%, 20%, 10%, 5% (by dry weight) of the volume of the peptide preparation. That a particular peptide preparation contains an isolated or purified peptide can be shown, for example, by the appearance of a single band following sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis of the protein preparation and Coomassie Brilliant Blue staining or the like of the gel. In a preferred embodiment, peptides and polynucleotides of the present invention are isolated or purified.

[0032] The terms "polypeptide", "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is a modified residue, or a non-naturally occurring residue, such as an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers.

[0033] The term "amino acid" as used herein refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that similarly function to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those modified after translation in cells (e.g., hydroxyproline, gamma-carboxyglutamate, and O-phosphoserine). The phrase "amino acid analog" refers to compounds that have the same basic chemical structure (an alpha-carbon bound to a hydrogen, a carboxy group, an amino group, and an R group) as a naturally occurring amino acid but have a modified R group or modified backbones (e.g., homoserine, norleucine, methionine, sulfoxide, methionine methyl sulfonium). The phrase "amino acid mimetic" refers to chemical compounds that have different structures but similar functions to general amino acids.

[0034] Amino acids may be referred to herein by their commonly known three letter symbols or the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission.

[0035] The terms "gene", "polynucleotide" and "nucleic acid" are used interchangeably herein and, unless otherwise specifically indicated, are referred to by their commonly accepted single-letter codes.

The terms "agent" and "composition" are used interchangeably herein to refer to a product that includes specified ingredients in specified amounts, as well as any product that results, directly or indirectly, from combination of the specified ingredients in the specified amounts. Such term in relation to pharmaceutical composition, is intended to encompass a product including the active ingredient(s), and the inert ingredient(s) that make up the carrier, as well as any product which results, directly or indirectly, from

combination, complexation or aggregation of any two or more of the ingredients, or from dissociation of one or more of the ingredients, or from other types of reactions or interactions of one or more of the ingredients. Accordingly, the pharmaceutical compositions of the present invention encompass any composition made by admixing a compound of the present invention and a pharmaceutically or physiologically acceptable carrier.

[0036] The term "active ingredient" herein refers to a substance in a composition that is biologically or physiologically active. Particularly, in the context of a pharmaceutical composition, the term "active ingredient" refers to a component substance that shows an objective pharmacological effect. For example, in case of pharmaceutical compositions for use in the treatment or prevention of cancer, active ingredients in the compositions may lead to at least one biological or physiologically action on cancer cells and/or tissues directly or indirectly. Preferably, such action may include reducing or inhibiting cancer cell growth, damaging or killing cancer cells and/or tissues, and so on. Typically, indirect effect of active ingredients is inductions of immune responses mediated by MHC Class II molecules. Before being formulated, the "active ingredient" may also be referred to as "bulk", "drug substance" or "technical product". The phrase "pharmaceutically acceptable carrier" or "physiologically acceptable carrier", as used herein, means a pharmaceutically or physiologically acceptable material, composition, substance or vehicle, including, but are not limited to, a liquid or solid filler, diluent, excipient, solvent or encapsulating material.

[0037] Unless otherwise defined, the term "cancer" refers to cancers expressing DEPDC1 gene, including, for example, bladder cancer and breast cancer. Cancer expressing DEPDC1 gene is also referred to as cancer expressing DEPDC1, or cancer expressing the gene encoding DEPDC1.

[0038] Unless otherwise defined, the terms "T lymphocyte" and "T cell" are used interchangeably herein.

[0039] Unless otherwise defined, the term "cytotoxic T lymphocyte", "cytotoxic T cell" and "CTL" are used interchangeably herein and, unless otherwise specifically indicated, refer to a sub-group of T lymphocytes that are capable of recognizing non-self cells (e.g., tumor cells, virus-infected cells) and inducing the death of such cells. CTLs are differentiated from $CD8^+$ T lymphocytes and can recognize peptides presented by MHC class I molecules.

[0040] Unless otherwise defined, the term "HLA-A24" refers to the HLA-A24 type containing the subtypes, examples of which include, but are not limited to, HLA-A*24:01, HLA-A*24:02, HLA-A*24:03, HLA-A*24:04, HLA-A*24:07, HLA-A*24:08, HLA-A*24:20, HLA-A*24:25 and HLA-A*24:88.

[0041] Unless otherwise defined, "HLA-A2", as used herein, representatively refers to the

subtypes, examples of which include, but are not limited to, HLA-A*02:01, HLA-A*02:02, HLA-A*02:03, HLA-A*02:04, HLA-A*02:05, HLA-A*02:06, HLA-A*02:07, HLA-A*02:10, HLA-A*02:11, HLA-A*02:13, HLA-A*02:16, HLA-A*02:18, HLA-A*02:19, HLA-A*02:28 and HLA-A*02:50.

[0042] Unless otherwise defined, the terms "T helper type 1 cell" and "Th1 cell" are used interchangeably herein and, unless otherwise specifically indicated, refer to a sub-group of $CD4^+$ T lymphocytes that are capable of recognizing peptides presented by an MHC class II molecules, and associated with cellular immunity. Unless otherwise defined, the terms "Th cell", "$CD4^+$ T cell" and "$CD4^+$ helper T cell" are also used interchangeably herein. Th1 cells secrete a variety of cytokines (such as IFN-gamma, IL-2, TNF-beta, GM-CSF, TNF-alpha, and so on) to help activation and/or stimulation of other immune cells relating to cellular immunity (e.g, CTL, macrophage).

[0043] Unless otherwise defined, the terms "HLA-DR53" refers to the subtypes, examples of which include, but are not limited to, HLA-DRB4*01:01, DRB4*01:02, DRB4*01:03, DRB4*01:04, DRB4*01:05, DRB4*01:06 and DRB4*01:07.

[0044] Unless otherwise defined, the term "HLA-DR4" refers to the subtypes, examples of which include, but are not limited to, HLA-DRB1*04:01, HLA-DRB1*04:02, HLA-DRB1*04:03, HLA-DRB1*04:04, HLA-DRB1*04:05, HLA-DRB1*04:06, HLA-DRB1*04:07, HLA-DRB1*04:08, HLA-DRB1*04:09, HLA-DRB1*04:10 and HLA-DRB1*04:11.

[0045] Unless otherwise defined, the term "HLA-DR15" refers to the subtypes, examples of which include, but are not limited to, HLA-DRB1*15:01, HLA-DRB1*15:02, HLA-DRB1*15:03, HLA-DRB1*15:04, HLA-DRB1*15:05, HLA-DRB1*15:06, HLA-DRB1*15:07, HLA-DRB1*15:08, HLA-DRB1*15:09, HLA-DRB1*15:10 and HLA-DRB1*15:11.

[0046] Unless otherwise defined, the term "HLA-DP5" refers to the subtypes, examples of which include, but are not limited to, HLA-DPB1*05:01.

[0047] Unless otherwise defined, the phrase "immune response mediated with an MHC class II molecule" refers to immune responses induced by presentation of peptide by MHC class II molecule. Herein, "immune response mediated with an MHC class II antigen" includes immune responses induced by $CD4^+$ T cells, in particular, Th1 cells. Examples of such immune responses include, but not limited to, production of cytokines (such as IFN-gamma, IL-2, TNF-beta, GM-CSF, TNF-alpha, and so on) and activation and/or stimulation of other immune cells (such as CTL, macrophage, and so on).

[0048] Unless otherwise defined, the phrase "Th1 cell specific to DEPDC1" refers to a Th1 cell that is specifically activated with an APC presenting a peptide derived from DEPDC1, but not with other APCs.

[0049] Unless otherwise defined, the phrase "DEPDC1-specific CTL" refers to a CTL that specifically shows cytotoxicity against a target cell expressing DEPDC1.

Unless otherwise defined, when used in the context of peptides, the phrase "CTL inducibility" refers to an ability of a peptide to induce a CTL when presented on an APC.

Unless otherwise defined, the term "kit" as used herein, is used in reference to a combination of reagents and other materials. It is contemplated herein that the kit may include microarray, chip, marker, and so on. It is not intended that the term "kit" be limited to a particular combination of reagents and/or materials.

[0050] In the context of the present invention, the term "antibody" refers to immunoglobulins and fragments thereof that are specifically reactive to a designated protein or peptide thereof. Examples of antibodies can include human antibodies, primatized antibodies, chimeric antibodies, bispecific antibodies, humanized antibodies, antibodies fused to other proteins or radiolabels, and antibody fragments. Furthermore, an antibody herein is used in the broadest sense and specifically covers intact monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments so long as they exhibit the desired biological activity. An "antibody" indicates all classes (e.g., IgA, IgD, IgE, IgG and IgM).

[0051] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

[0052] II. Peptides

Peptides of the present invention described in detail below may be referred to as "DEPDC1 peptide(s)" or " DEPDC1 polypeptide(s)".

To demonstrate that peptides derived from DEPDC1 function as an antigen recognized by Th1 cells, peptides derived from DEPDC1 (SEQ ID NO: 8 or 10) were analyzed to determine whether they were antigen epitopes promiscuously restricted by MHC class II molecules. Candidates of promiscuous MHC class II binding peptides derived from DEPDC1 were identified based on their binding affinities to HLA-DR53, HLA-DR4, HLA-DR15 and HLA-DP5. After in vitro stimulation of $CD4^+$ T-cells by dendritic cells (DCs) loaded with these peptides, Th1 cells were successfully established using each of the following peptides:

$DEPDC1_{191-213}$/ RYVILIYLQTILGVPSLEEVINP (SEQ ID NO: 1),

$DEPDC1_{292-313}$/ TFEYYELFVNILVVCGYITVSD (SEQ ID NO: 2),

$DEPDC1_{301-329}$/ NILVVCGYITVSDRSSGIHKIQDDPQSSK (SEQ ID NO: 3), and

$DEPDC1_{613-634}$/ NRRKLQLLMRMISRMSQNVDMP (SEQ ID NO: 4).

[0053] These established Th1 cells noted above showed potent specific Th1 cell activity in response to stimulation of APCs pulsed with respective peptides. Furthermore, the aforementioned peptides could stimulate Th1 cells restricted by several HLA-DR and

HLA-DP molecules (e.g., HLA-DR53, HLA-DR4, HLA-DR15 and HLA-DP5) which are frequently observed in the Japanese population. These results herein demonstrate that DEPDC1 is an antigen recognized by Th1 cells and that the peptides are epitope peptides of DEPDC1 promiscuously restricted by several HLA-class II molecules (such as HLA-DR53, HLA-DR4, HLA-DR15 and HLA-DP5).

[0054] Some of the above identified peptides additionally contain an amino acid sequence of a CTL epitope having an ability to induce a CTL specific to DEPDC1 and, as demonstrated herein, such peptides can induce CTLs specific to DEPDC1 as well as Th1 cells. Accordingly, those peptides may be suitable peptides for induction of immune responses against cancer expressing DEPDC1. Since the DEPDC1 gene is over-expressed in most cancer tissues, including, for example, bladder cancer and breast cancer, it represents a good target for immunotherapy.

[0055] Accordingly, the present invention provides peptides having ability to induce Th1 cells specific to DEPDC1. The peptides of the present invention can bind to at least one MHC class II molecule and be presented on APCs. Alternatively, the fragment of the peptides of the present invention may bind to at least one MHC class II molecule and be presented on APCs. Those fragments of the peptides may be produced by processing within APCs. In preferred embodiments, the peptides of the present invention or fragment thereof have abilities to bind to two or more kinds of MHC class II molecules (e.g., HLA-DR53, HLA-DR4, HLA-DR15 and HLA-DP5). In other words, the peptides of the present invention may have an ability to induce Th1 cells that are restricted by two or more kinds of MHC class II molecules. In another embodiment, the peptides of the present invention include an amino acid sequence of a peptide having DEPDC1-specific CTL inducibility. The typical examples of such peptides having DEPDC1-specific CTL inducibility include peptides having an amino acid sequence of SEQ ID NO: 5 or 6.

[0056] Since the binding groove in an MHC class II molecule is open at both ends, MHC class II binding peptides are allowed to have flexibility in their length. The core binding motif for MHC class II molecule is composed of 9 amino acid residues, and MHC class II binding peptides generally have other amino acid residues flanking with the core binding motif. The number of flanking amino acid residues is not restricted. Thus, all amino acid residues of SEQ ID NO: 1, 2, 3 or 4 are not indispensable for binding to an MHC class II molecule. Accordingly, the peptide of the present invention can be a peptide having ability to induce a Th1 cell, such peptide including an amino acid sequence selected from the group consisting of:

(a) an amino acid sequence having more than 9 contiguous amino acids from the amino acid sequence of SEQ ID NO: 1, 2, 3 or 4; and

(b) an amino acid sequence of (a) in which one, two or several amino acids are sub-

stituted, deleted, inserted, and/or added.

The length of an MHC class II binding peptides is generally 10-30 amino acids. In that the amino acid sequences of SEQ ID NOs: 1 to 4 are composed of a part of the amino acid sequence of DEPDC1 (SEQ ID NO: 8 or 10), the peptides of the present invention can be a following peptide of [1] to [5]:

[1] An isolated peptide having 10-30 amino acids in length and including a part of the amino acid sequence of SEQ ID NO: 8 or 10, wherein such peptide comprises an amino acid sequence selected from the group consisting of:

(a) a contiguous amino acid sequence having more than 9 amino acids in length selected from the amino acid sequence of SEQ ID NO: 1, 2, 3 or 4; and

(b) an amino acid sequence of (a) in which one, two or several amino acids are substituted, deleted, inserted, and/or added,

wherein such peptide has ability to induce Th1 cell(s);

[2] The isolated peptide of [1], wherein the peptide or fragment thereof has abilities to bind to at least two kinds of MHC class II molecules;

[3] The isolated peptide of [2], wherein the MHC class II molecules are selected from the group consisting of HLA-DR53, HLA-DR4, HLA-DR15 and HLA-DP5;

[4] The isolated peptide of any one of [1] to [3], wherein said peptide comprises an amino acid sequence of a peptide having DEPDC1-specific CTL inducibility; and

[5] The isolated peptide of [4], wherein said peptide comprises the amino acid sequence selected from the group consisting of:

(a) an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 to 4; and

(b) an amino acid sequence of (a) in which one, two or several amino acids are substituted, deleted, inserted, and/or added.

[0057] Th1 cells induced by the peptide of the present invention are specific to DEPDC1.

Therefore, in some embodiments, the present invention provides peptides of less than 30 amino acid residues consisting of a partial amino acid sequence of the amino acid sequence of SEQ ID NO: 8 or 10, wherein the peptides comprise the amino acid sequence of SEQ ID NO: 1, 2, 3 or 4.

[0058] Generally, software programs presently available on the Internet, such as those described in Wang P et al., 2008. PLoS Comput Biol. 4(4):e1000048. 11:568; and Wang P et al., 2010. BMC Bioinformatics. can be used to calculate the binding affinities between various peptides and HLA antigens in silico. Binding affinity with HLA antigens can be measured as described, for example, in Nielsen M and Lund O. 2009. BMC Bioinformatics. 10:296 ; Nielsen M et al., 2007. BMC Bioinformatics. 8:238 ; Bui HH, et al., 2005. Immunogenetics. 57:304-14; Sturniolo T et al., 1999. Nat Biotechnol. 17(6):555-61 and Nielsen M, et al., 2008. PLoS Comput Biol. 4(7)

e1000107. Thus, the present invention encompasses peptide fragments of DEPDC1 which are predicted to bind with HLA antigens identified using such known programs.

[0059] As described above, since MHC class II binding peptides have flexibility in their length, the amino acid sequence of SEQ ID NO: 1, 2, 3 or 4 can be optionally flanked with additional amino acid residues so long as the resulting peptide retains the requisite Th1 cell inducibility. Such peptides having Th1 cell inducibility are typically, less than about 30 amino acids, often less than about 29 amino acids, and usually less than about 28 or 27 amino acids. Amino acid sequence(s) flanking the amino acid sequence selected from among SEQ ID NOs: 1 to 4 are not limited and can be composed of any kind of amino acids, so long as such flanking amino acid sequences do not impair the Th1 cell inducibility of the original peptide. In typical embodiments, such flanking amino acid sequence(s) may be selected from among the amino acid sequence of SEQ ID NO: 8 or 10 adjacent to the amino acid sequence of SEQ ID NO: 1, 2, 3 or 4; however, the present invention is not limited thereto. As such, the present invention also provides peptides having Th1 cell inducibility and an amino acid sequence selected from among SEQ ID NOs: 1 to 4.

[0060] On the other hand, since a core binding motif for an MHC class II molecule is composed of 9 amino acid residues, the full length of the amino acid sequence of SEQ ID NO: 1, 2, 3 or 4 is not indispensible for binding to an MHC class II molecule and induction of Th1 cells. Thus, a peptide of the present invention can take the form of a peptide having more than 9 contiguous amino acids from the amino acid sequence of SEQ ID NO: 1, 2, 3 or 4, provided said peptide retains the requisite Th1 cell inducibility. Peptides having Th1 cell inducibility are typically, more than about 10 amino acids, often more than 11 or 12 amino acids, and usually more than 13 or 14 amino acids. Accordingly, the peptides of the present invention can be peptides having Th1 cell inducibility and an amino acid sequence having more than 9, 10, 11, 12, 13 or 14 contiguous amino acids from the amino acid sequence of SEQ ID NO: 1, 2, 3 or 4.

[0061] It is generally known that the modification of one, two, or more amino acids in a protein will not influence the function of the protein, and in some cases will even enhance the desired function of the original protein. In fact, modified peptides (i.e., peptides composed of an amino acid sequence in which one, two or several amino acid residues have been modified (i.e., substituted, added, deleted or inserted) as compared to an original reference sequence) have been known to retain the biological activity of the original peptide (Mark, et al., Proc Natl Acad Sci USA 1984, 81: 5662-6; Zoller and Smith, Nucleic Acids Res 1982, 10: 6487-500; Dalbadie-McFarland et al., Proc Natl Acad Sci USA 1982, 79: 6409-13). Thus, in one embodiment, the peptides of the present invention may have both Th1 cell inducibility and an amino acid sequence selected from among SEQ ID NO: 1 to 4, in which one, two or even more amino acids

are added, inserted, deleted and/or substituted. Alternatively, the peptides of the present invention may have both of Th1 cell inducibility and an amino acid sequence in which one, two or several amino acids are added, inserted, deleted and/or substituted in the amino acid sequence of SEQ ID NO: 1, 2, 3 or 4. That is, in some embodiments, the peptides of the present invention may have both of ability to induce Th1 cell and an amino acid sequence in which one, two or several modifications selected from the group consisting of addition, insertion, deletion and substitution are made to the amino acid sequence of SEQ ID NO: 1, 2, 3 or 4.

[0062] Those of skilled in the art recognize that individual additions or substitutions to an amino acid sequence which alter a single amino acid or a small percentage of amino acids tend to result in the conservation of the properties of the original amino acid side-chain. As such, they are often referred to as "conservative substitutions" or "conservative modifications", wherein the alteration of a protein results in a modified protein having a function analogous to the original protein. Conservative substitution tables providing functionally similar amino acids are well known in the art. Examples of properties of amino acid side chains are hydrophobic amino acids (A, I, L, M, F, P, W, Y, V), hydrophilic amino acids (R, D, N, C, E, Q, G, H, K, S, T), and side chains having the following functional groups or characteristics in common: an aliphatic side-chain (G, A, V, L, I, P); a hydroxyl group containing side-chain (S, T, Y); a sulfur atom containing side-chain (C, M); a carboxylic acid and amide containing side-chain (D, N, E, Q); a base containing side-chain (R, K, H); and an aromatic containing side-chain (H, F, Y, W). In addition, the following eight groups each contain amino acids that are conservative substitutions for one another:

1) Alanine (A), Glycine (G);
2) Aspartic acid (D), Glutamic acid (E);
3) Asparagine (N), Glutamine (Q);
4) Arginine (R), Lysine (K);
5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V);
6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W);
7) Serine (S), Threonine (T); and
8) Cysteine (C), Methionine (M) (see, e.g., Creighton, Proteins 1984).

[0063] Such conservatively modified peptides are also considered to be the peptides of the present invention. However, the peptides of the present invention are not restricted thereto and can include non-conservative modifications, so long as the modified peptide retains the Th1 cell inducibility of the original peptide. Furthermore, modified peptides should not exclude Th1 cell inducible peptides of polymorphic variants, interspecies homologues, and alleles of DEPDC1.

[0064] To retain the requisite Th1 cell inducibility, one can modify (insert, add, deletion

and/or substitute) a small number (for example, 1, 2 or several) or a small percentage of amino acids. Herein, the term "several" means 5 or fewer amino acids, for example, 4 or 3 or fewer. The percentage of amino acids to be modified is preferably 20% or less, more preferably, 15% of less, even more preferably 10% or 8%, less or 1 to 5%.

[0065] Homology analysis of preferred peptides of the present invention, namely SEQ ID NOs: 1 to 4 ($DEPDC1_{191-213}$-LP, $DEPDC1_{292-313}$-LP, $DEPDC1_{301-329}$-LP, and $DEPDC1_{613-634}$-LP), confirm that these peptides do not have significant homology with peptides derived from any other known human gene products. Thus, the possibility of these peptides generating unknown or undesired immune responses when used for immunotherapy is significantly lowered. Accordingly, these peptides are expected to be highly useful for eliciting immunity against DEPDC1 in cancer patients.

[0066] When used in the context of immunotherapy, the peptides of the present invention or fragment thereof should be presented on the surface of an APC, preferably as a complex with an HLA class II antigen. Therefore, it is preferable to select peptides that not only induce Th1 cells but also possess high binding affinity to the HLA class II antigen. To that end, the peptides can be modified by substitution, insertion, deletion and/or addition of the amino acid residues to yield a modified peptide having improved binding affinity.

[0067] The present invention also contemplates the addition of one to two amino acids to either or both of the N and C-terminus of the described peptides. Such modified peptides having high HLA antigen binding affinity and retained Th1 cell inducibility are also included in the present invention.

[0068] For example, the present invention provides an isolated peptide of less than 31, 30, 29, 28, 27, or 26 amino acids in length which binds an HLA class II antigen, has Th1 cell inducibility, and comprises the amino acid sequence in which one, two or several amino acid(s) are modified in the amino acid sequence selected from the group consisting of SEQ ID NOs: 1 to 4.

[0069] These peptides may also be processed in an APC to be presented as a processed fragment thereon, when these peptides are contacted with, or introduced into APC. For example, the peptide of the present invention may be processed into a fragment composed of usually 11-26 (typically 15-25) amino acid residues to be presented on a surface of an APC.

[0070] However, when the peptide sequence is identical to a portion of the amino acid sequence of an endogenous or exogenous protein having a different function, negative side effects such as autoimmune disorders and/or allergic symptoms against specific substances may be induced. Therefore, it may be desirable to first perform homology searches using available databases to avoid situations in which the sequence of the peptide matches the amino acid sequence of another protein. When it becomes clear

from the homology searches that no peptide identical to or having 1 or 2 amino acid differences as compared to the objective peptide exists in nature, the objective peptide can be modified in order to increase its binding affinity with HLA antigens, and/or increase its Th1 cell inducibility and/or CTL inducibility without any danger of such side effects.

[0071] Although peptides having high binding affinity to the HLA class II antigens as described above are expected to be highly effective, the candidate peptides, which are selected according to the presence of high binding affinity as an indicator, are further examined for the presence of Th1 cell inducibility. Herein, the phrase "Th1 cell inducibility" indicates an ability of a peptide to confer an ability to induce a Th1 cell on an APC when contacted with the APC. Further, " Th1 cell inducibility" includes the ability of the peptide to induce Th1 cell activation and/or Th1 cell proliferation, promote Th1 cell mediated-cytokine productions including IFN-gamma production to help and/or stimulate other cells (e.g. CTL, macrophage).

[0072] Confirmation of Th1 cell inducibility is accomplished by inducing APCs carrying human MHC antigens (for example, B-lymphocytes, macrophages, and dendritic cells (DCs)), preferably DCs derived from human peripheral blood mononuclear leukocytes, and after stimulation with the peptides, mixing with CD4-positive T cells ($CD4^+$ T cells), and then measuring the IFN-gamma produced and released by $CD4^+$ T cells. Alternatively, Th1 cell inducibility of the peptide can be assessed based on CTL activation by Th1 cells. For example, $CD4^+$ T cells are co-cultured with DCs stimulated with a test peptide, and then mixing with CTLs and target cells for CTLs. The target cells can be radiolabeled with ^{51}Cr and such, and cytotoxic activity of CTLs activated by the cytokines secreted from Th1 cells can be calculated from radioactivity released from the target cells. Alternatively, Th1 cells inducibility can be assessed by measuring IFN-gamma produced and released by Th1 cells in the presence of APCs stimulated with a test peptide, and visualizing the inhibition zone on the media using anti-IFN-gamma monoclonal antibodies.

[0073] In addition to the above-described modifications, the peptides of the present invention can also be linked to other substances, so long as the resulting linked peptide retains the Th1 cell inducibility of the original peptide. Examples of suitable substances include, for example: peptides, lipids, sugar and sugar chains, acetyl groups, natural and synthetic polymers, etc. The peptides of the present invention can contain modifications such as glycosylation, side chain oxidation, or phosphorylation, etc., provided the modifications do not destroy the biological activity of the original peptide. These kinds of modifications can be performed to confer additional functions (e.g., targeting function, and delivery function) or to stabilize the peptide.

[0074] For example, to increase the in vivo stability of a peptide, it is known in the art to

introduce D-amino acids, amino acid mimetics or unnatural amino acids; this concept can also be adapted to the peptides of the present invention. The stability of a peptide can be assayed in a number of ways. For instance, peptidases and various biological media, such as human plasma and serum, can be used to test stability (see, e.g., Verhoef et al., Eur J Drug Metab Pharmacokin 1986, 11: 291-302).

[0075] The peptides of the present invention may be presented on the surface of an APC as complexes in combination with HLA class II antigens and then induce Th1 cells. Therefore, the peptides forming complexes with HLA class II antigens on the surface of an APC are also included in the present invention. The APCs presenting the peptides of the present invention can be inoculated as vaccines.

[0076] The type of HLA antigens contained in the above complexes must match that of the subject requiring treatment and/or prevention. For example, in the Japanese population, HLA-DR53, HLA-DR4, HLA-DR15 and HLA-DP5 are prevalent and therefore would be appropriate for treatment of a Japanese patient. Typically, in the clinic, the type of HLA antigen of the patient requiring treatment is investigated in advance, which enables the appropriate selection of peptides having binding ability to the particular HLA class II antigen. In preferred embodiments, the peptides of the present invention can induce Th1 cells in a promiscuous manner. Herein, when a peptide can induce Th1 cells restricted by at least two different kinds of MHC class II molecules, the Th1 cell inducibility of the peptide is referred to as "promiscuous". In other word, when a peptide is recognized by at least two different kinds of MHC class II molecules, such antigen recognition is deemed "promiscuous". When used in the context of peptides, the phrase "recognized by at least two different kinds of MHC class II molecules" indicates that the peptide or fragment thereof can bind to at least two different kinds of MHC class II molecules. For example, $DEPDC1_{191-213}$-LP (SEQ ID NO: 1) is recognized by HLA-DR53 and HLA-DR4, $DEPDC1_{292-313}$-LP (SEQ ID NO: 2) is recognized by HLA-DR4, $DEPDC1_{301-329}$-LP (SEQ ID NO: 3) is recognized by HLA-DP5, and HLA-DR4, and $DEPDC1_{613-634}$-LP (SEQ ID NO: 4) is recognized by HLA-DP5 and HLA-DR15. Therefore, these peptides are typical examples of "promiscuous" epitope.

[0077] When using HLA-DR53 or HLA-DR4 positive APCs, the peptides having the amino acid sequence of SEQ ID NO: 1 are preferably used. When using HLA-DR4 positive APCs, the peptides having the amino acid sequence of SEQ ID NO: 2 are preferably used. When using HLA-DP5 or HLA-DR4 positive APCs, the peptides having the amino acid sequence of SEQ ID NO: 3 are preferably used. When using HLA-DP5 or HLA-DR15 positive APCs, the peptides having the amino acid sequence of SEQ ID NO: 4 are preferably used.

[0078] Accordingly, in preferred embodiments, peptides having the amino acid sequence of

SEQ ID NO: 1 may be used for the induction of Th1 cells in a subject that has been identified as having HLA-DR53 or HLA-DR4 prior to the induction. Likewise, peptides having the amino acid sequence of SEQ ID NO: 2 may be used for the induction of Th1 cells in a subject that has been identified as having HLA-DR4 prior to the induction. Likewise, peptides having the amino acid sequence of SEQ ID NO: 3 may be used for the induction of Th1 cells in a subject that has been identified as having HLA-DP5 or HLA-DR4 prior to the induction. Likewise, peptides having the amino acid sequence of SEQ ID NO: 4 may be used for the induction of Th1 cells in a subject that has been identified as having HLA-DP5 or HLA-DR15 prior to the induction.

[0079] III. Preparation of DEPDC1 Peptides

The peptides of the present invention can be prepared using well known techniques. For example, the peptides of the present invention can be prepared synthetically, using recombinant DNA technology or chemical synthesis. The peptide of the present invention can be synthesized individually or as longer polypeptides composed of two or more peptides. The peptides of the present invention can be then isolated, i.e., purified, so as to be substantially free of other naturally occurring host cell proteins and fragments thereof, or any other chemical substances.

[0080] The peptides of the present invention may contain modifications, such as glycosylation, side chain oxidation, or phosphorylation; provided the modifications do not destroy the biological activity of the original reference peptides. Other illustrative modifications include incorporation of D-amino acids or other amino acid mimetics. These modifications can be used, for example, to increase the serum half life of the peptides.

[0081] Peptides of the present invention can be obtained through chemical synthesis based on the selected amino acid sequence. Examples of conventional peptide synthesis methods that can be adapted for the synthesis include:

(i) Peptide Synthesis, Interscience, New York, 1966;

(ii) The Proteins, Vol. 2, Academic Press, New York, 1976;

(iii) Peptide Synthesis (in Japanese), Maruzen Co., 1975;

(iv) Basics and Experiment of Peptide Synthesis (in Japanese), Maruzen Co., 1985;

(v) Development of Pharmaceuticals (second volume) (in Japanese), Vol. 14 (peptide synthesis), Hirokawa, 1991;

(vi) WO99/67288; and

(vii) Barany G. & Merrifield R.B., Peptides Vol. 2, "Solid Phase Peptide Synthesis", Academic Press, New York, 1980, 100-118.

