



(86) Date de dépôt PCT/PCT Filing Date: 2015/06/11
(87) Date publication PCT/PCT Publication Date: 2015/12/17
(45) Date de délivrance/Issue Date: 2023/07/04
(85) Entrée phase nationale/National Entry: 2016/12/09
(86) N° demande PCT/PCT Application No.: US 2015/035371
(87) N° publication PCT/PCT Publication No.: 2015/191881
(30) Priorité/Priority: 2014/06/11 (US62/010,736)

(51) Cl.Int./Int.Cl. A61K 39/395 (2006.01),
A61K 31/198 (2006.01), C07K 16/18 (2006.01)

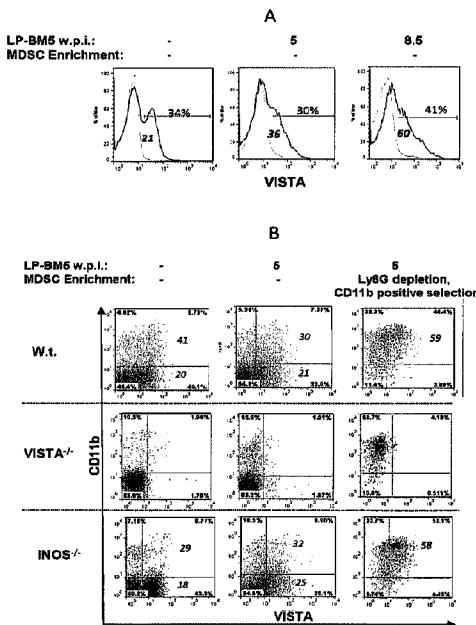
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(54) Titre : UTILISATION D'ANTAGONISTES ET D'AGONISTES VISTA POUR SUPPRIMER OU AMELIORER
L'IMMUNITE HUMORALE

(54) Title: USE OF VISTA AGONISTS AND ANTAGONISTS TO SUPPRESS OR ENHANCE HUMORAL IMMUNITY



(57) Abrégé/Abstract:

The present invention is directed to the use of VISTA agonists alone or in association with other immune inhibitors, preferably another inhibitor of humoral immunity such as an iNOS/NO promoter or nitric oxide or a PD-1 or PD-L1 agonist or a CD40 antagonist for the treatment or prevention of conditions wherein the suppression of humoral immunity is therapeutically beneficial. The present invention is further directed to the use of VISTA antagonists alone or in association with other immune agonists, preferably another enhancer of humoral immunity such as iNOS/NO inhibitor or a PD-1 or PD-L1 antagonist or anti-CTLA-4 antibody or a CD40 agonist for the treatment or prevention of conditions wherein the enhancement of humoral immunity is therapeutically beneficial. Also, the invention relates to the use of VISTA antagonists alone or in association with another immune agonist, e.g., an iNOS/NO inhibitor or a CD40 agonist to promote the efficacy of therapeutic and prophylactic vaccines, e.g., antitumor, and antiviral vaccines.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

CORRECTED VERSION

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
17 December 2015 (17.12.2015)

(10) International Publication Number
WO 2015/191881 A9

(51) International Patent Classification:
A61K 39/395 (2006.01) *C07K 16/18* (2006.01)

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(21) International Application Number:
PCT/US2015/035371

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(22) International Filing Date:
11 June 2015 (11.06.2015)

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(25) Filing Language: English

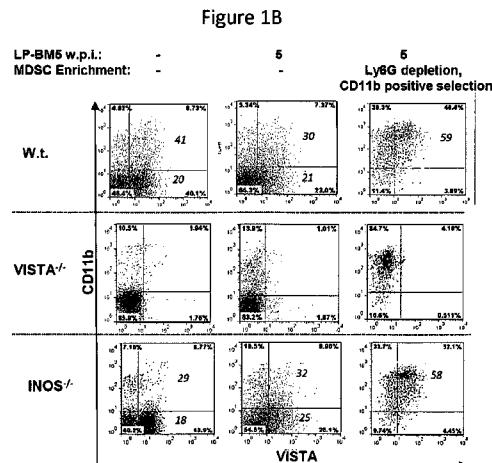
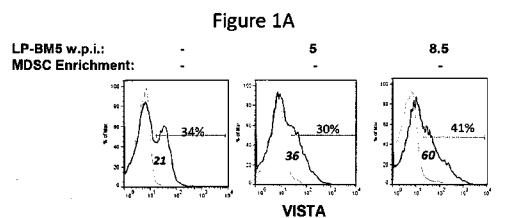
(26) Publication Language: English

(30) Priority Data:
62/010,736 11 June 2014 (11.06.2014) US

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH,

[Continued on next page]

(54) Title: USE OF VISTA AGONISTS AND ANTAGONISTS TO SUPPRESS OR ENHANCE HUMORAL IMMUNITY



(57) Abstract: The present invention is directed to the use of VISTA agonists alone or in association with other immune inhibitors, preferably another inhibitor of humoral immunity such as an iNOS/NO promoter or nitric oxide or a PD-1 or PD-L1 agonist or a CD40 antagonist for the treatment or prevention of conditions wherein the suppression of humoral immunity is therapeutically beneficial. The present invention is further directed to the use of VISTA antagonists alone or in association with other immune agonists, preferably another enhancer of humoral immunity such as iNOS/NO inhibitor or a PD-1 or PD-L1 antagonist or anti-CTLA-4 antibody or a CD40 agonist for the treatment or prevention of conditions wherein the enhancement of humoral immunity is therapeutically beneficial. Also, the invention relates to the use of VISTA antagonists alone or in association with another immune agonist, e.g., an iNOS/NO inhibitor or a CD40 agonist to promote the efficacy of therapeutic and prophylactic vaccines, e.g., antitumor, and antiviral vaccines.

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WO 2015/191881 A9

GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(88) Date of publication of the international search report: 3 March 2016

(48) Date of publication of this corrected version: 21 April 2016

(15) Information about Correction:
see Notice of 21 April 2016

Published:

— *with international search report (Art. 21(3))*

USE OF VISTA AGONISTS AND ANTAGONISTS TO SUPPRESS OR ENHANCE HUMORAL IMMUNITY

RELATED APPLICATIONS

[0001] This application claims priority to US Provisional Serial No. 62/010,736 filed on June 11, 2014.

FIELD OF THE INVENTION

[0002] This invention relates to the discovery that VISTA modulates (suppresses) B cell responsiveness. Based on this discovery this invention relates to the use of VISTA agonists alone or in association with other immune suppressers such as iNOS/NO inhibitor to suppress B cell responsiveness and to treat conditions wherein reduced B cell responsiveness is therapeutically beneficial. Also, this invention relates to the use of VISTA antagonists alone or in to promote B cell responsiveness and to treat conditions wherein enhanced B cell responsiveness is therapeutically beneficial.

[0003] Inhibition by myeloid derived suppressor cells (MDSC) against T-cell responses is well established in tumor microenvironments. The present inventors demonstrated (2013, J. Viral. 87:2058–2071) induction of monocytic MDSCs during infection of susceptible B6 mice by LP-BM5 retrovirus, which causes a profound immunodeficiency. These MDSCs inhibited not only T-, but also B-cell, responsiveness in ex vivo suppression assays. Whereas MDSC inhibition of stimulated T-cell proliferation and IFN-gamma production was almost completely iNOS/NO dependent, MDSC suppression of B-cell responses was only ~50% dependent on iNOS/NO – as shown by using iNOS inhibitors and iNOS k.o. mice as a source of MDSCs.

[0004] Here, we further studied additional suppressive mechanism(s) in MDSC inhibition of B- cell responsiveness by examining VISTA, a newly described negative checkpoint regulator. Using anti-VISTA blocking antibody, and LP-BM5 infected VISTA-/ MDSCs, MDSC suppression of B-cell responses was partly dependent on MDSC expressed VISTA. Combining the use of reagents to block both iNOS/NO and VISTA lead to an additive, if not synergistic, abrogation of MDSC suppression of B-cell responsiveness. These results were compatible with a role for MDSC in LP-BM5-induced immunodeficiency and highlight involvement of multiple and unique suppressive pathways in the under-studied area of MDSC suppression of B-cell responses.

BRIEF DESCRIPTION OF THE INVENTION

[0005] As mentioned, this invention relates to the discovery that VISTA modulates (suppresses) B cell responsiveness. Based on this discovery this invention relates to the use of VISTA agonists to suppress B cell responsiveness and to treat conditions wherein reduced B cell responsiveness is therapeutically beneficial. Also, this invention relates to the use of VISTA antagonists to promote B cell responsiveness and to treat conditions wherein enhanced B cell responsiveness is therapeutically beneficial.

[0006] In particular agonistic anti-human VISTA antibodies or antibody fragments or VISTA polypeptides, e.g., VISTA fusion proteins are used to treat human conditions wherein decreased B cell responsiveness is desired. In particular this may be therapeutically desired in treating autoimmune, allergic or inflammatory conditions wherein B cell responses are involved in disease pathology.

[0007] Also, antagonistic anti-human VISTA antibodies or antibody fragments or VISTA polypeptides, e.g., VISTA fragments and conjugates may be used alone or in association with an antigen and potentially another immune agonist in order to treat human conditions wherein increased B cell responsiveness is desired. In particular this may be therapeutically desired in treating cancer, infectious diseases and in promoting the efficacy of vaccines, e.g., prophylactic and therapeutic vaccines for eliciting protective B cell immune responses to a desired antigen, e.g., a tumor antigen, autoantigen, or an antigen specific to an infectious agent or a cell that is infected by an infectious agent.

[0008] VISTA is an Immunoglobulin (Ig) family ligand, designated V-domain Immunoglobulin Suppressor of T cell Activation (VISTA) (GenBank: JN602184)₇₅. Key features of VISTA include the following. VISTA bears limited homology to PD-L1, but does not belong to the B7 family due to its unique structure. VISTA is exclusively expressed within the hematopoietic compartment, with very high levels of expression on CD11b.sup.high myeloid cells, and lower expression levels on CD4+ and CD8+ T cells, and Tregs. A soluble VISTA-Ig fusion protein or VISTA expressed on APCs, acts as a ligand to suppress CD4+ and CD8+ T cell proliferation and cytokine production, via an unidentified receptor independent of PD-1. An anti-VISTA mAb (13F3) reversed VISTA-mediated T cell suppression *in vitro* and suppressed tumour growth in multiple murine tumour models by enhancing the anti-tumour T cell responses. VISTA over-expression on tumour cells impaired

protective anti-tumour immunity in vaccinated hosts. VISTA KO mice develop an inflammatory phenotype, which points towards a loss of peripheral tolerance. See U.S. Pat. Nos. 8,236,304 and 8,231,872, Published International Applications WO/2011/120013 and WO/2006/116181, U.S. Published Application Nos. 2008/0287358, 2011/0027278, and 2012/0195894, and U.S. Provisional Patent Application Ser Nos. 60/674,567, filed Apr. 25, 2005, 61/663,431, filed Jun. 22, 2012, Ser. No. 61/663,969, filed Jun. 25, 2012, 61/390,434, filed Oct. 6, 2010, 61/436,379, filed Jan. 26, 2011, and 61/449,882, filed Mar. 7, 2011.

[0009] Therefore, it is generally known that VISTA is an immune checkpoint protein ligand that critically regulates T cell related immune responses. However, prior to the present invention it was not known that VISTA further regulates B cell responsiveness.

[00010] Inhibition by myeloid derived suppressor cells (MDSC) against T-cell responses is well established in tumor microenvironments. However, their effects on B cell responses is much less established. Here, we demonstrate that an additional suppressive mechanism(s) in MDSC inhibition of B- cell responsiveness involves VISTA, a negative checkpoint regulator. Using anti-VISTA blocking antibody, and LP-BM5 infected VISTA-/- MDSCs, MDSC suppression of B-cell responses was partly dependent on MDSC expressed VISTA. Combining the use of reagents to block both iNOS/NO and VISTA led to an additive, if not synergistic, abrogation of MDSC suppression of B-cell responsiveness. These results indicate that MDSCs play an active role in LP-BM5-induced immunodeficiency and highlight involvement of multiple and unique suppressive pathways in the under-studied area of MDSC suppression of B-cell responses.

EXEMPLARY EMBODIMENTS OF THE INVENTION

[00011] The present invention provides methods of inhibiting or reversing VISTA-mediated inhibition of humoral immunity in a subject in need thereof comprising administering a VISTA antagonist alone or in association with another immune agonist, e.g., in a subject comprising a cancer or infectious disease condition wherein B cell immunity is suppressed.

[00012] The present invention also provides methods of promoting or increasing VISTA-mediated inhibition of humoral immunity in a subject in need thereof comprising administering a VISTA agonist alone or in association with another immune antagonist, e.g.,

in a subject comprising an autoimmune, allergic, inflammatory or infectious condition wherein B cells or antibody responses are involved in disease pathology.

[00013] The present invention also provides methods of suppressing B cell proliferation or B cell responses including but not limited to antigen-specific antibody responses in a subject in need thereof comprising administering a VISTA agonist, e.g., a subject comprising an autoimmune, allergic, inflammatory or infectious condition wherein B cells or antibody responses are involved in disease pathology.

[00014] The present invention also provides methods of increasing B cell proliferation or B cell responses including but not limited to antigen-specific antibody responses in a subject in need thereof comprising administering a VISTA agonist e.g., a subject comprising a cancer or infectious disease condition wherein B cell immunity is suppressed

[00015] The present invention also provides methods of promoting humoral immune responses elicited against an antigen or therapeutic antibody comprising administering such antigen or antibody in a therapeutic regimen that includes the use of a VISTA antagonist, e.g., wherein the antigen is a tumor antigen, autoantigen, allergen, or an infectious agent antigen or the antibody is specific to a tumor antigen, autoantigen, allergen, or an infectious agent antigen.

[00016] The present invention also provides methods of promoting humoral immune responses elicited by a therapeutic or prophylactic vaccine comprising administering such vaccine in a therapeutic regimen that includes the use of a VISTA antagonist, e.g., wherein the vaccine contains a tumor antigen, autoantigen, allergen, or an infectious agent antigen.

[00017] The present invention also provides methods of promoting humoral immune responses using a VISTA antagonist and an iNOS/NO inhibitor, e.g., an arginine derivative, optionally NG -nitro-L-arginine methyl ester, NG -ethyl-L-arginine, N-iminoethyl-L-arginine, L-NG -methyl arginine and NG -nitro-L-arginine or the inhibitor is NG -nitro-L-arginine methyl ester or lovastatin, a sodium salt of phenylacetic acid (NaPA), FPT inhibitor II, N-acetyl cysteine (NAC), and cAMP or any of the iNOS/NO inhibitors disclosed in US 6,586,474; 6,545,170; 6,593,372; 6,787,668; 6,809,117; 6,591,889; 7,196,118; 7,049,058.

[00018] The present invention also provides methods of inhibiting humoral immune responses using a VISTA agonist and an iNOS/NO promoter e.g. Nitric oxide or a compound that comprises nitric oxide.

[00019] In any of the foregoing embodiments the VISTA antagonist may include an antagonistic anti-VISTA antibody or a fragment of VISTA.

[00020] . In any of the foregoing embodiments the VISTA agonist may include an agonistic anti-human VISTA antibody or antibody fragment or a VISTA-Ig conjugate or multimeric form of VISTA, e.g., wherein the VISTA-Ig comprises an IgG1, IgG2, IgG3 or IgG4 Fc region or fragment thereof which optionally may be modified in order to impair or increase at least one effector function, or may comprise a human or humanized antibody

[00021] . In any of the foregoing embodiments the VISTA antagonist or agonist may be used in association with a PD-1, or PD-L1 agonist or a PD-1, or PD-L1 antagonist, e.g., an anti-PD-1 antibody or anti-PD-L1 antibody or PD-1 fusion protein or a cell which expresses PD-1 or PD-L1.

[00022] In any of the foregoing embodiments a VISTA antagonist and iNOS/NO inhibitor are administered together.

[00023] In any of the foregoing embodiments, a VISTA agonist and iNOS/NO promoter are administered together.

[00024] In any of the foregoing embodiments a VISTA antagonist and a iNOS/NO inhibitor are administered separately in a therapeutic regimen to promote B cell immunity.

[00025] In any of the foregoing embodiments a VISTA antagonist compound and a iNOS/NO inhibitor compound are administered in amounts sufficient to elicit a synergistic effect on humoral immunity relative to either of these compounds administered alone.

[00026] In any of the foregoing embodiments a VISTA agonist compound and a iNOS/NO promoter or nitric oxide are administered in amounts sufficient to elicit a synergistic effect on humoral immunity relative to either of these compounds administered alone.

BRIEF DESCRIPTION OF THE FIGURES

[00027] Figure 1A-B shows that spleen cell VISTA expression increases with in vivo LP-BM5 infection.

[00028] Figure 2A-C shows that Ly6C+CD11b+ purified spleen cells have differing mechanistic requirements for suppressing in vitro B and T cell proliferation.

[00029] Figure 3 shows that naïve VISTA-/- responder spleen cells mixed with a-VISTA pre-treated Ly6C+CD11b+ MDSCs at the responder to suppressor (R:S) ratio of 3:1, were stimulated for three days with α -CD40 and IL-4.

