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SOLICITUD

PATENTE DE INVENCION

EXPEDIENTE No.

05-116 756

TÍTULO Uso de B 7-113 Como un agente
Immuno regulador

CLASIFICACIÓN INTERNACIONAL A61K38/00 ; A61P37/00.

SOLICITANTE WYETH

DOMICILIO Madison, Estados Unidos

APODERADO Dilia Rodriguez D'Almeida

BOGOTÁ, D. C., Nov-17/05

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☒ Patente de invención

☐ Patente de Modelo de Utilidad

☒ Capítulo I.

☐ Capítulo II.

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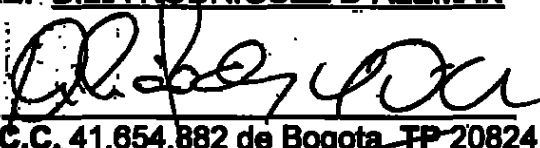
ANEX S

- ☒ Comprobante de pago de la tasa de presentación de la solicitud
- ☐ Comprobante de pago por reivindicación de prioridad
- ☐ Documento que acredita la existencia y representación legal cuando el solicitante sea persona Jurídica
- ☐ Poderes, si fuera el caso
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- ☒ Copia de la solicitud Internacional. (Resumen, descripción, reivindicación, Dibujos).
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(54) Title: USE OF B7-1/3 AS AN IMMUNOREGULATORY AGENT

(57) Abstract: The present disclosure relates to the fields of immunology and clinical immunology, and more particularly to the use
of B7-family ligands and agonists and antagonists thereof in modulation of immune response. The invention provides methods for
modulation of lymphocyte activation involving the use of B7-1/3, including B7-1/3 VC and B7-1/3 VCVC, and related molecules
such as, for example, antibodies and nucleic acids.

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USE OF B7-H3 AS AN IMMUNOREGULATORY AGENT

DESCRIPTION OF THE INVENTION

Field of the Invention

[0001] The present disclosure relates to the fields of immunology and clinical immunology, and more particularly to the use of B7-family ligands and agonists and antagonists thereof in modulation of immune responses.

Background of the Invention

[0002] Lymphocyte activation is a multi-step process requiring several signaling events between the lymphocyte and an "accessory" cell—an antigen presenting cell (APC) in the case of T cell activation, or a helper T cell for B cell activation). For lymphocyte activation to occur, two types of signals must be delivered to a resting lymphocyte. The primary type (stimulatory) confers specificity to the immune response and is mediated by the antigen-specific receptor (TcR—on T cells, and BcR—on cells) upon recognition of the antigenic peptide-MHC complex. The second type (costimulatory) is responsible for the magnitude of the response. This signal is mediated via "accessory" receptors expressed on the surface of the lymphocyte. The requirement for costimulation allows lymphocyte activation to be strictly regulated. Costimulation is in turn regulated by the inhibitory receptors on these cells that can deliver negative signals which counteract the positive costimulatory signals.

[0003] Mounting evidence suggests that a large number of structurally related ligands and receptors belonging to the immunoglobulin (Ig) superfamily of molecules interact and balance signals used during the process of lymphocyte activation (Frauwirth et al. (2002) J. Clin. Invest., 109:295-299). One such structurally related group includes ligands of the B7 family (B7-1, B7-2, ICOS-L, PD-L1, PD-L2, and B7-H3), which share similar domain structure having one Ig-V-like ("V") domain and one Ig-C-like ("C") domain which reside in the

extracellular portion of the molecule (Sharpe et al. (2002) *Nature Rev. Immunol.*, 2:116-126). In exon deletion and crystallographic studies of B7 proteins, it has been demonstrated that receptor-ligand interaction occurs via the V domains (Ostrov et al. (2000) *Science*, 290:816-819), while the C domains serve as structural support means for the V domains.

[0004] B7-1 and B7-2 can deliver either positive signals (through their low affinity cognate receptor CD28) or negative signals (through the high affinity receptor CTLA4). ICOS-L interaction with ICOS receptor results in positive signaling whereas PD-L1 delivers negative signals through its receptor, PD-1 (Carreno et al. (2002) *Annual Rev. Immunol.*, 20:29-53).

[0005] The present disclosure relates to the modulation of immune responses regulated by the newest member of the B7 family of ligands, B7-H3. Human B7-H3 was originally identified as a B7-like protein which shares 20-27% amino acid identity with other B7 family members and has one V and one C domain (Chapoval et al. (2001) *Nat. Immunol.*, 2:269-274). However, a more detailed analysis of partial B7-H3 EST clones demonstrated a variation in the gene exon structure in mammalian species (Sun et al. (2002) *J. Immunol.*, 168:6294-6297). In particular, in primates, B7-H3 cDNA exists in two forms: one encodes a single set single set of V and C domains ("VC form"), and the other encodes a duplicated set of V and C domains ("VCVC form"). In contrast to the primate B3-H3, rodent B7-H3 cDNA exists only in a single form as VC.

[0006] B7-H3 VC has been initially characterized as a costimulatory ligand in both human (Chapoval et al. (2001) *Nat. Immunol.*, 2:269-274 and U.S. Patent Application Pub. No. 2002/0168762) and mouse (Sun et al. (2002) *J. Immunol.*, 168:6294-6297). In particular, it has been reported that costimulation of human T cells with B7-H3 VC results in enhanced T cell proliferation, induction of cytotoxic T cells, and increased gamma interferon transcript expression. Additionally, binding experiments in cell-based assays suggest that B7-H3 VC binds to a receptor expressed on activated T cells, which is not CTLA-4, ICOS, or PD-1.

[0007] In general, a need exists to provide therapeutic methods for immune system-related disorders and conditions. Appropriate modulation of immune responses can be accomplished by manipulation of the B7-H3 pathway.

SUMMARY OF THE INVENTION

[0008] It is one of the objects of the present invention to provide methods and compositions for modulation of immune responses. Additional objects of the invention will be set forth in part in the following description and in part will be understood from the description or may be learned by practice of the invention.

[0009] The present invention is based, in part, on the discovery and demonstration that the VCVC form of B7-H3 accounts for the majority of B7-H3 transcripts seen across multiple tissues while the VC form of B7-H3 is only a minor transcript. The invention is further based, in part, on the discovery and demonstration that both forms of B7-H3, VC and VCVC, exhibit an inhibitory effect on lymphocyte activation as evidenced by decreased proliferation of T cells and cytokine secretion by these cells in the presence of B7-H3. The invention is yet further based, in part, on the discovery of specific regions within the B7-H3 genes that are currently undergoing purifying evolutionary selection.

[0010] In one aspect, the present disclosure provides *in vitro*, *in vivo*, and *ex vivo* methods of modulating immune responses, including methods of treating humans or animals. In some embodiments, such methods comprise a step of contacting a lymphocyte, such as a T cell, with a B7-H3 agent, wherein the B7-H3 agent may be (a) a derivative of B7-H3 such as a soluble form of B7-H3; (b) an antibody against B7-H3; (c) an antibody against a B7-H3 receptor; or (d) a nucleic acid comprising at least a portion of the B7-H3 mRNA or a complement thereto. In certain embodiments, the B7-H3 agent is coupled with the primary (stimulatory, antigen-specific) signal.

[0011] In particular embodiments, the methods of the invention are used to treat or prevent immune disorders susceptible to treatment with such compositions. Specifically, such disorders include but are not limited to immunologic disorders, including autoimmune disorders (e.g., rheumatoid arthritis (RA), psoriasis, multiple sclerosis (MS), inflammatory bowel disease (IBD), Crohn's disease, systemic lupus erythematosus (SLE), type 1 diabetes), transplant rejection, graft-versus-host disease (GVHD), hyperproliferative immune disorders, cancers, immunosuppressive disorders, various infectious diseases, etc. Thus, in certain embodiments, the methods of the invention comprise identifying a subject in need

of inhibiting lymphocyte activation, and administering a B7-H3 agent that is an agonist to the subject. In other embodiments, the methods comprise identifying a subject in need of enhancing lymphocyte activation, and administering a B7-H3 agent that is an antagonist to the subject.

[0012] Antibodies used in the methods of the invention fall into two groups: (1) antibodies against B7-H3 and (2) antibodies against a B7-H3 receptor. These antibodies may: (a) specifically bind to B7-H3 thereby blocking the interaction of B7-H3 with its receptor; (b) specifically bind to a B7-H3 receptor thereby blocking its interaction with B7-H3; or (c) perform both (a) and (b). Depending on the desired effect, the antibodies may be used in alternative configurations to either enhance or inhibit immune responses. In some embodiments, antibodies are administered to antagonize the biological activity of naturally expressed B7-H3.

[0013] The disclosure further provides methods that involve compositions comprising soluble forms of B7-H3. In some embodiments, a soluble form of B7-H3 comprises less than full length B7-H3 and does not include the transmembrane and the intracellular domains of B7-H3. In further embodiments, a soluble form of B7-H3 comprises at least one V domain of B7-H3, and optionally at least one C domain of B7-H3. A soluble form may comprise at least 2, 3, 4, or 5 V domains, and optionally at least 1, 2, 3, 4, or 5 C domains. In yet further embodiments, a soluble form of B7-H3 may comprise: (a) a first amino acid sequence derived from the extracellular domain of B7-H3 and (b) a second amino acid sequence derived from the constant region of an antibody. The first amino acid sequence is derived from all or a portion of the B7-H3 extracellular domain and (a) competitively inhibits binding of a naturally occurring form of B7-H3 to its receptor and/or (b) has a negative costimulatory activity. In some embodiments, the first amino acid sequence comprises a sequence as set out in SEQ ID NO:15. In certain embodiments, the first amino acid sequence is identical to or is substantially identical to amino acids 23-244 of SEQ ID NO:14, or amino acids 23-462 of SEQ ID NO:12. In an illustrative embodiment, the soluble form of B7-H3 comprises a sequence as in SEQ ID NO:12 or SEQ ID NO:14.

[0014] The disclosure also provides methods involving therapeutic and nontherapeutic uses of nucleic acids or polypeptides encoded by such nucleic acids, where the nucleotide sequence of such nucleic acid is selected from: (a) a

nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, or a portion thereof; and (b) a nucleic acid that is at least 60, 80, 100, 120, or 140 nucleotides long and hybridizes to the nucleic acid of (a) under defined conditions, wherein the nucleic acid encodes an expression product having a negative costimulatory activity. In certain embodiments, such a nucleic acid encodes an amino acid sequence as in SEQ ID NO:15. In an illustrative embodiment, the nucleic acid comprises a sequence substantially as in SEQ ID NO:11 or SEQ ID NO:13.

[0015] The methods of the invention also encompass the use of short interfering RNAs and antisense nucleic acids to reduce the expression of B7-H3 in order to enhance immune response.

[0016] The invention also encompasses vectors that contain any of the foregoing nucleic acids and host cells containing any such vector.

[0017] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed.

BRIEF DESCRIPTION OF THE FIGURES

[0018] FIG. 1A depicts human and mouse B7-H3 sequence comparisons. Sequence alignment of deduced translated human B7-H3 VC, human B7-H3 VCVC, and mouse B7-H3 gene products. Dark bars above sequence alignment denote exon domains demarcated by genomic sequences. Arrows below peptide sequence denote corresponding nucleic acid positions used in oligonucleotide primers used for cross-species amplification.

[0019] FIG. 1B depicts genomic organization of human and mouse B7-H3 gene loci. Assemblies are based on selected portions of Celera human genomic axis GA_x2HTBL4SSTP and Celera mouse genomic axis GA_x5J8B7W7NM9. Bars denote relative locations of Alu and SVA complex repeats, simple repeats, transcript exon structure, and domain name.

[0020] FIG. 2A shows results of B7-H3 RT-PCR on human samples. Three separate panels of first strand human cDNA were amplified with PCR primers common to both B7-H3 VC and B7-H3 VCVC sequences and detected with

oligonucleotides to V₁ domain. Expected sizes of amplified products are as indicated.

[0021] FIG. 2B shows results of B7-H3 RT-PCR on mouse samples. A mouse panel consisting of adult and embryonic cDNA was analyzed for the presence of B7-H3 transcripts. Predominant specific hybridization of a ~1 kb band is consistent with the presence of the single VC form of mouse B7-H3.

[0022] FIG. 3A-3B demonstrate a costimulatory effect of B7 as measured by proliferation of activated T cells. B7-H3 activation of T cells results in attenuation of proliferation and cytokine production. FIG. 3A depicts results of proliferation assays for CHO.HLA-DR2 cells expressing GFP, B7-1, B7-2, whereas FIG. 3B shows results of proliferation assays for CHO.HLA-DR2 cells expressing GFP, B7-H3 VCVC, or B7-H3 VC. CHO.HLA-DR2 transfectants (1.25×10^4 cells/well) were incubated with CD4⁺ T cells (10^5 cells/well) in the presence of soluble anti-CD3 antibody (1 μ g/ml) and titrated concentrations of anti-CD28 antibody. Proliferation was measured at 72 hours. Responses for CD4⁺ T cells plus CHO.HLA-DR2 transfectants in the absence of anti-CD3 antibody were below 300 CPM.

[0023] FIG. 4 demonstrates inhibitory effect of B7-H3 on cytokine production by activated T cells. CHO.HLA-DR2 cells expressing GFP, B7-H3 VCVC, or B7-H3 VC (1.25×10^4 cells/well) were incubated with CD4⁺ T cells (10^5 cells/well) in the presence of soluble anti-CD3 antibody (1 μ g/ml) and titrated concentrations of anti-CD28 antibody (0.5 ng/ml). Supernatants were harvested from T cells cultures stimulated with either CHO.HLA-DR2 GFP, B7-H3 VCVC, or B7-H3 VC in the presence of anti-CD3 (1 μ g/ml) and anti-CD28 (0.5 ng/ml) antibodies. Cytokine production was measured at 72 hours using multiplex ELISA screening.

[0024] FIG. 5 demonstrates that B7-H3 VC and B7-H3 VCVC deliver a negative signal to human CD4⁺ T cells as measured by inhibition of cell proliferation. Purified CD4⁺ cells (10^5 cells/well) were activated with anti-CD3 antibody (1 μ g/ 10^7 microspheres) and B7-H3-Ig (VCVC or VC; 4 μ g/ 10^7 microspheres) on CIS or TRANS microspheres. CIS microspheres coated with both anti-CD3 antibody (1 μ g/ 10^7 microspheres) and B7-H3-Ig (VCVC or VC) at 4 μ g/ 10^7 microspheres. TRANS microspheres consisted of a mix of two types of

microspheres: (a) microspheres coated with anti-CD3 antibody ($1 \mu\text{g}/10^7$ microspheres) and (b) microspheres coated with B7-H3 (VCVC or VC). To maintain equal microsphere-to-cell ratio, microspheres coated control murine Ig were added to achieve a total protein concentration of $5 \mu\text{g}/10^7$ beads. Proliferation was measured at 72 hours.

[0025] FIGS. 6A-6C demonstrate that B7-H3 VC and B7-H3 VCVC deliver a negative signal to human CD4^+ T cells as measured by inhibition of cytokine secretion. Purified CD4^+ cells (10^5 cells/well) were activated with anti-CD3 antibody ($1 \mu\text{g}/10^7$ microspheres) and B7-H3-Ig (VCVC or VC; $4 \mu\text{g}/10^7$ microspheres) on CIS or TRANS microspheres. CIS microspheres coated with both anti-CD3 ($1 \mu\text{g}/10^7$ microspheres) and B7-H3-Ig (VCVC or VC) at $4 \mu\text{g}/10^7$ microspheres. TRANS microspheres consisted of a mix of two types of microspheres: (a) microspheres coated with anti-CD3 antibody ($1 \mu\text{g}/10^7$ microspheres) and (b) microspheres coated with B7-H3 (VCVC or VC). To maintain equal microsphere-to-cell ratio, microspheres coated control murine Ig were added to achieve a total protein concentration of $5 \mu\text{g}/10^7$ beads. The amount of cytokines in the supernatants was measured at 72 hours using multiplex ELISA screening: $\text{TNF-}\alpha$ (FIG. 6A), $\text{IFN-}\gamma$ (FIG. 6B), and GM-CSF (FIG. 6C).

BRIEF DESCRIPTION OF THE SEQUENCES

[0026] SEQ ID NO:1 and SEQ ID NO:2 represent, respectively, nucleic acid and amino acid full-length sequences of human B7-H3 VC.

[0027] SEQ ID NO:3 and SEQ ID NO:4 represent, respectively, nucleic acid and amino acid full-length sequences of mouse B7-H3.

[0028] SEQ ID NO:5 and SEQ ID NO:6 represent, respectively, nucleic acid and amino acid full-length sequences of human B7-H3 VCVC.

[0029] SEQ ID NO:7 represents an amino acid sequence of human B7-H3 (amino acid 28-139 of B7-H3 VC or B7-H3 VCVC).

[0030] SEQ ID NO:8 represents amino acids conserved between the V1 and V2 regions of human B7-H3 VCVC, i.e., between amino acids 28-139 and 246-357 of SEQ ID NO:6.

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[0031] SEQ ID NO:9 and SEQ ID NO:10 represent respectively nucleic acid and amino acid sequences of a fusion polypeptide containing the oncostatin M signal sequence (amino acids 1-22 of SEQ ID NO:10), the extracellular domain of human B7-H3 VC (amino acids 23-244 of SEQ ID NO:10), and the constant region of mouse IgG_{2am} (amino acids 245-482 of SEQ ID NO:10).

[0032] SEQ ID NO:11 and SEQ ID NO:12 represent respectively nucleic acid and amino acid sequences of a fusion polypeptide containing the oncostatin M signal sequence (amino acids 1-22 of SEQ ID NO:12), the extracellular domain of human B7-H3 VCVC (amino acids 23-462 of SEQ ID NO:12), and the constant region of mouse IgG_{2am} (amino acids 463-700 of SEQ ID NO:12).

[0033] SEQ ID NO:13 and SEQ ID NO:14 represent, respectively, nucleic acid and amino acid sequences of a fusion polypeptide containing the oncostatin M signal sequence (amino acids 1-22 of SEQ ID NO:14), the extracellular domain of mouse B7-H3 VC (amino acids 23-244 of SEQ ID NO:14), and the constant region of mouse IgG_{2am} (amino acids 245-482 of SEQ ID NO:14).

[0034] SEQ ID NO:15 represents conserved amino acids in the Ig V-like domain(s) of mammalian B7-H3.

[0035] SEQ ID NOs:16-22 represent individual highly conserved regions in the Ig V-like domain(s) of mammalian B7-H3.

[0036] SEQ ID NOs:23-35 represent PCR primers employed for isolation of B7-H3 sequences as described in the Examples.

DETAILED DESCRIPTION OF THE INVENTION

1. Definitions

[0037] In order for the present invention to be more readily understood, certain terms are defined herein. Additional definitions are set forth throughout the detailed description.

[0038] The term "antibody," as used herein, refers to an immunoglobulin or a part thereof, and encompasses any polypeptide comprising an antigen-binding site regardless of the source, method of production, and other characteristics. The term includes but is not limited to polyclonal, monoclonal, monospecific, polyspecific, non-specific, humanized, single-chain, chimeric, synthetic,

recombinant, hybrid, mutated, and CDR-grafted antibodies. The term "antigen-binding domain" refers to the part of an antibody molecule that comprises the area specifically binding to or complementary to a part or all of an antigen. Where an antigen is large, an antibody may only bind to a particular part of the antigen. The "epitope," or "antigenic determinant" is a portion of an antigen molecule that is responsible for specific interactions with the antigen-binding domain of an antibody. An antigen-binding domain may be provided by one or more antibody variable domains (e.g., a so-called Fd antibody fragment consisting of a V_H domain). An antigen-binding domain comprises an antibody light chain variable region (V_L) and an antibody heavy chain variable region (V_H).

[0039] The term "anti-B7-H3 antibody," or "antibody against B7-H3," refers to any antibody that specifically binds to at least one epitope of at least one B7-H3 form, including but not limited to B7-H3 VC and B7-H3 VCVC. The terms "anti-B7-H3 receptor antibody" and "antibody against a B7-H3 receptor" refer to any antibody that specifically binds to at least one epitope of a receptor for B7-H3.

[0040] As used herein, the term "B7-H3," unless otherwise stated, refers to any and all forms of B7-H3, including but not limited to VC and VCVC. The term "B7-H3 agent" refers to any compound capable of modulating biological activity of B7-H3. The term "modulating" and its cognates refer to a reduction or an increase in biological activity of B7-H3, e.g., the activity associated with the effect exerted by naturally expressed B7-H3 on a lymphocyte expressing a B7-H3 receptor. A reduction or an increase in biological activity is preferably at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more. A B7-H3 agent that causes such a reduction is referred to as "antagonist," while a B7-H3 agent that causes such an increase is referred to as "agonist." It will be understood that an antagonist of B7-H3 would counteract the "negative costimulatory signal" exerted by naturally expressed B7-H3 on a lymphocyte expressing a B7-H3 receptor, whereas an agonist would enhance such a "negative costimulatory signal." Therefore, an antagonist of B7-H3 generally causes an increase in lymphocyte activation (e.g., as measured by cell proliferation and/or cytokine secretion), whereas an agonist of B7-H3 generally cause a decrease in the same.

[0041] The term "biological activity" refers to a function or set of functions (or the effect to which the function is attributed to) performed by a molecule in a

biological system, which may be *in vivo* or *in vitro*. Biological activity may be assessed by, for example, the effect on lymphocyte proliferation, survival, and function (e.g., cytokine secretion), cluster of differentiation marker expression, gene expression at the transcriptional, translational, or post-translational levels, or the effect on autoantibody production, etc.

[0042] The term **"costimulation"** and its cognates refer to signaling events between receptor/ligand pairs of cell surface molecules on the responder lymphocyte and an "accessory" cell (e.g., an antigen presenting cell (APC) in the case of T cell activation, or a helper T cell for B cell activation) to allow lymphocyte activation. The terms **"negative costimulation," "negative costimulatory signal," "inhibitory signal," "negative costimulatory activity,"** and their cognates refer to signaling events that inhibit lymphocyte activation relative to that in the absence of such signals. It will be understood that an activated T cell may be a helper cell (i.e., CD4⁺), a cytotoxic, or a suppressor cell (i.e., CD8⁺). Negative costimulatory activity can be measured using standard techniques and, without limitation, as described in the Examples. In particular, the presently disclosed B7-H3 agents inhibit lymphocyte activation, which can be measured by (a) cell proliferation and/or (b) cytokine secretion.

[0043] The term **"derivative," "derived from,"** and their cognates, when used in reference to an amino acid or a nucleotide sequence, refers to a sequence that is identical or substantially identical to all or a portion of a parent sequence and can be actually obtained from the parent sequence, for example, by way of amino acid or nucleotide substitution, deletion, or addition, or other modifications.

[0044] The term **"hybridization under defined conditions"** refers to conditions for hybridization and washes under which nucleotide sequences that are significantly identical or homologous to each other remain bound to each other. The conditions are such that sequences, which are at least 50, 100, 150, 300, or more nucleotides long and at least 70%, more preferably at least 80%, even more preferably at least 85-90% identical, remain bound to each other. The percent identity can be determined as described in Altschul et al. (1997) Nucleic Acids Res., 25:3389-3402. Nonlimiting examples of low, moderate, and high stringency hybridization conditions are provided in subsequent sections.

[0045] The term "immunologic disorder" refers to disorders and conditions in which an immune response is aberrant. The aberrant response can be due to (a) abnormal proliferation, maturation, survival, differentiation, or function of immune cells such as, for example, T or B cells. Such disorders include but are not limited to autoimmune disorders (e.g., rheumatoid arthritis (RA), psoriasis, multiple sclerosis (MS), inflammatory bowel disease (IBD), Crohn's disease, systemic lupus erythematosus (SLE), type I diabetes), transplant rejection, graft-versus-host disease (GVHD), hyperproliferative immune disorders, and immunosuppressive disorders. In particular, the disclosure provides methods that involve compositions comprising B7-H3 agents such as soluble forms of B7-H3 or antibodies against B7-H3 or against its receptor.

[0046] The term "isolated" refers to a molecule that is substantially free of its natural environment. For instance, an isolated protein is substantially free of cellular material or other proteins from the cell or tissue source from which it is derived. The term "isolated" also refers to preparations where the isolated protein is sufficiently pure to be administered as a pharmaceutical composition, or at least 70-80% (w/w) pure, more preferably, at least 80-90% (w/w) pure, even more preferably, 90-95% pure; and, most preferably, at least 95%, 96%, 97%, 98%, 99%, or 100% (w/w) pure. The term "isolated," as used herein, also refers to preparations that are substantially endotoxin-free, i.e., the endotoxin levels are below 500, 300, 200, 100, 50, 10, 5, 1, 0.5, 0.1, 0.05, 0.01 EU/ml, or below a detectable level.

[0047] The term "mammal" refers to any animal classified as such, including humans.

[0048] The term "primary stimulatory signal" refers to a stimulatory signal delivered to a lymphocyte that confers specificity to the immune response and is mediated by the antigen-specific receptor (TcR—on T cells, and BcR—on cells) upon recognition of the antigenic peptide-MHC complex.

[0049] The terms "treatment," "therapeutic method," and their cognates refer to both therapeutic treatment and prophylactic/preventative measures. Those in need of treatment may include individuals already having a particular medical disorder as well as those at risk for the disorder (i.e., those who are likely to ultimately acquire the disorder). A therapeutic method results in prevention or

amelioration of symptoms or an otherwise desired biological outcome and may be evaluated by improved clinical signs (e.g., PASI as described in the Examples), delayed onset of disease, reduced/elevated levels of lymphocytes and/or antibodies, etc.

[0050] The terms "therapeutic compound" and "therapeutic," as used herein, refer to any compound capable of ameliorating clinical manifestations of a disorder, or to produce a desired biological outcome.

[0051] The terms "therapeutically effective dose" and "therapeutically effective amount" refer to that amount of a compound that results in prevention or amelioration of symptoms in a patient or a desired biological outcome, e.g., improved clinical signs (e.g., PASI as described in the Examples), delayed onset of disease, reduced/elevated levels of lymphocytes and/or antibodies, etc. The effective amount can be determined as described in the subsequent sections.

[0052] The term "specifically binding" and its cognates mean that two molecules form a complex that is relatively stable under physiologic conditions. Specific binding is characterized by a high affinity and a low to moderate capacity. Nonspecific binding usually has a low affinity with a moderate to high capacity. Typically, the binding is considered specific when the affinity constant K_a is higher than 10^6 M^{-1} , or preferably higher than 10^8 M^{-1} . If necessary, nonspecific binding can be reduced without substantially affecting specific binding by varying the binding conditions. Such conditions are known in the art, and a skilled artisan using routine techniques can select appropriate conditions. The conditions are usually defined in terms of protein concentration, ionic strength of the solution, temperature, time allowed for binding, concentration of unrelated molecules (e.g., serum albumin, milk casein), etc.

[0053] The phrase "substantially identical" means that a relevant amino acid sequence is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to a given sequence. By way of example, such sequences may be variants derived from various species, or they may be derived from the given sequence by truncation, deletion, amino acid substitution or addition. Percent identity between two amino acid sequences is determined by standard alignment algorithms such as, for example, Basic Local Alignment Tool (BLAST) described in Altschul et al. (1990) J. Mol. Biol., 215:403-410, the algorithm of Needleman et al.

(1970) J. Mol. Biol., 48:444-453; the algorithm of Meyers et al. (1988) Comput. Appl. Biosci., 4:11-17; or Tatusova et al. (1999) FEMS Microbiol. Lett., 174:247-250, etc. Such algorithms are incorporated into the BLASTN, BLASTP and "BLAST 2 Sequences" programs (see www.ncbi.nlm.nih.gov/BLAST). When utilizing such programs, the default parameters can be used. For example, for nucleotide sequences the following settings can be used for "BLAST 2 Sequences": program BLASTN, reward for match 2, penalty for mismatch -2, open gap and extension gap penalties 5 and 2 respectively, gap x_dropoff 50, expect 10, word size 11, filter ON. For amino acid sequences the following settings can be used for "BLAST 2 Sequences": program BLASTP, matrix BLOSUM62, open gap and extension gap penalties 11 and 1 respectively, gap x_dropoff 50, expect 10, word size 3, filter ON.

[0054] The terms "polynucleotide," "oligonucleotide," and "nucleic acid" refer to deoxyribonucleic acid (DNA) and, where appropriate, to ribonucleic acid (RNA), or peptide nucleic acid (PNA). The term should also be understood to include nucleotide analogs, and single or double stranded polynucleotides (e.g., siRNA). Examples of polynucleotides include but are not limited to plasmid DNA or fragments thereof, viral DNA or RNA, antisense RNA, etc. The term "plasmid DNA" refers to double stranded DNA that is circular. "Antisense," as used herein, refers to a nucleic acid capable of hybridizing to a portion of a coding and/or noncoding region of mRNA by virtue of sequence complementarity, thereby interfering with translation from the mRNA. The terms "siRNA" and "RNAi" refer to a nucleic acid which is a double stranded RNA that has the ability to induce degradation of mRNA thereby "silencing" gene expression. Typically, siRNA is at least 15-50 nucleotides long, e.g., 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length.

[0055] The term "V domain" (singular or plural), unless specifically stated, refers to the first Ig-like variable domain (V_1) and/or the second Ig-like domain (V_2) in the protein or genomic sequence of B7-H3, regardless of the species of origin (e.g., any sequence comprising SEQ ID NO:15 and a nucleotide sequence encoding it; or a sequence substantially identical to SEQ ID NO:7 and a nucleotide sequence encoding it). Likewise, the term "C domain" (singular or plural), unless specifically stated, refers to the first Ig-like constant domain (C_1) and/or the second Ig-like constant domain (C_2) in the protein or genomic sequence of B7-H3,

regardless of the species of origin. Unless context requires otherwise, references to V and C domains should be understood to encompass the protein domains, nucleotide sequences encoding therefor, and pseudo-exon sequences corresponding to the coding sequences (e.g., C ψ and V ψ of rodent genomic sequences).

2. B7-H3 Agents

[0056] In one aspect, the present invention relates to the use of B7-H3 agents in modulation of immune responses. The present invention is based, in part, on the discovery and demonstration that the VCVC form of B7-H3 accounts for the majority of B7-H3 transcripts seen across multiple tissues while the VC form of B7-H3 is only a minor transcript. The invention is further based, in part, on the discovery and demonstration that both forms of B7-H3, VC and VCVC, exhibit an inhibitory effect on T cell activation as evidenced by decreased proliferation and cytokine secretion by the cells in the presence of B7-H3. The invention is yet further based, in part, on the discovery of specific regions within the B7-H3 genes that are currently undergoing purifying evolutionary selection.

[0057] Portions of mouse, human, monkey, and hamster genomic V-exons were aligned using ClustalW of the Align module of Vector NTI version 8.0. Regions that exhibited 100% sequence identity in alignment greater or equal to nine nucleotides were chosen as the most highly conserved nucleotide positions. These eleven conserved regions are represented in SEQ ID NO:15.

[0058] In certain embodiments, compositions used in the methods of the invention comprise a B7-H3 agent that antagonizes or agonizes the biological activity of naturally occurring B7-H3. In some embodiments, the B7-H3 agent is proteinaceous, i.e., it comprises amino acids linked by peptide bonds. Proteinaceous B7-H3 agents include but are not limited to soluble forms of B7-H3, including B7-H3-Ig fusions, antibodies against B7-H3, and antibodies against a B7-H3 receptor. In other embodiments, compositions used in the methods of the invention comprise nonproteinaceous B7-H3 agents, such as nucleic acids, small molecule inhibitors, etc. In particular, the presently disclosed B7-H3 agents modulate lymphocyte activation as measured by one or more of the following: (a)

lymphocyte proliferation; and (b) cytokine secretion (e.g., interleukin (IL)-10, tumor necrosis factor (TNF)- α , interferon (IFN)- γ , and granulocyte-macrophage-colony stimulating factor (GM-CSF)). In some embodiments, B7-H3 agents possess pharmacokinetic properties that make it suitable for therapeutic use, e.g., sufficiently long circulatory half-life and/or acceptable protection from proteolytic degradation.

2.1 Antibodies

[0059] Antibodies used in the methods of the invention fall into two groups: (1) antibodies against B7-H3 and (2) antibodies against a B7-H3 receptor. In various embodiments, antibodies used in the methods of the invention specifically bind to at least one of: (a) B7-H3; (b) B7-H3 receptor; (c) V domain in B7-H3; (d) C domain in B7-H3; and (e) polypeptide comprising SEQ ID NO:15, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:7. Such antibodies may (a) specifically bind to B7-H3 thereby blocking the interaction of B7-H3 with its receptor; (b) specifically bind to a B7-H3 receptor thereby blocking its interaction with B7-H3; or (c) perform both (a) and (b). Depending on the desired effect, the antibodies may be used in alternative configurations to either enhance (as an antagonist of B7-H3 biological activity) or inhibit immune responses (as an agonist of B7-H3 biological activity) as described in subsequent sections.

[0060] Antibodies can be made, for example, by traditional hybridoma techniques (Kohler and Milstein (1975) *Nature*, 256:495-499), recombinant DNA methods (U.S. Pat. No. 4,816,567), or phage display techniques using antibody libraries (Clackson et al. (1991) *Nature*, 352:624-628; Marks et al. (1991) *J. Mol. Biol.*, 222:581-597). For various other antibody production techniques, see, e.g., *Antibodies: A Laboratory Manual*, eds. Harlow et al., Cold Spring Harbor Laboratory, 1988; and *Antibody Engineering*, 2nd ed., Oxford University Press, ed. Borrebaeck, 1995. For administration to humans, antibodies may be fully human or humanized. In certain embodiments, antibodies may have an altered or mutated Fc region as described in subsequent sections.

2.2 Soluble Forms of B7-H3

[0061] The methods of the invention involve a use of soluble forms of B7-H3 that inhibit lymphocyte activation. In some embodiments, a soluble form of B7-H3 comprises less than full length B7-H3 and does not include the transmembrane and the intracellular domains of B7-H3. Such a soluble form may also not include a signal sequence. For illustration only, and not to be limiting, these domains can be delineated in human and mouse B7-H3 as depicted in FIG. 1A.

[0062] In certain embodiments, a soluble form comprises an amino acid sequence as in SEQ ID NO:15 or SEQ ID NO:7. In yet further embodiments, a soluble form of B7-H3 comprises at least one V domain of B7-H3, and optionally at least one C domain of B7-H3. A soluble form may comprise at least 2, 3, 4, or 5 V domains, and optionally at least 1, 2, 3, 4, or 5 C domains.

[0063] In further embodiments, a soluble form of B7-H3 may comprise (a) a first amino acid sequence derived from the extracellular domain of B7-H3 and (b) a second amino acid sequence derived from the constant region of an antibody. The first amino acid sequence is derived from all or a portion of the B7-H3 extracellular domain and (a) competitively inhibits binding of a naturally occurring form of B7-H3 to its receptor and/or (b) has a negative costimulatory activity.

[0064] In some embodiments, the first amino acid sequence comprises a sequence as set out in SEQ ID NO:15. In certain embodiments, the first amino acid sequence is identical to or is substantially identical to amino acids 23-244 of SEQ ID NO:14, or amino acids 23-462 of SEQ ID NO:12. In an illustrative embodiment, the soluble form of B7-H3 comprises a sequence as in SEQ ID NO:12 or SEQ ID NO:14.

[0065] The second amino acid sequence may be derived from the constant region of an antibody, such as the Fc portion. In some embodiments, the second amino acid sequence is derived from the Fc portion of an IgG. In related embodiments, the Fc portion is derived from IgG that is IgG₁, IgG₄, or another IgG isotype. In nonlimiting illustrative embodiments, the second sequence is derived from mouse IgG_{2am}.

[0066] In certain embodiments, the second amino acid sequence is linked to the C-terminus or the N-terminus of the first amino acid sequence, with or without being linked by a linker sequence. The exact length and sequence of the linker and its orientation relative to the linked sequences may vary. The linker may, for example, comprise one or more Gly-Ser. The linker may be at least 2, at least 10, at least 20, at least 30, amino acids long and is selected based on properties desired such as solubility, length, steric separation, immogenicity, etc.

2.3 Derivatives of Proteinaceous B7-H3 Agents

[0067] Derivatives of proteinaceous B7-H3 agents (including soluble forms of B7-H3, antibodies against B7-H3, and antibodies against B7-H3 receptor) can be made by altering their amino acids sequences by substitutions, additions, and/or deletions/truncations or by introducing chemical modification that result in functionally equivalent or molecules. It will be understood by one of ordinary skill in the art that certain amino acids in a sequence of any protein may be substituted for other amino acids without adversely affecting the activity of the protein.

[0068] Various changes may be made in the amino acid sequences of the proteinaceous B7-H3 agents of the invention or DNA sequences encoding therefor without appreciable loss of their biological activity, function, or utility. The use of such derivatives is within the scope of the present invention. In a specific embodiment, the derivative is functionally active, i.e., capable of exhibiting one or more activities associated with the extracellular domain of the naturally occurring B7-H3, e.g., as set out in SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs (see Table 1). Furthermore, various amino acids are commonly substituted with neutral amino acids, e.g., alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine (see, e.g., MacLennan et al. (1998) Acta Physiol. Scand. Suppl. 643:55-67; Sasaki et al. (1998) Adv. Biophys. 35:1-24).

TABLE 1

Original Residues	Exemplary Substitutions	Typical Substitutions
Ala (A)	Val, Leu, Ile	Val
Arg (R)	Lys, Gln, Asn	Lys
Asn (N)	Gln	Gln
Asp (D)	Glu	Glu
Cys (C)	Ser, Ala	Ser
Gln (Q)	Asn	Asn
Gly (G)	Pro, Ala	Ala
His (H)	Asn, Gln, Lys, Arg	Arg
Ile (I)	Leu, Val, Met, Ala, Phe, Norleucine	Leu
Leu (L)	Norleucine, Ile, Val, Met, Ala, Phe	Ile
Lys (K)	Arg, 1,4-Diamino-butyrlic Acid, Gln, Asn	Arg
Met (M)	Leu, Phe, Ile	Leu
Phe (F)	Leu, Val, Ile, Ala, Tyr	Leu
Pro (P)	Ala	Gly
Ser (S)	Thr, Ala, Cys	Thr
Thr (T)	Ser	Ser
Trp (W)	Tyr, Phe	Tyr
Tyr (Y)	Trp, Phe, Thr, Ser	Phe
Val (V)	Ile, Met, Leu, Phe, Ala, Norleucine	Leu

[0069] B7-H3 agents may be chemically coupled, or conjugated, to other proteins and pharmaceutical agents. Such modifications may be designed to alter the pharmacokinetics and/or biodistribution of the resultant composition. The B7-H3-Ig and antibodies of the invention may also be glycosylated, pegylated, or

linked to another nonproteinaceous polymer, e.g., polyethylene glycol, polypropylene glycol, or polyoxyalkylenes, in the manner set forth in U.S. Patent Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192; or 4,179,337. The B7-H3-Ig and antibodies may be chemically modified by covalent conjugation to a polymer to increase their circulating half-life, for example. Exemplary polymers, and methods to attach them to peptides, are also shown in U.S. Patent Nos. 4,766,106; 4,179,337; 4,495,285; and 4,609,546.

[0070] B7-H3 agents that comprise the Fc portion of an antibody, such as B7-H3-Ig fusions, or antibodies of used in the methods of the invention may further be modified in the Fc region to minimize the effector function. Such modifications include changing specific amino acid residues which alter binding to an Fc receptor (Lund et al. (1991) J. Immun., 147:2657-2662 and Morgan et al. (1995) Immunology, 86:319-324), or changing the species from which the constant region is derived. Antibodies and B7-H3-Ig fusions may have mutations in the C_H2 region of the heavy chain that reduce effector function, i.e., Fc receptor binding and complement activation. For example, antibodies and B7-H3-Ig fusions may have mutations such as those described in U.S. Patent Nos. 5,624,821 and 5,648,260. In the IgG₁ or IgG₂ heavy chain, for example, such mutations may be made at amino acid residues corresponding to amino acids 234 and 237 in the full-length sequence of IgG₁ or IgG₂. Antibodies and B7-H3-Ig fusions may also have mutations that stabilize the disulfide bond between the two heavy chains of an immunoglobulin, such as mutations in the hinge region of IgG₄, as disclosed in Angal et al. (1993) Mol. Immunol., 30:105-108.

[0071] In certain embodiments, additional fusions of any of B7-H3-Ig of the invention to amino acid sequences derived from other proteins may be constructed for use in the methods of the invention. Desirable fusion sequences may be derived from proteins having biological activity different from that of B7-H3, for example, cytokines, growth and differentiation factors, enzymes, hormones, other receptor components, etc.

[0072] The B7-H3 agents (proteinaceous and nonproteinaceous) may also be tagged with a detectable or functional label. Detectable labels include radiolabels such as ¹³¹I or ⁹⁹Tc, which may be attached using conventional chemistry. Detectable labels further include enzyme labels, e.g., horseradish

peroxidase or alkaline phosphatase and detectable moieties such as biotin or avidin.

[0073] Derivatives can be produced by various techniques well known in the art, including recombinant and synthetic methods (Maniatis (1990) Molecular Cloning, A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; and Bodansky et al. (1995) The Practice of Peptide Synthesis, 2nd ed., Springer Verlag, Berlin, Germany).

2.4 Nucleic Acids

[0074] The disclosure also provides methods involving therapeutic and nontherapeutic uses of nucleic acids or polypeptides encoded by such nucleic acids, wherein the nucleotide sequence of such nucleic acid is chosen from (a) a nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, or a portion thereof; and (b) a nucleic acid that is at least 60, 80, 100, 120, or 140 nucleotides long and hybridizes to the nucleic acid of (a) under defined conditions, wherein the nucleic acid encodes an expression product having a negative costimulatory activity.

[0075] In certain embodiments, such a nucleic acid encodes an amino acid sequence as in SEQ ID NO:15. In an illustrative embodiment, the nucleic acid comprises a sequence substantially as in SEQ ID NOs:11 or SEQ ID NO:13. In other embodiments, such a nucleic acid includes a nucleotide sequence that differs from SEQ ID NO:11 or SEQ ID NO:13 in that it has at least one synonymous substitution, i.e., the codon having the substitution encodes the same or a functionally equivalent amino acid residue as in SEQ ID NO:11 or SEQ ID NO:13.

[0076] In one embodiment, the defined conditions are low stringency conditions. In another embodiment, the defined conditions are moderate stringency conditions. In yet another embodiment, the defined conditions are high stringency conditions.

[0077] Appropriate hybridization conditions can be easily selected by those skilled in the art as exemplified in Ausubel et al. (1995), Current Protocols in Molecular Biology, John Wiley & Sons, sections 2, 4, and 6. Additionally, stringent conditions are described in Sambrook et al. (1989) Molecular Cloning: A

Laboratory Manual, 2nd ed., Cold Spring Harbor Press, chapters 7, 9, and 11. A nonlimiting example of defined conditions of low stringency is as follows. Filters containing DNA are pretreated for 6 h at 40°C in a solution containing 35% formamide, 5 x SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.1% PVP, 0.1% Ficoll, 1% BSA, and 500 µg/ml denatured salmon sperm DNA. Hybridizations are carried out in the same solution with the following modifications: 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 µg/ml salmon sperm DNA, 10% (wt/vol) dextran sulfate, and 5-20x10⁶ ³²P-labeled probe is used. Filters are incubated in hybridization mixture for 18-20 h at 40°C and then washed for 1.5 h at 55°C in a solution containing 2 x SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS. The wash solution is replaced with fresh solution and incubated an additional 1.5 h at 60°C. Filters are blotted dry and exposed for autoradiography. Other conditions of low stringency well known in the art may be used (e.g., as employed for cross-species hybridizations).

