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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
28 September 2006 (28.09.2006)

PCT

(10) International Publication Number
WO 2006/102408 A2

(51) International Patent Classification:
A61K 48/00 (2006.01)

(21) International Application Number:
PCT/US2006/010398

(22) International Filing Date: 22 March 2006 (22.03.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/664,001 22 March 2005 (22.03.2005) US

(71) Applicant *(for all designated States except US)*: **CHILDREN'S HOSPITAL MEDICAL CENTER** [US/US]; 3333 Burnet Avenue, Cincinnati, Ohio 45229-3039 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **KARP, Christopher, L.** [DE/US]; 3333 Burnet Avenue, Cincinnati, Ohio 45229 (US). **DIVANOVIC, Senad** [US/US]; 3333 Burnet Avenue, Cincinnati, Ohio 45229 (US).

(74) Agents: **ALBAINY-JENEI, Stephen, R.** et al.; Frost Brown Todd LLC, 2200 PNC Center, 201 East Fifth Street, Cincinnati, Ohio 45202 (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, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, 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, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, 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:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2006/102408 A2

(54) **Title:** METHODS AND COMPOSITIONS FOR THE MODULATION OF IMMUNE RESPONSES AND AUTOIMMUNE DISEASES

(57) **Abstract:** The present invention relates to a regulation of inflammation and immune responses. The present invention relates to a method of treating a condition comprising administering a pharmaceutically effective amount of an activator of RP 105. The condition is typically associated with TLR-4 activation and cytokine production. Conditions addressed by the invention include sepsis, septic shock, inflammation, rheumatoid arthritis and Crohn's disease. The invention also provides the use of an activator of RP 105 in the manufacture of a medicament for use in the treatment of a condition associated with cytokine production and methods for identifying an activator of RP 105, which is also suitable for use in the treatment of a condition associated with stimulus-induced cytokine production. More specifically the patent relates to the use of RP105 as a specific inhibitor of TLR4 signaling and as a physiological regulator of TLR4 signaling for the treatment of TLR4-mediated inflammation and immune-related diseases. This invention also relates to treating an animal having a disease or condition associated with Toll-like receptor (4).

METHODS AND COMPOSITIONS FOR THE MODULATION OF IMMUNE RESPONSES AND AUTOIMMUNE DISEASES

FIELD OF THE INVENTION

[0001] The present invention relates to regulation of inflammation and immune responses. The present invention relates to a method of treating a condition comprising administering a pharmaceutically effective amount of a compound that upregulates RP105-mediated inhibition of TLR inhibition, through either upregulating RP105 expression or activity. More specifically the patent relates to the use of RP105 as a specific inhibitor of TLR4 signaling and as a physiological regulator of TLR4 signaling for the treatment of TLR4-mediated inflammation and TLR4-driven immune-processes.

BACKGROUND OF THE INVENTION

[0002] The field of innate immunity has undergone a recent renaissance, fueled largely by the molecular identification of critical receptors and signaling pathways involved in pathogen recognition. Study of the Toll-like receptor (TLR) family has led the way. Activation of TLR signaling by conserved microbial molecular signatures promotes the induction of both innate and adaptive immune responses (1, 2). It has long been clear that such immune responses need to be kept under tight control. Responses that are delayed or of insufficient vigor can lead to a failure to control

infection. On the other hand, excessive or inappropriate inflammation can be harmful or even fatal. The hyper-inflammatory responses that characterize sepsis provide a paradigmatic example, as do the more localized inappropriate inflammatory processes leading to inflammatory bowel disease and arthritis (3-7).

[0003] The eleven known members of the mammalian TLR family are characterized structurally by an extracellular leucine-rich repeat (LRR) domain, a conserved pattern of juxtamembrane cysteine residues, and an intracytoplasmic signaling domain (Toll/IL-1 resistance [TIR]) that is highly conserved across the TLRs as well as the receptors for IL-1 and IL-18 (2, 8). The TLR-like molecule RP105 was originally cloned as a B cell-specific molecule able to drive B cell proliferation (9, 10). Like TLRs, RP105 has a conserved extracellular LRR domain and a TLR-like pattern of juxtamembrane cysteines (9-13). Unlike the TLRs, however, RP105 lacks a TIR domain, containing a mere 6 to 11 intracytoplasmic amino acids. In parallel with TLR4, whose surface expression and signaling depends upon co-expression of the molecule MD-2, surface expression of RP105 is dependent upon the co-expression of the MD-2 homologue, MD-1 (14-17).

[0004] Phylogenetic analysis demonstrates that RP105 is actually a specific homologue of TLR4. Further, RP105 is not B cell-specific as originally proposed: we have found that its expression directly mirrors that of TLR4 on antigen-presenting cells. In Toll and TLR4, mutation of the conserved juxtamembrane cysteine residues, or deletion of the extracellular portion altogether, results in a constitutively active molecule (18-20). This suggests that the activation of Toll/TLRs is normally restrained through extracellular protein/protein interactions, likely through the LRR

domain (21). On the other hand, deletions or mutations in the TIR domain of Toll/TLRs can yield inactive or dominant negative molecules (18, 22, 23). Thus, RP105 has the structure of an inhibitory TLR4.

[0005] RP105 specifically inhibits TLR4 signaling when co-expressed in cells and RP105 is a physiological regulator of TLR4 signaling and of TLR4-driven proinflammatory responses *in vivo*. Although the activation of proinflammatory responses through TLRs is critical for both innate and adaptive immunity, excessive or inappropriate inflammation can itself be maladaptive. RP105 inhibits TLR signaling and this regulation of TLR expression provides a point of control (48-50) since RP105 acts specifically to inhibit TLR4 signaling.

[0006] The present invention now provides methods of using RP105 as a specific inhibitor of TLR4 signaling to treat TLR4-mediated pathogenic processes and diseases.

BRIEF SUMMARY OF THE INVENTION

[0007] The present invention relates to the modulation of inflammation and immune responses. Methods and compositions for suppressing a pathological inflammatory or immune response are disclosed. The methods involve administering an effective amount of an agent that upregulates RP105-mediated inhibition of TLR inhibition, through either upregulating RP105 expression or activity. The methods are useful in the treatment of diseases marked by excessive or inappropriate inflammatory processes, including autoimmune diseases such as rheumatoid arthritis, juvenile rheumatoid arthritis, spondyloarthropathy, multiple sclerosis and inflammatory bowel disease; pathological systemic responses to a variety of injuries, such as systemic inflammatory response syndrome and adult respiratory distress syndrome; and localized and systemic infectious processes such as sepsis and meningitis..

[0008] Specifically, the present invention provides for methods and compositions for upregulating the expression of RP105, or for the engagement of or signaling through RP105, in order to inhibit Toll-like receptor signaling. More specifically, the patent relates to the use of RP105 as a specific inhibitor of TLR4 signaling and as a physiological regulator of TLR4 signaling for the treatment of TLR4-driven inflammatory processes and TRLR4-driven immune-immune pathology. This invention also relates to treating an animal having a disease or condition associated with Toll-like receptor 4 by agonists of RP105.

[0009] In another aspect, the invention involves a method for treating a subject having a disease or condition associated with Toll-like receptor 4 comprising administering to

the subject a therapeutically or prophylactically effective amount of one or more compounds capable of activating RP105 so that the action and/or expression of Toll-like receptor 4 is inhibited.

[0010] Consequently, broadly stated, the present invention provides a method of modulating an immune response comprising modulating the expression or activity of RP105. In one aspect, the present invention provides a method of suppressing an immune response comprising administering an effective amount of an activator of RP105 to an animal in need of such treatment.

[0011] In one embodiment, excessive or inappropriate inflammatory or proinflammatory responses through TLRs are treated by modulation of RP105.

[0012] In a further embodiment, the present invention provides a method of preventing or treating an autoimmune disease comprising administering an effective amount of an activator of RP105 to an animal having, suspected of having, or susceptible to having an autoimmune disease.

[0013] The invention also includes pharmaceutical compositions containing one or more activators of RP105 for use in inducing tolerance in autoimmune, inflammatory or infectious disease

[0014] In preferred embodiments of the above methods and compositions, the RP105 activator is an antibody that binds RP105, a small molecule that signals through RP105, or a compound that upregulates RP105 expression. Also in a preferred embodiment of the invention, the activator of RP105 is administered in combination a nucleic acid sequence encoding an RP105 protein.