[00030] Figure 4 shows that purified monocytic MDSCs (Ly6C+CD11b+) from 5 week LP-BM5 infected mice of the indicated strains, show differing patterns of suppression of naïve VISTA-/- spleen cell proliferation as assessed by flow cytometry.

[00031] Figure 5A-B shows monocytic suppression of B cell proliferation is dependent on two main mechanisms iNOS/NO and VISTA.

DETAILED DESCRIPTION OF THE INVENTION

[00032] Before describing the invention in detail the following definitions are provided.

[00033] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as those commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein may be used in the invention or testing of the present invention, suitable methods and materials are described herein. The materials, methods and examples are illustrative only, and are not intended to be limiting.

[00034] As used in the description herein and throughout the claims that follow, the meaning of "a," "an," and "the" includes plural reference unless the context clearly dictates otherwise.

[00035] The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia. More particular examples of such cancers include squamous cell cancer, lung cancer (including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung), cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer (including gastrointestinal cancer), pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma

and various types of head and neck cancer, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenström's Macroglobulinemia); chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblastic leukemia; multiple myeloma and post-transplant lymphoproliferative disorder (PTLD).

[00036] Exemplary cancers amenable for treatment by the present invention include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include colorectal, bladder, ovarian, melanoma, squamous cell cancer, lung cancer (including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung), cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer (including gastrointestinal cancer), pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma and various types of head and neck cancer, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenström's Macroglobulinemia); chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblastic leukemia; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), and Meigs' syndrome. Preferably, the cancer is selected from the group consisting of colorectal cancer, breast cancer, colorectal cancer, rectal cancer, non-small cell lung cancer, non-Hodgkin's lymphoma (NHL), renal cell cancer, prostate cancer, liver cancer, pancreatic cancer, soft-tissue sarcoma, Kaposi's sarcoma, carcinoid carcinoma, head and neck cancer, melanoma, ovarian cancer, mesothelioma, and multiple myeloma. In an exemplary

embodiment the cancer is an early or advanced (including metastatic) bladder, ovarian or melanoma. In another embodiment the cancer is colorectal cancer. The cancerous conditions amenable for treatment of the invention include metastatic cancers wherein VISTA expression by myeloid derived suppressor cells suppress antitumor responses and anti-invasive immune responses. The method of the present invention is particularly suitable for the treatment of vascularized tumors.

[00037] The invention is also suitable for treating cancers in combination with chemotherapy or radiotherapy or other biologics and for enhancing the activity thereof, i.e., in individuals wherein VISTA expression by myeloid derived suppressor cells suppress antitumor responses and the efficacy of chemotherapy or radiotherapy or biologic efficacy. Any chemotherapeutic agent exhibiting anticancer activity can be used according to the present invention. Preferably, the chemotherapeutic agent is selected from the group consisting of alkylating agents, antimetabolites, folic acid analogs, pyrimidine analogs, purine analogs and related inhibitors, vinca alkaloids, epipodophyllotoxins, antibiotics, L-Asparaginase, topoisomerase inhibitor, interferons, platinum coordination complexes, anthracenedione substituted urea, methyl hydrazine derivatives, adrenocortical suppressant, adrenocorticosteroids, progestins, estrogens, antiestrogen, androgens, antiandrogen, and gonadotropin-releasing hormone analog. More preferably, the chemotherapeutic agent is selected from the group consisting of 5-fluorouracil (5-FU), leucovorin (LV), irinotecan, oxaliplatin, capecitabine, paclitaxel and docetaxel. Two or more chemotherapeutic agents can be used in a cocktail to be administered in combination with administration of the anti-VEGF antibody. One preferred combination chemotherapy is fluorouracil-based, comprising 5-FU and one or more other chemotherapeutic agent(s). Suitable dosing regimens of combination chemotherapies are known in the art and described in, for example, Saltz et al. (1999) Proc. ASCO 18:233a and Douillard et al. (2000) Lancet 355:1041-7. The biologic may be another immune potentiators such as antibodies to PD-L1, PD-L2, CTLA-4 and PD-L1, PD-L2, CTLA-4 fusion proteins as well as cytokines, growth factor antagonists and agonists, hormones and anti-cytokine antibodies.

[00038] "Activating receptor," as used herein, refers broadly to immune cell receptors that bind antigen, complexed antigen (e.g., in the context of MHC molecules), Ig-fusion proteins, ligands, or antibodies. Activating receptors but are not limited to T cell receptors (TCRs), B

cell receptors (BCRs), cytokine receptors, LPS receptors, complement receptors, and Fc receptors. For example, T cell receptors are present on T cells and are associated with CD3 molecules. T cell receptors are stimulated by antigen in the context of MHC molecules (as well as by polyclonal T cell activating reagents). T cell activation via the TCR results in numerous changes, e.g., protein phosphorylation, membrane lipid changes, ion fluxes, cyclic nucleotide alterations, RNA transcription changes, protein synthesis changes, and cell volume changes. For example, T cell receptors are present on T cells and are associated with CD3 molecules. T cell receptors are stimulated by antigen in the context of MHC molecules (as well as by polyclonal T cell activating reagents). T cell activation via the TCR results in numerous changes, e.g., protein phosphorylation, membrane lipid changes, ion fluxes, cyclic nucleotide alterations, RNA transcription changes, protein synthesis changes, and cell volume changes.

[00039] "Antigen presenting cell," as used herein, refers broadly to professional antigen presenting cells (e.g., B lymphocytes, monocytes, dendritic cells, and Langerhans cells) as well as other antigen presenting cells (e.g., keratinocytes, endothelial cells, astrocytes, fibroblasts, and oligodendrocytes).

[00040] "Amino acid," as used herein refers broadly to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified (e.g., hydroxyproline, γ -carboxyglutamate, and O-phosphoserine.) Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid (i.e., a carbon that is bound to a hydrogen, a carboxyl group, an amino group), and an R group (e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium.) Analogs may have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid.

[00041] "Anergy" or "tolerance," as used herein refers broadly to refractivity to activating receptor-mediated stimulation. Refractivity is generally antigen-specific and persists after

exposure to the tolerizing antigen has ceased. For example, anergy in T cells (as opposed to unresponsiveness) is characterized by lack of cytokine production, e.g., IL-2. T cell anergy occurs when T cells are exposed to antigen and receive a first signal (a T cell receptor or CD-3 mediated signal) in the absence of a second signal (a costimulatory signal). Under these conditions, reexposure of the cells to the same antigen (even if reexposure occurs in the presence of a costimulatory molecule) results in failure to produce cytokines and, thus, failure to proliferate. Anergic T cells can, however, mount responses to unrelated antigens and can proliferate if cultured with cytokines (e.g., IL-2). For example, T cell anergy can also be observed by the lack of IL-2 production by T lymphocytes as measured by ELISA or by a proliferation assay using an indicator cell line. Alternatively, a reporter gene construct can be used. For example, anergic T cells fail to initiate IL-2 gene transcription induced by a heterologous promoter under the control of the 5' IL-2 gene enhancer or by a multimer of the AP1 sequence that can be found within the enhancer (Kang et al. (1992) *Science* 257:1134). Modulation of a costimulatory signal results in modulation of effector function of an immune cell. Thus, the term "PD-L3 OR VISTA activity" includes the ability of a PD-L3 OR VISTA polypeptide to bind its natural binding partner(s), the ability to modulate immune cell costimulatory or inhibitory signals, and the ability to modulate the immune response. Modulation of an inhibitory signal in an immune cell results in modulation of proliferation of and/or cytokine secretion by an immune cell.

[00042] "Antibody", as used herein, refers broadly to an "antigen-binding portion" of an antibody (also used interchangeably with "antibody portion," "antigen-binding fragment," "antibody fragment"), as well as whole antibody molecules. The term "antigen-binding portion", as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen (e.g., VISTA (PD-L3)). The antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of antigen-binding fragments encompassed within the term "antigen-binding portion" of an antibody include (a) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (b) a F(ab')2 fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (c) a Fd fragment consisting of the VH and CH1 domains; (d) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody; (e) a dAb fragment (Ward, et al. (1989) *Nature* 341: 544-546), which consists of a

VH domain; and (f) an isolated complementarily determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv). See e.g., Bird, et al. (1988) *Science* 242: 423-426; Huston, et al. (1988) *Proc Natl. Acad. Sci. USA* 85: 5879-5883; and Osbourn, et al. (1998) *Nat. Biotechnol.* 16: 778. Single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. Any VH and VL sequences of specific scFv can be linked to human immunoglobulin constant region cDNA or genomic sequences, in order to generate expression vectors encoding complete IgG molecules or other isotypes. VH and VL can also be used in the generation of Fab, Fv, or other fragments of immunoglobulins using either protein chemistry or recombinant DNA technology. Other forms of single chain antibodies, such as diabodies are also encompassed. Diabodies are bivalent, bispecific antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites. See e.g., Holliger, et al. (1993) *Proc Natl. Acad. Sci. USA* 90: 6444-6448; Poljak, et al. (1994) *Structure* 2: 1121-1123.

[00043] Still further, an antibody or antigen-binding portion thereof (antigen-binding fragment, antibody fragment, antibody portion) may be part of a larger immunoadhesion molecules, formed by covalent or noncovalent association of the antibody or antibody portion with one or more other proteins or peptides. Examples of immunoadhesion molecules include use of the streptavidin core region to make a tetrameric scFv molecule (Kipriyanov, et al. (1995) *Hum. Antibodies Hybridomas* 6: 93-101) and use of a cysteine residue, a marker peptide and a C-terminal polyhistidine tag to make bivalent and biotinylated scFv molecules. Kipriyanov, et al. (1994) *Mol Immunol.* 31: 1047-1058. Antibody portions, such as Fab and F(ab')2 fragments, can be prepared from whole antibodies using conventional techniques, such as papain or pepsin digestion, respectively, of whole antibodies. Moreover, antibodies, antibody portions and immunoadhesion molecules can be obtained using standard recombinant DNA techniques, as described herein.

[00044] Antibodies may be polyclonal, monoclonal, xenogeneic, allogeneic, syngeneic, or modified forms thereof, e.g., humanized, chimeric. Preferably, antibodies of the invention bind specifically or substantially specifically to VISTA (PD-L3) molecules. The terms "monoclonal antibodies" and "monoclonal antibody composition", as used herein, refer to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of an antigen, whereas the term "polyclonal antibodies" and "polyclonal antibody composition" refer to a population of antibody molecules that contain multiple species of antigen binding sites capable of interacting with a particular antigen. A monoclonal antibody composition, typically displays a single binding affinity for a particular antigen with which it immunoreacts.

[00045] "Antigen," as used herein, refers broadly to a molecule or a portion of a molecule capable of being bound by an antibody which is additionally capable of inducing an animal to produce an antibody capable of binding to an epitope of that antigen. An antigen may have one epitope, or have more than one epitope. The specific reaction referred to herein indicates that the antigen will react, in a highly selective manner, with its corresponding antibody and not with the multitude of other antibodies which may be evoked by other antigens. In the case of a desired enhanced immune response to particular antigens of interest, antigens include, but are not limited to, infectious disease antigens for which a protective immune response may be elicited are exemplary.

[00046] "Allergic disease," as used herein, refers broadly to a disease involving allergic reactions. More specifically, an "allergic disease" is defined as a disease for which an allergen is identified, where there is a strong correlation between exposure to that allergen and the onset of pathological change, and where that pathological change has been proven to have an immunological mechanism. Herein, an immunological mechanism means that leukocytes show an immune response to allergen stimulation.

[00047] "Antisense nucleic acid molecule," as used herein, refers broadly to a nucleotide sequence which is complementary to a "sense" nucleic acid encoding a protein (e.g., complementary to the coding strand of a double-stranded cDNA molecule) complementary to an mRNA sequence or complementary to the coding strand of a gene. Accordingly, an antisense nucleic acid molecule can hydrogen bond to a sense nucleic acid molecule.

[00048] "Asthma," as used herein, refers broadly to a disorder of the respiratory system characterized by inflammation, narrowing of the airways and increased reactivity of the airways to inhaled agents. Asthma is frequently, although not exclusively, associated with atopic or allergic symptoms.

[00049] "Apoptosis," as used herein, refers broadly to programmed cell death which can be characterized using techniques which are known in the art. Apoptotic cell death can be characterized by cell shrinkage, membrane blebbing, and chromatin condensation culminating in cell fragmentation. Cells undergoing apoptosis also display a characteristic pattern of internucleosomal DNA cleavage.

[00050] "Autoimmunity" or "autoimmune disease or condition," as used herein, refers broadly to a disease or disorder arising from and directed against an individual's own tissues or a co-segregate or manifestation thereof or resulting condition therefrom.

[00051] "B cell receptor" (BCR), as used herein, refers broadly to the complex between membrane Ig (mIg) and other transmembrane polypeptides (e.g., Ig α . and Ig β) found on B cells. The signal transduction function of mIg is triggered by crosslinking of receptor molecules by oligomeric or multimeric antigens. B cells can also be activated by anti-immunoglobulin antibodies. Upon BCR activation, numerous changes occur in B cells, including tyrosine phosphorylation.

[00052] "Cancer," as used herein, refers broadly to any neoplastic disease (whether invasive or metastatic) characterized by abnormal and uncontrolled cell division causing malignant growth or tumor (e.g., unregulated cell growth.)

[00053] "Chimeric antibody," as used herein, refers broadly to an antibody molecule in which the constant region, or a portion thereof, is altered, replaced or exchanged so that the antigen binding site (variable region) is linked to a constant region of a different or altered class, effector function and/or species, or an entirely different molecule which confers new properties to the chimeric antibody, e.g., an enzyme, toxin, hormone, growth factor, drug, the variable region or a portion thereof, is altered, replaced or exchanged with a variable region having a different or altered antigen specificity.

[00054] "Coding region," as used herein, refers broadly to regions of a nucleotide sequence comprising codons which are translated into amino acid residues, whereas the term

"noncoding region" refers to regions of a nucleotide sequence that are not translated into amino acids (e.g., 5' and 3' untranslated regions).

[00055] "Conservatively modified variants," as used herein, applies to both amino acid and nucleic acid sequences, and with respect to particular nucleic acid sequences, refers broadly to conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. "Silent variations" are one species of conservatively modified nucleic acid variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of 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) may be modified to yield a functionally identical molecule.

[00056] "Complementarity determining region," "hypervariable region," or "CDR," as used herein, refers broadly to one or more of the hyper-variable or complementarily determining regions (CDRs) found in the variable regions of light or heavy chains of an antibody. See Kabat, et al. (1987) "Sequences of Proteins of Immunological Interest" National Institutes of Health, Bethesda, Md. These expressions include the hypervariable regions as defined by Kabat, et al. (1983) "Sequences of Proteins of Immunological Interest" U.S. Dept. of Health and Human Services or the hypervariable loops in 3-dimensional structures of antibodies. Chothia and Lesk (1987) J Mol. Biol. 196: 901-917. The CDRs in each chain are held in close proximity by framework regions and, with the CDRs from the other chain, contribute to the formation of the antigen binding site. Within the CDRs there are select amino acids that have been described as the selectivity determining regions (SDRs) which represent the critical contact residues used by the CDR in the antibody-antigen interaction. Kashmari (2005) Methods 36: 25-34.

[00057] "Control amount," as used herein, refers broadly to a marker can be any amount or a range of amounts to be compared against a test amount of a marker. For example, a control amount of a marker may be the amount of a marker in a patient with a particular disease or

condition or a person without such a disease or condition. A control amount can be either in absolute amount (e.g., microgram/10 or a relative amount (e.g., relative intensity of signals).

[00058] "Costimulatory receptor," as used herein, refers broadly to receptors which transmit a costimulatory signal to an immune cell, e.g., CD28 or ICOS. As used herein, the term "inhibitory receptors" includes receptors which transmit a negative signal to an immune cell.

[00059] "Costimulate," as used herein, refers broadly to the ability of a costimulatory molecule to provide a second, non-activating, receptor-mediated signal (a "costimulatory signal") that induces proliferation or effector function. For example, a costimulatory signal can result in cytokine secretion (e.g., in a T cell that has received a T cell-receptor-mediated signal.) Immune cells that have received a cell receptor-mediated signal (e.g., via an activating receptor) may be referred to herein as "activated immune cells."

[00060] "Cytoplasmic domain," as used herein, refers broadly to the portion of a protein which extends into the cytoplasm of a cell.

[00061] "Diagnostic," as used herein, refers broadly to identifying the presence or nature of a pathologic condition. Diagnostic methods differ in their sensitivity and specificity. The "sensitivity" of a diagnostic assay is the percentage of diseased individuals who test positive (percent of "true positives"). Diseased individuals not detected by the assay are "false negatives." Subjects who are not diseased and who test negative in the assay are termed "true negatives." The "specificity" of a diagnostic assay is 1 minus the false positive rate, where the "false positive" rate is defined as the proportion of those without the disease who test positive. While a particular diagnostic method may not provide a definitive diagnosis of a condition, it suffices if the method provides a positive indication that aids in diagnosis.