[0078] A nonlimiting example of defined conditions of moderate stringency is as follows. Prehybridization of filters containing DNA is carried out for 7 h to overnight at 50°C in buffer composed of 5 x SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 µg/ml denatured salmon sperm DNA. Filters are hybridized for 18-36 h at 50°C in the prehybridization mixture containing 100 µg/ml denatured salmon sperm DNA and 5-20x10⁶ cpm of ³²P-labeled probe. Washing of filters is done at 37°C for 1 h in a solution containing 2 x SSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA. This is followed by a wash in 0.1 x SSC at 50°C for 45 minutes. Other conditions of moderate stringency well known in the art may be used.

[0079] A nonlimiting example of defined conditions of high stringency is as follows. Prehybridization of filters containing DNA is carried out for 8 h to overnight at 65°C in buffer composed of 6 x SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 µg/ml denatured salmon sperm DNA. Filters are hybridized for 48 h at 65°C in the prehybridization mixture containing 100 µg/ml denatured salmon sperm DNA and 5-20x10⁶ cpm of ³²P-labeled probe. Washing of filters is done at 37°C for 1 h in a solution containing 2 x SSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA. This is followed by a wash in

0.1 x SSC at 50°C for 45 minutes. Other conditions of high stringency well known in the art may be used.

[0080] B7-H3 agents may be obtained, isolated, and/or purified from their natural environment, in substantially pure or homogeneous form, or in the case of nucleic acid, free or substantially free of nucleic acid or genes origin other than the sequence encoding a polypeptide with the required function. Systems for cloning and expression of a polypeptide in a variety of different host cells are well known. Suitable host cells include bacteria, mammalian cells, and yeast and baculovirus systems. Mammalian cell lines available in the art for expression of a heterologous polypeptide include Chinese hamster ovary cells, HeLa cells, baby hamster kidney cells, NS0 mouse melanoma cells and many others. A common bacterial host is *E. coli*. For other cells suitable for producing, e.g., B7-H3-Ig, see Gene Expression Systems, eds. Fernandez et al., Academic Press, 1999.

[0081] Suitable vectors can be chosen or constructed, containing appropriate regulatory sequences, including promoter sequences, terminator sequences, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. Vectors may be plasmids or viral, e.g., phage, or phagemid, as appropriate. For further details see, e.g., Molecular Cloning: A Laboratory Manual, Sambrook et al., 2nd ed., Cold Spring Harbor Laboratory Press, 1989. Many known techniques and protocols for manipulation of nucleic acid, for example, in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, and analysis of proteins, are described in detail in Current Protocols in Molecular Biology, eds. Ausubel et al., 2nd ed., John Wiley & Sons, 1992.

[0082] A nucleic acid can be fused to other sequences encoding additional polypeptide sequences, for example, sequences that function as a marker or reporter. Examples of marker or reporter genes include β -lactamase, chloramphenicol acetyltransferase (CAT), adenosine deaminase (ADA), aminoglycoside phosphotransferase (responsible for neomycin (G418) resistance), dihydrofolate reductase (DHFR), hygromycin-B-phosphotransferase (HPH), thymidine kinase (TK), lacZ (encoding β -galactosidase), xanthine guanine phosphoribosyltransferase (XGPRT), and many others known in the art.

[0083] The methods of the invention also encompass the use of short interfering RNAs (siRNA) and antisense oligonucleotides to reduce the expression of B7-H3 in order to enhance immune response. siRNA may be produced using standard techniques as described in Hannon (2002) Nature, 418:244-251; McManus et al. (2002) Nat. Reviews, 3:737-747; Heasman (2002) Dev. Biol., 243:209-214; Stein (2001) J. Clin. Invest., 108:641-644; and Zamore (2001) Nat. Struct. Biol., 8(9):746-750. Antisense nucleic acids may be produced using standard techniques as described in Antisense Drug Technology: Principles, Strategies, and Applications, 1st ed., ed. Crooke, Marcel Dekker, 2001.

3. Methods of Use

3.1 Methods of Modulating Immune Responses

[0084] The disclosed B7-H3 agents can act as either agonists or antagonists of naturally expressed B7-H3, depending on the method of their use. The B7-H3 agents can be used to prevent, diagnose, or treat medical disorders in mammals (such as in humans). *In vitro* application of B7-H3 agents can be useful, for example, in production of activated lymphocytes for use in either studies on immune cell function or, for example, or for testing the biological activity of other B7-H3 agents. Such methods are detailed in the Examples.

[0085] In one aspect, the present invention relates to the use of B7-H3 and agonists and antagonists thereof in modulation of immune responses. The methods of the invention involve contacting a lymphocyte, e.g., T or B cell, with a B7-H3 agent in order to modulate (i.e., costimulate or inhibit) lymphocyte activation. In particular, the presently disclosed B7-H3 agents modulate lymphocyte activation as measured by one or more of the following: (a) lymphocyte proliferation; (b) cytokine secretion (e.g., interleukin (IL)-10, tumor necrosis factor (TNF)- α , interferon (IFN)- γ , and granulocyte-macrophage-colony stimulating factor (GM-CSF)). The methods can be performed *in vitro*, *in vivo*, or *ex vivo*.

[0086] The contacting step can occur before, during, or after activation of the lymphocyte. T cell activation can be effected, for example, by exposing the T cell to an antibody that binds to the TcR or one of the polypeptides of the CD3 complex that is physically associated with the TCR (e.g., using an anti-CD3

antibody; U.S. Patent Nos. 6,405,696 and 5,316,763). Alternatively, the T cell can be exposed to either an alloantigen (e.g., a MHC alloantigen) on, for example, an antigen presenting cell (APC) (e.g., a dendritic cell, a macrophage, a monocyte, or a B cell) or an antigenic peptide produced by processing of a protein antigen by any of the above APC and presented to the T cell by MHC molecules on the surface of the APC. The T cell can be a CD4⁺ T cell or a CD8⁺ T cell. The B7-H3 agent can be added to the solution containing the cells, or it can be expressed on the surface of an APC, e.g., an APC presenting an alloantigen or an antigen peptide bound to an MHC molecule.

[0087] Furthermore, B7-H3 agents can be used to treat a subject at risk of or susceptible to a disorder or having a disorder associated with aberrant B7-H3 expression or function. Thus, in certain embodiments, the methods of the invention comprise identifying a subject in need of inhibiting lymphocyte activation, and administering a B7-H3 agonist to the subject. In other embodiments, the methods comprise identifying a subject in need of enhancing lymphocyte activation, and administering a B7-H3 antagonist to the subject.

[0088] When diminished immune response is desirable, B7-H3 agents may be used as agonists of B7-H3 in order to enhance the B7-H3-associated attenuation of the immune response. For example, B7-H3 agents can be used in methods of the invention for induction of tolerance to a specific antigen (e.g., a therapeutic protein). In one embodiment, tolerance is induced against a specific antigen by co-administration of antigen and a B7-H3 agent. For example, patients that received Factor VIII or Factor IX frequently generate antibodies to this protein, therefore co-administration of a B7-H3 agonist (e.g., B7-H3-Ig and nucleic acids encoding B7-H3 or its functional fragments) in combination with recombinant Factor VIII or Factor IX is expected to result in the downregulation of immune responses to this clotting factor. Additionally, a reduction in the level of immune response may be desirable, for example, in certain types of allergy or allergic reactions, autoimmune diseases (e.g., rheumatoid arthritis, psoriasis, type I diabetes mellitus, multiple sclerosis, inflammatory bowel disease, Crohn's disease, and systemic lupus erythematosus), tissue, skin and organ transplant rejection, and graft-versus-host disease (GVHD).

[0089] In certain embodiments, to achieve an agonistic effect, co-presentation, or coupling, (i.e., physical proximity) between positive (i.e., mediated by an antigen receptor, e.g., TcR or BcR) and negative (i.e., B7-H3) signals may be necessary. This may be achieved by immobilizing a B7-H3 agent on a support matrix which also carries a primary stimulatory molecule (e.g., anti-CD3 antibody). In such cases, the preferred distance is less than or comparable to the size of a naturally occurring antigen-presenting cell, i.e., less than 100 μm ; more preferably, less than 50 μm ; and most preferably, less than 20 μm . Alternatively, a B7-H3 agent can be coupled with a primary stimulatory molecule, e.g., by cross-linking via antibodies.

[0090] In some embodiments, the positive (activating) and the negative (inhibiting) signals are provided by a ligand or antibodies immobilized on solid support matrix, or a carrier. In various embodiments, the solid support matrix may be composed of polymer such as activated agarose, dextran, cellulose, polyvinylidene fluoride (PVDF). Alternatively, the solid support matrix may be based on silica or plastic polymers, e.g., as nylon, dacron, polystyrene, polyacrylates, polyvinyls, teflons, etc.

[0091] The matrix can be implanted into the spleen of a patient. Alternatively, the matrix may be used for the *ex vivo* incubation of T cells obtained from a patient, which are then separated and implanted back into the patient. The matrix may also be made from a biodegradable material such polyglycolic acid, polyhydroxyalkanoate, collagen, or gelatin so that they can be injected into the patient's peritoneal cavity, and dissolve after some time following the injection. The carrier can be shaped to mimic a cell (e.g., bead or microsphere).

[0092] Under certain circumstances, it may be desirable to elicit or enhance a patient's immune response in order to treat an immune disorder or cancer. The disorders being treated or prevented by the disclosed methods include but are not limited to infections with microbes (e.g., bacteria), viruses (e.g., systemic viral infections such as influenza, skin diseases such as herpes or shingles, and HIV), or parasites; and cancer (e.g., melanoma and prostate cancers).

[0093] In such circumstances, B7-H3 agents may be used to inhibit or reduce the downregulatory activity associated with B7-H3. In particular, B7-H3

antagonists (e.g., anti-B7-H3 antibody, antibody against a B7-H3 receptor, siRNA, and antisense nucleic acids to B7-H3) can be used for stimulation of T cell activation. In various embodiments, antibodies against B7-H3 or against a B7-H3 receptor inhibit binding of B7-H3 to cells expressing such a receptor with an IC_{50} of less than 10 nM, and more preferably less than 5 nM, and most preferably less than 1 nM. IC_{50} can be measured using standard techniques known in the art.

[0094] The compositions of the present invention are administered in therapeutically effective amounts. Generally, a therapeutically effective amount may vary with the subject's age, condition, and sex, as well as the severity of the medical condition of the subject. A therapeutically effective amount of proteinaceous B7-H3 agents ranges from 0.001 to 30 mg/kg, preferably from 0.01 to 25 mg/kg, from 0.1 to 20 mg/kg, or from 1 to 10 mg/kg body weight. The dosage may be adjusted, as necessary, to suit observed effects of the treatment. The antibodies and soluble forms of B7-H3 may given as a bolus dose. Continuous infusion may also be used after the bolus dose. The appropriate dose and regimen is chosen based on clinical indications by a treating physician.

[0095] Immune cells (e.g., activated T cells) can also be isolated from a patient and incubated *ex vivo* with a B7-H3 agent. For example, peripheral blood mononuclear cells (PBMC) can be withdrawn from a subject or a suitable donor and exposed *ex vivo* to an activating stimulus (see above) and a B7-H3 agent (whether in soluble form or attached to a solid support). The PBMC containing activated T cells are then introduced into the same or a different subject. Alternatively, isolated cells can be transfected with a nucleic acid and such transfected cell may then reintroduced into the subject. While such cells would preferably be hemopoietic cells (e.g., bone marrow cells, macrophages, monocytes, dendritic cells, T cells, or B cells) they could also be of another cell type including, without limitation, fibroblasts, epithelial cells, endothelial cells, keratinocytes. The use of hemopoietic cells may be advantageous in that such cells would be expected to home to, among others, lymphoid tissue (e.g., lymph nodes or spleen). In addition, if APC are used, the APC expressing the exogenous B7-H3 can be the same APC that presents an alloantigen or antigenic peptide to the relevant T cell. The B7-H3 agents can be secreted by the APC or expressed on its surface. Prior to returning the recombinant APC to the subject, they can optionally be exposed to sources of

antigens or antigenic peptides of interest, e.g., those of tumors, infectious microorganisms, or autoantigens.

[0096] In some embodiments, B7-H3 agents are used to treat or prevent immune disorders susceptible to treatment with compositions of the invention which include but are not limited to immunologic disorders (e.g., rheumatoid arthritis (RA), psoriasis, multiple sclerosis (MS), inflammatory bowel disease (IBD), Crohn's disease, systemic lupus erythematosus (SLE), type I diabetes, transplant rejection, graft-versus-host disease (GVHD), hyperproliferative immune disorders, etc.), cancers, immunosuppressive disorders, and various infectious diseases. In particular, the disclosure provides methods that involve compositions comprising B7-H3 derivatives such as soluble forms of B7-H3 or antibodies against B7-H3 or against its receptor.

3.2 Screening Methods

[0097] B7-H3 agents can also be used in screening methods to identify therapeutic agents. A compound to be tested can be, for example, an anti-B7-H3 antibody, an antibody against a B7-H3 receptor, or a small organic molecule. In such a screening assay, a first binding mixture is formed by combining B7-H3-Ig and a cell expressing a B7-H3 receptor (e.g., an activated T cell); and the amount of binding between the two in the first binding mixture (M_0) is measured. A second binding mixture is also formed by combining B7-H3-Ig, a cell expressing a B7-H3 receptor, and an agent to be tested, and the amount of binding in the second binding mixture (M_1) is measured.

[0098] The amounts of binding in the first and second binding mixtures are then compared, for example, by calculating the M_1/M_0 ratio. The tested compound is considered to be capable of modulating a B7-H3-associated downregulation of immune responses if a decrease in binding in the second binding mixture as compared to the first binding mixture is observed. The formulation and optimization of binding mixtures is within the level of skill in the art, such binding mixtures may also contain buffers and salts necessary to enhance or to optimize binding, and additional control assays may be included in the screening assay of the invention. Compounds found to reduce the B7-H3 binding by at least 10% (i.e., $M_1/M_0 < 0.9$),

preferably greater than 30% may thus be identified and then, if desired, secondarily screened for the capacity to ameliorate a disorder in other assays or animal models as described below. The strength of the binding can be measured using, for example, an enzyme-linked immunoadsorption assay (ELISA), radio-immunoassay (RIA), surface plasmon resonance-based technology (e.g., Biacore), all of which are techniques well known in the art.

[0100] The tested compound may then be further tested *in vitro* as described in the Examples or in an animal model (see, generally, Immunologic Defects in Laboratory Animals, eds. Gershwin et al., Plenum Press, 1981), for example, such as the following: the SWR X NZB (SNF1) mouse model (Uner et al. (1998) J. Autoimmune. Dis., 11(3): 233-240), the KRN mouse (K/BxN) model (Ji et al. (1999) Immunol. Rev., 169: 139); NZB X NZW (B/W) mice, a model for SLE (Riemekasten et al. (2001) Arthritis Rheum., 44(10): 2435-2445); experimental autoimmune encephalitis (EAE) in mouse, a model for multiple sclerosis (Tuohy et al. (1988) J. Immunol., 141:1126-1130, Sobel et al. (1984) J. Immunol. 132:2393-2401, and Traugott (1989) Cell Immunol., 119:114-129); the NOD mouse model of diabetes (Baxter et al. (1991) Autoimmunity, 9(1):61-67), etc.).

[0101] Preliminary doses as, for example, determined according to animal tests, and the scaling of dosages for human administration is performed according to art-accepted practices. Toxicity and therapeutic efficacy can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD₅₀/ED₅₀. Compositions that exhibit large therapeutic indices are preferable.

[0102] The therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (i.e., the concentration of the therapeutic which achieves a half-maximal inhibition of symptoms) as determined in cell culture assays or animal models. Levels in plasma may be measured, for example, by high performance liquid chromatography or ELISA. The effects of any particular dosage can be monitored by a suitable bioassay.

Examples of dosages are: $0.1 \times IC_{50}$, $0.5 \times IC_{50}$, $1 \times IC_{50}$, $5 \times IC_{50}$, $10 \times IC_{50}$, $50 \times IC_{50}$, and $100 \times IC_{50}$.

[0103] The data obtained from the cell culture assays or animal studies can be used in formulating a range of dosage for use in humans. Therapeutically effective dosages achieved in one animal model can be converted for use in another animal, including humans, using conversion factors known in the art (see, e.g., Freireich et al. (1966) Cancer Chemother. Reports, 50(4):219-244 and Table 2 for Equivalent Surface Area Dosage Factors).

TABLE 2

To: From:	Mouse (20 g)	Rat (150 g)	Monkey (3.5 kg)	Dog (8 kg)	Human (60 kg)
Mouse	1	1/2	1/4	1/6	1/12
Rat	2	1	1/2	1/4	1/7
Monkey	4	2	1	3/5	1/3
Dog	6	4	3/5	1	1/2
Human	12	7	3	2	1

4. Pharmaceutical Compositions, Methods of Administration, and Dosage

[0104] The disclosure provides pharmaceutical compositions comprising B7-H3 agents. Such compositions may be suitable for pharmaceutical use and administration to patients. The compositions typically comprise one or more antibodies of the present invention and a pharmaceutically acceptable excipient. The phrase "pharmaceutically acceptable excipient" includes any and all solvents, dispersion media, coatings, antibacterial agents and antifungal agents, isotonic agents, and absorption delaying agents, and the like, that are compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. The compositions may also contain other active compounds providing supplemental, additional, or

enhanced therapeutic functions. The pharmaceutical compositions may also be included in a container, pack, or dispenser together with instructions for administration.

[0105] A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Methods to accomplish the administration are known to those of ordinary skill in the art. The administration may, for example, be intravenous, intraperitoneal, intramuscular, intracavity, subcutaneous or transdermal. It may also be possible to obtain compositions which may be topically or orally administered, or which may be capable of transmission across mucous membranes.

[0106] Solutions or suspensions used for intradermal or subcutaneous application typically include one or more of the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerin, propylene glycol, or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates; and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. Such preparations may be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[0107] Pharmaceutical compositions suitable for injection include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It should be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, and sodium chloride in the composition. The carrier can be a solvent or dispersion medium

containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and/or by the use of surfactants. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate, and gelatin.

[0108] Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For oral administration, the antibodies can be combined with excipients and used in the form of tablets, troches, or capsules. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches, and the like can contain any of the following ingredients, or compounds of a similar nature; a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

[0109] Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration may be accomplished, for example, through the use of lozenges, nasal sprays, inhalers, or suppositories. For example, in case of antibodies and Ig fusion proteins that comprise the Fc portion, compositions may be capable of transmission across mucous membranes in intestine, mouth, or lungs (e.g., via the FcRn receptor-mediated pathway as described in U.S. Patent No. 6,030,613). For transdermal administration, the active compounds may be formulated into ointments, salves, gels, or creams as generally known in the art. For administration by inhalation, the antibodies may be delivered in the form of an aerosol spray from pressured container or dispenser, which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

[0110] In certain embodiments, the presently disclosed B7-H3 agents are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. Liposomal suspensions containing the presently disclosed antibodies can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

[0111] It may be advantageous to formulate oral or parenteral compositions in a dosage unit form for ease of administration and uniformity of dosage. The term "dosage unit form" as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

[0112] Toxicity and therapeutic efficacy of the composition of the invention can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD₅₀/ED₅₀. Compositions that exhibit large therapeutic indices are preferred.

[0113] For any composition used in the present invention, the therapeutically effective dose can be estimated initially from cell culture assays. Examples of suitable bioassays include DNA replication assays, cytokine release assays, transcription-based assays, binding assays, creatine kinase assays, assays based on the differentiation of pre-adipocytes, assays based on glucose uptake in adipocytes, immunological assays other assays as, for example, described in the Examples. The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (i.e., the concentration of the therapeutic

that achieves a half-maximal inhibition of symptoms). Circulating levels in plasma may be measured, for example, by high performance liquid chromatography. The effects of any particular dosage can be monitored by a suitable bioassay. The dosage lies preferably within a range of circulating concentrations with little or no toxicity. The dosage may vary depending upon the dosage form employed and the route of administration utilized.

[0114] The following Examples do not in any way limit the scope of the invention. One of ordinary skill in the art will recognize the numerous modifications and variations that may be performed without altering the spirit or scope of the present invention. Such modifications and variations are encompassed within the scope of the invention. The entire contents of all references, patents, and published patent applications cited throughout this application are herein incorporated by reference.

EXAMPLES

Example 1: Isolation of genomic DNA for B7-H3

[0115] B7-H3 RT-PCR was performed using oligonucleotides corresponding to the regions containing the initiation methionine and termination codons of human B7-H3 (Genbank accession No. AF302102) using the following PCR conditions.

[0116] PCR enzymes used in this study include KOD Hot Start (Novagen, Madison, WI), Advantage™ 2 (Clontech, Palo Alto, CA), and Platinum Taq (Invitrogen, Carlsbad, CA) enzymes according to manufacturer's protocols. When necessary, reaction conditions were supplemented to a final concentration of 1 M Betaine and 3% DMSO for robust amplification. Primers PW264 (SEQ ID NO:23) with imbedded attB1/Kozak and PW265 (SEQ ID NO:24) with imbedded attB2 sites, were used to amplify human B7-H3 coding sequences from first strand cDNA of spleen, lymph node, heart, liver, pancreas, and placenta as templates (Clontech). Human B7-H3 VCVC coding region sequences were obtained corresponding to sequences represented in existing database entries (Celera Human and Mouse Genomic Assemblies, Celera Genomics, Rockville, MD): AX357960, AX097550, AX047072, AX097556, and AX136363 among others.

Human B7-H3 VC form was constructed by deletion of human B7-H3 VCV C₁-V₂ domains, matching the coding sequence of NM_025240. PW270 (SEQ ID NO:26) and PW271 (SEQ ID NO:27) were used to amplify mouse B7-H3 sequences from mouse embryo first strand cDNA. PCR products with a size of 951 bp were subcloned, clearly revealing the correct splicing of the 7 predicted bona fide exons with a 100% accuracy, without inclusion of any pseudo-exons sequences in any analyzed clone. Mouse B7-H3 coding sequences corresponded to existing database entries BC019436, AX370312, and NM_133983. Primers PW284 (SEQ ID NO:29) and PW267 (SEQ ID NO:25) were used to semi-quantitatively assess the relative contribution of human B7-H3 VC (690 bp) and VCV (1344 bp) transcripts in cDNA panels (Clontech). Southern blots were performed by alkaline transfer of DNA onto Zetaprobe™ GT membrane (BioRad, Hercules, CA) and hybridized using ³²P end-labeled PW278 (SEQ ID NO:28) as detection oligonucleotide (Ling et al. (2001) J. Immunol., 166:7300-7308).

[0117] Monkey and hamster genomic DNAs were isolated from COS and CHO cell lines (Ling et al. (1999) Genomics 60:341-355). Genomic PCR was performed using PW358 (SEQ ID NO:30) and PW359 (SEQ ID NO:31) as primers based on nucleotides conserved between human and mouse B7-H3 sequences (amino acids 60-66, 216-221, and 278-284, 434-439 of SEQ ID NO:6). Amplification reactions were performed in duplicate and multiple subcloned products were analyzed by sequencing. Orientation of V-intron-C domains within monkey genomic DNA was determined by PCR using PW381 (SEQ ID NO:33) and PW384 (SEQ ID NO:34), and within hamster genomic DNA using PW358 (SEQ ID NO:30) and PW378 (SEQ ID NO:32).

[0118] DNA analysis was performed as follows. An aliquot (0.25-0.5 µg) of plasmid DNA was combined with 1 µl of 5 µM primer and 3 µl of 2 fold diluted ABI PRISM™ BigDye™ Terminator Cycle Sequencing Ready Reaction Kit mix (Version 3.0). The volumes were adjusted to 10 µl with 10 mM Tris-HCl (pH 8.0), and amplification reactions were performed on PTC-225 cycler (MJ Research, Waltham, MA) for 25 cycles (96°C for 10 seconds, 50°C for 5 seconds and 60°C for 4 minutes). 10 µl of water was added to the reactions and the excess dye was removed by gel filtration on a 96-well Millipore filter plate with G-50 beads. The

samples were heat-denatured for 2 min at 90-95°C and electrophorezed on ABI3100 Genetic Analyzer (Applied Biosystems, Foster City, CA) under conditions recommended by the manufacturer. Manual sequence editing was performed using Sequencher™ 4.1 (Gene Codes, Ann Arbor, MI).

[0119] Sequence determination of the major amplification product resulted in a 1605 bp sequence distinct from B7-H3 and consistent with other EST and patent database entries. Whereas B7-H3 contained single V and C domain (B7-H3 VC form), the variant clones contained a duplicated V and C domains (B7-H3 VCVC form). To determine whether these clones were gene products independent from B7-H3, the genomic organization of the VCVC clones was analyzed by human genomic database query (Celera Genomics). The resulting match corresponded to one chromosome 15 genomic axis GA_x2HTBL4SSTP-04, suggesting a single genetic origin of both B7-H3 VC and B7-H3 VCVC variants. **FIG. 1B** depicts genomic organization of human and mouse B7-H3 gene loci. Assemblies are based on selected portions of Celera human genomic axis GA_x2HTBL4SSTP and Celera mouse genomic axis GA_x5J8B7W7NM9. Bars denote relative locations of Alu and SVA complex repeats, simple repeats, transcript exon structure, and domain name. The genomic organization of B7-H3 VCVC revealed 9 exons encoding leader domain, V₁ domain, C₁ domain, V₂ domain, C₂ domain, transmembrane domain and three cytoplasmic domains. Approximately 13.5 kb of genomic sequence separated initiation methionine on exon 1 from termination codon on exon 9 of the human B7-H3 locus. Exon delineation of human B7-H3 VC to the B7-H3 locus revealed alternative splicing from exon 2 (V₁) to exon 5 (C₂), resulting in the deletion of the C₁ and V₂ domains from this gene product.

[0120] Sequence analysis, alignments, and phylogenetic guide tree generation were performed using the Align module (Clustal W) of Vector NTI™ version 7.1 (Informax Inc, North Bethesda, MD). Sequence alignment and phylogram analysis of primate sequences revealed a greater degree of sequence similarity between intraspecies V-intron-C DNA sequences than interspecies V-intron-C DNA sequences, i.e., human V₁-intron-C₁ is more similar to human V₂-intron-C₂ than monkey V-intron-C. Indeed, 100% identity was observed between the monkey C domain sequences analyzed. Intraspecies clustering is further supported by alignment of the non-coding intra-VC intron sequence where higher

sequence conservation is observed than the duplicated V exons (97% vs. 94%). Although conserved nucleotides were found in intron sequences of all four species, no conserved nucleotide pairs were found to be shared exclusively between rodent and primate species, (data not shown) arguing against a common "pre-duplicated" primate VCVC molecule ancestral to both primates and rodents. Sequences flanking human B7-H3 V-intron-C domains revealed no such sequence conservation, suggesting the duplication in primates is very recent or the V-intron-C region is extremely protected from mutation. Thus, these data strongly support a model of multiple independent emergence of tandem VC repeats within human and monkey species.

Example 2: Relative transcript contribution between B7-H3 splice variants

[0121] RT-PCR was performed on various human tissue samples followed by hybridization with radiolabeled oligonucleotide probes (FIG. 2A). Two unambiguous bands were detected with one migrating with a relative mobility corresponding to 1344 bp, consistent with the predicted size of the B7-H3 VCVC amplification product. A minor band was also detected corresponding to 690 bp, consistent with the predicted size of a VC amplification product. RT-PCR of three cDNA panels revealed this pattern in every tissue examined, except for leukocyte/PBL, where no amplification products were detected. Phosphorimage quantitation of hybridized regions indicated a ratio between 12.7:1 (brain) and 92.1:1 (kidney). In no case was the hybridization signal to the smaller B7-H3 VC product found to be greater than those seen for the B7-H3 VCVC product. Given that the amplification reactions favor smaller amplification products, the relative abundance of larger B7-H3 VCVC products over that of the smaller B7-H3 VC products suggest that B7-H3 VCVC is the dominant transcript species of naturally expressed human B7-H3 gene product in human tissues. The rarity of the B7-H3 VC form is consistent with the observed single 4.1 kb B7-H3 band in previously reported Northern blots, most probably representing the predominant B7-H3 VCVC product (Chapoval et al. (2001) Nat. Immunol., 2:269-274). In comparison, PCR-Southern blots of mouse B7-H3 show expression in all tissues examined with a dominant band at approximately 1 kb, consistent with the predicted 951 bp

amplified product (FIG. 2B). Other B7-H3 hybridization signals were also detected in mouse embryo, heart and skeletal muscle tissues. Unlike primates, rodent sequences have only the single VC form, due to codon degeneration of putative C₁ and V₂ exons.

[0122] As demonstrated below, both human B7-H3 VC and VCVC forms have similar biological activity in vitro, suggesting that the tandemly duplicated exons are functionally equivalent in cell based assays. Such functional redundancy may explain the tolerance of C₁-V₂ exon loss without an adverse effect in physiology.

Example 3: Comparative genomic analysis of mouse and human B7-H3

[0123] Given that the segmental duplication of VC domains appears to be unique to B7-H3 amongst the B7-family of proteins, we next examined whether the sequence of B7-H3 was distinct from those of other costimulatory ligands based on codon base substitution. One method of determining the rates of molecular evolution is by the measurement of predicted mutation rates between synonymous and nonsynonymous sites within codons. Additional rodent versus primate sequence comparisons were performed for the V and C exons of other known ligands: B7-1, B7-2, GL50, PD-L1, and PD-L2. These ligands share structural similarities in which V and C domains reside in the extracellular portion of each molecule. The presence of shared structural motifs between these disparate molecules have propagated the notion that these molecules were derived from an ancestral sequence bearing V and C sequences. To determine the relative rates of divergence of V and C domains between mouse and humans, relative frequencies of nucleotide substitutions in synonymous and nonsynonymous codons were determined. Calculation of synonymous and nonsynonymous mutation frequencies were performed using Wisconsin Package GCG 10.0 Diverge module (GCG, Madison, WI). For B7-H3 alignments, exons corresponding to human V₁ and C₂ domains were aligned with mouse V and C domains. Of all the V and C domains examined, the ratio of synonymous substitutions vs. nonsynonymous mutations was less than 1 except for the V domain of B7-H3. The B7-H3 molecule had the lowest levels of synonymous mutations ($d_s=0.129$ substitutions per site) of all V

domains while simultaneously also had the lowest levels of nonsynonymous mutations ($d_N=0.026$ substitutions per site) of all C domains. As a result, the B7-H3 molecule is distinct among costimulatory ligands in having the highest $d_N:d_S$ ratio in the V domain while concurrently having the lowest $d_N:d_S$ ratio in the C domain. The cross-domain divergence comparison between human V_2 and mouse V domain revealed a $d_S=0.433$ and $d_N=0.037$, while human C_1 and mouse C domains revealed a $d_S=0.393$ and $d_N=0.034$. The lower synonymous mutation rate seen between human V_1 and mouse V than human V_2 and mouse V implies that human V_1 and mouse V domains are orthologous. Assuming a linear mechanism of sequence evolution, dichotomy of nucleotide substitution rates between adjacent exons of the same molecule indicates that different selection processes occur between the V domains and C domains of B7-H3.

[0124] Based on mathematical models of molecular evolution, cases where synonymous substitution rates are greater than nonsynonymous substitution rates for a particular coding sequence reflects purifying selection. Purifying selection occurs when physiological constraints limits the levels of amino acid variation within the gene product. Purifying selection is evident in all B7-family V and C domains examined with the exception of the B7-H3 V_1 domain. For B7-H3 V_1 , d_S is less than half that of B7-2 V and approximately one eighth that of PD-L1 V. Although B7-H3 V_1 d_N is low, it still exceeds that of B7-H3 V d_S for $d_N:d_S$ ratio of 1.18. Cases in which $d_N:d_S$ ratio is greater than 1 are unusual, and have been attributed to sequences undergoing positive selection for rapidly evolving function. It is also notable that the B7-H3 C_2 exon exhibits a $d_N:d_S$ ratio of 0.063, the lowest of all exons examined, and is from one-fifth to one-fifteenth the levels seen for other C domains examined. Therefore, exons comprising B7-H3 were/are currently being actively maintained in a manner disparate to other costimulatory ligands.

Example 4: B7-H3 VC and VCVC downregulate T cell activation

[0125] Based on the single VC unit observed in rodent B7-H3, we sought to determine whether the B7-H3 VC and VCVC forms found in humans had similar function in cell-based assay. The ability of B7-H3 VC and B7-H3 VCVC to

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downregulate T cell activation was observed for both proliferation and cytokine production levels. The experiments were conducted as follows.

[0126] Human B7-H3 VC or VCVC construct entry vectors were cloned into bicistronic retroviral vectors encoding IRES-GFP. The recipient destination retroviral vectors were originally derived from GFP-RV vector (Ranganath (1998) J. Immunol., 161:3822-3826) and modified for Gateway recombination using a attB1-ccdB-attB2 cassette (Invitrogen). Virus-containing supernatants were generated and used to infect CHO.HLA-DR2 cells as previously described (Carter et al. (2002) Eur. J. Immunol., 32:634-643). CHO.HLA-DR2.B7-H3 VC and CHO.HLA-DR2.B7-H3 VCVC were selected by cell sorting based on GFP expression. A CHO.HLA-DR2 transfectant expressing similar GFP levels was used as a control in cellular assays. Generation of CHO.HLA-DR2.B7.1 and CHO.HLA-DR2.B7.2 has been described previously (Anderson et al. (2000) Nature Medicine, 6(2):211-214). CHO.HLA-DR2 transfectants were fixed in 0.2% paraformaldehyde at room temperature (RT) for 4 min and fixation quenched in 1 M lysine at RT for 4 min. Cells were washed once with PBS, resuspended in culture media (RPMI1640, 10% FCS) and used as antigen presenting cells in T cell proliferation assays.

[0127] Human CD4⁺ T cells were purified by negative selection from peripheral lymphocytes as described previously (Blair et al. (1998) J. Immunol., 160:12-15). CD4⁺ T cells (10⁵ cells/well) were cultured in flat-bottom 96-well plates with paraformaldehyde-fixed CHO.HLA-DR2 transfectants (1.25 x 10⁴ cells/well) in the presence of soluble anti-CD3 antibody (1 µg/ml, UCHT1, Pharmingen, San Diego, CA) and various concentrations of soluble anti-CD28 antibody (CD28.2, Pharmingen). Proliferation was determined by pulsing cultures with 1 Ci [³H]-thymidine per well for the last 5-12 hours of a 72-hour incubation period. To measure cytokine production, supernatants were harvested at 72 hours, and samples assayed by multiplex ELISA screening (Pierce Boston, Woburn, MA).

[0128] In order to determine functional activity of the B7-H3 VC and VCVC forms, the following cell-based assays were conducted. Purified human CD4⁺ T cells were stimulated with paraformaldehyde-treated CHO.HLA-DR2 transfectants in the presence of constant amounts of soluble anti-CD3 antibody and increasing

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concentrations of soluble anti-CD28 antibody. Activation of purified T cells with anti-CD3 in the presence of CHO.HLA-DR2-GFP transfectants resulted in no proliferation; proliferation levels were enhanced in the presence of anti-CD3 and anti-CD28 antibodies (FIGS. 3A-3B); stimulation of T cells with anti-CD3 antibody in the presence of CHO.HLA-DR2.B7-1 or B7-2 resulted in proliferation above the levels obtained with CHO.HLA-DR2-GFP control cells (FIG. 3A). Soluble anti-CD28 antibody enhanced the GFP control, but not the B7-1 and B7-2, responses. In contrast, stimulation of T cells in the presence of B7-H3 VC or B7-H3 VCVC led to decreased proliferative responses (FIG. 3B). With anti-CD3 (1 g/ml) and low costimulation (5 ng/ml of anti-CD28 antibody), cytokine production was significantly reduced upon B7-H3 VC and B7-H3 VCVC stimulation. IL-10 (~81%), TNF- α (~69%), IFN- γ (~85%), and GM-CSF (~65%) levels were dramatically reduced in cultures stimulated with either B7-H3 VC or VCVC cultures relative to GFP controls (FIG. 4). Negligible amounts of IL-1A, IL-2, IL-4, IL-6, and IL-13 were detected in these assay conditions. These findings indicate that neither B7-H3 VC nor B7-H3 VCVC function as a costimulatory molecule and suggest that B7-H3 VC and B7-H3 VCVC cell surface molecules engage receptors on T cells that serve as negative regulators of activation. Addition of anti-CD28 antibody at concentrations as high as 200 ng/ml could only partially rescue the B7-H3 inhibitory effect on proliferation.

Example 5: B7-H3 VC and VCVC downregulate T cell activation

[0129] To further characterize B7-H3 function, experiments were performed on a single (CIS) or a separate (TRANS) surface to determine whether B7-H3 downregulation of T cell responses requires coordinate TCR/B7-H3 receptor engagement. CIS beads contained anti-CD3 antibodies and purified fusion proteins B7-H3 VC-Ig or B7-H3 VCVC-Ig, whereas TRANS beads contained either anti-CD3 antibody and B7-H3 VC-Ig or B7-H3 VCVC-Ig.

[0130] Bead stimulation of T cells was performed as follows. Anti-CD3 (UCHT1, Pharmingen), human B7-H3 VC-Ig, human B7-H3 VCVC-Ig and control Ig were covalently attached to polyurethane-coated tosyl-activated Dynabeads (Dyna, Lake Success, NY). Beads were prepared with a constant sub-optimal anti-CD3

antibody concentration (1 μ g, 20% of the total bound protein) and B7-H3-Ig or control Ig (4 μ g, 80% of total bound protein (Bennett et al. (2003) J. Immunol., 170:711-718). Beads have a binding capacity of 5 μ g per 10^7 beads. CIS beads contain both anti-CD3 and B7-H3 on the same bead, TRANS beads consist of two types of beads, one containing anti-CD3 antibody and the other containing B7-H3-Ig (Bennett et al. (2003) J. Immunol., 170:711-718). To maintain equal bead-to-cell ratios under CIS and TRANS conditions, beads coated with control IgG were added to CIS cultures. Protein-coated beads were added to purified CD4+ T cells (10^5 cells/well) in flat bottomed 96-well microtiter plates at a ratio of 1:1. Proliferation was determined by pulsing cultures with 1 Ci [3 H]-thymidine per well for the last 6-16 hour of a 72-hour incubation period.

[0131] As shown in FIG. 5, inhibition of proliferation was only observed when cells were activated with CIS beads. Altogether, these findings suggest that the B7-H3 receptor and the TCR need to be in close proximity for the downregulation of T cell activation. These data suggest that for the B7-H3 receptor pathway to modulate a T cell response, both activating and inhibitory signals must emanate from the same cell.

[0132] The amount of cytokines in the supernatants was measured at 72 hours using multiplex ELISA screening: TNF- α (FIG. 6A), IFN- γ (FIG. 6B), and GM-CSF (FIG. 6C). The ability of B7-H3 VC and B7-H3 VCVC to downregulate T cell activation was assessed by cytokine production levels. With anti-CD3 (1 g/ml) and low costimulation (5 ng/ml anti-CD28 antibody), cytokine production was significantly reduced upon B7-H3 VC and B7-H3 VCVC stimulation (FIGS. 6A-6C). IL-10 (~81%), TNF- α (~69%), IFN- γ (~85%), and GM-CSF (~65%) levels were dramatically reduced in cultures stimulated with either B7-H3 VC or VCVC cultures relative to GFP controls. Negligible amounts of IL-1A, IL-2, IL-4, IL-6, and IL-13 were detected in these assay conditions. These findings indicate that neither B7-H3 VC nor B7-H3 VCVC function as a costimulatory molecule and suggest that B7-H3 VC and B7-H3 VCVC cell surface molecules engage receptors on T-cells that serve as negative regulators of activation.

[0133] Results show that TCR/B7H3 (VC or VCVC) activation of T cells leads to downregulation of T cell responses. Proliferation and cytokine production

is decreased in TCR/B7-H3 activated T cells relative to cells activated by TCR alone. This data suggests that engagement of the B7-H3 receptor on T cell delivers a negative signal. Results also suggest that physical proximity between TCR and the B7-H3 receptor may be required in order to downregulate T cell activation via the B7-H3 receptor.

[0134] The ability of B7-H3 VC and VCVC to downregulate CD4⁺ T-cell activation is reminiscent of negative signals produced by engagement of CTLA4 by either of B7-proteins or by PD-1 by either of the PD-L1 and PD-L2 proteins. Furthermore, the experiments with B7-H3 coupled to a solid matrix indicates that both TCR and B7-H3 receptor signals are delivered by the same cell. Similar requirements have been described for negative signaling by either CTLA-4 or PD-1 (Griffin et al. (2000) J. Immunol., 164:4433; and Bennett et al. (2003) J. Immunol., 170:711-718). Finally, both human B7-H3 VC and human B7-H3 VCVC molecules appear to be redundant in their ability to modulate CD4 T-cell responses.

Example 6: Therapeutic efficacy in psoriasis patients

[0135] Modulation of immune response regulated by B7-H3 is useful in instances where an immunosuppressive effect or augmentation of immune response is desired. B7-H3 agonists (e.g., soluble forms of B7-H3) may be used to prevent and/or to reduce severity and/or symptoms of diseases or conditions that involve an aberrantly elevated immune response, including response to self antigens as, for example, in autoimmune disorders. B7-H3 antagonists, on the other hand, may be administered to subjects having an undesirably low immune response as for example it may occur in cancers or immunosuppressive disorders.

[0136] Psoriasis is considered to a typical T-cell-mediated autoimmune disease. Psoriasis is a chronic inflammatory skin disease mediated, in part, through IFN- γ production by activated lesional T cells (Th₁ skewed).

[0137] Most commonly, soluble proteins are administered in an outpatient setting by weekly administration at 0.1-10 mg/kg dose by slow intravenous (IV) infusion. The appropriate therapeutically effective dose of an antagonist is selected by a treating clinician and would range approximately from 1 μ g/kg to 20 mg/kg,

from 1 µg/kg to 10 mg/kg, from 1 µg/kg to 1 mg/kg, from 10 µg/kg to 1 mg/kg, from 10 µg/kg to 100 µg/kg, from 100 µg/kg to 1 mg/kg, and from 500 µg/kg to 5 mg/kg.

[0138] To evaluate the effects on skin T cells, an antibody against B7-H3, an antibody against a B7-H3 receptor, or B7-H3-Ig is administered for 12 consecutive weeks to randomized groups of psoriasis patients with normalized disease severity (minimum PASI (Psoriasis Activity and Severity Index) score of 12). To assess clinical improvement in patients over time and to monitor their response to therapy, the PASI scoring system can be used (Fredriksson et al. (1978) *Dermatologica*, 157:238-244; and Marks et al. (1989) *Arch. Dermatol.*, 1989; 125:235-240).

[0139] In brief, elements of the PASI score include: (1) body regions affected as percent of body surface area (BSA); (2) extent to which body region is affected (on a scale of 1-10); and (3) extent of psoriatic changes (erythema, infiltration, desquamation) on a scale of 0-4). PASI score is calculated as follows: $((0.1 \times (\text{erythema head}) + (\text{infiltration head}) + (\text{desquamation head})) \times (\text{extent of head affected})) + ((0.2 \times ((\text{erythema trunk}) + (\text{infiltration trunk}) + (\text{desquamation trunk})) \times (\text{extent of trunk affected})) + ((0.3 \times ((\text{erythema upper extremities}) + (\text{infiltration upper extremities}) + (\text{desquamation upper extremities})) \times (\text{extent of upper extremities affected})) + ((0.4 \times ((\text{erythema lower extremities}) + (\text{infiltration lower extremities}) + (\text{desquamation lower extremities})) \times (\text{extent of lower extremities affected}))$. The minimum score is 0, while the maximum score = 72. A reduction in PASI score is indicative of the effective treatment. It is anticipated that at least 50% of individuals receiving the treatment would exhibit a reduction in PASI score and an improvement in their condition.