[0015] The invention also includes pharmaceutical compositions containing an RP105 protein or a nucleic acid sequence encoding an RP105 protein for use in suppressing an immune response. Such compositions can include other molecules that can suppress the immune response such as activators of RP105.

[0016] The invention further includes screening assays for identifying substances that modulate RP105 expression or activity. Such substances may be useful in the therapeutic methods and compositions of the invention. The invention also includes diagnostic kits and methods for detecting conditions associated with increased, decreased or abnormal expression of RP105.

[0017] Further provided are methods for treating a condition involving Toll-like receptor-induced diseases or pathology comprising administering to a patient an effective amount of an agent that activates RP105. Preferably, the Toll-like receptor is Toll-like receptor 4. In another embodiment, the method further comprises administering a therapeutic steroid. In another embodiment, the agent that down-regulates the Toll-like receptor decreases the endogenous amount of intracellular or extracellular cytokine.

[0018] The present invention relates to methods for inhibiting Toll-like receptor-4 ("TLR-4") activity and treatment of Toll-like receptor 4 induced inflammatory bowel disease and related gastrointestinal pathologies. In a further aspect, the present invention concerns the treatment of Toll-like receptor 4 induced inflammatory bowel disease, including ulcerative colitis, Crohn's disease, indeterminate colitis, infectious colitis, drug or chemical-induced colitis, diverticulitis, and ischemic colitis. In

another embodiment, the present methods provide for the treatment of Toll-like receptor 4 -dependent colitis.

[0019] It is one object of the present invention to provide methods for inhibiting the biological activity of TLR-4, as, for example, by inhibiting its expression or signaling. It is a further object of the invention to provide methods of treating those diseases in which inhibiting TLR-4 would have a beneficial effect.

[0020] In one embodiment, the Toll-like receptor 4 induced disease is one or more of systemic lupus erythematosis, sceleroderma, Sjogren's syndrome, multiple sclerosis and other demyelinating diseases, rheumatoid arthritis, juvenile rheumatoid arthritis, myocarditis, uveitis, Reiter's syndrome, gout, osteoarthritis, polymyositis, primary biliary cirrhosis, inflammatory bowel disease, Crohn's disease, ulcerative colitis, aplastic anemia, Addison's disease, insulin-dependent diabetes mellitus, and other diseases.

[0021] In an alternate embodiment, methods of the present invention are used to inhibit the Toll-like receptor 4 induced disease including atherosclerosis, transplant atherosclerosis, vein-graft atherosclerosis, stent restenosis, and angioplasty restenosis, and to thereby treat the cardiovascular diseases that atherosclerosis causes (hereinafter "vascular diseases"). These methods may be used in any patient who could benefit from reducing atherosclerosis that is already present, from inhibiting atherosclerosis that has yet to form, or from both reducing existing atherosclerosis and inhibiting new atherosclerosis. Such patients include those suffering from, for example, angina pectoris and its subtypes (*e.g.*, unstable angina and variant angina); ischemias affecting organs such as the brain, heart, bone, and intestines, and conditions

associated with the ischemias, such as stroke, transient ischemic attacks, heart attack, osteonecrosis, colitis, poor kidney function, and congestive heart failure; poor blood circulation to the extremities and the complications of poor blood circulation, such as slow wound healing, infections, and claudication; atherosclerosis itself, including restenosis following angioplasty or stenting of atherosclerotic lesions; vein-graft atherosclerosis following bypass surgery; transplant atherosclerosis; and other diseases caused by or associated with atherosclerosis.

[0022] In another embodiment, such diseases include, for example, vascular disease such as atherosclerosis and thrombosis, restenosis after angioplasty and/or stenting, and vein-graft disease after bypass surgery.

[0023] In another aspect, the invention involves a method for treating a condition involving a cytokine-induced diseases or pathology by administering to a patient an effective amount of an agent that down-regulates a Toll-like receptor (TLR). In one embodiment, the agent that down-regulates the Toll-like receptor does so by decreasing the endogenous amount of intracellular or extracellular cytokines.

[0024] In one embodiment, the cytokine-mediated disease is selected from acquired immune deficiency syndrome, acute and chronic pain, acute purulent meningitis, adult respiratory distress syndrome (ARDS), Alzheimer's disease, aphthous ulcers, arthritis, asthma, atherosclerosis, atherosclerosisatopic dermatitis, bone resorption diseases, cachexia, chronic obstructive pulmonary disease, congestive heart failure, contact dermatitis, Crohn's disease, dermatoses with acute inflammatory components, diabetes, endotoxemia, glomerulonephritis, graft versus host disease, granulocyte transfusion, Guillain-Barre syndrome, inflammatory bowel disease, leprosy,

leukopherisis, malaria, multiple organ injury secondary to trauma, multiple sclerosis, myocardial infarction, necrotizing enterocolitis and syndromes associated with hemodialysis, osteoarthritis, osteoporosis, psoriasis, reperfusion injury, restenosis following percutaneous transluminal coronary angioplasty, rheumatoid arthritis, sarcoidosis, scleroderma, sepsis, septic shock, stroke, systemic lupus erythrematosis, thermal injury, toxic shock syndrome, traumatic arthritis, and ulcerative colitis.

[0025] In another embodiment, the present invention provides for methods of treating a disease mediated by cytokines which comprises administering to a patient in need of such treatment a therapeutically effective amount of one or more activators of RP105. In another embodiment, the present invention provides for methods of treating a gastrointestinal disorder in a patient in need thereof comprising administering to the patient a therapeutically effective amount of one or more activators of RP105. In an additional embodiment, the gastrointestinal disorder is an inflammatory bowel disease, Crohn's disease, gastritis, irritable bowel syndrome, ulcerative colitis, a peptic ulcer, a stress ulcer, a bleeding ulcer, gastric hyperacidity, dyspepsia, gastroparesis, Zollinger-Ellison syndrome, gastroesophageal reflux disease, a bacterial infection, short-bowel (anastomosis) syndrome, a hypersecretory state associated with systemic mastocytosis or basophilic leukemia or hyperhistaminemia.

[0026] In another aspect, the cytokine involved in the TLR-4-mediated disease or pathology is a proinflammatory cytokine. In still another aspect, the proinflammatory cytokine is selected from the group consisting of TNF-α, IL-1, IL-1β, IL-6, and IL-8.

[0027] Consequently, broadly stated, the present invention provides a method of modulating an immune response comprising modulating the expression or activity of RP105.

[0028] In one aspect, the present invention provides a method of suppressing an immune response comprising administering an effective amount of a modulator of RP105 activation or expression to an animal in need of such treatment.

[0029] In one embodiment, the present invention provides a method of inducing immune suppression or tolerance to a transplanted organ or tissue in a recipient animal comprising administering an effective amount of an activator of RP105 activation or expression to the recipient animal prior to the transplantation of the organ or tissue.

[0030] In another embodiment, the present invention provides a method of preventing or inhibiting graft versus host disease in a recipient animal receiving an organ or tissue transplant comprising administering an effective amount of an activator of RP105 activation or expression to the organ or tissue prior to the transplantation in the recipient animal.

[0031] In yet another embodiment, the present invention provides a method of preventing or inhibiting fetal loss comprising administering an effective amount of an activator of RP105 activation or expression to an animal in need thereof.

[0032] In a further embodiment, the present invention provides a method of preventing or treating an autoimmune disease comprising administering an effective

amount of an activator of RP105 activation or expression to an animal having, suspected of having, or susceptible to having an autoimmune disease.

[0033] In yet a further embodiment, the present invention provides a method of preventing or treating an allergy comprising administering an effective amount of an activator of RP105 activation or expression to an animal having or suspected of having an allergy.

[0034] The invention also includes pharmaceutical compositions containing one or more activators of RP105 activation or expression for use in inducing tolerance in transplantation, allergy or autoimmune disease or for preventing or treating fetal loss.

[0035] In preferred embodiments of the above methods and compositions, the RP105 activator is an antibody that binds RP105 or an antisense oligonucleotide that activates the expression of RP105. Also in a preferred embodiment of the invention, the inhibitor of RP105 is administered in combination with an MD-1 protein or a nucleic acid sequence encoding an MD-1 protein.