[00062] "Diagnosing," as used herein refers broadly to classifying a disease or a symptom, determining a severity of the disease, monitoring disease progression, forecasting an outcome of a disease and/or prospects of recovery. The term "detecting" may also optionally encompass any of the foregoing. Diagnosis of a disease according to the present invention may, in some embodiments, be affected by determining a level of a polynucleotide or a polypeptide of the present invention in a biological sample obtained from the subject, wherein the level determined can be correlated with predisposition to, or presence or absence

of the disease. It should be noted that a "biological sample obtained from the subject" may also optionally comprise a sample that has not been physically removed from the subject.

[00063] "Effective amount," as used herein, refers broadly to the amount of a compound, antibody, antigen, or cells that, when administered to a patient for treating a disease, is sufficient to effect such treatment for the disease. The effective amount may be an amount effective for prophylaxis, and/or an amount effective for prevention. The effective amount may be an amount effective to reduce, an amount effective to prevent the incidence of signs/symptoms, to reduce the severity of the incidence of signs/symptoms, to eliminate the incidence of signs/symptoms, to slow the development of the incidence of signs/symptoms, to prevent the development of the incidence of signs/symptoms, and/or effect prophylaxis of the incidence of signs/symptoms. The "effective amount" may vary depending on the disease and its severity and the age, weight, medical history, susceptibility, and pre-existing conditions, of the patient to be treated. The term "effective amount" is synonymous with "therapeutically effective amount" for purposes of this invention.

[00064] "Extracellular domain," as used herein refers broadly to the portion of a protein that extend from the surface of a cell.

[00065] "Expression vector," as used herein, refers broadly to any recombinant expression system for the purpose of expressing a nucleic acid sequence of the invention in vitro or in vivo, constitutively or inducibly, in any cell, including prokaryotic, yeast, fungal, plant, insect or mammalian cell. The term includes linear or circular expression systems. The term includes expression systems that remain episomal or integrate into the host cell genome. The expression systems can have the ability to self-replicate or not, i.e., drive only transient expression in a cell. The term includes recombinant expression cassettes which contain only the minimum elements needed for transcription of the recombinant nucleic acid.

[00066] "Family," as used herein, refers broadly to the polypeptide and nucleic acid molecules of the invention is intended to mean two or more polypeptide or nucleic acid molecules having a common structural domain or motif and having sufficient amino acid or nucleotide sequence homology as defined herein. Family members can be naturally or non-naturally occurring and can be from either the same or different species. For example, a family can contain a first polypeptide of human origin, as well as other, distinct polypeptides

of human origin or alternatively, can contain homologues of non-human origin (e.g., monkey polypeptides.) Members of a family may also have common functional characteristics.

[00067] "Fc receptor" (FcRs) as used herein, refers broadly to cell surface receptors for the Fc portion of immunoglobulin molecules (Igs). Fc receptors are found on many cells which participate in immune responses. Among the human FcRs that have been identified so far are those which recognize IgG (designated Fc γ R), IgE (Fc. ϵ psilon.R1), IgA (Fc α R), and polymerized IgM/A (Fc μ R). FcRs are found in the following cell types: Fc ϵ RI (mast cells), Fc. ϵ psilon.RII (many leukocytes), Fc α R (neutrophils), and Fc μ R (glandular epithelium, hepatocytes). Hogg (1988) Immunol Today 9: 185-86. The widely studied Fc γ Rs are central in cellular immune defenses, and are responsible for stimulating the release of mediators of inflammation and hydrolytic enzymes involved in the pathogenesis of autoimmune disease. Unkeless (1988) Annu. Rev. Immunol. 6: 251-87. The Fc γ Rs provide a crucial link between effector cells and the lymphocytes that secrete Ig, since the macrophage/monocyte, polymorphonuclear leukocyte, and natural killer (NK) cell Fc γ Rs confer an element of specific recognition mediated by IgG. Human leukocytes have at least three different receptors for IgG: hFc γ RI (found on monocytes/macrophages), hFc γ .RII (on monocytes, neutrophils, eosinophils, platelets, possibly B cells, and the K562 cell line), and Fc γ III (on NK cells, neutrophils, eosinophils, and macrophages).

[00068] With respect to T cells, transmission of a costimulatory signal to a T cell involves a signaling pathway that is not inhibited by cyclosporin A. In addition, a costimulatory signal can induce cytokine secretion (e.g., IL-2 and/or IL-10) in a T cell and/or can prevent the induction of unresponsiveness to antigen, the induction of anergy, or the induction of cell death in the T cell.

[00069] "Framework region" or "FR," as used herein, refers broadly to one or more of the framework regions within the variable regions of the light and heavy chains of an antibody. See Kabat, et al. (1987) "Sequences of Proteins of Immunological Interest" National Institutes of Health, Bethesda, Md. These expressions include those amino acid sequence regions interposed between the CDRs within the variable regions of the light and heavy chains of an antibody.

[00070] "Heterologous," as used herein, refers broadly to portions of a nucleic acid indicates that the nucleic acid comprises two or more subsequences that are not found in the

same relationship to each other in nature. For instance, the nucleic acid is typically recombinantly produced, having two or more sequences from unrelated genes arranged to make a new functional nucleic acid (e.g., a promoter from one source and a coding region from another source.) Similarly, a heterologous protein indicates that the protein comprises two or more subsequences that are not found in the same relationship to each other in nature (e.g., a fusion protein).

[00071] "High affinity," as used herein, refers broadly to an antibody having a KD of at least 10^{-8} M, more preferably at least 10^{-9} M and even more preferably at least 10^{-10} M for a target antigen. However, "high affinity" binding can vary for other antibody isotypes. For example, "high affinity" binding for an IgM isotype refers to an antibody having a K_D of at least 10^{-7} M, more preferably at least 10^{-8} M.

[00072] "Homology," as used herein, refers broadly to a degree of similarity between a nucleic acid sequence and a reference nucleic acid sequence or between a polypeptide sequence and a reference polypeptide sequence. Homology may be partial or complete. Complete homology indicates that the nucleic acid or amino acid sequences are identical. A partially homologous nucleic acid or amino acid sequence is one that is not identical to the reference nucleic acid or amino acid sequence. The degree of homology can be determined by sequence comparison. The term "sequence identity" may be used interchangeably with "homology."

[00073] "Host cell," as used herein, refers broadly to refer to a cell into which a nucleic acid molecule of the invention, such as a recombinant expression vector of the invention, has been introduced. Host cells may be prokaryotic cells (e.g., *E. coli*), or eukaryotic cells such as yeast, insect (e.g., SF9), amphibian, or mammalian cells such as CHO, HeLa, HEK-293, e.g., cultured cells, explants, and cells *in vivo*. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It should be understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

[00074] "Humanized antibody," as used herein, refers broadly to include antibodies made by a non-human cell having variable and constant regions which have been altered to more

closely resemble antibodies that would be made by a human cell. For example, by altering the non-human antibody amino acid sequence to incorporate amino acids found in human germline immunoglobulin sequences. The humanized antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo), for example in the CDRs. The term "humanized antibody", as used herein, also includes antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

[00075] "Hybridization," as used herein, refers broadly to the physical interaction of complementary (including partially complementary) polynucleotide strands by the formation of hydrogen bonds between complementary nucleotides when the strands are arranged antiparallel to each other.

[00076] "IgV domain" and "IgC domain" as used herein, refer broadly to Ig superfamily member domains. These domains correspond to structural units that have distinct folding patterns called Ig folds. Ig folds are comprised of a sandwich of two beta sheets, each consisting of antiparallel beta strands of 5-10 amino acids with a conserved disulfide bond between the two sheets in most, but not all, domains. IgC domains of Ig, TCR, and MHC molecules share the same types of sequence patterns and are called the C1 set within the Ig superfamily. Other IgC domains fall within other sets. IgV domains also share sequence patterns and are called V set domains. IgV domains are longer than C-domains and form an additional pair of beta strands.

[00077] "Immune cell," as used herein, refers broadly to cells that are of hematopoietic origin and that play a role in the immune response. Immune cells include lymphocytes, such as B cells and T cells; natural killer cells; and myeloid cells, such as monocytes, macrophages, eosinophils, mast cells, basophils, and granulocytes.

[00078] "Immunoassay," as used herein, refers broadly to an assay that uses an antibody to specifically bind an antigen. The immunoassay may be characterized by the use of specific binding properties of a particular antibody to isolate, target, and/or quantify the antigen.

[00079] "Immune response," as used herein, refers broadly to T cell-mediated and/or B cell-mediated immune responses that are influenced by modulation of T cell costimulation. Exemplary immune responses include B cell responses (e.g., antibody production) T cell

responses (e.g., cytokine production, and cellular cytotoxicity) and activation of cytokine responsive cells, e.g., macrophages. As used herein, the term "downmodulation" with reference to the immune response includes a diminution in any one or more immune responses, while the term "upmodulation" with reference to the immune response includes an increase in any one or more immune responses. It will be understood that upmodulation of one type of immune response may lead to a corresponding downmodulation in another type of immune response. For example, upmodulation of the production of certain cytokines (e.g., IL-10) can lead to downmodulation of cellular immune responses.

[00080] "Inflammatory conditions or inflammatory disease," as used herein, refers broadly to chronic or acute inflammatory diseases.

[00081] "Inhibitory signal," as used herein, refers broadly to a signal transmitted via an inhibitory receptor molecule on an immune cell. A signal antagonizes a signal via an activating receptor (e.g., via a TCR, CD3, BCR, or Fc molecule) and can result, e.g., in inhibition of: second messenger generation; proliferation; or effector function in the immune cell, e.g., reduced phagocytosis, antibody production, or cellular cytotoxicity, or the failure of the immune cell to produce mediators (e.g., cytokines (e.g., IL-2) and/or mediators of allergic responses); or the development of anergy.

[00082] "Isolated," as used herein, refers broadly to material removed from its original environment in which it naturally occurs, and thus is altered by the hand of man from its natural environment. Isolated material may be, for example, exogenous nucleic acid included in a vector system, exogenous nucleic acid contained within a host cell, or any material which has been removed from its original environment and thus altered by the hand of man (e.g., "isolated antibody"). For example, "isolated" or "purified," as used herein, refers broadly to a protein, DNA, antibody, RNA, or biologically active portion thereof, that is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the biological substance is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of VISTA (PD-L3) protein in which the protein is separated from cellular components of the cells from which it is isolated or recombinantly produced.

[00083] An "isolated antibody", as used herein, is intended to refer to an antibody that is substantially free of other antibodies having different antigenic specificities (e.g., an isolated

antibody that specifically binds PD-L3 OR VISTA is substantially free of antibodies that specifically bind antigens other than PD-L3 OR VISTA). Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

[00084] "K-assoc" or "Ka", as used herein, refers broadly to the association rate of a particular antibody-antigen interaction, whereas the term "Kdiss" or "Kd," as used herein, refers to the dissociation rate of a particular antibody-antigen interaction. The term "KD", as used herein, is intended to refer to the dissociation constant, which is obtained from the ratio of Kd to Ka (i.e., Kd/Ka) and is expressed as a molar concentration (M). KD values for antibodies can be determined using methods well established in the art.

[00085] "Label" or a "detectable moiety" as used herein, refers broadly to a composition detectable by spectroscopic, photochemical, biochemical, immunochemical, chemical, or other physical means.

[00086] "Low stringency," "medium stringency," "high stringency," or "very high stringency conditions," as used herein, refers broadly to conditions for nucleic acid hybridization and washing. Guidance for performing hybridization reactions can be found in Ausubel, et al. (2002) Short Protocols in Molecular Biology (5.sup.th Ed.) John Wiley & Sons, NY. Exemplary specific hybridization conditions include but are not limited to: (1) low stringency hybridization conditions in 6.times. Sodium chloride/sodium citrate (SSC) at about 45.degree. C., followed by two washes in 0.2.times.SSC, 0.1% SDS at least at 50.degree. C. (the temperature of the washes can be increased to 55.degree. C. for low stringency conditions); (2) medium stringency hybridization conditions in 6.times.SSC at about 45.degree. C., followed by one or more washes in 0.2.times.SSC, 0.1% SDS at 60.degree. C.; (3) high stringency hybridization conditions in 6.times.SSC at about 45.degree. C., followed by one or more washes in 0.2.times.SSC, 0.1% SDS at 65.degree. C.; and (4) very high stringency hybridization conditions are 0.5M sodium phosphate, 7% SDS at 65.degree. C., followed by one or more washes at 0.2.times.SSC, 1% SDS at 65.degree. C.

[00087] "Mammal," as used herein, refers broadly to any and all warm-blooded vertebrate animals of the class Mammalia, including humans, characterized by a covering of hair on the skin and, in the female, milk-producing mammary glands for nourishing the young. Examples of mammals include but are not limited to alpacas, armadillos, capybaras, cats, camels, chimpanzees, chinchillas, cattle, dogs, goats, gorillas, hamsters, horses, humans, lemurs,

llamas, mice, non-human primates, pigs, rats, sheep, shrews, squirrels, tapirs, and voles. Mammals include but are not limited to bovine, canine, equine, feline, murine, ovine, porcine, primate, and rodent species. Mammal also includes any and all those listed on the Mammal Species of the World maintained by the National Museum of Natural History, Smithsonian Institution in Washington D.C.

[00088] "Naturally-occurring nucleic acid molecule," as used herein, refers broadly to refers to RNA or DNA molecule having a nucleotide sequence that occurs in nature (e.g., encodes a natural protein).

[00089] "Nucleic acid" or "nucleic acid sequence," as used herein, refers broadly to a deoxy-ribonucleotide or ribonucleotide oligonucleotide in either single- or double-stranded form. The term encompasses nucleic acids, i.e., oligonucleotides, containing known analogs of natural nucleotides. The term also encompasses nucleic-acid-like structures with synthetic backbones. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions) and complementary sequences, as well as the sequence explicitly indicated. The term nucleic acid is used interchangeably with gene, cDNA, mRNA, oligonucleotide, and polynucleotide.

[00090] "Oligomerization domain", as used herein, refers broadly to a domain that when attached to a VISTA extracellular domain or fragment thereof, facilitates oligomerization. Said oligomerization domains comprise self-associating α -helices, for example, leucine zippers, that can be further stabilized by additional disulfide bonds. The domains are designed to be compatible with vectorial folding across a membrane, a process thought to facilitate *in vivo* folding of the polypeptide into a functional binding protein. Examples thereof are known in the art and include by way of example coiled GCN4, and COMP.

[00091] The α helical coiled coil is probably the most widespread subunit oligomerization motif found in proteins. Accordingly, coiled coils fulfill a variety of different functions. In several families of transcriptional activators, for example, short leucine zippers play an important role in positioning the DNA-binding regions on the DNA. Ellenberger, et al. (1992) Cell 71: 1223-1237. Coiled coils are also used to form oligomers of intermediate filament proteins. Coiled-coil proteins furthermore appear to play an important role in both vesicle and viral membrane fusion. Skehel and Wiley (1998) Cell 95: 871-874. In both cases hydrophobic sequences, embedded in the membranes to be fused, are located at the same end

of the rod-shaped complex composed of a bundle of long α -helices. This molecular arrangement is believed to cause close membrane apposition as the complexes are assembled for membrane fusion. The coiled coil is often used to control oligomerization. It is found in many types of proteins, including transcription factors include, but not limited to GCN4, viral fusion peptides, SNARE complexes and certain tRNA synthetases, among others. Very long coiled coils are found in proteins such as tropomyosin, intermediate filaments and spindle-pole-body components. Coiled coils involve a number of α -helices that are supercoiled around each other in a highly organized manner that associate in a parallel or an antiparallel orientation. Although dimers and trimers are the most common. The helices may be from the same or from different proteins. The coiled-coil is formed by component helices coming together to bury their hydrophobic seams. As the hydrophobic seams twist around each helix, so the helices also twist to coil around each other, burying the hydrophobic seams and forming a supercoil. It is the characteristic interdigitation of side chains between neighboring helices, known as knobs-into-holes packing, that defines the structure as a coiled coil. The helices do not have to run in the same direction for this type of interaction to occur, although parallel conformation is more common. Antiparallel conformation is very rare in trimers and unknown in pentamers, but more common in intramolecular dimers, where the two helices are often connected by a short loop. In the extracellular space, the heterotrimeric coiled-coil protein laminin plays an important role in the formation of basement membranes. Other examples are the thrombospondins and cartilage oligomeric matrix protein (COMP) in which three (thrombospondins 1 and 2) or five (thrombospondins 3, 4 and COMP) chains are connected. The molecules have a flower bouquet-like appearance, and the reason for their oligomeric structure is probably the multivalent interaction of the C-terminal domains with cellular receptors. The yeast transcriptional activator GCN4 is 1 of over 30 identified eukaryotic proteins containing the basic region leucine zipper (bZIP) DNA-binding motif. Ellenberger, et al. (1992) Cell 71: 1223-1237. The bZIP dimer is a pair of continuous alpha helices that form a parallel coiled-coil over their carboxy-terminal 34 residues and gradually diverge toward their amino termini to pass through the major groove of the DNA binding site. The coiled-coil dimerization interface is oriented almost perpendicular to the DNA axis, giving the complex the appearance of the letter T. bZIP contains a 4-3 heptad repeat of hydrophobic and nonpolar residues that pack together in a parallel alpha-helical coiled-coil.