[0140] The specification is most thoroughly understood in light of the teachings of the references cited within the specification which are hereby incorporated by reference. The embodiments within the specification provide an illustration of embodiments of the invention and should not be construed to limit the scope of the invention. The skilled artisan readily recognizes that many other embodiments are encompassed by the invention. All publications and patents cited and sequences identified by accession or database reference numbers in this disclosure are incorporated by reference in their entirety. To the extent the material

incorporated by reference contradicts or is inconsistent with the present specification, the present specification will supercede any such material. The citation of any references herein is as not an admission that such references are prior art to the present invention.

[0141] Unless otherwise indicated, all numbers expressing quantities of ingredients, cell culture, treatment conditions, and so forth used in the specification, including claims, are to be understood as being modified in all instances by the term "about." Accordingly, unless otherwise indicated to the contrary, the numerical parameters are approximations and may vary depending upon the desired properties sought to be obtained by the present invention. Unless otherwise indicated, the term "at least" preceding a series of elements is to be understood to refer to every element in the series. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

We claim:

1. A method of inhibiting activation of a lymphocyte, the method comprising contacting the lymphocyte with a B7-H3 agonist and allowing the agonist to inhibit the activation of the lymphocyte.
2. The method as in claim 1, wherein the B7-H3 agonist is a soluble form of B7-H3.
3. The method as in claim 3, wherein the B7-H3 agonist comprises SEQ ID NO:15.
4. The method as in claim 2, wherein the soluble form comprises at least one V domain of B7-H3.
5. The method as in claim 4, wherein the V domain comprises: (a) SEQ ID NO:7 or (b) an amino acid sequence which is substantially identical to SEQ ID NO:7.
6. The method as in claim 4, wherein the soluble form of B7-H3 further comprises at least one C domain of B7-H3.
7. The method as in claim 4, wherein the soluble form of B7-H3 further comprises an Fc region of an antibody.
8. The method as in claim 7, wherein the soluble form of B7-H3 comprises: (a) an amino acid sequence chosen from SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, or SEQ ID NO:22; or (b) an amino acid sequence which is substantially identical to at least one of the sequences chosen from SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, or SEQ ID NO:22.
9. The method as in claim 7, wherein the soluble form of B7-H3 comprises: (a) an amino acid sequence chosen from SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:14; or (b) an amino acid sequence which is substantially identical to at least one of the sequences chosen from SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:14.
10. The method as in claim 3, wherein the B7-H3 agonist is coupled with a primary stimulatory molecule.

11. The method as in claim 10, wherein the soluble form of B7-H3 and the primary stimulatory molecule are spaced by no more than 100 μm .
12. The method as in claim 1, wherein the B7-H3 antagonist is a nucleic acid encoding amino acid of SEQ ID NO:15.
13. A method of enhancing activation of a lymphocyte, the method comprising contacting the lymphocyte with a B7-H3 antagonist and allowing the antagonist to enhance the activation of the lymphocyte.
14. The method as in claim 13, wherein the lymphocyte is human.
15. The method as in claim 13, wherein the B7-H3 antagonist is an antibody to B7-H3 or an antibody against a B7-H3 receptor.
16. The method as in claim 13, wherein the B7-H3 antagonist is an antisense nucleic acid or a siRNA.
17. The method as any one of claims 1 through 16, wherein the lymphocyte is a T cell.
18. The method as in claims 17, wherein the T cell is a CD4^+ T cell.
19. The method as any one of claims 1 through 16, wherein the lymphocyte is in a mammal.
20. The method as in claim 19, wherein the mammal is afflicted with or is at risk for at least one of: an immunologic disorder, a cancer, or an infectious disease.
21. The method as in claim 19, wherein the mammal is treated with Factor VIII or Factor IX.

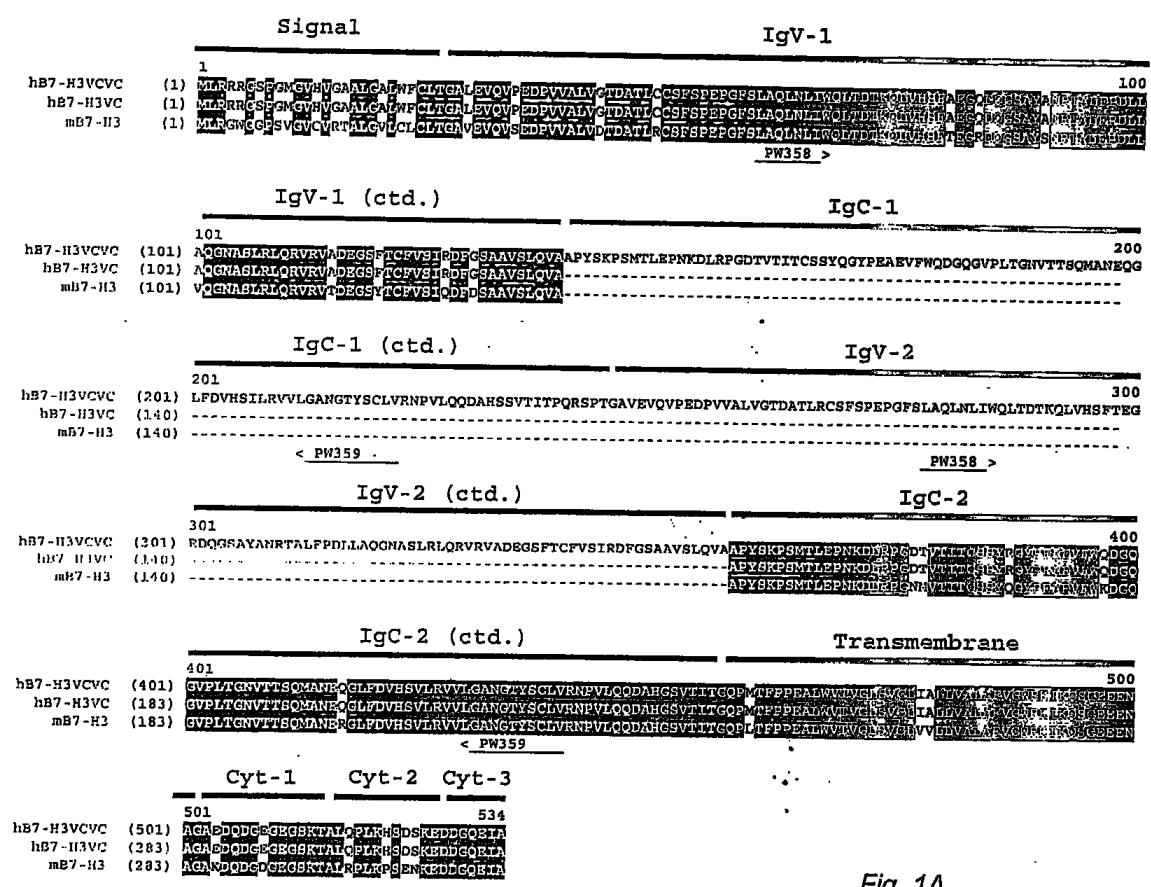


Fig. 1A

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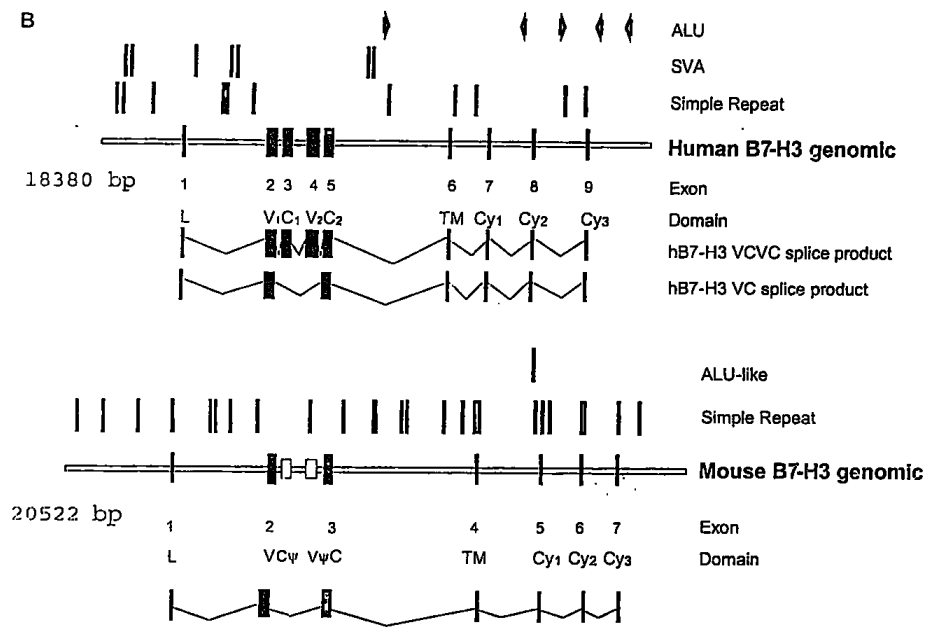


Fig. 1B

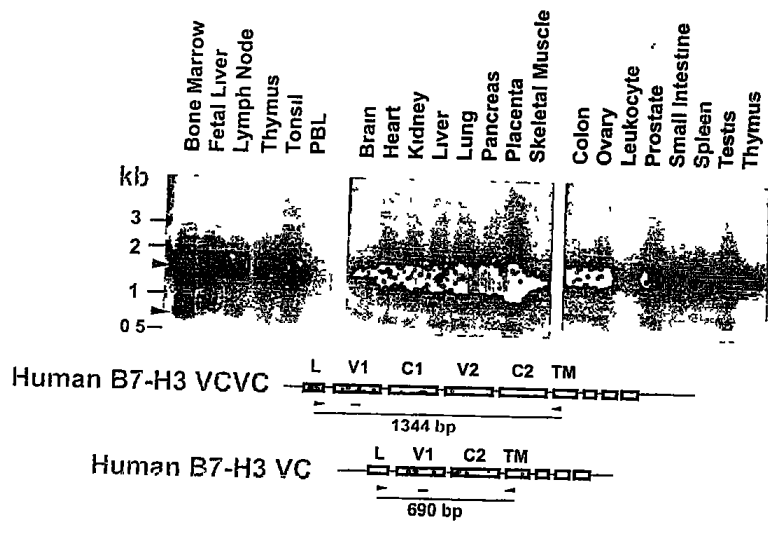


Fig 2A

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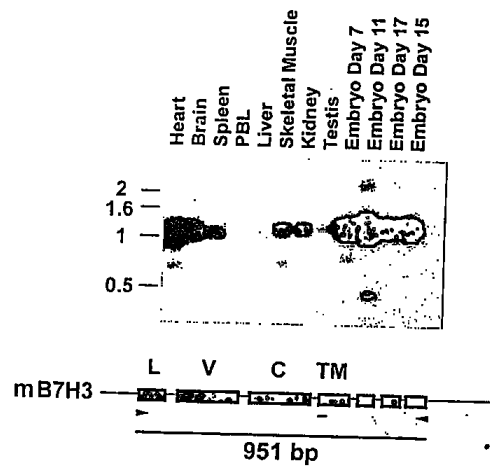


Fig. 2B

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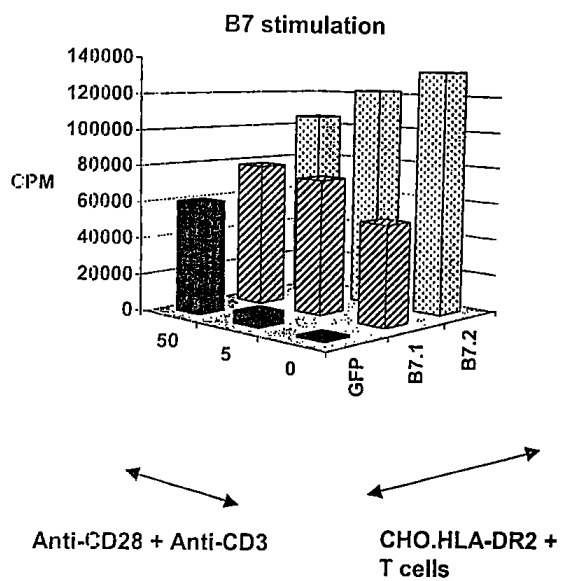


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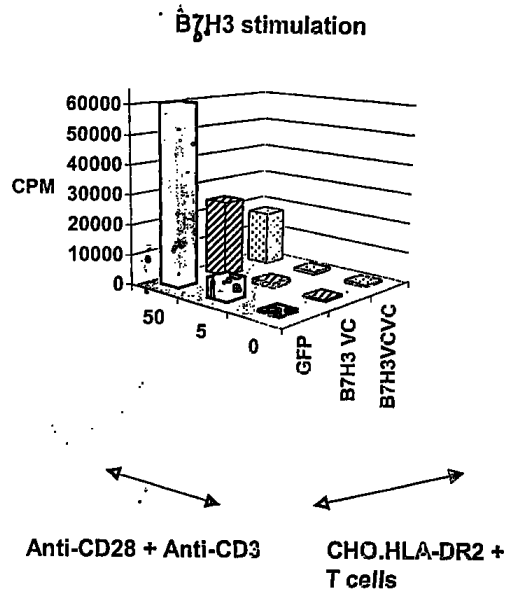


Fig. 3B

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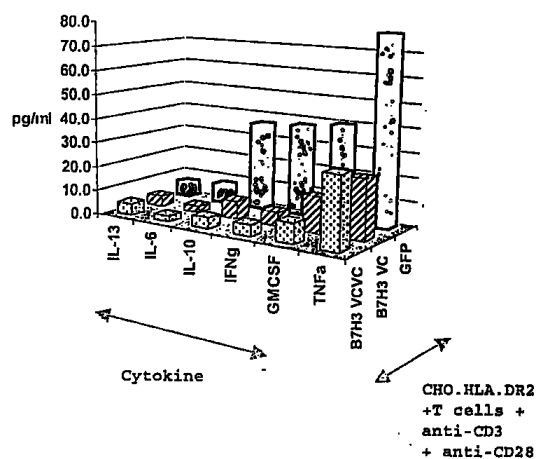


Fig. 4

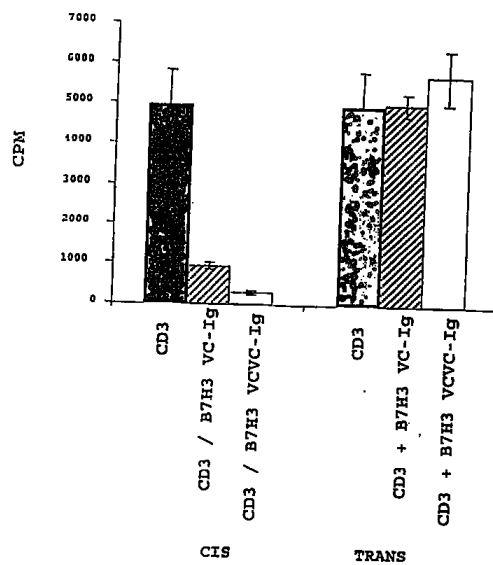


Fig. 5

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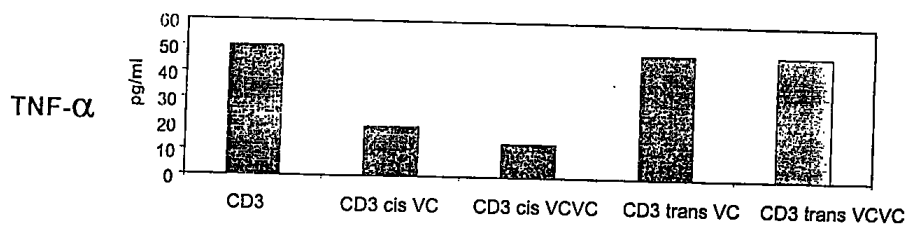


Fig. 6A

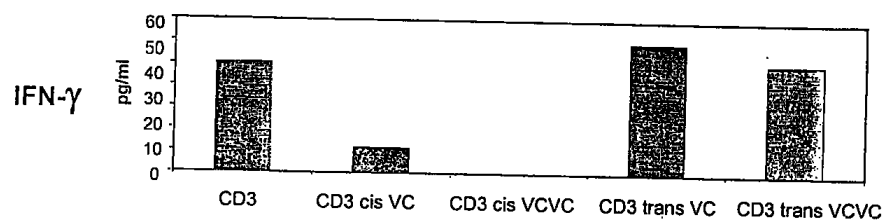


Fig. 6B

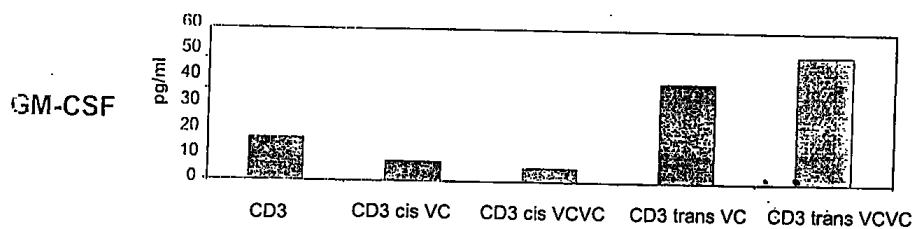


Fig. 6C

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cctctgaaac cctctgaaaa caaagaagat gacggacaag aaattgcttg a 951

<210> 4
<211> 316
<212> PRT
<213> Mouse

<400> 4

Met Leu Arg Gly Trp Gly Gly Pro Ser Val Gly Val Cys Val Arg Thr
1 5 10 15

Ala Leu Gly Val Leu Cys Leu Cys Leu Thr Gly Ala Val Glu Val Gln
20 25 30

Val Ser Glu Asp Pro Val Val Ala Leu Val Asp Thr Asp Ala Thr Leu
35 40 45

Arg Cys Ser Phe Ser Pro Glu Pro Gly Phe Ser Leu Ala Gln Leu Asn
50 55 60

Leu Ile Trp Gln Leu Thr Asp Thr Lys Gln Leu Val His Ser Phe Thr
65 70 75 80

Glu Gly Arg Asp Gln Gly Ser Ala Tyr Ser Asn Arg Thr Ala Leu Phe
85 90 95

Pro Asp Leu Leu Val Gln Gly Asn Ala Ser Leu Arg Leu Gln Arg Val
100 105 110

Arg Val Thr Asp Glu Gly Ser Tyr Thr Cys Phe Val Ser Ile Gln Asp
115 120 125

Phe Asp Ser Ala Ala Val Ser Leu Gln Val Ala Ala Pro Tyr Ser Lys
130 135 140

Pro Ser Met Thr Leu Glu Pro Asn Lys Asp Leu Arg Pro Gly Asn Met
145 150 155 160

Val Thr Ile Thr Cys Ser Ser Tyr Gln Gly Tyr Pro Glu Ala Glu Val
165 170 175

Phe Trp Lys Asp Gly Gln Gly Val Pro Leu Thr Gly Asn Val Thr Thr
180 185 190

Ser Gln Met Ala Asn Glu Arg Gly Leu Phe Asp Val His Ser Val Leu
195 200 205

Arg Val Val Leu Gly Ala Asn Gly Thr Tyr Ser Cys Leu Val Arg Asn
210 215 220

Pro Val Leu Gln Gln Asp Ala His Gly Ser Val Thr Ile Thr Gly Gln
225 230 235 240

Pro Leu Thr Phe Pro Pro Glu Ala Leu Trp Val Thr Val Gly Leu Ser
 245 250 255

Val Cys Leu Val Val Leu Leu Val Ala Leu Ala Phe Val Cys Trp Arg
 260 265 270

Lys Ile Lys Gln Ser Cys Glu Glu Glu Asn Ala Gly Ala Glu Asp Gln
 275 280 285

Asp Gly Asp Gly Glu Gly Ser Lys Thr Ala Leu Arg Pro Leu Lys Pro
 290 295 300

Ser Glu Asn Lys Glu Asp Asp Gly Gln Glu Ile Ala
 305 310 315

<210> 5
 <211> 1605
 <212> DNA
 <213> Homo sapiens

<400> 5
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 ctggtgggca ccgatgccac cctgtgctgc tccttctccc ctgagcctgg cttcagcctg 180
 gcacagctca acctcatctg gcagctgaca gatacctaac agctggtgca cagctttgct 240
 gagggccagg accagggcag cgcctatgcc aaccgcacgg cctcttctcc ggacctgctg 300
 gcacagggca acgcattcct gaggtgcag cgcgtgcgtg tggcggacca gggcagcttc 360
 acctgcttcg tgagcatccg ggatttcggc agcgtgccc tcagcctgca ggtggccgct 420
 cctactcga agcccagcat gaccctggag cccaacaagg acctgcggcc aggggacacg 480

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gcaggagctg aggaccagga tggggaggga gaaggctcca agacagccct gcagcctctg 1560
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67

<211> 534
<212> PRT
<213> Homo sapiens

<400> 6

Met Leu Arg Arg Arg Gly Ser Pro Gly Met Gly Val His Val Gly Ala
1 5 10 15

Ala Leu Gly Ala Leu Trp Phe Cys Leu Thr Gly Ala Leu Glu Val Gln
20 25 30

Val Pro Glu Asp Pro Val Val Ala Leu Val Gly Thr Asp Ala Thr Leu
35 40 45

Cys Cys Ser Phe Ser Pro Glu Pro Gly Phe Ser Leu Ala Gln Leu Asn
50 55 60

Leu Ile Trp Gln Leu Thr Asp Thr Lys Gln Leu Val His Ser Phe Ala
65 70 75 80

Glu Gly Gln Asp Gln Gly Ser Ala Tyr Ala Asn Arg Thr Ala Leu Phe
85 90 95

Pro Asp Leu Leu Ala Gln Gly Asn Ala Ser Leu Arg Leu Gln Arg Val
100 105 110

Arg Val Ala Asp Glu Gly Ser Phe Thr Cys Phe Val Ser Ile Arg Asp
115 120 125

Phe Gly Ser Ala Ala Val Ser Leu Gln Val Ala Ala Pro Tyr Ser Lys
130 135 140

68

Pro Ser Met Thr Leu Glu Pro Asn Lys Asp Leu Arg Pro Gly Asp Thr
145 150 155 160

Val Thr Ile Thr Cys Ser Ser Tyr Gln Gly Tyr Pro Glu Ala Glu Val
165 170 175

Phe Trp Gln Asp Gly Gln Gly Val Pro Leu Thr Gly Asn Val Thr Thr
180 185 190

Ser Gln Met Ala Asn Glu Gln Gly Leu Phe Asp Val His Ser Ile Leu
195 200 205

Arg Val Val Leu Gly Ala Asn Gly Thr Tyr Ser Cys Leu Val Arg Asn
210 215 220

Pro Val Leu Gln Gln Asp Ala His Ser Ser Val Thr Ile Thr Pro Gln
225 230 235 240

Arg Ser Pro Thr Gly Ala Val Glu Val Gln Val Pro Glu Asp Pro Val
245 250 255

Val Ala Leu Val Gly Thr Asp Ala Thr Leu Arg Cys Ser Phe Ser Pro
260 265 270

Glu Pro Gly Phe Ser Leu Ala Gln Leu Asn Leu Ile Trp Gln Leu Thr
275 280 285

Asp Thr Lys Gln Leu Val His Ser Phe Thr Glu Gly Arg Asp Gln Gly
290 295 300

Ser Ala Tyr Ala Asn Arg Thr Ala Leu Phe Pro Asp Leu Leu Ala Gln
305 310 315 320

Gly Asn Ala Ser Leu Arg Leu Gln Arg Val Arg Val Ala Asp Glu Gly
325 330 335

Ser Phe Thr Cys Phe Val Ser Ile Arg Asp Phe Gly Ser Ala Ala Val
340 345 350

Ser Leu Gln Val Ala Ala Pro Tyr Ser Lys Pro Ser Met Thr Leu Glu
355 360 365

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

Ser Tyr Arg Gly Tyr Pro Glu Ala Glu Val Phe Trp Gln Asp Gly Gln
385 390 395 400

Gly Val Pro Leu Thr Gly Asn Val Thr Thr Ser Gln Met Ala Asn Glu
405 410 415

Gln Gly Leu Phe Asp Val His Ser Val Leu Arg Val Val Leu Gly Ala
420 425 430

Asn Gly Thr Tyr Ser Cys Leu Val Arg Asn Pro Val Leu Gln Gln Asp
435 440 445

Ala His Gly Ser Val Thr Ile Thr Gly Gln Pro Met Thr Phe Pro Pro
450 455 460

Glu Ala Leu Trp Val Thr Val Gly Leu Ser Val Cys Leu Ile Ala Leu

465

470

475

480

Leu Val Ala Leu Ala Phe Val Cys Trp Arg Lys Ile Lys Gln Ser Cys
485 490 495

Glu Glu Glu Asn Ala Gly Ala Glu Asp Gln Asp Gly Glu Gly Glu Gly
500 505 510

Ser Lys Thr Ala Leu Gln Pro Leu Lys His Ser Asp Ser Lys Glu Asp
515 520 525

Asp Gly Gln Glu Ile Ala
530

<210> 7

<211> 112

<212> PRT

<213> Homo sapience

<400> 7

Ala Leu Glu Val Gln Val Pro Glu Asp Pro Val Val Ala Leu Val Gly
1 5 10 15

Thr Asp Ala Thr Leu Cys Cys Ser Phe Ser Pro Glu Pro Gly Phe Ser
20 25 30

Leu Ala Gln Leu Asn Leu Ile Trp Gln Leu Thr Asp Thr Lys Gln Leu
35 40 45

Val His Ser Phe Ala Glu Gly Gln Asp Gln Gly Ser Ala Tyr Ala Asn
50 55 60

Arg Thr Ala Leu Phe Pro Asp Leu Leu Ala Gln Gly Asn Ala Ser Leu
65 70 75 80

Arg Leu Gln Arg Val Arg Val Ala Asp Glu Gly Ser Phe Thr Cys Phe
85 90 95

Val Ser Ile Arg Asp Phe Gly Ser Ala Ala Val Ser Leu Gln Val Ala
100 105 110

<210> 8

<211> 112

<212> PRT

<213> Homo sapiens

<220>

<221> MISC_FEATURE

<222> (53)..(53)

<223> A, or T, or any other amino acid

<220>

<221> MISC_FEATURE

<222> (56)..(56)

<223> Q, or R, or any other amino acid

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> L, or V, or any other amino acid

<220>

<221> MISC_FEATURE

<222> (22)..(22)

<223> C, or R, or any other amino acid

<400> 8

Ala Xaa Glu Val Gln Val Pro Glu Asp Pro Val Val Ala Leu Val Gly
 1 5 10 15

Thr Asp Ala Thr Leu Xaa Cys Ser Phe Ser Pro Glu Pro Gly Phe Ser
 20 25 30

Leu Ala Gln Leu Asn Leu Ile Trp Gln Leu Thr Asp Thr Lys Gln Leu
 35 40 45

Val His Ser Phe Xaa Glu Gly Xaa Asp Gln Gly Ser Ala Tyr Ala Asn
 50 55 60

Arg Thr Ala Leu Phe Pro Asp Leu Leu Ala Gln Gly Asn Ala Ser Leu
 65 70 75 80

Arg Leu Gln Arg Val Arg Val Ala Asp Glu Gly Ser Phe Thr Cys Phe
 85 90 95

Val Ser Ile Arg Asp Phe Gly Ser Ala Ala Val Ser Leu Gln Val Ala
 100 105 110

<210> 9

<211> 1670

<212> DNA

<213> Chimera

<400> 9

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accgatgcca ccctgtgctg ctctttctcc cctgagcctg gcttcagcct ggcacagctc 180
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gaccagggca gcgcctatgc caaccgcacg gccctcttcc eggacctgct ggcacagggc 300
aacgcatccc tgaggctgca gcgcgtgcgt gtggcggacg agggcagctt cacctgcttc 360
gtgagcatcc gggatttcgg cagcgtgcc gtcagcctgc aggtggccgc tccctactcg 420
aagcccagca tgaccctgga gccaacaag gacctgcggc caggggacac ggtgaccatc 480
acgtgctcca gctaccgggg ctaccctgag gctgaggtgt tctggcagga tgggcagggt 540
gtgcccctga ctggcaacgt gaccacgtcg cagatggcca acgagcaggg cttgtttgat 600
gtgcacagcg tcttgccgggt ggtgctgggt gcgaatggca cctacagctg cctgggtgcgc 660
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ttccccccag aggcagggtc ggggtccgag cccgcggac cgacaatcaa gccctgtcct 780
ccatgcaa at gcccaggtaa gtcactagac cagagctcca ctcccgggag aatggtaagt 840
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tgtactcatg atctccctga gcccacatag cacatgtgtg gtggtggatg tgagcgagga 1020
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acaaacccat agagaggatt acaacagtac tctccgggtg gtcagtgcc tcccatcca 1140
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ggggctggga tgggcataag gataaaggct tgtgtggaca gccttctgct tcagccatga 1320
cctttgtgta tgtttctacc ctcacagggt cagtaagagc tccacaggta tatgtcttgc 1380

74

ctccaccaga agaagagatg actaagaaac aggtcactct gacctgcatg gtcacagact 1440
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 tggaaaagaa gaactgggtg gaaagaaata gctactcctg ttcagtgggc cacgaggggc 1620
 tgcacaatca ccacacgact aagagcttct cccggactcc gggtaaata 1670

<210> 10

<211> 482

<212> PRT

<213> Chimera

<400> 10

Met Gly Val Leu Leu Thr Gln Arg Thr Leu Leu Ser Leu Val Leu Ala
 1 5 10 15

Leu Leu Phe Pro Ser Met Ala Ser Met Leu Glu Val Gln Val Pro Glu
 20 25 30

Asp Pro Val Val Ala Leu Val Gly Thr Asp Ala Thr Leu Cys Cys Ser
 35 40 45

Phe Ser Pro Glu Pro Gly Phe Ser Leu Ala Gln Leu Asn Leu Ile Trp
 50 55 60

Gln Leu Thr Asp Thr Lys Gln Leu Val His Ser Phe Ala Glu Gly Gln
 65 70 75 80

Asp Gln Gly Ser Ala Tyr Ala Asn Arg Thr Ala Leu Phe Pro Asp Leu
 85 90 95

75

Leu Ala Gln Gly Asn Ala Ser Leu Arg Leu Gln Arg Val Arg Val Ala
100 105 110

Asp Glu Gly Ser Phe Thr Cys Phe Val Ser Ile Arg Asp Phe Gly Ser
115 120 125

Ala Ala Val Ser Leu Gln Val Ala Ala Pro Tyr Ser Lys Pro Ser Met
130 135 140

Thr Leu Glu Pro Asn Lys Asp Leu Arg Pro Gly Asp Thr Val Thr Ile
145 150 155 160

Thr Cys Ser Ser Tyr Arg Gly Tyr Pro Glu Ala Glu Val Phe Trp Gln
165 170 175

Asp Gly Gln Gly Val Pro Leu Thr Gly Asn Val Thr Thr Ser Gln Met
180 185 190

Ala Asn Glu Gln Gly Leu Phe Asp Val His Ser Val Leu Arg Val Val
195 200 205

Leu Gly Ala Asn Gly Thr Tyr Ser Cys Leu Val Arg Asn Pro Val Leu
210 215 220

Gln Gln Asp Ala His Gly Ser Val Thr Ile Thr Gly Gln Pro Met Thr
225 230 235 240

Phe Pro Pro Glu Ala Gly Ser Gly Ser Glu Pro Arg Gly Pro Thr Ile
245 250 255

Lys Pro Cys Pro Pro Cys Lys Cys Pro Ala Pro Asn Leu Glu Gly Gly
 260 265 270

Pro Ser Val Phe Ile Phe Pro Pro Lys Ile Lys Asp Val Leu Met Ile
 275 280 285

Ser Leu Ser Pro Ile Val Thr Cys Val Val Val Asp Val Ser Glu Asp
 290 295 300

Asp Pro Asp Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu Val His
 305 310 315 320

Thr Ala Gln Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Leu Arg
 325 330 335

Val Val Ser Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly Lys
 340 345 350

Ala Phe Ala Cys Ala Val Asn Asn Lys Asp Leu Pro Ala Pro Ile Glu
 355 360 365

Arg Thr Ile Ser Lys Pro Lys Gly Ser Val Arg Ala Pro Gln Val Tyr
 370 375 380

Val Leu Pro Pro Pro Glu Glu Glu Met Thr Lys Lys Gln Val Thr Leu
 385 390 395 400

Thr Cys Met Val Thr Asp Phe Met Pro Glu Asp Ile Tyr Val Glu Trp
 405 410 415

77

Thr Asn Asn Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr Glu Pro Val
420 425 430

Leu Asp Ser Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg Val Glu
435 440 445

Lys Lys Asn Trp Val Glu Arg Asn Ser Tyr Ser Cys Ser Val Val His
450 455 460

Glu Gly Leu His Asn His His Thr Thr Lys Ser Phe Ser Arg Thr Pro
465 470 475 480

Gly Lys

<210> 11

<211> 2324

<212> DNA

<213> Chimera

<400> 11

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accgatgcca cctgtgtctg ctctttctcc cctgagcctg gcttcagcct ggcacagctc	180
aacctcatct ggcagctgac agataccaaa cagctgggtgc acagctttgc' tgagggccag	240
gaccagggca gcgcctatgc caaccgcacg gccctcttcc cggacctgct ggcacagggc	300
aacgcattcc tgaggctgca gcgcgtgcgt gtgggggacg aggcacagctt cacctgcttc	360
ctgagcatcc gggatttcgg cagcgtctgc gttagcctgc aggtggcgcg tcctactcg	420
aagcccagca tgaccctgga gcccaacaag gaactgcggc caggggacac ggtgaccatc	480

acgtgctcca gctaccaggg ctacctgag gctgaggtgt tctggcagga tgggcaggg 540
gtgcccctga ctggcaacgt gaccacgtcg cagatggcca acgagcaggg cttgtttgat 600
gtgcacagca tcttgccgggt ggtgctgggt gcaaatggca cctacagctg cctgggtgcgc 660
aaccctgtgc tgcagcagga tgcgcacagc tctgtcacca tcacacccca gagaagcccc 720
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79

agatgtccag atcagctggt ttgtgaacaa cgtggaagta cacacagctc agacacaaac 1740
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 agaagaactg ggtggaaaga aatagctact cctgttcagt ggtccacgag ggtctgcaca 2280
 atcaccacac gactaagagc ttctcccgga ctccgggtaa atga 2324

<210> 12
 <211> 700
 <212> PRT
 <213> Chimera

<400> 12

Met Gly Val Leu Leu Thr Gln Arg Thr Leu Leu Ser Leu Val Leu Ala
 1 5 10 15

Leu Leu Phe Pro Ser Met Ala Ser Met Leu Glu Val Gln Val Pro Glu
 20 25 30

Asp Pro Val Val Ala Leu Val Gly Thr Asp Ala Thr Leu Cys Cys Ser
 35 40 45

Phe Ser Pro Glu Pro Gly Phe Ser Leu Ala Gln Leu Asn Leu Ile Trp
50 55 60

Gln Leu Thr Asp Thr Lys Gln Leu Val His Ser Phe Ala Glu Gly Gln
65 70 75 80

Asp Gln Gly Ser Ala Tyr Ala Asn Arg Thr Ala Leu Phe Pro Asp Leu
85 90 95

Leu Ala Gln Gly Asn Ala Ser Leu Arg Leu Gln Arg Val Arg Val Ala
100 105 110

Asp Glu Gly Ser Phe Thr Cys Phe Val Ser Ile Arg Asp Phe Gly Ser
115 120 125

Ala Ala Val Ser Leu Gln Val Ala Ala Pro Tyr Ser Lys Pro Ser Met
130 135 140

Thr Leu Glu Pro Asn Lys Asp Leu Arg Pro Gly Asp Thr Val Thr Ile
145 150 155 160

Thr Cys Ser Ser Tyr Gln Gly Tyr Pro Glu Ala Glu Val Phe Trp Gln
165 170 175

Asp Gly Gln Gly Val Pro Leu Thr Gly Asn Val Thr Thr Ser Gln Met
180 185 190

Ala Asn Glu Gln Gly Leu Phe Asp Val His Ser Ile Leu Arg Val Val
195 200 205

Leu Gly Ala Asn Gly Thr Tyr Ser Cys Leu Val Arg Asn Pro Val Leu
210 215 220

Gln Gln Asp Ala His Ser Ser Val Thr Ile Thr Pro Gln Arg Ser Pro
225 230 235 240

Thr Gly Ala Val Glu Val Gln Val Pro Glu Asp Pro Val Val Ala Leu
245 250 255

Val Gly Thr Asp Ala Thr Leu Arg Cys Ser Phe Ser Pro Glu Pro Gly
260 265 270

Phe Ser Leu Ala Gln Leu Asn Leu Ile Trp Gln Leu Thr Asp Thr Lys
275 280 285

Gln Leu Val His Ser Phe Thr Glu Gly Arg Asp Gln Gly Ser Ala Tyr
290 295 300

Ala Asn Arg Thr Ala Leu Phe Pro Asp Leu Leu Ala Gln Gly Asn Ala
305 310 315 320

Ser Leu Arg Leu Gln Arg Val Arg Val Ala Asp Glu Gly Ser Phe Thr
325 330 335

Cys Phe Val Ser Ile Arg Asp Phe Gly Ser Ala Ala Val Ser Leu Gln
340 345 350

Val Ala Ala Pro Tyr Ser Lys Pro Ser Met Thr Leu Glu Pro Asn Lys
355 360 365

Asp Leu Arg Pro Gly Asp Thr Val Thr Ile Thr Cys Ser Ser Tyr Arg

370 375 380

Gly Tyr Pro Glu Ala Glu Val Phe Trp Gln Asp Gly Gln Gly Val Pro
385 390 395 400

Leu Thr Gly Asn Val Thr Thr Ser Gln Met Ala Asn Glu Gln Gly Leu
405 410 415

Phe Asp Val His Ser Val Leu Arg Val Val Leu Gly Ala Asn Gly Thr
420 425 430

Tyr Ser Cys Leu Val Arg Asn Pro Val Leu Gln Gln Asp Ala His Gly
435 440 445

Ser Val Thr Ile Thr Gly Gln Pro Met Thr Phe Pro Pro Glu Ala Gly
450 455 460

Ser Gly Ser Glu Pro Arg Gly Pro Thr Ile Lys Pro Cys Pro Pro Cys
465 470 475 480

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

Pro Pro Lys Ile Lys Asp Val Leu Met Ile Ser Leu Ser Pro Ile Val
500 505 510

Thr Cys Val Val Val Asp Val Ser Glu Asp Asp Pro Asp Val Gln Ile
515 520 525

Ser Trp Phe Val Asn Asn Val Glu Val His Thr Ala Gln Thr Gln Thr
530 535 540

His Arg Glu Asp Tyr Asn Ser Thr Leu Arg Val Val Ser Ala Leu Pro
545 550 555 560

Ile Gln His Gln Asp Trp Met Ser Gly Lys Ala Phe Ala Cys Ala Val
565 570 575

Asn Asn Lys Asp Leu Pro Ala Pro Ile Glu Arg Thr Ile Ser Lys Pro
580 585 590

Lys Gly Ser Val Arg Ala Pro Gln Val Tyr Val Leu Pro Pro Pro Glu
595 600 605

Glu Glu Met Thr Lys Lys Gln Val Thr Leu Thr Cys Met Val Thr Asp
610 615 620

Phe Met Pro Glu Asp Ile Tyr Val Glu Trp Thr Asn Asn Gly Lys Thr
625 630 635 640

Glu Leu Asn Tyr Lys Asn Thr Glu Pro Val Leu Asp Ser Asp Gly Ser
645 650 655

Tyr Phe Met Tyr Ser Lys Leu Arg Val Glu Lys Lys Asn Trp Val Glu
660 665 670

Arg Asn Ser Tyr Ser Cys Ser Val Val His Glu Gly Leu His Asn His
675 680 685

His Thr Thr Lys Ser Phe Ser Arg Thr Pro Gly Lys
690 695 700

<210> 13

<211> 1670

<212> DNA

<213> Mouse

<400> 13

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acggatgcca ccctacgctg ctcccttttcc ccagagcctg gcttcagtct ggcacagctc 180

aacctcatct ggcagctgac agacaccaaa cagctggtgc acagcttcac ggagggccgg 240

gaccaaggca gtgcctactc caaccgcaca gcgctcttcc ctgacctgtt ggtgcaaggc 300

aatgcgtcct tgaggctgca gcgcgtccga gtaaccgacg agggcagcta cacctgcttt 360

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gctataaaca tccttgcaact agaggataag ccatgtacag atccatttcc atctctctc 900

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85

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 <211> 482
 <212> PRT
 <213> Mouse

<400> 14

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 1 5 10 15

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 20 25 30

Asp Pro Val Val Ala Leu Val Asp Thr Asp Ala Thr Leu Arg Cys Ser
 35 40 45

Phe Ser Pro Glu Pro Gly Phe Ser Leu Ala Gln Leu Asn Leu Ile Trp
50 55 60

Gln Leu Thr Asp Thr Lys Gln Leu Val His Ser Phe Thr Glu Gly Arg
65 70 75 80

Asp Gln Gly Ser Ala Tyr Ser Asn Arg Thr Ala Leu Phe Pro Asp Leu
85 90 95

Leu Val Gln Gly Asn Ala Ser Leu Arg Leu Gln Arg Val Arg Val Thr
100 105 110

Asp Glu Gly Ser Tyr Thr Cys Phe Val Ser Ile Gln Asp Phe Asp Ser
115 120 125

Ala Ala Val Ser Leu Gln Val Ala Ala Pro Tyr Ser Lys Pro Ser Met
130 135 140

Thr Leu Glu Pro Asn Lys Asp Leu Arg Pro Gly Asn Met Val Thr Ile
145 150 155 160

Thr Cys Ser Ser Tyr Gln Gly Tyr Pro Glu Ala Glu Val Phe Trp Lys
165 170 175

Asp Gly Gln Gly Val Pro Leu Thr Gly Asn Val Thr Thr Ser Gln Met
180 185 190

Ala Asn Glu Arg Gly Leu Phe Asp Val His Ser Val Leu Arg Val Val
195 200 205

87

Leu Gly Ala Asn Gly Thr Tyr Ser Cys Leu Val Arg Asn Pro Val Leu
210 215 220

Gln Gln Asp Ala His Gly Ser Val Thr Ile Thr Gly Gln Pro Leu Thr
225 230 235 240

Phe Pro Pro Glu Ala Gly Ser Gly Ser Glu Pro Arg Gly Pro Thr Ile
245 250 255

Lys Pro Cys Pro Pro Cys Lys Cys Pro Ala Pro Asn Leu Glu Gly Gly
260 265 270

Pro Ser Val Phe Ile Phe Pro Pro Lys Ile Lys Asp Val Leu Met Ile
275 280 285

Ser Leu Ser Pro Ile Val Thr Cys Val Val Val Asp Val Ser Glu Asp
290 295 300

Asp Pro Asp Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu Val His
305 310 315 320

Thr Ala Gln Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Leu Arg
325 330 335

Val Val Ser Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly Lys
340 345 350

Ala Phe Ala Cys Ala Val Asn Asn Lys Asp Leu Pro Ala Pro Ile Glu
355 360 365

Arg Thr Ile Ser Lys Pro Lys Gly Ser Val Arg Ala Pro Gln Val Tyr

370 375 380

Val Leu Pro Pro Pro Glu Glu Glu Met Thr Lys Lys Gln Val Thr Leu
385 390 395 400

Thr Cys Met Val Thr Asp Phe Met Pro Glu Asp Ile Tyr Val Glu Trp
405 410 415

Thr Asn Asn Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr Glu Pro Val
420 425 430

Leu Asp Ser Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg Val Glu
435 440 445

Lys Lys Asn Trp Val Glu Arg Asn Ser Tyr Ser Cys Ser Val Val His
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Glu Gly Leu His Asn His His Thr Thr Lys Ser Phe Ser Arg Thr Pro
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Gly Lys

<210> 15

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<222> (67)..(79)

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<222> (86)..(102)

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<222> (7)..(17)

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Xaa Asp Ala Thr Leu Cys Cys Ser Phe Xaa Xaa Glu Pro Gly Phe Ser
20 25 30

Leu Ala Gln Leu Asn Leu Ile Trp Gln Leu Thr Asp Thr Lys Gln Leu
35 40 45

Val His Ser Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Ala Asn
50 55 60

Arg Thr Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu
65 70 75 80

Arg Leu Gln Arg Val Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
85 90 95

Xaa Xaa Xaa Xaa Xaa Xaa Gly Ser Ala Ala Xaa Ser Leu Gln Val Ala
100 105 110

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<400> 16

Val Gln Val

1

<210> 17
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<400> 17

Asp Ala Thr Leu Cys Cys Ser Phe
1 5

<210> 18
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Glu Pro Gly Phe Ser Leu Ala Gln Leu Asn Leu Ile Trp Gln Leu Thr
1 5 10 15

Asp Thr Lys Gln Leu Val His Ser
20

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<211> 4
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92

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Ala Asn Arg Thr

1

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<211> 6

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Leu Arg Leu Gln Arg Val

1

5

<210> 21

<211> 4

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<213> Artificial sequence

<220>

<223> Conserved among mammals

<400> 21

Gly Ser Ala Ala

1

<210> 22

<211> 5

<212> PRT

<213> Artificial sequence

<220>

<223> Conserved among mammals

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Ser Leu Gln Val Ala

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<223> L or V, or another amino acid

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<222> (22)..(22)

<223> C or R, or another amino acid

<220>

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<222> (53)..(53)

<223> A or T, or another amino acid

<220>

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<222> (56)..(56)

<223> Q or R, or another amino acid

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Thr Asp Ala Thr Leu Xaa Cys Ser Phe Ser Pro Glu Pro Gly Phe Ser
20 25 30

Leu Ala Gln Leu Asn Leu Ile Trp Gln Leu Thr Asp Thr Lys Gln Leu
35 40 45

Val His Ser Phe Xaa Glu Gly Xaa Asp Gln Gly Ser Ala Tyr Ala Asn
50 55 60

Arg Thr Ala Leu Phe Pro Asp Leu Leu Ala Gln Gly Asn Ala Ser Leu
65 70 75 80

Arg Leu Gln Arg Val Arg Val Ala Asp Glu Gly Ser Phe Thr Cys Phe
85 90 95

Val Ser Ile Arg Asp Phe Gly Ser Ala Ala Val Ser Leu Gln Val Ala
100 105 110

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<211> 55

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<223> Primer

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55

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<211> 50
<212> DNA
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<220>
<223> Primer

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ctctggggggg aatgtcatag gc

22

<210> 27
<211> 55
<212> DNA
<213> Artificial sequence

<220>
<223> Primer

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55

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<211> 50
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<223> Primer

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agctttgctg agggccagga c

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<212> DNA

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<223> Primer

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<210> 31

<211> 21

<212> DNA

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<400> 31

ctggcacagc tcaacctcat c

21

<210> 32
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<400> 32
accaggcagc ttaggtgcc

20

<210> 33
<211> 22
<212> DNA
<213> Artificial sequence

<220>
<223> Primer

<400> 33
ctgtgatggt gactgagccg tg

22

<210> 34
<211> 21
<212> DNA
<213> Artificial sequence

<220>
<223> Primer

<400> 34
cgcgtagcgtg tggcggatga g

21

<210> 35
<211> 22
<212> DNA

<213> Artificial sequence

<220>

<223> Primer

<400> 35

tacaggaatc agcactgggt tc

22

IN THE EUROPEAN PATENT OFFICE

Applicant: WYETH et al.