[0036] As stated above, activating RP105 can be used to induce immune suppression. Consequently, inhibiting RP105 can be used in preventing immune suppression or inducing an immune response.

[0037] Therefore, in another aspect, the present invention provides a method of immune suppression comprising administering an effective amount of an RP105 protein or a nucleic acid sequence encoding an RP105 protein to an animal in need thereof.

[0038] The invention also includes pharmaceutical compositions containing an RP105 protein or a nucleic acid sequence encoding an RP105 protein for use in suppressing an immune response. Such compositions can include other molecules that can activate the immune response such as activators of MD-1 activity or expression.

[0039] The invention further includes screening assays for identifying substances that modulate RP105 expression or activity. Such substances may be useful in the therapeutic methods and compositions of the invention. The invention also includes diagnostic kits and methods for detecting conditions associated with increased, decreased or abnormal expression of RP105.

[0040] In one embodiment, the present invention provides for a method for screening a test compound for the potential to prevent, stabilize, or treat an autoimmune or inflammatory disease comprising the steps of: a) contacting a first cell sample from a first subject that has, or is at risk for developing, an autoimmune or inflammatory disease with the test compound; b) contacting a second cell sample from a second subject that does not have, or is not predisposed to developing, an autoimmune or inflammatory disease with the test compound, wherein the first and second subjects are of the same species and the first and second cell samples are contacted with the test compound in the same manner; and c) measuring RP105 inhibition of TLR4 in the first and second samples, wherein the compound is determined to have the potential if TLR4 inhibition in the first sample is decreased relative to the second sample. In one embodiment, RP105 inhibition of TLR4 is measured by the ability of the lipopolysaccharide (LPS) signaling complex to bind LPS. In another embodiment, RP105 activation or expression is measured.

[0041] In another embodiment, the present invention provides for a method for screening a test compound for the potential to prevent, stabilize, or treat an autoimmune or inflammatory disease comprising the steps of: a) contacting a population of cells from a subject that has, or is at risk for developing, an autoimmune or inflammatory disease with the test compound; b) contacting a second cell element from the subject with the test compound, wherein the cells and the second cell element are contacted with the test compound in the same manner; and c) measuring RP105 activation or expression of the cells, wherein the test compound is determined to have the potential if the RP105 activation or expression of the cell increases relative to the second cell element. In another embodiment, the cells are autoimmune cells. In another embodiment, the cells are leukocytes.

[0042] In another embodiment, the present invention provides for a method for diagnosing an autoimmune or inflammatory disease, or a predisposition to the disease, in a subject comprising the steps of: a) obtaining a first cell sample from a first subject; b) obtaining a second cell sample from a second subject of the same species as the first subject, wherein the second subject does not have, or is not at risk for developing, the autoimmune disease; c) contacting the first cell sample and the second cell sample with a compound that preferentially increases the activation or expression of RP105, wherein both of the first and second samples are contacted with the compound in the same manner; and d) measuring the activation or expression of RP105 in the first cell sample and in the second cell sample, wherein a decrease in activation or expression of RP105 in the first sample relative to the activation or

expression of RP105 in the second sample indicates that the first subject has, or is predisposed to developing, the autoimmune disease.

[0043] In another embodiment, the present invention provides for a method for diagnosing an autoimmune disease, or a predisposition to the disease, in a subject comprising the steps of: a) contacting autoimmune cells from a subject that has an autoimmune disease, or is at risk for developing an autoimmune disease, with the test compound; b) contacting a second cell element from the subject with the test compound, wherein the autoimmune cells and the second cell element are contacted with the compound in the same manner; and c) measuring the activation of RP105 of the autoimmune cells, wherein the subject has, or is at risk for developing, the autoimmune disease if the activation or expression of RP105 of the autoimmune cells decreases relative to the second cell element.

[0044] In another embodiment, the present invention provides for a method for the stratification of a human patient into a therapeutic subgroup for an autoimmune disease comprising the steps of: a) contacting a cell sample from the patient with a compound that preferentially decreases the activation of RP105 of leukocytes; b) measuring the activation of RP105 of the leukocytes; and c) placing the patient into a therapeutic subgroup based on the amount of decrease of the activation or expression of RP105.

[0045] In another embodiment, the present invention provides for a method for monitoring a therapy for a human that has an autoimmune disease, or is at risk for developing the disease, comprising the steps of: i) obtaining a first cell sample from the patient and contacting the first cell sample with a compound that preferentially

decreases the activation of RP105 of leukocytes; ii) measuring the activation of RP105 of leukocytes in the first cell sample; iii) obtaining a second cell sample from the patient and contacting the second cell sample with the compound, wherein the second cell sample is obtained at least 12 hours after obtaining the first cell sample; iv) measuring the activation of RP105 of leukocytes in the second cell sample; and v) determining the efficacy of the therapy based on leukocyte RP105 activation or expression, wherein an increase in the activation or expression indicates that the therapy is efficacious.

[0046] In another embodiment, the present invention provides for a pharmaceutical composition for use in suppressing an immune response comprising an activator of RP105/MD-1 complex formation in admixture with a suitable diluent or carrier. In one embodiment, the activator is an antibody that binds MD-1, RP105, or both.

[0047] In another embodiment, the present invention provides for a screening method for identifying an immunostimulatory compound, comprising: contacting a functional RP105 with a test compound under conditions which, in absence of the test compound, permit a negative control response mediated by a RP105 signal transduction pathway; detecting a test response mediated by the RP105 signal transduction pathway; and determining the test compound is an immunostimulatory compound when the test response exceeds the negative control response.

[0048] In another embodiment, the present invention provides for a screening method for identifying an immunostimulatory compound, comprising: contacting a functional RP105 with a test compound under conditions which, in presence of a reference immunostimulatory compound, permit a reference response mediated by a RP105

signal transduction pathway; detecting a test response mediated by the RP105 signal transduction pathway; and determining the test compound is an immunostimulatory compound when the test response equals or exceeds the reference response.

[0049] In another embodiment, the present invention provides for a screening method for identifying a compound that modulates RP105 signaling activity, comprising: contacting a functional RP105 with a test compound and a reference immunostimulatory compound under conditions which, in presence of the reference immunostimulatory compound alone, permit a reference response mediated by a RP105 signal transduction pathway; detecting a test-reference response mediated by the RP105 signal transduction pathway; determining the test compound is an agonist of RP105 signaling activity when the test-reference response exceeds the reference response; and determining the test compound is an antagonist of RP105 signaling activity when the reference response exceeds the test-reference response.

[0050] In another embodiment, the present invention provides for a screening method for identifying species specificity of an immunostimulatory compound, comprising: measuring a first species-specific response mediated by a RP105 signal transduction pathway when a functional RP105 of a first species is contacted with a test compound; measuring a second species-specific response mediated by the RP105 signal transduction pathway when a functional RP105 of a second species is contacted with the test compound; and comparing the first species-specific response with the second species-specific response.

[0051] In one embodiment, the screening method is performed on a plurality of test compounds. In another embodiment, the response mediated by the RP105 signal

transduction pathway is measured quantitatively. In another embodiment, the functional RP105 is expressed in a cell. In another embodiment, the cell is an isolated mammalian cell that naturally expresses the functional RP105.