Ellenberger, et al. (1992) *Cell* 71: 1223-1237. The stability of the dimer results from the side-by-side packing of leucines and nonpolar residues in positions a and d of the heptad repeat, as well as a limited number of intra- and interhelical salt bridges, shown in a crystal structure of the GCN4 leucine zipper peptide. Ellenberger, et al. (1992) *Cell* 71: 1223-1237. Another example is CMP (matrilin-1) isolated from bovine tracheal cartilage as a homotrimer of subunits of Mr 52,000 (Paulsson & Heinegard (1981) *Biochem J.* 197: 367-375), where each subunit consists of a vWFA1 module, a single EGF domain, a vWFA2 module and a coiled coil domain spanning five heptads. Kiss, et al. (1989) *J. Biol. Chem.* 264:8126-8134; Hauser and Paulsson (1994) *J. Biol. Chem.* 269: 25747-25753. Electron microscopy of purified CMP showed a bouquet-like trimer structure in which each subunit forms an ellipsoid emerging from a common point corresponding to the coiled coil. Hauser and Paulsson (1994) *J. Biol. Chem.* 269: 25747-25753. The coiled coil domain in matrilin-1 has been extensively studied. The trimeric structure is retained after complete reduction of interchain disulfide bonds under non-denaturing conditions. Hauser and Paulsson (1994) *J. Biol. Chem.* 269: 25747-25753. Yet another example is Cartilage Oligomeric Matrix Protein (COMP). A non-collagenous glycoprotein, COMP, was first identified in cartilage. Hedbom, et al. (1992) *J. Biol. Chem.* 267:6132-6136. The protein is a 524 kDa homopentamer of five subunits which consists of an N-terminal heptad repeat region (cc) followed by four epidermal growth factor (EGF)-like domains (EF), seven calcium-binding domains (T3) and a C-terminal globular domain (TC). According to this domain organization, COMP belongs to the family of thrombospondins. Heptad repeats (abcdefg)_n with preferentially hydrophobic residues at positions a and d form helical coiled-coil domains. Cohen and Parry (1994) *Science* 263: 488-489. Recently, the recombinant five-stranded coiled-coil domain of COMP (COMPcc) was crystallized and its structure was solved at 0.2 nm resolution. Malashkevich, et al. (1996) *Science* 274: 761-765.

[00092] "Operatively linked", as used herein, refers broadly to when two DNA fragments are joined such that the amino acid sequences encoded by the two DNA fragments remain in-frame.

[00093] "Paratope," as used herein, refers broadly to the part of an antibody which recognizes an antigen (e.g., the antigen-binding site of an antibody.) Paratopes may be a small region (e.g., 15-22 amino acids) of the antibody's Fv region and may contain parts of

the antibody's heavy and light chains. See Goldsby, et al. *Antigens* (Chapter 3) *Immunology* (5.sup.th Ed.) New York: W.H. Freeman and Company, pages 57-75.

[00094] "Patient," as used herein, refers broadly to any animal who is in need of treatment either to alleviate a disease state or to prevent the occurrence or reoccurrence of a disease state. Also, "Patient" as used herein, refers broadly to any animal who has risk factors, a history of disease, susceptibility, symptoms, signs, was previously diagnosed, is at risk for, or is a member of a patient population for a disease. The patient may be a clinical patient such as a human or a veterinary patient such as a companion, domesticated, livestock, exotic, or zoo animal. The term "subject" may be used interchangeably with the term "patient."

[00095] "Polypeptide," "peptide" and "protein," are used interchangeably and refer broadly to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an analog or mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymer. Polypeptides can be modified, e.g., by the addition of carbohydrate residues to form glycoproteins. The terms "polypeptide," "peptide" and "protein" include glycoproteins, as well as non-glycoproteins.

[00096] "Promoter," as used herein, refers broadly to an array of nucleic acid sequences that direct transcription of a nucleic acid. As used herein, a promoter includes necessary nucleic acid sequences near the start site of transcription, such as, in the case of a polymerase II type promoter, a TATA element. A promoter also optionally includes distal enhancer or repressor elements, which can be located as much as several thousand base pairs from the start site of transcription. A "constitutive" promoter is a promoter that is active under most environmental and developmental conditions. An "inducible" promoter is a promoter that is active under environmental or developmental regulation.

[00097] "Prophylactically effective amount," as used herein, refers broadly to the amount of a compound that, when administered to a patient for prophylaxis of a disease or prevention of the reoccurrence of a disease, is sufficient to effect such prophylaxis for the disease or reoccurrence. The prophylactically effective amount may be an amount effective to prevent the incidence of signs and/or symptoms. The "prophylactically effective amount" may vary

depending on the disease and its severity and the age, weight, medical history, predisposition to conditions, preexisting conditions, of the patient to be treated.

[00098] "Prophylaxis," as used herein, refers broadly to a course of therapy where signs and/or symptoms are not present in the patient, are in remission, or were previously present in a patient. Prophylaxis includes preventing disease occurring subsequent to treatment of a disease in a patient. Further, prevention includes treating patients who may potentially develop the disease, especially patients who are susceptible to the disease (e.g., members of a patient population, those with risk factors, or at risk for developing the disease).

[00099] "Recombinant" as used herein, refers broadly with reference to a product, e.g., to a cell, or nucleic acid, protein, or vector, indicates that the cell, nucleic acid, protein or vector, has been modified by the introduction of a heterologous nucleic acid or protein or the alteration of a native nucleic acid or protein, or that the cell is derived from a cell so modified. Thus, for example, recombinant cells express genes that are not found within the native (non-recombinant) form of the cell or express native genes that are otherwise abnormally expressed, under expressed or not expressed at all.

[000100] "Signal sequence" or "signal peptide," as used herein, refers broadly to a peptide containing about 15 or more amino acids which occurs at the N-terminus of secretory and membrane bound polypeptides and which contains a large number of hydrophobic amino acid residues. For example, a signal sequence contains at least about 10-30 amino acid residues, preferably about 15-25 amino acid residues, more preferably about 18-20 amino acid residues, and even more preferably about 19 amino acid residues, and has at least about 35-65%, preferably about 38-50%, and more preferably about 40-45% hydrophobic amino acid residues (e.g., Valine, Leucine, Isoleucine or Phenylalanine). A "signal sequence," also referred to in the art as a "signal peptide," serves to direct a polypeptide containing such a sequence to a lipid bilayer, and is cleaved in secreted and membrane bound polypeptides.

[000101] "Specifically (or selectively) binds" to an antibody or "specifically (or selectively) immunoreactive with," or "specifically interacts or binds," as used herein, refers broadly to a protein or peptide (or other epitope), refers, in some embodiments, to a binding reaction that is determinative of the presence of the protein in a heterogeneous population of proteins and other biologics. For example, under designated immunoassay conditions, the specified antibodies bind to a particular protein at least two times greater than the background (non-

specific signal) and do not substantially bind in a significant amount to other proteins present in the sample. Typically a specific or selective reaction will be at least twice background signal or noise and more typically more than about 10 to 100 times background.

[000102] "Specifically hybridizable" and "complementary" as used herein, refer broadly to a nucleic acid can form hydrogen bond(s) with another nucleic acid sequence by either traditional Watson-Crick or other non-traditional types. The binding free energy for a nucleic acid molecule with its complementary sequence is sufficient to allow the relevant function of the nucleic acid to proceed, e.g., RNAi activity. Determination of binding free energies for nucleic acid molecules is well known in the art. See, e.g., Turner, et al. (1987) CSH Symp. Quant. Biol. LII: 123-33; Frier, et al. (1986) PNAS 83: 9373-77; Turner, et al. (1987) J. Am. Chem. Soc. 109: 3783-85. A percent complementarity indicates the percentage of contiguous residues in a nucleic acid molecule that can form hydrogen bonds (e.g., Watson-Crick base pairing) with a second nucleic acid sequence (e.g., about at least 5, 6, 7, 8, 9, 10 out of 10 being about at least 50%, 60%, 70%, 80%, 90%, and 100% complementary, inclusive). "Perfectly complementary" or 100% complementarity refers broadly all of the contiguous residues of a nucleic acid sequence hydrogen bonding with the same number of contiguous residues in a second nucleic acid sequence. "Substantial complementarity" refers to polynucleotide strands exhibiting about at least 90% complementarity, excluding regions of the polynucleotide strands, such as overhangs, that are selected so as to be noncomplementary. Specific binding requires a sufficient degree of complementarity to avoid non-specific binding of the oligomeric compound to non-target sequences under conditions in which specific binding is desired, i.e., under physiological conditions in the case of in vivo assays or therapeutic treatment, or in the case of in vitro assays, under conditions in which the assays are performed. The non-target sequences typically may differ by at least 5 nucleotides.

[000103] "Signs" of disease, as used herein, refers broadly to any abnormality indicative of disease, discoverable on examination of the patient; an objective indication of disease, in contrast to a symptom, which is a subjective indication of disease.

[000104] "Solid support," "support," and "substrate," as used herein, refers broadly to any material that provides a solid or semi-solid structure with which another material can be attached including but not limited to smooth supports (e.g., metal, glass, plastic, silicon, and ceramic surfaces) as well as textured and porous materials.

[000105] "Subjects" as used herein, refers broadly to anyone suitable to be treated according to the present invention include, but are not limited to, avian and mammalian subjects, and are preferably mammalian. Any mammalian subject in need of being treated according to the present invention is suitable. Human subjects of both genders and at any stage of development (i.e., neonate, infant, juvenile, adolescent, adult) can be treated according to the present invention. The present invention may also be carried out on animal subjects, particularly mammalian subjects such as mice, rats, dogs, cats, cattle, goats, sheep, and horses for veterinary purposes, and for drug screening and drug development purposes. "Subjects" is used interchangeably with "patients."

[000106] "Substantially free of chemical precursors or other chemicals," as used herein, refers broadly to preparations of VISTA protein in which the protein is separated from chemical precursors or other chemicals which are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of VISTA protein having less than about 30% (by dry weight) of chemical precursors or non-VISTA chemicals, more preferably less than about 20% chemical precursors or non-VISTA chemicals, still more preferably less than about 10% chemical precursors or non-VISTA chemicals, and most preferably less than about 5% chemical precursors or non-VISTA (PD-L3) chemicals.

[000107] "Symptoms" of disease as used herein, refers broadly to any morbid phenomenon or departure from the normal in structure, function, or sensation, experienced by the patient and indicative of disease.

[000108] "T cell," as used herein, refers broadly to CD4+ T cells and CD8+ T cells. The term T cell also includes both T helper 1 type T cells and T helper 2 type T cells.

[000109] "Treg cell" (sometimes also referred to as suppressor T cells) as used herein refers to a subpopulation of T cells which modulate the immune system and maintain tolerance to self-antigens and can abrogate autoimmune diseases. Foxp3+CD4+CD25+ regulatory T cells (Tregs) are critical in maintaining peripheral tolerance under normal physiological conditions, and suppress anti-tumour immune responses in cancer.

[000110] "Therapy," "therapeutic," "treating," or "treatment", as used herein, refers broadly to treating a disease, arresting, or reducing the development of the disease or its clinical symptoms, and/or relieving the disease, causing regression of the disease or its clinical

symptoms. Therapy encompasses prophylaxis, treatment, remedy, reduction, alleviation, and/or providing relief from a disease, signs, and/or symptoms of a disease. Therapy encompasses an alleviation of signs and/or symptoms in patients with ongoing disease signs and/or symptoms (e.g., inflammation, pain). Therapy also encompasses "prophylaxis". The term "reduced", for purpose of therapy, refers broadly to the clinical significant reduction in signs and/or symptoms. Therapy includes treating relapses or recurrent signs and/or symptoms (e.g., inflammation, pain). Therapy encompasses but is not limited to precluding the appearance of signs and/or symptoms anytime as well as reducing existing signs and/or symptoms and eliminating existing signs and/or symptoms. Therapy includes treating chronic disease ("maintenance") and acute disease. For example, treatment includes treating or preventing relapses or the recurrence of signs and/or symptoms (e.g., inflammation, pain).

[000111] "Transmembrane domain," as used herein, refers broadly to an amino acid sequence of about 15 amino acid residues in length which spans the plasma membrane. More preferably, a transmembrane domain includes about at least 20, 25, 30, 35, 40, or 45 amino acid residues and spans the plasma membrane. Transmembrane domains are rich in hydrophobic residues, and typically have an alpha-helical structure. In an embodiment, at least 50%, 60%, 70%, 80%, 90%, 95% or more of the amino acids of a transmembrane domain are hydrophobic, e.g., leucines, isoleucines, tyrosines, or tryptophans. Transmembrane domains are described in, for example, Zagotta, et al. (1996) *Annu. Rev. Neurosci.* 19:235-263.

[000112] "Transgenic animal," as used herein, refers broadly to a non-human animal, preferably a mammal, more preferably a mouse, in which one or more of the cells of the animal includes a "transgene". The term "transgene" refers to exogenous DNA which is integrated into the genome of a cell from which a transgenic animal develops and which remains in the genome of the mature animal, for example directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal.

[000113] "Tumor," as used herein, refers broadly to at least one cell or cell mass in the form of a tissue neoformation, in particular in the form of a spontaneous, autonomous and irreversible excess growth, which is more or less disinhibited, of endogenous tissue, which growth is as a rule associated with the more or less pronounced loss of specific cell and tissue functions. This cell or cell mass is not effectively inhibited, in regard to its growth, by itself

or by the regulatory mechanisms of the host organism, e.g., colorectal cancer, melanoma or carcinoma. Tumor antigens not only include antigens present in or on the malignant cells themselves, but also include antigens present on the stromal supporting tissue of tumors including endothelial cells and other blood vessel components.

[000114] "Unresponsiveness," as used herein, refers broadly to refractivity of immune cells to stimulation, e.g., stimulation via an activating receptor or a cytokine. Unresponsiveness can occur, e.g., because of exposure to immunosuppressants or high doses of antigen.

[000115] "Variable region" or "VR," as used herein, refers broadly to the domains within each pair of light and heavy chains in an antibody that are involved directly in binding the antibody to the antigen. Each heavy chain has at one end a variable domain (VH) followed by a number of constant domains. Each light chain has a variable domain (VL) at one end and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain.

[000116] "Vector," as used herein, refers broadly to a nucleic acid molecule capable of transporting another nucleic acid molecule to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Vectors are referred to herein as "recombinant expression vectors" or simply "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions. The techniques and procedures are generally performed according

to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. See, e.g., Sambrook, et al. (2001) *Molec. Cloning: Lab. Manual* [3.sup.rd Ed] Cold Spring Harbor Laboratory Press. Standard techniques may be used for recombinant DNA, oligonucleotide synthesis, and tissue culture, and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques may be performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein.

[000117] The nomenclatures utilized in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art. Standard techniques may be used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

[000118] “VISTA agonist” herein includes any compound which directly or indirectly promotes the expression or activity of VISTA, particularly its suppressive effects on B cell proliferation and B cell responses, e.g., antigen-specific antibody responses. This includes agonistic anti-VISTA antibodies and antibody fragments, antibody or an antibody fragment thereof, a peptide, a glycoalkoid, an antisense nucleic acid, a ribozyme, a retinoid, an avemir, a small molecule, or any combination thereof. Preferred VISTA agonists include VISTA-Ig polypeptides, e.g., wherein the VISTA polypeptide or a fragment thereof is attached to the entire Fc region or a fragment of Fc region of an antibody, e.g., a human IgG1, IgG2, IgG3 or IgG4. Other exemplary VISTA agonists include multimeric forms of VISTA, i.e., which comprise more than one VISTA polypeptide or fragment thereof, e.g., 2, 3, 4, 5 or 6 VISTA polypeptides which typically are attached via oligomerization or multimerization domains or sequences. Such sequences are known and available. Also VISTA agonists include transfected cells which express VISTA on their surface and nucleic acids which promote the expression of VISTA. In most instances such agonists will be specific to human VISTA.

[000119] “VISTA antagonist” herein includes any compound which directly or indirectly inhibits or blocks the expression or activity of VISTA, particularly its suppressive effects on B cell proliferation and B cell responses, e.g., antigen-specific antibody responses. This includes antagonistic anti-VISTA antibodies and antibody fragments, antibody or an antibody fragment thereof, a peptide, a glycoalkoid, an antisense nucleic acid, a ribozyme, a retinoid,

an avemir, a small molecule, or any combination thereof. Preferred VISTA antagonists include anti-human VISTA antibodies and antibody fragments which block or inhibit the inhibitory effects of MSDCs on B cell proliferation or B cell responsiveness, e.g., antigen specific antibody responses. Other preferred VISTA antagonists include nucleic acids which block or inhibit the expression of VISTA such as antisense nucleic acids, and interfering RNAs or siRNAs or peptide nucleic acids (“PNAs”). Other exemplary VISTA antagonists include fragments of VISTA which block the binding of VISTA to a cell, e.g., an immune cell, and cells which express VISTA polypeptides which block the binding of VISTA to immune cells.

[000120] Host control of the extent of pathogenesis clearly reflects immunoregulatory responses, including, for example, control mechanisms resulting in overzealous negative immune regulation in the context of the tumor microenvironment. In virus infections, while in most cases viral hijacking of immunoregulatory cells and molecules only indirectly promotes increased pathogenesis by decreasing host responsiveness, it is possible that misdirected immunoregulatory systems could directly serve as the effector cells/molecules proximally causing disease.