International Application No.: PCT/US2004/011767

International Filing Date: 16 April 2004

European Patent Office
P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk
TEL: 011-31-70-340-2040

Attention: Valérie Marmoy, Authorized Officer

**RESPONSE TO INVITATION TO FURNISH NUCLEOTIDE AND/OR AMINO ACID
SEQUENCE LISTING COMPLYING WITH WIPO STANDARD ST25**

Enclosed are the following documents in response to the INVITATION mailed
October 18, 2004:

1. STATEMENT ACCOMPANYING SEQUENCE LISTING
2. SEQUENCE LISTING in written form
3. SEQUENCE LISTING in computer readable form (diskette)

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

Dated: November 16, 2004

By: 

Arthur S. Garrett
Reg. No. 20,338

IN THE INTERNATIONAL BUREAU OF WIPO

Applicant: WYETH, et al.

International Application No.: PCT/US2004/011767

International Filing Date: April 16, 2004

STATEMENT

It is hereby stated that the information recorded in computer readable form
(attached diskette) is identical to the attached written SEQUENCE LISTING.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

Dated: November 16, 2004

By: 

Rhonda B. Hinton

101 \$

sequence listing
SEQUENCE LISTING

<110> Wyeth
Ling, Vincent
Carreno, Beatriz M.
Collins, Mary

<120> B7-H3 AS AN IMMUNOREGULATORY AGENT

<130> 08702.134-00304

<160> 34

<170> PatentIn version 3.1

<210> 1

<211> 951

<212> DNA

<213> Homo sapiens

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gcactgctgg tggccctggc tttcgtgtgc tggagaaaga tcaaacagag ctgtgaggag 840

sequence listing

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<210> 2

<211> 316

<212> PRT

<213> Homo sapiens

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Ala Leu Gly Ala Leu Trp Phe Cys Leu Thr Gly Ala Leu Glu Val Gln
20 25 30

Val Pro Glu Asp Pro Val Val Ala Leu Val Gly Thr Asp Ala Thr Leu
35 40 45

Cys Cys Ser Phe Ser Pro Glu Pro Gly Phe Ser Leu Ala Gln Leu Asn
50 55 60

Leu Ile Trp Gln Leu Thr Asp Thr Lys Gln Leu Val His Ser Phe Ala
65 70 75 80

Glu Gly Gln Asp Gln Gly Ser Ala Tyr Ala Asn Arg Thr Ala Leu Phe
85 90 95

Pro Asp Leu Leu Ala Gln Gly Asn Ala Ser Leu Arg Leu Gln Arg Val
100 105 110

Arg Val Ala Asp Glu Gly Ser Phe Thr Cys Phe Val Ser Ile Arg Asp
115 120 125

Phe Gly Ser Ala Ala Val Ser Leu Gln Val Ala Ala Pro Tyr Ser Lys
130 135 140

Pro Ser Met Thr Leu Glu Pro Asn Lys Asp Leu Arg Pro Gly Asp Thr
145 150 155 160

Val Thr Ile Thr Cys Ser Ser Tyr Arg Gly Tyr Pro Glu Ala Glu Val
165 170 175

Phe Trp Gln Asp Gly Gln Gly Val Pro Leu Thr Gly Asn Val Thr Thr
180 185 190

Ser Gln Met Ala Asn Glu Gln Gly Leu Phe Asp Val His Ser Val Leu
195 200 205

sequence listing

103

103

Arg Val Val Leu Gly Ala Asn Gly Thr Tyr Ser Cys Leu Val Arg Asn
210 215 220

Pro Val Leu Gln Gln Asp Ala His Gly Ser Val Thr Ile Thr Gly Gln
225 230 235 240

Pro Met Thr Phe Pro Pro Glu Ala Leu Trp Val Thr Val Gly Leu Ser
245 250 255

Val Cys Leu Ile Ala Leu Leu Val Ala Leu Ala Phe Val Cys Trp Arg
260 265 270

Lys Ile Lys Gln Ser Cys Glu Glu Glu Asn Ala Gly Ala Glu Asp Gln
275 280 285

Asp Gly Glu Gly Glu Gly Ser Lys Thr Ala Leu Gln Pro Leu Lys His
290 295 300

Ser Asp Ser Lys Glu Asp Asp Gly Gln Glu Ile Ala
305 310 315

<210> 3

<211> 951

<212> DNA

<213> Mouse

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sequence listing

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cctctgaaac cctctgaaaa caaagaagat gacggacaag aaattgcttg a 951

104

<210> 4
<211> 316
<212> PRT
<213> Mouse

<400> 4

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20 25 30

Val Ser Glu Asp Pro Val Val Ala Leu Val Asp Thr Asp Ala Thr Leu
35 40 45

Arg Cys Ser Phe Ser Pro Glu Pro Gly Phe Ser Leu Ala Gln Leu Asn
50 55 60

Leu Ile Trp Gln Leu Thr Asp Thr Lys Gln Leu Val His Ser Phe Thr
65 70 75 80

Glu Gly Arg Asp Gln Gly Ser Ala Tyr Ser Asn Arg Thr Ala Leu Phe
85 90 95

Pro Asp Leu Leu Val Gln Gly Asn Ala Ser Leu Arg Leu Gln Arg Val
100 105 110

Arg Val Thr Asp Glu Gly Ser Tyr Thr Cys Phe Val Ser Ile Gln Asp
115 120 125

Phe Asp Ser Ala Ala Val Ser Leu Gln Val Ala Ala Pro Tyr Ser Lys
130 135 140

Pro Ser Met Thr Leu Glu Pro Asn Lys Asp Leu Arg Pro Gly Asn Met
145 150 155 160

Val Thr Ile Thr Cys Ser Ser Tyr Gln Gly Tyr Pro Glu Ala Glu Val
165 170 175

Phe Trp Lys Asp Gly Gln Gly Val Pro Leu Thr Gly Asn Val Thr Thr
180 185 190

Ser Gln Met Ala Asn Glu Arg Gly Leu Phe Asp Val His Ser Val Leu
195 200 205

sequence listing

105

105

Arg Val Val Leu Gly Ala Asn Gly Thr Tyr Ser Cys Leu Val Arg Asn
210 215 220

Pro Val Leu Gln Gln Asp Ala His Gly Ser Val Thr Ile Thr Gly Gln
225 230 235 240

Pro Leu Thr Phe Pro Pro Glu Ala Leu Trp Val Thr Val Gly Leu Ser
245 250 255

Val Cys Leu Val Val Leu Leu Val Ala Leu Ala Phe Val Cys Trp Arg
260 265 270

Lys Ile Lys Gln Ser Cys Glu Glu Glu Asn Ala Gly Ala Glu Asp Gln
275 280 285

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Ser Glu Asn Lys Glu Asp Asp Gly Gln Glu Ile Ala
305 310 315

<210> 5

<211> 1605

<212> DNA

<213> Homo sapiens

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ggcaccgatg ccaccctgcg ctgctccttc tccccgagc ctggcttcag cctggcacag 840

106

sequence listing

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 gcaggagctg aggaccagga tggggagggga gaaggctcca agacagccct gcagcctctg 1560
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<210> 6

<211> 534

<212> PRT

<213> Homo sapiens

<400> 6

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 Val Pro Glu Asp Pro Val Val Ala Leu Val Gly Thr Asp Ala Thr Leu
 35 40 45
 Cys Cys Ser Phe Ser Pro Glu Pro Gly Phe Ser Leu Ala Gln Leu Asn
 50 55 60
 Leu Ile Trp Gln Leu Thr Asp Thr Lys Gln Leu Val His Ser Phe Ala
 65 70 75 80
 Glu Gly Gln Asp Gln Gly Ser Ala Tyr Ala Asn Arg Thr Ala Leu Phe
 85 90 95
 Pro Asp Leu Leu Ala Gln Gly Asn Ala Ser Leu Arg Leu Gln Arg Val
 100 105 110

107

sequence listing

Arg Val Ala Asp Glu Gly Ser Phe Thr Cys Phe Val Ser Ile Arg Asp
 115 120 125

Phe Gly Ser Ala Ala Val Ser Leu Gln Val Ala Ala Pro Tyr Ser Lys
 130 135 140

Pro Ser Met Thr Leu Glu Pro Asn Lys Asp Leu Arg Pro Gly Asp Thr
 145 150 155 160

Val Thr Ile Thr Cys Ser Ser Tyr Gln Gly Tyr Pro Glu Ala Glu Val
 165 170 175

Phe Trp Gln Asp Gly Gln Gly Val Pro Leu Thr Gly Asn Val Thr Thr
 180 185 190

Ser Gln Met Ala Asn Glu Gln Gly Leu Phe Asp Val His Ser Ile Leu
 195 200 205

Arg Val Val Leu Gly Ala Asn Gly Thr Tyr Ser Cys Leu Val Arg Asn
 210 215 220

Pro Val Leu Gln Gln Asp Ala His Ser Ser Val Thr Ile Thr Pro Gln
 225 230 235 240

Arg Ser Pro Thr Gly Ala Val Glu Val Gln Val Pro Glu Asp Pro Val
 245 250 255

Val Ala Leu Val Gly Thr Asp Ala Thr Leu Arg Cys Ser Phe Ser Pro
 260 265 270

Glu Pro Gly Phe Ser Leu Ala Gln Leu Asn Leu Ile Trp Gln Leu Thr
 275 280 285

Asp Thr Lys Gln Leu Val His Ser Phe Thr Glu Gly Arg Asp Gln Gly
 290 295 300

Ser Ala Tyr Ala Asn Arg Thr Ala Leu Phe Pro Asp Leu Leu Ala Gln
 305 310 315 320

Gly Asn Ala Ser Leu Arg Leu Gln Arg Val Arg Val Ala Asp Glu Gly
 325 330 335

Ser Phe Thr Cys Phe Val Ser Ile Arg Asp Phe Gly Ser Ala Ala Val
 340 345 350

Ser Leu Gln Val Ala Ala Pro Tyr Ser Lys Pro Ser Met Thr Leu Glu
 355 360 365

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

807

108

sequence listing

Ser Tyr Arg Gly Tyr Pro Glu Ala Glu Val Phe Trp Gln Asp Gly Gln
385 390 395 400

Gly Val Pro Leu Thr Gly Asn Val Thr Thr Ser Gln Met Ala Asn Glu
405 410 415

Gln Gly Leu Phe Asp Val His Ser Val Leu Arg Val Val Leu Gly Ala
420 425 430

Asn Gly Thr Tyr Ser Cys Leu Val Arg Asn Pro Val Leu Gln Gln Asp
435 440 445

Ala His Gly Ser Val Thr Ile Thr Gly Gln Pro Met Thr Phe Pro Pro
450 455 460

Glu Ala Leu Trp Val Thr Val Gly Leu Ser Val Cys Leu Ile Ala Leu
465 470 475 480

Leu Val Ala Leu Ala Phe Val Cys Trp Arg Lys Ile Lys Gln Ser Cys
485 490 495

Glu Glu Glu Asn Ala Gly Ala Glu Asp Gln Asp Gly Glu Gly Glu Gly
500 505 510

Ser Lys Thr Ala Leu Gln Pro Leu Lys His Ser Asp Ser Lys Glu Asp
515 520 525

Asp Gly Gln Glu Ile Ala
530

<210> 7

<211> 112

<212> PRT

<213> Homo sapience

<400> 7

Ala Leu Glu Val Gln Val Pro Glu Asp Pro Val Val Ala Leu Val Gly
1 5 10 15

Thr Asp Ala Thr Leu Cys Cys Ser Phe Ser Pro Glu Pro Gly Phe Ser
20 25 30

Leu Ala Gln Leu Asn Leu Ile Trp Gln Leu Thr Asp Thr Lys Gln Leu
35 40 45

Val His Ser Phe Ala Glu Gly Gln Asp Gln Gly Ser Ala Tyr Ala Asn
50 55 60

109 \$

sequence listing

Arg Thr Ala Leu Phe Pro Asp Leu Leu Ala Gln Gly Asn Ala Ser Leu
 65 70 75 80
 Arg Leu Gln Arg Val Arg Val Ala Asp Glu Gly Ser Phe Thr Cys Phe
 85 90 95
 Val Ser Ile Arg Asp Phe Gly Ser Ala Ala Val Ser Leu Gln Val Ala
 100 105 110

<210> 8
 <211> 1670
 <212> DNA
 <213> Chimera

<400> 8
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 accgatgcca ccctgtgctg ctcttctctcc cctgagcctg gcttcagcct ggcaacagctc 180
 aacctcatct ggcagctgac agataccaaa cagctgggtgc acagctttgc tgagggccag 240
 gaccagggca gcgcctatgc caaccgcacg gccctcttcc cggacctgct ggcacagggc 300
 aacgcattccc tgaggctgca gcgcgtgcgt gtggcggacg agggcagctt cacctgcttc 360
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 aagcccagca tgacctgga gcccaacaag gacctgcggc caggggacac ggtgaccatc 480
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 gtgcacagcg tcctgcgggt ggtgctgggt gcgaatggca cctacagctg cctgggtgcgc 660
 aaccccgtgc tgcagcagga tgcgcacggc tctgtcacca tcacagggca gcctatgaca 720
 tcccccccag aggcagggtc ggggtccgag ccccgcgga cgcacaatcaa gccctgtcct 780
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 tgtactcatg atctccctga gcccatagt cacatgtgtg gtgggtggatg tgagcgagga 1020
 tgaccagat gtccagatca gctggtttgt gaacaacgtg gaagtacaca cagctcagac 1080
 acaaaccat agagaggatt acaacagtac tctccgggtg gtcagtgcc tccccatcca 1140
 gcaccaggac tggatgagtg gcaaggcttt cgcattgcgc gtcaacaaca aagacctccc 1200
 agcggccatc gagagaacca tctcaaaacc caaagggtgag agctgcagcc tgactgcatg 1260
 ggggctggga tgggcataag gataaagggtc tgtgtggaca gccttctgct tcagccatga 1320
 cctttgtgta tgtttctacc ctacaggggt cagtaagagc tccacaggta tatgtcttgc 1380

110

sequence listing

110

ctccaccaga agaagagatg actaagaaac aggtcactct gacctgcatg gtcacagact 1440
 tcatgcctga agacatttac gtggagtggg ccaacaacgg gaaaacagag ctaaactaca 1500
 agaacactga accagtcctg gactctgatg gttcttactt catgtacagc aagctgagag 1560
 tggaaaagaa gaactgggtg gaaagaaata gctactcctg ttcagtgggc cacgagggtc 1620
 tgcacaatca ccacacgact aagagcttct cccggactcc gggtaaatga 1670

<210> 9
 <211> 482
 <212> PRT
 <213> Chimera

<400> 9

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

111 #

sequence listing

Asp Gly Gln Gly Val Pro Leu Thr Gly Asn Val Thr Thr Ser Gln Met
180 185 190

Ala Asn Glu Gln Gly Leu Phe Asp Val His Ser Val Leu Arg Val Val
195 200 205

Leu Gly Ala Asn Gly Thr Tyr Ser Cys Leu Val Arg Asn Pro Val Leu
210 215 220

Gln Gln Asp Ala His Gly Ser Val Thr Ile Thr Gly Gln Pro Met Thr
225 230 235 240

Phe Pro Pro Glu Ala Gly Ser Gly Ser Glu Pro Arg Gly Pro Thr Ile
245 250 255

Lys Pro Cys Pro Pro Cys Lys Cys Pro Ala Pro Asn Leu Glu Gly Gly
260 265 270

Pro Ser Val Phe Ile Phe Pro Pro Lys Ile Lys Asp Val Leu Met Ile
275 280 285

Ser Leu Ser Pro Ile Val Thr Cys Val Val Val Asp Val Ser Glu Asp
290 295 300

Asp Pro Asp Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu Val His
305 310 315 320

Thr Ala Gln Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Leu Arg
325 330 335

Val Val Ser Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly Lys
340 345 350

Ala Phe Ala Cys Ala Val Asn Asn Lys Asp Leu Pro Ala Pro Ile Glu
355 360 365

Arg Thr Ile Ser Lys Pro Lys Gly Ser Val Arg Ala Pro Gln Val Tyr
370 375 380

Val Leu Pro Pro Pro Glu Glu Glu Met Thr Lys Lys Gln Val Thr Leu
385 390 395 400

Thr Cys Met Val Thr Asp Phe Met Pro Glu Asp Ile Tyr Val Glu Trp
405 410 415

Thr Asn Asn Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr Glu Pro Val
420 425 430

Leu Asp Ser Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg Val Glu
435 440 445

sequence listing

Lys Lys Asn Trp Val Glu Arg Asn Ser Tyr Ser Cys Ser Val Val His
 450 455 460
 Glu Gly Leu His Asn His His Thr Thr Lys Ser Phe Ser Arg Thr Pro
 465 470 475 480

Gly Lys

- <210> 10
- <211> 2324
- <212> DNA
- <213> Chimera

<400> 10
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 agcatggcca gcatgctgga ggtccaggtc cctgaagacc cagtgggtggc actggtgggc 120
 accgatgcca ccctgtgctg ctcccttctcc cctgagcctg gcttcagcct ggcacagctc 180
 aacctcatct ggcagctgac agataccaaa cagctgggtgc acagctttgc tgagggccag 240
 gaccagggca gcgcctatgc caaccgcacg gccctcttcc cggacctgct ggcacagggc 300
 aacgcatccc tgaggctgca gcgcgtgctg gtggcggacg agggcagctt cacctgcttc 360
 gtgagcatcc gggatttcgg cagcgtgccc gtcagcctgc aggtggccgc tccctactcg 420
 aagcccagca tgaccctgga gcccaacaag gacctgcggc caggggacac ggtgaccatc 480
 acgtgctcca gctaccaggg ctaccctgag gctgaggtgt tctggcagga tgggcagggt 540
 gtgcccctga ctggcaacgt gaccacgtcg cagatggcca acgagcaggg cttgtttgat 600
 gtgcacagca tcctgcgggt ggtgctgggt gcaaattggc cctacagctg cctggtgcgc 660
 aaccccgctg tgcagcagga tgcgcacagc tctgtcacca tcacaccca gagaagcccc 720
 acaggagccg tggaggtcca ggtccctgag gacccggtgg tggccctagt gggcaccgat 780
 gccaccctgc gctgctcctt ctcccccgag cctggcttca gcctggcaca gctcaacctc 840
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 ggcagcgcct atgccaaccg cacggccctc ttcccggacc tgctggcaca aggcaatgca 960
 tccctgaggc tgcagcgcgt gcgtgtggcg gacgaggga gcttcacctg cttcgtgagc 1020
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 agcatgaccc tggagcccaa caaggacctg cggccagggg acacggtgac catcacgtgc 1140
 tccagctacc ggggctaccc tgaggctgag gtgttctggc aggatgggca ggggtgtgcc 1200
 ctgactggca acgtgaccac gtcgcagatg gccaacgagc agggcttggt tgatgtgcac 1260
 agcgtcctgc ggggtggtgct ggggtgcgaat ggcacctaca gctgcctggt gcgcaacccc 1320
 gtgctgcagc aggatgcgca cggctctgtc accatcacag ggcagcctat gacattcccc 1380

113

sequence listing

ccagaggcag ggtcgggggtc cgagccccgc ggaccgacaa tcaagccctg tcctccatgc 1440
 aaatgcccag gtaagtcact agaccagagc tccactcccg ggagaatggt aagtgtata 1500
 aacatccctg cactagagga taagccatgt acagatccat ttccatctct cctcatcagc 1560
 acctaacctc gaggggtggac catccgtctt catcttccct ccaaagatca aggatgtact 1620
 catgatctcc ctgagcccca tagtcacatg tgtgggtggtg gatgtgagcg aggatgaccc 1680
 agatgtccag atcagctggt ttgtgaacaa cgtggaagta cacacagctc agacacaaac 1740
 ccatagagag gattacaaca gtactctccg ggtggtcagt gccctcccca tccagcacca 1800
 ggactggatg agtggcaagg ctttcgcatg cgccgtcaac aacaaagacc tcccagcgcc 1860
 catcgagaga accatctcaa aacccaaagg tgagagctgc agcctgactg catgggggct 1920
 gggatgggca taaggataaa ggtctgtgtg gacagccttc tgcttcagcc atgacctttg 1980
 tgtatgtttc taccctcaca gggtcagtaa gagctccaca ggtatatgtc ttgcctccac 2040
 cagaagaaga gatgactaag aaacaggta ctctgacctg catggtcaca gacttcatgc 2100
 ctgaagacat ttacgtggag tggaccaaca acgggaaaac agagctaaac tacaagaaca 2160
 ctgaaccagt cctggactct gatggttctt acttcatgta cagcaagctg agagtggaaa 2220
 agaagaactg ggtggaaaga aatagctact cctgttcagt ggtccacgag ggtctgcaca 2280
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<210> 11
 <211> 700
 <212> PRT
 <213> Chimera

<400> 11
 Met Gly Val Leu Leu Thr Gln Arg Thr Leu Leu Ser Leu Val Leu Ala
 1 5 10 15
 Leu Leu Phe Pro Ser Met Ala Ser Met Leu Glu Val Gln Val Pro Glu
 20 25 30
 Asp Pro Val Val Ala Leu Val Gly Thr Asp Ala Thr Leu Cys Cys Ser
 35 40 45
 Phe Ser Pro Glu Pro Gly Phe Ser Leu Ala Gln Leu Asn Leu Ile Trp
 50 55 60
 Gln Leu Thr Asp Thr Lys Gln Leu Val His Ser Phe Ala Glu Gly Gln
 65 70 75 80
 Asp Gln Gly Ser Ala Tyr Ala Asn Arg Thr Ala Leu Phe Pro Asp Leu
 85 90 95

sequence listing

114

Leu Ala Gln Gly Asn Ala Ser Leu Arg Leu Gln Arg Val Arg Val Ala
100 105 110

Asp Glu Gly Ser Phe Thr Cys Phe Val Ser Ile Arg Asp Phe Gly Ser
115 120 125

Ala Ala Val Ser Leu Gln Val Ala Ala Pro Tyr Ser Lys Pro Ser Met
130 135 140

Thr Leu Glu Pro Asn Lys Asp Leu Arg Pro Gly Asp Thr Val Thr Ile
145 150 155 160

Thr Cys Ser Ser Tyr Gln Gly Tyr Pro Glu Ala Glu Val Phe Trp Gln
165 170 175

Asp Gly Gln Gly Val Pro Leu Thr Gly Asn Val Thr Thr Ser Gln Met
180 185 190

Ala Asn Glu Gln Gly Leu Phe Asp Val His Ser Ile Leu Arg Val Val
195 200 205

Leu Gly Ala Asn Gly Thr Tyr Ser Cys Leu Val Arg Asn Pro Val Leu
210 215 220

Gln Gln Asp Ala His Ser Ser Val Thr Ile Thr Pro Gln Arg Ser Pro
225 230 235 240

Thr Gly Ala Val Glu Val Gln Val Pro Glu Asp Pro Val Val Ala Leu
245 250 255

Val Gly Thr Asp Ala Thr Leu Arg Cys Ser Phe Ser Pro Glu Pro Gly
260 265 270

Phe Ser Leu Ala Gln Leu Asn Leu Ile Trp Gln Leu Thr Asp Thr Lys
275 280 285

Gln Leu Val His Ser Phe Thr Glu Gly Arg Asp Gln Gly Ser Ala Tyr
290 295 300

Ala Asn Arg Thr Ala Leu Phe Pro Asp Leu Leu Ala Gln Gly Asn Ala
305 310 315 320

Ser Leu Arg Leu Gln Arg Val Arg Val Ala Asp Glu Gly Ser Phe Thr
325 330 335

Cys Phe Val Ser Ile Arg Asp Phe Gly Ser Ala Ala Val Ser Leu Gln
340 345 350

Val Ala Ala Pro Tyr Ser Lys Pro Ser Met Thr Leu Glu Pro Asn Lys
355 360 365

sequence listing

115

Asp Leu Arg Pro Gly Asp Thr Val Thr Ile Thr Cys Ser Ser Tyr Arg
370 375 380

Gly Tyr Pro Glu Ala Glu Val Phe Trp Gln Asp Gly Gln Gly Val Pro
385 390 395 400

Leu Thr Gly Asn Val Thr Thr Ser Gln Met Ala Asn Glu Gln Gly Leu
405 410 415

Phe Asp Val His Ser Val Leu Arg Val Val Leu Gly Ala Asn Gly Thr
420 425 430

Tyr Ser Cys Leu Val Arg Asn Pro Val Leu Gln Gln Asp Ala His Gly
435 440 445

Ser Val Thr Ile Thr Gly Gln Pro Met Thr Phe Pro Pro Glu Ala Gly
450 455 460

Ser Gly Ser Glu Pro Arg Gly Pro Thr Ile Lys Pro Cys Pro Pro Cys
465 470 475 480

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

Pro Pro Lys Ile Lys Asp Val Leu Met Ile Ser Leu Ser Pro Ile Val
500 505 510

Thr Cys Val Val Val Asp Val Ser Glu Asp Asp Pro Asp Val Gln Ile
515 520 525

Ser Trp Phe Val Asn Asn Val Glu Val His Thr Ala Gln Thr Gln Thr
530 535 540

His Arg Glu Asp Tyr Asn Ser Thr Leu Arg Val Val Ser Ala Leu Pro
545 550 555 560

Ile Gln His Gln Asp Trp Met Ser Gly Lys Ala Phe Ala Cys Ala Val
565 570 575

Asn Asn Lys Asp Leu Pro Ala Pro Ile Glu Arg Thr Ile Ser Lys Pro
580 585 590

Lys Gly Ser Val Arg Ala Pro Gln Val Tyr Val Leu Pro Pro Pro Glu
595 600 605

Glu Glu Met Thr Lys Lys Gln Val Thr Leu Thr Cys Met Val Thr Asp
610 615 620

Phe Met Pro Glu Asp Ile Tyr Val Glu Trp Thr Asn Asn Gly Lys Thr
625 630 635 640

sequence listing

116

Glu Leu Asn Tyr Lys Asn Thr Glu Pro Val Leu Asp Ser Asp Gly Ser
645 650 655

Tyr Phe Met Tyr Ser Lys Leu Arg Val Glu Lys Lys Asn Trp Val Glu
660 665 670

Arg Asn Ser Tyr Ser Cys Ser Val Val His Glu Gly Leu His Asn His
675 680 685

His Thr Thr Lys Ser Phe Ser Arg Thr Pro Gly Lys
690 695 700

<210> 12

<211> 1670

<212> DNA

<213> Mouse

<400> 12
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gtgcccttga ctggcaatgt gaccacatcc cagatggcca acgagcgggg cttgttcgat 600
gttcacagcg tgctgagggg ggtgctgggt gctaaccgca cctacagctg cctggtacgc 660
aaccgggtgt tgcagcaaga tgctcacggc tcagtcacca tcacagggca gccctgaca 720
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ccatgcaaat gccaggtaa gtcactagac cagagctcca ctccgggag aatggttaagt 840
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tgtactcatg atctccctga gcccatagt cacatgtgtg gtggtggatg tgagcgagga 1020
tgaccagat gtccagatca gctggtttgt gaacaacgtg gaagtacaca cagctcagac 1080
acaaacccat agagaggatt acaacagtac tctccgggtg gtcagtgcc tccccatcca 1140
gcaccaggac tggatgagtg gcaaggcttt cgcattgcgc gtcaacaaca aagacctccc 1200

sequence listing

agcgcccatc gagagaacca tctcaaaacc caaagggtgag agctgcagcc tgactgcatg 1260
 ggggctggga tgggcataag gataaagggtc tgtgtggaca gccttctgct tcagccatga 1320
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 ctccaccaga agaagagatg actaagaaac aggtcactct gacctgcatg gtcacagact 1440
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 agaacactga accagtcctg gactctgatg gttcttactt catgtacagc aagctgagag 1560
 tggaaaagaa gaactgggtg gaaagaaata gctactcctg ttcagtgggtc cacgaggggtc 1620
 tgcacaatca ccacacgact aagagcttct cccggactcc gggtaaata 1670

<210> 13

<211> 482

<212> PRT

<213> Mouse

<400> 13

Met Gly Val Leu Leu Thr Gln Arg Thr Leu Leu Ser Leu Val Leu Ala
 1 5 10 15

Leu Leu Phe Pro Ser Met Ala Ser Met Val Glu Val Gln Val Ser Glu
 20 25 30

Asp Pro Val Val Ala Leu Val Asp Thr Asp Ala Thr Leu Arg Cys Ser
 35 40 45

Phe Ser Pro Glu Pro Gly Phe Ser Leu Ala Gln Leu Asn Leu Ile Trp
 50 55 60

Gln Leu Thr Asp Thr Lys Gln Leu Val His Ser Phe Thr Glu Gly Arg
 65 70 75 80

Asp Gln Gly Ser Ala Tyr Ser Asn Arg Thr Ala Leu Phe Pro Asp Leu
 85 90 95

Leu Val Gln Gly Asn Ala Ser Leu Arg Leu Gln Arg Val Arg Val Thr
 100 105 110

Asp Glu Gly Ser Tyr Thr Cys Phe Val Ser Ile Gln Asp Phe Asp Ser
 115 120 125

Ala Ala Val Ser Leu Gln Val Ala Ala Pro Tyr Ser Lys Pro Ser Met
 130 135 140

Thr Leu Glu Pro Asn Lys Asp Leu Arg Pro Gly Asn Met Val Thr Ile
 145 150 155 160

sequence listing

118

Thr Cys Ser Ser Tyr Gln Gly Tyr Pro Glu Ala Glu Val Phe Trp Lys
165 170 175

Asp Gly Gln Gly Val Pro Leu Thr Gly Asn Val Thr Thr Ser Gln Met
180 185 190

Ala Asn Glu Arg Gly Leu Phe Asp Val His Ser Val Leu Arg Val Val
195 200 205

Leu Gly Ala Asn Gly Thr Tyr Ser Cys Leu Val Arg Asn Pro Val Leu
210 215 220

Gln Gln Asp Ala His Gly Ser Val Thr Ile Thr Gly Gln Pro Leu Thr
225 230 235 240

Phe Pro Pro Glu Ala Gly Ser Gly Ser Glu Pro Arg Gly Pro Thr Ile
245 250 255

Lys Pro Cys Pro Pro Cys Lys Cys Pro Ala Pro Asn Leu Glu Gly Gly
260 265 270

Pro Ser Val Phe Ile Phe Pro Pro Lys Ile Lys Asp Val Leu Met Ile
275 280 285

Ser Leu Ser Pro Ile Val Thr Cys Val Val Val Asp Val Ser Glu Asp
290 295 300

Asp Pro Asp Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu Val His
305 310 315 320

Thr Ala Gln Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Leu Arg
325 330 335

Val Val Ser Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly Lys
340 345 350

Ala Phe Ala Cys Ala Val Asn Asn Lys Asp Leu Pro Ala Pro Ile Glu
355 360 365

Arg Thr Ile Ser Lys Pro Lys Gly Ser Val Arg Ala Pro Gln Val Tyr
370 375 380

Val Leu Pro Pro Pro Glu Glu Glu Met Thr Lys Lys Gln Val Thr Leu
385 390 395 400

Thr Cys Met Val Thr Asp Phe Met Pro Glu Asp Ile Tyr Val Glu Trp
405 410 415

Thr Asn Asn Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr Glu Pro Val
420 425 430

sequence listing

119

57

Leu Asp Ser Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg Val Glu
435 440 445

Lys Lys Asn Trp Val Glu Arg Asn Ser Tyr Ser Cys Ser Val Val His
450 455 460

Glu Gly Leu His Asn His His Thr Thr Lys Ser Phe Ser Arg Thr Pro
465 470 475 480

Gly Lys

<210> 14

<211> 112

<212> PRT

<213> Artificial sequence

<220>

<223> Conserved regions in mammalian B7-H3 V1 exon

<220>

<221> Misc_feature

<222> (1)..(3)

<223> ALE, or another amino acid sequence

<220>

<221> Misc_feature

<222> (26)..(27)

<223> SP, or another amino acid sequence

<220>

<221> Misc_feature

<222> (52)..(62)

<223> FAEGQDQGSAY, or another amino acid sequence

<220>

<221> Misc_feature

<222> (67)..(79)

021

sequence listing
<223> ALFPDLLAQGNAS, or another amino acid sequence

120

<220>

<221> Misc_feature

<222> (86)..(102)

<223> RVADEGSFTCFVSIRDF, or another amino acid sequence

<220>

<221> Misc_feature

<222> (107)..(107)

<223> V, or another amino acid sequence

<220>

<221> Misc_feature

<222> (7)..(17)

<223> PEDPVVALVGT, or another amino acid sequence

<400> 14

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1 5 10 15

Xaa Asp Ala Thr Leu Cys Cys Ser Phe Xaa Xaa Glu Pro Gly Phe Ser
20 25 30

Leu Ala Gln Leu Asn Leu Ile Trp Gln Leu Thr Asp Thr Lys Gln Leu
35 40 45

Val His Ser Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Ala Asn
50 55 60

Arg Thr Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu
65 70 75 80

Arg Leu Gln Arg Val Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
85 90 95

Xaa Xaa Xaa Xaa Xaa Xaa Gly Ser Ala Ala Xaa Ser Leu Gln Val Ala
100 105 110

<210> 15

<211> 3

<212> PRT

<213> Artificial sequence

<220>

<223> Conserved among mammals

<400> 15

Val Gln Val
1

<210> 16

<211> 8

<212> PRT

<213> Artificial sequence

<220>

<223> Conserved among mammals

<400> 16

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sequence listing

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22

RECEIVED

PATENT COOPERATION TREATY

PCT/US2004/011767

DEC 07 2004

From the INTERNATIONAL BUREAU

128

Finnegan, Henderson, Farabow,
Garrett & Dunner, LLP

PCT

FIRST NOTICE INFORMING THE APPLICANT OF
THE COMMUNICATION OF THE INTERNATIONAL
APPLICATION (TO DESIGNATED OFFICES WHICH
DO NOT APPLY THE 30 MONTH TIME LIMIT
UNDER ARTICLE 22(1))

To:

GARRETT, Arthur, S.
Finnegan, Henderson, Farabow, Garrett & Dunner, L.L.P.
1300 I Street, N.W.
Washington, DC 20005-3315
ETATS-UNIS D'AMERIQUE

(PCT Rule 47.1(c))

Date of mailing (day/month/year) 18 November 2004 (18.11.2004)		IMPORTANT NOTICE	
Applicant's or agent's file reference 8702.134-304			
International application No. PCT/US2004/011767	International filing date (day/month/year) 16 April 2004 (16.04.2004)	Priority date (day/month/year) 17 April 2003 (17.04.2003)	
Applicant WYETH et al			

- ATTENTION:** For any designated Office(s), for which the time limit under Article 22(1), as in force from 1 April 2002 (30 months from the priority date), **does apply**, please see Form PCT/IB/308(Second and Supplementary Notice) (to be issued promptly after the expiration of 28 months from the priority date).
- Notice is hereby given that the following designated Office(s), for which the time limit under Article 22(1), as in force from 1 April 2002, **does not apply**, has/have requested that the communication of the international application, as provided for in Article 20, be effected under Rule 93bis.1. The International Bureau has effected that communication on the date indicated below:
04 November 2004 (04.11.2004)

CH

In accordance with Rule 47.1(c-bis)(i), those Offices will accept the present notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

- The following designated Offices, for which the time limit under Article 22(1), as in force from 1 April 2002, **does not apply**, have not requested, as at the time of mailing of the present notice, that the communication of the international application be effected under Rule 93bis.1:

FI, LU, SE, TZ, UG, ZM

In accordance with Rule 47.1(c-bis)(ii), those Offices accept the present notice as conclusive evidence that the Contracting State for which that Office acts as a designated Office does not require the furnishing, under Article 22, by the applicant of a copy of the international application.

4. TIME LIMITS for entry into the national phase

For the designated Office(s) listed above, and unless a demand for international preliminary examination has been filed before the expiration of 19 months from the priority date (see Article 39(1)), the applicable time limit for entering the national phase will, subject to what is said in the following paragraph, be 20 MONTHS from the priority date.

In practice, time limits other than the 20-month time limit will continue to apply, for various periods of time, in respect of certain of the designated Offices listed above. For regular updates on the applicable time limits (20 or 21 months, or other time limit), Office by Office, refer to the *PCT Gazette*, the *PCT Newsletter* and the *PCT Applicant's Guide*, Volume II, National Chapters, all available from WIPO's Internet site, at <http://www.wipo.int/pct/en/index.html>.

It is the applicant's sole responsibility to monitor all these time limits.

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Dorothee Mülhausen
Facsimile No.+41 22 740 14 35	Facsimile No.+41 22 338 87 40

Form PCT/IB/308(First Notice) (January 2004)



Office Européen des Brevets
Europäisches Patentamt
European Patent Office
Storage and Retrieval of Amino acid and Nucleotide Data

DG1
DG1
DG1

AM/ESM/ROB/H

129

Ms. Valérie Marmoy
P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk

Tel. : + 31 70 340 23 66
Fax : + 31 70 340 39 92

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER L.L.P.
1300 I Street, N.W.
Washington, DC 20005
UNITED STATES OF AMERICA

RECEIVED

DEC 09 2004

Finnegan, Henderson, Farabow,
Garrett & Dunner, L.L.P.

24 November 2004,

Refer: International application : PCT/US 2004/001767
Your Ref.: 8702.134-304

Dear Sir,

Unfortunately, according to our verification the Sequence Listing on electronical medium which has been sent to us is not correctly done.

1. We observed that in the sequence listing you sent on 18 November Item <160> 34, in the application as originally filed(Item <160> 35).

SEQ.ID.NOs 8 is not the same.

Please check and correct the Sequence listing.

For further information, please see Supplement 2 to Official Journal Nr. 11/1998 .
Internet page. <http://www.european-patent-office.org/filingsoft/index.htm>

The applicant is herewith invited to file a correct Sequence Listing in computer readable form.

Concerning above mentioned application, we herewith kindly remind you that the ISA expects to receive the requested documents before 8 December 2004.

The ISA hereby invites the applicant to submit a sequence listing, with appropriate annotations for each sequence [where applicable], both on paper and in computer readable form, accompanied by the appropriate statements.

Relating to this, we remind you that if these requirements are not met or not met in due time, the EPO does not perform the international search where a meaningful search cannot be carried out (Rule 13^{ter}.1(c)PCT). In this case the international search report is replaced in full or in part by the statement under Article 17(2)(a)(ii)PCT.

✓
12/9/04
H.C.

Moreover Rule 13^{ter} (f) prescribes that a subsequently filed sequence listing, which is not a correction within the meaning of rule 26.4 PCT and which is not a rectification within the meaning of Rule 91.1.PCT of a sequence listing, shall not form part of the international application. In accordance herewith, the furnishing of a subsequently filed sequence listing does not give rise to an opportunity either to amend the description, claims and figures with a view to refer to said subsequently filed sequence listing or add it to the application as originally filed. The subsequently furnished listing will therefore normally not be forwarded to the international Bureau for publication purposes.

130

23

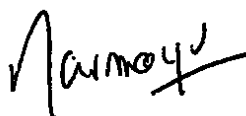
The computer readable form of the Sequence Listing in ASCII format (text only) is mandatory. For further questions do not hesitate to contact us.

Should you not be able to send us the sequence listing, please let us also know your response in the best delays.

Please send sequence listing in computer-readable form preferably to the European Patent Office, Strand Program, Directorate Documentation, Ms V. Marmoy, Room S13C10, Patentlaan 2, NL 2288 EE Rijswijk, The Netherlands.

Yours faithfully

Marmoy Valérie



IN THE EUROPEAN PATENT OFFICE

Applicant: WYETH et al.

International Application No.: PCT/US2004/011767

International Filing Date: 16 April 2004

European Patent Office
Strand Program
Directorate Documentation
Attn: Ms. V. Marmoy
Roon S13CJ10, Patentlaan 2
NL 2288 EE Rijswijk, The Netherlands
TEL: 011-31-70-340-2040

**RESPONSE TO INVITATION CORRECTING SEQUENCE LISTING COMPLYING
WITH WIPO STANDARD ST25**

Enclosed are the following documents in response to the INVITATION mailed
November 24, 2004:

1. STATEMENT ACCOMPANYING SEQUENCE LISTING
2. SEQUENCE LISTING in written form
3. SEQUENCE LISTING in computer readable form (diskette)

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

Dated: December 13, 2004

By: 
Arthur S. Garrett
Reg. No. 20,338

132 

Agent Reference No: 8702.134-304

IN THE EUROPEAN PATENT OFFICE

Applicant: WYETH, et al.