[0052] In another embodiment, the cell is an isolated mammalian cell that does not naturally express the functional RP105, and wherein the cell comprises an expression vector for RP105. In another embodiment, the cell is a human fibroblast. In another embodiment, the cell comprises an expression vector comprising an isolated nucleic acid which encodes a reporter construct selected from the group of interleukins, NF-kappaB, interferons, and TNFs. In another embodiment, the functional RP105 is part of a cell-free system. In another embodiment, the reference immunostimulatory compound is a nucleic acid. In another embodiment, the nucleic acid is a CpG nucleic acid. In another embodiment, the reference immunostimulatory compound is a small molecule. In another embodiment, the test compound is a part of a combinatorial library of compounds. In another embodiment, the test compound is a nucleic acid. In another embodiment, the the nucleic acid is a CpG nucleic acid. In another embodiment, the test compound is a small molecule. In another embodiment, the test compound is a polypeptide. In another embodiment, the response mediated by a RP105 signal transduction pathway is induction of a reporter gene under control of a promoter response element selected from the group consisting of ISRE, IL-6, IL-8, IL-12 p40, IFN-alpha, IFN-beta, IFN-omega, TNF, IP-10, and I-TAC. In another embodiment, the response mediated by a RP105 signal transduction pathway is selected from the group consisting of (a) induction of a reporter gene under control of a minimal promoter responsive to a transcription factor selected from the group

consisting of AP1, NF-kappaB, ATF2, IRF3, and IRF7; (b) secretion of a chemokine; and (c) secretion of a cytokine. In another embodiment, the response mediated by a RP105 signal transduction pathway is secretion of a type 1 IFN. In another embodiment, the response mediated by a RP105 signal transduction pathway is secretion of a chemokine. In another embodiment, the contacting a functional RP105 with a test compound further comprises, for each test compound, contacting with the test compound at each of a plurality of concentrations. In another embodiment, the e detecting is performed 6-12 hours following the contacting. In another embodiment, the detecting is performed 16-24 hours following the contacting.

[0053] The present compounds may also be used in co-therapies, partially or completely, in place of other conventional anti-inflammatories, such as together with steroids, cyclooxygenase-2 inhibitors, NSAIDs, DMARDS, antibiotics, immunosuppressive agents, 5-lipoxygenase inhibitors, LTB_4 antagonists and LTA_4 hydrolase inhibitors and anti-cell adhesion molecules such as anti E-selectin.

[0054] In one embodiment, this aspect additionally involves administering a therapeutic steroid to the patient. By way of non-limiting example, therapeutic steroids may include, for example, corticoids, glucocorticoids, dexamethasone, prednisone, prednisalone, and betamethasone.

[0055] These methods may employ the compounds of this invention in a monotherapy or in combination with an anti-inflammatory or immunosuppressive agent. Such combination therapies include administration of the agents in a single dosage form or in multiple dosage forms administered at the same time or at different times.

[0056] The above summary of the present invention is not intended to describe each embodiment or every implementation of the present invention. Advantages and attainments, together with a more complete understanding of the invention, will become apparent and appreciated by referring to the following detailed description and claims taken in conjunction with the accompanying drawings.

[0057] Throughout this document, all temperatures are given in degrees Celsius, and all percentages are weight percentages unless otherwise stated. All publications mentioned herein are incorporated herein by reference for the purpose of describing and disclosing the compositions and methodologies, which are described in the publications which might be used in connection with the presently described invention. The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such a disclosure by virtue of prior invention.

BRIEF DESCRIPTION OF THE FIGURES

[0058] Figure 1. RP105 phylogeny. Phylogenetic relationships among the human TLRs as inferred by sequence alignment using ClustalW software. Similar results were obtained with PileUp software. Horizontal branch lengths are proportional to the degree of inferred evolutionary change.

[0059] Figure 2. RP105 expression by human peripheral blood leukocytes. Flow cytometric analysis of PBMC from healthy human donors. (*a*) B cells; (*b*) monocytes; (*c*) myeloid DC; (*d*) plasmacytoid DC. Myeloid DC were identified as lineage negative ($CD3^-/CD14^-/CD19^-/CD20^-/CD56^-$), $HLA\text{-}DR^+$, $CD11c^+$. Plasmacytoid DC were identified as lineage negative, $HLA\text{-}DR^+$, $CD11c^-$, $BDCA\text{-}4^-$. Data are representative of an experimental $n > 15$ for monocytes; $n = 3$ for the other cell types.

[0060] Figure 3. RP105 expression by murine leukocytes. Flow cytometric analysis of cell populations. (*a*) splenic B cells (representative of an experimental $n >$ 50). (*b*) resident peritoneal macrophages ($n = 5$). (*c*) splenic $CD11c^+CD11b^+$ $CD4^-$ and $CD11c^+CD11b^+CD4^+$ DC ($n = 3$). (*d*) splenic $CD11c^+CD11b^-$ $CD8\alpha^+$DC ($n = 3$). (*e*) splenic plasmacytoid DC ($CD19^+$B220+$CD11c^+GR\text{-}1^+$) [$n = 2$]. (*f*) bone marrow-derived DC. ($n = 6$). Analysis of splenic DC subsets was performed after 10 d of *in vivo* treatment with flt3L.

[0061] Figure 4. Suppression of TLR4 signaling in HEK293 cells by RP105 expression. (*a*) Lack of activity of RP105 as a signaling receptor for LPS in HEK293 cells. HEK293 cells stably expressing CD14 were transiently

transfected with cDNA encoding MD-1, MD-2, TLR4, empty vector control cDNA (EV) and/or RP105. Cells were subsequently stimulated with re-purified *E. coli* K235 LPS (10 ng/ml). (*b*) Dose-dependent suppression of LPS-driven IL-8 production by RP105. HEK293 cells stably expressing CD14 and TLR4 were transiently transfected with MD-1 and MD-2 along with the indicated concentrations of RP105 and/or EV cDNA, and secondarily stimulated with LPS (10 ng/ml). (*c*) RP105-mediated suppression of LPS-induced NF-κB activation. HEK293 cells stably expressing MD-2 and TLR4 were transiently co-transfected with an NF-κB-firefly luciferase reporter plasmid, a TK-renilla luciferase reporter plasmid and MD-1, along with EV (open bars) or RP105 (filled bars). Cells were stimulated with the indicated concentrations of LPS. * $p < 0.03$, ‡ $p < 0.004$, compared with RP105-deficient cells. Means +/- SE of triplicate cultures in a single experiment are depicted, representative of an experimental $n = 4$ (*a* and *b*); $n = 2$ (*c*). NS: no stimulation.

[0062] Figure 5. Specificity of RP105-mediated suppression. (*a*) Lack of suppression of IL-1R signaling. HEK293 cells stably expressing CD14 and TLR4 (open bars) or CD14, TLR4 and RP105 (filled bars) were transiently transfected with MD-1 and MD-2, and subsequently stimulated with re-purified *E. coli* K235 LPS (10 ng/ml) or IL-1β (100 ng/ml). (*b*) Lack of suppression of TLR2 signaling. HEK293 cells stably expressing CD14 and TLR2 were transiently transfected with MD-1 and EV (open bars) or MD-1 and RP105 (filled bars) and subsequently stimulated with Zymosan A (10 μg/ml) or IL-1β (100

ng/ml).). * $p < 0.0001$, ‡ $p = 0.05$, compared with RP105-deficient cells. Means +/- SE of triplicate cultures in a single experiment are depicted, representative of an experimental $n = 2$-4.

[0063] Figure 6. Necessity of MD-1 for RP105-mediated suppression of TLR4 signaling. (*a*) MD-1 dependence of RP105 mediated suppression. HEK293 cells stably expressing CD14 and TLR4 were transiently transfected with MD-2; along with EV, MD-1 and/or RP105 as indicated. Cells were subsequently stimulated with re-purified *E. coli* K235 LPS (10 ng/ml). (*b*) Association of MD-1 expression with surface expression of RP105. HEK293 cells were further analyzed for surface and intracellular RP105 expression by FACS. *$p <$ 0.008, compared with RP105-deficient cells. Means +/- SE of triplicate cultures in a single experiment are depicted, representative of an experimental $n = 4$ (*a*); $n = 2$ (*b*).

[0064] Figure 7. Altered cytokine production by dendritic cells from RP105$^{-/-}$ mice following *in vitro* stimulation with TLR4-specific LPS. Bone marrow-derived DC from wild type [▒] or RP105$^{-/-}$ [•] mice were stimulated with repurified *E. coli* K235 LPS. (*a*) TNF-α. (*b*) IL-12p70. (*c*) IL-6. * $p < 0.05$, ‡ $p < 0.001$, § $p < 0.01$. Means + SE of triplicate cultures in a single experiment, representative of an $n = 8$ (*a*); $n = 4$ (*b* and *c*).

[0065] Figure 8. Ability of heterologous TLR4 signaling to overcome RP105-mediated inhibition of TLR4 signaling. Bone marrow-derived DC from wild type [▒] or RP105$^{-/-}$ [•] mice were stimulated with: (*a*) non-repurified *E. coli* K235 LPS; or (*b*) repurified *E. coli* K235 LPS plus varying concentrations of

Pam_3Cys. * $p < 0.05$, ‡ $p < 0.001$. Means + SE of triplicate cultures in a single experiment; representative of an experimental $n = 4$.