[0082] Alternatively, the peptides of the present invention can be obtained adapting any known genetic engineering method for producing peptides (e.g., Morrison J, J Bac-

teriology 1977, 132: 349-51; Clark-Curtiss & Curtiss, Methods in Enzymology (eds. Wu et al.) 1983, 101: 347-62). For example, first, a suitable vector harboring a polynucleotide encoding the objective peptide in an expressible form (e.g., downstream of a regulatory sequence corresponding to a promoter sequence) is prepared and transformed into a suitable host cell. The host cell is then cultured to produce the peptide of interest. The peptide of the present invention can also be produced in vitro adopting an in vitro translation system.

[0083] IV. Polynucleotides

The present invention also provides a polynucleotide which encodes any of the aforementioned peptides of the present invention. These include polynucleotides derived from the natural occurring DEPDC1 gene (GenBank Accession No. NM_001114120 (SEQ ID NO: 7) or NM_017779 (SEQ ID NO: 9)) as well as those having a conservatively modified nucleotide sequence thereof. Herein, the phrase "conservatively modified nucleotide sequence" refers to sequences which encode identical or essentially identical amino acid sequences. Due to the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG, and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a peptide also describes every possible silent variation of the nucleic acid. One of ordinary skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid that encodes a peptide is implicitly described in each disclosed sequence.

[0084] The polynucleotide of the present invention can be composed of DNA, RNA and derivatives thereof. As is well known in the art, a DNA is suitably composed of bases such as A, T, C and G, and T is replaced by U in an RNA. One of skill will recognize that non-naturally occurring bases may be included in polynucleotides, as well.

[0085] The polynucleotide of the present invention can encode multiple peptides of the present invention with or without intervening amino acid sequences in between. For example, the intervening amino acid sequence can provide a cleavage site (e.g., enzyme recognition sequence) of the polynucleotide or the translated peptides. Furthermore, the polynucleotide can include any additional sequences to the coding sequence encoding the peptide of the present invention. For example, the polynucleotide can be a recombinant polynucleotide that includes regulatory sequences

required for the expression of the peptide or can be an expression vector (plasmid) with marker genes and such. In general, such recombinant polynucleotides can be prepared by the manipulation of polynucleotides through conventional recombinant techniques using, for example, polymerases and endonucleases.

[0086] Both recombinant and chemical synthesis techniques can be used to produce the polynucleotides of the present invention. For example, a polynucleotide can be produced by insertion into an appropriate vector, which can be expressed when transfected into a competent cell. Alternatively, a polynucleotide can be amplified using PCR techniques or expression in suitable hosts (see, e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York, 1989). Alternatively, a polynucleotide can be synthesized using the solid phase techniques, as described in Beaucage SL & Iyer RP, Tetrahedron 1992, 48: 2223-311; Matthes et al., EMBO J 1984, 3: 801-5.

[0087] V. Antigen-Presenting Cells (APCs)

The present invention also provides antigen-presenting cells (APCs) that present complexes formed between HLA class II antigens and the peptides of the present invention or fragment thereof on its surface. The APCs that are obtained by contacting the peptides of the present invention can be derived from patients who are subject to treatment and/or prevention, and can be administered as vaccines by themselves or in combination with other drugs including the peptides of the present invention, Th1 cells or CTLs.

[0088] The APCs are not limited to a particular kind of cells and include dendritic cells (DCs), Langerhans cells, macrophages, B cells, and activated T cells, which are known to present proteinaceous antigens on their cell surface so as to be recognized by lymphocytes. Since a DC is a representative APC having the strongest Th1 cell-inducing activity among APCs, DCs find use as the APCs of the present invention.

[0089] Moreover, in preferred embodiments, the peptides of the present invention can also induce CTL response mediated with the MHC class I antigen, as well as Th1 cell response mediated with the MHC class II antigen. In general, it is well known that the length of epitope recognized by the MHC-class I antigen is shorter (e.g. 8-10 amino acid residues) than that of MHC-class II (15 or more). Therefore, processed products of some peptides of the present invention may lead to induce CTL. In fact, DEPDC1 $_{292\text{-}313}$-LP (SEQ ID NO: 2) can induce a CTL that recognizes the fragment (EYYELFVNI: SEQ ID NO: 5) and DEPDC1$_{292\text{-}313}$-LP (SEQ ID NO: 2) or DEPDC1 $_{301\text{-}329}$-LP (SEQ ID NO: 3) can induce a CTL that recognizes the fragment (ILVVCGYITV: SEQ ID NO: 6). Accordingly, such peptides of the present invention can induce not only Th1 cells but also CTLs after processing of them in APCs. In other words, APCs contacted with the above peptides of the present invention present such

peptides with MHC class II antigens and concurrently process them to present fragments thereof with MHC-class I antigens. Consequently, both of Th1 cells and CTLs can be induced by using the above peptides of the present invention.

[0090] For example, an APC can be obtained by inducing DCs from peripheral blood mononuclear cells and then contacting (stimulating) them with the peptides of the present invention in vitro, ex vivo or in vivo. When the peptides of the present invention are administered to the subjects, APCs that present the peptides of the present invention or fragments thereof are induced in the body of the subject. Herein, the phrase "inducing an APC" includes contacting (stimulating) an APC with the peptides of the present invention to present complexes formed between HLA class II antigens and the peptides of the present invention or fragments thereof on their surface. Alternatively, after introducing the peptides of the present invention to APCs to allow the APCs to present the peptides or fragments thereof, the APCs can be administered to the subject as a vaccine. For example, the ex vivo administration can include steps of:

(a) collecting APCs from a first subject:

(b) contacting the APCs of step (a), with the peptide of the present invention and

(c) administering the peptide-loaded APCs of step (b) to a second subject.

[0091] The first subject and the second subject may be the same individual, or can be different individuals. Alternatively, according to the present invention, use of the peptides of the present invention for manufacturing a pharmaceutical composition inducing APCs is provided. In addition, the present invention provides a method or process for manufacturing a pharmaceutical composition inducing APCs, wherein the method comprises the step for admixing or formulating the peptide of the present invention with a pharmaceutically acceptable carrier. Further, the present invention also provides the peptides of the present invention for use in inducing APCs. The APCs obtained by step (b) can be administered to the subject as a vaccine.

[0092] In one aspect of the present invention, the APCs of the present invention have a high level of Th1 cell inducibility. Herein, in the phrase "high level of Th1 cell inducibility", the high level is relative to the level of that of an APC contacted with no peptide or a peptide which can not induce a Th1 cell. Herein, when used in the context of APCs, the phrase "Th1 cell inducibility" indicates an ability of an APC to induce a Th1 cell when contacted with a $CD4^+$ T cell. Such APCs having a high level of Th1 cell inducibility can be also prepared by a method which includes the step of transferring polynucleotides that encode the peptides of the present invention to APCs in vitro. The polynucleotides to be introduced can be in the form of DNAs or RNAs. Examples of methods for introduction include, without particular limitations, various methods conventionally performed in this field, such as lipofection, electroporation,

and calcium phosphate method. More specifically, it can be performed as described in Cancer Res 1996, 56: 5672-7; J Immunol 1998, 161: 5607-13; J Exp Med 1996, 184: 465-72; Published Japanese Translation of International Publication No. 2000-509281. By transferring the gene into APCs, the gene undergoes transcription, translation, and such in the cell, and then the obtained protein is processed by MHC Class I or Class II, and proceeds through a presentation pathway to present peptides. Alternatively, the APCs of the present invention can be prepared by a method which induces the step of contacting APCs with the peptide of the present invention.

[0093] In preferred embodiments, the APCs of the present invention can be APCs that present complexes of an MHC class II molecule selected from among HLA-DR53 and HLA-DR4 and the peptide of the present invention (including an amino acid sequence of SEQ ID NO: 1) on their surface. In another embodiment, the APCs of the present invention can be APCs that present complexes of an MHC class II molecule selected from among HLA-DR4 and the peptide of the present invention (including an amino acid sequence of SEQ ID NO: 2) on their surface. In another embodiment, the APCs of the present invention can be APCs that present complexes of an MHC class II molecule of HLA-DP5 and HLA-DR4 and the peptide of the present invention (including an amino acid sequence of SEQ ID NO: 3) on their surface. In another embodiment, the APCs of the present invention can be APCs that present complexes of an MHC class II molecule of HLA-DP5 and HLA-DR15 and the peptide of the present invention (including an amino acid sequence of SEQ ID NO: 4) on their surface. Preferably, HLA-DR53, HLA-DR4, HLA-DR15 and HLA-DP5 may be HLA-DRB4*01:03, HLA-DRB1*04:05, HLA-DRB1*15:02, and HLA-DPB1*05:01, respectively.

[0094] VI. T Helper Type 1 Cells (Th1 cells)

A Th1 cell induced against any of the peptides of the present invention strengthens immune responses of any of effector cells including CTLs targeting cancer cells in vivo, and thus serve as vaccines, in a fashion similar to the peptides per se. Thus, the present invention also provides isolated Th1 cells that are specifically induced or activated by any of the peptides of the present invention.

[0095] Such Th1 cells can be obtained by (1) administering one or more peptides of the present invention to a subject, and then collecting Th1 cells from the subject, (2) contacting (stimulating) APCs and $CD4^+$ T cells, or peripheral blood mononuclear cells in vitro with the peptides of the present invention, and then isolating Th1 cells, (3) contacting $CD4^+$ T cells or peripheral blood mononuclear cells in vitro with the APCs of the present invention, or (4) introducing a polynucleotide encoding both of TCR subunits or polynucleotides encoding each of TCR subunits into a $CD4^+$ T cell, wherein the TCR can bind to a complex of an MHC class II molecule and the peptide

of the present invention. Such APCs for the method of (3) can be prepared by the methods described above. Details of the method of (4) are described below in section "VII. T Cell Receptor (TCR)".

[0096] The Th1 cells that have been induced by stimulation with APCs of the present invention can be derived from patients who are subject to treatment and/or prevention, and can be administered by themselves or in combination with other drugs including the peptides of the present invention for the purpose of regulating effects. The obtained Th1 cells can activate and/or stimulate immune cells responsible for cellular immunity (e.g., CTL, macrophage). Such immune cells that can be activated by the Th1 cells of the present invention include CTLs that show cytotoxicity against target cells such as cancer cells. For example, target cells for such CTLs may be cells that endogenously express DEPDC1 (e.g., cancer cells), or cells that are transfected with the DEPDC1 gene. In preferred embodiments, the peptides of the present invention can contain at least one amino acid sequence of a CTL epitope peptide and also induce CTLs against DEPDC1 expressing cells such as cancer cells, in addition to Th1 cells. In this case, the peptide of the present invention can induce Th1 cells and CTLs simultaneously or sequentially in vivo, and the induced Th1 cells can effectively activate the induced CTLs. Accordingly, such peptides containing at least one amino acid sequence of a CTL epitope peptide are suitable peptides for cancer immunotherapy.

[0097] Furthermore, the Th1 cells of the present invention secrete various cytokines (e.g. IFN-gamma) which activate and/or stimulate any CTLs against other target cells in an antigen independent manner. Accordingly, the Th1 cells of the present invention can also contribute to enhance CTL activity targeting cells expressing a TAA other than DEPDC1. Thus, the Th1 cells of the present invention are useful for immunotherapy for not only tumor expressing DEPDC1, but also tumor expressing other TAAs, as well as the peptides and APCs of the present invention.

[0098] In some embodiments, the Th1 cells of the present invention are Th1 cells that recognize cells presenting complexes of an HLA-DR or HLA-DP antigen and the peptide of the present invention. In the context of Th1 cells, the phrase "recognize a cell" refers to binding of a complex of an MHC class II molecule and the peptide of the present invention on the cell surface via its TCR and being activated in an antigen specific manner. Herein, the phrase "activated in antigen specific manner" refers to being activated in response to a particular MHC class II molecule and peptide and cytokine production from the activated Th1 cells are induced. In preferred embodiments, HLA-DR and HLA-DP may be selected from the group consisting of HLA-DR53, HLA-DR4, HLA-DR15 and HLA-DP5.

[0099] VII. T Cell Receptor (TCR)

The present invention also provides a composition containing one or more polynu-

cleotides encoding one or more polypeptides that are capable of forming a subunit of a T cell receptor (TCR), and methods of using the same. Such TCR subunits have the ability to form TCRs that confer specificity for DEPDC1 to $CD4^+$ T cells against APCs presenting DEPDC1 peptides. By using the known methods in the art, the nucleic acids of alpha- and beta- chains as the TCR subunits of Th1 cells induced by the peptides of the present invention can be identified (WO2007/032255 and Morgan et al., J Immunol, 171, 3288 (2003)). The derivative TCRs can bind APCs displaying DEPDC1 peptides with high avidity, and optionally mediate efficient cytokine productions.

[0100] The polynucleotide/polynucleotides encoding the TCR subunits (i.e., a single polynucleotide encoding both of the TCR subunits or multiple polynucleotides each encoding a separate TCR subunits) can be incorporated into suitable vectors e.g. retroviral vectors. These vectors are well known in the art. The polynucleotides or the vectors containing them usefully can be transferred into a $CD4^+$ T cell, for example, a $CD4^+$ T cell from a patient. Advantageously, the present invention provides an off-the-shelf composition allowing rapid modification of a patient's own T cells (or those of another subject) to rapidly and easily produce modified T cells having excellent cancer cell killing properties.

[0101] For example, TCR-alpha and -beta subunits, each having a CDR3 consisting of the amino acid sequence of SEQ ID NO: 11 and 21, respectively, were increased in Th cells stimulated by DEPDC1-LP2 ($DEPDC1_{292-313}$-LP) in an HLA-DR4 restricted manner (Fig. 6). Therefore, polynucleotides encoding them may be preferable for Th1 cell induction of the present invention. Likewise, Th1 cell expressing TCR formed between alpha and beta subunits, each having a CDR3 consisting of the amino acid sequence of SEQ ID NO: 11 and 21, respectively is also preferred embodiments of the present invention. In general. antigen specificity of TCR mainly depends on their CDR 3. Accordingly, by replacing CDR3 of alpha and beta subunit with SEQ ID NO: 11 and 21, respectively, DEPDC1-LP2 specific TCR can also be re-constructed from known TCR. Such TCR having trans- planted CDR3 from another subunits may be referred as chimeric TCR. Method for trans-planting specific CDRs is well known in the art.

[0102] The present invention further provides Th1 cells which are prepared by transduction with the polynucleotide encoding both of the TCR subunits or polynucleotides encoding each of TCR subunits, wherein the TCR subunit can bind to the DEPDC1 peptide (e.g. SEQ ID NO: 1 in the context of HLA-DR53 or HLA-DR4, SEQ ID NO: 2 in the context of HLA-DR4, SEQ ID NO: 3 in the context of HLA-DP5 or HLA-DR4, SEQ ID NO: 4 in the context of HLA-DP5 or HLA-DR15). The transduced Th1 cells are capable of homing to cancer cells in vivo, and can be expanded by well known culturing methods in vitro (e.g., Kawakami et al., J Immunol., 142, 3452-3461 (1989)).

The Th1 cells prepared as described above can be used to form an immunogenic composition useful in treating or the prevention of cancer in a patient in need of therapy or protection.

[0103] VIII. Pharmaceutical Agents or Compositions

To the extent that the methods and compositions of the present invention find utility in the context of the "treatment" of cancer, a treatment is deemed "efficacious" if it leads to clinical benefit such as, reduction in expression of DEPDC1 gene, or a decrease in size, prevalence, or metastatic potential of the cancer in the subject. When the treatment is applied prophylactically, "efficacious" means that it retards or prevents cancers from forming or prevents or alleviates a clinical symptom of cancer. Efficaciousness is determined in association with any known method for diagnosing or treating the particular tumor type.

To the extent that the methods and compositions of the present invention find utility in the context of the "prevention" and "prophylaxis" of cancer, such terms are interchangeably used herein to refer to any activity that reduces the burden of mortality or morbidity from disease. Prevention and prophylaxis can occur "at primary, secondary and tertiary prevention levels." While primary prevention and prophylaxis avoid the development of a disease, secondary and tertiary levels of prevention and prophylaxis encompass activities aimed at the prevention and prophylaxis of the progression of a disease and the emergence of symptoms as well as reducing the negative impact of an already established disease by restoring function and reducing disease-related complications. Alternatively, prevention and prophylaxis include a wide range of prophylactic therapies aimed at alleviating the severity of the particular disorder, e.g. reducing the proliferation and metastasis of tumors, reducing angiogenesis.

[0104] In the context of the present invention, the treatment and/or prophylaxis of cancer and/or the prevention of postoperative recurrence thereof include any of the following steps, such as surgical removal of cancer cells, inhibition of the growth of cancerous cells, involution or regression of a tumor, induction of remission and suppression of occurrence of cancer, tumor regression, and reduction or inhibition of metastasis. Effectively treating and/or the prophylaxis of cancer decreases mortality and improves the prognosis of individuals having cancer, decreases the levels of tumor markers in the blood, and alleviates detectable symptoms accompanying cancer. For example, reduction or improvement of symptoms constitutes effective treatment and/or prophylaxis, including 10%, 20%, 30% or more reduction, or stable disease.

[0105] As described above, the Th1 cells induced by the peptides of the present invention can help immune cells responsible for cellular immunity. Such immune cells include CTLs against not only cancer cells expressing DEPDC1, but also cancer cells expressing other TAAs, since cytokines secreted by Th1 cells can affect CTLs in antigen

independent manner. Accordingly, the present invention provides a pharmaceutical agent or composition comprising at least one peptide of the present invention. In the pharmaceutical agent or composition, such peptide is present in a therapeutically or pharmaceutically effective amount.

[0106] A pharmaceutical agent or composition of the present invention is useful for helping, stimulating and/or enhancing any immune cells responsible for cellular immunity (e.g., CTLs, macrophage), since Th1 cells induced by the agent or composition of the present invention can secrete cytokines that affects any immune cells responsible for cellular immunity. Therefore, the agent or composition of the present invention is useful for any purposes of enhancing or promoting immune responses mediated with such immune cells including CTLs. For example, the present invention provides agent or compositions comprising at least one of the peptide of the present invention, for use in treatment and/or prevention of cancer since the agent or composition of the present invention can enhance or promote immune responses against cancer or tumor mediated with such immune cells. The amount of the peptide in such agent or composition may be an amount that is effective in significantly enhancing or stimulating immunological response in a subject carrying a cancer expressing DEPDC1.

[0107] The present invention also provides an agent or composition for enhancing or stimulating immunological responses mediated with an MHC class I antigen, such as HLA-A2 and HLA-A24. In another embodiment, the present invention further provides a use of the peptide of the present invention for manufacturing an agent or composition for enhancing or stimulating an immunological response mediated with an MHC class I antigen.

[0108] In preferred embodiments, DEPDC1 derived peptides identified in the course of the present invention can induce Th1 cells, as well as CTLs against DEPDC1-expressing cells. Accordingly, the present invention also provides agents or compositions comprising at least one of the peptide of the present invention, for use in the induction of CTLs against cancer or tumor expressing DEPDC1.

[0109] Moreover, the agent or composition comprising at least one of the peptides of the present invention can be used in enhancing or promoting immune responses mediated by MHC class II molecules.

[0110] Since DEPDC1 expression is specifically elevated in several cancer types, including bladder cancer, breast cancer and several other malignancies, as compared with normal tissue (WO2006/085684, WO2007/013665, Kanehira M, et al., Oncogene 2007; 26: 6448-55, and our microarray data (data not shown)), the peptides of the present invention or polynucleotides encoding the peptides can be used for the treatment and/or prophylaxis of cancer or tumor, and/or for the prevention of postoperative recurrence thereof. Thus, the present invention provides a pharmaceutical agent or a

composition for treating and/or for the prophylaxis of cancer or tumor, and/or prevention of postoperative recurrence thereof, which comprises one or more of the peptides of the present invention, or polynucleotides encoding the peptides as an active ingredient. Alternatively, the present peptides can be expressed on the surface of any of the foregoing cells, such as APCs for the use as pharmaceutical agents or compositions. In addition, the aforementioned Th1 cells can also be used as active ingredients of the present pharmaceutical agents or compositions.

[0111] In another embodiment, the present invention also provides the use of an active ingredient selected from among:

(a) a peptide of the present invention,

(b) a polynucleotide encoding such a peptide as disclosed herein in an expressible form,

(c) an APC presenting on its surface a peptide of the present invention or fragment thereof, and

(d) a Th1 cell of the present invention

in manufacturing a pharmaceutical composition or agent for treating cancer or tumor.

[0112] Alternatively, the present invention further provides an active ingredient selected from among:

(a) a peptide of the present invention,

(b) a polynucleotide encoding such a peptide as disclosed herein in an expressible form,

(c) an APC presenting on its surface a peptide of the present invention or fragment thereof, and

(d) a Th1 cell of the present invention

for use in treating cancer or tumor.

[0113] Alternatively, the present invention further provides a method or process for manufacturing a pharmaceutical composition or agent for treating cancer or tumor, wherein the method or process includes the step of formulating a pharmaceutically or physiologically acceptable carrier with an active ingredient selected from among:

(a) a peptide of the present invention,

(b) a polynucleotide encoding such a peptide as disclosed herein in an expressible form,

(c) an APC presenting on its surface a peptide of the present invention or fragment thereof, and

(d) a Th1 cell of the present invention

as active ingredients.

[0114] In another embodiment, the present invention also provides a method or process for manufacturing a pharmaceutical composition or agent for treating cancer or tumor,

wherein the method or process includes the step of admixing an active ingredient with a pharmaceutically or physiologically acceptable carrier, wherein the active ingredient is selected from among:

(a) a peptide of the present invention,

(b) a polynucleotide encoding such a peptide as disclosed herein in an expressible form,

(c) an APC presenting on its surface a peptide of the present invention or fragment thereof, and

(d) a Th1 cell of the present invention.

[0115] Alternatively, the pharmaceutical composition or agent of the present invention may be used for either or both of the prophylaxis of cancer or tumor and prevention of post-operative recurrence thereof.

[0116] The present pharmaceutical agents or compositions find use as a vaccine. In the context of the present invention, the phrase "vaccine" (also referred to as an immunogenic composition) refers to a composition that has the function to induce anti-tumor immunity upon inoculation into animals.

[0117] The pharmaceutical agents or compositions of the present invention can be used to treat and/or prevent cancers or tumors, and/or prevent postoperative or metastatic recurrence thereof in subjects or patients. Examples of such subjects include humans as well as other mammals including, but not limited to, mouse, rat, guinea-pig, rabbit, cat, dog, sheep, goat, pig, cattle, horse, monkey, baboon, and chimpanzee, particularly a commercially important animal or a domesticated animal.

[0118] In the course of the present invention, the peptides having an amino acid sequence selected from among SEQ ID NOs: 1 to 4 have been found to be promiscuous Th1 cell epitopes restricted by several HLA-DR and/or HLA-DP molecules (e.g., HLA-DR53, HLA-DR4, HLA-DR15 and HLA-DP5) and can be candidates that can induce potent and specific immune response against cancer due to immune responses mediated with MHC class II molecules. Therefore, the present pharmaceutical agents or compositions which include any of these peptides having the amino acid sequences of SEQ ID NOs: 1 to 4 are particularly suited for the administration to subjects that have at least one selected from among HLA-DR53, HLA-DR4, HLA-DR15 and HLA-DP5 as an MHC class II molecule. The same applies to pharmaceutical agents or compositions which contain polynucleotides encoding any of these peptides.

[0119] Alternatively, in preferred embodiments, a peptide identified in the course of the present invention can also induce CTLs specific to DEPDC1, when the peptide is applied to a subject having HLA-A2 or HLA-A24. Accordingly, through the administration of the peptide of the present invention, it is further expected that CTL response against cancer expressing DEPDC1 can be induced in addition to Th1 cell

induction. Moreover, the peptide of the present invention can not only induce CTL response against DEPDC1-expressing cells via processing thereof, but also enhance it by Th1 cell induction mediated thereby. Accordingly, in order to achieve inductions of both of Th1 cells and DEPDC1-specific CTLs in the same subject, for example, the subject to be treated preferably has HLA-DR4 or HLA-DP5 as an MHC class II molecule and HLA-A2 as an MHC class I molecule, when administering peptides having the amino acid sequence of SEQ ID NO: 2 or 3. In order to achieve inductions of both of Th1 cells and DEPDC1-specific CTLs in the same subject, for example, the subject to be treated preferably has HLA-DR4 as an MHC class II molecule and HLA-A24 as an MHC class I molecule, when administering peptides having the amino acid sequence of SEQ ID NO:2.

[0120] In the present invention, it was confirmed that peptides of the present invention promote an immunological response mediated by an MHC class II antigen, in particular, in an HLA type-restricted manner in the combinations as shown below:

DEPDC1-LP1: HLA-DR53 and HLA-DR4

DEPDC1-LP2: HLA-DR4

DEPDC1-LP3: HLA-DP5 and HLA-DR4

DEPDC1-LP4: HLA-DP5 and HLA-DR15

[0121] Therefore, DEPDC1-LP1, -LP2, -LP3 and -LP4 and a peptide comprising any one of the amino acid sequences of SEQ ID NO: 1-4 are useful for treating cancer expressing DEPDC1 in a patient who has at least one HLA allele selected from their corresponding HLA sub-types, shown in the above combinations. Alternatively, the present invention provides a pharmaceutical composition for treating a cancer expressing DEPDC1 in a patient, wherein the composition comprises any one of peptide selected from the group consisting of the peptides of the present invention, and wherein the patient has at least one HLA allele selected from the peptide's corresponding HLA sub-types, shown in the above combinations.

[0122] Further, the present invention also provides use of a peptide selected from the group consisting of the peptides of the present invention for manufacturing a composition for treating a cancer expressing DEPDC1 in a patient who has at least one HLA allele selected from the peptide's corresponding HLA sub-types, shown in the above combinations. Moreover, in some embodiments, the present invention provides a peptide selected from the group consisting of the peptides of the present invention for use in treatment of cancer expressing DEPDC1 in a patient who has at least one HLA allele selected from the peptide's corresponding HLA sub-types, shown in the above combinations. In further embodiments, the present invention provides a method for treating a cancer expressing DEPDC1 in a patient, which method comprises a step of administering a peptide selected from the group consisting of the peptides of the present

invention to the patient, wherein the patient has at least one HLA allele selected from the peptide's HLA sub-types in the above combinations.

[0123] In addition, in some embodiments, the present invention provides a method for manufacturing or formulating a pharmaceutical composition for treating a cancer expressing DEPDC1 in a patient, wherein the composition comprises any one of peptide selected from the group consisting of the peptides of the present invention, and wherein the patient has at least one HLA allele selected from the peptide's corresponding HLA sub-types, shown in the above combinations. The method of the present invention, for example, may comprise a step for admixing or formulating any one of peptide selected from the group consisting of the peptides of the present invention, and pharmaceutically acceptable carrier.

[0124] As discussed above, it is well known that Th1 cells are important for induction of effective tumor immunity in tumor-bearing hosts. Peptides of the present invention have an ability to induce Th1 cells in an HLA restricted manner, the specific HLA restriction pattern for each of the peptides of the present invention being shown above. Accordingly, the present invention provides a composition for promoting or enhancing a Th1 cell response for cancer expressing DEPDC1 in a patient, wherein the composition comprises any one of peptide selected from the group consisting of the peptides of the present invention, and wherein the patient has at least one HLA allele selected from the peptide's corresponding HLA sub-types, shown above.

[0125] Further, the present invention also provides use of a peptide selected from the group consisting of the peptides of the present invention for manufacturing a composition for promoting or enhancing a Th1 cell response for cancer expressing DEPDC1 in a patient who has at least one HLA allele selected from the peptide's corresponding HLA sub-types, shown in the above combinations. Moreover, in some embodiments, the present invention provides a peptide selected from the group consisting of the peptides of the present invention for use in promoting or enhancing a Th1 cell response for cancer expressing DEPDC1 in a patient who has at least one HLA allele selected from the peptide's corresponding HLA sub-types, shown in the above combinations. In further embodiments, the present invention provides a method for promoting or enhancing a Th1 cell response for cancer expressing DEPDC1 in a patient, which method comprises a step of administering a peptide selected from the group consisting of the peptides of the present invention to the patient, wherein the patient has at least one HLA allele selected from the peptide's HLA sub-types in the above combinations.

[0126] In addition, in some embodiments, the present invention provides a method for manufacturing or formulating a pharmaceutical composition for promoting or enhancing a Th1 cell response for cancer expressing DEPDC1 in a patient, wherein the composition comprises any one of peptide selected from the group consisting of the peptides of the

present invention, and wherein the patient has at least one HLA allele selected from the peptide's corresponding HLA sub-types, shown in the above combinations. The method of the present invention, for example, may comprise a step for admixing or formulating any one of peptide selected from the group consisting of the peptides of the present invention, and pharmaceutically acceptable carrier.

[0127] In another embodiment, the present invention provides an immunological cancer therapy dependent on Th1 cell induction. The therapeutic strategy provided by the present invention is applicable to and effective for any cancers independent of DEPDC1 expression, as long as immune cells activated by cytokines secreted from Th1 cells target objective cancer cells.

[0128] Cancers or tumors to be treated by the pharmaceutical agents or compositions of the present invention include any kinds of cancers or tumors expressing DEPDC1, including, but are not limited to, for example, bladder cancer, breast cancer.

[0129] The present pharmaceutical agents or compositions can contain in addition to the aforementioned active ingredients, other peptides that have the ability to induce Th1 cells or CTLs, other polynucleotides encoding the other peptides, other cells that present the other peptides or fragment thereof, and the like. Examples of such "other" peptides having the ability to induce Th1 cells or CTLs include, but are not limited to, peptides derived from cancer specific antigens (e.g., identified TAAs).