[000121] An immune regulatory cell type well studied over the last decade is the myeloid-derived suppressor cell (MDSC) (rev in, Ostrand-Rosenberg, 2010; Youn and Gabrilovich, 2010). Like CD4+FoxP3+ Treg cells, MDSCs are viewed as primarily acting in a negative fashion with respect to protective T-cell immune responses, particularly in various tumor systems. In addition, there is a small but growing literature providing evidence that MDSC regulatory control can limit autoimmune disease processes (Bowen and Olson, 2009). MDSCs, although their ultimate definition is based on their (immature) myeloid derivation and their suppressive function, have a generally accepted surface phenotype of positivity for Gr-1 and CD11b in murine systems. Although MDSC populations are typically heterogeneous, there are two phenotypically distinguishable cell-surface phenotypic murine MDSC subsets. Using mAbs to separate the specificities that anti-Gr-1 recognizes: the granulocytic/PMN-like MDSC subset is Ly6G+/hi Ly6C+/lo whereas the monocytic MDSC subset is Ly6G+/lo Ly6C+/hi (rev. in Ostrand-Rosenberg, 2010; Peranzoni et al., 2010; Youn and Gabrilovich, 2010). Based primarily on studies of a broad spectrum of tumor microenvironments, MDSCs have also been differentially characterized by the mechanisms

by which they cause responder T-cell suppression, including: arginase; iNOS/NO; other oxygen and nitrogen reactive species; and other mechanisms (rev. in LaFace and Talmadge). Although some reviews of MDSCs indicate that B-cell responses can be sensitive to MDSC regulation, and MDSCs can exhibit broad, non-MHC-restricted responses to polyclonal activators and mitogens, the primary literature on B-cell targeting by MDSCs is clearly limited.

[000122] MDSCs have also been less well studied in various infectious diseases: bacterial (Gabrilovich and Nagaraj, 2009), yeast/fungal (Mencacci et al., 2002), protozoan parasitic (Voisin et al., 2004), helminthic (Brys et al., 2005) and viral infections: e.g. influenza A virus (Jeisy-Scott et al., 2011), chronic, murine hepatitis B virus (Chen et al., 2011); and both vesicular stomatitis virus (Willmon et al., 2011), and Herpes Simplex virus 1 engineered strains (Walker et al., 2011). But, there are only a few recent reports on the presence or function of MDSC populations in the context of retroviral infections, and ensuing diseases such as acquired immunodeficiency (Vollbrecht et al, 2012, comment Macantangay et al. 2012; Green et al., 2013; Qin et al., 2013; Garg and Spector, 2014).

[000123] Retroviruses are proficient in co-opting various immunoregulatory mechanisms. HIV-1 and SIV have been shown to cause the premature expression of PD-1 on effector T cells, which can push antiviral CD8+ cytolytic T lymphocytes (CTL) effector cells to an inappropriately early down regulation, akin to the normal T-cell contraction phase that occurs at the latter stages of viral clearance (Day et al., 2006; Velu et al., 2009). With murine Friend retrovirus (FV) altered expression of PD-1 and Tim-3 has been reported to have varying effects on retroviral load and pathogenesis (Takamura et al., 2010; Zelinsky et al., 2011), including “exhaustion”, or the relatively “function-less” T-cell phenotype observed in other viral systems (Day et al., 2006; Velu et al., 2009). Viral infections, can also alter immunoregulatory cells, such as CD4+ FoxP3+ T-regulatory (Treg) cells, a major control point of anti-tumor immunity and autoimmunity (rev. in Sakaguchi, 2004; Piccirillo and Shevach, 2004). In the LP-BM5 murine retrovirus system studied here, an early report provided evidence in support of a direct role of CD25+CD4+ T cells, considered to be Tregs based on other assessed markers but not including FoxP3, in mediating LP-BM5 pathogenesis (Beilharz et al., 2004). However, subsequent reports supported an indirect role for true CD4+FoxP3+ Treg cells in LP-BM5 infection/pathogenesis, limiting (along with PD-

1/PD-L1) the development of a protective CD8+ CTL response (Li and Green, 2006; Li and Green 2011).

[000124] After infection with the LP-BM5 retrovirus isolate certain inbred strains of mice, such as the highly susceptible C57BL/6 (B6) strain, develop a disease syndrome which includes immunodeficiency. Beginning at approximately 5 weeks post infection, a profound immunodeficiency is readily apparent, including severely damped T- and B-cell responses, leading to the full array of disease features (Simard et al., 1997). Consequently, there is an increased susceptibility to disease progression and sometimes death when exposed to environmental pathogens that normally cause limited infections. At later time points LP-BM5-infected, B6 mice develop B-cell lymphomas. Because these features of LP-BM5-induced disease are similar to many of those seen in HIV-infected individuals, this syndrome has been designated murine AIDS (MAIDS).

[000125] The mechanism of LP-BM5 retroviral pathogenesis is not completely understood. Inoculation of LP-BM5 into B6 mice genetically deficient in, or subjected to prior in vivo antibody depletion of, either CD4+ T cells or B cells leads to infection but not to virus-induced disease (Yetter et al., 1988). Relative to this requirement for “pathogenic CD4+ T-effector cells, we determined that CD154/CD40 interactions are necessary for both the induction and progression of LP-BM5 pathogenesis. In vivo treatment with □-CD154 (CD40 ligand) monoclonal antibody (mAb) either at the initiation of, or 3-4 weeks after, infection of B6 mice leads to substantial inhibition of standard MAIDS parameters including: splenomegaly, hyper-gammaglobulinemia, immune cell subset phenotypic changes, and B- and T-cell immunodeficiency (Green et al., 1996; Green et al., 1998). The extent of LP-BM5-induced disease was also controlled by PD-1/PD-L1 and IL-10, which served to down-regulate the pathogenic CD4+ T cells, thus leading to diminished disease (Li and Green, 2006). PD-1 ligation and CD4+ T reg cells also combined to inhibit the generation of a protective CD8+ CTL response in B6 mice (Li and Green, 2011). An expansion of a CD11b+ Fc γ III/II+ myeloid cell population is also a prominent feature of LP-BM5 induced pathogenesis. Based on this cell surface phenotype we recently assessed the possible involvement of retrovirus-induced MDSCs in this system. Indeed, we identified an LP-BM5-induced MDSC population with a monocyteoid cell-surface phenotype (Gr-1+, Ly6C+, Ly6G-, CD11b+) that had strong ex vivo inhibitory activity effective against immune responses to

stimuli used standardly to measure LP-BM5-induced immunodeficiency. By use of several informative B6 k.o. strains we had previously shown to exhibit varying levels of sensitivity to LP-BM5 pathogenesis, we established a direct correlation between in vivo LP-BM5 induced disease severity and ex vivo MDSC inhibitory activity. (Green et al., 2013).

[000126] Also recently, a new immunoglobulin (Ig) superfamily member that belongs to the subset of critical immune negative regulator ligands, VISTA (V-domain Ig suppressor of T cell activation) has been defined (Wang et al., 2011; LeMercier et al., 2014; Lines et al., 2014). VISTA bears homology to the B7 family ligand PD-L1, particularly for its extracellular domain. As primarily expressed on hematopoietic cells, VISTA can be highly up-regulated on myeloid antigen-presenting cells (APCs) and T cells. Several lines of evidence indicate that VISTA serves to inhibit T cell responses in autoimmunity and anti-tumor immunity. First, VISTA-specific mAb interferes with VISTA-induced suppression of T cell responses by VISTA-expressing APCs in vitro. Second, anti-VISTA treatment exacerbates the development of T cell-mediated experimental autoimmune encephalomyelitis in mice. Third, a soluble VISTA-Ig fusion protein, or VISTA expression on APCs, inhibits T cell proliferation and cytokine production in vitro. Fourth, VISTA overexpression on tumor cells interferes directly with protective antitumor immunity in vivo in mice. These early reports collectively show that VISTA serves as a novel immunoregulatory molecule, with functional activities that are non-redundant with other Ig superfamily members such as PD-L1, and thus plays a likely key role in the development and regulation of both autoimmunity and T-cell immune surveillance against tumor cells, albeit with essentially opposite consequences with respect to disease severity.

[000127] In this application we further assess the LP-BM5 retrovirus-induced monocytic MDSC population, just described (Green et al., 2013), with respect to the possible involvement of VISTA in the suppressive mechanism(s) of these MDSCs. Specifically, given that MDSC inhibition of T-cell proliferation and IFN-gamma producing responses is near totally dependent on iNOS/NO (Green et al., 2013), we compared VISTA involvement for T-cell targets of MDSCs vs for B-cell targets, where we previously have only been able to account for ~50% of the MDSC suppressive mechanism as due to iNOS/NO. Our results indicate a differential role for MDSC expressed VISTA in suppression of T- vs B-cell

responsiveness, underscoring the heterogeneity of MDSC function and the possibility of different MDSC subpopulations.

EXPERIMENTAL EXAMPLES

Materials and Methods Used in Examples

[000128] Mice. Seven week old male C57BL/6 (B6) mice were purchased from the National Institutes of Health (Bethesda, MD), housed in the Dartmouth Medical School Animal facility, and used when approximately, 8-10 weeks of age. Fully backcrossed-to-B6, iNOS k.o. mouse breeding pairs were obtained from the Jackson Labs (Bar Harbor, ME), and were originally derived as previously described (Laubach et al., 1995). Also backcrossed to B6: VISTA k.o. breeding pairs, derived as reported were obtained from

[000129] Cell purification. For experimental suppressor cell populations, splenocyte suspensions from either three or four LP-BM5-infected mice (5 w.p.i.) were pooled and labeled with \square -Ly6G coupled paramagnetic beads, with subsequent column purification according to the manufacturer's protocol (MACS, Miltenyi Biotec, Auburn, CA). The flow-through from the first separation was labeled with \square -CD11b coupled paramagnetic beads and subjected to column purification yielding a positively selected cell population which was $> 75\%$ CD11b+Ly6C+.

[000130] LP-BM5 virus inoculations. LP-BM5 was prepared in our lab as previously described (Klinken et al., 1988). To produce LP-BM5 virus stocks, G6 cells, originally generously provided by Drs. Janet Hartley and Herbert Morse (NIH), as a cloned cell line from SC-1 cells infected with the LP-BM5 virus preparation, were used in a co-culture with non-infected SC-1 cells. Mice were infected intraperitoneally with 5x10⁴ ecotropic plaque forming units as determined by a standard retroviral XC plaque assay (Rowe et al., 1970).

[000131] 3H-Thymidine incorporation proliferation assays. For responder cells, 5x10⁵ non-infected spleen cells, which were either non-fractionated or CD19+ purified, were plated into 96 well flat bottom plates along with 1.6x10⁵ (unless otherwise indicated) CD11b+ Ly6G+, or CD11b+Ly6C+, enriched suppressor cells obtained from spleens of 5-w.p.i. LP-BM5 mice of the indicated w.t. B6 or B6 knock-out strains. All wells were plated in triplicate with media containing 5% FCS, L glutamine, antibiotics, and a final concentration of either 10.ig/ml LPS, 50.ig/ml ct-CD40 plus 10ng/ml IL-4, or 2.ig/ml Con A. For the inhibition of suppression of proliferation assays, non-fractionated or CD11b+Ly6C+ enriched

suppressor cells were pretreated for one hour before the start of the co-culture with 0.8 mM of either the NOS inhibitor NG-monomethyl-L-Arginine (L-NMMA), the negative-control enantiomer NG-monomethyl-D-arginine (D-NMMA) (A.G. Scientific, San Diego, CA), or 80 ug/ml purified ct-VISTA (13F3) or control HIg. After 66 hours all wells were pulsed with 1mCi 3H-thymidine (Perkin Elmer, Waltham, MA) and harvested 6 hours later for assessment of thymidine incorporation by scintillation counting (Perkin Elmer, Waltham, MA). Data for B- and T-cell responder cell stimulation are expressed either as the percent suppression. For the percent suppression calculation, first, the percent residual responsiveness (R) was calculated as follows: $R = (\text{cpm of co-cultured responder cells and experimental suppressor cells}) \div (\text{cpm of co-cultured responder cells and control, non-infected suppressor cells}) \times 100\%$. Standard deviations of the mean were determined for triplicate wells, and were statistically compared by the two-tailed, two sample equal variance, Student T test.

[000132] CFSE dilution proliferation assays. Naïve responder splenocytes were labeled with 5 .iM CFSE (Cell trace, CFSE proliferation Kit, Molecular Probes, Eugene, OR) in PBS/0.5% BSA for 10 minutes at 37° C followed by the addition of cold 10% FBS in RPMI for 5 minutes on ice. After three cold 10% FCS/RPMI washes, cell cultures were set up in the same manner as that for the 3H-thymidine incorporation proliferation assays described above. On culture day 4, wells were stained with monoclonal antibody (mAb): ct-CD19-PerCP (Biolegend, San Diego, CA) and were analyzed on a FACSCalibur™ flow cytometer (BD bioscience, San Jose, CA).

[000133] Flow cytometry. 5×10^5 spleen cells were incubated with an unlabeled mAb directed against the Fcγ III/II receptors, (2.4G2, Biolegend, San Jose, CA) for 10 min on ice followed by surface staining for 25 min on ice with FITC-, PE-, APC-, or PerCP-conjugated antibodies. Stained cells were analyzed, as quantified by log amplification, on a FACSCalibur flow cytometer using CellQuest software (BD Bioscience, San Jose, CA). To detect the expression of the following murine antigens the indicated mAbs were employed: VISTA (13F4) purified/ PE streptavidin, CD4 (RM4-5), CD19 (6D5), CD11b (M1/70), Ly6G (1A8), Ly6C (HK1.4). Appropriate FITC-, PE-, PerCP- or APC-conjugated Ig isotypes of irrelevant specificity were used to control for each experimental mAb.

EXAMPLE 1: VISTA Expression by Spleen Cells

[000134] This example relates to the experiments in Figure 1. In these experiments VISTA expression by spleen cells was shown to increase with *in vivo* LP-BM5 infection. Figure 1(A) contains flow cytometric analysis showing the pattern of VISTA expression for spleen cells from uninfected or 5 or 8.5 week infected W.t. B6 mice. Percentages indicate cells positive for VISTA expression; italicized values indicate the MFI of the VISTA positive cells. Figure 1(B) shows spleen cell surface VISTA expression VISTA for CD11b+ cells for the following groups: cells obtained, without subsequent purification, from the indicated uninfected or infected mouse strains; and purified Ly6C+CD11b+ spleen cells from 5 wpi-LP-BM5 mice. The presented pattern of results is representative of two additional experiments.

EXAMPLE 2 : Ly6C+CD11b+ purified spleen cells have differing mechanistic requirements for suppressing *in vitro* B and T cell proliferation

[000135] This example relates to the experiments in Figure 2. These experiments demonstrate that Ly6C+CD11b+ purified spleen cells have differing mechanistic requirements for suppressing *in vitro* B and T cell proliferation. Figure 2(A and B) show the results of experiments wherein naïve B6 responder spleen cells mixed with a-VISTA and LNMMMA pre-treated Ly6C+CD11b+ MDSCs at the responder to suppressor (R:S) ratio of 3:1, and were stimulated for three days with (A) a-CD40 and IL-4 or (B) Con A.

[000136] In the figure 3H -thymidine incorporation is converted to % suppression (see materials and methods). The presented pattern of results is representative of three additional experiments for each stimulation. Figure 2C shows the average + standard deviation of 4 independent experiments showing the % of blockade of suppression by a-VISTA pretreatment of Ly6C+CD11b+ MDSCs before culturing for three days with naïve W.t. B6 responders. Significance levels: *, P < 0.05; **, P < 0.01, ***, P < 0.001.

EXAMPLE 3: Effect of VISTA Antagonist on MDSC- Mediated Inhibition of Spleen Cell Proliferation

[000137] This example relates to the experiments in Figure 3. In these experiments naïve VISTA-/- responder spleen cells were mixed with α-VISTA pre-treated Ly6C+CD11b+ MDSCs at the responder to suppressor (R:S) ratio of 3:1, and were stimulated for three days

with a-CD40 and IL-4. The presented pattern of results is representative of three additional experiments. *, P < 0.05; **, P < 0.01; ***, P < 0.001; ns., P > 0.05.

EXAMPLE 4: MDSC Suppression of Spleen Cell Proliferation

[000138] This example relates to the experiments in Figure 4. In these experiments purified monocytic MDSCs (Ly6C+CD11b+) from 5 week LP-BM5 infected mice of the indicated strains, show differing patterns of suppression of naïve VISTA-/- spleen cell proliferation as assessed by flow cytometry. CFSE labeled VISTA-/- responder cells were cultured for three days in the presence (+) or absence (-) of ct-CD40 + IL-4, and with or without monocytic MDSCs (a) (-); (b) (+) (grey shaded curves); (c) (+), W.t. MDSCs (in black open curves); (d) (+), DNMMA treated W.t. MDSCs (black open curves); (e) (+), LNMMA treated W.t. MDSCs; (f) (+), iNOS-/- MDSCs (dotted line curves); (g) (+), (h) (+), VISTA-/- MDSCs, vs w.t. MDSCs; Cells from all cultures were stained at the termination of a four day incubation, with ct-CD19 fluorochrome conjugated mAb and CFSE dilution was also assessed by FACS. CFSE labeled VISTA-/- Responders: % = the percentage e of cells in the first proliferation peak; italicized numbers = MFI of CFSE+ cells. The presented pattern of results is representative of one additional experiment.