[0066] Figure 9. Elevated production of TNF-α by $RP105^{-/-}$ mice following LPS challenge *in vivo*. Wild type mice ($n = 17$) (open bars) or $RP105^{-/-}$ mice ($n = 18$) (filled bars) were challenged intraperitoneally with 25 μg of re-purified *E. coli* K235 LPS. Serum was harvested 60 min later. * $p < 0.01$.

[0067] Figure 10. Genotyping and phenotyping of $RP105^{-/-}$ mice. FACS analysis of peripheral blood cells.

[0068] Figure 11. RP105 phylogeny. Phylogenetic relationships among the mouse TLRs as inferred by sequence alignment using ClustalW software.

DETAILED DESCRIPTION OF THE INVENTION

[0069] Before the present device and methods for tissue augmentation is described, it is to be understood that this invention is not limited to the specific methodology, devices, formulations, and compositions described as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention, which will be limited only by the appended claims.

[0070] It must be noted that as used herein and in the appended claims, the singular forms "a", "and", and "the" include plural referents unless the context clearly dictates otherwise. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention belongs. Although any methods, devices and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, the preferred methods, devices and materials are now described.

[0071] As used herein, the following terms shall have the definitions given below.

[0072] The term "activation" as used herein to describe the mechanism of action of an activator of RP105, means a mechanism wherein a compound upregulates RP105-mediated inhibition of TLR activity, through upregulating RP105 expression, increasing engagement of or signaling activity with TLR and/or other means that provide for a decrease in the activity of TLR.

[0073] The term "administration" of the pharmaceutically active compounds and the pharmaceutical compositions defined herein includes systemic use, as by injection (especially parenterally), intravenous infusion, suppositories and oral administration thereof, as well as topical application of the compounds and compositions. Oral administration is particularly preferred in the present invention.

[0074] "Ameliorate" or "amelioration" means a lessening of the detrimental effect or severity of the cell adhesion disorder in the subject receiving therapy, the severity of the response being determined by means that are well known in the art.

[0075] "Chemokines" are chemotactic cytokines that are released by a wide variety of cells to attract macrophages, T-cells, eosinophils, basophils, neutrophils and endothelial cells to sites of inflammation and tumor growth. There are two main classes of chemokines, the CXC-chemokines and the CC-chemokines. The class depends on whether the first two cysteines are separated by a single amino acid (CXC-chemokines) or are adjacent (CC-chemokines). The CXC-chemokines include interleukin-8 (IL-8), neutrophil-activating protein-1 (NAP-1), neutrophil-activating protein-2 (NAP-2), GROα, GROβ, GROγ, ENA-78, GCP-2, IP-10, MIG and PF4. CC chemokines include RANTES, MIP-1α, MIP-2β, monocyte chemotactic protein-1 (MCP-1), MCP-2, MCP-3 and eotaxin.

[0076] By "compatible" herein is meant that the components of the compositions which comprise the present invention are capable of being commingled without interacting in a manner which would substantially decrease the efficacy of the pharmaceutically active compound under ordinary use conditions.

[0077] By "corticosteroid" is meant any naturally occurring or synthetic steroid hormone that can be derived from cholesterol and is characterized by a hydrogenated cyclopentanoperhydrophenanthrene ring system. Naturally occurring corticosteriods are generally produced by the adrenal cortex. Synthetic corticosteriods may be halogenated. Functional groups required for activity include a double bond at Δ4, a C3 ketone, and a C20 ketone. Corticosteroids may have glucocorticoid and/or mineralocorticoid activity.

[0078] Exemplary corticosteroids include, for example, dexamethasone, betamethasone, triamcinolone, triamcinolone acetonide, triamcinolone diacetate, triamcinolone hexacetonide, beclomethasone, dipropionate, beclomethasone dipropionate monohydrate, flumethasone pivalate, diflorasone diacetate, fluocinolone acetonide, fluorometholone, fluorometholone acetate, clobetasol propionate, desoximethasone, fluoxymesterone, fluprednisolone, hydrocortisone, hydrocortisone acetate, hydrocortisone butyrate, hydrocortisone sodium phosphate, hydrocortisone sodium succinate, hydrocortisone cypionate, hydrocortisone probutate, hydrocortisone valerate, cortisone acetate, paramethasone acetate, methylprednisolone, methylprednisolone acetate, methylprednisolone sodium succinate, prednisolone, prednisolone acetate, prednisolone sodium phosphate, prednisolone tebutate, clocortolone pivalate, flucinolone, dexamethasone 21-acetate, betamethasone 17-valerate, isoflupredone, 9-fluorocortisone, 6-hydroxydexamethasone, dichlorisone, meclorisone, flupredidene, doxibetasol, halopredone, halometasone, clobetasone, diflucortolone, isoflupredone acetate, fluorohydroxyandrostenedione, beclomethasone, flumethasone, diflorasone, fluocinolone, clobetasol, cortisone, paramethasone,

clocortolone, prednisolone 21-hemisuccinate free acid, prednisolone metasulphobenzoate, prednisolone terbutate, and triamcinolone acetonide 21-palmitate. By "low dose corticosteroid" is meant a dose that is less than a dose that would typically be given to a patient for treatment of inflammation. Exemplary low doses of corticosteroids are as follows: cortisol: 12 mg/day; cortisone: 15 mg/day; prednisone: 3 mg/day; methylprednisolone: 2.5 mg/day; triameinolone: 2.5 mg/day; betamethasone: 250 μg/day; dexamethasone: 450 μg/day; hydrocortisone: 9 mg/day.

[0079] The term "patient" or "subject," as used herein, is intended to encompass any mammal, animal or human subject, which may benefit from treatment with the compounds, compositions and methods of the present invention.

[0080] "Pharmaceutically-acceptable" shall mean that the pharmaceutically active compound and other ingredients used in the pharmaceutical compositions and methods defined herein are suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response, and the like, commensurate with a reasonable benefit/risk ratio.

[0081] The term "prodrug" indicates a therapeutic agent that is prepared in an inactive form that is converted to an active form (*i.e.*, drug) within the body or cells thereof by the action of endogenous enzymes or other chemicals and/or conditions.

[0082] The phrase "safe and effective amount" means a sufficient amount of pharmaceutically active compound to effect the activation of RP105 and inhibition of TLR-4. Within the scope of sound medical judgment, the required dosage of a pharmaceutically active agent or of the pharmaceutical composition containing that

active agent will vary with the severity of the condition being treated, the duration of the treatment, the nature of adjunct treatment, the age and physical condition of the patient, the specific active compound employed, and like considerations discussed more fully hereinafter. In arriving at the "safe and effective amount" for a particular compound, these risks must be taken into consideration, as well as the fact that the compounds described herein provide pharmaceutical activity at lower dosage levels than conventional compounds.

[0083] "Toll-like receptors" or "TLRs" are type I transmembrane proteins containing repeated leucine-rich motifs in their extracellular domains. At least eight mammalian TLR proteins have been identified, Toll-like receptors 1-8. Ligand engagement of the TLRs results in activation of NF-κB and induction of the cytokines and co-stimulatory molecules required for the activation of the adaptive immune response. Human Toll-like receptor 4 (also known as TLR4 and hToll), the human homolog to the Drosophilae protein known as Toll, was cloned from a human fetal liver/spleen library, characterized, and mapped to chromosome 9q32-33. Toll-like receptor 4 mRNA expression can be detected in the cells of the immune system: monocytes, macrophages, dendritic cells, γΔ T-cells, Th1 and Th2 αβ T-cells, and B-cells. Expression has also been detected in the cardiac myocytes and placenta.

[0084] "Treat," "treating," "treatment," and "therapy" as used herein refer to any curative therapy, prophylactic therapy, ameliorative therapy and preventative therapy of a subject. "Ameliorate" or "amelioration" means a lessening of the detrimental effect or severity of a disease or pathology in the subject receiving therapy, the severity of the response being determined by means that are well known in the art.