[0130] If necessary, the pharmaceutical agents or compositions of the present invention can optionally include other therapeutic substances as an additional active ingredient, so long as the substance does not inhibit the antitumoral effect of the active ingredient, e.g., any of the present peptides. For example, formulations can include anti-inflammatory agents, pain killers, chemotherapeutics, and the like. In addition to including other therapeutic substances in the medicament itself, the medicaments of the present invention can also be administered sequentially or concurrently with the one or more other pharmacologic agents. The amounts of medicament and pharmacologic agent depend, for example, on what type of pharmacologic agent(s) is/are used, the disease being treated, and the scheduling and routes of administration.

[0131] Those of skill in the art will recognize that, in addition to the ingredients particularly mentioned herein, the pharmaceutical agents or compositions of the present invention can include other agents conventional in the art having regard to the type of formulation in question (e.g., fillers, binders, diluents, excipients, etc.).

[0132] In one embodiment of the present invention, the present pharmaceutical agents or compositions can be included in articles of manufacture and kits containing materials useful for treating the pathological conditions of the disease to be treated, e.g., cancer. The article of manufacture can include a container of any of the present pharmaceutical agents or compositions with a label. Suitable containers include bottles, vials, and test

tubes. The containers can be formed from a variety of materials, such as glass or plastic. The label on the container should indicate the agent is used for treating or prevention of one or more conditions of the disease. The label can also indicate directions for administration and so on.

[0133] In addition to the container described above, a kit including a pharmaceutical agent or composition of the present invention can optionally further include a second container housing a pharmaceutically-acceptable diluent. It can further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

[0134] The pharmaceutical agents or compositions can, if desired, be packaged in a pack or dispenser device that can contain one or more unit dosage forms containing the active ingredient. The pack can, for example, include metal or plastic foil, such as a blister pack. The pack or dispenser device can be accompanied by instructions for administration.

[0135] (1) Pharmaceutical agents or compositions containing the peptides as the active ingredient:

The peptide of the present invention can be administered directly as a pharmaceutical agent or composition, or if necessary, that has been formulated by conventional formulation methods. In the latter case, in addition to the peptides of the present invention, carriers, excipients, and such that are ordinarily used for drugs can be included as appropriate without particular limitations. Examples of such carriers include, but are not limited to, sterilized water, physiological saline, phosphate buffer, culture fluid and such. Furthermore, the pharmaceutical agents or compositions can contain as necessary, stabilizers, suspensions, preservatives, surfactants and such. The pharmaceutical agents or compositions of the present invention can be used for anticancer purposes.

[0136] The peptides of the present invention can be prepared in a combination, composed of two or more of peptides of the present invention to induce Th1 cells in vivo. The peptide combination can take the form of a cocktail or can be conjugated to each other using standard techniques. For example, the peptides can be chemically linked or expressed as a single fusion polypeptide sequence. The peptides in the combination can be the same or different.

[0137] By administering the peptides of the present invention, the peptides or fragments thereof are presented at a high density by the HLA class II antigens on APCs, then Th1 cells that specifically react toward the complex formed between the displayed peptide and the HLA class II antigen are induced. Alternatively, APCs (e.g., DCs) are removed from subjects and then stimulated by the peptides of the present invention to obtain APCs that present any of the peptides of this invention or fragments thereof on their

surface. These APCs can be readministered to the subjects to induce Th1 cells in the subjects, and as a result, aggressiveness towards the tumor-associated endothelium can be increased.

[0138] The pharmaceutical agents or compositions for the treatment and/or prevention of cancer or tumor that include a peptide of the present invention as the active ingredient, can also include an adjuvant known to effectively establish cellular immunity. Alternatively, the pharmaceutical agents or compositions can be administered with other active ingredients or can be administered by formulation into granules. An adjuvant refers to a compound that enhances the immune response against the protein when administered together (or successively) with the protein having immunological activity. Adjuvants contemplated herein include those described in the literature (Clin Microbiol Rev 1994, 7: 277-89). Examples of suitable adjuvants include, but are not limited to, aluminum phosphate, aluminum hydroxide, alum, cholera toxin, salmonella toxin, Incomplete Freund's adjuvant (IFA), Complete Freund's adjuvant (CFA), ISCOMatrix, GM-CSF, CpG, O/W emulsion, and the like.

[0139] Furthermore, liposome formulations, granular formulations in which the peptide is bound to few-micrometers diameter beads, and formulations in which a lipid is bound to the peptide may be conveniently used.

[0140] In another embodiment of the present invention, the peptides of the present invention may also be administered in the form of a pharmaceutically acceptable salt. Examples of preferred salts include salts with an alkali metal, salts with a metal, salts with an organic base, salts with an organic acid (e.g., acetic acid, formic acid, propionic acid, fumaric acid, maleic acid, succinic acid, tartaric acid, citric acid, malic acid, oxalic acid, benzoic acid, methanesulfonic acid and so on) and salts with an inorganic acid (e.g., hydrochloric acid, phosphoric acid, hydrobromic acid, sulfuric acid and so on). As used herein, the phrase "pharmaceutically acceptable salt" refers to those salts that retain the biological effectiveness and properties of the compound and that are obtained by reaction with inorganic acids or bases such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid and the like.

[0141] In some embodiments, the pharmaceutical agents or compositions of the present invention may further include a component which primes Th1 cells and optionally CTLs. Lipids have been identified as agents capable of priming Th1 cells and optionally CTLs in vivo against viral antigens. For example, palmitic acid residues can be attached to the epsilon- and alpha-amino groups of a lysine residue and then linked to a peptide of the present invention. The lipidated peptide can then be administered either directly in a micelle or particle, incorporated into a liposome, or emulsified in an adjuvant. As another example of lipid priming of Th1 cell and optionally CTL

responses, E. coli lipoproteins, such as tripalmitoyl-S-glycerylcysteinlyseryl- serine (P3CSS) can be used to prime Th1 cells and optionally CTLs when covalently attached to an appropriate peptide (see, e.g., Deres et al., Nature 1989, 342: 561-4).

[0142] Examples of suitable methods of administration include, but are not limited to, oral, intradermal, subcutaneous, intramuscular, intraosseous, peritoneal, and intravenous injection, or such, and systemic administration or local administration to the vicinity of the targeted sites (i.e., direct injection). The administration can be performed by single administration or boosted by multiple administrations. A pharmaceutically or therapeutically effective amount of the peptide of the present invention can be administered to a subject in need of treatment of cancer expressing DEPDC1. Alternatively, an amount of the peptide of the present invention sufficient to enhance or stimulate immunological response mediated with Th1 cells, and/or to induce CTLs against cancer or tumor expressing DEPDC1 can be administered to a subject carrying a cancer expressing DEPDC1. The dose of the peptides of the present invention can be adjusted appropriately depending on a disease to be treated, a patient's age and weight, a method of administration, and such. The dose of the peptide may be ordinarily 0.001 mg to 1000 mg, for example, 0.01 mg to 100 mg, for example, 0.1 mg to 10 mg, for example, 0.5 mg to 5 mg, and the peptide can be administered once in a few days to a few months. One skilled in the art can readily determine suitable and optimal dosages.

[0143] (2) Pharmaceutical agents or compositions containing polynucleotides as the active ingredient:

The pharmaceutical agents or compositions of the present invention can also contain polynucleotides encoding the peptides disclosed herein in an expressible form. Herein, the phrase "in an expressible form" means that the polynucleotide, when introduced into a cell, will be expressed in vivo as a polypeptide that induces anti-tumor immunity. In an illustrative embodiment, the nucleic acid sequence of the polynucleotide of interest includes regulatory elements necessary for expression of the polynucleotide. The polynucleotide(s) can be equipped with sequences useful to achieve stable insertion into the genome of the target cell (see, e.g., Thomas KR & Capecchi MR, Cell 1987, 51: 503-12 for a description of homologous recombination cassette vectors). See, e.g., Wolff et al., Science 1990, 247: 1465-8; U.S. Patent Nos. 5,580,859; 5,589,466; 5,804,566; 5,739,118; 5,736,524; 5,679,647; and WO 98/04720. Examples of DNA-based delivery technologies include "naked DNA", facilitated (bupivacaine, polymers, peptide-mediated) delivery, cationic lipid complexes, and particle-mediated ("gene gun") or pressure-mediated delivery (see, e.g., U.S. Patent No. 5,922,687).

[0144] The peptides of the present invention can also be expressed by viral or bacterial vectors. Examples of expression vectors include attenuated viral hosts, such as

vaccinia or fowlpox. This approach involves the use of vaccinia virus, e.g., as a vector to express nucleotide sequences that encode the peptide. Upon introduction into a host, the recombinant vaccinia virus expresses the immunogenic peptide, and thereby elicits an immune response. Vaccinia vectors and methods useful in immunization protocols are described in, e.g., U.S. Patent No. 4,722,848. Another vector is BCG (Bacille Calmette Guerin). BCG vectors are described in Stover et al., Nature 1991, 351: 456-60. A wide variety of other vectors useful for therapeutic administration or immunization e.g., adeno and adeno-associated virus vectors, retroviral vectors, Salmonella typhi vectors, detoxified anthrax toxin vectors, and the like, will be apparent. See, e.g., Shata et al., Mol Med Today 2000, 6: 66-71; Shedlock et al., J Leukoc Biol 2000, 68: 793-806; Hipp et al., In Vivo 2000, 14: 571-85.

[0145] Delivery of a polynucleotide into a subject can be either direct, in which case the subject is directly exposed to a polynucleotide-carrying vector, or indirect, in which case, cells are first transformed with the polynucleotide of interest in vitro, then the cells are transplanted into the subject. These two approaches are known, respectively, as in vivo and ex vivo gene therapies.

[0146] For general reviews of the methods of gene therapy, see Goldspiel et al., Clinical Pharmacy 1993, 12: 488-505; Wu and Wu, Biotherapy 1991, 3: 87-95; Tolstoshev, Ann Rev Pharmacol Toxicol 1993, 33: 573-96; Mulligan, Science 1993, 260: 926-32; Morgan & Anderson, Ann Rev Biochem 1993, 62: 191-217; Trends in Biotechnology 1993, 11(5): 155-215. Methods commonly known in the art of recombinant DNA technology which can also be used for the present invention are described in eds. Ausubel et al., Current Protocols in Molecular Biology, John Wiley & Sons, NY, 1993; and Krieger, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY, 1990.

[0147] Like administration of peptides, administration of polynucleotides may be performed by oral, intradermal, subcutaneous, intravenous, intramuscular, intraosseous, and/or peritoneal injection, or such, and via systemic administration or local administration to the vicinity of the targeted sites finds use. The administration can be performed by single administration or boosted by multiple administrations. A pharmaceutically or therapeutically effective amount of the polynucleotide of the present invention can be administered to a subject in need of treatment of cancer expressing DEPDC1. Alternatively, an amount of the polynucleotide of the present invention sufficient to enhance or stimulate immunological response mediated with Th1 cells, and/or to induce CTLs against cancer or tumor expressing DEPDC1 can be administered to a subject carrying a cancer expressing DEPDC1. The dose of the polynucleotide in the suitable carrier or cells transformed with the polynucleotide encoding the peptides of the present invention can be adjusted appropriately depending on a disease to be treated, a patient's

age and weight, a method of administration, and such. The dose of the peptide may be ordinarily 0.001 mg to 1000 mg, for example, 0.01 mg to 100 mg, for example, 0.1 mg to 10 mg, for example, 0.5 mg to 5 mg, and the peptide can be administered once every a few days to once every a few months. One skilled in the art can readily determine suitable and optimal dosages.

[0148] IX. Methods Using the Peptides, APCs or Th1 Cells

The peptides of the present invention and polynucleotides encoding such peptides can be used for inducing APCs and Th1 cells of the present invention. The APCs of the present invention can be also used for inducing Th1 cells of the present invention. The peptides, polynucleotides, and APCs can be used in combination with any other compounds so long as the compounds do not inhibit their Th1 cell inducibility. Thus, any of the aforementioned pharmaceutical agents or compositions of the present invention can be used for inducing Th1 cells, and in addition thereto, those including the peptides or polynucleotides of the present invention can be also used for inducing APCs as discussed below.

[0149] (1) Method of inducing antigen-presenting cells (APCs):

The present invention provides methods of inducing APCs using the peptides of the present invention or polynucleotides encoding the peptides. The induction of APCs can be performed as described above in section "V. Antigen-presenting cells". The present invention also provides a method for inducing APCs having Th1 cell inducibility, the induction of which has been also mentioned under the item of "V. Antigen-presenting cells", supra.

[0150] Alternatively, the present invention provides a method for preparing an APC which has ability to induce a Th1 cell, wherein the method can include one of the following steps:

(a) contacting an APC with a peptide of the present invention in vitro, ex vivo or in vivo; and

(b) introducing a polynucleotide encoding a peptide of the present invention into an APC.

Alternatively, the present invention provides methods for inducing an APC having Th1 cell inducibility, wherein the methods include the step selected from the group consisting of:

(a) contacting an APC with the peptide of the present invention, and

(b) introducing the polynucleotide encoding the peptide of the present invention into an APC.

[0151] The methods of the present invention can be carried out in vitro, ex vivo or in vivo. Preferably, the methods of the present invention can be carried out in vitro or ex vivo. In preferred embodiment, APCs used for induction of APCs having Th1 cell in-

ducibility can be preferably APCs expressing at least one selected from among HLA-DR53, HLA-DR4, HLA-DR15 and HLA-DP5 as an MHC class II molecule. Such APCs can be prepared by the methods well-known in the arts from peripheral blood mononuclear cells (PBMCs) obtained from a subject having at least one selected from among HLA-DR53, HLA-DR4, HLA-DR15 and HLA-DP5 as an MHC class II molecule. The APCs induced by the method of the present invention can be APCs that present a complex of the peptide of the present invention or fragment thereof and HLA class II antigen (e.g., HLA-DR53, HLA-DR4, HLA-DR15 and HLA-DP5) on their surface. When APCs induced by the method of the present invention are administered to a subject in order to induce immune responses against cancer in the subject, the subject is preferably the same one from whom APCs are derived. However, the subject may be a different one from the APC donor so long as the subject has the same HLA type with the APC donor.

[0152] In another embodiment, the present invention provides agents or compositions for use in inducing an APC having Th1 cell inducibility, and such agents or compositions include one or more peptides or polynucleotides of the present invention.

[0153] In another embodiment, the present invention provides the use of the peptide of the present invention or the polynucleotide encoding the peptide in the manufacture of an agent or composition formulated for inducing APCs.

[0154] Alternatively, the present invention further provides the peptide of the present invention or the polynucleotide encoding the peptide for use in inducing an APC having Th1 cell inducibility.

[0155] In preferred embodiments, the peptides of the present invention can induce not only Th1 response but also CTL response after being processed in a APC. Accordingly, in preferred embodiments, APCs prepared by the method of the present invention can be also useful for inducing CTLs against DEPDC1 expressing cells, including cancer cells. For example, when induced by the peptides containing the amino acid sequence of SEQ ID NO: 5, APCs expressing HLA-A2 are suitable for inducing DEPDC1-specific CTLs. Alternatively, when induced by the peptides containing the amino acid sequence of SEQ ID NO: 6, APCs expressing HLA-A24 are suitable for inducing DEPDC1-specific CTLs.

[0156] (2) Method of inducing Th1 cells:

Furthermore, the present invention provides methods for inducing Th1 cells using the peptides of the present invention, polynucleotides encoding the peptides or APCs presenting the peptides of the present invention or fragments thereof. The present invention also provides methods for inducing Th1 cells using a polynucleotide encoding a polypeptide that is capable of forming a TCR subunit recognizing a complex of the peptides of the present invention and HLA class II antigens. Preferably,

the methods for inducing Th1 cells comprise at least one step selected from the group consisting of:

(a) contacting a CD4-positive T cell with an APC that presents on its surface a complex of an HLA class II antigen and the peptide of the present invention or fragment thereof, and

(b) introducing a polynucleotide encoding both of TCR subunits or polynucleotides encoding each of TCR subunits, wherein the TCR can recognize or bind to a complex of the peptide of the present invention or fragment thereof and an HLA class II antigen, into a CD4-positive T cell.

[0157] When the peptides of the present invention are administered to a subject, Th1 cells are induced in the body of the subject, and immune responses mediated by MHC class II molecules (e.g., immune responses targeting cancer cells) are enhanced. Alternatively, the peptides and polynucleotides encoding the peptides can be used for an ex vivo therapeutic method, in which subject-derived APCs and CD4-positive cells, or peripheral blood mononuclear leukocytes are contacted (stimulated) with the peptides of the present invention in vitro, and after inducing Th1 cells, the activated Th1 cells are returned to the subject. For example, the method can include the steps of:

(a) collecting APCs from subject:

(b) contacting the APCs of step (a), with the peptide of the present invention:

(c) mixing the APCs of step (b) with $CD4^+$ T cells, and co-culturing for inducing Th1 cells: and

(d) collecting $CD4^+$ T cells from the co-culture of step (c).

Furthermore, Th1 cells can be induced by introducing a polynucleotide encoding both of TCR subunits or polynucleotides encoding each of TCR subunits, wherein the TCR can bind to a complex of the peptide of the present invention or fragment thereof and an HLA class II antigen, into CD4-positive T cells. Such transduction can be performed as described above in section "VII. T Cell Receptor (TCR)".

[0158] The methods of the present invention can be carried out in vitro, ex vivo or in vivo. Preferably, the methods of the present invention can be carried out in vitro or ex vivo. CD4 positive T cells used for induction of Th1 cells can be prepared by well-known methods in the art from PBMCs obtained from a subject. In preferred embodiments, the donor for CD4-positive T cells can be a subject having at least one selected from among HLA-DR53, HLA-DR4, HLA-DR15 and HLA-DP5 as an MHC class II molecule. The Th1 cells induced by the methods of the present invention can be Th1 cells that can recognize APCs presenting a complex of the peptide of the present invention or fragment thereof and HLA class II antigen on its surface. When Th1 cells induced by the method of the present invention are administered to a subject in order to induce immune responses against cancer in the subject (or immune responses mediated

by MHC class I molecules), the subject is preferably the same one from whom CD4-positive T cells are derived. However, the subject may be a different one from the CD4-positive T cell donor so long as the subject has the same HLA type with the CD4-positive T cell donor.

[0159] In preferred embodiments, the peptides of the present invention can induce CTLs against DEPDC1 expressing cells, as well as Th1 cells. Therefore, the present invention further provides a method for inducing a CTL, which comprises at least one step selected from the group consisting of:

(a) co-culturing both of a CD4-positive T cell and a CD8-positive T cell with APCs contacted with the peptide of the present invention; and

(b) co-culturing a CD8-positive T cell with an APC contacted with the peptide of the present invention.

[0160] In such methods of inducing CTLs, the peptides of the present invention are processed in APCs to produce CTL epitope peptides, and produced CTL epitope peptides are presented on APC's surface.

[0161] Alternatively, according to the present invention, use of the peptides of the present invention for manufacturing a pharmaceutical agent or composition inducing Th1 cells is provided. In addition, the present invention provides a method or process for manufacturing a pharmaceutical agent or composition inducing Th1 cells, wherein the method comprises the step for admixing or formulating the peptide of the present invention with a pharmaceutically acceptable carrier. Further, the present invention also provides the peptide of the present invention for inducing Th1 cells.

[0162] The $CD4^+$ T cells induced by the method of the present invention can be administered to a subject as a vaccine.

[0163] In the context of the present invention, cancer overexpressing DEPDC1 can be treated with these active ingredients. Examples of such cancers include, but are not limited to, bladder cancer and breast cancer. Accordingly, prior to the administration of the vaccines or pharmaceutical compositions comprising the active ingredients, it is preferable to confirm whether the expression level of DEPDC1 in the cancer cells or tissues to be treated is enhanced as compared with normal cells of the same organ. Thus, in one embodiment, the present invention provides a method for treating cancer (over)expressing DEPDC1, which method may include the steps of:

(i) determining the expression level of DEPDC1 in cancer cells or tissue(s) obtained from a subject with the cancer to be treated;

(ii) comparing the expression level of DEPDC1 with normal control; and

(iii) administrating at least one component selected from the group consisting of (a) to (d) described above to a subject with cancer overexpressing DEPDC1 compared with normal control.

[0164] Alternatively, the present invention may provide a vaccine or pharmaceutical composition that includes at least one component selected from the group consisting of (a) to (d) described above, for use in administrating to a subject having cancer overexpressing DEPDC1. In other words, the present invention further provides a method for identifying a subject to be treated with a DEPDC1 polypeptide of the present invention, such method including the step of determining an expression level of DEPDC1 in subject-derived cancer cells or tissue(s), wherein an increase of the level compared to a normal control level of the gene indicates that the subject has cancer which may be treated with the DEPDC1 polypeptide of the present invention. Methods of treating cancer of the present invention are described in more detail below.

[0165] Further, in preferred embodiments, the HLA type of a subject may be identified before administering the peptides of the present invention. For example, peptides having the amino acid sequence of SEQ ID NO: 1 are preferably administered to a subject identified as having HLA-DR53 or HLA-DR4. Alternatively, peptides having the amino acid sequence of SEQ ID NO: 2 are preferably administered to a subject identified as having HLA-DR4. Alternatively, peptides having the amino acid sequence of SEQ ID NO: 3 are preferably administered to a subject identified as having HLA-DP5 or HLA-DR4. Alternatively, peptides having the amino acid sequence of SEQ ID NO: 4 are preferably administered to a subject identified as having HLA-DP5 or HLA-DR15.

[0166] Any subject-derived cell or tissue can be used for the determination of DEPDC1-expression so long as it includes the objective transcription or translation product of DEPDC1. Examples of suitable samples include, but are not limited to, bodily tissues and fluids, such as blood, sputum and urine. Preferably, the subject-derived cell or tissue sample contains a cell population including an epithelial cell, more preferably a cancerous epithelial cell or an epithelial cell derived from tissue suspected to be cancerous. Further, if necessary, the cell may be purified from the obtained bodily tissues and fluids, and then used as the subjected-derived sample.

[0167] A subject to be treated by the present method is preferably a mammal. Exemplary mammals include, but are not limited to, e.g., human, non-human primate, mouse, rat, dog, cat, horse, and cow.

[0168] According to the present invention, the expression level of DEPDC1 in cancer cells or tissues obtained from a subject may be determined. The expression level can be determined at the transcription (nucleic acid) product level, using methods known in the art. For example, the mRNA of DEPDC1 may be quantified using probes by hybridization methods (e.g., Northern hybridization). The detection may be carried out on a chip or an array. The use of an array is preferable for detecting the expression level of DEPDC1. Those skilled in the art can prepare such probes utilizing the sequence in-

formation of DEPDC1. For example, the cDNA of DEPDC1 may be used as the probes. If necessary, the probes may be labeled with a suitable label, such as dyes, fluorescent substances and isotopes, and the expression level of the gene may be detected as the intensity of the hybridized labels.

[0169] Furthermore, the transcription product of DEPDC1 (e.g., SEQ ID NO: 7 or 9) may be quantified using primers by amplification-based detection methods (e.g., RT-PCR). Such primers may be prepared based on the available sequence information of the gene.

[0170] Specifically, a probe or primer used for the present method hybridizes under stringent, moderately stringent, or low stringent conditions to the mRNA of DEPDC1. As used herein, the phrase "stringent (hybridization) conditions" refers to conditions under which a probe or primer will hybridize to its target sequence, but not to other sequences. Stringent conditions are sequence-dependent and will be different under different circumstances. Specific hybridization of longer sequences is observed at higher temperatures than shorter sequences. Generally, the temperature of a stringent condition is selected to be about 5 degrees C lower than the thermal melting point (Tm) for a specific sequence at a defined ionic strength and pH. The Tm is the temperature (under a defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to their target sequence hybridize to the target sequence at equilibrium. Since the target sequences are generally present at excess, at Tm, 50% of the probes are occupied at equilibrium. Typically, stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30 degrees C for short probes or primers (e.g., 10 to 50 nucleotides) and at least about 60 degrees C for longer probes or primers. Stringent conditions may also be achieved with the addition of destabilizing agents, such as formamide.

[0171] Alternatively, the translation product may be detected for the diagnosis of the present invention. For example, the quantity of DEPDC1 protein (SEQ ID NO: 9 or 11) may be determined. Methods for determining the quantity of the protein as the translation product include immunoassay methods that use an antibody specifically recognizing the protein. The antibody may be monoclonal or polyclonal. Furthermore, any fragment or modification (e.g., chimeric antibody, scFv, Fab, $F(ab')_2$, Fv, etc.) of the antibody may be used for the detection, so long as the fragment or modified antibody retains the binding ability to the DEPDC1 protein. Methods to prepare these kinds of antibodies for the detection of proteins are well known in the art, and any method may be employed in the present invention to prepare such antibodies and equivalents thereof.

[0172] As another method to detect the expression level of DEPDC1 gene based on its

translation product, the intensity of staining may be measured via immunohistochemical analysis using an antibody against the DEPDC1 protein. Namely, in this measurement, strong staining indicates increased presence/level of the protein and, at the same time, high expression level of DEPDC1 gene.

[0173] The expression level of a target gene, e.g., the DEPDC1 gene, in cancer cells can be determined to be increased if the level increases from the control level (e.g., the level in normal cells) of the target gene by, for example, 10%, 25% or 50%; or increases to more than 1.1 fold, more than 1.5 fold, more than 2.0 fold, more than 5.0 fold, more than 10.0 fold, or more.

[0174] The control level may be determined at the same time as the cancer cells, by using a sample(s) previously collected and stored from a subject/subjects whose disease state(s) (cancerous or non-cancerous) is/are known. In addition, normal cells obtained from non-cancerous regions of an organ that has the cancer to be treated may be used as normal control. Alternatively, the control level may be determined by a statistical method based on the results obtained by analyzing previously determined expression level(s) of DEPDC1 gene in samples from subjects whose disease states are known. Furthermore, the control level can be derived from a database of expression patterns from previously tested cells. Moreover, according to an aspect of the present invention, the expression level of DEPDC1 gene in a biological sample may be compared to multiple control levels determined from multiple reference samples. It is preferred to use a control level determined from a reference sample derived from a tissue type similar to that of the subject-derived biological sample. Moreover, it is preferred to use the standard value of the expression levels of DEPDC1 gene in a population with a known disease state. The standard value may be obtained by any method known in the art. For example, a range of mean +/- 2 S.D. or mean +/- 3 S.D. may be used as the standard value.

[0175] In the context of the present invention, a control level determined from a biological sample that is known to be non-cancerous is referred to as a "normal control level". On the other hand, if the control level is determined from a cancerous biological sample, it is referred to as a "cancerous control level". Difference between a sample expression level and a control level can be normalized to the expression level of control nucleic acids, e.g., housekeeping genes, whose expression levels are known not to differ depending on the cancerous or non-cancerous state of the cell. Exemplary control genes include, but are not limited to, beta-actin, glyceraldehyde 3 phosphate dehydrogenase, and ribosomal protein P1.

[0176] When the expression level of DEPDC1 gene is increased as compared to the normal control level, or is similar/equivalent to the cancerous control level, the subject may be diagnosed with cancer to be treated.

[0177] More specifically, the present invention provides a method of (i) diagnosing whether a subject has the cancer to be treated, and/or (ii) selecting a subject for cancer treatment, which method includes the steps of:

(a) determining the expression level of DEPDC1 in cancer cells or tissue(s) obtained from a subject who is suspected to have the cancer to be treated;

(b) comparing the expression level of DEPDC1 with a normal control level;

(c) diagnosing the subject as having the cancer to be treated, if the expression level of DEPDC1 is increased as compared to the normal control level; and

(d) selecting the subject for cancer treatment, if the subject is diagnosed as having the cancer to be treated, in step (c).

[0178] Alternatively, such a method includes the steps of:

(a) determining the expression level of DEPDC1 in cancer cells or tissue(s) obtained from a subject who is suspected to have the cancer to be treated;

(b) comparing the expression level of DEPDC1 with a cancerous control level;

(c) diagnosing the subject as having the cancer to be treated, if the expression level of DEPDC1 is similar or equivalent to the cancerous control level; and

(d) selecting the subject for cancer treatment, if the subject is diagnosed as having the cancer to be treated, in step (c).

[0179] In some embodiments, such a method may further comprise the step of identifying, after or before the steps (a)-(d) defined above, a subject having an HLA selected from the group consisting of HLA-DR53, HLA-DR4, HLA-DR15 and HLA-DP5. Cancer therapy according to the present invention is preferable for a subject that suffers from cancer overexpressing DEPDC1 and has any one of HLA-DR53, HLA-DR4, HLA-DR15 and HLA-DP5. Methods for HLA typing are well known in the art. For example, PCR-based methods for typing HLA alleles are well known. Antibodies specific for each HLA molecule are also appropriate tools for identifying HLA types of a subject.

[0180] The present invention also provides a kit for determining a subject suffering from cancer that can be treated with the DEPDC1 polypeptide of the present invention, which may also be useful in assessing and/or monitoring the efficacy of a particular cancer therapy, more particularly a cancer immunotherapy. Examples of suitable cancers include, but are not limited to, bladder cancer and breast cancer. More particularly, the kit preferably includes at least one reagent for detecting the expression of the DEPDC1 gene in a subject-derived cancer cell, such reagent being selected from the group of:

(a) a reagent for detecting an mRNA of the DEPDC1 gene;

(b) a reagent for detecting the DEPDC1 protein; and

(c) a reagent for detecting the biological activity of the DEPDC1 protein.

[0181] Examples of reagents suitable for detecting an mRNA of the DEPDC1 gene include nucleic acids that specifically bind to or identify the DEPDC1 mRNA, such as oligonucleotides that have a complementary sequence to a portion of the DEPDC1 mRNA. These kinds of oligonucleotides are exemplified by primers and probes that are specific to the DEPDC1 mRNA. These kinds of oligonucleotides may be prepared based on methods well known in the art. If needed, the reagent for detecting the DEPDC1 mRNA may be immobilized on a solid matrix. Moreover, more than one reagent for detecting the DEPDC1 mRNA may be included in the kit.