EXAMPLE 5: MDSC Suppression of B cell Proliferation

[000139] This example relates to the experiments in Figure 5. In these experiments monocytic suppression of B cell proliferation was shown to be dependent on two main mechanisms iNOS/NO and VISTA. In the experiment in 5A purified Ly6C+CD11b+ MDSCs from 5 w.p. LP-BM5 infected W.t. or VISTA-/- mice were pretreated with ct-VISTA blocking antibody or control HIg, LNMMA or control DNMMA and cultured for three days with VISTA-/- responder cells and ct-CD40 and IL-4. In the experiment in 5(B) W.t. Ly6C+CD11b+ MDSCs were pretreated with the same treatment regimens as shown in panel (A) with the inclusion of a combined regimen containing both ct-VISTA and LNMMA. The % suppression was calculated for both (A) and (B) by cpm responder 3H thymidine incorporation. The presented pattern of results is representative of two additional experiments (A) and one additional experiment (B). *, P < 0.05; **, P < 0.01; ns., P > 0.05.

RESULTS AND CONCLUSIONS

[000140] Because MDSCs expand following LP-BM5 retrovirus infection, VISTA cell-surface expression was first assessed p.i. Although at 5 w.p.i. the percentage of VISTA+ spleen cells had not expanded, VISTA MFI was increased and the shape of the positive peak changed (Fig. 1A). This change was consistent with both the reported dominance of CD4 T-cell expression of VISTA in uninfected B6 mice (Wang et al., 2011); and with additional cell types, such as CD11b+ VISTA+ cells expanding upon in vivo stimulation: here, LP-BM5 infection, which we have reported expands MDSCs (our ref). At 8.5 w.p.i. the increased VISTA expression was more pronounced (Fig 1A). Further staining for VISTA and CD11b co-expression by cells from uninfected; vs 5 w.p.i., W.t., VISTA -/-, and iNOS-/- ; B6 mice confirmed the specificity of the anti-VISTA mAb; and corroborated expanded VISTA expression in the highly enriched monocytic MDSC population (Fig 1B) (previously shown to also express Ly6C (our ref and data not shown here). Again, as predicted by the studies by Wang et al (2011), the other major VISTA+ population was the CD4 T-cell compartment (data not shown).

[000141] The functionality of VISTA expression by the monocytic MDSCs was next evaluated in blocking experiments. First, possible VISTA mechanistic involvement was compared to the known differential role of iNOS/NO in MDSC suppression (Fig 2A/B). Whereas, MDSC suppression of T-cell responsiveness was essentially completely dependent on iNOS/NO, as revealed by the iNOS inhibitor LNMMA (vs control DNMMA); for B-cell responsiveness, LNMMA blocked MDSC suppression by ~50% (as previously shown, Green et al., 2013). In sharp contrast, anti-VISTA mAb blocked MDSC suppression of B-cell responsiveness again by ~50%, whereas MDSC suppression of T-cell responsiveness was essentially unaffected. Indeed, over several independent experiments, the range of blocking by anti-VISTA centered around 65%, but closer to a delta of ~55% if the small non-specific effect of control hamster immunoglobulin (HIg) was considered. Thus, for suppression of B-cell responses VISTA appeared to serve in a distinguishable manner relative to iNOS/NO.

[000142] To demonstrate unequivocally that the blocking effects of anti-VISTA were specifically directed to the MDSCs, the anti-VISTA blocking experiments were repeated with the use of VISTA-/- responder cells (Fig. 3). Again, highly significant ($p = 0.0001$) but partial, blocking by anti-VISTA (but not HIg) was observed. These results confirmed the

dependency on MDSC VISTA for blocking, and for simplicity, and clarity, all following experiments employed VISTA-/- responder cells.

[000143] To independently corroborate these findings and to compare these two molecular mechanisms, CFSE-preloaded responder cells were stimulated in the presence of monocytic MDSCs of various wild-type vs k.o. B6 origin, and flow cytometric analyses performed with identification of CD19+ B-cells (Fig. 4). In Fig. 4A, w.t. MDSC suppression of stimulated B cells was clearly observed (panels a, b, vs c) and by inclusion of LNMMA (not DNMMA) the proliferation curve was partially restored towards that of control B cells stimulated in the absence of MDSCs (panels d, e). Again, this partial dependence on iNOS/NO was revealed both by the percentage of cells shown in the first peak of proliferation and the CFSE MFI value attained collectively by the first and all subsequent proliferation peaks. The alternative use of iNOS-/- MDSCs (in the absence of inhibitors) underscored this CFSE proliferation profile by the congruence of the overlay of the iNOS-/- MDSC, v.s. w.t. MDSC + LNMMA, curves in Fig. 4B (panels e vs f). However, importantly, when VISTA-/- MDSCs were employed two results were prominent: 1) partial blocking of MDSC suppression due to the loss of MDSC VISTA expression (Fig. 4 panel g); and 2) the very different blocking profiles of the proliferation curves resulting from removal of MDSC VISTA vs LNMMA blockade of iNOS/NO, each compared to w.t. MDSC suppression without inhibitors (Fig. 4, panel h). These findings confirmed the distinguishable nature of the VISTA, vs the iNOS/NO, - dependent components of MDSC suppression of B-cell responsiveness and suggested the independence of these two mechanisms.

[000144] Additional combined “treatment” experiments were conducted to assess the role of these two pathways in MDSC suppression of B cells. First, we employed MDSC of w.t. vs VISTA-/- origin in the presence of the iNOS/NO inhibitor LNMMA. With w.t. MDSC (as a control) their suppression of B-cell responsiveness was partially blocked by either anti-VISTA mAb or LNMMA (Fig. 5A), in agreement with our results above. In contrast in the same experiment using the same responder cells and reagents, MDSC of VISTA-/- origin suppressed B-cell responsiveness in a manner that was blockable only by LNMMA (Fig. 5A). And the extent of blocking was now essentially 100%, indicating retention of only the iNOS/NO pathway. These data were further consistent with only two main mechanistic pathways of MDSC suppression. In a second approach w.t. MDSCs were treated with anti-

VISTA, LNMMA, or a combination of these two blocking reagents (Fig. 5B). Only with the combined treatment, in which both VISTA and iNOS/NO involvement was interfered with, was there a blockade of suppression that was essentially complete. Collectively (Fig. 5A/B) this additive, if not synergistic, blocking of MDSC suppression re-iterated the iNOS/NO and VISTA dependencies as defining two main mechanisms of suppression of B-cell responses by monocytic MDSCs from LP-BM5-infected mice.

[000145] In conclusion, we describe here, for the first time to our knowledge, the involvement of the novel negative checkpoint regulator VISTA in MDSC suppression -- in particular, of B-cell responsiveness. Our observation that this VISTA-dependent suppressive mechanism is a player only in MDSC suppression of B-cell, not T-cell, responses contributes to the novelty of our findings. Thus, MDSC suppression of B-cell reactivity has been a very under-studied area. We have previously published (Green et al., 2013) that LP-BM5 retrovirus-induced MDSC suppression depends neither on PD-L1 nor PD-1 (nor IL-10). Therefore, the present results on participation of a VISTA-dependent pathway, along with the iNOS/NO mechanism(s), underscores the uniqueness of VISTA-related function versus its closest relative by sequence homology, PD-L1 (Wang et al., 2011). Taken together with the differential involvement of VISTA in suppression of B cells versus T cells by the same population of monocytic MDSC from LP-BM5 retrovirus-infected mice, these results raise important further questions, ranging from the possibility of the existence of functional/phenotypic MDSC subpopulations with different mechanisms, and targets of suppression, to a role for MDSC subsets in the profound and broad immunodeficiency of T- and B-cell responses characteristic of LP-BM5 induced MAIDS.

THERAPEUTIC APPLICATIONS OF THE INVENTION

[000146] Based on VISTA's effect on B cell responsiveness, in particular its suppressive effect on B cell responsiveness mediated by MSDCs, VISTA antagonists may be used alone or in association with other immune activators or agonists, particularly those which enhance humoral immunity such as CD40 agonists such as agonistic anti-CD40 antibodies or CD40L polypeptides in order to treat or prevent conditions where increased B cell responsiveness is therapeutically desired. Such conditions include in particular cancer wherein B cell responses, e.g., antibody responses are involved in disease pathology or in eliciting prophylactic or therapeutic immunity.. Examples of such cancer conditions include but are

not limited to carcinoma, lymphoma, blastoma, sarcoma (including liposarcoma), neuroendocrine tumors, mesothelioma, schwannoma, meningioma, adenocarcinoma, melanoma, ovarian cancer, breast cancer, lung cancer, brain cancer, digestive organ cancers, and leukemia or lymphoid malignancies.

[000147] More particular examples of such cancers include hematologic malignancies, such as non-Hodgkin's lymphomas (NHL). NHL cancers include but are not limited to Burkitt's lymphoma (BL), small lymphocytic lymphoma/chronic lymphocytic leukemia (SLL/CLL), mantle cell lymphoma (MCL), follicular lymphoma (FL), diffuse large B-cell lymphoma (DLCL), marginal zone lymphoma (MZL), hairy cell leukemia (HCL) and lymphoplasmacytic leukemia (LPL), extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT), nodal marginal zone B cell lymphoma, mediastinal large cell lymphoma, intravascular large cell lymphoma, primary effusion lymphoma, precursor B-lymphoblastic leukemia/lymphoma, precursor T- and NK-cells lymphoma (precursor T lymphoblastic lymphoma, blastic NK cell lymphoma), tumors of the mature T and NK cells, including peripheral T-cell lymphoma and leukemia (PTL), adult T-cell leukemia/T-cell lymphomas and large granular lymphocytic leukemia, T-cell chronic lymphocytic leukemia/prolymphocytic leukemia, T-cell large granular lymphocytic leukemia, aggressive NK-cell leukemia, extranodal T/NK cell lymphoma, enteropathy-type T-cell lymphoma, hepatosplenic T-cell lymphoma, anaplastic large cell lymphoma (ALCL), angiocentric and angioimmunoblastic T-cell lymphoma, mycosis fungoides/Sézary syndrome, and cutaneous T-cell lymphoma (CTCL). Other cancers that may be treatable by VISTA antagonists include but are not limited to Hodgkin's lymphoma, tumors of lymphocyte precursor cells, including B-cell acute lymphoblastic leukemia/lymphoma (B-ALL), and T-cell acute lymphoblastic leukemia/lymphoma (T-ALL), thymoma, Langerhans cell histiocytosis, multiple myeloma (MM), myeloid neoplasias such as acute myelogenous leukemias (AML), including AML with maturation, AML without differentiation, acute promyelocytic leukemia, acute myelomonocytic leukemia, and acute monocytic leukemias, myelodysplastic syndromes, and chronic myeloproliferative disorders (MDS), including chronic myelogenous leukemia (CML). Other cancers that may be treatable with VISTA antagonists include but are not limited to tumors of the central nervous system such as glioma, glioblastoma, neuroblastoma, astrocytoma, medulloblastoma, ependymoma, and

retinoblastoma; solid tumors of the head and neck (e.g. nasopharyngeal cancer, salivary gland carcinoma, and esophageal cancer), lung (e.g. small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung), digestive system (e.g. gastric or stomach cancer including gastrointestinal cancer, cancer of the bile duct or biliary tract, colon cancer, rectal cancer, colorectal cancer, and anal carcinoma), reproductive system (e.g. testicular, penile, or prostate cancer, uterine, vaginal, vulval, cervical, ovarian, and endometrial cancer), skin (e.g. melanoma, basal cell carcinoma, squamous cell cancer, actinic keratosis), liver (e.g. liver cancer, hepatic carcinoma, hepatocellular cancer, and hepatoma), bone (e.g., osteoclastoma, and osteolytic bone cancers) additional tissues and organs (e.g. pancreatic cancer, bladder cancer, kidney or renal cancer, thyroid cancer, breast cancer, cancer of the peritoneum, and Kaposi's sarcoma), and tumors of the vascular system (e.g. angiosarcoma and hemangiofibromatoma).

[000148] Other specific cancer Indications that may be treated include but are not limited to all non-Hodgkin's lymphomas (NHL), especially refractory/resistant NHL, chronic lymphocytic leukemia (CLL), B-cell acute lymphoblastic leukemia/lymphoma (B-ALL), mantle cell lymphoma (MCL), and multiple myeloma (MM).

[000149] Further based on VISTA's effect on B cell responsiveness, in particular its suppressive effect on B cell responsiveness mediated by MSDCs, VISTA antagonists may be used to treat or prevent infectious conditions where increased B cell responsiveness is therapeutically desired. Such conditions include in particular diseases caused by pathogens such as viruses, bacteria, fungi, protozoa, and parasites. Infectious diseases may be caused by viruses including adenovirus, cytomegalovirus, dengue, Epstein-Barr, hanta, hepatitis A, hepatitis B, hepatitis C, herpes simplex type I, herpes simplex type II, human immunodeficiency virus (HIV), human papilloma virus (HPV), influenza, measles, mumps, papova virus, polio, respiratory syncytial virus (RSV), rinderpest, rhinovirus, rotavirus, rubella, SARS virus, smallpox, viral meningitis, and the like. Infectious diseases may also be caused by bacteria including *Bacillus anthracis*, *Borrelia burgdorferi*, *Campylobacter jejuni*, *Chlamydia trachomatis*, *Clostridium botulinum*, *Clostridium tetani*, *Diphtheria*, *E. coli*, *Legionella*, *Helicobacter pylori*, *Mycobacterium rickettsia*, *Mycoplasma neisseria*, *Pertussis*, *Pseudomonas aeruginosa*, *S. pneumonia*, *Streptococcus*, *Staphylococcus*, *Vibrio cholerae*, *Yersinia pestis*, and the like. Infectious diseases may also be caused by fungi such as

Aspergillus fumigatus, Blastomyces dermatitidis, Candida albicans, Coccidioides immitis, Cryptococcus neoformans, Histoplasma capsulatum, Penicillium marneffei, and the like. Infectious diseases may also be caused by protozoa and parasites such as chlamydia, kokzidioia, leishmania, malaria, rickettsia, trypanosoma, and the like.

[000150] Further based on VISTA's effect on B cell responsiveness, in particular its suppressive effect on B cell responsiveness mediated by MSDCs, VISTA antagonists may be used to "boost" the efficacy of therapeutic vaccines. In this application of the invention a subject will be actively or passively immunized against a particular antigen by the administration of one or more antigens or one or more antibodies, typically human or humanized specific thereto in combination with at least one VISTA antagonist, e.g., an anti-VISTA antibody or an antagonistic VISTA polypeptide or conjugate comprising such peptide or a nucleic acid which antagonizes VISTA activity or expression such as an antisense nucleic acid .

[000151] The VISTA antagonist may be administered prior, concurrent or after administration of the vaccine containing a desired antigen or antibody. Such antigen or antibody and the VISTA antagonist may be in the same or different formulation. Most typically they are comprised in different formulations. The VISTA antagonist is administered in an amount and under dosing conditions such that it promotes B cell immune responses to be elicited against the desired antigen or by the therapeutic antibody. This antigen may comprise a tumor antigen, an infectious agent antigen, e.g., a bacterial, viral, yeast, fungal, or parasite antigen or may comprise an allergen or an autoantigen. Similarly, the administered antibody may be specific to a tumor antigen, an infectious agent antigen, e.g., a bacterial, viral, yeast, fungal, or parasite antigen, allergen or an autoantigen.

[000152] Also based on VISTA's effect on B cell responsiveness, in particular its suppressive effect on B cell responsiveness mediated by MSDCs, VISTA agonists may be used to treat or prevent conditions where decreased B cell responsiveness is therapeutically desired. Such conditions include in particular autoimmune conditions wherein B cell responses are involved in disease pathology, inflammatory conditions wherein B cell responses are involved in disease pathology, and allergic conditions wherein B cell responses are involved in disease pathology.

[000153] Examples of such autoimmune conditions treatable with VISTA agonists include the following: allogenic islet graft rejection, alopecia areata, ankylosing spondylitis, antiphospholipid syndrome, autoimmune Addison's disease, antineutrophil cytoplasmic autoantibodies (ANCA), autoimmune diseases of the adrenal gland, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune myocarditis, autoimmune neutropenia, autoimmune oophoritis and orchitis, autoimmune thrombocytopenia, autoimmune urticaria, Behçet's disease, bullous pemphigoid, cardiomyopathy, Castleman's syndrome, celiac sprue-dermatitis, chronic fatigue immune dysfunction syndrome, chronic inflammatory demyelinating polyneuropathy, Churg-Strauss syndrome, cicatricial pemphigoid, CREST syndrome, cold agglutinin disease, Crohn's disease, dermatomyositis, discoid lupus, essential mixed cryoglobulinemia, factor VIII deficiency, fibromyalgia-fibromyositis, glomerulonephritis, Grave's disease, Guillain-Barre, Goodpasture's syndrome, graft-versus-host disease (GVHD), Hashimoto's thyroiditis, hemophilia A, idiopathic pulmonary fibrosis, idiopathic thrombocytopenia purpura (ITP), IgA neuropathy, IgM polyneuropathies, immune mediated thrombocytopenia, juvenile arthritis, Kawasaki's disease, lichen planus, lupus erythematosus, Meniere's disease, mixed connective tissue disease, multiple sclerosis, type 1 diabetes mellitus, myasthenia gravis, pemphigus vulgaris, pernicious anemia, polyarteritis nodosa, polychondritis, polyglandular syndromes, polymyalgia rheumatica, polymyositis and dermatomyositis, primary agammaglobulinemia, primary biliary cirrhosis, psoriasis, psoriatic arthritis, Reynaud's phenomenon, Reiter's syndrome, rheumatoid arthritis, sarcoidosis, scleroderma, Sjögren's syndrome, solid organ transplant rejection, stiff-man syndrome, systemic lupus erythematosus, Takayasu arteritis, temporal arteritis/giant cell arteritis, thrombotic thrombocytopenia purpura, ulcerative colitis, uveitis, vasculitides such as dermatitis herpetiformis vasculitis, vitiligo, and Wegner's granulomatosis.