[0085] As used herein, unless otherwise indicated, the terms "bacterial infection(s)" include bacterial infections that occur in mammals, fish and birds as well as disorders related to bacterial infections that may be treated or prevented by administering antibiotics such as the compounds of the present invention. Such bacterial infections, and disorders related to such infections, include the following: pneumonia, otitis media, sinusitus, bronchitis, tonsillitis, and mastoiditis related to infection by Streptococcus pneumonia, Haemophilus influenzae, Moraxella catarrhalis, Staphylococcus aureus, or Peptostreptococcus spp.; pharynigitis, rheumatic fever, and glomerulonephritis related to infection by Streptococcus pyogenes, Groups C and G streptococci, Clostridium diptheriae, or Actinobacillus haemolyticum; respiratory tract infections related to infection by Mycoplasma pneumoniae, Legionella pneumophila, Streptococcus pneumoniae, Haemophilus influenzae, or Chlamydia pneumoniae; uncomplicated skin and soft tissue infections, abscesses and osteomyelitis, and puerperal fever related to infection by Staphylococcus aureus, coagulase-positive staphylococci (*i.e.*, S. epidermidis, S. hemolyticus, *etc.*), Streptococcus pyogenes, Streptococcus agalactiae, Streptococcal groups C-F (minute-colony streptococci), viridans streptococci, Corynebacterium minutissimum, Clostridium spp., or Bartonella henselae; uncomplicated acute urinary tract infections related to infection by Staphylococcus saprophyticus or Enterococcus spp.; urethritis and cervicitis; and sexually transmitted diseases related to infection by Chlamydia trachomatis, Haemophilus ducreyi, Treponema pallidum, Ureaplasma urealyticum, or Neiserria gonorrheae; toxin diseases related to infection by S. aureus (food poisoning and Toxic shock syndrome), or Groups A, B, and C streptococci; ulcers related to infection by Helicobacter pylori; systemic febrile syndromes related to infection by Borrelia

recurrentis; Lyme disease related to infection by Borrelia burgdorferi; conjunctivitis, keratitis, and dacrocystitis related to infection by Chlamydia trachomatis, Neisseria gonorrhoeae, S. aureus, S. pneumoniae, S. pyogenes, H. influenzae, or Listeria spp.; disseminated Mycobacterium avium complex (MAC) disease related to infection by Mycobacterium avium, or Mycobacterium intracellulare; gastroenteritis related to infection by Campylobacter jejuni; odontogenic infection related to infection by viridans streptococci; persistent cough related to infection by Bordetella pertussis; gas gangrene related to infection by Clostridium perfringensor Bacteroides spp.; and atherosclerosis related to infection by Helicobacter pylori or Chlamydia pneumoniae. Bacterial infections and protozoa infections and disorders related to such infections that may be treated or prevented in animals include the following: bovine respiratory disease related to infection by P. haem., P. multocida, Mycoplasma bovis, or Bordetella spp.; cow enteric disease related to infection by E. coli (*i.e.*, coccidia, cryptosporidia, *etc.*); dairy cow mastitis related to infection by Staph. aureus, Strep. uberis, Strep. agalactiae, Strep. dysgalactiae, Klebsiella spp., Corynebacterium, or Enterococcus spp.; swine respiratory disease related to infection by A. pleuro., P. multocida, or Mycoplasma spp.; swine enteric disease related to infection by E coli, Lawsonia intracellularis, Salmonella, or Serpulina hyodyisinteriae; cow footrot related to infection by Fusobacterium spp.; cow metritis related to infection by E coli; cow hairy warts related to infection by Fusobacterium necrophorum or Bacteroides nodosus; cow pink-eye related to infection by Moraxella bovis; urinary tract infection in dogs and cats related to infection by E coli; skin and soft tissue infections in dogs and cats related to infection by Staph. epidermidis, Staph. intermedius, coagulase neg. Staph. or P. multocida; and dental or mouth infections in dogs and cats related to

infection by Alcaligenes spp., Bacteroides spp., Clostridium spp., Enterobacter spp., Eubacterium, Peptostreptococcus, Porphyromonas, or Prevotella. Other bacterial infections and disorders related to such infections that may be treated or prevented in accord with the method of the present invention.

[0086] Accordingly the invention provides a method of treating a condition associated with cytokine production in a mammal comprising administering a pharmaceutically effective amount of an activator of a member of RP105. The invention also provides the use of an activator of RP105 in the manufacture of a medicament for use in the treatment of a condition associated with cytokine production

[0087] In one embodiment the cytokine is TNF, preferably TNFα. In another embodiment the cytokine is IL-1, preferably IL-1β. The cytokine-associated condition may be any one of sepsis, septic shock, inflammation, rheumatoid arthritis or Crohn's disease. The cytokine-associated condition may be irritable bowel disease (IBD). The cytokine-associated condition may be ulcerative colitis.

[0088] In a preferred embodiment the cytokine-associated condition is induced by a Toll-related receptor (TRR) ligand. In another preferred embodiment the cytokine-associated condition is induced by lipopolysaccharide (LPS). Gram-negative bacteria may induce the condition. Gram-positive bacteria may induce the condition. In another preferred embodiment the cytokine-associated condition is induced by zymosan. The condition may induced by yeast. The TRR ligand may be a microbial factor.

[0089] The invention also provides a pharmaceutical formulation comprising a pharmaceutically acceptable carrier and an activator of RP105 identifiable by a method of the invention.

[0090] The invention also provides the use of an activator of RP105 to study the inhibition of sepsis, septic shock and/or inflammation caused by a TRR-ligand, preferably LPS, *in vitro*.

[0091] The invention provides a method of treating sepsis or septic shock in a subject administering a pharmaceutically effective amount of an activator of RP105.

[0092] The invention provides a method of treating inflammation in a subject comprising administering a pharmaceutically effective amount of an activator of RP105. The invention provides a RP105 activator for use as a medicament. The invention provides a RP105 activator for use in the manufacture of a medicament to treat sepsis, septic shock and/or inflammation. The invention provides a method or activator of the invention wherein the activator is a vaccine, an antibody or a fragment thereof which is capable of binding RP105 or a fragment thereof.

[0093] The invention provides a method of treating sepsis, septic shock and/or inflammation in a subject comprising administering a pharmaceutically effective amount of an activator of a member of RP105. The invention provides a method of treating rheumatoid arthritis in a subject comprising administering a pharmaceutically effective amount of an activator of a member of RP105. The invention provides a method of treating Crohn's disease in a subject comprising administering a pharmaceutically effective amount of an activator of a member of RP105. The

invention provides a method of treating a condition induced by a TRR ligand in a subject comprising administering a pharmaceutically effective amount of an activator of a member of RP105.

[0094] The invention provides a method of treating a condition induced by a TLR4 ligand in a subject comprising administering a pharmaceutically effective amount of an activator of a member of RP105. The invention provides a method of treating a condition induced by a pathogen in a subject comprising administering a pharmaceutically effective amount of an activator of a member of RP105. The invention provides a method of treating a condition induced by bacteria, or a factor derived there from in a subject comprising administering a pharmaceutically effective amount of an activator of a member of RP105.

[0095] All of the results of the inventors demonstrate that RP105 is an immune modulating molecule that has utility in a wide range of applications. Accordingly, the present invention includes all uses that relate to the realization of the immune modulatory properties of RP105 including, but not limited to, the development of therapeutic and diagnostic assays and compositions as well as the preparation and/or isolation of other molecules that modulate RP105 that may be useful in the therapeutic and diagnostic assays and compositions of the invention.

Inducing Immune Suppression

[0096] In one aspect, the present invention provides a method of suppressing an immune response comprising administering an effective amount of an activator of RP105 to an animal in need of such treatment. The invention includes a use of an

effective amount of an activator of RP105 to suppress an immune response or to prepare a medicament to suppress an immune response.

[0097] The term "an activator of RP105" means any molecule or compound that can activate the expression of the RP105 gene or that can activate the activity of the RP105 protein.

[0098] In another embodiment, the activator of RP105 is an RP105 specific antibody. Antibodies to RP105 may be prepared using techniques known in the art such as those described by Kohler and Milstein, Nature 256, 495 (1975) and in U.S. Pat. Nos. RE 32,011; 4,902,614; 4,543,439; and 4,411,993, which are incorporated herein by reference. (See also Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses, Plenum Press, Kenneft, McKearn, and Bechtol (eds.), 1980, and Antibodies: A Laboratory Manual, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988, which are also incorporated herein by reference). Within the context of the present invention, antibodies are understood to include monoclonal antibodies, polyclonal antibodies, antibody fragments (*e.g.*, Fab, and F(ab').sub.2) and recombinantly produced binding partners.