[0182] On the other hand, examples of reagents suitable for detecting the DEPDC1 protein include antibodies to the DEPDC1 protein. The antibody may be monoclonal or polyclonal. Furthermore, any fragment or modification (e.g., chimeric antibody, scFv, Fab, $F(ab')_2$, Fv, etc.) of the antibody may be used as the reagent, so long as the fragment or modified antibody retains the binding ability to the DEPDC1 protein. Methods to prepare these kinds of antibodies for the detection of proteins are well known in the art, and any method may be employed in the present invention to prepare such antibodies and equivalents thereof. Furthermore, the antibody may be labeled with signal generating molecules via direct linkage or an indirect labeling technique. Labels and methods for labeling antibodies and detecting the binding of the antibodies to their targets are well known in the art, and any labels and methods may be employed for the present invention. Moreover, more than one reagent for detecting the DEPDC1 protein may be included in the kit.

[0183] The kit may contain more than one of the aforementioned reagents. For example, tissue samples obtained from subjects without cancer or suffering from cancer, may serve as useful control reagents. A kit of the present invention may further include other materials desirable from a commercial and user standpoint, including buffers, diluents, filters, needles, syringes, and package inserts (e.g., written, tape, CD-ROM, etc.) with instructions for use. These reagents and such may be retained in a container with a label. Suitable containers include bottles, vials, and test tubes. The containers may be formed from a variety of materials, such as glass or plastic.

[0184] As an embodiment of the present invention, when the reagent is a probe against the DEPDC1 mRNA, the reagent may be immobilized on a solid matrix, such as a porous strip, to form at least one detection site. The measurement or detection region of the porous strip may include a plurality of sites, each containing a nucleic acid (probe). A test strip may also contain sites for negative and/or positive controls. Alternatively, control sites may be located on a strip separated from the test strip. Optionally, the different detection sites may contain different amounts of immobilized nucleic acids, i.e., a higher amount in the first detection site and lesser amounts in subsequent sites. Upon the addition of a test sample, the number of sites displaying a detectable signal

provides a quantitative indication of the amount of DEPDC1 mRNA present in the sample. The detection sites may be configured in any suitably detectable shape and are typically in the shape of a bar or dot spanning the width of a test strip.

[0185] The kit of the present invention may further include a positive control sample or DEPDC1 standard sample. The positive control sample of the present invention may be prepared by collecting DEPDC1 positive samples and then assaying their DEPDC1 levels. Alternatively, a purified DEPDC1 protein or polynucleotide may be added to cells that do not express DEPDC1 to form the positive sample or the DEPDC1 standard sample. In the present invention, purified DEPDC1 may be a recombinant protein. The DEPDC1 level of the positive control sample is, for example, more than the cut off value.

[0186] X. Antibodies:

The present invention further provides antibodies that bind to the peptide of the present invention. Preferred antibodies specifically bind to the peptide of the present invention and will not bind (or will bind weakly) to other peptides. Alternatively, antibodies bind to the peptide of the invention as well as the homologs thereof. Antibodies against the peptide of the invention can find use in cancer diagnostic and prognostic assays, as well as imaging methodologies. Similarly, such antibodies can find use in the treatment, diagnosis, and/or prognosis of other cancers, to the extent DEPDC1 is also expressed or over-expressed in a cancer patient. Moreover, intracellularly expressed antibodies (e.g., single chain antibodies) may therapeutically find use in treating cancers in which the expression of DEPDC1 is involved, examples of which include, but are not limited to, bladder cancer and breast cancer.

[0187] The present invention also provides various immunological assay for the detection and/or quantification of DEPDC1 protein (SEQ ID NO: 8 or 10) or fragments thereof including a polypeptide composed of amino acid sequences selected from among SEQ ID NOs: 1 to 4. Such assays may include one or more anti-DEPDC1 antibodies capable of recognizing and binding a DEPDC1 protein or fragments thereof, as appropriate. In the present invention, anti-DEPDC1 antibodies binding to DEPDC1 polypeptide preferably recognize a polypeptide composed of amino acid sequences selected from among SEQ ID NOs: 1 to 4, preferably to the exclusion of other peptides. The binding specificity of antibody can be confirmed with inhibition test. That is, when the binding between an antibody to be analyzed and full-length of DEPDC1 polypeptide is inhibited under presence of any fragment polypeptides having an amino acid sequence selected from among SEQ ID NOs: 1 to 4, the antibody is deemed to "specifically bind" the fragment. In the context of the present invention, such immunological assays are performed within various immunological assay formats well known in the art, including but not limited to, various types of radio-im-

munoassays, immuno-chromatograph technique, enzyme-linked immunosorbent assays (ELISA), enzyme-linked immunofluorescent assays (ELIFA), and the like.

[0188] Related immunological but non-antibody assays of the invention may also include T cell immunogenicity assays (inhibitory or stimulatory) as well as MHC binding assays. In addition, immunological imaging methods capable of detecting cancers expressing DEPDC1 are also provided by the invention, including, but not limited to, radioscintigraphic imaging methods using labeled antibodies of the present invention. Such assays can clinically find use in the detection, monitoring, and prognosis of DEPDC1 expressing cancers, examples of which include, but are not limited to, bladder cancer and breast cancer.

[0189] The present invention also provides antibodies that bind to a peptide of the invention. An antibody of the invention can be used in any form, such as monoclonal or polyclonal antibodies, and include antiserum obtained by immunizing an animal such as a rabbit with the peptide of the invention, all classes of polyclonal and monoclonal antibodies, human antibodies and humanized antibodies produced by genetic recombination.

[0190] A peptide of the invention used as an antigen to obtain an antibody may be derived from any animal species, but preferably is derived from a mammal such as a human, mouse, or rat, more preferably from a human. A human-derived peptide may be obtained from the nucleotide or amino acid sequences disclosed herein.

According to the present invention, complete and partial peptides of polypeptide of the present invention may serve as immunization antigens. Examples of suitable partial peptide include, for example, the amino (N)-terminal or carboxy (C)-terminal fragment of a peptide of the present invention.

[0191] Herein, an antibody is defined as a protein that reacts with either the full length or a fragment of a DEPDC1 peptide. In a preferred embodiment, antibody of the present invention can recognize fragment peptides of DEPDC1 having an amino acid sequence selected from among SEQ ID NOs: 1 to 4. Methods for synthesizing oligopeptide are well known in the arts. After the synthesis, peptides may be optionally purified prior to use as immunogen. In the present invention, the oligopeptide (e.g., 24 mer or 26 mer) may be conjugated or linked with carriers to enhance the immunogenicity. Keyhole-limpet hemocyanin (KLH) is well known as the carrier. Method for conjugating KLH and peptide are also well known in the arts.

[0192] Alternatively, a gene encoding a peptide of the invention or fragment thereof may be inserted into a known expression vector, which is then used to transform a host cell as described herein. The desired peptide or fragment thereof may be recovered from the outside or inside of host cells by any standard method, and may subsequently be used as an antigen. Alternatively, whole cells expressing the peptide or their lysates or a

chemically synthesized peptide may be used as the antigen.

[0193] Any mammalian animal may be immunized with the antigen, though preferably the compatibility with parental cells used for cell fusion is taken into account. In general, animals of Rodentia, Lagomorpha or Primate family may be used. Animals of the family Rodentia include, for example, mouse, rat and hamster. Animals of the family Lagomorpha include, for example, rabbit. Animals of the Primate family include, for example, a monkey of Catarrhini (old world monkey) such as Macaca fascicularis, rhesus monkey, sacred baboon and chimpanzees.

[0194] Methods for immunizing animals with antigens are known in the art. Intraperitoneal injection or subcutaneous injection of antigens is a standard method for immunization of mammals. More specifically, antigens may be diluted and suspended in an appropriate amount of phosphate buffered saline (PBS), physiological saline, etc. If desired, the antigen suspension may be mixed with an appropriate amount of a standard adjuvant, such as Freund's complete adjuvant, made into emulsion and then administered to mammalian animals. Preferably, it is followed by several administrations of antigen mixed with an appropriately amount of Freund's incomplete adjuvant every 4 to 21 days. An appropriate carrier may also be used for immunization. After immunization as above, serum may be examined by a standard method for an increase in the amount of desired antibodies.

[0195] Polyclonal antibodies against the peptides of the present invention may be prepared by collecting blood from the immunized mammal examined for the increase of desired antibodies in the serum, and by separating serum from the blood by any conventional method. Polyclonal antibodies include serum containing the polyclonal antibodies, as well as the fraction containing the polyclonal antibodies may be isolated from the serum. Immunoglobulin G or M can be prepared from a fraction which recognizes only the peptide of the present invention using, for example, an affinity column coupled with the peptide of the present invention, and further purifying this fraction using protein A or protein G column.

[0196] To prepare monoclonal antibodies for use in the context of the present invention, immune cells are collected from the mammal immunized with the antigen and checked for the increased level of desired antibodies in the serum as described above, and are subjected to cell fusion. The immune cells used for cell fusion may preferably be obtained from spleen. Other preferred parental cells to be fused with the above immunocyte include, for example, myeloma cells of mammalians, and more preferably myeloma cells having an acquired property for the selection of fused cells by drugs.

The above immunocyte and myeloma cells can be fused according to known methods, for example, the method of Milstein et al. (Galfre and Milstein, Methods Enzymol 73: 3-46 (1981)).

[0197] Resulting hybridomas obtained by cell fusion may be selected by cultivating them in a standard selection medium, such as HAT medium (hypoxanthine, aminopterin and thymidine containing medium). The cell culture is typically continued in the HAT medium for several days to several weeks, the time being sufficient to allow all the other cells, with the exception of the desired hybridoma (non-fused cells), to die. Then, the standard limiting dilution may be performed to screen and clone a hybridoma cell producing the desired antibody.

[0198] In addition to the above method, wherein a non-human animal is immunized with an antigen for preparing hybridoma, human lymphocytes such as those infected by EB virus may be immunized with a peptide, peptide expressing cells or their lysates in vitro. Then, the immunized lymphocytes may be fused with human-derived myeloma cells that are capable of indefinitely dividing, such as U266, to yield a hybridoma producing a desired human antibody that is able to bind to the peptide can be obtained (Unexamined Published Japanese Patent Application No. Sho 63-17688).

[0199] The obtained hybridomas may then be subsequently transplanted into the abdominal cavity of a mouse and the ascites extracted. The obtained monoclonal antibodies can be purified by, for example, ammonium sulfate precipitation, a protein A or protein G column, DEAE ion exchange chromatography or an affinity column to which the peptide of the present invention is coupled. An antibody of the present invention can be used not only for purification and detection of a peptide of the present invention, but also as a candidate for agonists and antagonists of a peptide of the present invention.

Alternatively, an immune cell, such as an immunized lymphocyte, producing antibodies may be immortalized by an oncogene and used for preparing monoclonal antibodies.

[0200] Monoclonal antibodies thus obtained can be also recombinantly prepared using genetic engineering techniques (see, for example, Borrebaeck and Larrick, Therapeutic Monoclonal Antibodies, published in the United Kingdom by MacMillan Publishers LTD (1990)). For example, a DNA encoding an antibody may be cloned from an immune cell, such as a hybridoma or an immunized lymphocyte producing the antibody, inserted into an appropriate vector, and introduced into host cells to prepare a recombinant antibody. The present invention also provides for recombinant antibodies prepared as described above.

[0201] An antibody of the present invention may be a fragment of an antibody or modified antibody, so long as it binds to one or more of the peptides of the invention. For instance, the antibody fragment may be Fab, $F(ab')_2$, Fv or single chain Fv (scFv), in which Fv fragments from H and L chains are ligated by an appropriate linker (Huston et al., Proc Natl Acad Sci USA 85: 5879-83 (1988)). More specifically, an antibody fragment may be generated by treating an antibody with an enzyme, such as papain or

pepsin. Alternatively, a gene encoding the antibody fragment may be constructed, inserted into an expression vector and expressed in an appropriate host cell (see, for example, Co et al., J Immunol 152: 2968-76 (1994); Better and Horwitz, Methods Enzymol 178: 476-96 (1989); Pluckthun and Skerra, Methods Enzymol 178: 497-515 (1989); Lamoyi, Methods Enzymol 121: 652-63 (1986); Rousseaux et al., Methods Enzymol 121: 663-9 (1986); Bird and Walker, Trends Biotechnol 9: 132-7 (1991)).

[0202] An antibody may be modified by conjugation with a variety of molecules, such as polyethylene glycol (PEG). The present invention provides for such modified antibodies. The modified antibody can be obtained by chemically modifying an antibody. These modification methods are conventional in the field.

[0203] Alternatively, an antibody of the present invention may be obtained as a chimeric antibody, between a variable region derived from nonhuman antibody and the constant region derived from human antibody, or as a humanized antibody, including the complementarity determining region (CDR) derived from nonhuman antibody, the frame work region (FR) and the constant region derived from human antibody. Such antibodies can be prepared according to known technology. Humanization can be performed by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody (see, e.g., Verhoeyen et al., Science 239:1534-1536 (1988)). Accordingly, such humanized antibodies are chimeric antibodies, wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species.

[0204] Fully human antibodies including human variable regions in addition to human framework and constant regions can also be used. Such antibodies can be produced using various techniques known in the art. For example, in vitro methods involve use of recombinant libraries of human antibody fragments displayed on bacteriophage (e.g., Hoogenboom & Winter, J. Mol. Biol. 227:381 (1991). Similarly, human antibodies can be made by introducing of human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. This approach is described, e.g., in U.S. Patent Nos. 6,150,584; 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016.

[0205] Antibodies obtained as above may be purified to homogeneity. For example, the separation and purification of the antibody can be performed according to the separation and purification methods used for general proteins. For example, the antibody may be separated and isolated by the appropriately selected and combined use of column chromatographies, such as affinity chromatography, filter, ultrafiltration, salting-out, dialysis, SDS polyacrylamide gel electrophoresis and isoelectric focusing (Antibodies: A Laboratory Manual. Ed Harlow and David Lane, Cold Spring Harbor Laboratory (1988)), but are not limited thereto. A protein A column and protein G

column can be used as the affinity column. Exemplary protein A columns to be used include, for example, Hyper D, POROS and Sepharose F.F. (Pharmacia).

[0206] Examples of suitable chromatography techniques, with the exception of affinity chromatography, include, for example, ion-exchange chromatography, hydrophobic chromatography, gel filtration, reverse phase chromatography, adsorption chromatography and the like (Strategies for Protein Purification and Characterization: A Laboratory Course Manual. Ed Daniel R. Marshak et al., Cold Spring Harbor Laboratory Press (1996)). The chromatographic procedures can be carried out by liquid-phase chromatography, such as HPLC and FPLC.

[0207] For example, measurement of absorbance, ELISA, enzyme immunoassay (EIA), radioimmunoassay (RIA) and/or immunofluorescence (IF) may be used to measure the antigen binding activity of the antibody of the invention. In ELISA, the antibody of the present invention is immobilized on a plate, a peptide of the invention is applied to the plate, and then a sample containing a desired antibody, such as culture supernatant of antibody producing cells or purified antibodies, is applied. Then, a secondary antibody that recognizes the primary antibody and is labeled with an enzyme, such as alkaline phosphatase, is applied, and the plate is incubated. Next, after washing, an enzyme substrate, such as p-nitrophenyl phosphate, is added to the plate, and the absorbance is measured to evaluate the antigen binding activity of the sample. A fragment of the peptide, such as a C-terminal or N-terminal fragment, may be used as the antigen to evaluate the binding activity of the antibody. BIAcore (Pharmacia) may be used to evaluate the activity of the antibody according to the present invention.

[0208] The above methods allow for the detection or measurement of the peptide of the invention, by exposing the antibody of the invention to a sample assumed to contain the peptide of the invention, and detecting or measuring the immune complex formed by the antibody and the peptide.

Because the method of detection or measurement of the peptide according to the invention can specifically detect or measure a peptide, the method can find use in a variety of experiments in which the peptide is used. For example, when the peptide of the present invention in cancer cells or tissues obtained from a patient is detected, it is expected that Th1 cells (or CTL cells) against them would be effective tools for cancer immunotherapy,

[0209] XI. Vectors and Host Cells

The present invention also provides for vectors and host cells into which a nucleotide encoding the peptide of a present invention is introduced. A vector of the present invention finds utility as a carrier of nucleotides, especially a DNA, of the present invention in host cell, to express the peptide of the present invention, or to administer the nucleotide of the present invention for gene therapy.

[0210] When E. coli is selected as the host cell and the vector is amplified and produced in a large amount in E. coli (e.g., JM109, DH5-alpha, HB101 or XL1Blue), the vector should have an "ori" suitable for amplification in E. coli and a marker gene suited for selecting transformed E. coli (e.g., a drug-resistance gene selected by a drug such as ampicillin, tetracycline, kanamycin, chloramphenicol or the like). For example, M13-series vectors, pUC-series vectors, pBR322, pBluescript, pCR-Script, etc., can be used. In addition, pGEM-T, pDIRECT and pT7 can also be used for subcloning and extracting cDNA as well as the vectors described above. When a vector is used to produce the protein of the present invention, an expression vector can find use. For example, an expression vector to be expressed in E. coli should have the above characteristics to be amplified in E. coli. When E. coli, such as JM109, DH5 alpha, HB101 or XL1 Blue, are used as a host cell, the vector should have a promoter, for example, lacZ promoter (Ward et al., Nature 341: 544-6 (1989); FASEB J 6: 2422-7 (1992)), araB promoter (Better et al., Science 240: 1041-3 (1988)), T7 promoter or the like, that can efficiently express the desired gene in E. coli. In that respect, pGEX-5X-1 (Pharmacia), "QIAexpress system" (Qiagen), pEGFP and pET (in this case, the host is preferably BL21 which expresses T7 RNA polymerase), for example, can be used instead of the above vectors. Additionally, the vector may also contain a signal sequence for peptide secretion. An exemplary signal sequence that directs the peptide to be secreted to the periplasm of the E. coli is the pelB signal sequence (Lei et al., J Bacteriol 169: 4379 (1987)). Means for introducing of the vectors into the target host cells include, for example, the calcium chloride method, and the electroporation method.

[0211] In addition to E. coli, for example, expression vectors derived from mammals (for example, pcDNA3 (Invitrogen) and pEGF-BOS (Nucleic Acids Res 18(17): 5322 (1990)), pEF, pCDM8), expression vectors derived from insect cells (for example, "Bac-to-BAC baculovirus expression system" (GIBCO BRL), pBacPAK8), expression vectors derived from plants (e.g., pMH1, pMH2), expression vectors derived from animal viruses (e.g., pHSV, pMV, pAdexLcw), expression vectors derived from retroviruses (e.g., pZIpneo), expression vector derived from yeast (e.g., "Pichia Expression Kit" (Invitrogen), pNV11, SP-Q01) and expression vectors derived from Bacillus subtilis (e.g., pPL608, pKTH50) can be used for producing the polypeptide of the present invention.

[0212] In order to express the vector in animal cells, such as CHO, COS or NIH3T3 cells, the vector should carry a promoter necessary for expression in such cells, for example, the SV40 promoter (Mulligan et al., Nature 277: 108 (1979)), the MMLV-LTR promoter, the EF1 alpha promoter (Mizushima et al., Nucleic Acids Res 18: 5322 (1990)), the CMV promoter and the like, and preferably a marker gene for selecting transformants (for example, a drug resistance gene selected by a drug (e.g., neomycin,

G418)). Examples of known vectors with these characteristics include, for example, pMAM, pDR2, pBK-RSV, pBK-CMV, pOPRSV and pOP13.

[0213] XII. Monitoring of Th1 response mediated by peptides

Through deep cDNA sequence analyses of TCR-alpha and -beta genes, it was revealed that the frequency of a particular pair of the genes was increased in Th cells stimulated by the peptides of the present invention. Therefore, when such a pair of genes is detected in a subject after vaccination of the peptides of the present invention it implies that peptide specific Th1 response in a subject was induced. Accordingly, the increase in a particular pair of genes in Th cells stimulated by the peptides of the present invention can be useful as a surrogate marker for monitoring or detecting Th1 response in a subject after the stimulation. In the context of the present invention, "peptide specific Th1 response" is understood to mean that TCR formed between the pair of alpha and beta subunits specifically recognizes a complex formed between the peptide of the present invention and HLA molecule. As discussed above, ability of Th1 cell induction of the peptides defined with specific sequence of the present invention can be maintained, even after amino acid modification(s). Therefore, in addition to stimulation by the specific peptides, even when Th1 cell is induced from mutated peptide, their antigen specificity is considered to "peptide specific", as long as TCR thereof specifically recognizes such complex formed by the original peptide.

[0214] For example, TCR-alpha and -beta subunits, each having a CDR3 consisting of the amino acid sequence of SEQ ID NO: 11 and 21, respectively, were increased in Th cells stimulated by DEPDC1-LP2 ($DEPDC1_{292-313}$-LP) in an HLA-DR4 restricted manner (Fig. 6). Therefore, their accumulation in the Th cell population of a subject implies that Th1 response mediated by DEPDC1-LP2 was successfully induced in the subject vaccinated with DEPDC1-LP2. Presence of CDR3s consisting of the amino acid sequences of SEQ ID NO: 11 and 21 can be detected by antibody based analyses. In an alternative embodiment of the present application, a specific pair of TCR-alpha and -beta subunits can also be estimated by detecting the polynucleotides encoding them. For example, the polynucleotide encoding each of the CDR3s consisting of the amino acid sequences of SEQ ID NO: 11 and 21 is represented by the nucleotide sequence of SEQ ID NO: 31 and 32, respectively. Accordingly, each of cDNAs synthesized from mRNAs encoding alpha and beta subunits of DEPDC1-specific TCR may comprise the nucleotide sequence of SEQ ID NO: 31 and 32, respectively. Such polynucleotides can be detected by PCR based analysis.

[0215] In a preferred embodiment, the present invention provides a method for monitoring, assessing or evaluating a peptide-specific Th1 response in a subject immunized with the peptide comprising the steps of:

(a) providing a sample obtained from the subject administered said peptide, wherein

said sample comprises T cells;

(b) detecting the presence of T cells expressing the TCR which binds to a complex of an MHC class II molecule and said peptide or a fragment thereof in said sample; and

(c) indicating an induction of Th1 response specific to said peptide when the presence of said T cells is detected in (b).

For example, by detecting alpha- and beta- subunits consisting of an amino acid sequence of SEQ ID NO: 11 and 21, induction of T cell specific to DEPDC1-LP2 ($DEPDC1_{292-313}$-LP) in an HLA-DR4 restricted manner can be detected.

[0216] In the present invention, any biological sample obtained from a subject can be used for monitoring the Th1 response, as long as Th1 cells are included in the sample. For example, for the purposes of the present invention, blood or blood derived samples can be used as biological samples. In the present invention, blood derived samples include cell populations comprising T cells. Methods for obtaining cell populations comprising T cells are well known for the skilled persons. Alternatively, in some embodiments, tissues or lymph nodes infiltrated with T cells are also useful as biological samples.

[0217] In some embodiments of the present invention, cell populations comprising T cells can be fixed for antibody based analyses in evaluating TCR subunits. After contacting the fixed cells, antibodies recognize CDR3s of alpha and beta subunits in the fixed cells, and when the antibodies bind both of the TCR subunits on a single cell, then TCR-antibody complexes formed with the specific pair of subunits can be detected. Alternatively, in nucleotide sequence based analyses, accumulation of amplicons synthesized from polynucleotides encoding TCR subunits in a single cell implies that a particular pair of the subunits is present. Alternatively, a specific pair of polynucleotides encoding TCR subunits for each of the peptides to be vaccinated can be monitored by deep cDNA sequence analysis as shown in Fang H, et al., Oncoimmunology 3: e968467, 2015.

[0218] In this respect, it is well known that structural diversity of TCRs on Th1 cells mainly depends on their CDR3s. Consequently, antigen specificity thereby also depends on CDR3. Therefore, the presence of a specific pair of alpha and beta subunits on a single cell can be monitored through CDR3 detection.

In some embodiments of the present invention, for monitoring or evaluating Th1 response, a specific pair of TCR-alpha and -beta subunits can be detected at least once or more times after vaccination(s). When the pair is increased in a time dependent manner through the monitoring, it means that peptide mediated Th1 response in a subject is sufficiently induced. Alternatively, when the specific pair is detected at least once, it shows that Th1 response in a subject occurred.

[0219] Hereinafter, the present invention is described in more detail with reference to specific Examples. However, while the following materials, methods and examples

may serve to assist one of ordinary skill in making and using certain embodiments of the present invention, there are only intended to illustrate aspects of the present invention and thus in no way to limit the scope of the present invention. As one of ordinary skill in the art will readily recognize, methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention.

Examples

[0220] Materials and Methods

Cell lines

The research protocol for collecting and using PBMCs from healthy donors (HDs) and patients with cancer was approved by the Institutional Review Board of Kumamoto University. PBMCs were obtained from HDs and patients with cancer after obtaining written informed consent. Human monocyte-derived DCs and murine BM-derived DCs were generated as previously reported (Tomita Y,et al., Cancer Sci. 2011;102(1):71-78; Tomita Y, et al., Clin Cancer Res. 2013;19(16):4508-4520; Tomita Y, et al., Int J Cancer. 2014;134(2):352-366; Tomita Y, et al., Oncoimmunology. 2014;3:e28100; Sayem MA, et al., Oncoimmunology. 2016;5(1):e1062209; Hirayama M, et al., Oncoimmunology. 2016;5(6):e1123368).

Mouse fibroblast cell lines (L-cells), genetically engineered to express DR4 (DRB1*04:05), L-DR4; DR8 (DRB1*08:03), L-DR8; DR9 (DRB1*09:01), L-DR9; DR15 (DRB1*15:02), L-DR15; DR53 (DRB4*01:03), L-DR53; DP2 (DPA1*01:03/DPB1*02:01), L-DP2; DP4 (DPA1*01:03/DPB1*04:01), RM3-DP4; DP5 (DPA1*02:02/DPB1*05:01), L-DP5; and DP9 (DPB1*09:01), L-DP9 were provided by Dr. Alessandro Sette (La Jolla Institute for Allergy and Immunology, CA, USA), and used as APCs.

[0221] Prediction of HLA class II-binding peptides

To predict potential promiscuous HLA-class II binding human DEPDC1-derived peptides, the amino acid sequence of the human DEPDC1 protein was analyzed by a recently developed computer algorithm (IEDB analysis resource, consensus method, http://tools.immuneepitope.org/analyze/html/mhc_II_binding.html) (Wang P, et al., BMC Bioinformatics 2010;11:568. Wang P, et al.PLoS Comput Biol 2008;4:e1000048). The program analyzed 15 amino-acid-long sequences offset to encompass the entire DEPDC1 protein. The 22-29 amino-acid-long peptides, DEPDC1 $_{191\text{-}213}$LP (DEPDC1-LP1; RYVILIYLQTILGVPSLEEVINP (SEQ ID NO: 1)), DEPDC1$_{292\text{-}313}$-LP (DEPDC1-LP2; TFEYYELFVNILVVCGYITVSD (SEQ ID NO: 2)), DEPDC1$_{301\text{-}329}$-LP (DEPDC1-LP3; NILVVCGYITVSDRSSGIHKIQDDPQSSK (SEQ ID NO: 3)) and DEPDC1$_{613\text{-}634}$-LP (DEPDC1-LP4; NRRKLQLLMRMIS-

RMSQNVDMP (SEQ ID NO: 4)) with relatively lower consensus percentile ranks for multiple HLA-class II molecules encoded for by DRB1*04:05, DRB1*09:01, DRB1*15:02, DRB4*01:03 and DPB1*05:01 alleles, and two of which include DEPDC1-derived 9 or 10-mer CTL epitopes (DEPDC1-$A2_{302-311}$ and/or DEPDC1-$A24_{294-302}$), were selected and synthesized (Fig. 1 and Table 1).

[0222] [Table 1-1]

Binding algorithm scores of DEPDC1-derived peptides for HLA-class II molecules.

Amino acid residues position	Percentile Rank		
	HLA-DR9 (DRB1*09:01)	HLA-DR4 (DRB1*04:05)	HLA-DR15 (DRB1*15:02)
DEPDC1-LP1 ($DEPDC1_{191-213}$-LP)			
191-205	39.19	1.61	0.02
192-206	36.64	1.05	0.02
193-207	41.9	1.08	0.9
194-208	39.16	1.46	0.9
195-209	36.49	1.3	4.26
196-210	37.77	1.54	4.26
197-211	35.2	2.13	9.34
DEPDC1-LP2 ($DEPDC1_{292-313}$-LP)			
292-306	34.29	1.25	0.04
293-307	34.29	0.42	0.04
294-308	34.29	0.49	0.04
295-309	34.29	1.28	0.04
296-310	34.29	1.4	0.04
297-311	34.29	4.42	1.02
298-312	61.16	9.16	1.02
DEPDC1-LP3 ($DEPDC1_{301-329}$-LP)			
301-315	50.38	8.37	1.02
302-316	45.58	6.31	1.02
304-317	50.67	6.7	1.02
305-318	55.02	9.33	10.52
306-319	55.4	9.51	16.67
307-320	57.38	13.32	16.67
308-321	57.38	24.64	16.67
DEPDC1-LP4 ($DEPDC1_{613-634}$-LP)			
613-627	11.58	6.31	0.71
614-628	0.43	1.48	0.71
615-629	0.41	1.46	0.57
616-630	0.43	1.5	0.57
617-631	0.4	1.43	0.57
618-632	0.42	1.41	0.57
619-633	2.39	3.21	0.57

Peptide-binding algorithm scores for indicated HLA-class II molecules are shown for each 15-mer DEPDC1-derived overlapping peptide.

[0223] [Table 1-2]

Binding algorithm scores of DEPDC1-derived peptides for HLA-class II molecules.