[000154] Examples of such autoimmune conditions treatable with VISTA agonists include the following: acute respiratory distress syndrome (ARDS), acute septic arthritis, adjuvant arthritis, juvenile idiopathic arthritis, allergic encephalomyelitis, allergic rhinitis, allergic vasculitis, allergy, asthma, atherosclerosis, chronic inflammation due to chronic bacterial or viral infections, chronic obstructive pulmonary disease (COPD), coronary artery disease, encephalitis, inflammatory bowel disease, inflammatory osteolysis, inflammation associated with acute and delayed hypersensitivity reactions, inflammation associated with tumors,

peripheral nerve injury or demyelinating diseases, inflammation associated with tissue trauma such as burns and ischemia, inflammation due to meningitis, multiple organ injury syndrome, pulmonary fibrosis, sepsis and septic shock, Stevens-Johnson syndrome, undifferentiated arthropy, and undifferentiated spondyloarthropathy.

[000155] Examples of such allergic conditions treatable with VISTA agonists include by way of example Preferred allergic conditions treatable using a VISTA agonist include allergic contact dermatitis, alopecia universalis, asthma, anaphylactoid purpura asthma, atopic dermatitis, dermatitis herpetiformis, erythema elevatum diutinum, erythema marginatum, erythema multiforme; erythema nodosum, allergic granulomatosis, granuloma annulare, granulocytopenia, hypersensitivity pneumonitis, keratitis, nephrotic syndrome, overlap syndrome, pigeon breeder's disease, idiopathic polyneuritis, urticaria, uveitis, juvenile dermatomyositis, and vitiligo.

[000156] Other examples of conditions where decreased B cell responsiveness may be desired include allergic bronchopulmonary aspergillosis; Autoimmune hemolytic anemia; Acanthosis nigricans; Allergic contact dermatitis; Addison's disease; Atopic dermatitis; Alopecia areata; Alopecia universalis; Amyloidosis; Anaphylactoid purpura; Anaphylactoid reaction; Aplastic anemia; Angioedema, hereditary; Angioedema, idiopathic; Ankylosing spondylitis; Arteritis, cranial; Arteritis, giant cell; Arteritis, Takayasu's; Arteritis, temporal; Asthma; Ataxia-telangiectasia; Autoimmune oophoritis; Autoimmune orchitis; Autoimmune polyendocrine failure; Behçet's disease; Berger's disease; Buerger's disease; Bullous pemphigus; Candidiasis, chronic mucocutaneous; Caplan's syndrome; Post-myocardial infarction syndrome; Post-pericardiotomy syndrome; Carditis; Celiac sprue; Chagas' disease; Chediak-Higashi syndrome; Churg-Strauss disease; Cogan's syndrome; Cold agglutinin disease; CREST syndrome; Crohn's disease; Cryoglobulinemia; Cryptogenic fibrosing alveolitis; Dermatitis herpetiformis; Dermatomyositis; Diabetes mellitus; Diamond-Blackfan syndrome; DiGeorge syndrome; Discoid lupus erythematosus; Eosinophilic fasciitis; Episcleritis; Erythema elevatum diutinum; Erythema marginatum; Erythema multiforme; Erythema nodosum; Familial Mediterranean fever; Felty's syndrome; Fibrosis pulmonary; Glomerulonephritis, anaphylactoid; Glomerulonephritis, autoimmune; Glomerulonephritis, post-streptococcal; Glomerulonephritis, post-transplantation; Glomerulopathy, membranous; Goodpasture's syndrome; Graft-vs.-host disease;

Granulocytopenia, immune-mediated; Granuloma annulare; Granulomatosis, allergic; Granulomatous myositis; Grave's disease; Hashimoto's thyroiditis; Hemolytic disease of the newborn; Hemochromatosis, idiopathic; Henoch-Schönlein purpura; Hepatitis, chronic active and chronic progressive; Histiocytosis X; Hypereosinophilic syndrome; Idiopathic thrombocytopenic purpura; Job's syndrome; Juvenile dermatomyositis; Juvenile rheumatoid arthritis (Juvenile chronic arthritis); Kawasaki's disease; Keratitis; Keratoconjunctivitis sicca; Landry-Guillain-Barre-Strohl syndrome; Leprosy, lepromatous; Loeffler's syndrome; Lyell's syndrome; Lyme disease; Lymphomatoid granulomatosis; Mastocytosis, systemic; Mixed connective tissue disease; Mononeuritis multiplex; Muckle-Wells syndrome; Mucocutaneous lymph node syndrome; Mucocutaneous lymph node syndrome; Multicentric reticulohistiocytosis; Multiple sclerosis; Myasthenia gravis; Mycosis fungoides; Necrotizing vasculitis, systemic; Nephrotic syndrome; Overlap syndrome; Panniculitis; Paroxysmal cold hemoglobinuria; Paroxysmal nocturnal hemoglobinuria; Pemphigoid; Pemphigus; Pemphigus erythematosus; Pemphigus foliaceus; Pemphigus vulgaris; Pigeon breeder's disease; Pneumonitis, hypersensitivity; Polyarteritis nodosa; Polymyalgia rheumatica; Polymyositis; Polyneuritis, idiopathic; Portuguese familial polyneuropathies; Pre-eclampsia/eclampsia; Primary biliary cirrhosis; Progressive systemic sclerosis (Scleroderma); Psoriasis; Psoriatic arthritis; Pulmonary alveolar proteinosis; Pulmonary fibrosis, Raynaud's phenomenon/syndrome; Reidel's thyroiditis; Reiter's syndrome, Relapsing polychondritis; Rheumatic fever; Rheumatoid arthritis; Sarcoidosis; Scleritis; Sclerosing cholangitis; Serum sickness; Sézary syndrome; Sjögren's syndrome; Stevens-Johnson syndrome; Still's disease; Subacute sclerosing panencephalitis; Sympathetic ophthalmia; Systemic lupus erythematosus; Transplant rejection; Ulcerative colitis; Undifferentiated connective tissue disease; Urticaria, chronic; Urticaria, cold; Uveitis; Vitiligo; Weber-Christian disease; Wegener's granulomatosis; Wiskott-Aldrich syndrome. Other B cell related conditions treatable with VISTA agonists or antagonists include B-cell related conditions include disorders characterized by having too many B cells, too few B cells, overactive B cells, inactive B cells (or B cells with substantially reduced activity), or dysfunctional B cells. Exemplary human diseases known to involve B-cells include, but are not limited to, autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, Sjögren's syndrome, ANCA-associated vasculitis, anti-phospholipid syndrome, idiopathic

thrombocytopenia purpura (ITP), autoimmune haemolytic anaemia, Guillain-Barré syndrome, chronic immune polyneuropathy, autoimmune thyroiditis, type I (autoimmune) diabetes, membranous glomerulonephropathy, Goodpasture's disease, autoimmune gastritis, pernicious anaemia, pemphigus vulgaris, primary biliary cirrhosis, dermatomyositis-polymyositis, myasthenia gravis, systemic sclerosis (scleroderma), multiple sclerosis, interstitial lung disease, chronic fatigue syndrome, autoimmune thyroiditis due to pregnancy antineutrophil cytoplasmic antibodies (ANCA) and celiac disease); inflammatory diseases (e.g., immunoglobulin A neuropathy, Henoch-Schönlein purpura, chronic graft rejection, atopic dermatitis, Hashimoto's thyroiditis, Grave's disease, atrophic thyroiditis, Riedel's thyroiditis, asthma, lyme neuroborreliosis, ulcerative colitis, Crohn's disease, and allergy); and cancer (e.g., lymphoma, non-Hodgkin's lymphoma (NHL), aggressive NHL, relapsed aggressive NHL, relapsed indolent NHL, refractory NHL, refractory indolent NHL, chronic lymphocytic leukemia (CLL) including immunoglobulin-mutated CLL and immunoglobulin-unmutated CLL, small lymphocytic lymphoma, leukemia, hairy cell leukemia (HCL), acute lymphocytic leukemia (ALL), mantle cell lymphoma, intermediate grade/follicular NHL, intermediate grade diffuse NHL, high grade immunoblastic NHL, high grade lymphoblastic NHL, high grade small non-cleaved cell NH, bulky disease NHL, Burkitt's lymphoma, multiple myeloma, pre-B acute lymphoblastic leukemia and other malignancies that derive from early B cell precursors, hairy cell leukemia, null-acute lymphoblastic leukemia, Waldenström's macroglobulinemia, diffuse large B cell lymphoma (DLBCL) including germinal center B cell-like (GCB) DLBCL, activated B cell-like (ABC) DLBCL, and type 3 DLBCL, pro-lymphocytic leukemia, light chain disease, plasmacytoma, osteosclerotic myeloma, plasma cell leukemia, monoclonal gammopathy of undetermined significance (MGUS), smoldering multiple myeloma (SMM), indolent multiple myeloma (IMM), Hodgkin's lymphoma including classical and nodular lymphocyte pre-dominant type, lymphoplasmacytic lymphoma (LPL), and marginal-zone lymphoma including gastric mucosal-associated lymphoid tissue (MALT) lymphoma).

[000157] Scleroderma encompasses a heterogeneous group of diseases including but not limited to, limited cutaneous disease, diffuse cutaneous disease, sine scleroderma, undifferentiated connective tissue disease, overlap syndromes, localized scleroderma, morphea, linear scleroderma, en coup de sabre, scleredema adiutorum of Buschke,

scleromyxedema, chronic graft-vs.-host disease (GVHD), eosinophilic fasciitis, digital sclerosis in diabetes, and primary amyloidosis and amyloidosis associated with multiple myeloma. (Reviewed in: Harrison's Principles of Internal Medicine, 16.sup.th ed./editors, Dennis L. Kasper, et al. The McGraw-Hill Companies, Inc. 2005 New York, N.Y.).

[000158] SEQUENCES

MURINE VISTA SEQ ID NO:1

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gshlkanash dqpqkhglel
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gsmelqvqag kgsgstcmas
181 neqdsdsita aalatgaciv gilclplill lvykqrqvas
hrraqelvrm dsntqgieng
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tplsppgpgd vffpsldpvp
301 dspnseai