[0099] The therapeutic methods of the invention can be used to treat any condition wherein it is desirable to modulate RP105 expression or activity. Such conditions include, but are not limited to, , autoimmune disease, , inflammatory conditions, septic shock, organ dysfunction, neurodegenerative diseases (*e.g.*, Alzheimer's disease), stroke and spinal injury.

[00100] As stated previously, the method of the present invention may also be used to treat or prevent autoimmune disease. In an autoimmune disease, the immune system of the host fails to recognize a particular antigen as "self" and an immune reaction is mounted against the host's tissues expressing the antigen. Normally, the immune system is tolerant to its own host's tissues and autoimmunity can be thought of as a breakdown in the immune tolerance system.

[00101] Accordingly, in a further embodiment, the present invention provides a method of preventing or treating an autoimmune disease comprising administering an effective amount of an activator of RP105 to an animal having, suspected of having, or susceptible to having an autoimmune disease. The invention includes a use of an effective amount of an activator of RP105 to prevent or activate an autoimmune disease or to prepare a medicament to prevent or activate an autoimmune disease.

[00102] Autoimmune diseases that may be treated or prevented according to the present invention include, but are not limited to, type 1 insulin-dependent diabetes mellitus, adult respiratory distress syndrome, inflammatory bowel disease, dermatitis, meningitis, thrombotic thrombocytopenic purpura, Sjogren's syndrome, encephalitis, uveitic, leukocyte adhesion deficiency, rheumatoid arthritis, rheumatic fever, Reiter's syndrome, psoriatic arthritis, progressive systemic sclerosis, primary biniary cirrhosis, pemphigus, pemphigoid, necrotizing vasculitis, myasthenia gravis, multiple sclerosis, lupus erythematosus, polymyositis, sarcoidosis, granulomatosis, vasculitis, pernicious anemia, CNS inflammatory disorder, antigen-antibody complex mediated diseases, autoimmune haemolytic anemia, Hashimoto's thyroiditis, Graves disease, habitual spontaneous abortions, Reynard's syndrome, glomerulonephritis, dermatomyositis,

chronic active hepatitis, celiac disease, autoimmune complications of AIDS, atrophic gastritis, ankylosing spondylitis and Addison's disease.

[00103] The above described methods for suppressing an immune response using RP105 activators may be further enhanced by co-administering other immune modulators including but not limited to RP105, anti-fgl2, anti-B7, anti-CD80 or anti-CD86. Preferably, the RP105 activators are co-administered with an RP105 protein or a nucleic acid molecule encoding an RP105 protein as described in the PCT application no. WO00/12130 and U.S. Pat. No. 5,780,609, which are incorporated herein by reference in their entirety.

Preventing Immune Suppression

[00104] The term "RP105 protein" includes the full-length RP105 protein as well as fragments or portions of the protein. Preferred fragments or portions of the protein are those that are sufficient to induce an immune response or prevent immune suppression. The RP105 protein or the nucleic acid encoding the RP105 protein can be readily obtained by one of skill in the art. The RP105 protein or nucleic acid may be modified from the known sequences to make it more useful in the methods of the present invention.

[00105] In one embodiment, the RP105 protein is prepared as a soluble fusion protein. The fusion protein may contain the extracellular domain of RP105 linked to an immunoglobulin (Ig) Fc Region. The RP105 fusion may be prepared using techniques known in the art. Generally, a DNA sequence encoding the extracellular domain of RP105 is linked to a DNA sequence encoding the Fc of the Ig and expressed in an

appropriate expression system where the RP105-Fclg fusion protein is produced. The RP105 protein may be obtained from known sources or prepared using recombinant DNA techniques. The protein may have any of the known published sequences for RP105. For example, the sequences can be obtained from GenBank as described above. The protein may also be modified to contain amino acid substitutions, insertions and/or deletions that do not alter the immunomodulatory properties of the protein. Conserved amino acid substitutions involve replacing one or more amino acids of the RP105 amino acid sequence with amino acids of similar charge, size, and/or hydrophobicity characteristics. When only conserved substitutions are made the resulting analog should be functionally equivalent to the RP105 protein. Non-conserved substitutions involve replacing one or more amino acids of the RP105 amino acid sequence with one or more amino acids that possess dissimilar charge, size, and/or hydrophobicity characteristics.

[00106] Administration of an "effective amount" of the RP105 protein and nucleic acid of the present invention is defined as an amount effective, at dosages and for periods of time necessary to achieve the desired result. The effective amount of the RP105 protein or nucleic acid of the invention may vary according to factors such as the disease state, age, sex, and weight of the animal. Dosage regime may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

[00107] In one embodiment, the present invention provides a method of inducing fetal loss comprising administering an effective amount of an RP105 protein or a nucleic

acid sequence encoding an RP105 protein to an animal in need thereof. RP105 Modulators

[00108] The present invention also includes the isolation and/or identification of substances to modulate RP105 expression or activity. Such substances or RP105 modulators may be useful in the above-described therapeutic methods. Two examples of RP105 modulators include antibodies and antisense molecules that are described in detail above. Other RP105 modulators may be identified, for example, using the screening assays described below.

Substances that Bind RP105

[00109] Substances that affect RP105 activity can be identified based on their ability to bind to RP105.

[00110] Substances that can bind with the RP105 of the invention may be identified by reacting the RP105 with a substance that potentially binds to RP105, and assaying for complexes, for free substance, or for non-complexed RP105, or for activation of RP105.

[00111] Accordingly, the invention provides a method of identifying substances that can bind with RP105, comprising the steps of: (a) reacting RP105 and a test substance, under conditions that allow for formation of a complex between the RP105 and the test substance, and (b) assaying for complexes of RP105 and the test substance, for free substance or for non complexed RP105, wherein the presence of complexes indicates that the test substance is capable of binding RP105.

[00112] Conditions that permit the formation of substance and RP105 complexes may be selected having regard to factors such as the nature and amounts of the substance and the protein.

[00113] The substance-protein complex, free substance or non-complexed proteins may be isolated by conventional isolation techniques, for example, salting out, chromatography, electrophoresis, gel filtration, fractionation, absorption, polyacrylamide gel electrophoresis, agglutination, or combinations thereof. To facilitate the assay of the components, antibody against RP105 or the substance, or labeled RP105, or a labeled substance may be utilized. The antibodies, proteins, or substances may be labeled with a detectable substance as described above.

[00114] RP105, or the substance used in the method of the invention may be insolubilized. For example, RP105 or substance may be bound to a suitable carrier. Examples of suitable carriers are agarose, cellulose, dextran, Sephadex, Sepharose, carboxymethyl cellulose polystyrene, filter paper, ion-exchange resin, plastic film, plastic tube, glass beads, polyamine-methyl vinyl-ether-maleic acid copolymer, amino acid copolymer, ethylene-maleic acid copolymer, nylon, silk, *etc.* The carrier may be in the shape of, for example, a tube, test plate, beads, disc, sphere *etc.* The insolubilized protein or substance may be prepared by reacting the material with a suitable insoluble carrier using known chemical or physical methods, for example, cyanogen bromide coupling. The proteins or substance may also be expressed on the surface of a cell in the above assay. The invention also contemplates assaying for an antagonist or agonist of the action of RP105.

[00115] It will be understood that the agonists and antagonists that can be assayed using the methods of the invention may act on one or more of the binding sites on the protein or substance including agonist binding sites, competitive antagonist binding sites, non-competitive antagonist binding sites or allosteric sites.

[00116] The invention also makes it possible to screen for antagonists that activate the effects of an agonist of RP105. Thus, the invention may be used to assay for a substance that competes for the same binding site of RP105.

Peptide Mimetics

[00117] The present invention also includes peptide mimetics of the RP105 of the invention. For example, a peptide derived from a binding domain of RP105 will interact directly or indirectly with an associated molecule in such a way as to mimic the native binding domain. Such peptides may include competitive activators, enhancers, peptide mimetics, and the like. All of these peptides as well as molecules substantially homologous, complementary or otherwise functionally or structurally equivalent to these peptides may be used for purposes of the present invention.