International Application No.: PCT/US2004/011767

International Filing Date: April 16, 2004

STATEMENT

European Patent Office
Strand Program
Directorate Documentation
Attn: Ms. V. Marmoy
Room S13C10, Patentlaan 2
NL 2288 EE Rijswijk, The Netherlands

Via Facsimile
011.31.70.3403992

It is hereby stated that the information recorded in computer readable form
(attached diskette) is identical to the attached written SEQUENCE LISTING.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

Dated: November 16, 2004

By:


Rhonda B. Hinton

SEQUENCE LISTING

37

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Carreno, Beatriz M.
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95

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137

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 Pro Val Leu Gln Gln Asp Ala His Ser Ser Val Thr Ile Thr Pro Gln
 225 230 235 240
 Arg Ser Pro Thr Gly Ala Val Glu Val Gln Val Pro Glu Asp Pro Val
 245 250 255
 Val Ala Leu Val Gly Thr Asp Ala Thr Leu Arg Cys Ser Phe Ser Pro
 260 265 270
 Glu Pro Gly Phe Ser Leu Ala Gln Leu Asn Leu Ile Trp Gln Leu Thr
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 Asp Thr Lys Gln Leu Val His Ser Phe Thr Glu Gly Arg Asp Gln Gly
 290 295 300
 Ser Ala Tyr Ala Asn Arg Thr Ala Leu Phe Pro Asp Leu Leu Ala Gln
 305 310 315 320

Gly Asn Ala Ser Leu Arg Leu Gln Arg Val Arg Val Ala Asp Glu Gly
325 330 335

Ser Phe Thr Cys Phe Val Ser Ile Arg Asp Phe Gly Ser Ala Ala Val
340 345 350

Ser Leu Gln Val Ala Ala Pro Tyr Ser Lys Pro Ser Met Thr Leu Glu
355 360 365

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

Ser Tyr Arg Gly Tyr Pro Glu Ala Glu Val Phe Trp Gln Asp Gly Gln
385 390 395 400

Gly Val Pro Leu Thr Gly Asn Val Thr Thr Ser Gln Met Ala Asn Glu
405 410 415

Gln Gly Leu Phe Asp Val His Ser Val Leu Arg Val Val Leu Gly Ala
420 425 430

Asn Gly Thr Tyr Ser Cys Leu Val Arg Asn Pro Val Leu Gln Gln Asp
435 440 445

Ala His Gly Ser Val Thr Ile Thr Gly Gln Pro Met Thr Phe Pro Pro
450 455 460

Glu Ala Leu Trp Val Thr Val Gly Leu Ser Val Cys Leu Ile Ala Leu
465 470 475 480

Leu Val Ala Leu Ala Phe Val Cys Trp Arg Lys Ile Lys Gln Ser Cys
485 490 495

Glu Glu Glu Asn Ala Gly Ala Glu Asp Gln Asp Gly Glu Gly Glu Gly
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Ser Lys Thr Ala Leu Gln Pro Leu Lys His Ser Asp Ser Lys Glu Asp
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Asp Gly Gln Glu Ile Ala
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<210> 7
<211> 112
<212> PRT
<213> Homo sapience

<400> 7

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140 470

Leu Ala Gln Leu Asn Leu Ile Trp Gln Leu Thr Asp Thr Lys Gln Leu
35 40 45

Val His Ser Phe Ala Glu Gly Gln Asp Gln Gly Ser Ala Tyr Ala Asn
50 55 60

Arg Thr Ala Leu Phe Pro Asp Leu Leu Ala Gln Gly Asn Ala Ser Leu
65 70 75 80

Arg Leu Gln Arg Val Arg Val Ala Asp Glu Gly Ser Phe Thr Cys Phe
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Val Ser Ile Arg Asp Phe Gly Ser Ala Ala Val Ser Leu Gln Val Ala
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<210> 8
<211> 112
<212> PRT
<213> Homo sapiens

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<223> Q, or R, or any other amino acid

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<223> C, or R, or any other amino acid

<400> 8

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20 25 30

Leu Ala Gln Leu Asn Leu Ile Trp Gln Leu Thr Asp Thr Lys Gln Leu
35 40 45

Val His Ser Phe Xaa Glu Gly Xaa Asp Gln Gly Ser Ala Tyr Ala Asn
50 55 60

Arg Thr Ala Leu Phe Pro Asp Leu Leu Ala Gln Gly Asn Ala Ser Leu
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Arg Leu Gln Arg Val Arg Val Ala Asp Glu Gly Ser Phe Thr Cys Phe
85 90 95

Val Ser Ile Arg Asp Phe Gly Ser Ala Ala Val Ser Leu Gln Val Ala
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<211> 1670
<212> DNA
<213> Chimera

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142

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 <212> PRT
 <213> Chimera

<400> 10

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 35 40 45
 Phe Ser Pro Glu Pro Gly Phe Ser Leu Ala Gln Leu Asn Leu Ile Trp
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 Gln Leu Thr Asp Thr Lys Gln Leu Val His Ser Phe Ala Glu Gly Gln
 65 70 75 80
 Asp Gln Gly Ser Ala Tyr Ala Asn Arg Thr Ala Leu Phe Pro Asp Leu
 85 90 95
 Leu Ala Gln Gly Asn Ala Ser Leu Arg Leu Gln Arg Val Arg Val Ala
 100 105 110
 Asp Glu Gly Ser Phe Thr Cys Phe Val Ser Ile Arg Asp Phe Gly Ser
 115 120 125
 Ala Ala Val Ser Leu Gln Val Ala Ala Pro Tyr Ser Lys Pro Ser Met
 130 135 140
 Thr Leu Glu Pro Asn Lys Asp Leu Arg Pro Gly Asp Thr Val Thr Ile
 145 150 155 160
 Thr Cys Ser Ser Tyr Arg Gly Tyr Pro Glu Ala Glu Val Phe Trp Gln
 165 170 175
 Asp Gly Gln Gly Val Pro Leu Thr Gly Asn Val Thr Thr Ser Gln Met
 180 185 190
 Ala Asn Glu Gln Gly Leu Phe Asp Val His Ser Val Leu Arg Val Val
 195 200 205
 Leu Gly Ala Asn Gly Thr Tyr Ser Cys Leu Val Arg Asn Pro Val Leu
 210 215 220

Gln Gln Asp Ala His Gly Ser Val Thr Ile Thr Gly Gln Pro Met Thr
 225 230 235 240
 Phe Pro Pro Glu Ala Gly Ser Gly Ser Glu Pro Arg Gly Pro Thr Ile
 245 250 255
 Lys Pro Cys Pro Pro Cys Lys Cys Pro Ala Pro Asn Leu Glu Gly Gly
 260 265 270
 Pro Ser Val Phe Ile Phe Pro Pro Lys Ile Lys Asp Val Leu Met Ile
 275 280 285
 Ser Leu Ser Pro Ile Val Thr Cys Val Val Val Asp Val Ser Glu Asp
 290 295 300
 Asp Pro Asp Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu Val His
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 Thr Ala Gln Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Leu Arg
 325 330 335
 Val Val Ser Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly Lys
 340 345 350
 Ala Phe Ala Cys Ala Val Asn Asn Lys Asp Leu Pro Ala Pro Ile Glu
 355 360 365
 Arg Thr Ile Ser Lys Pro Lys Gly Ser Val Arg Ala Pro Gln Val Tyr
 370 375 380
 Val Leu Pro Pro Pro Glu Glu Glu Met Thr Lys Lys Gln Val Thr Leu
 385 390 395 400
 Thr Cys Met Val Thr Asp Phe Met Pro Glu Asp Ile Tyr Val Glu Trp
 405 410 415
 Thr Asn Asn Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr Glu Pro Val
 420 425 430
 Leu Asp Ser Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg Val Glu
 435 440 445
 Lys Lys Asn Trp Val Glu Arg Asn Ser Tyr Ser Cys Ser Val Val His
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 Glu Gly Leu His Asn His His Thr Thr Lys Ser Phe Ser Arg Thr Pro
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 Gly Lys

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144

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<212> DNA
<213> Chimera

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 <213> Chimera

<400> 12

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20 25 30

Asp Pro Val Val Ala Leu Val Gly Thr Asp Ala Thr Leu Cys Cys Ser
35 40 45

Phe Ser Pro Glu Pro Gly Phe Ser Leu Ala Gln Leu Asn Leu Ile Trp
50 55 60

Gln Leu Thr Asp Thr Lys Gln Leu Val His Ser Phe Ala Glu Gly Gln
65 70 75 80

Asp Gln Gly Ser Ala Tyr Ala Asn Arg Thr Ala Leu Phe Pro Asp Leu
85 90 95

Leu Ala Gln Gly Asn Ala Ser Leu Arg Leu Gln Arg Val Arg Val Ala
100 105 110

Asp Glu Gly Ser Phe Thr Cys Phe Val Ser Ile Arg Asp Phe Gly Ser
115 120 125

Ala Ala Val Ser Leu Gln Val Ala Ala Pro Tyr Ser Lys Pro Ser Met
130 135 140

Thr Leu Glu Pro Asn Lys Asp Leu Arg Pro Gly Asp Thr Val Thr Ile
145 150 155 160

Thr Cys Ser Ser Tyr Gln Gly Tyr Pro Glu Ala Glu Val Phe Trp Gln
165 170 175

Asp Gly Gln Gly Val Pro Leu Thr Gly Asn Val Thr Thr Ser Gln Met
180 185 190

145 4

Ala Asn Glu Gln Gly Leu Phe Asp Val His Ser Ile Leu Arg Val Val
195 200 205

Leu Gly Ala Asn Gly Thr Tyr Ser Cys Leu Val Arg Asn Pro Val Leu
210 215 220

Gln Gln Asp Ala His Ser Ser Val Thr Ile Thr Pro Gln Arg Ser Pro
225 230 235 240

Thr Gly Ala Val Glu Val Gln Val Pro Glu Asp Pro Val Val Ala Leu
245 250 255

Val Gly Thr Asp Ala Thr Leu Arg Cys Ser Phe Ser Pro Glu Pro Gly
260 265 270

Phe Ser Leu Ala Gln Leu Asn Leu Ile Trp Gln Leu Thr Asp Thr Lys
275 280 285

Gln Leu Val His Ser Phe Thr Glu Gly Arg Asp Gln Gly Ser Ala Tyr
290 295 300

Ala Asn Arg Thr Ala Leu Phe Pro Asp Leu Leu Ala Gln Gly Asn Ala
305 310 315 320

Ser Leu Arg Leu Gln Arg Val Arg Val Ala Asp Glu Gly Ser Phe Thr
325 330 335

Cys Phe Val Ser Ile Arg Asp Phe Gly Ser Ala Ala Val Ser Leu Gln
340 345 350

Val Ala Ala Pro Tyr Ser Lys Pro Ser Met Thr Leu Glu Pro Asn Lys
355 360 365

Asp Leu Arg Pro Gly Asp Thr Val Thr Ile Thr Cys Ser Ser Tyr Arg
370 375 380

Gly Tyr Pro Glu Ala Glu Val Phe Trp Gln Asp Gly Gln Gly Val Pro
385 390 395 400

Leu Thr Gly Asn Val Thr Thr Ser Gln Met Ala Asn Glu Gln Gly Leu
405 410 415

Phe Asp Val His Ser Val Leu Arg Val Val Leu Gly Ala Asn Gly Thr
420 425 430

Tyr Ser Cys Leu Val Arg Asn Pro Val Leu Gln Gln Asp Ala His Gly
435 440 445

Ser Val Thr Ile Thr Gly Gln Pro Met Thr Phe Pro Pro Glu Ala Gly
450 455 460

147

Ser Gly Ser Glu Pro Arg Gly Pro Thr Ile Lys Pro Cys Pro Pro Cys
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Lys Cys Pro Ala Pro Asn Leu Glu Gly Gly Pro Ser Val Phe Ile Phe
485 490 495

Pro Pro Lys Ile Lys Asp Val Leu Met Ile Ser Leu Ser Pro Ile Val
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Thr Cys Val Val Val Asp Val Ser Glu Asp Asp Pro Asp Val Gln Ile
515 520 525

Ser Trp Phe Val Asn Asn Val Glu Val His Thr Ala Gln Thr Gln Thr
530 535 540

His Arg Glu Asp Tyr Asn Ser Thr Leu Arg Val Val Ser Ala Leu Pro
545 550 555 560

Ile Gln His Gln Asp Trp Met Ser Gly Lys Ala Phe Ala Cys Ala Val
565 570 575

Asn Asn Lys Asp Leu Pro Ala Pro Ile Glu Arg Thr Ile Ser Lys Pro
580 585 590

Lys Gly Ser Val Arg Ala Pro Gln Val Tyr Val Leu Pro Pro Pro Glu
595 600 605

Glu Glu Met Thr Lys Lys Gln Val Thr Leu Thr Cys Met Val Thr Asp
610 615 620

Phe Met Pro Glu Asp Ile Tyr Val Glu Trp Thr Asn Asn Gly Lys Thr
625 630 635 640

Glu Leu Asn Tyr Lys Asn Thr Glu Pro Val Leu Asp Ser Asp Gly Ser
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Tyr Phe Met Tyr Ser Lys Leu Arg Val Glu Lys Lys Asn Trp Val Glu
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Arg Asn Ser Tyr Ser Cys Ser Val Val His Glu Gly Leu His Asn His
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His Thr Thr Lys Ser Phe Ser Arg Thr Pro Gly Lys
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<213> Mouse

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Page 15

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<211> 482
<212> PRT
<213> Mouse

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<400> 14

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20         25         30

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57

149

Asp Pro Val Val Ala Leu Val Asp Thr Asp Ala Thr Leu Arg Cys Ser
35 40 45

Phe Ser Pro Glu Pro Gly Phe Ser Leu Ala Gln Leu Asn Leu Ile Trp
50 55 60

Gln Leu Thr Asp Thr Lys Gln Leu Val His Ser Phe Thr Glu Gly Arg
65 70 75 80

Asp Gln Gly Ser Ala Tyr Ser Asn Arg Thr Ala Leu Phe Pro Asp Leu
85 90 95

Leu Val Gln Gly Asn Ala Ser Leu Arg Leu Gln Arg Val Arg Val Thr
100 105 110

Asp Glu Gly Ser Tyr Thr Cys Phe Val Ser Ile Gln Asp Phe Asp Ser
115 120 125

Ala Ala Val Ser Leu Gln Val Ala Ala Pro Tyr Ser Lys Pro Ser Met
130 135 140

Thr Leu Glu Pro Asn Lys Asp Leu Arg Pro Gly Asn Met Val Thr Ile
145 150 155 160

Thr Cys Ser Ser Tyr Gln Gly Tyr Pro Glu Ala Glu Val Phe Trp Lys
165 170 175

Asp Gly Gln Gly Val Pro Leu Thr Gly Asn Val Thr Thr Ser Gln Met
180 185 190

Ala Asn Glu Arg Gly Leu Phe Asp Val His Ser Val Leu Arg Val Val
195 200 205

Leu Gly Ala Asn Gly Thr Tyr Ser Cys Leu Val Arg Asn Pro Val Leu
210 215 220

Gln Gln Asp Ala His Gly Ser Val Thr Ile Thr Gly Gln Pro Leu Thr
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Phe Pro Pro Glu Ala Gly Ser Gly Ser Glu Pro Arg Gly Pro Thr Ile
245 250 255

Lys Pro Cys Pro Pro Cys Lys Cys Pro Ala Pro Asn Leu Glu Gly Gly
260 265 270

Pro Ser Val Phe Ile Phe Pro Pro Lys Ile Lys Asp Val Leu Met Ile
275 280 285

Ser Leu Ser Pro Ile Val Thr Cys Val Val Val Asp Val Ser Glu Asp
290 295 300

Asp Pro Asp Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu Val His

150

305 310 315 320

150

Thr Ala Gln Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Leu Arg
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Val Val Ser Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly Lys
340 345 350

Ala Phe Ala Cys Ala Val Asn Asn Lys Asp Leu Pro Ala Pro Ile Glu
355 360 365

Arg Thr Ile Ser Lys Pro Lys Gly Ser Val Arg Ala Pro Gln Val Tyr
370 375 380

Val Leu Pro Pro Pro Glu Glu Glu Met Thr Lys Lys Gln Val Thr Leu
385 390 395 400

Thr Cys Met Val Thr Asp Phe Met Pro Glu Asp Ile Tyr Val Glu Trp
405 410 415

Thr Asn Asn Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr Glu Pro Val
420 425 430

Leu Asp Ser Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg Val Glu
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Lys Lys Asn Trp Val Glu Arg Asn Ser Tyr Ser Cys Ser Val Val His
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Gly Lys

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<211> 112
<212> PRT
<213> Artificial sequence

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<223> Conserved among mammals

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<222> (26)..(27)
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75

151

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<222> (107)..(107)
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<221> Misc_feature
<222> (7)..(17)
<223> PEDPVVALVGT, or another amino acid sequence

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Leu Ala Gln Leu Asn Leu Ile Trp Gln Leu Thr Asp Thr Lys Gln Leu
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Val His Ser Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Ala Asn
50 55 60

Arg Thr Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu
65 70 75 80

Arg Leu Gln Arg Val Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
85 90 95

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Thr Asp Ala Thr Leu Xaa Cys Ser Phe Ser Pro Glu Pro Gly Phe Ser

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30

Leu Ala Gln Leu Asn Leu Ile Trp Gln Leu Thr Asp Thr Lys Gln Leu

35

40

45

Val His Ser Phe Xaa Glu Gly Xaa Asp Gln Gly Ser Ala Tyr Ala Asn

50

55

60

154

57

Arg Thr Ala Leu Phe Pro Asp Leu Leu Ala Gln Gly Asn Ala Ser Leu
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22

①

157

\$

USO DE B7-H3 COMO UN AGENTE INMUNO REGULATORIO

DESCRIPCIÓN DE LA INVENCION

Campo de la Invención

[0001] La presente Descripción se relaciona con los campos de inmunología e inmunología clínica, y más particularmente con el uso de ligandos de la familia B7 y agonistas y antagonistas de estos en la modulación de respuestas inmunes.

Antecedentes de la Invención

[0002] La activación del linfocito es un proceso multietapas que requiere que varios eventos de señalización entre el linfocito y una célula "accesoria" una célula que presenta antígeno (APC) en el caso de activación de célula T, o una célula T adyuvante para activación de célula B). Para que ocurra la activación del linfocito, se deben suministrar dos tipos de señales a un linfocito en descanso. El tipo primario (estimulador) le confiere especificidad a la respuesta inmune y es mediado por el receptor específico de antígeno (TcR-sobre células T y BcR-sobre células) luego del

reconocimiento del complejo péptido MHC antigénico. El segundo tipo (coestimulador) es responsable por la magnitud de la respuesta. La señal es mediada vía receptores "accesorios" expresados sobre la superficie del linfocito. El requisito para la coestimulación permite que la activación del linfocito sea estrictamente regulada. La coestimulación es a su vez regulada por los receptores inhibitorios sobre estas células que pueden suministrar señales negativas que contra actúan las señales coestimuladoras positivas.

[0003] La evidencia montada sugiere que un gran número de ligandos y receptores estructuralmente relacionados que pertenecen a la superfamilia de las inmunoglobulinas (Ig) de moléculas interactúan y balancean las señales utilizadas durante el proceso de activación del linfocito (Frauwirth et al. (2002) J. Clin. Invest., 109:295-299). Uno de tales grupos estructuralmente relacionados incluye ligandos de la familia (B7-1, B7-2, ICOS-L, PD-L1, PD-L2, y B7-H3), Cuando comparten una estructura de dominio similar que tiene un dominio similar a Ig-V ("V") y un dominio similar a Ig-C ("C") que reciben en la porción extracelular de la molécula (Sharpe et al. (2002) Nature Rev. Immunol., 2:116-126). En la supresión de un exón y en los estudios cristalográficos las proteínas B7, han demostrado que la interacción del receptor-ligando ocurre por vía de los dominios V (Ostrov et al.,

(2002 Science, 290:816-819), aunque los dominios C sirven como medios de soporte estructural para los dominios V.

[0004] Los B7-1 y los B7-2 pueden suministrar señales positivas (a través de su receptor cognato de afinidad baja CD28) o señales negativas (a través del receptor de alta afinidad CTLA4). La interacción ICOS-L con el receptor ICOS resulta en señalización positiva mientras que el PD-L1 suministra las señales negativas a través del receptor, PD-1 (Carreno et al., (2002) Annual Rev. Immunol., 20:29-53).

[0005] La presente descripción se relaciona con la modulación de respuestas inmunes reguladas por el miembro más nuevo de la familia de ligando B7, el B7-H3. El B7-H3 humano fue originalmente identificado como una proteína similar a B7 que comparte el 20-27% de la identidad de aminoácido con otros miembros de la familia B7 y tiene un dominio V y uno C (Chapoval et al., (2001) Nat. Immunol., 2:269-274). Un análisis más detallado de los clones B7-H3 EST demostró una variación en la estructura del exón del gen en especies de mamíferos (Sun et al., (2002) J. Immunol., 168:6294-6297). En particular, en primates, el B7-H3 cADN existe en dos formas: Una que codifica un conjunto simple de dominios V Y C ("forma VC"), y la otra codifica un conjunto duplicado de dominios V

y C ("forma VCVC"). Como contraste al B3-H3 de primates, el B7-H3 cADN existe solamente en una forma simple como VC.

[0006] El B7-H3 VC ha sido inicialmente caracterizado como un ligando coestimulador tanto en humanos (Chapoval et al. (2201) Nat. Immunol., 2:269-274 y Solicitud de Patente Estadounidense No. Pub. 2002/0168762) y ratones (Sun et al. (2002) J. Immunol., 168:6294-6297). En particular, se ha reportado que la coestimulación en las células T humanas con el B7-H3 VC da como resultado una proliferación mejorada de células T, inducción de células T citotóxicas, y expresión incrementada del transcripto interferón gama. Adicionalmente, los experimentos de unión en ensayos basados en células sugieren que el B7-H3 VC se une a un receptor expresado sobre células T activadas, que no es CTLA-4, ICOS, o PD-1.

[0007] En general, existe necesidad de suministrar métodos terapéuticos para los desórdenes y las condiciones relacionadas con el sistema inmune. La modulación apropiada de respuestas inmune se puede lograr mediante la manipulación de la senda B7-H3.

RESUMEN DE LA INVENCION

[0008] Es uno de los objetos de la presente invención suministrar métodos y composiciones para la modulación de respuestas inmune. Los objetos adicionales de la invención reestablecerán en parte en la siguiente descripción y en parte serán entendidos de la descripción o pueden ser aprendidos mediante la práctica de la invención.

[0009] La presente invención se basa, en parte, en el descubrimiento y demostración de que la forma VCVC del B7-H3 cuenta para la mayoría de los transcritos B7-H3 vistos a través de tejidos múltiples aunque la forma VC del B7-H3 es solo un transcripto menor. La invención se basa además, en parte, en el descubrimiento y demostración de que ambas formas de B7-H3, VC y VCVC exhiben un efecto inhibitorio de activación de linfocito como se evidenció por la proliferación disminuida de células T y la secreción de citosina por estas células en la presencia de B7-H3. La invención está todavía además basada en parte, en el descubrimiento de regiones específicas dentro de los genes B7-H3 que están habitualmente sufriendo selección evolucionaria purificante.

[0010] En un aspecto, la presente descripción suministra métodos *in vitro*, *in vivo*, y *ex vivo* de respuestas inmunes modulantes, que incluyen métodos para tratar humanos o animales. En algunas modalidades, tales métodos comprenden una etapa de poner en contacto un linfocito, tal como una célula T, con un agente B7-H3, en donde el agente B7-H3 puede ser (a) un derivado de B7-H3 tal como una forma soluble de B7-H3; (b) un anticuerpo contra B7-H3; (c) un anticuerpo contra un receptor de B7-H3; o (d) un ácido nucleico que comprende al menos una porción del B7-H3 mRNA o un complemento a este. En ciertas modalidades, el agente B7-H3 está acoplado con la señal primaria (estimuladora específica de antígeno).

[0011] En modalidades particulares, los métodos de la invención, se utilizan para tratar o prevenir desórdenes inmunes susceptibles al tratamiento con tales composiciones. Específicamente, tales desórdenes incluyen pero no están limitados a desórdenes inmunológicos, que incluyen desórdenes autoinmunes (por ejemplo artritis reumatoide (RA), soriasis, esclerosis múltiple (MS), enfermedad inflamatoria del intestino (IBD), enfermedad de Crohn, lupus sistémico eritematoso (SLE), diabetes tipo I), rechazo de transplante, enfermedad de injerto de versus huésped, (GVHD), desórdenes inmunes hiperproliferativos, cánceres, desórdenes

inmunosupresores, enfermedades infecciosas varias etc. Así, en ciertas modalidades, los métodos de la invención comprenden identificar un sujeto necesitado de inhibir la activación del linfocito, y administrar un agente B7-H3 que es un agonista al sujeto. En otras modalidades, los métodos comprenden identificar un sujeto necesitado de mejorar la activación del linfocito, y administrar un agente B7-H3 que es un antagonista al sujeto.

[0012] Los anticuerpos utilizados en los métodos de la invención caen en dos grupos: (1) anticuerpos contra B7-H3 y (2) anticuerpos contra el receptor B7-H3. Esos anticuerpos pueden: (a) unirse específicamente al B7-H3 que bloquea mediante este la interacción del B7-H3 con su receptor; (b) unir específicamente un receptor B7-H3 bloqueando mediante este su interacción con el B7-H3; o (c) desarrollar tanto (a) como (b). Dependiendo del efecto deseado, los anticuerpos se pueden utilizar en configuraciones alternativas para mejorar o inhibir respuestas inmunes. En algunas modalidades, los anticuerpos son administrados para antagonizar la actividad biológica de los B7-H3 naturalmente expresados.

[0013] La descripción suministra además métodos que involucran composiciones que comprenden formas solubles de B7-H3. En algunas modalidades, la forma soluble del B7-H3,

comprende menos del B7-H3 de longitud completa y no incluye la transmembrana y los dominios intracelulares de B7-H3. En modalidades adicionales, una forma soluble de B7-H3 comprende al menos un dominio V de B7-H3, y opcionalmente al menos un dominio C de B7-H3. Una forma soluble puede comprender al menos 2, 3, 4, y 5 dominios V, y opcionalmente al menos 1, 2 3, 4, o 5 dominios C. En modalidades aún adicionales, una forma soluble del B7-H3 puede comprender: (a) una primer secuencia de aminoácido derivada del dominio extracelular de del B7-H3 y (b) una segunda secuencia de aminoácido derivada de la región constante de un anticuerpo, La primer secuencia de aminoácido se deriva de toda o una porción del dominio extracelular del B7-H3 y (a) inhibe competitivamente la unión de una forma de B7-H3 de ocurrencia natural a su receptor y/o (b) tiene una actividad coestimuladora negativa. En algunas modalidades, la primer secuencia de aminoácido comprende una secuencia como SE reestableció en la ID DE SEC. NO. 15. En ciertas modalidades, la primer secuencia de aminoácido es idéntica a o sustancialmente idéntica a los aminoácidos 23-244 de la ID DE SEC. NO. 14 o los aminoácidos 23-462 de la ID DE SEC. NO. 12. En una modalidad ilustrativa, la forma soluble del B7-H3 comprende una secuencia como en ID DE SEC. NO. 12 o ID DE SEC. NO. 14.

[0014] La descripción también suministra métodos que involucran usos terapéuticos y no terapéuticos de ácidos nucleicos o polipéptidos codificados por tales ácidos nucleicos, donde la secuencia de nucleótido de tal ácido nucleico se selecciona de: (a) una secuencia de nucleótido de la ID DE SEC. NO. 1, ID DE SEC. NO. 3, ID DE SEC. NO. 5 a una porción de ésta; y (b) un ácido nucleico que es al menos 60, 80, 100, 120, o 140 nucleótidos de largo e hibridiza al ácido nucleico de (a) bajo condiciones definidas, en donde el ácido nucleico codifica un producto de expresión que tiene una actividad coestimuladora negativa. En ciertas modalidades, tales ácidos nucleicos codifican una secuencia de aminoácido como la ID DE SEC. NO. 15. En una modalidad ilustrativa, el ácido nucleico comprende una secuencia sustancialmente como la ID DE SEC. NO. 11 o la ID DE SEC. NO. 13.

[0015] Los métodos de la invención también comprenden el uso de ARN de interferencia corta y ácidos nucleicos contra sentido para reducir la expresión del B7-H3 con el fin de mejorar la respuesta inmune.

[0016] La invención también comprende vectores que contienen cualquiera de los ácidos nucleicos anteriores y células huéspedes que contienen tales vectores.

[0017] Se debe entender que tanto la descripción general anterior como la siguiente descripción detallada son solo ejemplos y explicativas y no son restrictivas de la invención, tal como se reivindica.

BREVE DESCRIPCIÓN DE LAS FIGURAS

[0018] La figura 1A describe comparaciones de secuencia de B7-H3 de humano y ratón. El alineamiento de la secuencia de productos de gen de B7-H3 VC humano, B7-H3 VCVC humano, y B7-H3 de ratón traducidos deducidos. Las barras oscuras por encima del alineamiento de secuencia denotan dominios de exón demarcados mediante secuencias genómicas. Las flechas por debajo de las secuencias de péptidos denotan posiciones de ácidos nucleicos correspondientes utilizadas en iniciadores de oligonucleótido utilizados para amplificación de especies cruzadas.

[0019] la figura 1B describe la organización genómica de los locus de genes B7-H3 de humano y de ratón. Los montajes están basados en posiciones seleccionadas del eje genómico humano de Celera GA_x2HTBL4SSTP y el eje genómico de ratón de Celera GA_x5J8B7W7NM9. Las barras denotan localizaciones de repeticiones complejas, repeticiones simples, estructura de

exón de transcripto, y nombre de dominio de complejos Alu y SVA.

[0020] **La figura 2A** muestra los resultados del RT-PCR DEL B7-H3 sobre muestras de humano. Tres paneles separados de la primer hebra de cADN de humano se amplificaron con los iniciadores PCR comunes a ambas secuencias B7-H3 VC y B7-H3 VCVC y detectadas con oligonucleótidos al dominio V1. Los tamaños esperados de los productos amplificados son como se indica.

[0021] **La figura 2B** muestra los resultados del RT-PCR del B7-H3 sobre muestras de ratón. Un panel de ratón que consiste de cADN adulto y embrionario se analizó para la presencia de transcritos B7-H3. La hibridización específica predominante de una banda ~ 1 kb es consistente con la presencia de la forma VC simple del B7-H3 de ratón.

[0022] **La figura 3A-3B** demuestra un efecto coestimulatorio del B7 medido mediante la proliferación de células T activadas. La activación de B7-H3 de las células T da como resultado la atenuación y proliferación de producción de citoquina. La **figura 3A** describe resultados de ensayos proliferación de células CHO.HLA-DR2 que expresan GFP, B7-1, B7-2, mientras la **figura 3B** muestra los resultados de ensayos

de proliferación para las células CHO.HLA-DR2 que expresan GFP, B7-H3 VCVC, o B7-H3 VC. Los transfectantes de CHO.HLA-DR2 ($1,25 \times 10^4$ células/pozo) se incubaron con células T CD4⁺ (10^5 células/pozo) en la presencia de anticuerpos anti-CD3 solubles (1 µg/ml) y concentraciones tituladas de anticuerpo CD28. La proliferación fue medida en 72 horas. Las respuestas para las células T CD4⁺ más los transfectantes CHO.HLA-DR2 en ausencia del anticuerpo anti-CD3 fueron 300 CPM por debajo.

[0023] La **figura 4** demuestra el efecto inhibitorio del B7-H3 sobre la producción de citoquina mediante células T activadas. Las células CHO.HLA-DR2 que expresan GFP, B7-H3 VCVC o B7-H3 VC ($1,25 \times 10^4$ células /pozo) se incubaron con células T CD4⁺ (10^5 células/pozo) en la presencia de un anticuerpo anti-CD3 soluble (1 µ/ml) y concentraciones tituladas de anticuerpo CD28 (0.5 ng/ml). Los supernadantes se cosecharon de cultivos de células T estimulados con CHO.HLA-DR2 GFP, B7-H3 VCVC, o B7-H3 VC en la presencia de anticuerpos anti-CD3 (1 µ/ml).y anti-CD28 (0.5 ng/ml). La producción de citoquina se inhibió a las 72 horas utilizando selección ELISA.

[0024] La **figura 5** demuestra que el B7-H3 VC y B7-H3-VCVC suministra una señal negativa a las células T CD4⁺ humanas medidas mediante la inhibición de la proliferación celular.

Las células T CD4⁺ más purificadas (10⁵ células/pozo) se activaron con anticuerpo anti-CD3 (1 µg/10⁷ microesfera) y B7-H3-Ig (VCVC o VC; 4 µg/10⁷ microesferas) sobre microesferas CIS o TRANS. Las microesferas CIS recubiertas con tanto anticuerpo anti-CD3 (1 µg/10⁷ microesferas) y B7-H3 Ig (VCVC o VC) a 4 µg/10⁷ microesferas. Las microesferas TRANS consistían de una mezcla de dos tipos de microesferas: (a) microesferas recubiertas con anticuerpo anti-CD3 1 µg/10⁷ microesferas) y (b) microesferas recubiertas con B7-H3 (VCVC o VC). Para mantener una proporción microesfera a célula igual, las microesferas recubiertas con Ig de murino de control se agregaron para lograr una concentración de proteína total de 5 µg/10⁷ glóbulos. La proliferación se midió a las 72 horas.

[0025] Las **figuras 6A-6C** demuestran que el B7-H3 VC y el B7-H3 VCVC suministran una señal negativa a las células T CD4⁺ de humano medidas mediante la inhibición de secreción de citoquina. Las células CD4⁺ purificadas (10⁵ células/pozo) se activaron con un anticuerpo anti-CD3 (1 µg/10⁷ microesferas) y B7-H3-Ig (VCVC o VC; 4 µg/10⁷ microesferas) sobre microesferas CIS o TRANS. Las microesferas CIS recubiertas con tanto anti-CD3 (1 µg/10⁷ microesferas) como B7-H3-Ig (VCVC o VC) a 4 µg/10⁷ microesferas. Las microesferas TRANS consistían de una mezcla de dos tipos de microesferas: (a)

microesferas recubiertas con anticuerpo anti-CD3 ($1 \mu\text{g}/10^7$ microesferas) y (b) microesferas recubiertas con B7-HA (VCVC o VC). Para mantener una proporción microesfera a célula igual, las microesferas recubiertas con el Ig de murino de control se agregaron para lograr una concentración de proteína total de $5 \mu\text{g}/10^7$ glóbulos. La cantidad de citoquinas en los supernadantes se midió a las 72 horas utilizando selección ELISA multiplex: TNF- α -(figura 6A), IFN- γ -(figura 6B), y GM-CSF (figura 6C).

BREVE DESCRIPCIÓN DE LAS SECUENCIAS

[0026] La ID DE SEC. NO. 1 y la ID DE SEC. NO. 2 representan, respectivamente, secuencias de longitud completa de ácido nucleico y aminoácido del B7-H3 VC de humano.

[0027] La ID DE SEC. NO. 3 y la ID DE SEC. NO. 4 representan, respectivamente, secuencias de longitud completa de ácido nucleico de aminoácido de B7-H3 de ratón.

[0028] La ID DE SEC. NO. 5 y la ID DE SEC. NO. 6 representan, respectivamente, secuencias de longitud completa de ácidos nucleico y aminoácido del B7-H3 VCVC de humano.

[0029] La ID DE SEC. NO. 7 representa una secuencia de aminoácido de B7-H3 de humano (28-139 de aminoácidos de B7-H3 de VC o B7-H3 VCVC).

[0030] La ID DE SEC. NO. 8 representa aminoácidos conservados entre las regiones V1 y V2 del B7-H3 VCVC de humano, es decir, entre los aminoácidos 28-139 y 246-357 de la ID DE SEC. NO. 6.

[0031] La ID DE SEC. NO. 9 Y la ID DE SEC. NO. 10 representan respectivamente secuencias de ácido nucleico y aminoácido de un polipéptido de fusión que contiene la secuencia de señal M de oncostatina (los aminoácidos 1-22 de la ID DE SEC. NO. 10), el dominio extracelular del B7-H3 VC de humano (aminoácidos 23-244 de la ID DE SEC. NO. 10), y la región constante del ratón YgG_{2am} (aminoácidos 245-482 de la ID DE SEC. NO. 10).

[0032] La ID DE SEC. NO. 11 y la ID DE SEC. NO. 12 representan respectivamente secuencias de ácido nucleico y aminoácido del polipéptido de fusión que contiene la secuencia de señal M de oncostatina (aminoácidos 1-22 de la ID DE SEC. NO. 12), el dominio extracelular de B7-H3 VCVC de humano (aminoácidos 23-462 de la ID DE SEC. NO. 12), y la

región constante del IgG_{2am} de ratón (aminoácidos 463-700 de la ID DE SEC. NO. 12).

[0033] La ID DE SEC. NO. 13 Y LA ID DE SEC. NO. 14 representan, respectivamente, secuencias de ácido nucleico y aminoácido de un polipéptido de fusión que contiene la secuencia de señal M de oncostatina (aminoácidos 1-22 de la ID DE SEC. NO. 14), el dominio extracelular de B7-H3 VC de ratón (aminoácidos 23-244 de la ID DE SEC. NO. 14, y la región constante de ratón IgG_{2am} (aminoácidos 245-482 de la ID DE SEC. NO. 14).

[0034] La ID DE SEC. NO. 15 representa los aminoácidos conservados en él o los dominios similares a -V del B7-H3 de mamífero.

[0035] La SEQ ID NOs:16-22 representan regiones a individuales altamente conservadas en el o los dominios similares a Ig-V de B7-H3 de mamífero.

[0036] La SEQ ID NOs:23-35 representan iniciadores PCR empleados para aislamiento de secuencias B7-H3 como se describe en los ejemplos.

DESCRIPCIÓN DETALLADA DE LA INVENCION

1. Definición

[0037] Con el fin de ser más fácilmente entendida la presente invención, se definen ciertos términos aquí. Las definiciones adicionales son como se establece a lo largo de la descripción.

[0038] El término "anticuerpo", como se utiliza aquí, se refiere a una inmunoglobulina o parte de esta, y comprende cualquier polipéptido que comprenda un ciclo que se une a antígenos sin importar la fuente, el método de producción, y otras características. El término incluye pero no está limitado a policlonal, monoclonal monoespecífico, poliespecífico, no específico, humanizado, de cadena simple, quimérico, sintético, recombinante, híbrido, mutante, y anticuerpos injertados CDR. El término "dominio de unión de antígeno" se refiere a parte de una molécula de anticuerpo que comprende el área que se une específicamente o que es complementaria con una parte o todo un antígeno. Donde un antígeno es largo, un anticuerpo puede solo unirse a una parte particular del antígeno. El "epítipo", o "determinante antigénico" es una porción de una molécula de antígeno que es responsable por las interacciones específicas con el dominio

de unión de antígeno de un anticuerpo. El dominio de unión de antígeno se puede suministrar mediante uno o más dominios variables de anticuerpos (por ejemplo un fragmento del así llamado anticuerpo Fd que consiste de un dominio V_H). El dominio de unión del antígeno comprende una región variable de cadena ligera de anticuerpo (V_L) y una región variable de cadena pesada de anticuerpo (V_H).

[0039] El término "**anticuerpo anti-B7-H3**" o "**anticuerpo contra B7-H3**", se refiere a un anticuerpo se une específicamente a al menos un epítipo de al menos una forma B7-H3 que incluye pero no está limitado a B7-H3 VC y B7-H3 VCVC. Los términos "**anticuerpo receptor anti-B7-H3**" y "**anticuerpo contra un receptor B7-H3**" se refieren a cualquier anticuerpo que se une específicamente al menos un epítipo de un receptor para B7-H3.

[0040] Como se utiliza aquí, el término "**B7-H3**", a menos que se establezca otra cosa, se refiere a cualquiera y todas las formas de B7-H3, que incluye pero ni está limitado a VC y VCVC. El término "**agente B7-H3**" se refiere a cualquier compuesto capaz remodelar actividad biológica del B7-H3. El término "**modular**" y sus análogos se refieren a una reducción a un incremento de la actividad biológica del B7-H3, por ejemplo, la actividad asociada con el efecto ejercido por el

B7-H3 naturalmente expresado sobre un linfocito que expresa un receptor de B7-H3. Una reducción o un incremento en la actividad biológica es preferiblemente al menos 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, o más. Un agente B7-H3 que origina tal reducción es denominado como "**antagonista**", mientras que un agente B7-H3 que origina tal incremento es denominado como "**agonista**". Se entenderá que el antagonista del B7-H3 contra actuaría la "señal coestimuladora negativa" ejercida por el B7-H3 naturalmente expresado en un linfocito que expresa un receptor de B7-H3 mientras que un agonista mejoraría tal "señal coestimuladora negativa". Por lo tanto, un antagonista de B7-H3 generalmente origina un incremento de la activación del linfocito (por ejemplo medido mediante la proliferación celular y/o la secreción de citoquina), mientras que el agonista de B7-H3 generalmente origina una disminución del mismo.

[0041] El término "**actividad biológica**" se refiere a una función o un conjunto de funciones (o al efecto al cual es atribuible la función) desarrollada por una molécula en un sistema biológico, que puede ser *in vivo* o *in vitro*. La actividad biológica puede ser evaluada mediante, por ejemplo, el efecto sobre la proliferación del linfocito, la supervivencia, y la función (por ejemplo secreción de citosina), agrupamiento de expresión de marcador de

diferenciación, expresión de gen en los niveles transcripcional, transduccional y post transduccional, o el efecto sobre la producción de anticuerpos, etc.

[0042] El término "coestimulación" y sus análogos se refiere a eventos de señalización entre los cuales el receptor/ligando de las moléculas de superficie de células sobre el linfocito respondedor y unas células "accesoria" (por ejemplo una célula que representa antígeno (APC) en el caso de activación de célula T, o una activación de célula T para célula B ayudante) para permitir la activación del linfocito. Los términos **"coestimulación negativa"**, **"señal coestimuladora negativa"**, **"señal inhibitoria"**, **"actividad coestimuladora negativa"**, y sus análogos se refiere a eventos de señalización que inhiben la activación de linfocito con relación a aquella en la ausencia de tales señales. Se entenderá que una célula T activada puede ser una célula ayudante (es decir $CD4^+$), una célula citotóxica o una célula supresora (es decir $CD8^+$). La actividad coestimuladora negativa se puede medir utilizando técnicas estándar y, sin limitación, como se describe en los ejemplos. Y en particular y sin limitación, como se describe en los ejemplos. En particular, los agentes B7-H3 actualmente descritos inhiben la activación de linfocito, que se puede

medir mediante (a) la proliferación celular y/o (b) de secreción de citosina.

[0043] El término "**derivada**", "**derivado de**", y sus análogos, cuando se utilizan con referencia a un aminoácido o a una secuencia de nucleótido, se refiere, a una secuencia que es idéntica o sustancialmente idéntica a toda o a una porción de la secuencia padre y puede ser de hecho obtenida de la secuencia padre, por ejemplo, por vía del ácido nucleico o la sustitución de nucleótido, supresión, o adición, u otras modificaciones.

[0044] El término "**hibridización bajo condiciones definidas**" se refiere a las condiciones para hibridización y lavado bajo las cuales las secuencias de nucleótido que son significativamente idénticas u homologas una a la otra permanecen unidas una a la otra. Se refiere a condiciones para hibridización y lavados bajo las cuales las secuencias de nucleótido que son significativamente idénticas u homologas una a la otra permanecen unidas una a la otra. Las condiciones son tales que las secuencias, que son al menos 50, 100, 150, 300, o más nucleótidos de largo y al menos 70%, más preferiblemente al menos 80%, aún más preferiblemente al menos 85-90% idénticas, permanecen unidas una a la otra. La identidad porcentual se puede determinar como se describe en

Altschul et al. (1997) Nucleic Acids Res., 25:3389-3402.