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MURINE VISTA SEQ ID NO:2

```

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gshlkanash dqpqkhglel
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MURINE VISTA SEQ ID NO:3

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 hrraqelvrm dssntqggen
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 301 pdspnseai

HUMAN VISTA SEQ ID NO:4

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 hgghqaants hdlaqrhgle
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 hgamelqvqt gkdapsncvv
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 epstplsppg pgdvvffpsld
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CLAIMS

1. Use of an antagonistic anti-VISTA antibody and an iNOS/NO inhibitor for alleviating VISTA-mediated suppression of B cell immunity by myeloid-derived suppressor cells (MDSCs) in a subject with a cancer or infectious disease condition characterized by VISTA-expressing MDSCs.
2. Use of an antagonistic anti-VISTA antibody and an iNOS/NO inhibitor in the manufacture of a medicament or medicaments for alleviating VISTA-mediated suppression of B cell immunity by myeloid-derived suppressor cells (MDSCs) in a subject with a cancer or infectious disease condition characterized by VISTA-expressing MDSCs.
3. The use of claim 2, wherein the antagonistic anti-VISTA antibody and the iNOS/NO inhibitor are in the same medicament.
4. The use of claim 2, wherein the antagonistic anti-VISTA antibody and the iNOS/NO inhibitor are in separate medicaments.
5. The use of claim 1, wherein said antagonistic anti-VISTA antibody and said iNOS/NO inhibitor are for use in combination as separate medicaments.
6. The use of any one of claims 1 to 5, which is for treating cancer.
7. The use of any one of claims 1 to 5, which is for treating an infectious condition.
8. The use of claim 6, further comprising use of a tumor antigen.
9. The use of claim 7, further comprising use of an infectious agent antigen.

10. The use of any one of claims 1 to 9, wherein the iNOS/NO inhibitor comprises NG-monomethyl-L-arginine (L-NMMA).
11. An antagonistic anti-VISTA antibody and an iNOS/NO inhibitor for use in alleviating VISTA-mediated suppression of B cell immunity by myeloid-derived suppressor cells (MDSCs) in a subject with a cancer or infectious disease condition characterized by VISTA-expressing MDSCs.
12. The antagonistic anti-VISTA antibody and iNOS/NO inhibitor of claim 11, wherein said antagonistic anti-VISTA antibody and said iNOS/NO inhibitor are for use in combination as separate medicaments.
13. The antagonistic anti-VISTA antibody and iNOS/NO inhibitor of claim 11 or 12, which is for treating cancer.
14. The antagonistic anti-VISTA antibody and iNOS/NO inhibitor of claim 11 or 12, which is for treating an infectious condition.
15. The antagonistic anti-VISTA antibody and iNOS/NO inhibitor of claim 13, further comprising use of a tumor antigen.
16. The antagonistic anti-VISTA antibody and iNOS/NO inhibitor of claim 14, further comprising use of an infectious agent antigen.
17. The antagonistic anti-VISTA antibody and iNOS/NO inhibitor of any one of claims 11 to 16, wherein the iNOS/NO inhibitor comprises NG-monomethyl-L-arginine (L-NMMA).

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Figure 1A

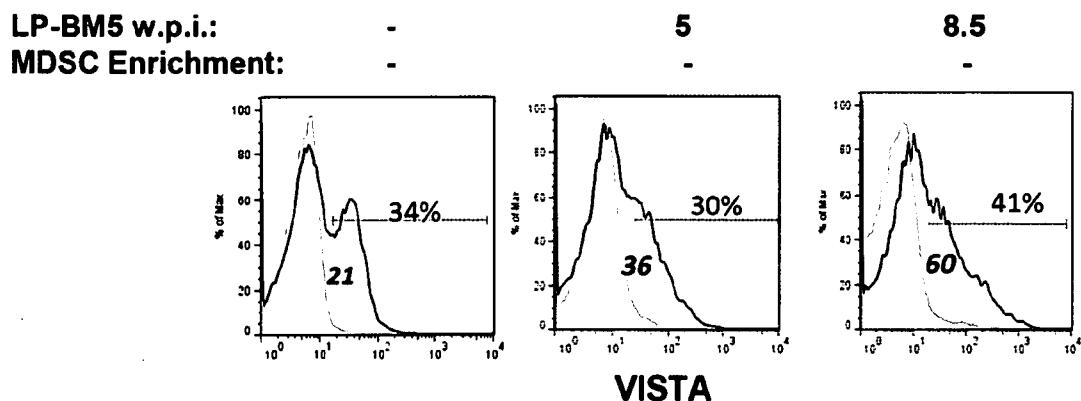
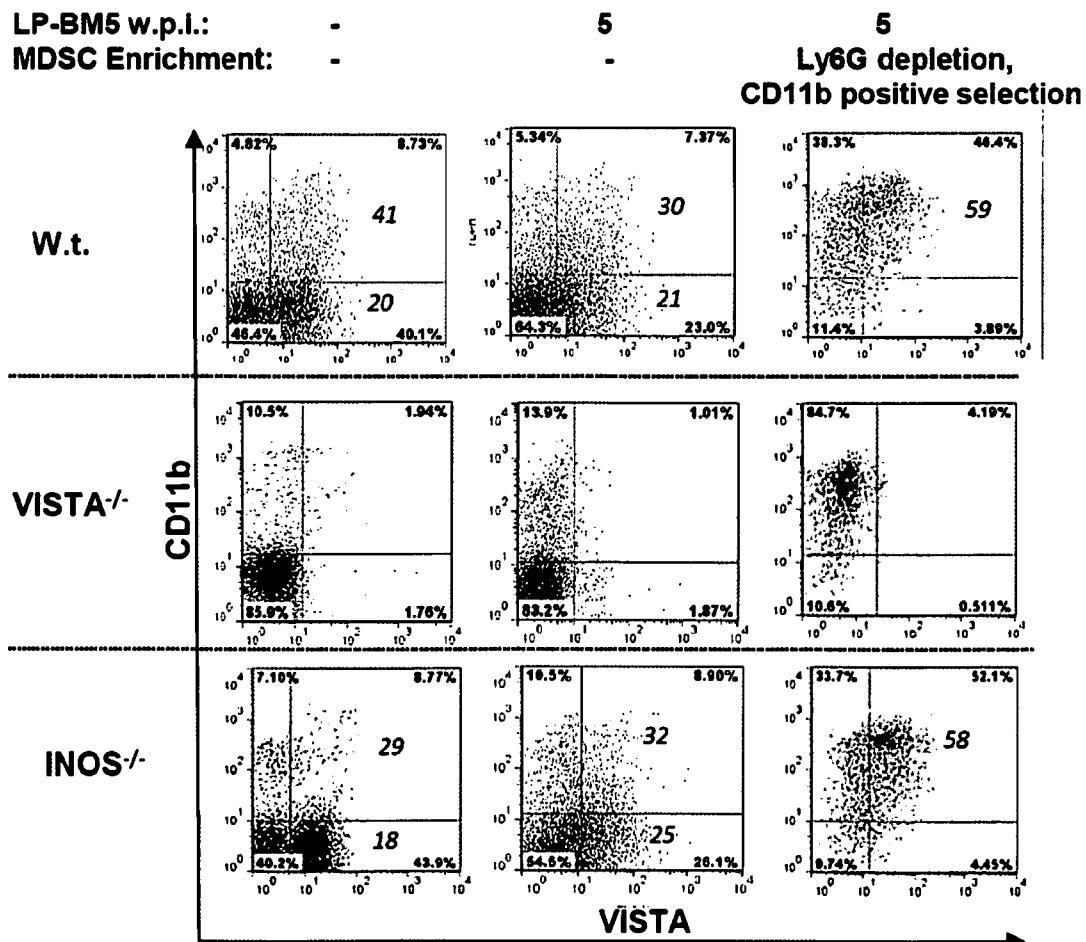
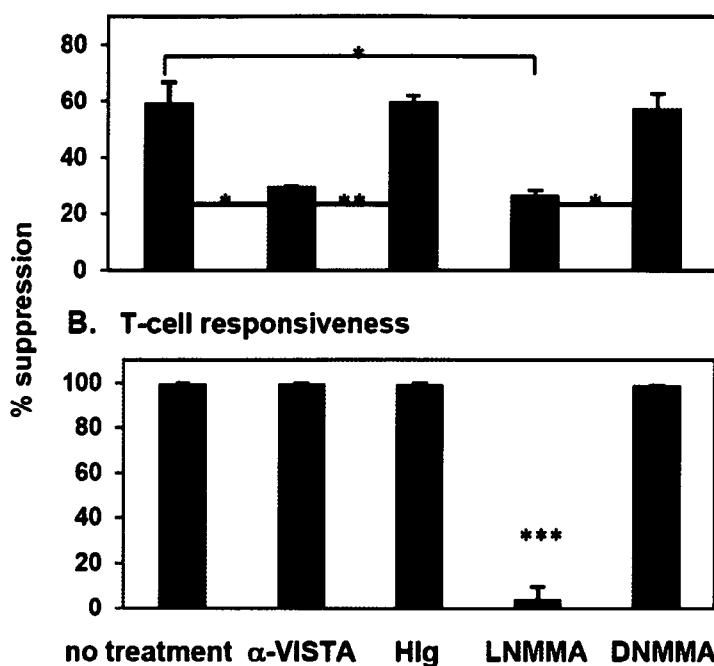
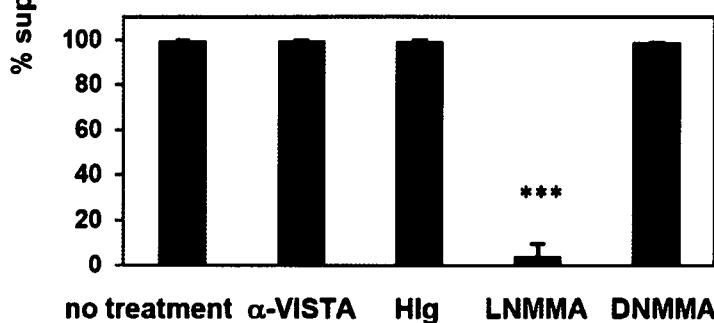
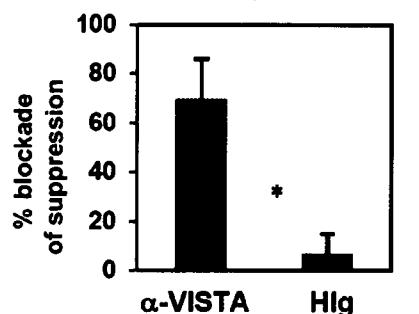


Figure 1B



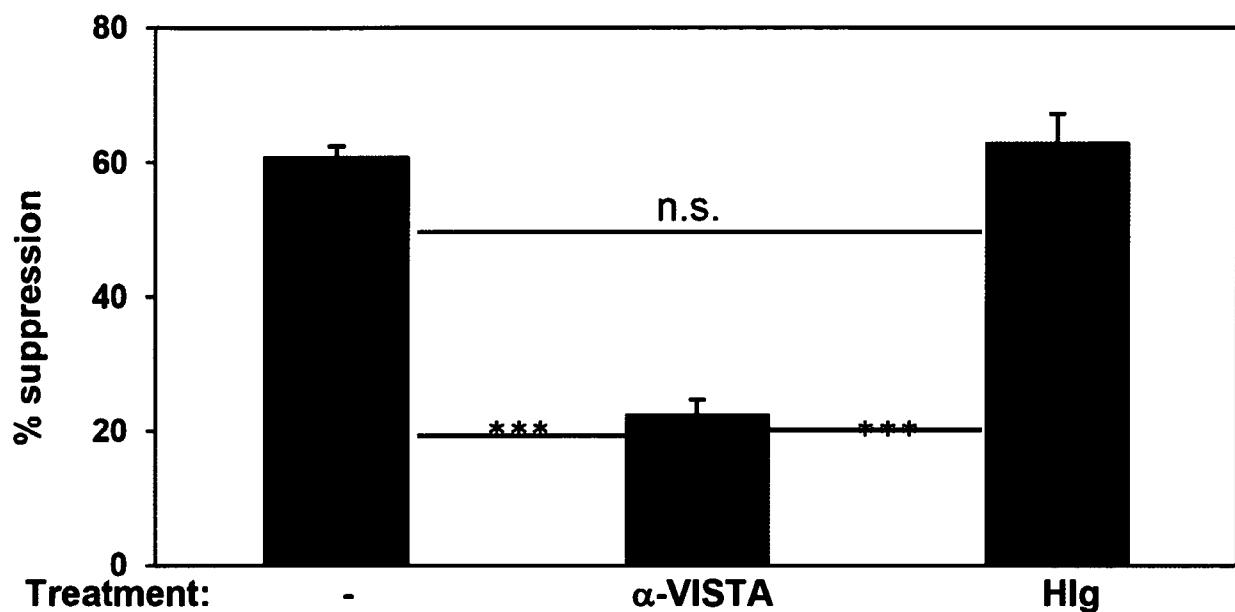
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Figure 2**A. B-cell responsiveness****B. T-cell responsiveness****C. B-cell responsiveness**

3/5

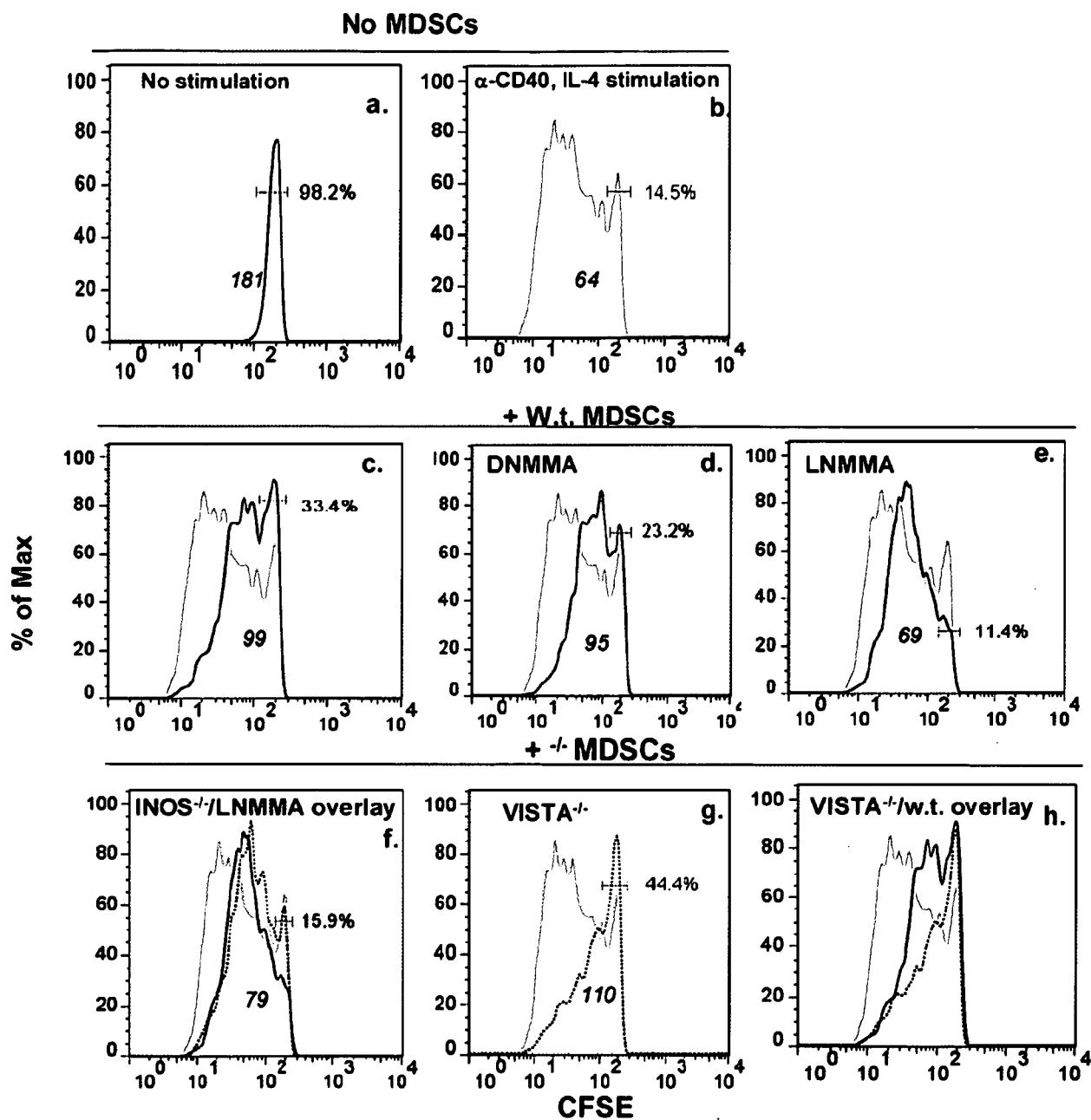
Figure 3

B-cell stimulation
VISTA k.o. responder cells



4/5

Figure 4



5/5

Figure 5A
B-cell responsiveness

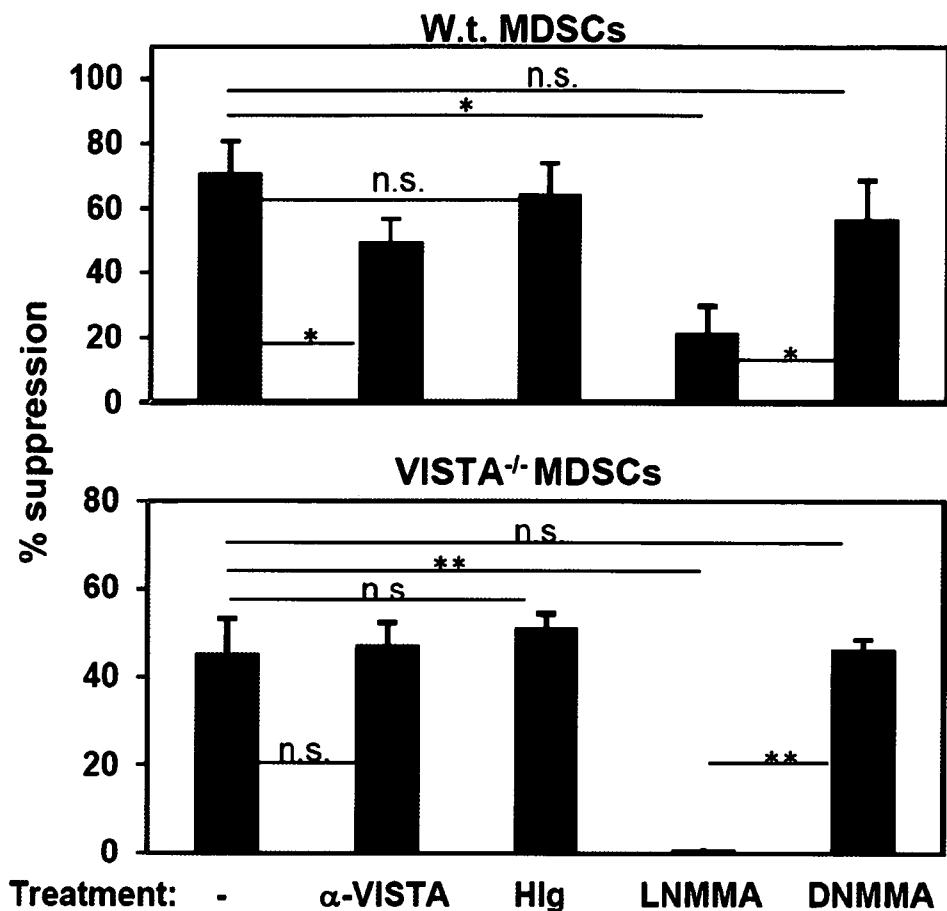
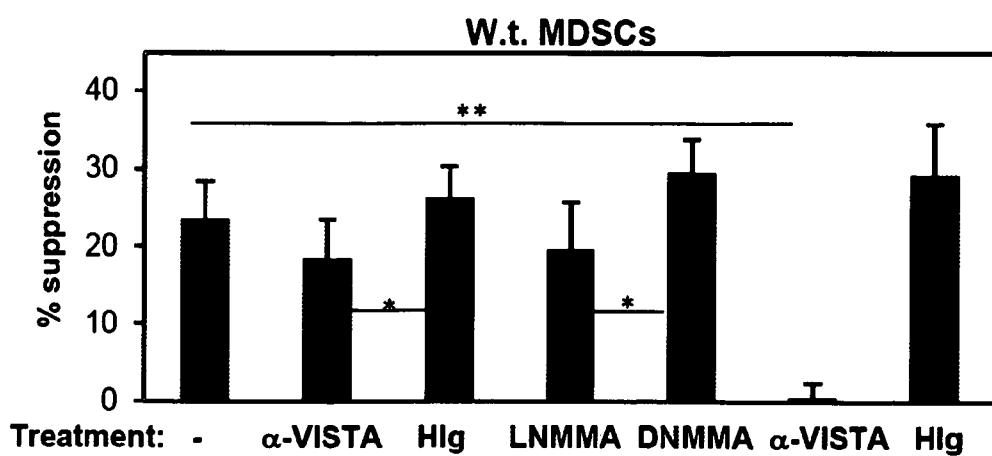
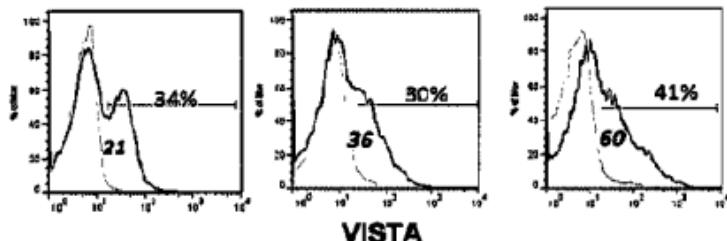


Figure 5B
B-cell responsiveness



A

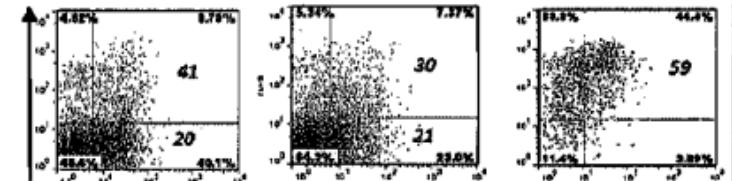
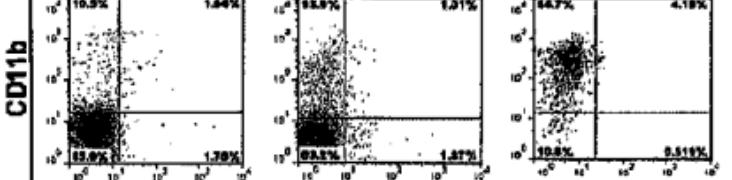
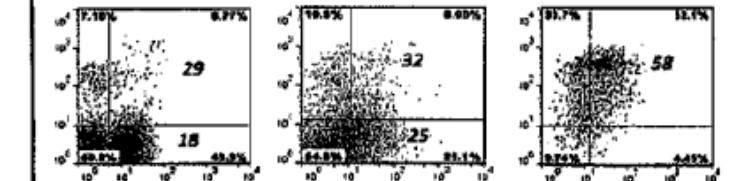
LP-BM5 w.p.i.: -
MDSC Enrichment: -

**B**

LP-BM5 w.p.i.: -
MDSC Enrichment: -

5 6
Ly6G depletion,
CD11b positive selection

W.t.

VISTA^{-/-}INOS^{-/-}

VISTA