Amino acid residues position	Percentile Rank		
	HLA-DR4 (*DRB1*04:05*)	HLA-DR53 (*DRB4*01:03*)	HLA-DP5 (*DPB1*05:01*)
DEPDC1-LP1 ($DEPDC1_{191-213}$-LP)			
191-205	1.61	1.37	7.16
192-206	1.05	0.93	9.22
193-207	1.08	0.86	11.02
194-208	1.46	1.28	15.08
195-209	1.3	2.39	22.6
196-210	1.54	6.47	37.35
197-211	2.13	8.9	46.71
198-212	3.03	16.91	42.28
199-213	5.98	39.66	43.02
DEPDC1-LP2 ($DEPDC1_{292-313}$-LP)			
292-306	1.25	59.11	1.47
293-307	0.42	62.86	1.59
294-308	0.49	61.35	3.84
295-309	1.28	63.33	7.84
296-310	1.4	61.19	19.73
297-311	4.42	60.51	30.15
298-312	9.16	58.58	30.15
299-313	5.38	55.45	40.79
DEPDC1-LP3 ($DEPDC1_{301-329}$-LP)			
301-315	8.37	46.01	52.62
302-316	6.31	44.97	66.5
303-317	6.7	49.69	68.94
304-318	6.7	52.53	68.55
305-319	9.33	55.8	72.39
306-320	9.51	62.45	75.06
307-321	13.32	67.03	78.3
308-322	24.64	74.1	80.03
309-323	49.5	81.68	81.98
310-324	49.54	86.99	81.62
311-325	58.13	86.51	83.83
312-326	19.73	33.76	83.78
313-327	19.69	18.96	89.74
314-328	20.44	10.33	90.87
315-329	20.16	6.81	90.17
DEPDC1-LP4 ($DEPDC1_{613-634}$-LP)			
613-627	6.31	0.48	3.52
614-628	1.48	0.22	3.52
615-629	1.46	0.17	29.41
616-630	1.5	0.18	40.94
617-631	1.43	0.16	45.53
618-632	1.41	0.3	49.03
619-633	3.21	0.79	59.37
620-634	3.44	1.77	69.46

Peptide-binding algorithm scores for indicated HLA-class II molecules are shown for

each 15-mer DEPDC1-derived overlapping peptide.

[0224] Synthetic peptides and recombinant proteins

Two human DEPDC1-derived SPs presented by HLA-A2 (DEPDC1-$A2_{302-311}$)(Kanehira M , et al., Oncogene 2007; 26: 6448-55) or HLA-A24 (DEPDC1-$A24_{294-302}$)(Harada Y, et al., Cancer Res 2010;70:5829-39), and four LPs (DEPDC1-LP1-4) were synthesized (MBL, Nagoya, Japan; purity >95%; Fig. 1B). A human immunodeficiency virus (HIV)-SPs that bind to HLA-A2 (HIV-A2) or HLA-A24 (HIV-A24) were used as negative control SPs (Kanehira M , et al., Oncogene 2007; 26: 6448-55; Tomita Y, et al., Cancer Sci 2011;102:697-705). Peptides were dissolved in dimethylsulfoxide at 10 mg/mL, and stored at -80 degrees C. The recombinant human whole DEPDC1 protein and MPHOSPH1 protein were generated in Escherichia coli BL21 transformed with a pET28a vector (Novagen, 69864-3) encoding DEPDC1 and MPHOSPH1 according to the manufacture's instruction. Both DEPDC1 and MPHOSPH1 protein were assessed by SDS-PAGE and purified by HisTrap FF columns (GE Healthcare, Little Chalfont, Buckinghamshire, UK). MPHOSPH1 protein was used as a negative control.

[0225] Generation of antigen-specific $CD4^+$ T-cells from healthy donors

The research protocol for collecting and using PBMCs from healthy donors was approved by the Institutional Review Board of Kumamoto University. The present inventors obtained PBMCs from seven healthy donors with written informed consents. Genotyping of HLA-A, DRB1, and DPB1 alleles was performed at the HLA Laboratory (Kyoto, Japan) (Table 2). Induction of antigen-specific $CD4^+$ T-cells was performed as described previously, with some modifications (Tomita Y, et al., Clin Cancer Res 2013; 19:4508-20 ; Tomita Y, et al. Int J Cancer 2014; 134:352-66 ; Tomita Y, et al., OncoImmunology 2014; 3:e28100 ; Sayem MA, et al., OncoImmunology 2016; 5:e1062209 ; Hirayama M, et al. OncoImmunology 2016; 5:6, e1123368 ; Inoue M, et al., Int J Cancer 2010;127:1393-403 ; Harao M, et al., Int J Cancer 2008;123:2616-25). $CD4^+$ T-cells were purified from PBMCs by positive selection with magnetic microbeads (Miltenyi Biotec, Auburn, CA, USA) (Inoue M, et al., Int J Cancer 2010;127:1393-403). Monocyte-derived dendritic cells (DCs) were generated from $CD14^+$ cells by in vitro culture, as described previously (Harao M, et al., Int J Cancer 2008;123:2616-25.), and used as antigen-presenting cells (APCs) to induce antigen-specific $CD4^+$ T-cells. DCs (1 X 10^4/well) were pulsed with 10 micro-g/mL LP for 3 h and irradiated (45 Gy), then mixed with $CD4^+$ T-cells (3 X 10^4/well) in 200 micro-L AIM-V supplemented with 5% human decomplemented plasma in each well of a 96-well, flat-bottomed culture plate. After 7 days, half of the medium was removed from each culture, and fresh medium (100 micro-L/well) containing irradiated (50 Gy) autologous PBMCs (1 X 10^5) pulsed with peptide (10 micro-g/mL)

and 5 ng/mL recombinant human interleukin 7 (rhIL-7) was added. Two days after the second stimulation with peptide, rhIL-2 was added to each well (10 IU/mL). One week later, the stimulated $CD4^+$ T-cells in each well were analyzed for specificity in IFN-gamma ELISPOT assays. The T-cells showing a specific response to the cognate peptide were transferred to 24-well plates and restimulated at weekly intervals with irradiated autologous PBMCs (1 X 10^6/well) pulsed with the peptide in medium supplemented with rhIL-2 (20 IU/mL) and rhIL-7 (5 ng/mL). In some instances, T-cells were cloned by limiting dilution for further studies as described previously (Tabata H, et al., Hum Immunol 1998;59:549-60).

[0226] [Table 2]

HLA-A, -DR, and *-DP* genotypes of healthy donors analyzed in this study.

Donor ID[a]	*HLA-A* genotype	*HLA-DRB1* genotype	*HLA-DPB1* genotype
HD1	*A*24:02 / -*	*DRB1*04:05 / 09:01*	*DPB1*05:01 / -*
HD2	*A*02:01 / 02:06*	*DRB1*04:05 / 09:01*	*DPB1*02:01 / 04:02*
HD3	*A*24:02 / 31:01*	*DRB1*08:03 / 14:05*	*DPB1*02:02 / 05:01*
HD4	*A*02:01 / 24:02*	*DRB1*04:05 / -*	*DPB1*05:01 / -*
HD5	*A*24:02 / -*	*DRB1*08:03 / 15:02*	*DPB1*02:01 / 09:01*
HD6	*A*31:01 / 33:03*	*DRB1*09:01 / 13:02*	*DPB1*03:01 / 04:01*
HD7	*A*24:02 / 31:01*	*DRB1*14:03 / 15:02*	*DPB1*05:01 / 09:01*

[a] Th cells specific to DEPDC1-LP were derived from PBMCs of healthy donors (HD1 though HD7).

[0227] Assessment of T-cell responses to peptides and proteins

The immune response of Th cells to irradiated APCs pulsed with peptides was assessed by IFN-gamma enzyme-linked immunospot (ELISPOT) assays (BD Biosciences) as described previously (Tomita Y, et al., Cancer Sci 2011;102:697-705). Briefly, the frequency of peptide-specific $CD4^+$ T-cells producing IFN-gamma per 3 X 10^4 bulk $CD4^+$ T-cells upon stimulation with peptide-pulsed irradiated PBMCs (3 X 10^4), or 1 X 10^4 bulk $CD4^+$ T-cells upon stimulation with peptide-pulsed HLA-DP or HLA-DR-expressing L-cells (5 X 10^4/well) was analyzed. To determine the restriction by HLA molecules involved in antigen presentation, blocking of antigen-induced IFN-gamma production was investigated by adding anti-HLA-DR monoclonal antibody (mAb) (L243, BioLegend), anti-HLA-DP mAb, (B7/21, Abcam), anti-human HLA-DQ mAb (SPV-L3, Abcam), or anti-HLA class I mAb, (W6/32, Abcam). All mAbs were used at a final concentration of 5 micro-g/mL. All assessments of IFN-gamma ELISPOT assays were carried out in triplicate or duplicate, and results are presented as means +/- SD.

[0228] Assessment of DEPDC1-LP-specific $CD4^+$ T-cell responses in UC patients.

PBMCs obtained from five urothelial carcinoma (UC) patients were cultured in the presence of a mixture of DEPDC1-LPs (20 micro-g/mL) in a final volume of 2 mL of the AIM-V medium (Invitrogen) supplemented with 5% human decomplemented plasma at 37 degrees C (2 X 10^6/well, 24-well plates). IL-2 (20 U/ml) and IL-7 (5 ng/ml) were added on days 0 and 2. After seven days of cell culture, the cells were collected, washed, and transferred in ELISPOT plates (5-10 X 10^4/well) with the individual DEPDC1-LP or control LP for 18 h. The numbers of DEPDC1-LP-specific $CD4^+$ T cells were counted as described above.

[0229] In vivo cross-priming assay

HLA-A2 and HLA-A24 (HHD) transgenic mice (Tgm) were kindly provided by Dr. F.A. Lemonnier (Firat H, et al. Eur J Immunol 1999;29:3112-21; Jung KO, et al., J Virol 2012;86:7616-24). Mice were intradermally injected at the base of the tail with DEPDC1-LP2 solution (100 micro-g/mouse) emulsified in incomplete Freund's adjuvant (IFA) at 7-days intervals. Seven days after the second vaccination with DEPDC1-LP2, $CD8^+$ T-cells were isolated from inguinal lymph nodes by positive selection with magnetic microbeads (Miltenyi Biotec, Auburn, CA, USA). The number of IFN-gamma producing $CD8^+$ T-cells (1 X 10^5/well) upon stimulation with DEPDC1-$A2_{302\text{-}311}$SP or DEPDC1-$A24_{294\text{-}302}$SP-pulsed bone marrow-derived DCs (BM-DCs, 2 X 10^4/well) was counted by ex vivo ELISPOT assay (Harao M, et al., Int J Cancer 2008;123:2616-25 ; Inoue M,et al., Immunol Lett 2009;126:67-72).

[0230] The cytokine analysis

DEPDC1-LP-specific bulk Th cells or Th clones (1 X 10^4/well) were cultured with autologous PBMCs (3 X 10^4/well) pulsed with cognate peptides in 96-well plates. Using the Bio-Plex system (Bio-Rad), cytokine levels in the culture supernatants after 24 h were measured according to the manufacturer's instructions as reported previously.

[0231] TCR sequencing

Total RNAs were isolated from DEPDC1-LP2 -specific T cells using RNeasy mini kit (Qiagen, Valencia, CA, USA). Sequencing libraries of TRAV and TRBV were prepared using protocol described previously (Fang H, et al., Oncoimmunology. 2014; 3(12): e968467; Choudhury NJ, et al., European urology focus. 2016; 2(4): 445-52) with some modifications, and subjected to sequencing on the Illumina Miseq platform, using 600 cycles Miseq Reagent Kit V3 (Illumina, Inc., San Diego, CA, USA). Sequence reads were then analyzed with the algorithm described previously (Fang H, et al., Oncoimmunology. 2014; 3(12): e968467). To identify V-(D)-J segments in individual TRAV and TRBV sequencing reads, each of the sequence reads in FASTQ files were mapped to the reference sequences derived from IMGT/GENE-DB (Giudicelli V, et al., Nucleic acids research. 2005; 33(Database issue): D256-61) using

Bowtie2 aligner (Version 2.1.0) (Langmead B, et al., Nature methods. 2012; 9(4): 357-9; Lefranc MP, et al. Nucleic acids research. 2005; 33(Database issue): D593-7). To define amino acid sequences of complementarity determining region 3 (CDR3) in TRAV and TRBV, a conserved cysteine encoded in the 3' portion of V segment and a conserved phenylalanine encoded in the 5' portion of the J segments that form the boundaries of CDR3 were identified (Fang H, et al., Oncoimmunology. 2014; 3(12): e968467). The nucleotide sequences between both conserved cysteine and phenylalanine were extracted to determine the amino acid sequence of CDR3 region.

[0232] TCR cloning and expression in TG40

The TCR cDNAs were amplified from the T cell lines using one-step multiplex RT-PCR as described previously (Hamana H, et al., Biochemical and biophysical research communications. 2016; 474(4): 709-14). The DNA sequences of the amplified PCR products were determined by direct sequencing. To analyze the antigen-specificity of the obtained TCR cDNAs, expression vectors for the TCRs were constructed, and then transferred to Plat-E Cells (generously provided by Professor Toshio Kitamura, University of Tokyo) to produce the recombinant retroviruses as described previously (Mou Z, et al. Science translational medicine. 2015; 7(310): 310ra167; Kobayashi E, et al. Nature medicine. 2013; 19(11): 1542-6). The produced TCRs were transferred to TG40 cells (Ohno H, et al. Journal of immunology (Baltimore, Md : 1950). 1991; 146(11): 3742-6) as described previously (Kobayashi E, et al. Nature medicine. 2013; 19(11): 1542-6). 1 X 10^5 cells of TCR-transferred TG40 cells were cocultured with 1 X 10^5 cells of restricting HLA-DR expressing L cells in the presence of 10 micro-g/mL of antigenic peptides overnight at 37 degrees C in 5% CO_2 atmosphere, and the expression of CD69 and CD137 on the cell surface was analyzed with flow cytometer.

[0233] Statistical analysis

The present inventors compared data by the two-tailed Student's t-test (bar graphs) or by the nonparametric Mann-Whitney U test (scatter-dot graph). Differences with a P value < 0.05 were considered statistically significant for all tests.

[0234] Results

Prediction and selection of potential promiscuous HLA class II-binding DEPDC1 long peptides containing CTL-epitopes

To identify potential promiscuous HLA-class II binding Th-cell epitopes of DEPDC1, the amino acid sequence of DEPDC1 was first examined using a recently developed computer algorithm (Fig. 1A, C, and Table 1) (Wang P, et al. BMC Bioinformatics 2010; 11:568; Wang P, et al., PLoS Comput Biol 2008; 4:e1000048). Two regions, $DEPDC1_{191-213}$-LP (DEPDC1-LP1) and $DEPDC1_{613-634}$-LP (DEPDC1-LP4) does not include a known CTL-epitope sequence. Other two peptides, $DEPDC1_{292-313}$-LP (DEPDC1-LP2) and $DEPDC1_{301-329}$-LP (DEPDC1-LP3) were proximal to the CTL

epitopes recognized by HLA-A2 or A24-restricted CTLs (Fig. 1B). Therefore, the present inventors synthesized 4 candidate LPs, DEPDC1-LP1-4, predicted to have strong binding affinity to frequent HLA-class II molecules in the Japanese population (HLA-DR4, HLA-DR9, HLA-DR15, HLA-DP2 and HLA-DP5), and two of them include 9 or 10-mer peptides recognized by HLA-A2- or -A24-restricted CTLs for subsequent analyses.

[0235] Identification of promiscuous DEPDC1-derived Th cells epitopes

$CD4^+$ T-cells isolated from PBMCs of healthy donors were stimulated at weekly intervals with autologous DCs and irradiated PBMCs pulsed with DEPDC1-LP1. After at least 3 rounds of stimulations, DEPDC1-LP1-specific responses of cultured $CD4^+$ T-cells were examined by IFN-gamma ELISPOT assays.

In an HLA-DR53 (DRB4*01:03)-positive healthy donor (HD1), the generated Th cells produced a significant amount of IFN-gamma in response to DEPDC1-LP1-pulsed PBMCs in an HLA-DR-dependent manner. The bulk Th cells specifically recognized L-DR53 cells pulsed with DEPDC1-LP1 in an HLA-DR-dependent manner, but not unpulsed L-DR53 cells, DEPDC1-LP1-pulsed L-DR4 cells and L-DR9 cells (Fig. 2A). These results indicated that DEPDC1-LP1 was presented by HLA-DR53. To investigate whether DEPDC1-LP1 induces responses in Th cells restricted by other HLA class II molecules, $CD4^+$ T-cells from other healthy donors were tested (HD4). The present inventors confirmed DEPDC1-LP1 generates HLA-DR4 (DRB1*04:05/-)-restricted Th cells (Fig. 2B). Thus, DEPDC1-LP1 binds to HLA-DR53 and HLA-DR4, suggesting DEPDC1-LP1 is a promiscuous Th-cell epitope presented by frequent HLA class II in the Japanese population. (Saito S,et al., Tissue Antigens 2000;56:522-9 ; Mack SJ, et al., Tissue Antigens 2000;55:383-400).

[0236] DEPDC1-LP2, which bears a known CTL-epitope, can generate Th cells. DEPDC1-LP2-iduced Th cells were derived from HD1. DEPDC1-LP2-specific Th cells from healthy donor HD1 (DRB1*04:05/09:01) specifically recognized L-DR4 cells (Fig. 2C) pulsed with DEPDC1-LP2 but not DEPDC1-LP2-pulsed L-DR9 and L-DR53. Then the present inventors confirmed that DEPDC1-LP2 could generate HLA-DR4-restricted bulk Th cells in this donor.

[0237] DEPDC1-LP3, which also bears a known CTL-epitope, can generate Th cells. DEPDC1-LP3-iduced Th cells were derived from HD3, and HD4. In an HLA-DP2 and DP5-positive healthy donor (HD3), the generated Th cells produced a significant amount of IFN-gamma in response to DEPDC1-LP3-pulsed PBMCs in an HLA-DP-dependent manner. Then The present inventors confirmed that DEPDC1-LP3 could generate HLA-DP5-restricted bulk Th cells in this donor (Fig. 2D). To investigate whether DEPDC1-LP3 can induce responses in Th cells restricted by other HLA class II molecules, $CD4^+$ T-cells from other healthy donor (HD4) were tested.

The present inventors confirmed that DEPDC1-LP3 could generate HLA-DR4-restricted bulk Th cells in this donor (Fig. 2E).

[0238] By doing similar experiments, the present inventors are also able to generate DEPDC1-LP4 specific and IFN-gamma producing cells. DEPDC1-LP4-iduced Th cells were derived from HD1, HD5 and HD7. In an DP5-positive healthy donor (HD1), the generated Th cells produced a significant amount of IFN-gamma in response to DEPDC1-LP4-pulsed PBMCs in an HLA-DP-dependent manner. Then the present inventors confirmed that DEPDC1-LP4 could generate HLA-DP5-restricted bulk Th cells in this donor (Fig. 2F). The present inventors also confirmed that DEPDC1-LP4 could generate HLA-DP5-restricted bulk Th cells in the other healthy donor (HD7) (Fig. 2H). To investigate whether DEPDC1-LP4 can induce responses in Th cells restricted by other HLA class II molecules, $CD4^+$ T-cells from other healthy donor (HD5) were tested. The present inventors confirmed that DEPDC1-LP4 could generate HLA-DR15-restricted bulk Th cells in this donor (Fig. 2G). These results indicated that DEPDC1-LP4 was presented at least by HLA-DP5 and HLA-DR15. These results demonstrate these four DEPDC1-LPs are promiscuous Th-cell epitopes as summarized in Table 3. Since these LPs were promiscuous Th-cell epitopes presented by frequently observed HLA class II in the Japanese population (Table 4) (Zarour HM, et al. Cancer research. 2002; 62(1): 213-8; Grabowska AK, et al. International journal of cancer. 2015; 136(1): 212-24), combination of these peptides would induce DEPDC1-specific Th-cell responses in PBMCs of many Japanese donors.

[0239]

[Table 3]

Identification of DEPDC1-derived and promiscuous HLA-class II-restricted $CD4^+$ T cell epitopes encompassing cytotoxic T lymphocyte (CTL) epitopes

Designation of long peptide (LP)	a.a. residue position	Sequence	a.a. length	Tcell donor	[a]Donors' HLA-class II alleles	Immune response	Restriction HLA-class II molecule
DEPDC1-LP1	191-213	RYVILIYLQTILGVPSLEEVINP	23	HD1	*DRB1*04:05/09:01,(DR53)*	Positive	DR53 (*DRB4*0103*)
				HD2	*DRB1*04:05/09:01*	Negative	
				HD3	*DRB1*08:03/14:05*	Negative	
				HD4	*DRB1*04:05/-,(DR53)*	Positive	DR4 (*DRB1*04:05*)
				HD7	*DRB1*14:03/15:02*	Negative	
DEPDC1-LP2	292-313	TF**EYYELFVN*/LVCGYITV***SD Underlined and bold sequences; [b]CTL epitope:A24-DEPDC1$_{294–302}$ Italic and bold sequences; [b]CTL epitope:A2-DEPDC1$_{302–311}$	22	HD1	*DRB1*04:05/09:01,(DR53)*	Positive	DR4 (*DRB1*04:05*)
				HD2	*DRB1*04:05/09:01*	Negative	
				HD3	*DRB1*08:03/14:05*	Negative	
				HD4	*DRB1*04:05/-,(DR53)*	Negative	
				HD6	*DRB1*09:01/13:02*	Negative	
				HD7	*DRB1*14:03/15:02*	Negative	
DEPDC1-LP3	301-329	N***/LVCGYITV***SDRSSGIHKIQDDPQSSK Italic and bold sequences; [b]CTL epitope:A2-DEPDC1$_{302–311}$	29	HD1	*DRB1*04:05/09:01,(DR53)*	Negative	
				HD3	*DPB1*02:02/05:01*	Positive	DP5 (*DPB1*05:01*)
				HD4	*DRB1*04:05/-,(DR53)*	Positive	DR4 (*DRB1*04:05*)
				HD6	*DRB1*09:01/13:02*	Negative	
				HD7	*DRB1*14:03/15:02*	Negative	
DEPDC1-LP4	613-634	NRRKLQLLMRMISRMSQNVDMP	22	HD1	*DPB1*02:02/05:01*	Positive	DP5 (*DPB1*05:01*)
				HD2	*DRB1*04:05/09:01*	Negative	
				HD3	*DRB1*08:03/14:05*	Negative	
				HD4	*DRB1*04:05/-,(DR53)*	Negative	
				HD5	*DRB1*08:03/15:02*	Positive	DR15 (*DRB1*15:02*)
				HD7	*DPB1*05:01/09:01*	Positive	DP5 (*DPB1*05:01*)

Summary of DEPDC1-LPs and the immune responses of Th cells reactive to DEPDC1-LPs in five HDs.

[a] The underlined HLA-class II alleles encode HLA-class II molecules presenting DEPDC1-LPs to Th cells in HDs; details of donors' HLA alleles are shown in Table 2.

[b] Underlined and italic bold sequences are CTL epitopes; DEPDC1-LP1 and DEPDC1-LP4 sequences were selected on high-affinity binding to HLA-class II molecules predicted by the algorithm;

DEPDC1-LP2 and DEPDC1-LP3 were selected on both the prediction of HLA-class II binding and the proximity to the known CTL epitopes. a.a.: amino acid, Negative: we could not obtain positive data and did not proceed further, LP: long peptide, HD: healthy donor.

[0240] [Table 4]

Antigen frequency of restriction HLA-class II molecules involved in the presentation of DEPDC1-LPs to Th cells in the Japanese population (http://www.hla.or.jp/).

HLA-class II type	Antigen frequency	Presenting peptides
DR4 (*DRB1*04:05*)	25.1%	DEPDC1-LP1, LP2, LP3
DR15 (*DRB1*15:02*)	20.0%	DEPDC1-LP4
DR53 (*DRB4*01:03*)	51.4%	DEPDC1-LP1
HLA-class I type		
A24 (*A*24:02*)	59.2%	
A2 (*A*02:01*)	21.6%	

The antigen frequency of each HLA allele in the Japanese population was referred from a previous report (Kobayashi, et al., Can res 2001;61(12):4773-8)

[0241] DEPDC1-LP2, DEPDC1-LP3 and DEPDC1-LP4 are naturally processed and presented by DCs

It was assessed whether DCs take up and process the DEPDC1 protein to stimulate DEPDC1-LPs-specific Th-clones. DCs loaded with recombinant DEPDC1 protein were prepared and used as APCs in IFN-gamma ELISPOT assays (Kanehira M, et al. Oncogene 2007;26:6448-55; Harao M, et al., Int J Cancer 2008; 123:2616-25). An HLA-DR4-restricted DEPDC1-LP2-reactive Th-clone efficiently recognized DCs loaded with DEPDC1 protein, but did not recognize control protein-loaded DCs, indicating this epitope was naturally processed from DEPDC1 protein and presented by HLA-DR4 molecules (Fig. 3A). An HLA-DR4-restricted DEPDC1-LP3-reactive Th-clone efficiently recognized DCs loaded with DEPDC1 protein in an HLA-DR-dependent manner, but did not recognize control protein-loaded DCs, indicating that this epitope was also naturally processed from DEPDC1 protein and presented by

HLA-DR4 molecules (Fig. 3B). Similarly, an HLA-DP5-restricted and DEPDC1-LP4-reactive Th-clone efficiently recognized DCs loaded with DEPDC1 protein in an HLA-DP-dependent manner, but did not recognize control protein-loaded DCs, indicating that this epitope was also naturally processed from DEPDC1 protein and presented by HLA-DP5 molecules (Fig. 3C). These results suggested that DEPDC1-LP2, DEPDC1-LP3 and DEPDC1-LP4 are naturally processed from DEPDC1 protein and presented by DCs.

[0242] DEPDC1-LP-induced Th cells produced Th1 cytokines.

To characterize DEPDC1-LP specific Th cells, the present inventors measured various cytokines secreted by the Th cells in response to stimulation with autologous PBMCs pulsed with respective cognate peptide. Relatively large amounts of Th1 cytokines, such as IFN-gamma, TNF-alpha, IL-2, and GM-CSF but not IL-4 or IL-17 were produced by DEPDC1-LP-specific Th cells after re-stimulation with the cognate peptides (Fig.5), suggesting that DEPDC1-LP had the capacity to induce Th1-polarized Th cells. Therefore, induction of the Th cells by DEPDC1-LP could be useful for anti-tumor therapy.

[0243] The DEPDC1-LP2-specific bulk Th cells expressed converged TCR-alpha and -beta genes.

DEPDC1-LP2 specific T cell repertoire was analyzed by deep cDNA sequencing of TCR-alpha and -beta genes using next-generation sequencer. After the several times of stimulation of bulk Th cells, the frequency of a particular pair of TCR-alpha and -beta gene usage was increased in both bulk Th cells. Two LPs-specific T cell clones established from the parental bulk T cell lines, which had been stimulated 4 or 5 times with the peptide, expressed the same pair of TCR-alpha and -beta genes as the bulk Th cells did (Fig. 6A and 6B). Introduction of the pair of TCR-alpha and -beta genes isolated from both DEPDC1-LP2-specific T cell clones, respectively, into murine TCR-negative T cell line TG40 successfully acquired the original antigenic peptide specificity and HLA-restriction of the parental Th cells, as revealed by the up-regulation of CD69 and CD137 detected by flow-cytometric analysis (Fig. 6C). These observation confirmed the clonality of the established T cells and specificity of these pairs of TCR-alpha and -beta chains.

[0244] Cross-presentation of DEPDC1-LPs efficiently primes DEPDC1-SPs-specific $CD8^+$ T-cells in vivo

The capacity of DEPDC1-LP2 to prime DEPDC1-$A2_{302-311}$-specific CTLs and DEPDC1-$A24_{294-302}$-specific CTLs was examined by an ex vivo IFN-gamma ELISPOT assay. HLA-A2 and HLA-A24 Tgm were immunized twice with DEPDC1-LP2 emulsified in IFA. The $CD8^+$ T-cells isolated from HLA-A2 or HLA-A24 Tgm vaccinated with DEPDC1-LP2 produced IFN-gamma specifically in response to

stimulation with BM-DCs pulsed with the DEPDC1-$A2_{302-311}$ SP or DEPDC1-$A24_{294-302}$ SP (Fig. 4A and 4B). These results suggested that after uptake of DEPDC1-LP2, APCs can cross-prime DEPDC1-$A2_{302-311}$ SP -specific CTL and DEPDC1-$A24_{294-302}$-specific CTL in vivo in HLA-A2 and HLA-A24 Tgm.

[0245] PBMCs isolated from UC patients recognized some of DEPDC1-LPs.

To examine the induction of DEPDC1-LPs-specific Th cell response in UC patients, the present inventors stimulated PBMCs isolated from advanced stage UC patients with LPs in vitro. Patient characteristics were indicated in Table 5. After stimulation, the frequency of individual DEPDC1-LP-specifc T cells was measured. DEPDC1-LP4 induced peptide-specific T cells from 2 patients. Peptide-specific IFN-gamma production by T cells was significantly inhibited by anti-HLA-DR mAb but not by anti-HLA-DP mAb nor anti-HLA class I mAb (Fig.7). These results demonstrated that IFN-gamma was produced by DEPDC1-LP -specific and HLA-DR-restricted $CD4^+$ T cells, suggesting that DEPDC1-LP4 induced antigen-specific Th cells not only from HDs but also from patients with UC.

[0246] [Table 5]

DEPDC1-LP-specific responses of PBMCs isolated from patients and the patients' *HLA* genotypes.

Patient ID[a]	Age/Sex	Specificity of LPs	HLA-class II restriction	*HLA-A**	*HLA-DRB1**	*HLA-DPB1**
Pt 4	74/F	DEPDC1-LP4	DR	*24:02 / 26:03*	*14:54 / 15:02*	*03:01 / 05:01*
Pt 6	57/F	DEPDC1-LP4	DR	*31:01 / 33:03*	*04:10 / 13:02*	*04:01 / 05:01*

[a] Summary of the DEPDC1-LP-specific immune responses of PBMCs derived from 2 patients with UC. Responses of DEPDC1-LPs-specific T helper (Th) cells were measured using an interferon (IFN)-gamma enzyme-linked immunospot assay (ELISPOT).

In brief, patient-derived PBMCs were stimulated with the indicated DEPDC1-LP. The responses were recorded as positive when the mean number of IFN-gamma spots both exceeded 25 and was greater than 2 times the background signal.