Ejemplos no limitativos de condiciones de hibridización con restricción baja, moderada y alta se suministran en las secciones subsecuentes.

[0045] El término "**desorden inmunológico**" se refiere a desórdenes y condiciones en las cuales es aberrante una respuesta inmune. La respuesta aberrante se puede deber a (a) proliferación anormal, maduración, supervivencia, diferenciación, o función de células inmunes tales como, por ejemplo, células T o B. Tales desórdenes incluyen pero no están limitados a desórdenes autoinmunes (artritis reumatoide (RA), psoriasis, esclerosis múltiple (MS), enfermedad inflamatoria del intestino (IBD, enfermedad de Crohn, grupos eritematosos por lupus sistémico (SLE), diabetes tipo I), rechazo de trasplante, enfermedad de injerto versus (GVHD), desórdenes inmunes hiperproliferativos, y desórdenes inmunosupresores. En particular, la descripción suministra métodos que involucran composiciones que comprenden agentes de B7-H3 tales como formas solubles de B7-H3 o anticuerpos contra B7-H3 o contra su receptor.

[0046] El término "**aislados**" se refiere a una molécula que es sustancialmente libre de su ambiente natural. Por ejemplo, una proteína aislada sustancialmente libre de material

celular y otras proteínas de la fuente de célula o tejido de la cual se deriva. El término "aislado" también se refiere a preparaciones donde la proteína aislada es suficientemente pura para ser administrada como una composición farmacéutica, o al menos 70-80% (p/p) pura, más preferiblemente, al menos 80-90% (p/p) pura, aún más preferiblemente, 90-95% pura; y, más preferiblemente al menos 95%, 96%, 97%, 98%, 99% o 100% (p/p) pura. El término "aislado", como se utiliza aquí, también se refiere a preparaciones que son sustancialmente libres de endotoxina, es decir, los niveles de endotoxina están por debajo de 500, 300, 200, 100, 50, 10, 5, 1, 0.5, 0.1, 0.05, 0.01 EU/ml, o por debajo del nivel detectable.

[0047] El término "**mamífero**" se refiere a cualquier animal clasificado como tal, incluyendo humanos.

[0048] El término "**señal estimuladora primaria**" se refiere a una señal estimuladora derivada de un linfocito que confiere especificidad a la respuesta inmune y es mediada por el receptor específico de antígeno (TcR-sobre células T, y BcR-sobre células) luego del reconocimiento del complejo péptido-MHC antigénico.

[0049] Los términos "**tratamiento**", "**método terapéutico**", y sus análogos se refiere a tanto a tratamiento terapéutico

como a medidas profilácticas/preventivas. Aquellos necesitados del tratamiento pueden incluir individuos que ya tienen un desorden médico particular así como también aquellos en riesgo de padecer el desorden (es decir, aquellos que tienen probabilidades de finalmente adquirir el desorden). Un método terapéutico como resultado de la prevención o el mejoramiento de los síntomas o de otra manera una consecuencia biológica deseada y puede ser evaluada mediante signos químicos mejorados, (por ejemplo PASI como se describe en los ejemplos), acceso retrasado de enfermedad, niveles reducidos/elevados de linfocitos y/o anticuerpos, etc.

[0050] Los términos **"compuesto terapéutico"** y **"terapéuticos"**, como se utilizan aquí, se refieren a cualquier compuesto capaz de mejorar las manifestaciones químicas de un desorden, o producir una consecuencia biológica deseada.

[0051] Los términos **"dosis terapéuticamente efectivas"** y **"cantidad terapéuticamente efectiva"** se refiere a aquella cantidad de un compuesto que da como resultado la prevención o mejoramiento de los síntomas en un paciente o de una consecuencia biológica deseada, por ejemplo, signos químicos mejorados (por ejemplo PASI como se describe en los

ejemplos), acceso retrasado de, enfermedad, niveles reducidos/elevados de linfocitos y/o anticuerpos, etc La cantidad efectiva se puede determinar, como se describe en las secciones frecuentes

[0052] El término "**específicamente unido**" y sus análogos significa que dos moléculas forman un complejo que es relativamente estable bajo condiciones fisiológicas La unión específica se caracteriza por una alta afinidad y una moderada capacidad La unión no específica usualmente tiene una baja afinidad con una capacidad moderada a alta Típicamente, la unión es considerada específica cuando la constante de afinidad K_a es mayor que 10^6 M^{-1} , o preferiblemente mayor de 10^8 M^{-1} Si es necesario, la unión no específica se puede reducir sin afectar sustancialmente la unión específica al variarlas condiciones de unión Tales condiciones son conocidas en la técnica, y un experto utilizando técnicas de rutina puede seleccionar las condiciones apropiadas Las condiciones son usualmente definidas en términos de concentración de proteína, tensión iónica de la solución, temperatura, tiempo permitido para la unión, concentración de moléculas no relacionadas (por ejemplo sero albúmina, caseína de leche), etc

[0053] La frase "**sustancialmente idéntica**" significa que una secuencia de aminoácido relevante es al menos 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, o 100% idéntica a una secuencia dada. Por vía de ejemplo, tales consecuencias pueden ser variantes derivadas de especies varias, o ellas pueden ser derivadas de la secuencia dada mediante ubicamiento, su presión, sustitución o adición de aminoácido. La identidad porcentual entre dos secuencias de aminoácido se determina mediante algoritmos de alineación estándar tales como, por ejemplo, Basic Local Alignment Tool (BLAST) descrito en Altschul et al. (1990) J. Mol. Biol. , 225:403-410, el algoritmo de Needleman et al. (1970) J. Mol. Biol. , 48:444-453; el algoritmo de Meyers et al. (1988) Comput. Appl. Biosci., 4:11-17; o Tatusova et al. (1999) FEMS Microbiol. Lett., 174:247-250, etc. Tales algoritmos reincorporan en posprogramas BLASTN, BLASTP y "BLAST 2 Sequences" (ver www.ncbi.nlm.nih.gov/BLAST) Cuando se utilizan tales programas, se pueden utilizar los parametros por defecto. Por ejemplo, para la secuencias de nucleótido se pueden utilizar las siguientes configuraciones para "BLAST 2 Sequences": el programa BLASTN, regreso por coincidencia 2, pena por no coincidencia -2, penas de espacio abierto y extensión de espacio 5 y 2 respectivamente, espacio x_espacio por caída 50, expectativa 10, tamaño de palabra 11, filtro PRENDIDO. Para las secuencias de aminoácido se puede

utilizar la siguiente configuración para "BLAST 2 Sequences":
programa BLASTP, matriz BLOSUM62, pena de espacio abierto
extensión de espacio 11 y 1 respectivamente, espacio x_caída
50, expectativa 10, tamaño de palabra 3, filtro PRENDIDO.

[0054] Los términos "**polinucleótido**", "**oligonucleótido**" y "**ácido nucleico**" se refiere a ácido desoxiribonucleico (ADN) y, cuando es apropiado, al ácido ribonucleico (ARN), o ácido nucleico péptido (ANP). El término también se debe entender por incluir los análogos de nucleótido, y los polinucleótidos de hebra simple o doble (por ejemplo siARN). Los ejemplos de polinucleótidos incluyen pero no están limitados a ADN de plásmido o fragmentos de éste, ADN o ARN viral, ARN antisentido, etc. El término "ADN de plásmido" se refiere a un ADN de hebra doble que es circular. "Antisentido", como reutiliza aquí, se refiere a un ácido nucleico capaz de hibridizar a una porción de una región codificante y/o no codificante de mRNA en virtud de la complementariedad de la secuencia, interfiriendo de esta manera con la traducción del mRNA. Los términos "siARN" y "ARNi" se refieren al ácido de nucleico que es un ARN de hebra doble que tiene la capacidad de inducir la degradación del mRNA "silenciando" de esta manera la extensión del gen. Típicamente, el siARN tiene al menos 15-50 nucleótidos largos, por ejemplo, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 nucleótidos de largo.

[0055] El término "**dominio V**" (singular o plural), a menos que se establezca otra cosa, se refiere al primer dominio variable similar a Ig (V₁) y/o al segundo dominio similar a Ig (V₂) en la secuencia de proteína o genómica del B7-H3, sin importar las especies de origen (por ejemplo cualquier secuencia que comprende la ID DE SEC. NO. 15 y una secuencia de nucleótidos que la codifican; a una secuencia sustancialmente idéntica a la ID DE SEC. NO. 7 y una secuencia de nucleótidos que la codifican). De manera similar, el término "**dominio C**" (singular o plural), a menos que específicamente se establezca, se refiere al primer dominio constante similar a Ig (C₁) y/o al segundo dominio constante similar a Ig (C₂) en la secuencia de proteína genómica del B7-H3. Sin importar las especies de origen. A menos que el contexto lo requiera de otra forma, las referencias a los dominios V y C se debe entender por comprender los dominios de proteína, las secuencias de nucleótidos, que codifican para estos, y las secuencias de pseudo-exón que corresponden a las secuencias codificantes (por ejemplo, C ψ y V ψ de las secuencias genéricas de roedor).

2. Agente B7-H3.

[0056] En un aspecto, la presente invención se relaciona con el uso de agentes B7-H3 en la modulación de respuestas inmunes. La presente invención se basa, en parte, en el descubrimiento y la demostración de que la forma VCVC del B7-H3 cuenta para la mayoría de los transcritos B7-H3 lisos a través de tejidos múltiples aunque la forma VC del B7-H3 es solo un transcrito menor. La invención se basa adicionalmente, en parte en el descubrimiento y demostración de que ambas formas de B7-H3, VC y VCVC, exhiben un efecto inhibitorio sobre la activación de las células T como se evidencian mediante la proliferación disminuida y la secreción de citocina mediante las células en la presencia de B7-H3. La invención se basa aún adicionalmente, en parte, en el descubrimiento de las regiones específicas dentro de los genes B7-H3 que sufren concurrentemente selección e evolucionaria purificante.

[0057] Las porciones de los exones V genómicos de ratón, humano, mono, y Hámster se alinearon utilizando ClustalW del modulo de Alineación del vector NTL versión 8.0. Las regiones que exhibieron 100% de identidad de secuencia en alineamiento mayor o igual a nueve nucleótidos se escogen como las posiciones de nucleótido más altamente conservadas. Estas

once regiones conservadas se representan en la ID de ID DE SEC. NO. 15.

[0058] En ciertas modalidades, las composiciones utilizadas en los métodos de la invención comprenden un agente B7-H3 que antagoniza o agoniza la actividad biológica del B7-H3 de ocurrencia natural. En algunas modalidades, el agente B7-H3 es proteinacea, es decir, comprende aminoácidos ligados mediante uniones de péptido. Los agentes B7-H3 proteinaceos incluyen pero no están limitados a formas solubles de B7-H3, que incluyen fusiones de B7-H3-Ig, anticuerpos contra B7-H3, y anticuerpos contra un receptor B7-H3. En otras modalidades, las composiciones utilizadas en los métodos de la invención comprenden agentes B7-H3 no proteinaceos, tales como ácidos nucleicos, inhibidores de moléculas pequeñas, etc. En particular, los agentes B7-H3 actualmente descritos modulan activación de linfocito medidos mediante uno o más de los siguientes: (a) proliferación de linfocito; y (b) secreción de citocina (por ejemplo interleucina (iL) -10, factor de necrosis tumoral (TNF) - α , interferón (IFN) - γ , y factor estimulante de granuloso-macrófago-colonia (GM-CSF)). En algunas modalidades, los agentes B7-H3 poseen propiedades farmacocinéticas que los hacen adecuados para uso terapéutico, por ejemplo, vida media

circulatoria suficientemente larga y/o protección aceptable proveniente de degradación proteolítica.

2.1 Anticuerpos

[0059] Los anticuerpos utilizados en el método de la invención caen en dos grupos: (1) anticuerpos contra el B7-H3 y (2) anticuerpos contra el receptor de B7-H3. En varias modalidades, los anticuerpos utilizados en el método de la invención se unen específicamente a al menos uno de: (a) B7-H3; (b) receptor de B7-H3; (c) dominio V en B7-H3; (d) dominio (c) en B7-H3; y (e) el polipéptido que comprende la ID DE SEC. NO. 15, ID DE SEC. NO. 2, ID DE SEC. NO. 4, ID DE SEC. NO. 6, O ID DE SEC. NO. 7. Tales anticuerpos pueden (a) unirse específicamente al B7-H3 bloqueando de esta manera la interacción del B7-H3 con su receptor; (b) unirse específicamente al receptor de B7-H3 bloqueando de esta manera su interacción con el B7-H3, o (c) desarrollar ambas (a) y (b). Dependiendo del efecto deseado, los anticuerpos se pueden utilizar en configuraciones alternativas para mejorar (como un antagonista de la actividad biológica de B7-H3) o inhibir respuestas inmunes (como un agonista de la actividad biológica de B7-H3) como se describe en las siguientes secciones).

[0060] Los anticuerpos se pueden hacer, por ejemplo, mediante técnicas de hibridoma tradicional (Kohler and Milstein (1975) Nature, 256: 495-499), métodos de ADN recombinante (U.S. Pat No. 4,816,567) o técnicas de despliegue de fago utilizando librerías de anticuerpo (Claxon et al. (1991) Nature, 352: 624-628; Marks et al. (1991) J. Mol, Biol., 222: 581-597). Para varias otras técnicas de producción de anticuerpo, ver, por ejemplo, anticuerpos: A Laboratory Manual eds. Harlow et al., Cold Spring Harbor Laboratory, 1988; y Antibody Engineering, 2nd ed., Oxford University Press, ed. Borrebaeck, 1995. Para la administración a humanos, los anticuerpos pueden ser completamente humanos o humanizados. En ciertas modalidades los anticuerpos pueden tener una región Fc alterada o mutada como se describe en las siguientes secciones.

2.2. Formas Solubles de B7-H3

[0061] Los métodos de la invención involucran el uso de formas solubles de B7-H3 que inhiben la activación del linfocito. En algunas modalidades, la forma soluble del B7-H3 comprende menos del B7-H3 de longitud completa y no incluye la transmembrana y los dominios intracelulares del B7-H3. Tal forma soluble también puede no incluir una secuencia de señal. Para solamente ilustración, y no para ser limitante,

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esos dominios se pueden delinear en B7-H3 de humano y ratón como se describe en la figura 1A.

[0062] En ciertas modalidades, una forma soluble comprende una secuencia de aminoácido como la ID DE SEC. NO. 15 O la ID DE SEC. NO. 7. En aún modalidades adicionales, una forma soluble de B7-H3 comprende al menos un dominio de B7-H3 y opcionalmente al menos un dominio C de B7-H3. Una forma soluble puede comprender al menos 2, 3, 4, o 5 dominios V, funcionalmente al menos 1, 2, 3, 4, o 5 dominios C.

[0063] En modalidades adicionales, una forma soluble de B7-H3 puede comprender (a) una primer secuencia de aminoácido derivada del dominio extracelular de B7-H3 y (b) una segunda secuencia de aminoácido derivada de la región constante de un anticuerpo. La primer secuencia de aminoácido se deriva de toda o una porción del dominio extracelular B7-H3 y (a) inhibe competitivamente la unión de una forma de ocurrencia natural de B7-H3 a su receptor y/o (b) tiene una actividad coestimuladora negativa.

[0064] En algunas modalidades, la primer secuencia de aminoácido comprende una secuencia como se establece en la ID DE SEC. NO. 15. En ciertas modalidades, la primer secuencia de aminoácido es idéntica o es sustancialmente idéntica al

los aminoácidos 23-244 de la ID DE SEC. NO. 14, o a los aminoácidos 23-462 de la ID DE SEC. NO. 12. En una modalidad ilustrativa, la forma soluble del B7-H3 comprende una secuencia como la ID DE SEC. NO. 12 o la ID DE SEC. NO. 14.

[0065] La segunda frecuencia de aminoácido se puede derivar de la región constante de un anticuerpo, tal como la porción Fc. En algunas modalidades, la segunda secuencia de aminoácido se deriva de la porción Fc de un IgG. En las modalidades relacionadas, la porción Fc se deriva del IgG que es IgG₁, IgG₄ u otro isotipo IgG. En modalidades ilustrativas no limitantes, la segunda secuencia se deriva del iGg_{2am} de ratón.

[0066] En ciertas modalidades, la segunda secuencia de aminoácido está ligada al terminal C o al terminal N de la primer secuencia de aminoácido, o con o sin estar ligada mediante una secuencia ligadora. La longitud exacta y la secuencia del ligador y su orientación con relación a la secuencia ligada pueden variar. El ligador puede, por ejemplo, comprender uno o más Gly-Ser. El ligador puede tener 2, al menos 10, al menos 20, al menos 30, aminoácidos de longitud y se selecciona con base en las propiedades deseadas tal como solubilidad, longitud, separación estérica, inmunogenicidad, etc.

2.3 Derivados de Agentes Proteinaceos B7-H3.

[0067] Los derivados de agentes B7-H3 proteinaceos (que incluyen las formas solubles de B7-H3, anticuerpos contra B7-H3, y anticuerpos contra receptor de B7-H3) se pueden hacer al alterar las secuencias de aminoácidos mediante sustituciones, adiciones, y/o supresiones/truncamientos o mediante la introducción de modificación química que da como resultado equivalentes funcionales o moléculas. Se entenderá por un experto en la técnica que ciertos aminoácidos en una secuencia de cualquier proteína pueden ser sustituidos por otros aminoácidos sin afectar adversamente la actividad de la proteína.

[0068] Se pueden hacer varios cambios en la secuencia de aminoácido de los agentes B7-H3 proteináceos de la invención o de las secuencias de ADN que codifican para éstos sin pérdida apreciable de su actividad biológica, función, o utilidad. El uso de tales derivados está dentro del alcance de la presente invención. En una modalidad específica, el derivado es funcionalmente activo, es decir, capaz de decidir una o más actividades asociadas con el dominio extracelular del B7-H3 de ocurrencia natural, por ejemplo, como se establece en la ID DE SEC. NO. 2, ID DE SEC. NO. 4, o ID DE

SEC. NO. 6. Los sustitutos para un aminoácido dentro de la secuencia se pueden seleccionar de otros miembros de la clase a los cuales pertenece el aminoácido (ver tabla 1). Adicionalmente, varios aminoácidos son comúnmente sustituidos con aminoácidos neutros, por ejemplo, alanina, leucina, isoleucina, valina, prolina, fenilalanina, triptofano, y metionina, (ver ejemplo MacLennan et al. (1998) Acta Physiol. Scand. Suppl. 643: 55-67; Sasaki et al. (1998) Adv. Biophys. 35:1-24.

Tabla 1

Residuos Originales	Sustituciones de Ejemplo	Sustituciones Típicas
Ala (A)	Val, Leu, Ile	Val
Arg (R)	Lys, Gln, Asn	Lys
Asn (N)	Gln	Gln
Asp (D)	Glu	Glu
Cys (C)	Ser, Ala	Ser
Gln (Q)	Asn	Asn
Gly (G)	Pro, Ala	Ala
His (H)	Asn, Gln, Lys, Arg	Arg
Ile (I)	Leu, Val, Met, Ala, Phe, Norleucine	Leu
Leu (L)	Norleucine, Ile, Val, Met, Ala, Phe	Ile
Lys (K)	Arg, 1,4-Diamino-butiric Acid, Gln, Asn	Arg
Met (M)	Leu, Phe, Ile	Leu
Phe (F)	Leu, Val, Ile, Ala, Tyr	Leu
Pro (P)	Ala	Gly
Ser (S)	Thr, Ala, Cys	Thr
Thr (T)	Ser	Ser
Trp (W)	Tyr, Phe	Tyr
Tyr (Y)	Trp, Phe, Thr, Ser	Phe
Val (V)	Ile, Met, Leu, Phe, Ala, Norleucine	Leu

[0069] Los agentes B7-H3 pueden ser químicamente acoplados, o conjugados, a otras proteínas y agentes farmacéuticos. Tales modificaciones se pueden diseñar para alterar la farmacocinética y/o biodistribución de la composición resultante. El B7-H3-Ig y los anticuerpos de la invención también pueden ser glicosilados, pegilados, o ligados a otro polímero no proteinaceo, por ejemplo, polietilenglicol, propilenglicol, o polioxialquilenos, como se establece en las Patentes U.S. No. 4,640,385; 4,496,689; 4,301, 144; 4,670,417; 4,791,192; o 4,179,337. El B7-H3 Ig y los anticuerpos pueden ser químicamente modificados mediante conjugación covalente a un polímero para incrementar su vida media circulante, por ejemplo. Los polímeros de ejemplo, y los métodos para unirlos a los péptidos, también son conocidos de las Patentes U.S. 4,766,106; 4,179,337; 4,495,285; 4,495,285;y 4,609,546.

[0070] Los agentes B7-H3 que comprenden la porción Fc de un anticuerpo, tal como las fusiones B7-H3-Ig, o los anticuerpos utilizados en los métodos de la invención pueden además ser modificados en la región Fc para minimizar la función del efectuator. Tales modificaciones incluyen cambiar residuos de aminoácidos específicos los cuales alteran la unión a un receptor Fc (Lund et al. (1991) J. Immun., 147:2657-2662 y Morgan et al. (1995) Immunology, 86:319-324),

o cambiar las especies de las cuales se deriva la región constante. Los anticuerpos y las fusiones B7-H3 Ig pueden tener mutación en la región C_H2 de la cadena pesada que reducen la función efectuada, es decir, la unión de receptor Fc y la activación del complemento. Por ejemplo, los anticuerpos y las funciones B7-H3-Ig pueden tener mutaciones tales como aquellas descritas en la Patente U.S. 5,624,821 y 5,648,260. En la cadena pesada IgG₁ o IgG₂, por ejemplo, tales mutaciones pueden ser hechas en los residuos de aminoácido que corresponden a los aminoácidos 234 y 237 en la secuencia de longitud completa del IgG₁ o IgG₂. Los anticuerpos y las fusiones B7-H3-Ig también pueden tener mutaciones que estabilizan el enlace disulfuro entre las dos cadenas pesadas de una inmunoglobulina, tal como mutaciones en la región de articulación del IgG₄, Como se describe en Angal et al. 819939 Mol. Immunol., 30:105-108.

[0071] En ciertas modalidades, las fusiones adicionales de cualquier B7-H3-Ig de la invención a la secuencia de aminoácido derivadas de otras proteínas se pueden construir para uso de los métodos de la invención. Las secuencias de fusión deseable se pueden derivar de proteínas que tienen actividad biológica diferente de aquella del B7-H3, por ejemplo, citocinas, factores de crecimiento y diferenciación, enzimas, hormonas otros componentes receptores, etc.

[0072] Los agentes B7-H3m (ptoteinaceos y no proteinaceos) pueden también ser etiquetados con un marcador detectable o funcional. Los marcadores detectables incluyen radiomarcadores tales como ^{131}I o ^{99}Tc , los cuales se pueden unir utilizando química convencional. Los marcadores detectables además incluyen marcadores de enzima, por ejemplo, peroxidasa de cola de caballo o alcalino fosfatasa y porciones detectables tales como biotina o avidita.

[0073] Los derivados se pueden producir mediante varias técnicas bien conocidas en el arte, que incluyen métodos recombinantes y sintéticos (Maniatis (1990) Molecular Cloning, A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; y Bodansky et al. (1995).

2.4 Acidos Nucleicos

[0074] La descripción también suministran métodos que involucran usos terapéuticos y no terapéuticos de ácido nucleicos y polipéptidos codificados portales ácidos nucleicos, en donde la secuencia de nucleótido de tal ácido nucleico es escogida de (a) una secuencia de nucleótido de la ID DE SEC. NO. 1, ID DE SEC. NO. 3, ID DE SEC. NO. 5, o una porción de estos; y (b) un ácido nucleico que tiene al menos

60, 80, 100, 120, o 140 nucleótidos de largo e hibridiza al ácido nucleico de (a) bajo condiciones definidas, en donde el ácido nucleico codifica un producto de expresión que tiene una actividad coestimuladora negativa.

[0075] En ciertas modalidades, tal ácido nucleico codifica una secuencia de aminoácido como la ID DE SEC. NO. 15. En una modalidad ilustrativa, el ácido nucleico comprende una secuencia sustancialmente como la ID DE SEC. NO. 11 o la ID DE SEC. NO. 13. En otras modalidades, tal como un ácido nucleico incluye una secuencia de nucleótido que difiere de las ID DE SEC. NO. 11 o la ID DE SEC. NO. 13 en que tiene al menos una sustitución sinónima, es decir, el codón que tiene una sustitución que codifica el mismo o un residuo aminoácido funcionalmente equivalente como en la ID DE SEC. NO. 11 o la ID DE SEC. NO. 13.

[0076] En una modalidad, las condiciones definidas son condiciones de baja restricción. En otra modalidad, las condiciones definidas son condiciones de moderada restricción. En aún otra modalidad, las condiciones definidas son condiciones de alta restricción.

[0077] Las condiciones de hibridización apropiadas pueden ser fácilmente seleccionadas por aquellos expertos en la

técnica como se ejemplificó en Ausubel et al. (1995), Current Protocols in Molecular Biology, John Wiley & Sons, sections 2,4 y 6. Adicionalmente, las condiciones rigurosas son descritas en Sambrook et al. (1989) Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Press, chapters 7,9, y 11. Un ejemplo no limitante de condiciones definidas de baja restricción es como sigue. Filtros que contienen ADN son pre-tratados durante 6 h a 40°C en una solución que contiene 35% de formamida, 5 x SSC, 50 mM Tris-HCL (Ph 7,5), 5 mM de EDTA, 0,1% PVP, 0,1% Ficoll, 1% BSA, Y 500 µg/ml de ADN de esperma de salmón desnaturalizado. Las hibridizaciones se llevan a cabo en la misma solución con las siguientes modificaciones: 0,02% PVP, 0,02% Ficoll, 0,2% BSA, 100 µg/ml de ADN de esperma de salmón, 10% (p/vol) sulfato de dextrano, y se utilizan $5-20 \times 10^6$ de una sonda marcada de ^{32}P . Los filtros son incubados en una mezcla de hibridización durante 18-20 h a 40°C y luego lavados durante 1,5 h a 55°C en una solución que contiene 2 x SSC, 25 mM de Tris-HCL (Ph 7,5), 5 mM EDTA, y 0,1% SDS. La solución lavada se reemplaza con solución fresca y se incuba durante 1,5 h adicionales a 60°C. Los filtros se secan por transferencia y son expuestos para autorradiografía. Se pueden utilizar otras condiciones de baja restricción bien conocidas en la técnica (por ejemplo como las empleadas para hibridizaciones de especies cruzadas).

[0078] Un ejemplo no limitante de condiciones definidas de baja restricción es como sigue. La prehibridización de filtros que contienen ADN se lleva a cabo durante 7 h a toda la noche a 50°C en un compuesto amortiguado de 5 x SSC, 50 mM Tris-HCL (pH 7,5), 1 mM EDTA 0,02% PVP, 0.02% Ficoll, 0, 02% BSA, y 500 µg/ml de ADN de esperma de salmón desnaturalizado. Los filtros son hibridizados durante 18-36 h 50°C en la mezcla de pre-hibridización que contiene 100 µg/ml de ADN de esperma de salmón desnaturalizado y 5-20 x 10⁶ cpm de una sonda marcadora de ³²P. El lavado de los filtros es hecho a 37°C durante 1 h en una solución que contiene 2 x SSC, 0,01% PVP, 0,01% de Ficoll, y 0,01% BSA. Esto es seguido por un lavado en 0,1 x SSC a 50°C durante 45 minutos. Se pueden utilizar otras condiciones de restricción moderada bien conocidas en la técnica.

[0079] Un ejemplo no limitante de condiciones definidas de alta restricción es como sigue. La pre-hibridización de los filtros que contienen ADN se lleva a cabo durante 8 h a toda la noche a 65°C en un amortiguador compuesto de 6 x SSC, 50 mM Tris-HCL (Ph 7,5), un mM EDTA, 0,02% PVP, 0,02% de Ficoll, y 0,02% BSA, Y 500 Mg/ml de ADN de esperma de salmón desnaturalizado. Los filtros son hibridizados durante 48 h a 65°C en la mezcla de pre-hibridización que contiene 100 µg/ml

de ADN de esperma de salmón desnaturalizado y $5-20 \times 10^6$ CPM de una sonda marcadora de ^{32}P . El lavado de los filtros se hace a 37°C durante 1 h en una solución que contiene 2 x SSC, 0,01% PVP, 0,01% de Ficoll, y 0,01% de BSA. Esto es seguido por un lavado en 0,1 x SSC a 50°C durante 45 minutos. O También se pueden utilizar otras condiciones de alta restricción bien conocidas en la técnica.

[0080] Se pueden obtener agentes B7-H3, aislados y/o purificados de su ambiente natural, en forma sustancialmente pura u homogénea, o en el caso de ácido nucleico, libre o sustancialmente libre de ácido nucleico o genes que den origen a otras secuencias que codifican el polipéptido con la función requerida. Los sistemas para clonar y la expresión de un polipéptido en una variedad de células huésped diferentes son bien conocidos. Las células huésped adecuadas incluyen bacterias, células de mamífero, y levaduras y sistemas de baculovirus. Las línea de célula de mamífero disponibles en la técnica para expresión de un polipéptido heterólogo incluyen células de ovario de hámster Chino, células HeLa, células de riñón de hamster nené, células de melanoma de ratón NSO y otras muchas. Un huésped bacterial común es *E. coli*. Para otras células adecuadas para producir, por ejemplo, B7-H3-Ig, ver Gene Expresión Systems, eds. Fernandez et al., Academic Press, 1999.

[0081] Los vectores adecuados se pueden escoger o construir, conteniendo secuencias regulatorias apropiadas, incluyendo secuencias promotoras, secuencias terminadoras, secuencias de poliadenilación, secuencias mejoradas, secuencias marcadoras y otras secuencias según sea apropiado. Los vectores pueden ser plásmidos o virales, por ejemplo, fagos, o fagemidos, según sea apropiado. Para detalles adicionales, ver, por ejemplo, Molecular Cloning: A Laboratory Manual, Sambrook et al., 2nd ed., Cold Spring Harbor Laboratory Press, 1989. Muchas técnicas conocidas y protocolos para la manipulación del ácido nucleico, por ejemplo, en la preparación de construcciones de ácido nucleico, mutagénesis, secuenciación, introducción de ADN en células y expresión de gen, y análisis de proteínas, se describen en detalle en los Current Protocols in Molecular Biology, eds. Ausubel et al., 2nd ed., John Wiley & Sons, 1992.

[0082] Un ácido nucleico se puede fusionar a otras secuencias que codifican secuencias de polipéptido adicionales, por ejemplo, secuencias que funcionan como un marcador o un reportero. Ejemplos de genes marcadores o reporteros incluyen β -lactama, clorofenicol acetiltransferasa (CAT), adenosin deaminasa (ADA), aminoglicosido

fosfotransferasa (responsable de resistencia a la neomicina) (G418)), dihidrofolato reductasa (DHFR), hidromicin-B-fosfotransferasa (HPH), timidita quinasa (TK), lacZ (que codifica β -galactosidasa), xantino guanina fosforibosiltransferasa (XGPRT), y muchas otras conocidas en la técnica.

[0083] Los métodos de la invención también comprenden el uso de ARN de interferencia corta (siARN) y oligonucleótidos contra sentido para reducir la expresión de B7-H3 con el fin de mejorar la respuesta inmune. El siARN se puede producir utilizando técnicas estándar como se describe en Hannon (2002) Nature, 418:244-251; McManus et al. (2002) Nat. Reviews, 3:737-747; Heasman (2002) Dev. Biol. 243:209-214; Stein (2001) J. Clin. Invest., 108:641-644; y Zamore (2001) Nat. Struct. Biol. 8(9):746-750. Los ácidos nucleico con trasentido se pueden producir utilizando técnicas estándar como se describe en Antisense Drug Technology: Principles Strategies, y Applications, 1st ed., Crooke, Marcel Dekker, 2001.

3. Métodos de Uso

3.1 Métodos de Modulación de Respuestas Inmunes.

[0084] Los agentes B7-H3 descritos pueden actuar bien sea como agonistas o antagonistas de B7-H3 expresado naturalmente, dependiendo del método de su uso. Los agentes B7-H3 se pueden utilizar para prevenir, diagnosticar, o tratar desórdenes médicos en mamíferos (tales como humanos). La aplicación *in vitro* de los agentes B7-H3 puede ser útil, por ejemplo, en la producción de linfocitos activados para uso en estudios sobre función de célula inmune, o, por ejemplo, para ensayar la actividad biológica de otros agentes B7-H3. Tales métodos son descritos en los ejemplos.

[0085] En un aspecto, la presente invención se relaciona con el uso de B7-H3 y agonistas y antagonistas de éstos en la modulación de respuestas inmunes. Los métodos de la invención involucran poner en contacto un linfocito, por ejemplo, una célula T o B, con un agente B7-H3 con el fin de modular (es decir coestimular o inhibir) la activación de linfocitos. En particular, los agentes B7-H3 actualmente descritos modulan la activación de linfocito medidos mediante uno o más de los siguientes: (a) proliferación de linfocito; (b) secreción de citosina (por ejemplo interleucina (IL)-10, factor de necrosis tumoral (TNF)- α , interferón (IFN)- γ , y factor de

estimulación de colonia de granulocito-macrófago (GM-CSF)). Los métodos se pueden desarrollar *in vitro*, *in vivo* o *ex vivo*.

[0086] La etapa de contactar puede ocurrir antes, durante, o después de la activación del linfocito. La activación de la célula T se puede efectuar, por ejemplo, al expresar la célula T a un anticuerpo que se une al TcR o uno de los polipéptidos del complejo CD3 que está físicamente asociado con el TCR (por ejemplo que utiliza un anti-CD3 Anticuerpo; Patentes Estadounidenses Nos. 6,405,696 y 5,316,763). Alternativamente, la célula T puede ser expuesta a cualquier aloantígeno (por ejemplo, un aloantígeno MHC) en, por ejemplo, una célula que presenta antígeno (APC) (por ejemplo, una célula dendrítica, un macrófago, un monocito, o una célula B) o un péptido antigénico producido mediante el procesamiento de un antígeno de proteína mediante cualquiera de los APC anteriores y presentado a la célula T mediante moléculas MHC sobre la superficie del APC. La célula T puede ser una célula T CD4⁺ o una célula T CD8⁺. El agente B7-H3 puede ser agregado a la solución que contiene las células, o esta se puede expresar sobre la superficie de un APC, por ejemplo, un APC que presenta un aloantígeno o un péptido antígeno unido a una molécula MHC.

[0087] Adicionalmente, agentes B7-H3 se pueden utilizar para tratar un sujeto en riesgo de o susceptible a un desorden o que tiene un desorden asociado con expresión o función B7-H3 aberrante. Así, en ciertas modalidades los métodos de la invención comprenden identificar un sujeto en necesidad de inhibir la activación de linfocito, y administrar un agonista B7-H3 al sujeto. En otras modalidades, los métodos comprenden identificar un sujeto en necesidad de mejorar la activación de linfocito y administrar un antagonista B7-H3 al sujeto.

[0088] Cuando la respuesta inmune disminuye es deseable, que los agentes B7-H3 puedan ser utilizados como agonistas de B7-H3 con el fin de mejorar la atenuación asociada con B7-H3 de la respuesta inmune. Por ejemplo, los agentes B7-H3 se pueden utilizar en métodos de la invención para la inducción de tolerancia a un antígeno específico (por ejemplo, una proteína terapéutica). En una modalidad, se induce tolerancia contra un antígeno específico mediante la co-administración de antígeno y un agente B7-H3. Por ejemplo, los pacientes que reciben el Factor VIII o Factor IX frecuentemente generan anticuerpos para esta proteína, por lo tanto la co-administración de un agonista B7-H3 (por ejemplo, ácidos nucleicos y B7-H3-Ig que codifica B7-H3 o sus fragmentos funcionales) en combinación con Factor VIII o Factor IX

recombinantes se espera que resulten en la sub-regulación de respuestas inmunes a este factor de coagulación. Adicionalmente, una reducción en el nivel de la respuesta inmune puede ser deseable, por ejemplo, en ciertos tipos de alergia o reacciones alérgicas, enfermedades autoinmunes (por ejemplo, artritis reumatoide, soriasis, diabetes melitus tipo I, esclerosis múltiple, enfermedad inflamatoria del intestino, enfermedad de Crohn, y lupus eritematoso sistémico), rechazo en transplante de tejido, piel y órganos, y enfermedad de injerto-versus-huésped (GVHD).

[0089] En ciertas modalidades, para alcanzar un efecto agonístico, la co-presentación, o acoplamiento, (es decir, proximidad física) entre las señales positivas (es decir, mediadas por un receptor antígeno, por ejemplo, TcR o BcR) y señales negativas (es decir, B7-H3) pueden ser necesarias. Esto se puede alcanzar al inmovilizar un agente B7-H3 sobre una matriz de soporte que también lleva una molécula estimuladora primaria (por ejemplo, anticuerpo anti-CD3). En tales casos, la distancia preferida es menor de o comparable con el tamaño de una célula que presenta antígeno de ocurrencia natural, es decir, menos de 100 μm ; más preferiblemente, menos de 50 μm ; y más preferiblemente, menos de 20 μm . Alternativamente, un agente B7-H3 se puede acoplar

con una molécula estimuladora primaria, por ejemplo, por reticulación por vía de anticuerpos.

[0090] En algunas modalidades, las señales positivas (de activación) y las señales negativas (de inhibición) se suministran mediante un ligando o anticuerpos inmovilizados sobre la matriz de soporte sólido, o un portador. En varias modalidades, la matriz de soporte sólido puede estar compuesta de polímeros tales como agarosa activada, dextran, celulosa, fluoruro de polivinilideno (PVDF). Alternativamente, la matriz de soporte sólido se puede basar sobre polímeros plásticos o de sílice, por ejemplo, como nylon, dacrón, poliestireno, poliacrilatos, polivinilos, teflones, etc.

[0091] La matriz puede ser implantada dentro del bazo de un paciente. Alternativamente, la matriz puede ser utilizada para la incubación ex vivo de células T obtenidas de un paciente, que luego son separadas e implantadas de nuevo al paciente. La matriz también puede ser hecha de un material biodegradable tal como ácido poliglicólico, polihidroxialcanoato, colágeno, o gelatina de tal forma que ellos pueden ser inyectados dentro de la cavidad peritoneal del paciente, y disueltos después de algún tiempo luego de la

inyección. Al portador se le puede dar forma que asemeje una célula (por ejemplo gota o microesfera).

[0092] Bajo ciertas circunstancias, puede ser deseable causar o mejorar la respuesta inmune del paciente con el fin de tratar un desorden inmune o cáncer. El desorden que se trata o evita mediante los métodos descritos incluye pero no está limitado a infecciones con microbios (por ejemplo, bacterias), virus (por ejemplo, infecciones virales sistémicas tales como influenza, enfermedades de la piel tales como herpes o herpes zoster, y VIH), o parásitos; y cáncer (por ejemplo, melanoma y cánceres de próstata).

[0093] En tales circunstancias, los agentes B7-H3 se pueden utilizar para inhibir o reducir la actividad subreguladora asociada con B7-H3. En particular, los agonistas B7-H3 (por ejemplo, anticuerpo anti-B7-H3, anticuerpo contra un receptor B7-H3, siARN, y ácidos nucleicos antisentido para B7-H3) se pueden utilizar para estimular la activación de la célula T. En varias modalidades, los anticuerpos contra B7-H3 o contra un receptor B7-H3 que inhibe la unión del B7-H3 a las células que expresan tal un receptor con una IC_{50} de menos de 10 nM, y más preferiblemente menos de 5 nM, y más preferiblemente

menos de 1 nM. El IC₅₀ puede ser medido utilizando técnicas estándar conocidas en la técnica.

[0094] Las composiciones de la presente invención se administran en cantidades terapéuticamente efectivas. Generalmente, una cantidad terapéuticamente efectiva puede variar con la edad del sujeto, condición, y sexo, así como también la severidad de la condición médica del sujeto. Una cantidad terapéuticamente efectiva de agentes proteínicos B7-H3 varía de 0,001 a 30 mg/kg, preferiblemente de 0,01 a 25 mg/kg, desde 0,1 a 20 mg/kg, o desde 1 a 10 mg/kg de peso corporal. La dosis se puede ajustar, según sea necesario, para ajustarse a los efectos observados del tratamiento. Los anticuerpos y formas solubles de B7-H3 pueden dar una dosis de bolo. La infusión continua también puede ser utilizada después de la dosis de bolo. La dosis apropiada y el régimen se escogen basados en indicaciones clínicas por un médico tratante.

[0095] Las células inmunes (por ejemplo, células T activadas) también pueden ser aisladas de un paciente e incubadas ex vivo con un agente B7-H3. Por ejemplo, células mononucleares sanguíneas periféricas (PBMC) pueden ser retiradas de un sujeto o un donador adecuado y expuestas ex vivo a un estímulo activador (ver anteriormente) y un agente

B7-H3 (si está en forma soluble o unida a un soporte soldado). El PBMC que contiene células T activadas son luego introducidas dentro del mismo sujeto o de un sujeto diferente. Alternativamente, las células aisladas pueden ser transfectadas con un ácido nucleico y tales células transfectadas pueden luego ser reintroducidas dentro del sujeto. Mientras tales células serían preferiblemente células hemopoyéticas (por ejemplo, células de médula de hueso, macrófagos, monolitos, células dendríticas, células T, o células B) ellas también pueden ser de otro tipo de célula que incluye, sin limitación, fibroblastos, células epitaliales, células endoteliales, queratinocitos. El uso de células hemopoyéticas puede ser ventajoso en que tales células se espera alojar a, entre otros, tejido linfoide (por ejemplo, nodos de plasma o bazo). Adicionalmente, si se utiliza APC, el APC que expresa el B7-H3 exógeno puede ser el mismo APC que presenta un péptido aloantígeno o antigénico a la célula T relevante. Los agentes B7-H3 pueden ser secretados por el APC o expresados en su superficie. Antes de regresar el APC recombinante al sujeto, ellos opcionalmente pueden ser expuestos a fuentes de antígenos o péptidos antigénicos de interés, por ejemplo, aquellos de tumores, microorganismos infecciosos o auto-antígenos.

[0096] En algunas modalidades, los agentes B7-H3 se utilizan para tratar o evitar desórdenes inmunes susceptibles de tratamiento con composiciones de la invención que incluyen pero no están limitados a desórdenes inmunológicos (por ejemplo, artritis reumatoide (RA), soriasis, esclerosis múltiple (MS), enfermedad inflamatoria del intestino (IBD), enfermedad de Crohn, lupus eritematoso sistémico (SLE), diabetes tipo I, rechazo de transplante, enfermedad de injerto versus huésped (GVHD), desórdenes inmuno hiperproliferativos, etc.), cánceres, desórdenes inmunosupresivos, y varias enfermedades infecciosas. En particular, la descripción suministra métodos que involucran composiciones que comprenden derivados B7-H3 tales como formas solubles de B7-H3 o anticuerpos contra B7-H3 o contra su receptor.

3.2 Métodos de Selección

[0097] Los agentes B7-H3 también se pueden utilizar en métodos de selección para identificar agentes terapéuticos. Un compuesto para ser ensayado puede ser, por ejemplo, un anticuerpo anti-B7-H3, un anticuerpo contra un receptor B7-H3, o una molécula orgánica pequeña. En tal un ensayo de selección, una primera mezcla de unión se forma al combinar B7-H3-Ig y una célula que expresa un receptor B7-H3 (por

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ejemplo, una célula T activada); y la cantidad de unión entre los dos en la primera mezcla de unión (M_0) se mide. Una segunda mezcla de unión también se forma al combinar B7-H3-Ig, una célula que expresa un receptor B7-H3, y un agente a ser ensayado, y la cantidad de unión en la segunda mezcla de unión (M_1) se mide.

[0098] Las cantidades de unión en la primera y segunda mezcla de unión son luego comparadas, por ejemplo, al calcular la proporción M_1/M_0 . El compuesto ensayado se considera es capaz de modular una subregulación asociada B7-H3 de respuestas inmunes si una reducción en la unión en la segunda mezcla de unión como se comparó con la primera mezcla de unión se observa. La formulación y optimización de mezclas de unión está dentro del nivel del experto en la técnica, de tal forma que las mezclas de unión pueden también contener amortiguadores y sales necesarias para mejorar u optimizar la unión, y ensayos de control adicionales se pueden incluir en los ensayos de selección de la invención. Los compuestos encontrados para reducir la unión B7-H3 mediante al menos el 10% (es decir, $M_1/M_0 < 0,9$), preferiblemente mayor del 30% puede así ser identificado y luego, si se desea, seleccionado por la capacidad de mejorar un desorden en otros ensayos o modelos animales como se describe adelante. La potencia de unión puede ser medida utilizando, por ejemplo, un ensayo de

inmunoadsorción ligada a enzima (ELISA), radio-inmunoensayo (RIA), tecnología basada en resonancia de superficie de plasmón (por ejemplo, Biacore), todas las cuales son técnicas bien conocidas en el arte.

[0100] El compuesto ensayado puede luego ser adicionalmente ensayado *in vitro* como se describe en los Ejemplos o en un modelo animal (ver, generalmente, Immunologic Defects in Laboratory Animals, eds. Gershwin et al., Plenum Press, 1981), por ejemplo, tal como el siguiente: el modelo de ratón SWR X NZB (SNF1) (Uner et al. (1998) J. Autoimmune. Dispositivo., 11(3): 233-240), el modelo (K/BxN) de ratón KRN (Ji et al. (1999) Immunol. Rev., 169: 139); ratones (B/W) NZB X NZW, un modelo para SLE (Riemekasten et al. (2001) Arthritis Rheum., 44(10): 2435-2445); encefalitis autoinmune experimental (EAE) en ratones, un modelo para esclerosis múltiple (Tuohy et al. (1988) J. Immunol., 141: 1126-1130, Sobel et al. (1984) J. Immunol. 132: 2393-2401, y Traugott (1989) Cell Immunol., 119: 114-129); el modelo de ratón NOD de diabetes (Baxter et al. (1991) Autoimmunity, 9(1): 61-67), etc.).

[0101] Dosis preliminares como, por ejemplo, determinadas de acuerdo con ensayos animales, y la escala de dosis para administración humana se desarrollan de acuerdo con las

prácticas aceptadas en la técnica. La toxicidad y eficacia terapéutica se pueden determinar mediante procedimientos farmacéuticos estándares en cultivos de célula o animales experimentales, por ejemplo, para determinar el LD_{50} (la dosis letal al 50% de la población) y el ED_{50} (la dosis terapéuticamente efectiva en el 50% de la población). La proporción de dosis entre los efectos tóxicos y terapéuticos es el índice terapéutico y este puede ser expresado como la proporción LD_{50}/ED_{50} . Las composiciones que exhiben índices terapéuticos grandes son preferibles.

[0102] La dosis terapéuticamente efectiva puede ser estimada inicialmente desde los ensayos de cultivo celular. Una dosis puede ser formulada en modelos animales para alcanzar un rango de concentración de plasma circulante que incluye el IC_{50} (es decir, la concentración del terapéutico que alcanza una inhibición máximo de la mitad de los síntomas) como se determina en ensayos de cultivo celular o en modelos animales. Los niveles en plasma pueden ser medidos, por ejemplo, mediante cromatografía líquida de alto desempeño o ELISA. Los efectos de cualquier dosis particular pueden ser monitoreados por un bioensayo adecuado. Ejemplos de dosis son: $0,1 \times IC_{50}$, $0,5 \times IC_{50}$, $1 \times IC_{50}$, $5 \times IC_{50}$, $10 \times IC_{50}$, $50 \times IC_{50}$, y $100 \times IC_{50}$.

[0103] Los datos obtenidos a partir de los ensayos de cultivo celular o estudios animales se pueden utilizar en la formulación de un rango de dosis para uso en humanos. Las dosis terapéuticamente efectivas alcanzadas en un modelo animal pueden ser convertidas para uso en otro animal, que incluye humanos, que utiliza factores de conversión conocidos en la técnica (ver, por ejemplo, Freireich et al. (1966) Cancer Chemother. Reports, 50(4): 219-244 y la Tabla 2 para Factores de Dosis de Área de Superficie Equivalente).

TABLA 2

A: De:	Ratón (20 g)	Rata (150 g)	Mono (3,5 kg)	Perro (8 kg)	Humano (60 kg)
Ratón	1	1/2	1/4	1/6	1/12
Rata	2	1	1/2	1/4	1/7
Mono	4	2	1	3/5	1/3
Perro	6	4	3/5	1	1/2
Humano	12	7	3	2	1

4. Composiciones Farmacéuticas, Métodos de Administración, y Dosis

[0104] La descripción suministra composiciones farmacéuticas que comprenden agentes B7-H3. Tales composiciones pueden ser adecuadas para uso farmacéutico y administración a pacientes. Las composiciones típicamente

comprenden uno o más anticuerpos de la presente invención y un excipiente farmacéuticamente aceptable. La frase "excipiente farmacéuticamente aceptable" incluye cualquiera y todos los solventes, medios de dispersión, recubrimientos, agentes anti-bacteriales y agentes antifungosos, agentes isotónicos, y agentes retardantes de absorción, y similares, que son compatibles con la administración farmacéutica. El uso de tales medios y agentes para sustancias farmacéuticamente activas son bien conocidos en la técnica. Las composiciones pueden también contener otros compuestos activos que suministran funciones terapéuticas mejoradas complementarias, o adicionales. Las composiciones farmacéuticas también pueden estar incluidas en un contenedor, empaque, o dispensador junto con instrucciones para la administración.

[0105] Una composición farmacéutica de la invención se formula para ser compatible con su ruta propuesta de administración. Los métodos para lograr la administración son conocidos por aquellos medianamente versados en la técnica. La administración puede, por ejemplo, ser intravenosa, intraperitoneal, intramuscular, intracavital, subcutánea o transdérmica. También puede ser posible obtener composiciones que pueden ser tópicamente u oralmente administradas, y que

pueden ser capaces de transmisión a través de las membranas de la mucosa.

[0106] Las soluciones o suspensiones utilizadas para aplicación intradermal o subcutánea típicamente incluyen uno o más de los siguientes componentes: un diluyente estéril tal como agua para inyección, solución salina, aceites fijos, polietilen glicoles, glicerina, propilen glicol, u otros solventes sintéticos; agentes antibacteriales tales como alcohol benzilo o parabenos metilo; antioxidantes tales como ácido ascórbico o bisulfito de sodio; agentes quelantes tales como ácido etilodiaminotetraacético; amortiguadores tales como acetatos, citratos o fosfatos; y agentes para el ajuste de la tonicidad tales como cloruro de sodio o dextrosa. El pH se puede ajustar con ácidos o bases, tales como ácido clorhídrico o hidróxido de sodio. Tales preparaciones se pueden incluir en ampollas, jeringas desechables o frascos de dosis múltiples hechos de vidrio o plástico.

[0107] Las composiciones farmacéuticas adecuadas para inyección incluye soluciones acuosas estériles o dispersiones y polvos estériles para la preparación extemporánea de soluciones inyectables estériles o dispersión. Para la administración intravenosa, portadores adecuados incluyen salina fisiológica, agua bacteriostática, o salina

amortiguada de fosfato (PBS). En todos los casos, la composición debe ser estéril y debe ser fluida hasta el punto en que sea fácil la aplicación con jeringa. Esta debe ser estable bajo las condiciones de fabricación y almacenamiento y debe ser preservada contra la acción contaminante de microorganismos tales como bacterias y hongos. La prevención de la acción de los microorganismos se puede alcanzar mediante varios agentes antibacteriales y antifungosos, por ejemplo, parabenos, clorobutanol, fenol, ácido ascórbico, timerosal, y similares. En muchos casos, será preferible incluir agentes isotónicos, por ejemplo, azúcares, polialcoholes tales como manitol, sorbitol, y cloruro de sodio en la composición. El portador puede ser un solventes o un medio de dispersión que contiene, por ejemplo, agua, etanol, poliol (por ejemplo, glicerol, propilenglicol, y polietilenglicol líquido, y similares) y mezclas adecuadas de estos. La fluidez apropiada se puede mantener, por ejemplo, mediante el uso de un recubrimiento tal como lecitina, mediante el mantenimiento del tamaño de partícula requerido en el caso de dispersión y/o mediante el uso de tensoactivos. La absorción prolongada de las composiciones inyectables se puede lograr al incluir en la composición un agente que retarda la absorción, por ejemplo, monoestearato de aluminio, y gelatina.

[0108] Las composiciones orales generalmente incluyen un diluyente inerte o un portador comestible. Ellas se pueden incluir en cápsulas de gelatina o comprimidas en tabletas. Para la administración oral, los anticuerpos pueden ser combinados con excipientes y utilizados en la forma de tabletas, pastillas, o cápsulas. Agentes de unión farmacéuticamente compatibles, y/o materiales adyuvantes se pueden incluir como parte de la composición. Las tabletas, pastillas, cápsulas, píldoras y similares pueden contener cualquiera de los siguientes ingredientes, o compuestos de una naturaleza similar; un ligador tal como celulosa microcristalina, goma tragacanto o gelatina; un excipiente tal como almidón o lactosa; un agente desintegrante tal como ácido algínico, Primogel, o almidón de maíz; un lubricante tal como estearato de magnesio o Esterotes; un glidante tal como dióxido de silicio coloidal; un agente endulzante tal como sucrosa o sacarina; o un agente saborizante tal como hierbabuena, salicilato de metilo, o saborizante de naranja.

[0109] La administración sistémica también puede ser mediante medios transdérmicos o de transmucosa. Para la administración transdérmica o de transmucosa, los penetrantes apropiados para la barrera a ser permeada se utilizan en la formulación. Tales líquidos penetrantes son generalmente conocidos en la técnica, e incluyen, por ejemplo,

detergentes, sales de bilis, y derivados de ácido fusídico. La administración de transmucosa se puede acompañar, por ejemplo, a través del uso de pastillas, aerosoles nasales, inhaladores, o supositorios. Por ejemplo, en el caso de los anticuerpos y las proteínas de fusión Ig que comprenden la porción Fc, las composiciones pueden ser capaces de transmisión a través de membranas de mucosa en el intestino, boca, o pulmones (por ejemplo, por vía de la trayectoria mediada por receptor FcRn como se describe en la Patente Estadounidense No. 6,030,613). Para administración transdérmica, los compuestos activos pueden ser formulados en ungüentos, pomadas, geles, o cremas como se conoce generalmente en la técnica. Para administración por inhalación, los anticuerpos pueden ser administrados en la forma de un rociado por aerosol desde contenedores presurizados o dispensadores, que contienen un gas propelente adecuado, por ejemplo, un gas tal como dióxido de carbono, o un nebulizador.

[0110] En ciertas modalidades, agentes B7-H3 descritos actualmente son preparados con portadores que protegerán el compuesto contra la eliminación rápida del cuerpo, tal como una formulación de liberación controlada, que incluye implantes y sistemas de administración microencapsulada. Los polímeros biocompatibles, biodegradables se pueden utilizar,

tales como acetato de vinil etileno, polianhídridos, ácido poliglicólico, colágeno, poliortoésteres, y ácido poliláctico. Métodos para la preparación de tales formulaciones serán evidentes para aquellos expertos en la técnica. Las suspensiones liposomales que contienen los anticuerpos descritos actualmente también se pueden utilizar como portadores farmacéuticamente aceptables. Estos pueden ser preparados de acuerdo con los métodos conocidos por aquellos expertos en la técnica, por ejemplo, como se describe en la Patente Estadounidense No. 4,522,811.

[0111] Esto puede ser ventajoso para formular composiciones orales o parenterales en una forma de dosis unitaria para facilidad de administración y uniformidad de dosis. El término "forma unitaria de dosis" como se utiliza aquí se refiere a unidades discretas físicamente adecuadas como dosis unitarias para el sujeto a ser tratado; cada unidad contiene una cantidad predeterminada de compuesto calculado para producir un efecto terapéutico deseado en asociación con el portador farmacéuticamente requerido.

[0112] La toxicidad y la eficacia terapéutica de la composición de la invención pueden ser determinados mediante procedimientos farmacéuticos estándar en cultivos celulares o animales experimentales, por ejemplo, para determinar el LD₅₀

(la dosis letal del 50% de la población) y el ED_{50} (la dosis terapéuticamente efectiva en 50% de la población). La proporción de dosis entre los efectos tóxicos y terapéuticos es el índice terapéutico y este se puede expresar como la proporción LD_{50}/ED_{50} . Las composiciones que exhiben índices terapéuticos grandes se prefieren.

[0113] Para cualquier composición utilizada en la presente invención, la dosis terapéuticamente efectiva puede ser estimada inicialmente a partir de ensayos de cultivo celular. Ejemplos de bioensayos adecuados incluyen ensayos de replicación de ADN, ensayos de liberación de citoquina, ensayos basados en transcripción, ensayos de unión, ensayos de creatina quinasa, ensayos basados en la diferenciación de pre-adipocitos, ensayos basados en la toma en adipositos, ensayos inmunológicos, otros ensayos como, por ejemplo, los descritos en los Ejemplos. Los datos obtenidos a partir de los ensayos de cultivo celular y los estudios en animales se pueden utilizar en la formulación de un rango de dosis para uso en humanos. Una dosis puede ser formulada en modelos animales para alcanzar una concentración de plasma circulante que está en el rango que incluye el IC_{50} (es decir, la concentración del terapéutico que alcanza una inhibición de máximo la mitad de los síntomas). Los niveles circulantes en plasma pueden ser medidos, por ejemplo, mediante

cromatografía líquida de alto desempeño. Los efectos de cualquier dosis particular pueden ser monitoreados mediante un bioensayo adecuado. Las dosis están preferiblemente dentro de un rango de concentraciones que circulan con poca o ninguna toxicidad. Las dosis pueden variar dependiendo de la forma de dosis empleada y la ruta de administración utilizada.

[0114] Los siguientes Ejemplos no limitan en ninguna forma el alcance de la invención. Una persona medianamente versada en la técnica reconocerá que numerosas modificaciones y variaciones se pueden desarrollar sin alterar el espíritu o alcance de la presente invención. Tales modificaciones y variaciones se abarcan dentro del alcance de la invención. Los contenidos completos de todas las referencias, patentes, y solicitudes de patente publicadas citadas a través de esta solicitud se incorporan aquí como referencia.

EJEMPLOS

Ejemplo 1: Aislamiento de ADN genómico para B7-H3

[0115] Se desarrolló RT-PCR de B7-H3 utilizando oligonucleótidos que corresponden a las regiones que contienen los codones de iniciación y terminación de

metionina de B7-H3 humano (Genbank acceso No. AF302102) que utiliza las siguientes condiciones PCR.

[0116] Las enzimas PCR utilizadas en este estudio incluyen enzimas KOD Hot Start (Novagen, Madison, WI), AdvantageTM 2 (Clontech, Palo Alto, CA), y Platinum Taq (Invitrogen, Carlsbad, CA) de acuerdo con los protocolos del fabricante. Cuando es necesario, las condiciones de reacción se complementan para una concentración final de Betaina 1M y 3% de DMSO para amplificación robusta. Los iniciadores PW264 (ID DE SEC. NO.: 23) con attB1/Kozak embebido y PW265 (ID DE SEC. NO.: 24) con sitios attB2 embebidos, se utilizaron para amplificar las secuencias que codifican B7-H3 humano a partir de primeras hebras de cADN de bazo, nodo de plasma, corazón, hígado, páncreas y placenta como plantillas (Clontech). Las secuencias de región que codifican VCVC de B7-H3 humano se obtuvieron al corresponderle las secuencias representadas en las entradas de bases de datos existentes (Celera Human and Mouse Genomic Assemblies, Celera Genomics, Rockville, MD): AX357960, AX097550, AX047072, AX097556, y AX136363 entre otros. La forma VC de B7-H3 humano fue construida al eliminar los dominios C₁-V₂ VCVC de B7-H3 humano, que casa con la secuencia de codificación de NM_025240. La PW270 (ID DE SEC. NO.: 26) y PW271 (ID DE SEC. NO.: 27) se utilizaron para amplificar las secuencias B7-H3 de ratón desde el cADN de

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primera hebra de embrión de ratón. Los productos PCR con un tamaño de 951 bp se subcloraron, que revela claramente el empalme correcto de los 7 exones auténticos predichos con una exactitud del 100%, sin la inclusión de cualesquier pseudo-exones en cualquier clon analizado. Las secuencias de codificación de B7-H3 de ratón correspondieron a las entradas de la base de datos existentes BC019436, AX370312, y NM_133983. Los iniciadores PW284 (ID DE SEC. NO.: 29) y PW267 (ID DE SEC. NO.: 25) se utilizaron para evaluar semicuantitativamente la contribución relativa del VC B7-H3 humano (690 bp) y las transcripciones VCVC (1344 bp) en paneles de cADN (Clontech). Se ejecutó transferencia Southern mediante transferencia alcalina de ADN sobre membrana GT Zetaprobe™ (BioRad, Hercules, CA) e hibridizó utilizando PW278 etiquetado de extremo ³²P (ID DE SEC. NO.: 28) como oligonucleótido de detección (Ling et al. (2001) J. Immunol., 166: 7300-7308).

[0117] Se aislaron ADN genómicos de hámster y mono de líneas celulares CHO y COS (Ling et al. (1999) Genomics 60: 341-355). Se desarrolló el PCR genómico utilizando PW358 (ID DE SEC. NO.: 30) y PW359 (ID DE SEC. NO.: 31) como iniciadores basados en nucleótidos conservados entre las secuencias B7-H3 de ratón y humano (aminoácidos 60-66, 216-221, y 278-284, 434-439 de la ID DE SEC. NO.: 6). Las

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reacciones de amplificación se desarrollaron por duplicado y múltiples productos subclonados se analizaron mediante secuenciación. La orientación de los dominios V-intrón-C dentro del ADN genómico de monos se determinó mediante PCR utilizando PW381 (ID DE SEC. NO.: 33) y PW384 (ID DE SEC. NO.: 34), y dentro del ADN genómico de hamster utilizando PW358 (ID DE SEC. NO.: 30) y PW378 (ID DE SEC. NO.: 32).

[0118] Se desarrolló análisis de ADN como sigue. Una alícuota (0,25-0,5 µg) de ADN de plásmido se obtuvo con 1 µl de 5 µM de iniciador y 3 µl de 2 pliegues diluido ABI PRISM™ BigDye™ Terminator Cycle Sequencing Ready Reaction Kit Mix (Versión 3.0). Los volúmenes se ajustaron a 10 µl con 10 mM de Tris-HCl (pH 8,0), y reacciones de amplificación se desarrollaron en un ciclizador PCT-225 (MJ Research, Waltham, MA) para 25 ciclos (96° C durante 10 segundos, 50° C durante 5 segundos y 60° C durante 4 minutos). Se agregó 10 µl de agua a las reacciones y el exceso de colorante se removió mediante filtración de gel en una placa de filtro Millipore de 96 pozos con gotas G-50. Las muestras se calentaron-desnaturalizadas durante 2 min a 90-95° C y se les hizo electroforesis en un ABI3100 Genetic Analyzer (Applied Biosystems, Foster City, CA) bajo condiciones recomendadas por el fabricante. Se desarrolló edición de la secuencia

manual utilizando Sequencher™ 4.1.4 (Gene Codes, Ann Arbor, MI).

[0119] La determinación de la secuencia del producto de amplificación mayor resultó en una secuencia de 1605 bp distinta de B7-H3 y consistente con otro EST y las entradas de bases de datos de patentes. Mientras B7-H3 contenía los dominios V y C sencillos (forma VC de B7-H3), los clones variantes contenían unos dominios V y C duplicados (forma VCVC de B7-H3). Para determinar si estos clones fueron productos de gen independientes de B7-H3, la organización genómica de los clones VCVC fue analizada mediante solicitudes a bases de datos genómicos humanos (Celera Genomics). Los casos resultantes que corresponde a un cromosoma GA_x2HTBL4SSTP-04 de eje genómico 15, que sugiere un origen genético único de ambas variantes VC B7-H3 y VCVC B7-H3. La Fig. 1B describe la organización genómica del gen loci B7-H3 humano y de ratón. Los ensambles se basan en porciones seleccionadas de GA_x2HTBL4SSTP de eje genómico humano Celera y GA_x5J8B7W7NM9 de eje genómico de ratón Celera. Las barras denotan ubicaciones relativas de las repeticiones complejas SVA y Alu, repeticiones simples, estructuras de transcripción de exón, y nombres de dominio. La organización genómica de VCVC B7-H3 reveló 9 exones que codifican el dominio líder, dominio V₁, dominio C₁, dominio

V₂, dominio C₂, dominio transmembrana y tres dominios citoplasmáticos. Aproximadamente 13,5 kb de la secuencia genómica separó la iniciación de metionina sobre el exón 1 desde el codón de terminación sobre el exón 9 del locus B7-H3 humano. La delineación de VC B7-H3 humano al locus B7-H3 reveló empalme alternativo desde el exón 2(V₁) al exón 5(C₂), que resulta en la eliminación de los dominios C₁ y V₂ desde este producto de gen.

[0120] El análisis de secuencia, alineamientos, y generación de tres guías filogenéticas se desarrollaron utilizando el módulo Align (Clustal W) de Vector NTI™ versión 7.1 (Informax Inc, North Bethesda, MD). La alineación de la secuencia y el análisis de filograma de las secuencias primadas revelaron un grado mayor de similitud de secuencia entre secuencias ADN V-intrón-C de intraespecies que secuencias de ADN V-Intrón-C de interespecies, es decir, el V₁-intrón-C₁ humano es más similar al V₂-intrón-C₂ humano que al V-intrón-C de mono. En cambio, se observó 100% de identidad entre las secuencias de dominio C de mono analizado. Los agrupamientos intraespecies se soportan adicionalmente por la alineación de la secuencia intrón intra-VC no codificante donde se observó la conservación de secuencia mayor que los exones V duplicados (97% versus 94%). Aunque los nucleótidos conservados se encontraron en

secuencias intrón de todas las cuatro especies, no se encontraron pares de nucleótidos conservados para ser compartidos exclusivamente entre las especies roedoras y de primates, (datos no mostrados) que arguyen contra una molécula ancestral VCVC de primate "pre-duplicado" común a ambos primates y roedores. Las secuencias de dominios V-intrón-C de B7-H3 de flanco humano revelaron ninguna conservación de tales secuencias, lo que sugiere que la duplicación en los primates es muy reciente o que la región V-intrón-C es extremadamente protegida de mutación. Así, estos datos soportan fuertemente un modelo de emergencia independiente múltiple de repeticiones VC en tándem dentro de especies de monos y humanos.

Ejemplo 2: Contribución de transcripción relativa entre variantes de empalme B7-H3

[0121] Se desarrolló RT-PCR en varias muestras de tejido humano seguido por hibridización con sondas de oligonucleótido radioetiquetado (Fig. 2A). Se detectaron dos bandas no ambiguas con una migración con una movilidad relativa correspondiente a 1344 bp, consistente con el tamaño predicho del producto de amplificación VCVC B7-H3. También se detectó una banda menor correspondiente a 690 bp, consistente con el tamaño predicho de un producto de amplificación VC.

RT-PCR de tres paneles de cADN reveló este patrón en cada tejido examinado, excepto para el leucocito/PBL, en donde no se detectaron productos de amplificación. La cuantificación de fosfoimagen de las regiones hibridizadas indicó una proporción entre 12,7:1 (cerebro) y 92,1:1 (riñón). En ningún caso se encontró que la señal de hibridización para el producto VC B7-H3 más pequeño fuera mayor que aquella vista para el producto VCVC B7-H3. Dado que las reacciones de amplificación favorecen los productos de amplificación más pequeños, la abundancia relativa de productos VCVC B7-H3 más grandes sobre aquellos de los productos VC B7-H3 más pequeños sugiere que el VCVC B7-H3 es la especie de transcripción dominante del producto de gen B7-H3 humano expresado naturalmente en tejidos humanos. La rareza de la forma VC B7-H3 es consistente con la banda B7-H3 de 4,1 kb sencilla observada en transcripciones Northern reportadas previamente, más probablemente representan el producto VCVC B7-H3 predominante (Chapoval et al. (2001) Nat. Immunol., 2: 269-274). En comparación, la transferencia Southern PCR de B7-H3 de ratón muestra la expresión en todos los tejidos examinados con una banda dominante en aproximadamente 1 kb, consistente con el producto amplificado de 951 bp predicho (Fig. 2B). También se detectaron otras señales de hibridización B7-H3 en embriones de ratón, tejidos musculares de corazón y esqueleto. A diferencia de los primates, las secuencias de

los roedores solo tienen la forma VC sencilla, debido a la degeneración del codón de exones putativos C₁ y V₂.

[0122] Como se demuestra adelante, ambas formas VCVC y VC de B7-H3 tienen similar actividad biológica en in vitro, lo que sugiere que los exones duplicados en tándem son funcionalmente equivalentes en ensayos basados en células. Tal redundancia funcional puede explicar la tolerancia de la pérdida de exón C₁-V₂ sin un efecto adverso en la fisiología.

Ejemplo 3: Análisis genómico comparativo de B7-H3 humano y de ratón

[0123] Dado que la duplicación segmental de los dominios VC parece ser única para el B7-H3 entre la familia B7 de proteínas, examinamos luego si la secuencia de B7-H3 fue distinta de aquellas de otros ligandos co-estimuladores basados en sustitución de base de codón. Un método para determinar las proporciones de evolución molecular es mediante la medición de las proporciones de mutación predichas entre los sitios sinónimos y no sinónimos dentro de los codones. Adicionalmente las comparaciones de las secuencias de primate versus roedores se desarrollaron para los exones V y C de otros ligandos conocidos: B7-1, B7-2, GL50, PD-L1, y PD-L2. Estos ligandos comparten similitudes

estructurales en las cuales los dominios V y C residen en la porción extracelular de cada molécula. La presencia de los motivos estructurales compartidos entre estas moléculas dispares ha propagado la noción de que estas moléculas se derivaron de una secuencia ancestral que lleva las secuencias V y C. Para determinar las proporciones relativas de la divergencia de los dominios V y C entre los humanos y ratones, las frecuencias relativas de las sustituciones de nucleótido en codones sinónimos y no sinónimos se determinaron. Se desarrolló el cálculo de las frecuencias de mutación de sinónimos y no sinónimos utilizando el Wisconsin Package GCG 10.0 Diverge module (GCG, Madison, WI). Para las alineaciones B7-H3, los exones que corresponden a los dominios V₁ y C₂ humano se alinearon con los dominios V y C de ratón. De todos los dominios V y C examinados, la proporción de sustituciones de sinónimo versus mutaciones de no sinónimo fueron menores de 1 excepto para el dominio V del B7-H3. La molécula B7-H3 tuvo los niveles más bajos de mutaciones de sinónimos ($d_s=0,129$ sustituciones por sitio) de todos los dominios V mientras simultáneamente también tiene los niveles más bajos de mutaciones no sinónimas ($d_N=0,026$ sustituciones por sitio) de todos los dominios C. Como un resultado, la molécula B7-H3 es distinta entre los ligandos co-estimuladores al tener la proporción $d_N:d_s$ más alta en el dominio V mientras simultáneamente tiene la proporción $d_N:d_s$

más baja en el dominio C. La comparación de divergencia de dominio transversal entre el dominio V de ratón y dominio V₂ humano reveló un $d_s=0,433$ y $d_N=0,037$, mientras los dominios C de ratón y C₁ humano revelaron un $d_s=0,393$ y $d_N=0,034$. La proporción de mutación de sinónimos inferiores vista entre los humanos V₁ y ratones V a diferencia de los humanos V₂ y ratones V implica que los dominios V de ratón y V₁ humano son ortólogos. Asumiendo un mecanismo lineal de la evolución de la secuencia, la dicotomía de los índices de sustitución de nucleótido entre exones adyacentes de la misma molécula indica que los procesos de selección diferentes ocurren entre los dominios V y los dominios C del B7-H3.

[0124] Basado en modelos matemáticos de evolución molecular, los casos en donde los índices de sustitución de sinónimo son mayores que los índices de sustitución de no sinónimo para una secuencia de codificación particular refleja la selección de purificación. La selección de purificación ocurre cuando las restricciones fisiológicas limitan los niveles de la variación de aminoácido dentro del producto de gen. La selección de purificación es evidente en todos los dominios V y C de la familia B7 examinados con la excepción del dominio V₁ de B7-H3. Para el V₁ de B7-H3, d_s es menos de la mitad que del V de B7-2 y aproximadamente un octavo que del V de PD-L1. Aunque el d_N del V₁ de B7-H3 es

bajo, este todavía excede aquel del d_s del V B7-H3 para la proporción $d_N:d_s$ de 1,18. No son usuales los casos en los que la proporción $d_N:d_s$ es mayor de 1, y se han atribuido a secuencias que experimentan selección positiva para la función de evolución rápida. También es notable que el exón C_2 de B7-H3 exhibe una proporción $d_N:d_s$ de 0,063, el más bajo de todos los exones examinados, y es de un quinto a un quinceavo de los niveles vistos para otros dominios C examinados. Por lo tanto, los exones que comprenden B7-H3 fueron/están actualmente siendo mantenidos activamente en una forma dispar a otros ligandos co-estimulatorios.

Ejemplo 4: Activación de célula T subregulada VCVC y VC de B7-H3

[0125] Basado en la unidad VC sencilla observada en roedores B7-H3, buscamos determinar si las formas VCVC y VC de B7-H3 encontradas en humanos tienen función similar en ensayos basados en células. La capacidad del VC de B7-H3 y VCVC de B7-H3 para la activación de células T subreguladas se observó para ambos niveles de producción de citoquina y proliferación. Los experimentos se conducen como sigue.

[0126] Se clonaron vectores de entrada en construcción VCVC o VC de B7-H3 humano en vectores retrovirales

bicistrónicos que codifican IRES-GFP. Los vectores retrovirales de destinación de recipiente se derivaron originalmente del vector GFP-RV (Ranganath (1998) J. Immunol., 161: 3822-3826) y modificado para recombinación Gateway utilizando un cassette attB1-ccdB-attB2 (Invitrogen). Se generaron supernadantes que contienen virus y se utilizaron para infectar células CHO.HLA-DR2 como se describió previamente (Carter et al. (2002) Eur. J. Immunol., 32: 634-643). Se seleccionaron VCVC de CHO.HLA-DR2.B7-H3 y VC de CHO.HLA-DR2.B7-H3 mediante clasificación celular basado en expresión GFP. Un CHO.HLA-DR2 transfectante que expresa niveles GFP similares se utilizaron como un control en ensayos celulares. La generación de CHO.HLA-DR2.B7.1 y CHO.HLA-DR2.B7.2 se ha descrito previamente (Anderson et al. (2000) Nature Medicine, 6(2): 211-214). Los transfectantes CHO.HLA-DR2 se fijaron en 0,2% de paraformaldehído a temperatura ambiente (RT) durante 4 min y la fijación se apagó en lisina 1M a RT durante 4 min. Las células se lavaron una vez con PBS, se resuspendieron en medio de cultivo (RPMI1640, 16% FCS) y se utilizó como células que presentan antígeno en ensayos de proliferación de células T.

[0127] Las células T CD4⁺ humanas se purificaron mediante selección negativa de linfocitos periféricos como se describió anteriormente (Blair et al. (1998) J. Immunol.,

160: 12-15). Se cultivaron células T CD4⁺ (10⁵ células/pozo) en placas de 96 pozos de fondo plano con transfectantes CHO.HLA-DR2 de paraformaldehído fijo (1,25 x 10⁴ células/pozo) en la presencia de anticuerpo anti-CD3 soluble (1 µg/ml, UCHT1, Pharmingen, San Diego, CA) y varias concentraciones de anticuerpo anti-CD28 soluble (CD28.2, Pharmingen). La proliferación se determinó mediante pulsación de cultivos con 1 Ci [³H]-timidina por pozo durante al menos 5-12 horas de un periodo de incubación de 72 horas. Para medir la producción de citoquina, se cultivaron supernadantes en 72 horas, y las muestras ensayadas mediante selección ELISA múltiplex (Pierce Boston, Woburn, MA).

[0128] Con el fin de determinar la actividad funcional de las formas VCVC y VC de B7-H3, se condujeron los siguientes ensayos basados en células. Se estimularon las células T CD4⁺ humanas purificadas con transfectantes CHO.HLA-DR2 tratadas con paraformaldehído en la presencia de cantidades constantes de anticuerpo anti-CD3 soluble e incrementar las concentraciones de anticuerpo anti-CD28 soluble. La activación de células T purificadas con anti-CD3 en la presencia de transfectantes CHO.HLA-DR2-GFP resulta en ninguna proliferación; los niveles de proliferación se mejoraron en la presencia de anticuerpos anti-CD3 y anti-CD28 (Fig. 3A-3B); la estimulación de las células T con anticuerpo

Ejemplo 5: Activación de células T subreguladas VCVC y VC de B7-H3

[0129] Para caracterizar adicionalmente la función B7-H3, se desarrollaron experimentos sobre una superficie (TRANS) separada o sencilla (CIS) para determinar si la subregulación B7-H3 de la célula T responde los requerimientos coordinados del enganche del receptor TCR/B7-H3. Gotas de CIS contenidas en anticuerpos anti-CD3 y proteínas de fusión purificadas VC-Ig de B7-H3 o VCVC-Ig de B7-H3, mientras que las gotas TRANS contenidas en el anticuerpo anti-CD3 y VC-Ig de B7-H3 o VCVC-Ig de B7-H3.

[0130] La estimulación de gotas de células T se desarrollaron como sigue. Anti-CD3 (UCHT1, Pharmigen), VC-Ig de B7-H3 humano, VCVC-Ig de B7-H3 humano e Ig de control se adhirieron covalentemente a Dynabeads activadas con tosilo recubiertas con poliuretano (Dynal, Lake Success, NY). Las gotas se prepararon con una concentración de anticuerpo anti-CD3 sub-óptima constante (1 µg, 20% de proteína de unión total) y B7-H3-Ig o Ig de control (4 µg, 80% de proteína de unión total (Bennett et al. (2003) J. Immunol., 170: 711-718). Las gotas tienen una capacidad de unión de 5 µg por 10⁷ gotas. Gotas CIS contienen anti-CD3 y B7-H3 en la misma gota, las gotas TRANS consisten de dos tipos de gotas, una que

contiene anticuerpo anti-CD3 y otra que contiene B7-H3-Ig (Bennett et al. (2003) J. Immunol., 170: 711-718). Para mantener proporciones iguales de gota a célula bajo condiciones CIS y TRANS, las gotas recubiertas con IgG de control se agregaron a los cultivos CIS. Las gotas recubiertas con proteínas se agregaron a células T CD4⁺ purificadas (10⁵ células/pozo) en placas de microtítulo de 96 pozos de fondo plano en una proporción de 1:1. La proliferación se determinó mediante pulsación de cultivos con 1 Ci [³H]-timidina por pozo durante al menos 6-16 horas de un periodo de incubación de 72 horas.

[0131] Como se muestra en la Fig. 5, la inhibición de la proliferación solo se observó cuando las células se activaron con gotas CIS. En conjunto, estos hallazgos sugieren que el receptor B7-H3 y el TCR necesitan estar en proximidad cercana para la subregulación de la activación de la célula T. Estos datos sugieren que para la trayectoria del receptor B7-H3 para modular una respuesta de célula T, la activación y las señales inhibitorias deben emanar de la misma célula.

[0132] La cantidad de citoquinas en los supernadantes se midió a 72 horas utilizando selección ELISA múltiplex: TNF- α (Fig. 6A), IFN- γ (Fig. 6B), y GM-CSF (Fig. 6C). La capacidad del VC de B7-H3 y el VCVC de B7-H3 para subregular la

anti-CD3 en la presencia de CHO.HLA-DR2.B7-1 o B7-2 resultó en la proliferación sobre los niveles obtenidos con las células de control CHO.HLA-DR2-GFP (Fig. 3A). Los anticuerpos anti-CD28 solubles mejoraron el control GFP, pero no las respuestas B7-1 y B7-2. Por el contrario, la estimulación de las células T en la presencia de VC de B7-H3 o VCVC de B7-H3 condujo a respuestas proliferativas reducidas (Fig. 3B). Con anti-CD3 (1 g/ml) y coestimulación baja (5 ng/ml de anticuerpo anti-CD28), la producción de citoquina fue significativamente reducida luego de estimulación con VCVC de B7-H3 y VC de B7-H3. Los niveles IL-10(~81%), TNF- α (~69%), IFN- γ (~85%), y GM-CSF(~65%) fueron dramáticamente reducidos en los cultivos estimulados ya sea con cultivos VCVC o VC de B7-H3 relacionados con controles GFP (Fig. 4). Cantidades insignificantes de IL-1A, IL-2, IL-4, IL-6, e IL-13 se detectaron en las condiciones de estos ensayos. Estos hallazgos indican que ni el VC de B7-H3 ni el VCVC de B7-H3 funcionan como una molécula coestimuladora y sugiere que las moléculas de superficie de célula VCVC de B7-H3 y VC de B7-H3 se enganchan en los receptores de células T que sirven como reguladores negativos de activación. La adición de anticuerpo anti-CD28 en concentraciones tan grandes como 200 ng/ml solo puede parcialmente rescatar el efecto inhibitorio del B7-H3 sobre la proliferación.

activación de células T fue evaluada mediante los niveles de producción de citoquina. Con anti-CD3 (1 g/ml) y coestimulación baja (5 ng/ml anticuerpo anti-CD28), la producción de citoquina fue significativamente reducida luego de la estimulación del VC de B7-H3 y VCVC de B7-H3 (Fig. 6A-6C). Los niveles IL-10 (~81%), TNF- α (~69%), IFN- γ (~85%), y GM-CSF (~65%) se redujeron dramáticamente en cultivos estimulados con VCVC o VC de B7-H3 relacionados con los controles GFP. Cantidades insignificantes de IL-1A, IL-2, IL-4, IL-6, e IL-13 se detectaron en estas condiciones de ensayo. Estos hallazgos indican que ni el VC de B7-H3 ni el VCVC de B7-H3 funcionan como una molécula coestimuladora y sugieren que las moléculas de superficie de célula VC de B7-H3 y VCVC de B7-H3 enganchan los receptores en las células T que sirven como reguladores negativos de activación.

[0133] Los resultados muestran que la activación TCR/B7H3 (VC o VCVC) de las células T conduce a una subregulación de las respuestas de células T. La proliferación y producción de citoquina se reduce en células T activadas con TCR/B7-H3 relacionadas con células activadas mediante TCR solo. Estos datos sugieren que el enganche del receptor B7-H3 en la célula T suministra una señal negativa. Los resultados también sugieren que la proximidad física entre el receptor TCR y el receptor B7-H3 se puede requerir con el fin de

subregular la activación de la célula T por vía del receptor B7-H3.

[0134] La capacidad del VCVC y el VC de B7-H3 para subregular la activación de célula T CD4⁺ es evocador de señales negativas producidas por el enganche del CTLA4 por cualquiera de las proteínas B7 o por PD-1 mediante cualquiera de las proteínas PD-L1 y PD-L2. Adicionalmente, los experimentos con B7-H3 acoplado a una matriz sólida indica que las señales de receptor B7-H3 y TCR se administran por la misma célula. Se han descrito requerimientos similares para la formación de señales negativas mediante CTLA-4 o PD-1 (Griffin et al. (2000) J. Immunol., 164: 4433; y Bennett et al. (2003) J. Immunol., 170: 711-718). Finalmente, ambas moléculas VCVC de B7-H3 humano y VC de B7-H3 humano parecen ser redundantes en su capacidad de modular las respuestas de célula T CD4.

Ejemplo 6: Eficacia terapéutica en pacientes con soriasis

[0135] La modulación de la respuesta inmune regulada por el B7-H3 es útil en instancias en donde un efecto inmunosupresivo o aumento de la respuesta inmune se desea. Los agonistas B7-H3 (por ejemplo, formas solubles de B7-H3) se pueden utilizar para evitar y/o reducir la severidad y/o

los síntomas de enfermedades o condiciones que involucren una respuesta inmune aberrantemente elevada, que incluye la respuesta de auto antígenos como, por ejemplo, un desorden autoinmune. Los antagonistas B7-H3, de otra parte, pueden ser administrados a los sujetos que tienen una respuesta inmune indeseablemente baja como por ejemplo esto puede ocurrir en cánceres o desórdenes inmunosupresivos.

[0136] Se considera a la soriasis una enfermedad autoinmune mediada por célula T típica. La soriasis es una enfermedad de la piel inflamatoria crónica mediada, en parte, a través de la producción de IFN- γ mediante células T lesionales activadas (Th₁ oblicuo).

[0137] Más comúnmente, las proteínas solubles se administran en una configuración de paciente externo mediante administración semanal en dosis de 0,1-10 mg/kg por infusión intravenosa lenta (IV). La dosis terapéuticamente efectiva apropiada de un antagonista se selecciona mediante un tratamiento clínico y puede variar aproximadamente de 1 μ g/kg a 20 mg/kg, de 1 μ g/kg a 10 mg/kg, de 1 μ g/kg a 1 mg/kg, de 10 μ g/kg a 1 mg/kg, de 10 μ g/kg a 100 μ g/kg, de 100 μ g/kg a 1 mg/kg, y de 500 μ g/kg a 5 mg/kg.

[0138] Para evaluar los efectos sobre las células T de piel, un anticuerpo contra B7-H3, un anticuerpo contra receptor B7-H3, o B7-H3-Ig se administra durante 12 semanas consecutivas a grupos aleatorizados de pacientes con soriasis con severidad de la enfermedad normalizada (clasificación mínima PASI (Actividad de Soriasis e Índice de Severidad) de 12). Para evaluar las mejoras clínicas en los pacientes durante el tiempo y para monitorear su respuesta a la terapia, el sistema de clasificación PASI puede ser utilizado (Fredriksson et al. (1978) Dermatológica, 157: 238-244; y Marks et al (1989) Arch. Dermatol., 1989; 125: 235-240).

[0139] En resumen, los elementos de la clasificación PASI incluyen: (1) regiones de cuerpo afectadas como porcentaje del área de superficie corporal (BSA); (2) alcance al que la región del cuerpo está afectado (en una escala de 1-10); y (3) alcance de los cambios soriáticos (eritema, infiltración, descamación) en una escala de 0-4). La clasificación PASI se calcula como sigue: $((0,1 \times (\text{cabeza eritema}) + (\text{cabeza de infiltración}) + (\text{cabeza de descamación})) \times (\text{alcance de cabeza afectada})) + ((0,2 \times ((\text{tronco eritema}) + (\text{tronco infiltración}) + (\text{tronco de descamación})) \times (\text{alcance de tronco afectado})) + ((0,3 \times ((\text{extremidades superiores con eritema}) + (\text{infiltración de extremidades superiores}) + (\text{descamación de extremidades superiores})) \times (\text{alcance de las extremidades$

superiores afectadas)) + ((0,4 x ((eritema de extremidades inferiores) + (infiltración de extremidades inferiores) + (descamación de extremidades inferiores)) x (alcance de las extremidades inferiores afectadas)). La clasificación mínima es 0, mientras que la clasificación máxima es = 72. Una reducción de la clasificación PASI es indicadora del tratamiento efectivo. Se anticipa que al menos el 50% de los individuos que reciben el tratamiento exhibirían una reducción en la clasificación PASI y una mejora en su condición.