[0247] Discussion

In a phase I/II clinical trial, combination immunotherapy of intravesical BCG inoculation, and DEPDC1-derived SPs vaccine demonstrated the promising clinical effect on recurrence prevention for non-muscle invasive bladder cancer as well as safety and good immunogenicity to induce CTL (Obara W, et al., Clin Oncol 2012;42: 591-600). Therefore, the present inventors attempted to identify the LPs that induce both antigen-specific Th1 cells and CTLs in order to further develop better peptide vaccine immunotherapy.

[0248] The present inventors identified four promiscuous DEPDC1-derived Th cell epitope peptides as summarized in Table 3. Two of them include HLA-A2 and/or A24-restricted CTL-epitopes. The present inventors confirmed that DEPDC1-LP2,

DEPDC1-LP3 and DEPDC1-LP4 were naturally processed from DEPDC1 protein and presented on cell surfaces of DCs in the context of HLA class II molecule (Fig. 3). Suggesting that the $CD4^+$ T-cells induced by these DEPDC1-LPs have the advantageous characteristic for cancer immunotherapy.

[0249] Since MHC class II molecules are highly polymorphic, in order to clinically apply the LPs activating HLA class II-restricted Th cells to cancer vaccine therapy, it should be considered that the peptides are capable of binding to multiple HLA class II molecules and have a wider range of adaptation (Kobayashi H, et al., Cancer research. 2001; 61(12): 4773-8; Zarour HM, et al., Cancer research. 2002; 62(1): 213-8; Grabowska AK, et al., International journal of cancer. 2015; 136(1): 212-24). The present inventors have demonstrated herein that DEPDC1-LPs were presented by HLA-DR4, HLA-DR9, HLA-DR15 (Fig. 1-1), HLA-DR53, HLA-DP2, and HLA-DP5 (Fig. 1-2), suggesting that vaccinations with a combination of DEPDC1 and -LPs could cover the majority of the Japanese population (Table 4). In fact, the present inventors observed the presence of DEPDC1-LP-specific Th cells in 2 UC patients suggesting that DEPDC1-LP may be broadly useful in many of the patients and further studies using larger number of cancer patients with various HLA-class II types should be investigated.

[0250] DEPDC1-LPs induced HLA-DP5, DR4, DR53 and DR15-restricted Th cells (Fig. 2 and Table 3). HLA-DP5, DR4, DR53 and DR15 alleles are very frequent HLA class II alleles in the Japanese and Pacific/ Asian populations, and HLA-A2 and A24 alleles are also observed at high frequencies in those populations (Table 4). In addition, DEPDC1-LP2 induced priming and expansion of DEPDC1-$A2_{302\text{-}311}$-specific CTLs and DEPDC1-$A24_{294\text{-}302}$-specific CTLs in vivo in HLA-A2 or A24 Tgm (Fig. 4), suggesting that DEPDC1-LP bearing DEPDC1-SP epitopes can induce both DEPDC1-specific Th cells and CTLs. Therefore, the present inventors suggest that these immunogenic DEPDC1-LPs could have the potential for inducing stronger anti-tumor responses.

[0251] Recent studies of LPs have shown that vaccines containing natural CTL-epitopes are superior to those composed of minimal CTL-epitopes in inducing anti-tumor CTL immunity because of long-lasting cross-presentation of the LPs (Brunsvig PF, et al., Clin Cancer Res 2011;17:6847-57; Bijker MS, et al., Eur J Immunol 2008;38:1033-42; Chauvin JM, et al., J Immunol 2012;188:2102-10). The DEPDC1-LP2 encompassing known CTL epitopes induced the CTL epitope-specific CTLs via the cross-presentation pathway in HLA-A2 or A24 Tgm in vivo (Fig. 4). Therefore, vaccination of the peptide may induce DEPDC1-LP2 specific both Th cells and CTLs simultaneously in cancer patients.

[0252] DEPDC1-LP1, and -LP4 efficiently induced those LP-specific Th cell responses (Fig. 2A-B and F-H), however, they do not contain known HLA class-I-restricted CTL

epitopes (Fig. 1B). Recently, it has reported that a phenomenon called epitope spreading is critical in induction of effective antitumor immune responses (Inderberg-Suso EM, et al., Oncoimmunology. 2012; 1(5): 670-86; Corbiere V, et al., Cancer research. 2011; 71(4): 1253-62). In fact, patients who received therapeutic cancer (minimal CTL epitope peptides) vaccines manifested clinical responses and long-term survival. In those patients, both Th-cell and CTL responses to other epitopes of the same proteins or several unrelated antigens not included in the vaccines were observed. This is because the vaccination induced CTLs specific to the target antigens, and as a result of damaging tumor cells, other TAAs were released, taken up by APCs and presented to induce other TAA-specific T cell-responses. If this is the case, vaccination of CTL epitope-free DEPDC1-LP1, -LP4 would induce LP-specific Th1 responses together with other antitumor Th1 and CTL responses. Furthermore, if the epitopes spread to include neoantigens, the aberrant peptides derived from proteins encoded by somatic missense mutant genes generated in cancer cells, the anti-tumor T cell responses would be more efficient since those neoantigens are non-self. In fact, recent studies have shown that there are many T cells specific to neoantigens, suggesting that the genes encoding for the antigens have mutations and spread epitopes may also include neoantigens. The cancer antigen vaccine therapies using neoantigens have attracted many attentions (Kreiter S, et al., Nature. 2015; 520(7549): 692-6; Linnemann C, et al., Nature medicine. 2015; 21(1): 81-5). Beside the induction of the epitope spreading, to induce and activate as many TAA-specific T cells as possible, which is a key to the success of cancer vaccine therapy, the vaccine formulation of DEPDC1-LPs and SPs may be a promising one to be investigated.

[0253] When the present inventors intend to use LPs harboring Th cell epitopes for cancer immunotherapy, whether anti-tumor Th1 cells could be induced in the patients' bodies is one major concern. It is reported that the phenotype of Th cells is affected by not only the surrounding cytokine milieu at the differentiation stage but also by the affinity/avidity of TCR and MHC-peptide complexes (Constant S, et al., The Journal of experimental medicine. 1995; 182(5): 1591-6; Yamane H, et al., The Journal of experimental medicine. 2005; 202(6): 793-804; van Panhuys N, et al., Immunity. 2014; 41(1): 63-74; Hosken NA, et al., The Journal of experimental medicine. 1995; 182(5): 1579-84). In other words, there is a tendency for $CD4^+$ T cells to differentiate into Th1 cells in recognition of high density of peptides-HLA class II complexes inducing a strong TCR-mediated signal, and in the opposite case, Th cells tend to differentiate into Th2 cells. The DEPDC1-LP-specific Th cells induced from healthy donors in this study were all Th1-type cells that predominantly produced IFN-gamma, TNF-alpha, GM-CSF, and IL-2 (Fig. 5). Therefore, the DEPDC1-LPs may preferentially trigger Th1 type Th cell differentiation possibly due to the higher affinity binding to HLA

class-II molecules, which could produce high density LP-HLA class II complexes to stimulate durable and potentially strong TCR-mediated activation signals.

[0254] Opposite to inducing various TAA-specific Th cells and CTLs by vaccination, direct introduction of genetically engineered T cells specific to TAA into cancer patients seems to be another effective antitumor therapy. In clinical trials, Rosenberg et al. introduced autologous T cells retrovirally transduced with TCR genes of NY-ESO-1-reactive CTLs into patients with metastatic synovial cell sarcoma and melanoma, who demonstrated objective clinical responses (Robbins PF, et al., Clinical cancer research : an official journal of the American Association for Cancer Research. 2015; 21(5): 1019-27). In this study, TCR-usage of DEPDC1-LP -specific Th cells extremely converged to one combination of TCRA and TCRB genes, respectively after repeated stimulation (Fig. 6A-B). Introduction of each pair of TCR genes into murine TCR-negative T cell line TG40 exhibited the original T cell specificity to their cognate ligands (Fig. 6C), suggesting that transfer of patients' autologous T cells expressing those TCR genes could be an effective therapy. The convergence of TCR repertoire of DEPDC1-LP-specific Th cells into those with particular CDR sequences seemed to be similar to the recent observation by Dash et al., and Glanville et al., in which the antigen specific TCRs share specifically enriched short sequence motifs in CDRs compared to random TCR repertoires that had not been selected for binding to the particular antigenic peptide (Dash P, et al., Nature. 2017; 547(7661): 89-93; Glanville J, et al. Nature. 2017; 547(7661): 94-8).

[0255] In conclusion, the present inventors identified four immunogenic DEPDC1-LPs that can induce Th cells. Two of them encompass HLA-A2-restricted and HLA-A24-restricted CTL epitopes. Our results suggest that DEPDC1-LPs provide a useful tool for propagation of both DEPDC1-specific Th1 cells and CTLs. These findings support a possible clinical trial of DEPDC1 peptide-based immunotherapy for several types of cancers such as bladder cancer. In addition, converged TCRs expressed on LPs-specific Th cells may be useful for monitoring of LPs-specific Th cells in vitro and possibly in vivo.

Industrial Applicability

[0256] The present invention describes Th1 cell epitope peptides derived from DEPDC1 that can induce potent anti-tumor immune responses and thus have applicability to a wide array of cancer types. Such peptides warrant further development as peptide vaccines against cancer, especially against cancers expressing DEPDC1. The peptides of the present invention can induce the Th1 cell response and thus cytokines secreted by Th1 cells can help or activate any immune cells responsible for cellular immunity in an antigen independent manner. Therefore, immunotherapeutic strategy provided by the

present invention can be applied to any diseases including cancers, as long as the disease can be improved via immune responses mediated by MHC class II molecules. In particular, Th1 cells of the present invention can improve immunological responses raised by CTLs. Therefore, the peptide of the present invention would be beneficial to enhance CTL response against diseases including cancers in a subject.

[0257] Moreover, in preferred embodiments, the peptides of the present invention can also induce CTLs against DEPDC1 expressing cells, as well as Th1 cells. Such peptide of the present invention can be also useful for the treatment of diseases associated with DEPDC1, e.g. cancers expressing DEPDC1, more particularly, bladder cancer and breast cancer.

[0258] While the present invention is herein described in detail and with reference to specific embodiments thereof, it is to be understood that the foregoing description is exemplary and explanatory in nature and is intended to illustrate the invention and its preferred embodiments. Through routine experimentation, one skilled in the art will readily recognize that various changes and modifications can be made therein without departing from the spirit and scope of the invention, the metes and bounds of which are defined by the appended claims.

Claims

[Claim 1] An isolated peptide having 10-30 amino acids in length and comprising a part of the amino acid sequence of SEQ ID NO: 8 or 10, wherein said peptide comprises an amino acid sequence selected from the group consisting of:
(a) a contiguous amino acid sequence having more than 9 amino acids in length selected from the amino acid sequence of SEQ ID NO: 1, 2, 3 or 4; and
(b) an amino acid sequence in which one, two or several amino acids are substituted, deleted, inserted, and/or added in the amino acid sequence of (a),
wherein said peptide has ability to induce T helper type 1 (Th1) cells.

[Claim 2] The isolated peptide of claim 1, wherein the peptide or fragment thereof has abilities to bind to at least two kinds of MHC class II molecules.

[Claim 3] The isolated peptide of claim 2, wherein the MHC class II molecules are selected from the group consisting of HLA-DR53, HLA-DR4, HLA-DR15 and HLA-DP5.

[Claim 4] The isolated peptide of any one of claims 1 to 3, wherein said peptide comprises an amino acid sequence of a peptide having DEPDC1-specific cytotoxic T lymphocyte (CTL) inducibility.

[Claim 5] The isolated peptide of claim 4, wherein said peptide comprises the amino acid sequence selected from the group consisting of:
(a) an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 to 4; and
(b) an amino acid sequence in which one, two or several amino acids are substituted, deleted, inserted, and/or added in the amino acid sequence of (a).

[Claim 6] An isolated polynucleotide encoding the peptide of any one of claims 1 to 5.

[Claim 7] A composition for inducing at least one of the cells selected from the group consisting of
(i) Th1 cells,
(ii) CTLs,
(iii) antigen-presenting cells (APCs) having an ability to induce Th1 cells, and
(iv) APCs having an ability to induce CTLs,

wherein the composition comprises one or more peptide(s) of any one of claims 1 to 5, or one or more polynucleotide(s) encoding them.

[Claim 8] A pharmaceutical composition, wherein the composition comprises at least one active ingredient selected from the group consisting of:
(a) one or more peptide(s) of any one of claims 1 to 5;
(b) one or more polynucleotide(s) of claim 6;
(c) one or more APC(s) presenting the peptide of any one of claims 1 to 5 or fragment thereof on their surface;
(d) one or more Th1 cells that recognize(s) an APC presenting the peptide of any one of claims 1 to 5 or fragment thereof on its surface; and
(e) combination of any two or more of (a) to (d) above; and is formulated for a purpose selected from the group consisting of:
(i) cancer treatment,
(ii) cancer prevention,
(iii) prevention of post-operative recurrence in cancer, and
(iv) combinations of any two or more of (i) to (iii) above.

[Claim 9] The pharmaceutical composition of claim 8, wherein said composition is formulated for administration to a subject that has at least one selected from the group consisting of HLA-DR53, HLA-DR4, HLA-DR15 and HLA-DP5 as an MHC class II molecule.

[Claim 10] The pharmaceutical composition of claim 8 or 9, wherein said composition further comprises one or more peptides having CTL inducibility.

[Claim 11] A composition for enhancing an immune response mediated with an MHC class II molecule, wherein the composition comprises at least one active ingredient selected from the group consisting of:
(a) one or more peptide(s) of any one of claims 1 to 5;
(b) one or more polynucleotide(s) of claim 6;
(c) one or more APC(s) presenting the peptide of any one of claims 1 to 5 or fragment thereof on their surface;
(d) one or more Th1 cell(s) that recognize(s) an APC presenting the peptide of any one of claims 1 to 5 or fragment thereof on its surface; and
(e) combination of any two or more of (a) to (d) above.

[Claim 12] A method for inducing an APC having an ability to induce a Th1 cell, said method comprising a step of contacting an APC with the peptide of any one of claims 1 to 5 in vitro, ex vivo or in vivo.

[Claim 13] A method for inducing an APC having an ability to induce a CTL, said method comprising a step selected from the group consisting of:
(a) contacting an APC with the peptide of any one of claims 1 to 5 in vitro, ex vivo or in vivo; and
(b) introducing a polynucleotide encoding the peptide of any one of claims 1 to 5 into an APC.

[Claim 14] A method for inducing a Th1 cell, said method comprising a step selected from the group consisting of:
(a) co-culturing a CD4-positive T cell with an APC that presents on its surface a complex of an MHC class II molecule and the peptide of any one of claims 1 to 5 or fragment thereof; and
(b) introducing a polynucleotide encoding both of T cell receptor (TCR) subunits, or polynucleotides encoding each of TCR subunits into a CD4-positive T cell, wherein the TCR can bind to a complex of an MHC class II molecule and the peptide of any one of claims 1 to 5 or fragment thereof presented on cell surface.

[Claim 15] A method for inducing a CTL, said method comprising the step selected from the group consisting of:
(a) co-culturing both of a CD4-positive T cell and a CD8-positive T cell with APCs contacted with the peptide of claim 4 or 5; and
(b) co-culturing a CD8-positive T cell with an APC contacted with the peptide of claim 4 or 5.

[Claim 16] A method for enhancing an immune response mediated by an MHC class II molecule, wherein the method comprises a step of administering to a subject at least one active ingredient selected from the group consisting of:
(a) one or more peptide(s) of any one of claims 1 to 5;
(b) one or more polynucleotide(s) of claim 6;
(c) one or more APC(s) presenting the peptide of any one of claims 1 to 5 or fragment thereof on their surface;
(d) one or more Th1 cell(s) that recognize(s) an APC presenting the peptide of any one of claims 1 to 5 or fragment thereof on its surface; and
(e) combination of any two or more of (a) to (d) above.

[Claim 17] An isolated APC that presents on its surface a complex of an MHC class II molecule and the peptide of any one of claims 1 to 5 or fragment thereof.

[Claim 18] The APC induced by the method of claim 12 or 13.

[Claim 19] An isolated Th1 cell that recognizes the peptide of any one of claims 1 to 5 or fragment thereof presented on a surface of an APC.

[Claim 20] The Th1 cell induced by the method of claim 14.

[Claim 21] The Th1 cell of claim 20, wherein the alpha and beta subunits of TCR comprises CDR3 consisting of the amino acid sequence of SEQ ID NO: 11 and 21, respectively.

[Claim 22] A method of inducing an immune response against cancer in a subject in need thereof, said method comprising the step of administering to the subject a composition comprising at least one active ingredient selected from the group consisting of:
(a) one or more peptide(s) of any one of claims 1 to 5;
(b) one or more polynucleotide(s) of claim 6;
(c) one or more APC(s) presenting the peptide of any one of claims 1 to 5 or fragment thereof on their surface;
(d) one or more Th1 cell(s) that recognize(s) an APC presenting the peptide of any one of claims 1 to 5 or fragment thereof on its surface; and
(e) combination of any two or more of (a) to (d) above.

[Claim 23] An antibody or immunologically active fragment thereof against the peptide of any one of claims 1 to 5.

[Claim 24] A vector comprising a nucleotide sequence encoding the peptide of any one of claims 1 to 5.

[Claim 25] A host cell transformed or transfected with the expression vector of claim 24.

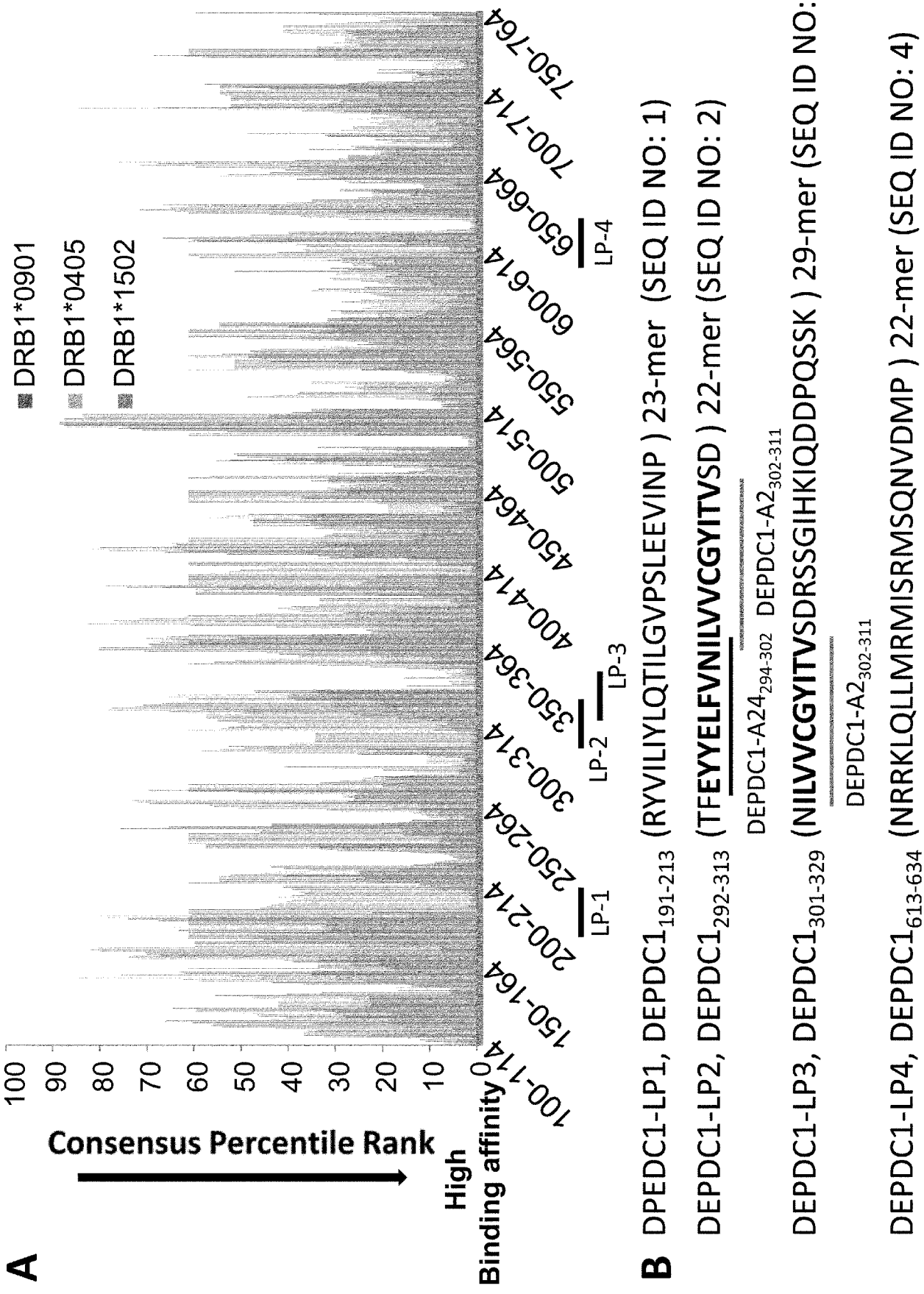
[Claim 26] A diagnostic kit comprising the peptide of any one of claims 1 to 5, the polynucleotide of claim 6 or the antibody of claim 23.

[Claim 27] A method for assessing and/or monitoring Th1 response specific to the peptide comprising the amino acid sequence of SEQ ID NO: 1, 2, 3 or 4 in a subject, said method comprising:
(a) providing a sample obtained from the subject administered said peptide, wherein said sample comprises T cells;
(b) detecting the presence of T cells expressing the TCR which binds to a complex of an MHC class II molecule and said peptide or a fragment thereof in said sample; and
(c) indicating an induction of Th1 response specific to said peptide when the presence of said T cells is detected in (b).

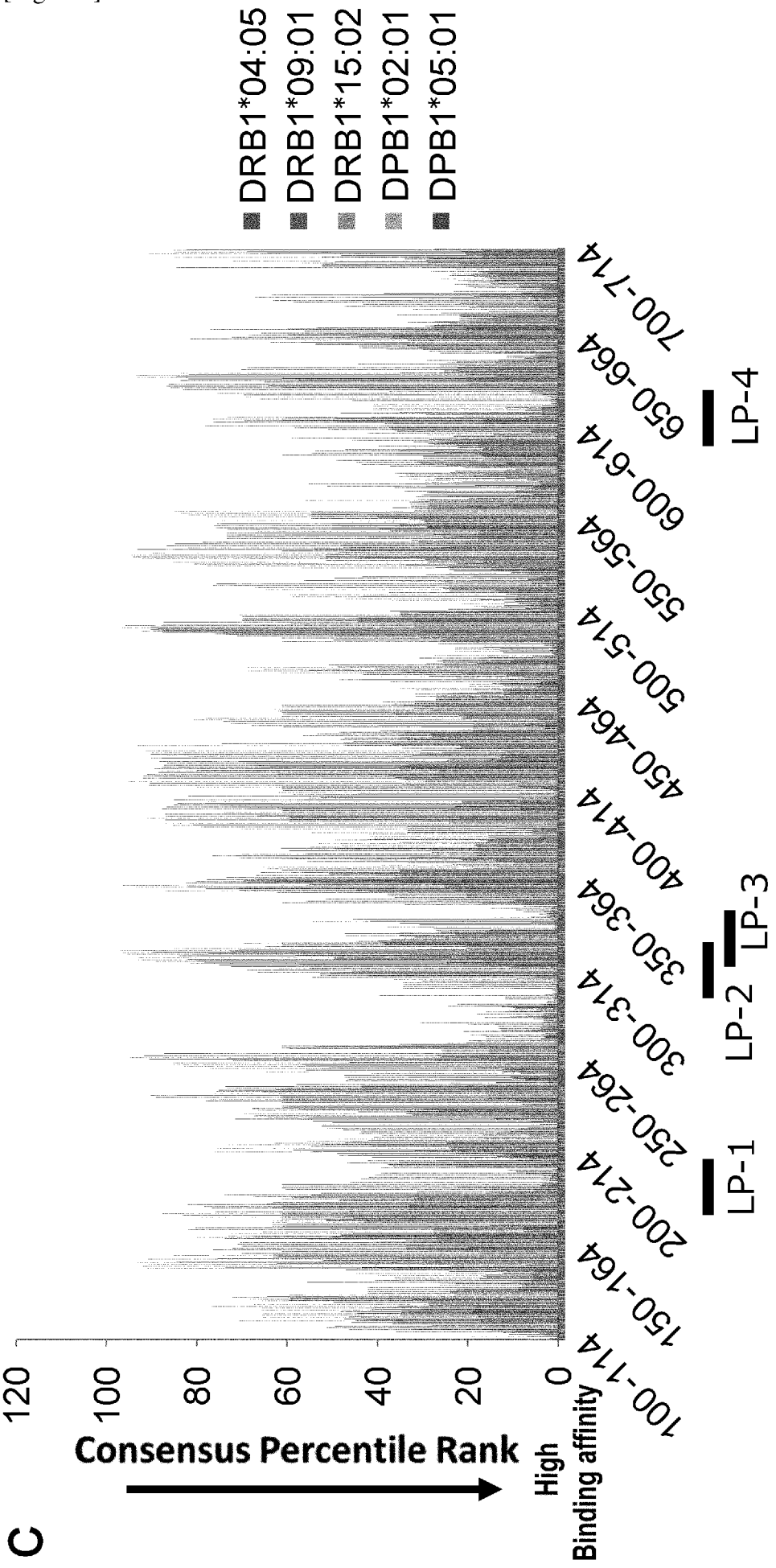
[Claim 28] The method of claim 27, wherein said TCR comprises a particular pair of alpha and beta subunits each comprising CDR3 consisting of the

amino acid sequence of SEQ ID NO: 11 and 21, respectively.

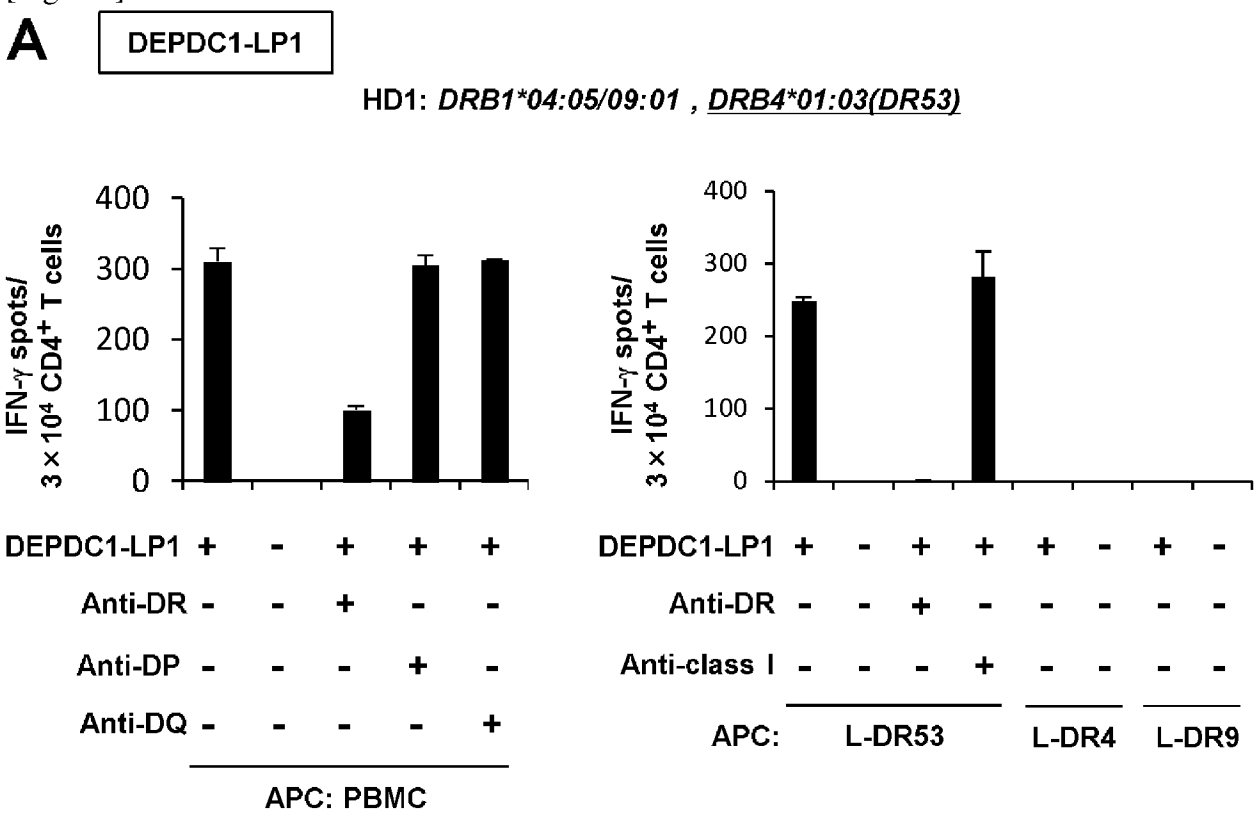
[Fig. 1-1]


A
DRB1*0901
DRB1*0405
DRB1*1502
Consensus Percentile Rank
High
Binding affinity
100
90
80
70
60
50
40
30
20
10
0
100-174
150-164
200-214
250-264
300-314
350-364
400-414
450-464
500-514
550-564
600-614
650-664
700-714
750-764
LP-1
LP-2
LP-3
LP-4
B DPEDC1-LP1, DEPDC1191-213 (RYVILIYLQTILGVPSLEEVINP) 23-mer (SEQ ID NO: 1)
DEPDC1-LP2, DEPDC1292-313 (TFEYYELFVNILVVCGYITVSD) 22-mer (SEQ ID NO: 2)
DEPDC1-A24294-302 DEPDC1-A2302-311
DEPDC1-LP3, DEPDC1301-329 (NILVVCGYITVSDRSSGIHKIQDDPQSSK) 29-mer (SEQ ID NO: 3)
DEPDC1-A2302-311
DEPDC1-LP4, DEPDC1613-634 (NRRKLQLLMRMISRMSQNVDMP) 22-mer (SEQ ID NO: 4)