[0140] La divulgación es más entendida a fondo en claridad de las enseñanzas de las referencias citadas dentro de la divulgación que se incorpora aquí como referencia. Las modalidades dentro de la divulgación suministran una ilustración de las modalidades de la invención y no debe ser construida para limitar el alcance de la invención. El experto reconoce fácilmente que muchas otras modalidades son abarcadas por la invención. Todas las publicaciones y patentes citadas y secuencias identificadas mediante números de referencia de bases de datos o números de acceso en esta descripción se incorporan como referencia en su totalidad. Para el alcance del material incorporado como referencia contradice o es inconsistente con la presente divulgación, la presente divulgación reemplazará cualquier tal material. La

citación de cualquier referencia aquí no es una admisión de que tales referencias son de la técnica anterior a la presente invención.

[0141] A menos que se indique otra cosa, todos los números que expresan cantidades de ingredientes, cultivos celulares, condiciones de tratamiento, y así sucesivamente utilizados en la divulgación, incluyendo reivindicaciones, se entiende que son modificadas en todas las instancias por el término "cerca de". De acuerdo con esto a menos que se indique otra cosa, los parámetros numéricos son aproximaciones y pueden variar dependiendo de las propiedades deseadas buscadas para ser obtenidas por la presente invención. A menos que se indique otra cosa, el término "al menos" que precede una serie de elementos se entiende que se refieren a cada elemento en las series. Aquellos expertos en la técnica reconocerán, o serán capaces de comprobar utilizando nada más que la rutina de experimentación, muchas modalidades equivalentes a las específicas de la invención descrita aquí. Tales equivalentes se pretenden estén abarcadas por las siguientes reivindicaciones.

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REIVINDICACIONES

1. Un método para inhibir la activación de un linfocito, el método comprende poner en contacto el linfocito con un agonista B7-H3 y permitir al agonista inhibir la activación del linfocito.
2. El método según la reivindicación 1, en donde el agonista B7-H3 es una forma soluble de B7-H3.
3. El método según la reivindicación 3, en donde el agonista B7-H3 comprende la ID DE SEC. NO.: 15.
4. El método según la reivindicación 2, en donde la forma soluble comprende al menos un dominio V de B7-H3.
5. El método según la reivindicación 4, en donde el dominio V comprende: (a) la ID DE SEC. NO.: 7 o (b) una secuencia de aminoácido que es sustancialmente idéntica a la ID DE SEC. NO.: 7.
6. El método según la reivindicación 4, en donde la forma soluble de B7-H3 comprende además al menos un dominio C de B7-H3.

7. El método según la reivindicación 4, en donde la forma soluble de B7-H3 comprende además una región Fc de un anticuerpo.
8. El método según la reivindicación 7, en donde la forma soluble de B7-H3 comprende: (a) una secuencia de aminoácido escogida de la ID DE SEC. NO.: 16, ID DE SEC. NO.: 17, ID DE SEC. NO.: 18, ID DE SEC. NO.: 19, ID DE SEC. NO.: 20, ID DE SEC. NO.: 21, o ID DE SEC. NO.: 22; o (b) una secuencia de aminoácido que es sustancialmente idéntica a al menos una de las secuencias escogidas de la ID DE SEC. NO.: 16, ID DE SEC. NO.: 17, ID DE SEC. NO.: 18, ID DE SEC. NO.: 19, ID DE SEC. NO.: 20, ID DE SEC. NO.: 21, o ID DE SEC. NO.: 22.
9. El método según la reivindicación 7, en donde el soluble de B7-H3 comprende: (a) una secuencia de aminoácido escogida de la ID DE SEC. NO.: 10, ID DE SEC. NO.: 12 o ID DE SEC. NO.: 14; o (b) una secuencia de aminoácido que es sustancialmente idéntica a al menos una de las secuencias escogidas de la ID DE SEC. NO.: 10, ID DE SEC. NO.: 12, o ID DE SEC. NO.: 14.

10. El método según la reivindicación 3, en donde el agonista B7-H3 se acopla con una molécula estimuladora primaria.
11. El método según la reivindicación 10, en donde la forma soluble de B7-H3 y la molécula estimuladora primaria están espaciadas por no más de 100 μm .
12. El método según la reivindicación 1, en donde el agonista B7-H3 es un ácido nucleico que codifica aminoácido de la ID DE SEC. NO.: 15.
13. Un método para mejorar la activación de un linfocito, el método comprende poner en contacto el linfocito con un antagonista B7-H3 y permitir al antagonista mejorar la activación del linfocito.
14. El método según la reivindicación 13, en donde el linfocito es humano.
15. El método según la reivindicación 13, en donde el antagonista B7-H3 es un anticuerpo para B7-H3 o un anticuerpo contra un receptor B7-H3.

16. El método según la reivindicación 13, en donde el antagonista B7-H3 es un ácido nucleico antisentido o un siARN.
17. El método según una cualquiera de las reivindicaciones 1 a 16, en donde el linfocito es una célula T.
18. El método según la reivindicación 17, en donde la célula T es una célula T CD4⁺.
19. El método según una cualquiera de las reivindicaciones 1 a 16, en donde el linfocito está en un mamífero.
20. El método según la reivindicación 19, en donde el mamífero está afligido con o está en riesgo de al menos una de: un desorden inmunológico, un cáncer, o una enfermedad infecciosa.
21. El método según la reivindicación 19, en donde el mamífero se trata con Factor VIII o Factor IX.

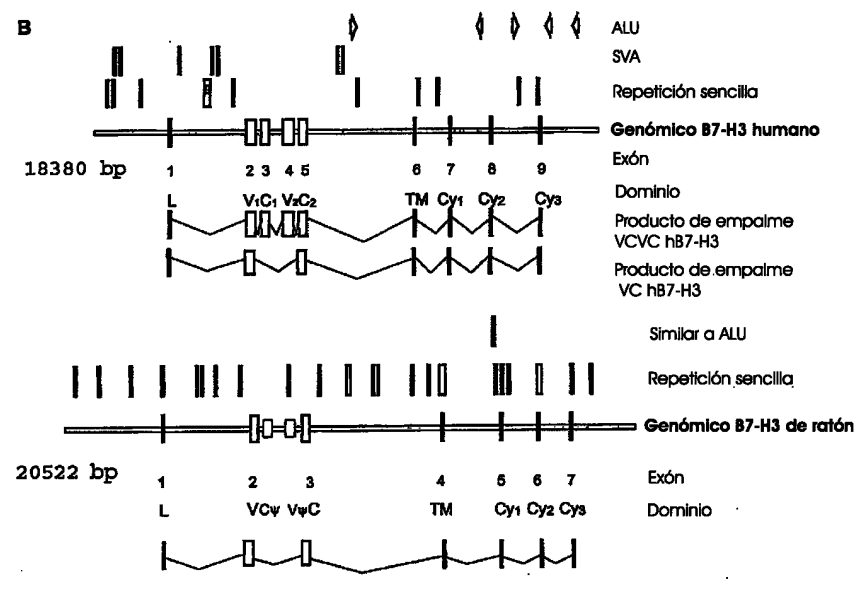


Fig. 1B

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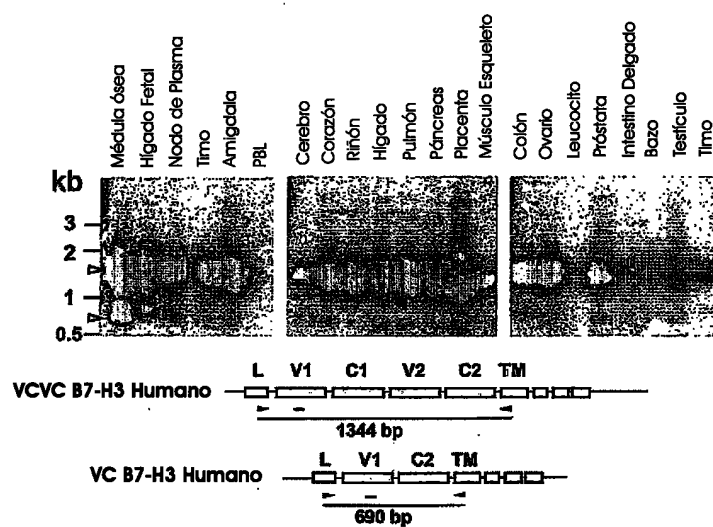


Fig. 2A

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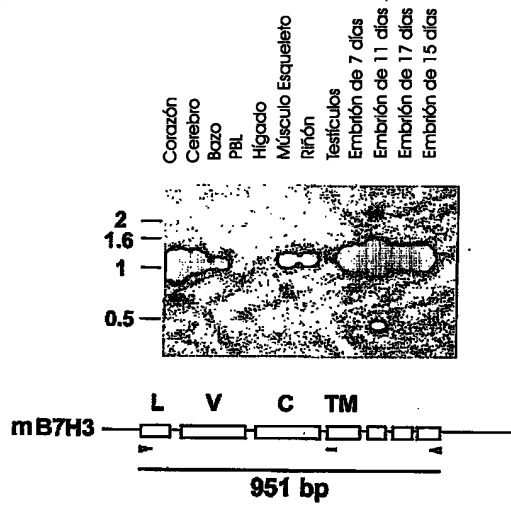


Fig. 2B

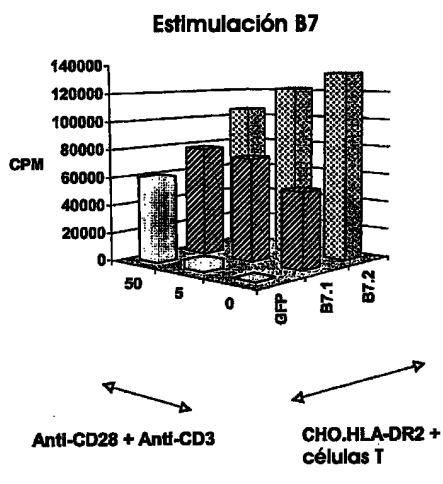


Fig. 3A

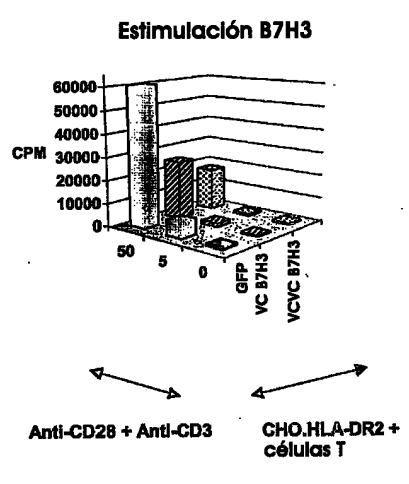


Fig. 3B

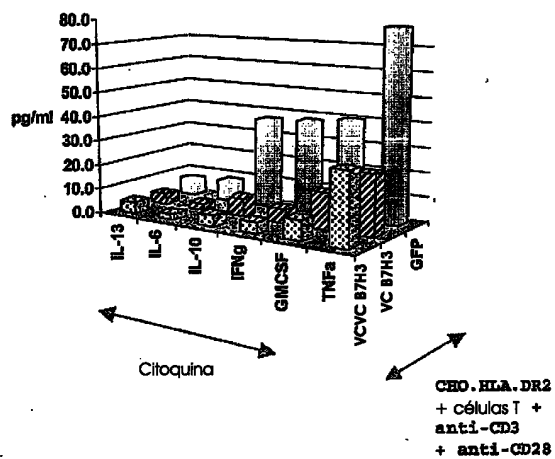


Fig. 4

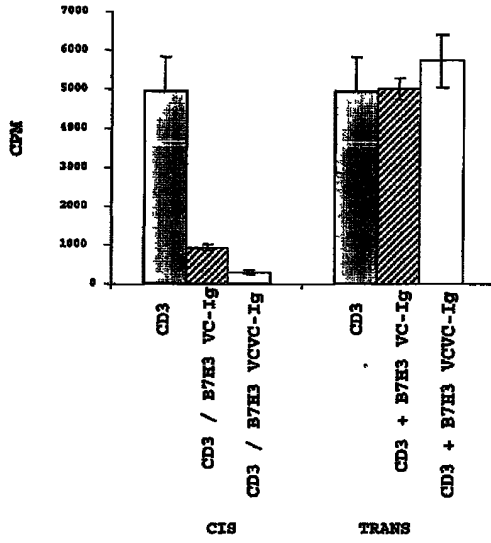


Fig. 5

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TNF- α

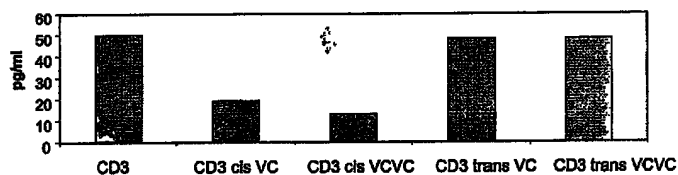


Fig. 6A

IFN- γ

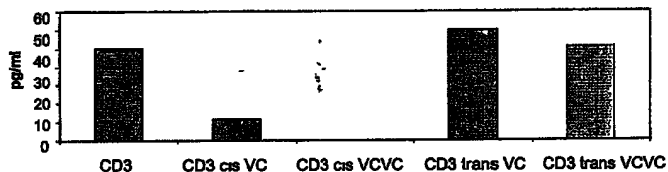


Fig. 6B

GM-CSF

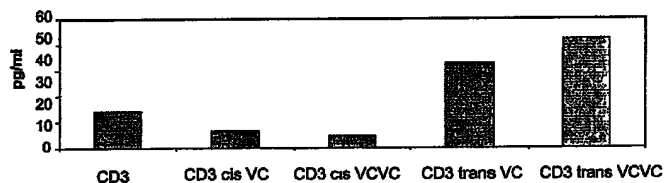


Fig. 6C

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

257

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference
(if desired) (12 characters maximum) 8702.134-304

Box No. I TITLE OF INVENTION USE OF B7-H3 AS AN IMMUNOREGULATORY AGENT	
Box No. II APPLICANT ☐ This person is also inventor	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.) WYETH Five Giralda Farms Madison, New Jersey 07940 United States of America	
Telephone No.	
Facsimile No.	
Teleprinter No.	
Applicant's registration No. with the Office	
State (that is, country) of nationality: US	State (that is, country) of residence: US
This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box	
Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.) LING, Vincent 18 Forsythe Drive Walpole, MA 02081 United States of America	
This person is: ☐ applicant only ☒ applicant and inventor ☐ inventor only (If this check-box is marked, do not fill in below.)	
Applicant's registration No. with the Office	
State (that is, country) of nationality: US	State (that is, country) of residence: US
This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box	
☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.	
Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE	
The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.) GARRETT, Arthur S. Finnegan, Henderson, Farabow, Garrett & Dunner, L.L.P. 1300 I Street, NW Washington, DC 20005 United States of America	
Telephone No. Carol Marshall - 202.408.4181	
Facsimile No. 202.408.4400	
Teleprinter No.	
Agent's registration No. with the Office 20,338	
☐ Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.	

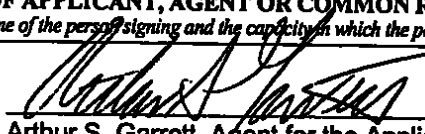
258

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)	
If none of the following sub-boxes is used, this sheet should not be included in the request.	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.) CARRENO, Beatriz M. 37 Nash Road Action, MA 01720 United States of America	This person is: ☐ applicant only ☒ applicant and inventor ☐ inventor only (If this check-box is marked, do not fill in below.) Applicant's registration No. with the Office
State (that is, country) of nationality: US	State (that is, country) of residence: US
This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.) COLLINS, Mary 54 Rathburn Road Natick, MA 01760 United States of America	This person is: ☐ applicant only ☒ applicant and inventor ☐ inventor only (If this check-box is marked, do not fill in below.) Applicant's registration No. with the Office
State (that is, country) of nationality: US	State (that is, country) of residence: US
This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)	This person is: ☐ applicant only ☐ applicant and inventor ☐ inventor only (If this check-box is marked, do not fill in below.) Applicant's registration No. with the Office
State (that is, country) of nationality:	State (that is, country) of residence:
This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)	This person is: ☐ applicant only ☐ applicant and inventor ☐ inventor only (If this check-box is marked, do not fill in below.) Applicant's registration No. with the Office
State (that is, country) of nationality:	State (that is, country) of residence:
This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box	
☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.	

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Box No. IX CHECK LIST; LANGUAGE OF FILING

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This international application contains: (a) in paper form, the following number of sheets: request (including declaration sheets) : 4 description (excluding sequence listing and/or tables related thereto) : 44 claims : 2 abstract : 1 drawings : 8 Sub-total number of sheets : 59 sequence listing : 40 tables related thereto : 0 <i>(for both, actual number of sheets if filed in paper form, whether or not also filed in computer readable form; see (c) below)</i> Total number of sheets : 99 (b) ☐ only in computer readable form (Section 801(a)(i)) (i) ☐ sequence listing (ii) ☐ tables related thereto (c) ☐ also in computer readable form (Section 801(a)(ii)) (i) ☐ sequence listing (ii) ☐ tables related thereto Type and number of carriers (diskette, CD-ROM, CD-R or other) on which are contained the ☒ sequence listing: 1 ☐ tables related thereto: <i>(additional copies to be indicated under items 9(ii) and/or 10(ii), in right column)</i>		This international application is accompanied by the following item(s) (mark the applicable check-boxes below and indicate in right column the number of each item): 1. ☒ fee calculation sheet : 1 2. ☐ original separate power of attorney : 3. ☐ original general power of attorney : 4. ☒ copy of general power of attorney; reference number, if any: : 1 5. ☐ statement explaining lack of signature : 6. ☐ priority document(s) identified in Box No. VI as item(s): : 7. ☐ translation of international application into (language): : 8. ☐ separate indications concerning deposited microorganism or other biological material : 9. ☒ sequence listing in computer readable form (indicate type and number of carriers) (i) ☐ copy submitted for the purposes of international search under Rule 13ter only (and not as part of the international application) : (ii) ☐ (only where check-box (b)(i) or (c)(i) is marked in left column) additional copies including, where applicable, the copy for the purposes of international search under Rule 13ter : (iii) ☒ together with relevant statement as to the identity of the copy or copies with the sequence listing mentioned in left column : 1 10. ☐ tables in computer readable form related to sequence listing (indicate type and number of carriers) (i) ☐ copy submitted for the purposes of international search under Section 802(b-quater) only (and not as part of the international application) : (ii) ☐ (only where check-box (b)(ii) or (c)(ii) is marked in left column) additional copies including, where applicable, the copy for the purposes of international search under Section 802(b-quater) : (iii) ☐ together with relevant statement as to the identity of the copy or copies with the tables mentioned in left column : 11. ☐ other (specify): :	
Figure of the drawings which should accompany the abstract:		Language of filing of the international application: English	
Box No. X SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE <i>Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).</i>  Arthur S. Garrett, Agent for the Applicant(s)			

1. Date of actual receipt of the purported international application:		2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:		
4. Date of timely receipt of the required corrections under PCT Article 11(2):		
5. International Searching Authority (if two or more are competent): ISA /	6. ☐ Transmittal of search copy delayed until search fee is paid	
For International Bureau use only Date of receipt of the record copy by the International Bureau:		

PATENT COOPERATION TREATY

From the RECEIVING OFFICE

PCT

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To:
ARTHUR S. GARRETT
FINNEGAN, HENDERSON, FARABOW, GARRETT
& DUNNER LLP
1300 I STREET, NW.
WASHINGTON, DISTRICT OF COLUMBIA 20005

NOTIFICATION OF THE INTERNATIONAL APPLICATION NUMBER AND OF THE INTERNATIONAL FILING DATE

(PCT Rule 20.5(c))

Applicant's or agent's file reference 8702.134-304		IMPORTANT NOTIFICATION	
International application No. PCT/US2004/011767	International filing date (day/month/year) 16 Apr 2004	Priority date (day/month/year) 17 Apr 2003	
Applicant WYETH			
Title of the invention USE OF B7-H3 AS AN IMMUNOREGULATORY AGENT			

1. The applicant is hereby notified that the international application has been accorded the international application number and the international filing date indicated above.

2. The applicant is further notified that the record copy of the international application:

- 26 Jul 2004
- ☒ was transmitted to the International Bureau on _____
- ☐ has not yet been transmitted to the International Bureau for the reason indicated below and a copy of this notification has been sent to the International Bureau*:
- ☐ because the necessary national security clearance has not yet been obtained.

☐ because (reason to be specified): _____

* The International Bureau monitors the transmittal of the record copy by the receiving Office and will notify the applicant (with Form PCT/IB/301) of its receipt. Should the record copy not have been received by the expiration of 14 months from the priority date, the International Bureau will notify the applicant (Rule 22.1(c)).

3. FOREIGN TRANSMITTAL LICENSE INFORMATION Completed by: LR

☐ Additional license for foreign transmittal not required. This subject matter is covered by a license already granted or the equivalent U.S. national application. Refer to that license for information concerning its scope.

☐ License for foreign transmittal not required. 37 CFR 5.11(e)(1) or 37 CFR 5.11(e)(2). However, a license may be required for additional subject matter. See 37 CFR 5.15(b).

☒ Foreign transmittal license granted. 35 U.S.C. 184; 37 CFR 5.11 on 21 Jul 2004 :
(date)

☐ 37 CFR 5.15(a) ☒ 37 CFR 5.15(b)

Name and mailing address of the receiving Office
Mail Stop PCT, Commissioner for Patents
P.O. Box 1450, Alexandria, VA 22313-1450
Facsimile No. 703-305-3230

Authorized officer
Latrice Russell
Telephone No. 703-306-5829

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF RECEIPT
OF SEARCH COPY

(PCT Rule 25.1)

To:

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.
Attn. Garrett, Arthur S.
1300 I Street, N.W.
Washington, DC 20005
UNITED STATES OF AMERICA

Date of mailing
(day/month/year)

17/08/2004

Applicant's or agent's file reference

8702.134-304

IMPORTANT NOTIFICATION

International application No.

PCT/US2004/011767

International filing date(day/month/year)

16/04/2004

Priority date (day/month/year)

17/04/2003

Applicant

WYETH

1. Where the International Searching Authority and the receiving Office are not the same office:

The applicant is hereby notified that the search copy of the international application was received by this International Searching Authority on the date indicated below.

Where the International Searching Authority and the receiving Office are the same office:

The applicant is hereby notified that the search copy of the international application was received on the date indicated below.

28/07/2004

(date of receipt).

2. ☐ The search copy was accompanied by a nucleotide and/or amino acid sequence listing or tables related thereto in computer readable form.

3. Time limit for establishment of international search report and written opinion of the International Searching Authority

The applicant is informed that the time limit for establishing the international search report and the written opinion of the International Searching Authority is three months from the date of receipt indicated above or nine months from the priority date, whichever time limit expires later (Rules 42.1 and 43bis.1(a))

4. A copy of this notification has been sent to the International Bureau and, where the first sentence of paragraph 1 applies, to the receiving Office.

Name and mailing address of the International Searching Authority

European Patent Office, P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016



Authorized officer

AUG 24 2004

ISA/EP

Finnegan, Henderson, Farabow,
Garrett & Dunner, L.L.P.

Form PCT/ISA/202 (January 2004)

10/11/04 8-21 02

PATENT COOPERATION TREATY

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PCT

From the INTERNATIONAL BUREAU

NOTIFICATION OF RECEIPT OF
RECORD COPY

(PCT Rule 24.2(a))

To:

GARRETT, Arthur, S.
Finnegan, Henderson, Farabow,
Garrett & Dunner, L.L.P.
1300 I Street, N.W.
Washington, DC 20005-3315
United States of America

Date of mailing (day/month/year) 18 August 2004 (18.08.2004)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference 8702.134-304	International application No. PCT/US2004/011767

The applicant is hereby notified that the International Bureau has received the record copy of the international application as detailed below.

Name(s) of the applicant(s) and State(s) for which they are applicants:

WYETH (for all designated States except US)
LING, Vincent et al (for US)

International filing date : 16 April 2004 (16.04.2004)
Priority date(s) claimed : 17 April 2003 (17.04.2003)
Date of receipt of the record copy
by the International Bureau : 26 July 2004 (26.07.2004)
List of designated Offices :

AP : BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW
EA : AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
EP : AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR
OA : BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
National : AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW

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AUG 31 2004

Finnegan, Henderson, Farabow,
Garrett & Dunner, L.L.P.

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. (41-22) 338.89.75	Authorized officer: Rosana REYES (Fax : 338 89 75) Telephone No. (41-22) 338 8471
--	---

/ Aug 8-31-04

PATENT COOPERATION TREATY

MY ESTY/KBH

265

From the INTERNATIONAL SEARCHING AUTHORITY

PCT 265

To:

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.
Attn. Garrett, Arthur S.
1300 I Street, N.W.
Washington, DC 20005
UNITED STATES OF AMERICA

INVITATION TO FURNISH NUCLEOTIDE AND/OR
AMINO ACID SEQUENCE LISTING AND/OR TABLES
RELATED THERETO COMPLYING WITH STANDARD
AND/OR TECHNICAL REQUIREMENTS

(PCT Rule 13ter.1(a) and (c) and Administrative Instructions,
Sections 208 and 802 and Annexes C and C-bis)

Date of mailing
(day/month/year)

18/10/2004

Applicant's or agent's file reference

8702.134-304

REPLY DUE

within 1 months/2006
from the above date of mailing

International application No.

PCT/US2004/011767

International filing date
(day/month/year)

16/04/2004

Applicant

WYETH

1. The applicant is hereby invited, within the time limit indicated above, to furnish to this Authority:

☐

a nucleotide and/or amino acid sequence listing in written form complying with the standard provided for in Annex C of the Administrative Instructions, accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

☐

a statement to the effect that the sequence listing in written form, already furnished to this Authority, does not go beyond the disclosure in the international application as filed.

☒

a nucleotide and/or amino acid sequence listing and/or tables related thereto in computer readable form complying with the standard and/or technical requirements provided for in Annex C and/or Annex C-bis of the Administrative Instructions, accompanied by a statement that the information recorded in computer readable form is identical to the written sequence listing and/or tables related thereto.

☐

a statement that the information recorded in computer readable form (that computer readable form having already been furnished to this Authority) is identical to the written sequence listing.

2. Failure to comply with this invitation may result in this Authority not carrying out the international search to the extent that no meaningful search can be carried out.

3. Further observations (if necessary):

IMPORTANT REMARK:

The statements are legally required [See Suppl.No.2 to Official Journal No.11/1998 (page 14, § 40 and page 64, § III.2)]

RECEIVED

OCT 26 2004

Finnegan, Henderson, Farabow,
Garrett & Dunner, LLP

Name and mailing address of the International Searching Authority



European Patent Office, P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Valérie Marmoy

J. Marmoy

/Ld 10/26/04

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

266

Applicant's or agent's file reference 8702.134-304	FOR FURTHER ACTION see Form PCT/ISA/220 as well as, where applicable, item 5 below.	
International application No. PCT/US2004/011767	International filing date (day/month/year) 16/04/2004	(Earliest) Priority Date (day/month/year) 17/04/2003
Applicant WYETH		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the language, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ The international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. ☒ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, see Box No. I.

2. ☒ Certain claims were found unsearchable (See Box II).**3. ☐ Unity of invention is lacking (see Box III).****4. With regard to the title,**

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the abstract,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the drawings,

- a. the figure of the drawings to be published with the abstract is Figure No. _____

☐ as suggested by the applicant.

☐ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

- b. ☒ none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2004/011767

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Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

a. type of material



a sequence listing



table(s) related to the sequence listing

b. format of material



in written format



in computer readable form

c. time of filing/furnishing



contained in the international application as filed



filed together with the international application in computer readable form



furnished subsequently to this Authority for the purpose of search

2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

national Application No
PCT/US2004/011767

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K38/00 A61K39/00 A61P37/00

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A61P

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

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

EPO-Internal, PAJ, BIOSIS, EMBASE, Sequence Search, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 02/10187 A (CHEN LIEPING ; MAYO FOUNDATION (US)) 7 February 2002 (2002-02-07) see pages 1-2, page 6 lines 5-10, pages 10-12, page 18, page 21 lines 10-19 and the examples	1-21
X	WO 03/014293 A (HEUER JOSEF GEORG ; OKRAGLY ANGELA JEANNINE (US); NA SONGQING (US); OU) 20 February 2003 (2003-02-20) see Seq. No.2, pages 5-6, 11, page 18-20 and example 11.	1-12
Y		1-21
	-/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

12 January 2005

Date of mailing of the international search report

15. 03. 2005

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Merckling-Ruiz, V

INTERNATIONAL SEARCH REPORT

national Application No

PCT/US2004/011767

269

269

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CHAPOVAL I ET AL.: "B7-H3: a costimulatory molecule for T cell activation and IFN-g production" NATURE IMMUNOLOGY, NATURE PUBLISHING GROUP,, GB, vol. 2, no. 3, March 2001 (2001-03), pages 269-274, XP002958730 ISSN: 1529-2908 cited in the application see abstract and page 273 last paragraph -----	1-21
Y	SUN M. ET AL.: "Characterization of mouse and human B7-H3 genes." J. IMMUNOL., vol. 168, 2002, pages 6294-6297, XP002313221 cited in the application see abstract and fig.4 -----	1-21
X	Biosis abstract No. PREV200400117815 Ling V. et al., FASEB J. vol.17 No.7 page C215 (14.04.2003) Genomic and functional analysis of primate an rodent B7-H3. XP009042456	1,2,6
Y	abstract -----	1-21

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2004/011767

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Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 1-21 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US2004/011767

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Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 0210187	A	07-02-2002	AU 8350701 A	13-02-2002
			WO 0210187 A1	07-02-2002
			US 2002168762 A1	14-11-2002

WO 03014293	A	20-02-2003	EP 1423402 A2	02-06-2004
			WO 03014293 A2	20-02-2003
			US 2004236088 A1	25-11-2004

PATENT COOPERATION TREATY

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From the
INTERNATIONAL SEARCHING AUTHORITY

PCT

To:

see form PCT/ISA/220

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (PCT Rule 43bis.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No. PCT/US2004/011767	International filing date (day/month/year) 16.04.2004	Priority date (day/month/year) 17.04.2003
--	--	--

International Patent Classification (IPC) or both national classification and IPC
A61K38/00, A61K39/00, A61P37/00

Applicant
WYETH

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and Industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII *Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for International preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Merckling-Ruiz, V Telephone No. +49 89 2399-8590 
---	--

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITYInternational application No.
PCT/US2004/011767

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Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).
2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:
 - a. type of material:
☒ a sequence listing
☐ table(s) related to the sequence listing
 - b. format of material:
☒ in written format
☒ in computer readable form
 - c. time of filing/furnishing:
☒ contained in the international application as filed.
☐ filed together with the international application in computer readable form.
☒ furnished subsequently to this Authority for the purposes of search.
3. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
4. Additional comments:

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2004/011767

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Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. -

because:

☒ the said international application, or the said claims Nos. 1-21 relate to the following subject matter which does not require an international preliminary examination (*specify*):

see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the whole application or for said claims Nos. -

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form

☐ has not been furnished

☐ does not comply with the standard

the computer readable form

☐ has not been furnished

☐ does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-bis of the Administrative Instructions.

☐ See separate sheet for further details

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US2004/011767

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**Box No. V Reasoned statement under Rule 43bis.1(a)(I) with regard to novelty, inventive step or
Industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Yes: Claims	13-21
	No: Claims	1-12
Inventive step (IS)	Yes: Claims	
	No: Claims	1-21
Industrial applicability (IA)	Yes: Claims	1-21 see separate sheet
	No: Claims	

2. Citations and explanations

see separate sheet

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No. **276**
PCT/US2004/011767

1. Reference is made to the following documents :

- D1: WO 02/10187 A (CHEN LIEPING ; MAYO FOUNDATION (US)) 7 February 2002 (2002-02-07)
D2: WO 03/014293 A (HEUER JOSEF GEORG ; OKRAGLY ANGELA JEANNINE (US); NA SONGQING (US); OU) 20 February 2003 (2003-02-20)
D3: CHAPOVAL I ET AL: "B7-H3: a costimulatory molecule for T cell activation and IFN-g production" NATURE IMMUNOLOGY, NATURE PUBLISHING GROUP,, GB, vol. 2, no. 3, March 2001 (2001-03), pages 269-274, XP002958730 ISSN: 1529-2908
D4: SUN M. ET AL.: "Characterization of mouse and human B7-H3 genes." J. IMMUNOL., vol. 168, 2002, pages 6294-6297, XP002313221
D5: Biosis abstract No. PREV200400117815 Ling V. et al., FASEB J. vol.17 No.7 page C215 (14.04.2003) Genomic and functional analysis of primate an rodent B7-H3. XP009042456

Unless specified otherwise, the relevant passages are the ones that are cited in the Search Report.

Regarding point III

2. Claims 1-21 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1 (iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

Regarding point V

3. For the assessment of the present claims 1-21 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The

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**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

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patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

4. The use of fragments of B7-H3 polypeptides (or analogues thereof) or polynucleotides for inhibiting the activation of T lymphocytes is disclosed in D2 and D5. D5 specifically discloses LP276 peptides and fusion proteins, said LP276 being substantially homologous to human B7-H3 (see sequence alignments of pages 18-20). Sequence ID No.2, corresponding to full-length LP276, has 97.9% identity with sequence No.12 of the present application. The LP276 polypeptides and fusion proteins that contain 4 IgG-like extracellular domains are also shown to inhibit the proliferation of T cells and the secretion of IFN- γ (pages 11 and 18-20).

In conclusion, the subject-matter of claims 1-12 is not new.

- 4.1 There is no explicit disclosure in the prior art of the use of B7-H3 antagonists for enhancing lymphocyte activation.
Claims 13-21 are novel.

5. The technical problem underlying the application is to provide immunomodulatory agents. The problem is "solved" by using B7-H3 agonists as inhibitors of lymphocyte activation (claims 1-12) and antagonists of B7-H3 to stimulate lymphocyte activation (claims 13-21).

- 5.1 Although claims 1-12 are not novel, it should be emphasized that these claims have a major fault in that the technical problem is not solved across the whole scope of the claims.

These claims (including dependent claims in which the wording "comprising sequence X" does not restrict the scope to sequence X itself) also encompass the whole B7-H3 polypeptide/polynucleotide. Nevertheless, it appears from the prior art that B7-H3 (entire polypeptide as in D3 or only the extracellular domain as in D1) does not inhibit

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**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

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PCT/US2004/011767

lymphocyte proliferation/activation but, on the contrary, stimulates lymphocyte activity. It is not only unclear what is meant by "B7-H3 agonist" in present claim 1 but it also seems that this claim is in contradiction with the teaching of the prior art.

5.2 For the reasons already developed in point 5.1, claims 13-21 also seem to be in contradiction with the teaching of the prior art. The wording "B7-H3 antagonist" is not clear and the prior art shows that the compounds that enhance lymphocyte activation are B7.H3 itself or the extracellular domain of B7-H3.

Moreover, D3 shows that an antisense directed against the B7-H3 gene (which can reasonably be classified as a B7-H3 antagonist) inhibits cytokine production by T lymphocytes (the contrary of the subject-matter of present claim 13).

Finally, not one single B7-H3 antagonist was actually manufactured in the application, no experiment was carried out with such an antagonist, so that the claimed technical effect is purely hypothetical. Claims 13-21 are not inventive.

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING
TRANSMITTAL OF COPY OF INTERNATIONAL
APPLICATION AS PUBLISHED OR REPUBLISHED

To: JAB/SRD/JZL

279

GARRETT, Arthur, S.
Finnegan, Henderson, Farabow, Garrett & Dunner, L.L.P.
1300 I Street, N.W.
Washington, DC 20005-3315
ETATS-UNIS D'AMERIQUEDate of mailing (day/month/year)
24 March 2005 (24.03.2005)Applicant's or agent's file reference
8702.134-304

IMPORTANT NOTICE

International application No.
PCT/US2004/011767International filing date (day/month/year)
16 April 2004 (16.04.2004)Priority date (day/month/year)
17 April 2003 (17.04.2003)

Applicant

WYETH et al

The International Bureau transmits herewith the following documents:

- ☐ copy of the international application as published by the International Bureau on under No. WO
- ☒ copy of international application as republished by the International Bureau on 24 March 2005 (24.03.2005) under No. WO 2004/093894
For an explanation as to the reason for this republication of the international application, reference is made to INID codes (15), (48) or (88) (as the case may be) on the front page of the attached document.

RECEIVED

MAR 29 2005

Finnegan, Henderson, Farabow,
Garrett & Dunner, L.L.P.The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Authorized officer

Dorothee Mülhausen

Facsimile No.+41 22 740 14 35

Facsimile No.+41 22 338 87 40

(19) World Intellectual Property
Organization
International Bureau



280

(43) International Publication Date
4 November 2004 (04.11.2004)

PCT

(10) International Publication Number
WO 2004/093894 A3

(51) International Patent Classification⁷: A61K 38/00,
39/00, A61P 37/00

(21) International Application Number:
PCT/US2004/011767

(22) International Filing Date: 16 April 2004 (16.04.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/463,342 17 April 2003 (17.04.2003) US

(71) Applicant (for all designated States except US): WYETH
[US/US]; Five Giralda Farms, Madison, NJ 07940 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): LING, Vincent
[US/US]; 18 Forsythe Drive, Walpole, MA 02081 (US).
CARRENO, Beatriz, M. [US/US]; 37 Nash Road, Acton,
MA 01720 (US). COLLINS, Mary [US/US]; 54 Rathburn
Road, Natick, MA 01760 (US).

(74) Agent: GARRETT, Arthur, S.; Finnegan, Henderson,
Farrabow, Garrett & Dunner, L.L.P., 1300 I Street, N.W.,
Washington, DC 20005-3315 (US).

(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,

AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,
GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE,
KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,
MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG,
PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM,
TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM,
ZW.

(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), Euro-
pean (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR,
GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK,
TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments

(88) Date of publication of the international search report:
24 March 2005

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: USE OF B7-H3 AS AN IMMUNOREGULATORY AGENT

(57) Abstract: The present disclosure relates to the fields of immunology and clinical immunology, and more particularly to the use of B7-family ligands and agonists and antagonists thereof in modulation of immune response. The invention provides methods for modulation of lymphocyte activation involving the use of B7-H3, including B7-H3 VC and B7-H3 VCVC, and related molecules such as, for example, antibodies and nucleic acids.

WO 2004/093894 A3

INTERNATIONAL SEARCH REPORT

Inte al Application No

PC 1, u32004/011767

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A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K38/00 A61K39/00 A61P37/00

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K A61P

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

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

EPO-Internal, PAJ, BIOSIS, EMBASE, Sequence Search, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	WO 03/014293 A (HEUER JOSEF GEORG ; OKRAGLY ANGELA JEANNINE (US); NA SONGQING (US); OU) 20 February 2003 (2003-02-20)	1-12
Y	see Seq. No.2, pages 5-6, 11, page 18-20 and example 11.	1-21
	----- -/-	

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents :

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

Z document member of the same patent family

Date of the actual completion of the international search

12 January 2005

Date of making of the international search report

25/01/2005

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
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Fax (+31-70) 340-3016

Authorized officer

Merckling-Ruiz, V

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US2004/011767

282

G.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CHAPOVAL I ET AL: "B7-H3: a costimulatory molecule for T cell activation and IFN-gamma production" NATURE IMMUNOLOGY, NATURE PUBLISHING GROUP,, GB, vol. 2, no. 3, March 2001 (2001-03), pages 269-274, XP002958730 ISSN: 1529-2908 cited in the application see abstract and page 273 last paragraph	1-21
Y	SUN M. ET AL.: "Characterization of mouse and human B7-H3 genes." J. IMMUNOL., vol. 168, 2002, pages 6294-6297, XP002313221 cited in the application see abstract and fig.4	1-21
X	Biosis abstract No. PREV200400117815 Ling V. et al., FASEB J. vol.17 No.7 page C215 (14.04.2003) Genomic and functional analysis of primate and rodent B7-H3. XP009042456	1,2,6
Y	abstract	1-21

INTERNATIONAL SEARCH REPORT

International application No. .

PCT/US2004/011767

283

283

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:
 - a. type of material

☒

a sequence listing

☐

table(s) related to the sequence listing
 - b. format of material

☒

in written format

☒

in computer readable form
 - c. time of filing/furnishing

☒

contained in the international application as filed

☐

filed together with the international application in computer readable form

☒

furnished subsequently to this Authority for the purpose of search
2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

national application No.
PCT/US2004/011767

284

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-21 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US2004/011767

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Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 0210187	A	07-02-2002	AU 8350701 A	13-02-2002
			WO 0210187 A1	07-02-2002
			US 2002168762 A1	14-11-2002
WO 03014293	A	20-02-2003	EP 1423402 A2	02-06-2004
			WO 03014293 A2	20-02-2003
			US 2004236088 A1	25-11-2004

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REQUISITOS NECESARIOS PARA PRESENTACION Y AGILIZACION DEL
TRAMITE

	USUARIO	RADICADOR
PATENTE DE INVENCION ✓	()	()
MODELO DE UTILIDAD	()	()
- Indicación de que se solicita la concesión de una patente	()	()
- Datos de identificación del solicitante o de la persona que se presenta la solicitud.	()	()
- Descripción de la invención.	()	()
- Dibujos de ser estos pertinentes.	()	()
- Comprobante de Pago de las tasas establecidas	()	()
COMPLETA ✓		
INCOMPLETA ()	()	()
DISEÑO INDUSTRIAL ()		
- Indicación de que se solicita el registro de Diseño Industrial	()	()
- Datos de identificación del solicitante o de la persona que presenta la solicitud	()	()
- Representación gráfica o fotografica del Diseño Industrial o muestra del Material que incorpora el diseño	()	()
- Comprobante de pago de las tasas establecidas	()	()
COMPLETA ()		
INCOMPLETA ()	()	()
ESQUEMA DE TRAZADO ()		
- Indicación de que solicita el registro de un Esquema de trazado.	()	()
- Datos de identificación del solicitante o de la persona que presenta La solicitud	()	()
- Representación gráfica del esquema de trazado	()	()
- Comprobante de pago de las tasas establecidas	()	()
COMPLETA ()		
INCOMPLETA ()	()	()

Firma Responsable

Fecha

REPUBLICA DE COLOMBIA
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

Bogotá,
2020

Doctor(a)
RODRIGUEZ D'ALEMAN DILIA MARIA

P-620

REFERENCIA	Radicación No	5 116756
	Solicitud internacional	US2004/011767
	Fecha inicio fase nacional	17/11/2005
	Trámite	11
	Evento	378
	Actuación	430
	Oficio No	1176

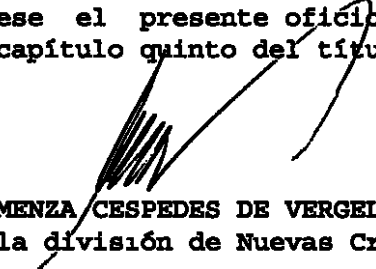
Como resultado del examen de forma, realizado con fundamento en el artículo 38 de la Decisión 486 de la Comisión de la Comunidad Andina, artículo 27 del Tratado de Cooperación en Materia de Patentes (PCT), regla 51 bis del Reglamento y Circular Única de la Superintendencia de Industria y Comercio, se le comunica que:

1 La solicitud no contiene la copia del documento en que consta la cesión del derecho, a la patente del inventor al solicitante o a su causante (Art 26-k) Decisión 486

2 La solicitud no contiene el poder debidamente otorgado, con el lleno de los requisitos exigidos por los artículos 63 y subsiguientes del Código de Procedimiento Civil. Adicionalmente, deberá presentar el documento que acredita la existencia y representación legal de la sociedad solicitante, cuando ésta fuere persona jurídica

En cumplimiento de lo dispuesto por el artículo 39 de la Decisión 486, se le requiere para que dentro del término de dos meses siguientes a la fecha de notificación del presente oficio, complete los requisitos anteriormente enunciados. En caso de no dar cumplimiento al presente requerimiento, la solicitud se considerará abandonada y perderá su prelación.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No 10 de 2001.


ALIX CARMENZA CESPEDES DE VERGEL
Jefe de la división de Nuevas Creaciones

El anterior requerimiento fue notificado por fijación en lista de fecha 03 FEB 2006 adpa1