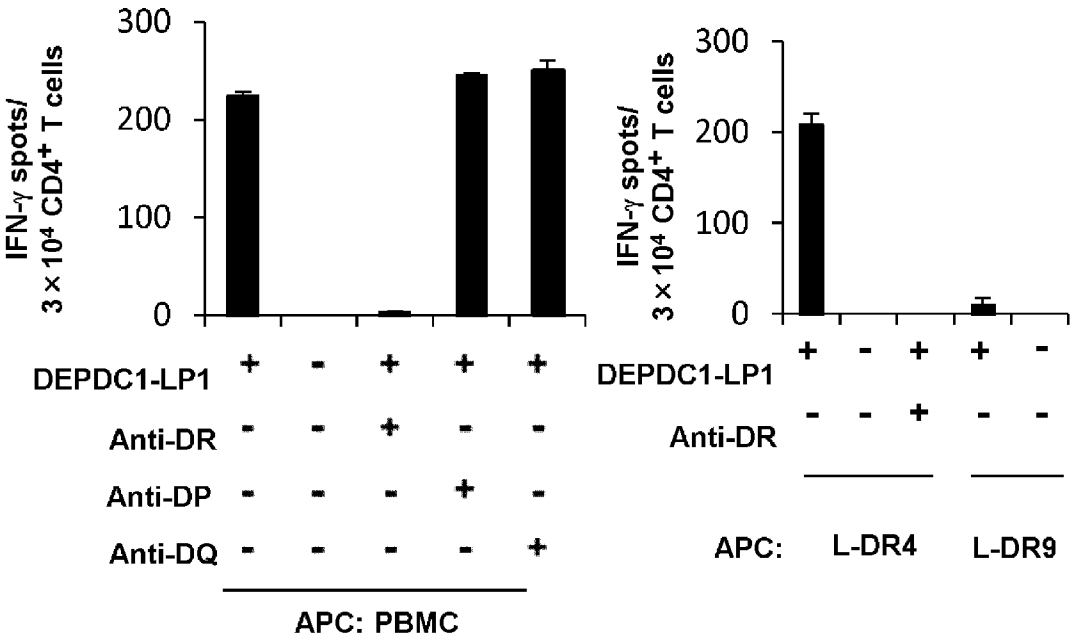
[Fig. 1-2]


DRB1*04:05
DRB1*09:01
DRB1*15:02
DPB1*02:01
DPB1*05:01
700-714
650-664
600-614
550-564
500-514
450-464
400-414
350-364
300-314
250-264
200-214
150-164
100-114
LP-4
LP-2
LP-3
LP-1
120
100
80
60
40
20
0
Consensus Percentile Rank
High
Binding affinity
C

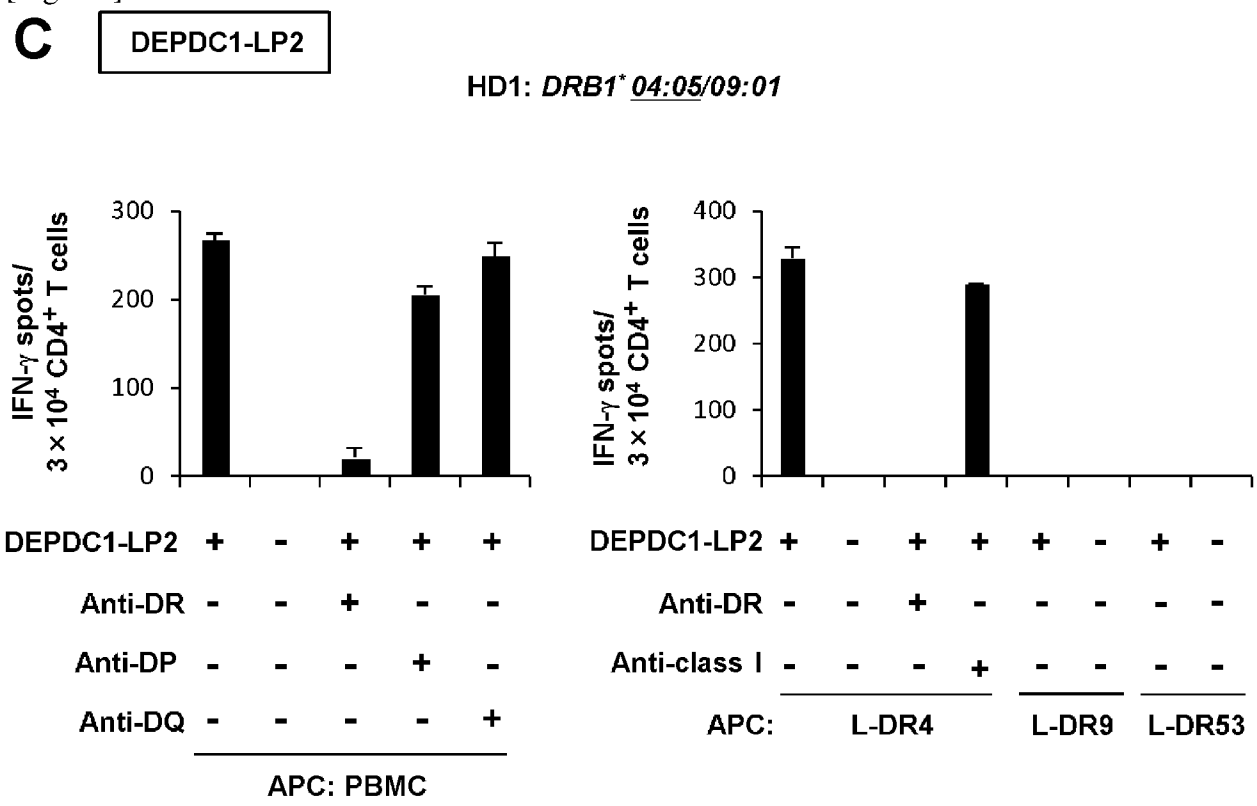
[Fig. 2A]


A
DEPDC1-LP1
HD1: DRB1*04:05/09:01 , DRB4*01:03(DR53)
IFN-γ spots/ 3 × 10⁴ CD4⁺ T cells
400
300
200
100
0
DEPDC1-LP1 + - + + +
Anti-DR - - + - -
Anti-DP - - - + -
Anti-DQ - - - - +
APC: PBMC
DEPDC1-LP1 + - + + + - + -
Anti-DR - - + - - - - -
Anti-class I - - - + - - - -
APC: L-DR53 L-DR4 L-DR9

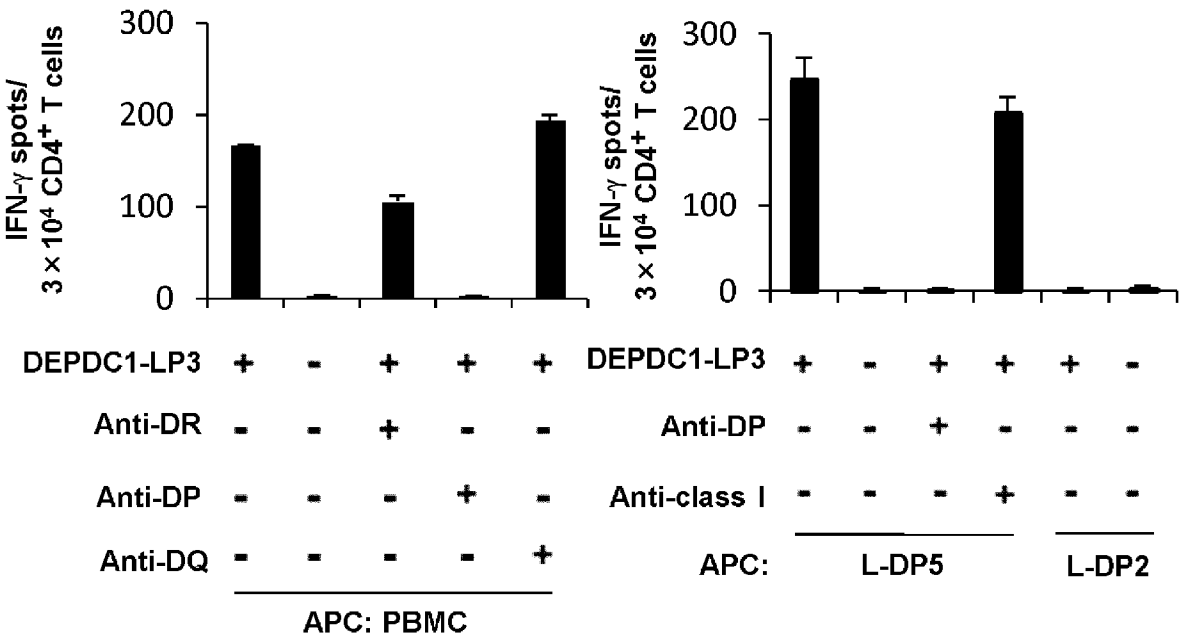
[Fig. 2B]


B
DEPDC1-LP1
HD4: DRB1* 04:05 / -
IFN-γ spots/ 3 × 10⁴ CD4⁺ T cells
300
200
100
0
DEPDC1-LP1 + - + + +
Anti-DR - - + - -
Anti-DP - - - + -
Anti-DQ - - - - +
APC: PBMC
DEPDC1-LP1 + - + + -
Anti-DR - - + - -
APC: L-DR4 L-DR9

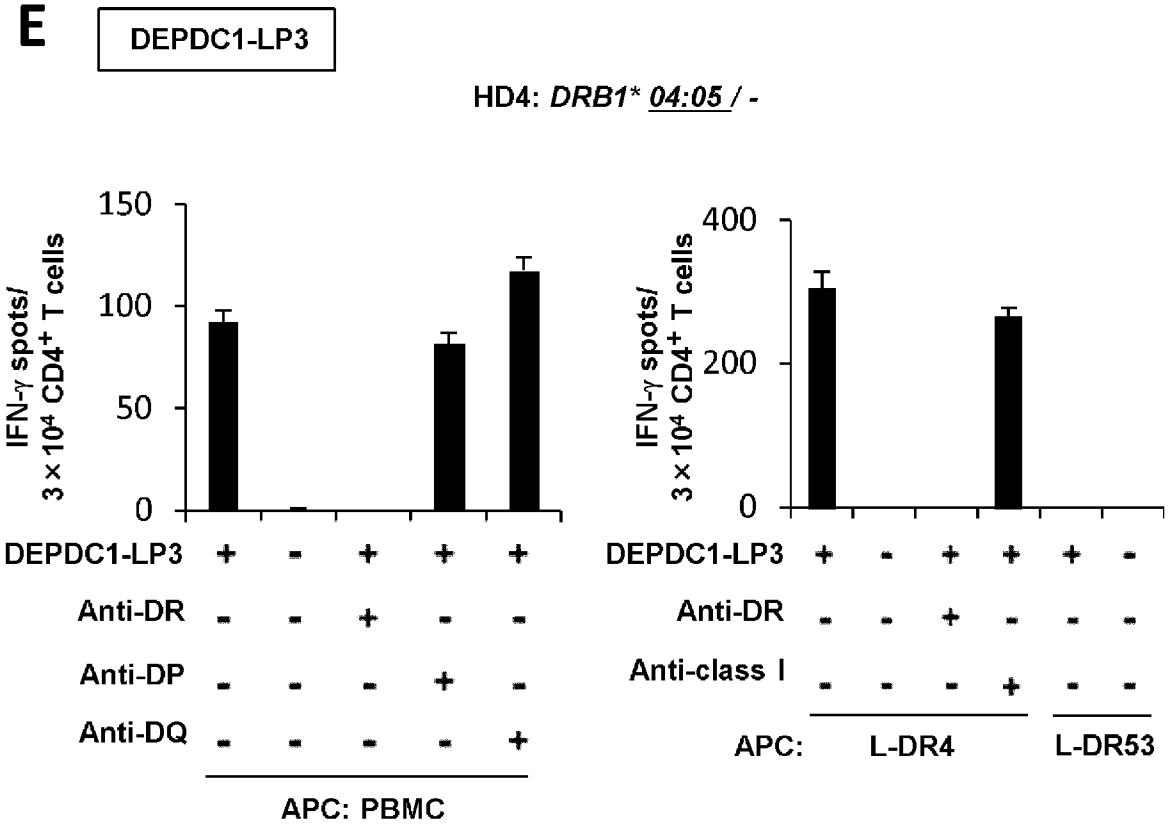
[Fig. 2C]


C
DEPDC1-LP2
HD1: DRB1* 04:05/09:01
IFN-γ spots/ 3 × 10⁴ CD4⁺ T cells
300
200
100
0
DEPDC1-LP2 + - + + +
Anti-DR - - + - -
Anti-DP - - - + -
Anti-DQ - - - - +
APC: PBMC
400
300
200
100
0
DEPDC1-LP2 + - + + + - + -
Anti-DR - - + - - - - -
Anti-class I - - - + - - - -
APC: L-DR4 L-DR9 L-DR53

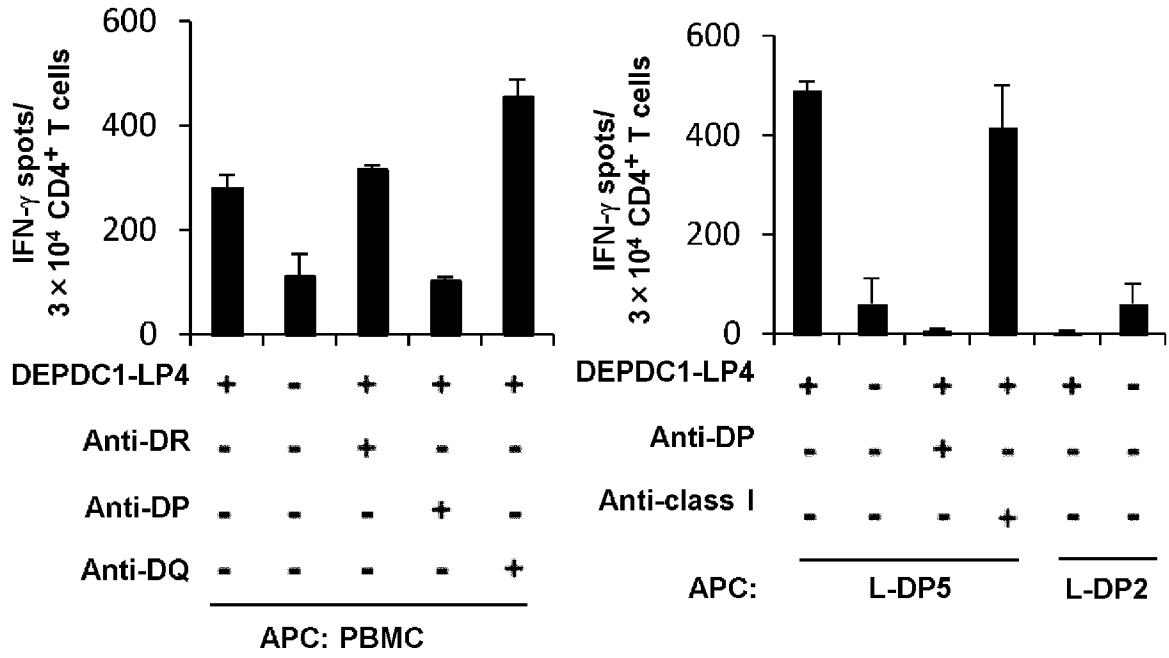
[Fig. 2D]


D
DEPDC1-LP3
HD3: DPB1* 02:02/05:01
IFN-γ spots/ 3 × 10⁴ CD4⁺ T cells
300
200
100
0
DEPDC1-LP3 + - + + +
Anti-DR - - + - -
Anti-DP - - - + -
Anti-DQ - - - - +
APC: PBMC
300
200
100
0
DEPDC1-LP3 + - + + + -
Anti-DP - - + - - -
Anti-class I - - - + - -
APC: L-DP5 L-DP2

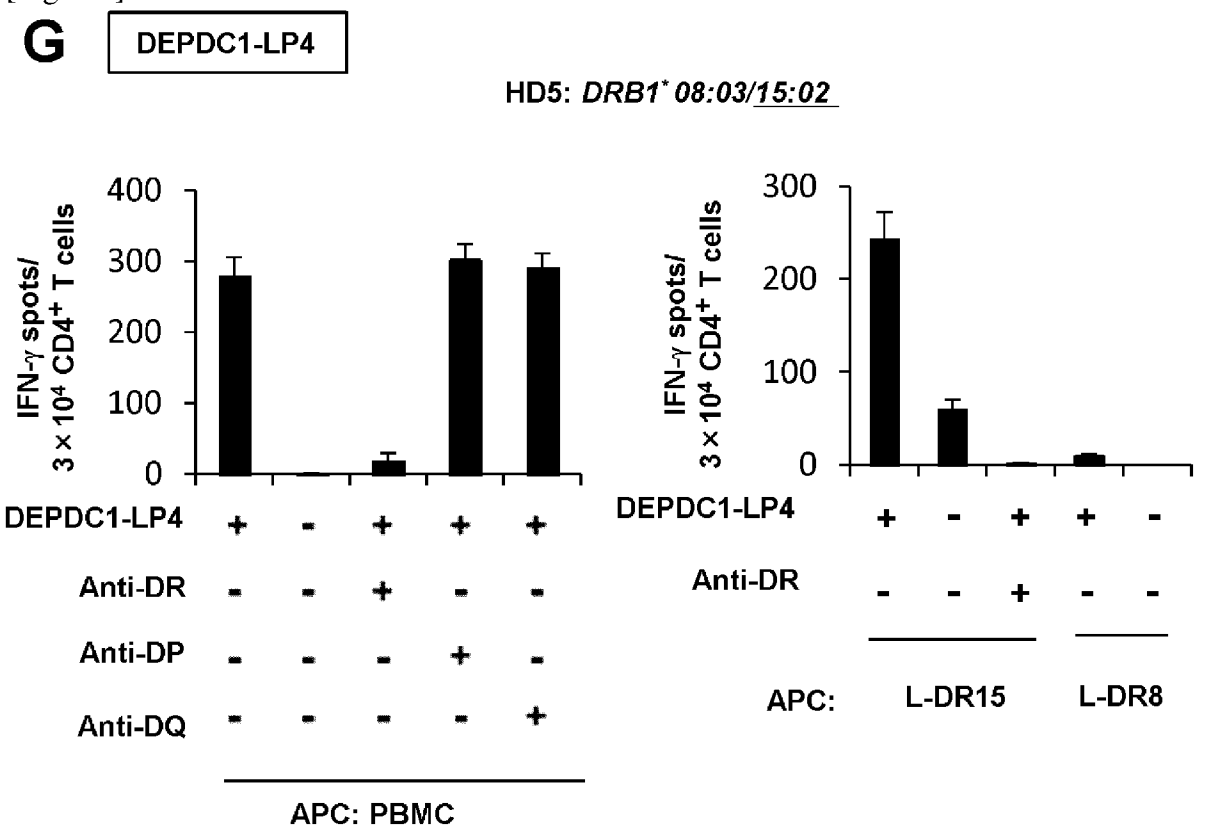
[Fig. 2E]


E
DEPDC1-LP3
HD4: DRB1* 04:05 / -
IFN-γ spots/ 3 × 10⁴ CD4⁺ T cells
150
100
50
0
DEPDC1-LP3 + - + + +
Anti-DR - - + - -
Anti-DP - - - + -
Anti-DQ - - - - +
APC: PBMC
400
200
0
DEPDC1-LP3 + - + + + -
Anti-DR - - + - - -
Anti-class I - - - + - -
APC: L-DR4 L-DR53

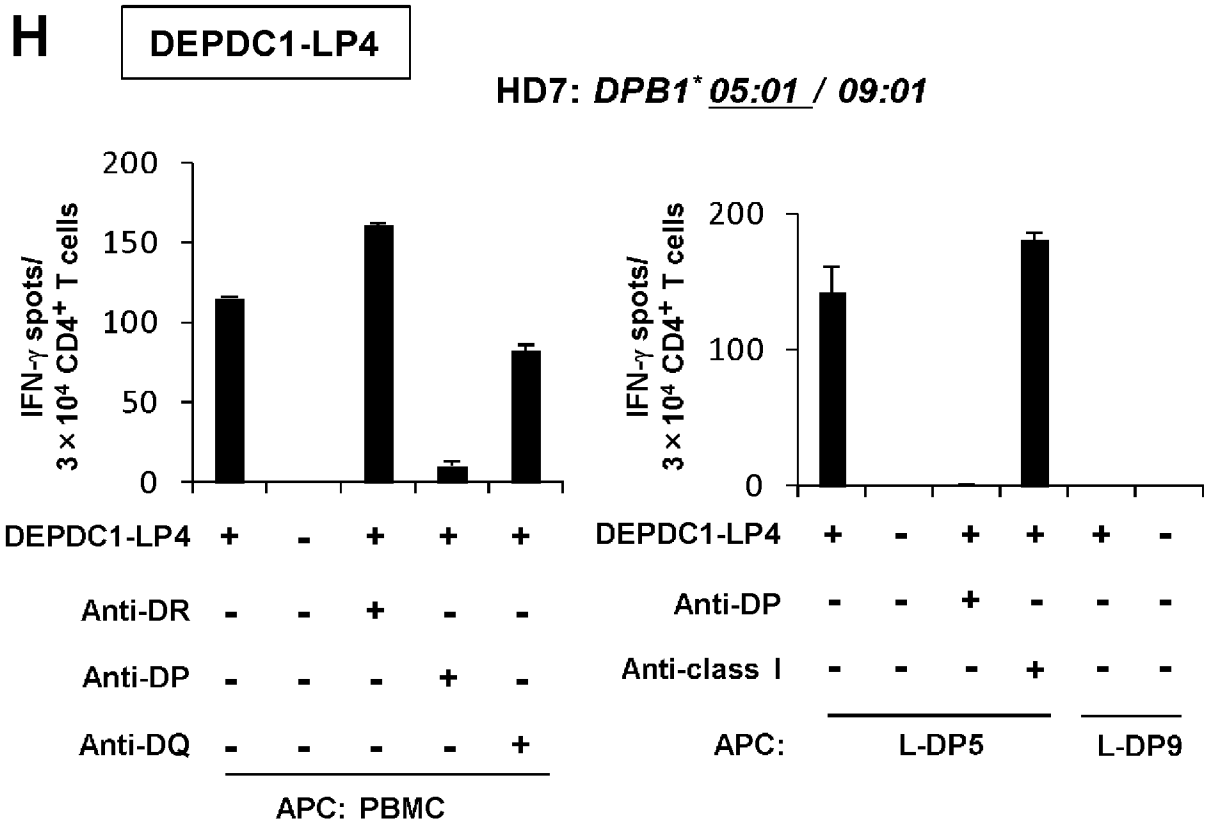
[Fig. 2F]


F
DEPDC1-LP4
HD1: DPB1* 05:01 / -
IFN-γ spots/ 3 × 10⁴ CD4⁺ T cells
600
400
200
0
DEPDC1-LP4 + - + + +
Anti-DR - - + - -
Anti-DP - - - + -
Anti-DQ - - - - +
APC: PBMC
600
400
200
0
DEPDC1-LP4 + - + + + -
Anti-DP - - + - - -
Anti-class I - - - + - -
APC: L-DP5 L-DP2

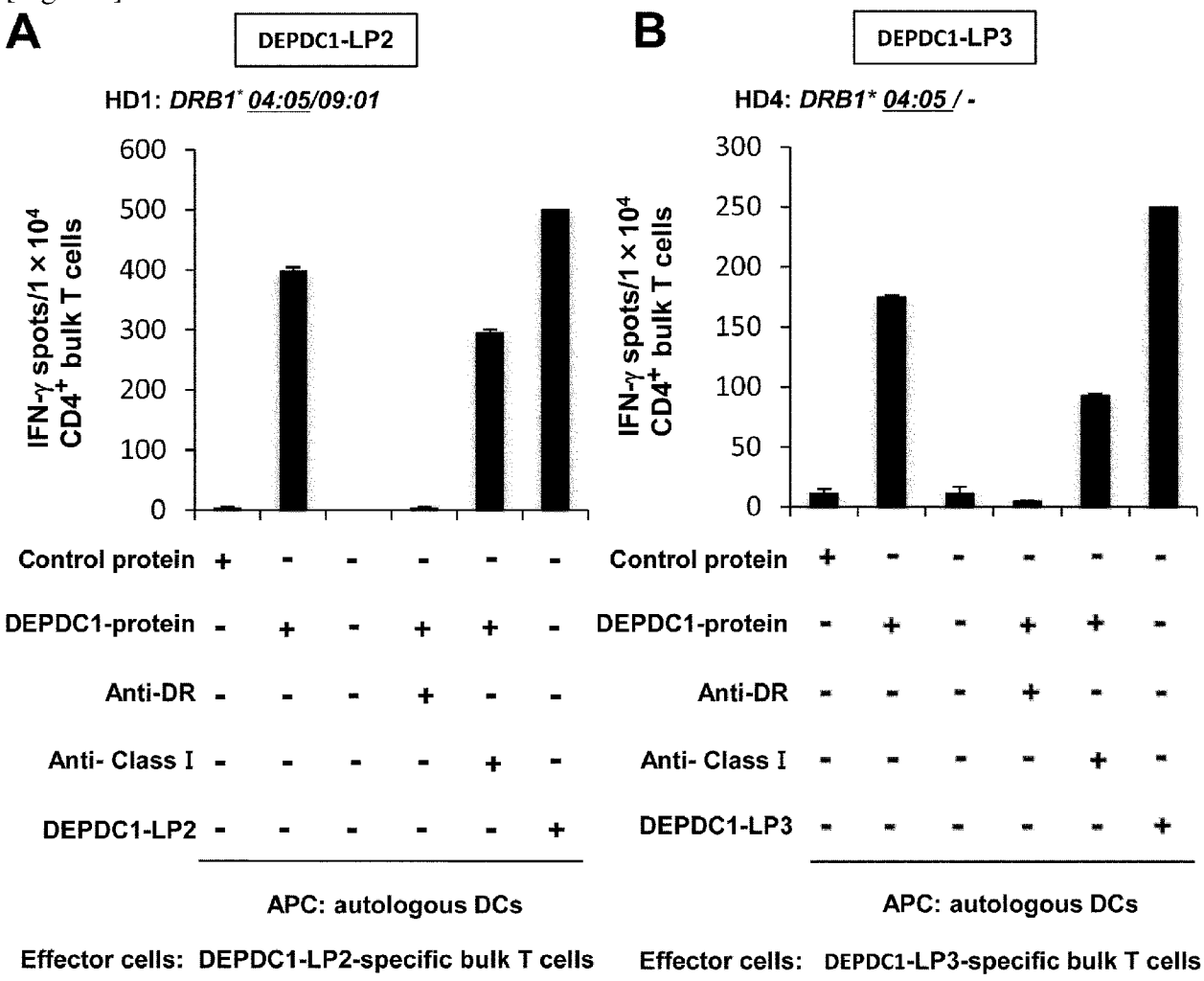
[Fig. 2G]


G
DEPDC1-LP4
HD5: DRB1* 08:03/15:02
IFN-γ spots/ 3 × 10⁴ CD4⁺ T cells
400
300
200
100
0
DEPDC1-LP4 + - + + +
Anti-DR - - + - -
Anti-DP - - - + -
Anti-DQ - - - - +
APC: PBMC
300
200
100
0
DEPDC1-LP4 + - + + -
Anti-DR - - + - -
APC: L-DR15 L-DR8

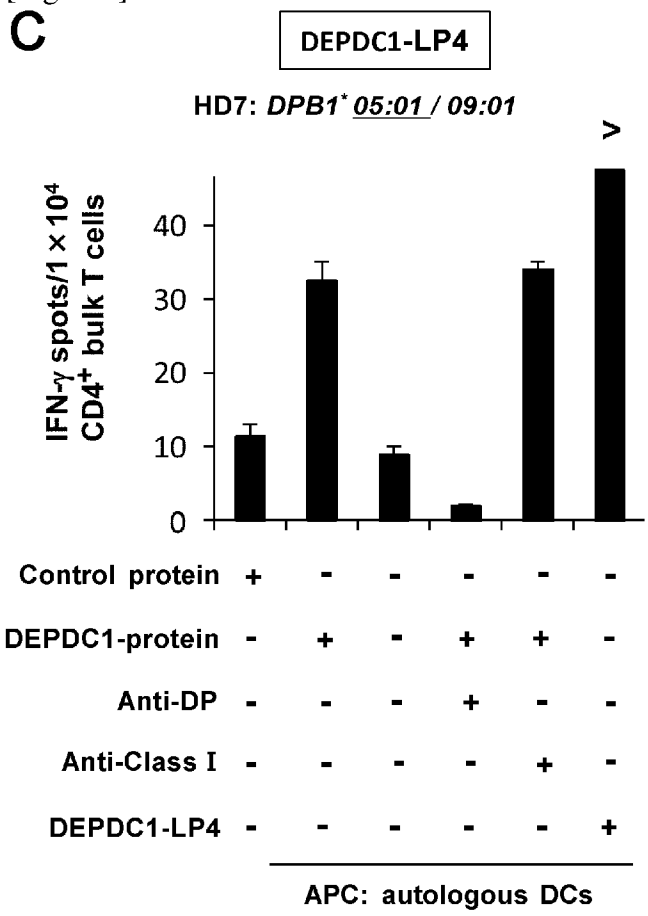
[Fig. 2H]


H
DEPDC1-LP4
HD7: DPB1* 05:01 / 09:01
IFN-γ spots/ 3 × 10⁴ CD4⁺ T cells
200
150
100
50
0
DEPDC1-LP4 + - + + +
Anti-DR - - + - -
Anti-DP - - - + -
Anti-DQ - - - - +
APC: PBMC
200
100
0
DEPDC1-LP4 + - + + + -
Anti-DP - - + - - -
Anti-class I - - - + - -
APC: L-DP5 L-DP9

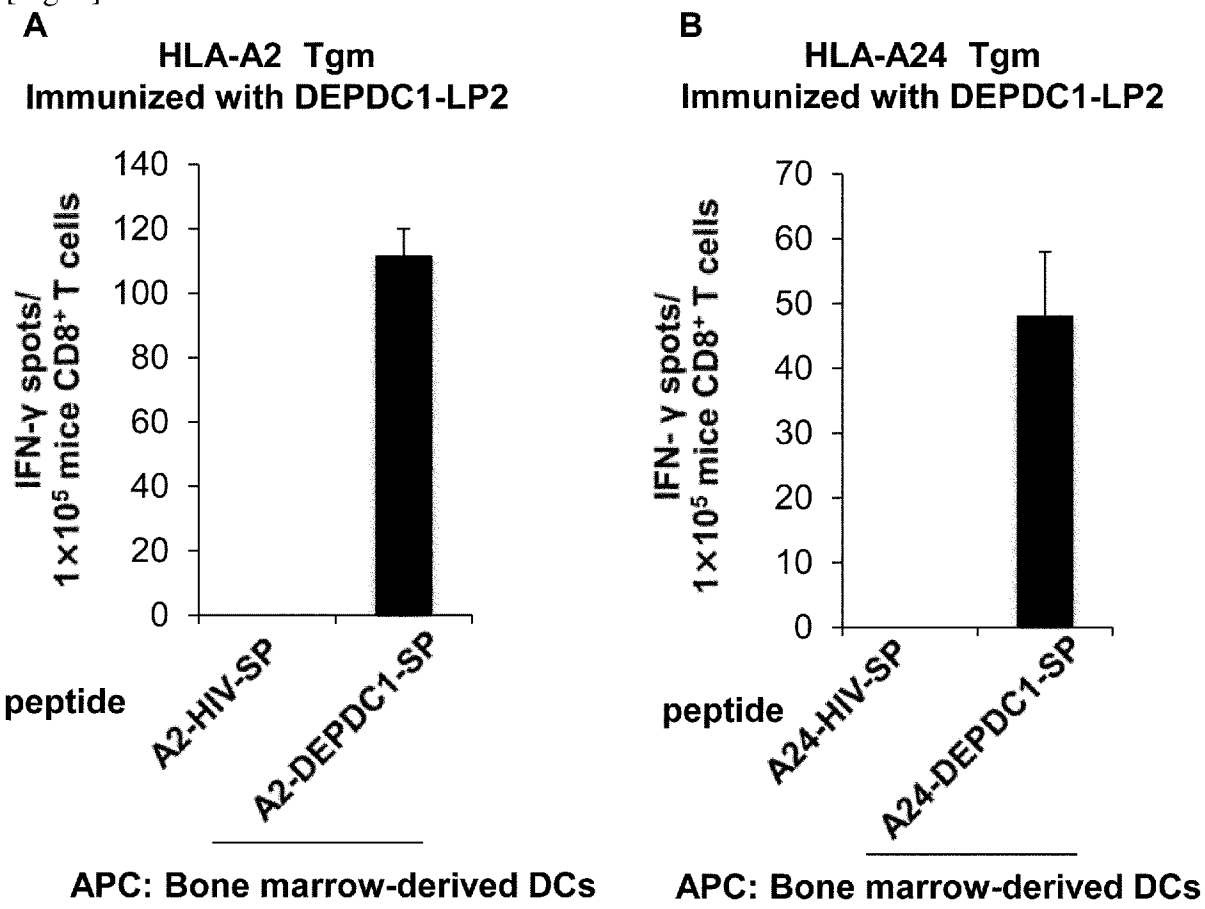
[Fig. 3-1]


A
DEPDC1-LP2
HD1: DRB1* 04:05/09:01
IFN-γ spots/1 × 10^4 CD4+ bulk T cells
600
500
400
300
200
100
0
Control protein + - - - - -
DEPDC1-protein - + - + + -
Anti-DR - - - + - -
Anti- Class I - - - - + -
DEPDC1-LP2 - - - - - +
APC: autologous DCs
Effector cells: DEPDC1-LP2-specific bulk T cells
B
DEPDC1-LP3
HD4: DRB1* 04:05 / -
IFN-γ spots/1 × 10^4 CD4+ bulk T cells
300
250
200
150
100
50
0
Control protein + - - - - -
DEPDC1-protein - + - + + -
Anti-DR - - - + - -
Anti- Class I - - - - + -
DEPDC1-LP3 - - - - - +
APC: autologous DCs
Effector cells: DEPDC1-LP3-specific bulk T cells

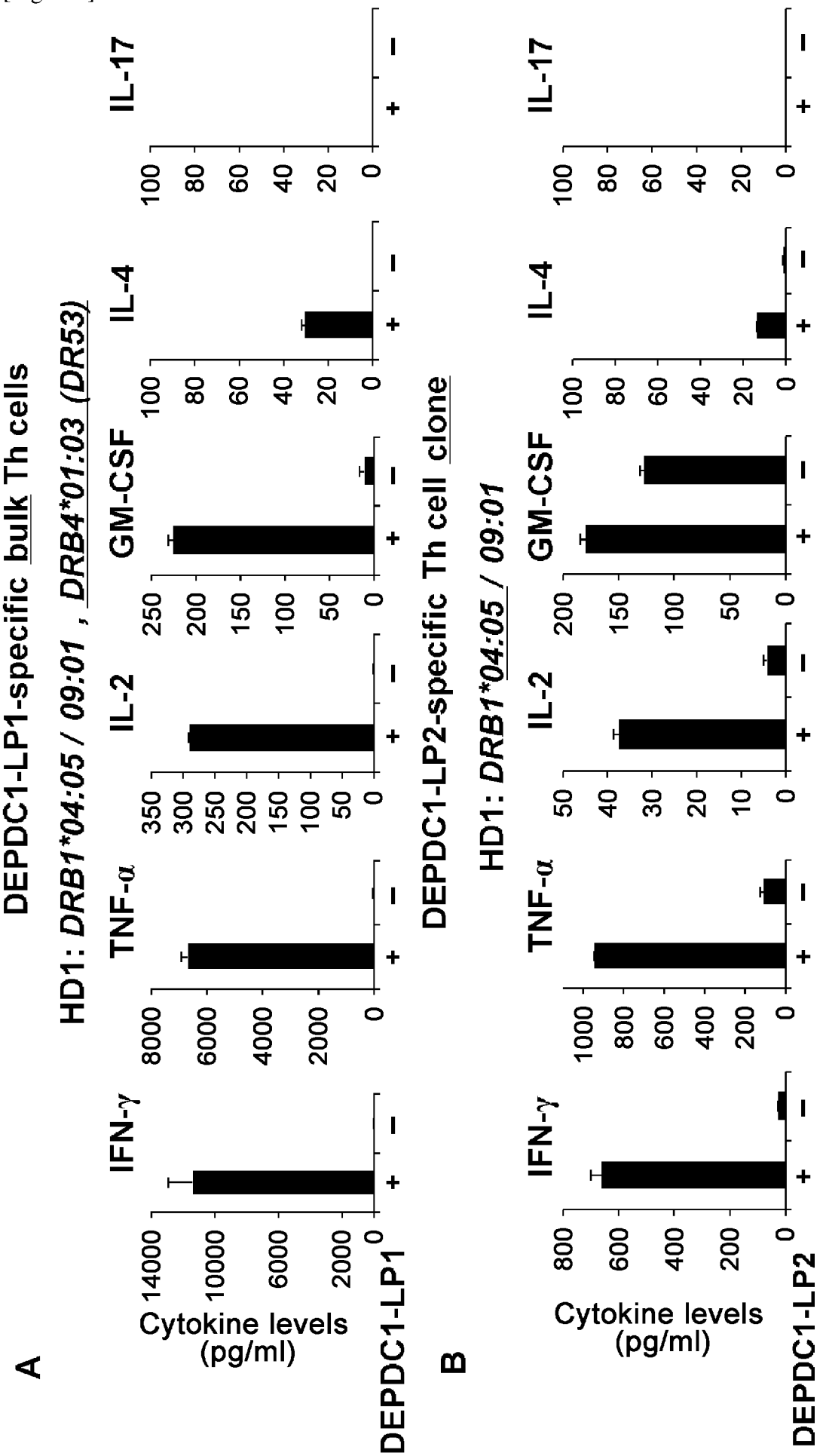
[Fig. 3-2]


C
DEPDC1-LP4
HD7: DPB1* 05:01 / 09:01
IFN-γ spots/1 × 10^4 CD4+ bulk T cells
0
10
20
30
40
Control protein + - - - - -
DEPDC1-protein - + - + + -
Anti-DP - - - + - -
Anti-Class I - - - - + -
DEPDC1-LP4 - - - - - +
APC: autologous DCs
Effector cells: DEPDC1-LP4-specific bulk T cells

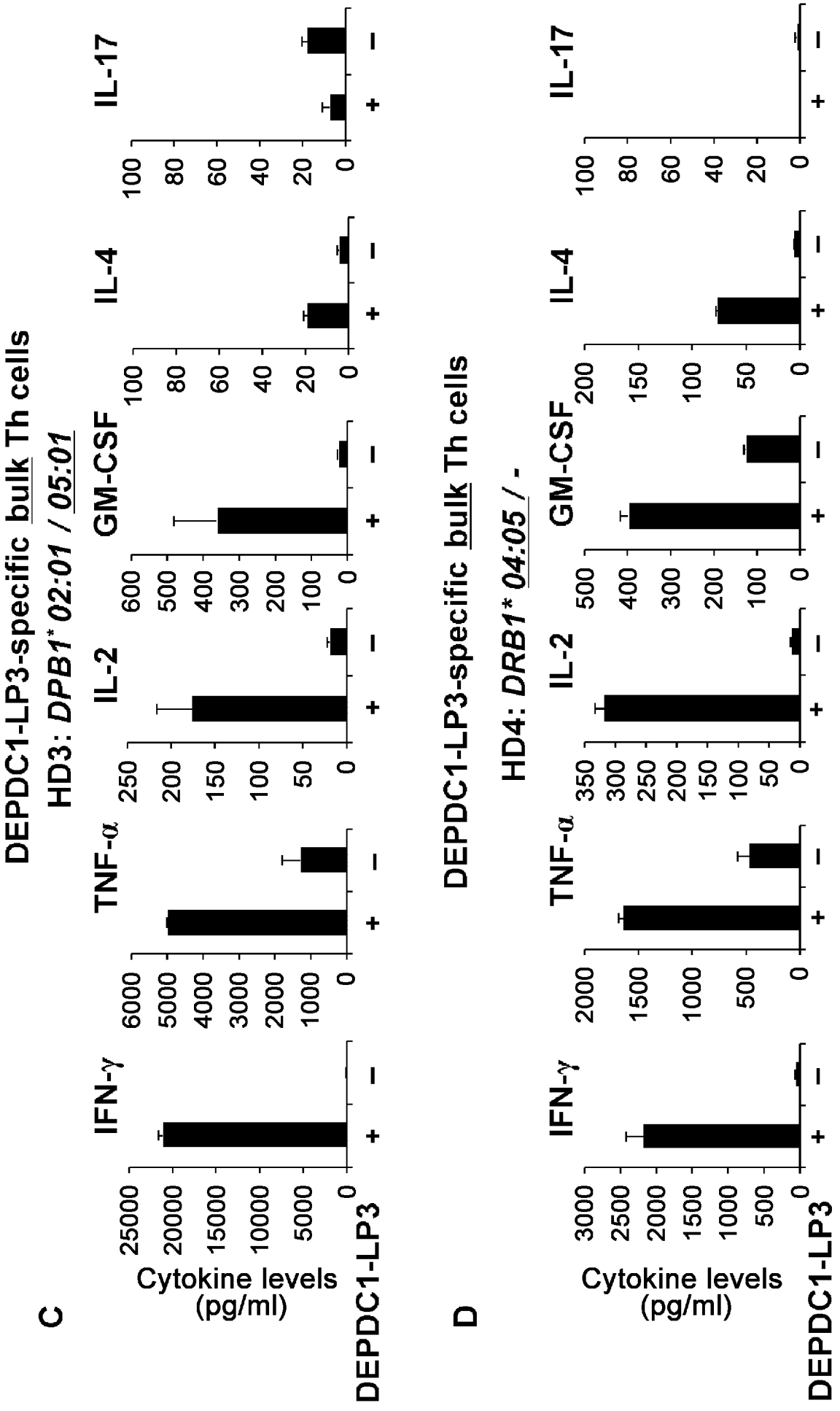
[Fig. 4]


A
HLA-A2 Tgm
Immunized with DEPDC1-LP2
IFN-γ spots/ 1×10^5 mice CD8+ T cells
0
20
40
60
80
100
120
140
peptide
A2-HIV-SP
A2-DEPDC1-SP
APC: Bone marrow-derived DCs
B
HLA-A24 Tgm
Immunized with DEPDC1-LP2
IFN-γ spots/ 1×10^5 mice CD8+ T cells
0
10
20
30
40
50
60
70
peptide
A24-HIV-SP
A24-DEPDC1-SP
APC: Bone marrow-derived DCs

[Fig. 5-1]


A
DEPDC1-LP1-specific bulk Th cells
HD1: DRB1*04:05 / 09:01 , DRB4*01:03 (DR53)
IFN-γ
TNF-α
IL-2
GM-CSF
IL-4
IL-17
Cytokine levels (pg/ml)
DEPDC1-LP1
B
DEPDC1-LP2-specific Th cell clone
HD1: DRB1*04:05 / 09:01
DEPDC1-LP2

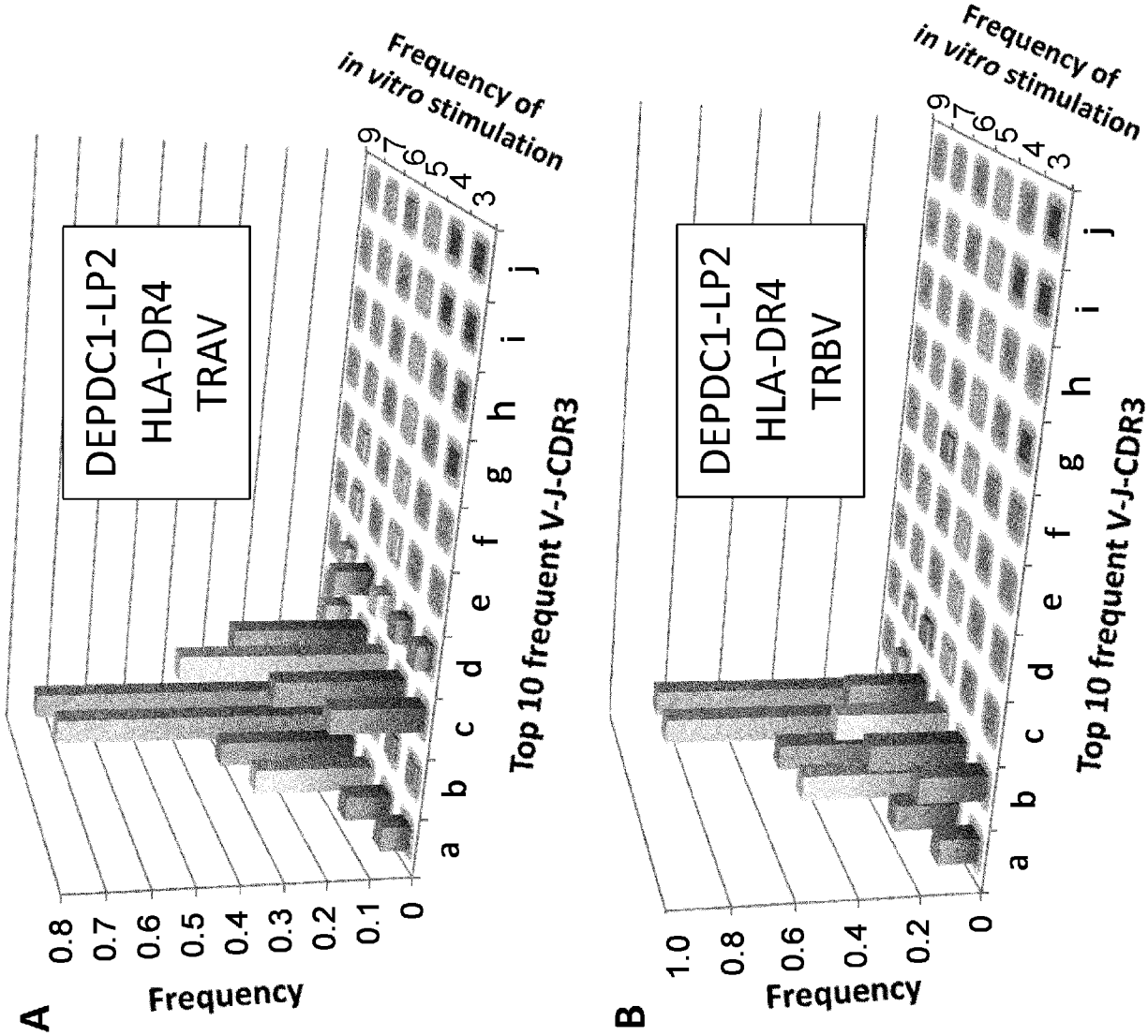
[Fig. 5-2]


C
DEPDC1-LP3-specific bulk Th cells
HD3: DPB1* 02:01 / 05:01
IFN-γ
TNF-α
IL-2
GM-CSF
IL-4
IL-17
Cytokine levels (pg/ml)
DEPDC1-LP3
+
−
D
DEPDC1-LP3-specific bulk Th cells
HD4: DRB1* 04:05 / -
IFN-γ
TNF-α
IL-2
GM-CSF
IL-4
IL-17
Cytokine levels (pg/ml)
DEPDC1-LP3
+
−

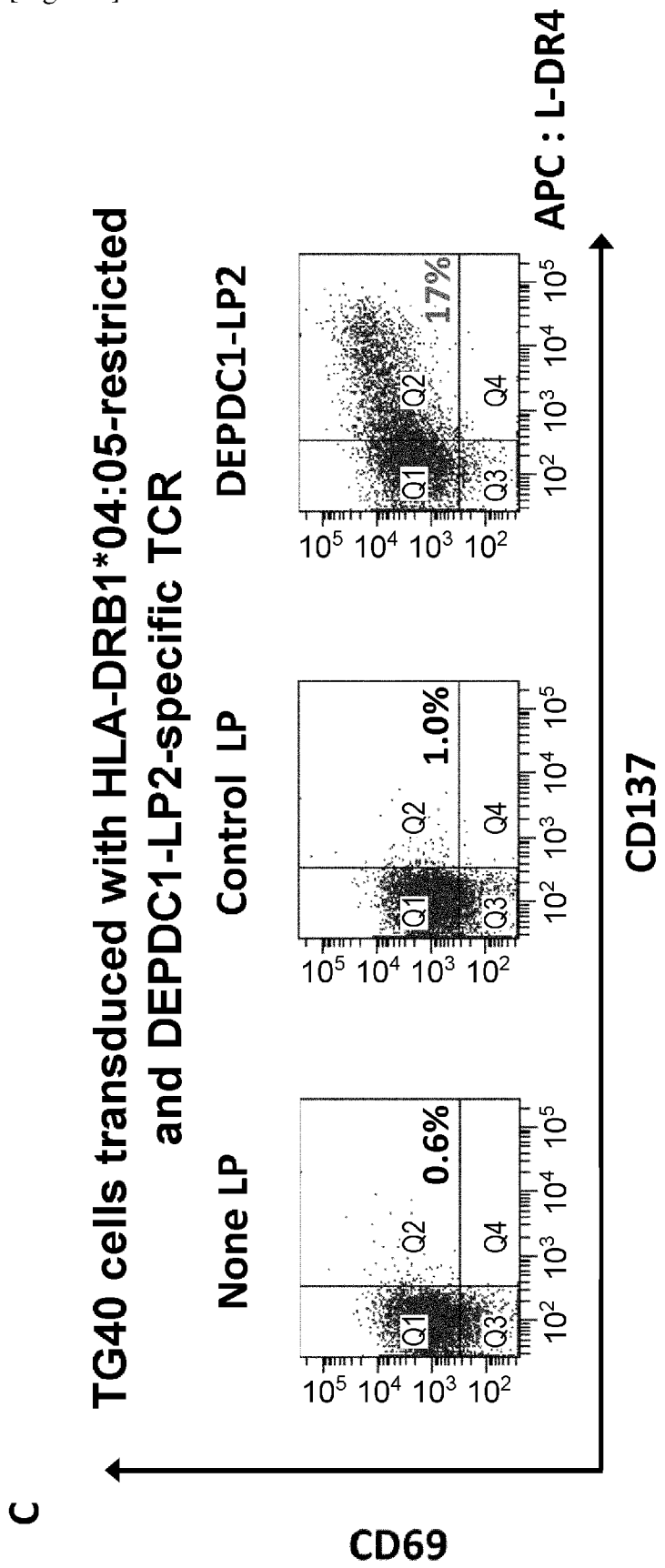
[Fig. 6-1]

Top 10 of functional TCRA (DEPDC1)				SEQ ID NO:
	TRAV	TRAJ	CDR3a	
a	TRAV8-1	TRAJ53	CAVNIRGSNYKLTF	11
b	TRAV38-2/DV8	TRAJ44	CALNTGTASKLTF	12
c	TRAV5	TRAJ13	CAESKGSGGYQKVTF	13
d	TRAV38-2/DV8	TRAJ31	CAYRSRNNNARLMF	14
e	TRAV21	TRAJ39	CCLFSQVRESKQVEDLMPRWNHQ DVVLYTLQLLSLVTQPPTSMLDLNN AGNMLTF	15
f	TRAV21	TRAJ39	CCLFSQVRESKQVEDLMPRWNHQ DVVLYTLQLLSLVTQPPTSLLDLNNA GNMLTF	16
g	TRAV8-1	TRAJ53	CTVNIRGSNYKLTF	17
h	TRAV21	TRAJ39	CCLFSQVRESKQVEDLMPRWNHQ DVVLYTLQLLSLVTQPPTFVLDLNNA GNMLTF	18
i	TRAV21	TRAJ39	CCLFSQVRESKQVEDLMPRWNHQ DVVLYTLQLLSLVTQPPTYVLDLNNA GNMLTF	19
j	TRAV8-2	TRAJ34	CVVSDPNTDKLIF	20

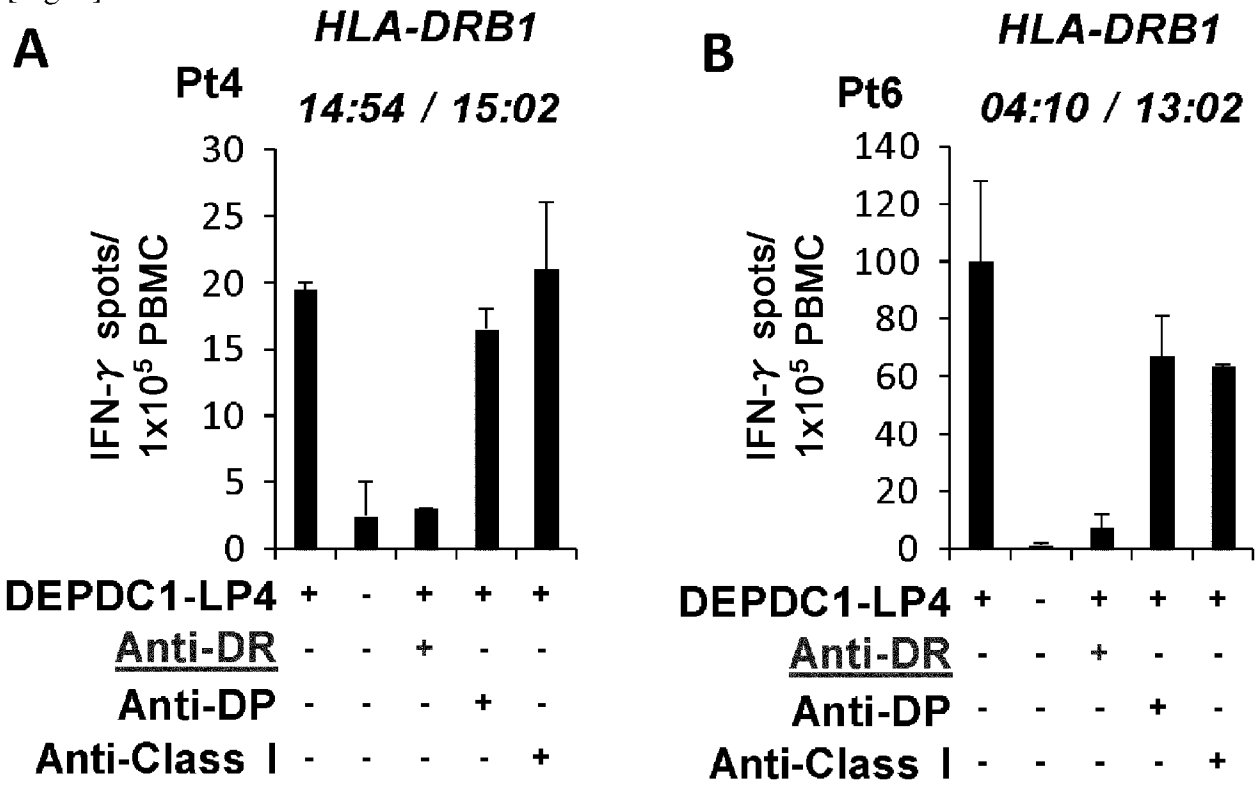
Top 10 of functional TCRB (DEPDC1)				SEQ ID NO:
	TRBV	TRBJ	CDR3b	
a	TRBV19	TRBJ2-1	CASSPQLAGGSYNEQFF	21
b	TRBV15	TRBJ2-1	CATSRDEASGSPSSYNEQFF	22
c	TRBV7-9	TRBJ2-1	CASNPLAGGPRDEQFF	23
d	TRBV19	TRBJ2-1	CASSPQLAGGSYNAQFF	24
e	TRBV19	TRBJ2-1	CASSPQLAGGSYNVQFF	25
f	TRBV18	TRBJ1-5	CASSPYQGQGDNQPQHF	26
g	TRBV19	TRBJ2-1	CASSPQLAGGSYKEQFF	27
h	TRBV5-1	TRBJ2-7	CASTPFGLAGDGEQYF	28
i	TRBV18	TRBJ1-1	CASSPTGPEAFF	29
j	TRBV19	TRBJ2-1	CASSPQLAGGSDNEQFF	30



[Fig. 6-2]


C
TG40 cells transduced with HLA-DRB1*04:05-restricted and DEPDC1-LP2-specific TCR
None LP
Control LP
DEPDC1-LP2
APC : L-DR4
CD137
CD69
Q1
Q2
Q3
Q4
0.6%
1.0%
17%
10^2 10^3 10^4 10^5

[Fig. 7]


A
HLA-DRB1
Pt4
14:54 / 15:02
IFN-γ spots/ 1x10^5 PBMC
30
25
20
15
10
5
0
DEPDC1-LP4 + - + + +
Anti-DR - - + - -
Anti-DP - - - + -
Anti-Class I - - - - +
B
HLA-DRB1
Pt6
04:10 / 13:02
IFN-γ spots/ 1x10^5 PBMC
140
120
100
80
60
40
20
0
DEPDC1-LP4 + - + + +
Anti-DR - - + - -
Anti-DP - - - + -
Anti-Class I - - - - +

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2017/040888

A. CLASSIFICATION OF SUBJECT MATTER

Int.Cl. See extra sheet

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Int.Cl. See extra sheet

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Published examined utility model applications of Japan 1922-1996
Published unexamined utility model applications of Japan 1971-2018
Registered utility model specifications of Japan 1996-2018
Published registered utility model applications of Japan 1994-2018

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

JSTPlus/JST7580 (JDreamIII), CA/MEDLINE/WPIDS/BIOSIS (STN), UniProt/GeneSeq

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	YATSUDA J et al., Establishment of HLA-DR4 transgenic mice for the identification of CD4+ T cell epitopes of tumor-associated antigens, PLoS One, 2013, Vol.8, e84908, Abstract, Fig.6	1-3,7-9,11, 12,14, 16-18,27 6,19-26,28
Y	WO 2014/010231 A1 (ONCOTHERAPY SCIENCE, INC.) 2014.01.16, Claims & US 2015/0190489 A1 & EP 2872530 A1 & JP 2015-524788 A	1-28
Y	NAKANE M et al., Identification of cancer-testis antigen (DEPDC1 and MPHOSPH1)-derived long peptides encompassing both CTL and HLA class II-restricted Th cell epitopes, The 45th Annual Meeting of The Japanese Society for Immunology, 2016.11.14, p.64, Whole Document	1-28

☑ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
07.02.2018	20.02.2018

Name and mailing address of the ISA/JP	Authorized officer	
Japan Patent Office 3-4-3, Kasumigaseki, Chiyoda-ku, Tokyo 100-8915, Japan	NISHIMURA, Akiko Telephone No. +81-3-3581-1101 Ext. 3488	4N 3435

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2017/040888

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2008/047473 A1 (ONCOTHERAPY SCIENCE, INC.) 2008.04.24, Claims, Tables & JP 2010-506826 A & US 2010/0028373 A1 & EP 2091965 A1	1-28
A	OBARA W et al., Cancer peptide vaccine therapy developed from oncoantigens identified through genome-wide expression profile analysis for bladder cancer, Jpn. J. Clin. Oncol., 2012, Vol.42, pp.591-600, Table1	1-28
A	WO 2014/188721 A1 (ONCOTHERAPY SCIENCE, INC.) 2014.11.27, Claims & JP 2016-525498 A & US 2016/0114018 A1 & EP 3004346 A1	1-28
A	WO 2014/010232 A1 (ONCOTHERAPY SCIENCE, INC.) 2014.01.16, Claims & JP 2015-524652 A & US 2015/0191516 A1 & EP 2872531 A1	1-28
P,A	WO 2017/026503 A1 (ONCOTHERAPY SCIENCE, INC.) 2017.02.16, Claims (no family)	1-28

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2017/040888

CLASSIFICATION OF SUBJECT MATTER
C12N15/09(2006.01)i, A61K31/7088(2006.01)i, A61K35/26(2015.01)i, A61K39/00(2006.01)i, A61P35/00(2006.01)i, C07K7/06(2006.01)i, C07K7/08(2006.01)i, C07K14/47(2006.01)i, C07K16/30(2006.01)i, C12N1/15(2006.01)i, C12N1/19(2006.01)i, C12N1/21(2006.01)i, C12N5/0783(2010.01)i, C12N5/0784(2010.01)i, C12N5/10(2006.01)i

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2017/040888

Minimum documentation searched

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