[00118] "Peptide mimetics" are structures that serve as substitutes for peptides in interactions between molecules (See Morgan *et al.*. (1989), Ann. Reports Med. Chem. 24:243-252 for a review). Peptide mimetics include synthetic structures that may or may not contain amino acids and/or peptide bonds but retain the structural and functional features of a peptide, or enhancer or activator of the invention. Peptide mimetics also include peptoids, oligopeptoids (Simon *et al.* (1972) Proc. Natl. Acad, Sci USA 89:9367); and peptide libraries containing peptides of a designed length

representing all possible sequences of amino acids corresponding to a peptide of the invention.

[00119] Peptide mimetics may be designed based on information obtained by systematic replacement of L-amino acids by D-amino acids, replacement of side chains with groups having different electronic properties, and by systematic replacement of peptide bonds with amide bond replacements. Local conformational constraints can also be introduced to determine conformational requirements for activity of a candidate peptide mimetic. The mimetics may include isosteric amide bonds, or D-amino acids to stabilize or promote reverse turn conformations and to help stabilize the molecule. Cyclic amino acid analogues may be used to constrain amino acid residues to particular conformational states. The mimetics can also include mimics of activator peptide secondary structures. These structures can model the 3-dimensional orientation of amino acid residues into the known secondary conformations of proteins. Peptoids may also be used which are oligomers of N-substituted amino acids and can be used as motifs for the generation of chemically diverse libraries of novel molecules.

[00120] Peptides of the invention may also be used to identify lead compounds for drug development. The structure of the peptides described herein can be readily determined by a number of methods such as NMR and X-ray crystallography. A comparison of the structures of peptides similar in sequence, but differing in the biological activities they elicit in target molecules can provide information about the structure-activity relationship of the target. Information obtained from the examination of structure-activity relationships can be used to design either modified peptides, or

other small molecules or lead compounds that can be tested for predicted properties as related to the target molecule. The activity of the lead compounds can be evaluated using assays similar to those described herein.

[00121] Information about structure-activity relationships may also be obtained from co-crystallization studies. In these studies, a peptide with a desired activity is crystallized in association with a target molecule, and the X-ray structure of the complex is determined. The structure can then be compared to the structure of the target molecule in its native state, and information from such a comparison may be used to design compounds expected to possess.

Drug Screening Methods

[00122] In accordance with one embodiment, the invention enables a method for screening candidate compounds for their ability to increase or decrease the activity of a RP105 protein. The method comprises providing an assay system for assaying RP105 activity, assaying the activity in the presence or absence of the candidate or test compound and determining whether the compound has increased or decreased RP105 activity.

[00123] Accordingly, the present invention provides a method for identifying a compound that affects RP105 protein activity or expression comprising: (a) incubating a test compound with a RP105 protein or a nucleic acid encoding a RP105 protein; and (b) determining an amount of RP105 protein activity or expression and comparing with a control (*i.e.* in the absence of the test substance), wherein a change in the

RP105 protein activity or expression as compared to the control indicates that the test compound has an effect on RP105 protein activity or expression.

[00124] In accordance with a further embodiment, the invention enables a method for screening candidate compounds for their ability to increase or decrease expression of a RP105 protein. The method comprises putting a cell with a candidate compound, wherein the cell includes a regulatory region of a RP105 gene operably joined to a reporter gene coding region, and detecting a change in expression of the reporter gene.

[00125] In one embodiment, the present invention enables culture systems in which cell lines which express the RP105 gene, and thus RP105 protein products, are incubated with candidate compounds to test their effects on RP105 expression. Such culture systems can be used to identify compounds that upregulate or downregulate RP105 expression or its function, through the interaction with other proteins.

[00126] Such compounds can be selected from protein compounds, chemicals and various drugs that are added to the culture medium. After a period of incubation in the presence of a selected test compound(s), the expression of RP105 can be examined by quantifying the levels of RP105 mRNA using standard Northern blotting procedure to determine any changes in expression as a result of the test compound. Cell lines transfected with constructs expressing RP105 can also be used to test the function of compounds developed to modify the protein expression. In addition, transformed cell lines expressing a normal RP105 protein could be mutagenized by the use of mutagenizing agents to produce an altered phenotype in which the role of mutated RP105 can be studied in order to study structure/function relationships of the protein products and their physiological effects.

[00127] Accordingly, the present invention provides a method for identifying a compound that affects the binding of an RP105 protein and an RP105 binding protein comprising: (a) incubating (i) a test compound; (ii) an RP105 protein and (iii) an RP105 binding protein under conditions which permit the binding of RP105 protein to the RP105 binding protein; and (b) assaying for complexes of RP105 protein and the RP105 binding protein and comparing to a control (*i.e.* in the absence of the test substance), wherein a reduction of complexes indicates that the compound has an effect on the binding of the RP105 protein to an RP105 binding protein.

[00128] All testing for novel drug development is well suited to defined cell culture systems, which can be manipulated to express RP105 and study the result of RP105 protein modulation. Animal models are also important for testing novel drugs and thus may also be used to identify any potentially useful compound affecting RP105 expression and activity and thus physiological function.

Compositions

[00129] The invention also includes pharmaceutical compositions containing substances that activate RP105 activators for use in immune suppression as well as pharmaceutical compositions containing substances that enhance RP105 for use in preventing immune suppression. Substances that activate RP105 include substances that activate RP105 gene expression as well as substances that activate RP105 activity. Such substances include antisense molecules to RP105, antibodies to RP105 as well as other substances or RP105 antagonists identified using the screening assays described herein.

[00130] Substances that enhance RP105 include substances that enhance RP105 expression and/or activity. Such substances include nucleic acid molecules encoding RP105, RP105 proteins and other substances or RP105 agonists identified using the screening assays described herein.

[00131] Such pharmaceutical compositions can be for intralesional, intravenous, topical, rectal, parenteral, local, inhalant or subcutaneous, intradermal, intramuscular, intrathecal, transperiton al, oral, and intracerebral use. The composition can be in liquid, solid or semisolid form, for example pills, tablets, creams, gelatin capsules, capsules, suppositories, soft gelatin capsules, gels, membranes, tubelets, solutions or suspensions.

[00132] The pharmaceutical compositions of the invention can be intended for administration to humans or animals. Dosages to be administered depend on individual needs, on the desired effect and on the chosen route of administration.

[00133] The pharmaceutical compositions can be prepared by per se known methods for the preparation of pharmaceutically acceptable compositions which can be administered to patients, and such that an effective quantity of the active substance is combined in a mixture with a pharmaceutically acceptable vehicle. Suitable vehicles are described, for example, in Remington's Pharmaceutical Sciences (Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., USA 1985).

[00134] On this basis, the pharmaceutical compositions include, albeit not exclusively, the active compound or substance in association with one or more pharmaceutically acceptable vehicles or diluents, and contained in buffered solutions with a suitable pH

and iso-osmotic with the physiological fluids. The pharmaceutical compositions may additionally contain other agents such as immunosuppressive drugs or antibodies to enhance immune tolerance or immunostimulatory agents to enhance the immune response.

[00135] In one embodiment, the pharmaceutical composition for use in inducing immune tolerance comprises an effective amount of an activator of RP105 in admixture with a pharmaceutically acceptable diluent or carrier. The RP105 activator is preferably an antisense oligonucleotide to RP105 or an antibody that binds to RP105. The pharmaceutical compositions may also contain other active agents such as other immune modulators including, but not limited to RP105, fgl2, B7, CD80 or CD86 including antagonists, agonists and modulators thereof. Preferably the compositions further contain an RP105 protein or a nucleic acid molecule encoding an RP105 protein.

[00136] Pharmaceutical compositions comprising nucleic acid molecules may be directly introduced into cells or tissues *in vivo* using delivery vehicles such as retroviral vectors, adenoviral vectors and DNA virus vectors. They may also be introduced into cells *in vivo* using physical techniques such as microinjection and electroporation or chemical methods such as co-precipitation and incorporation of DNA into liposomes. Recombinant molecules may also be delivered in the form of an aerosol or by lavage. The nucleic acid molecules of the invention may also be applied extracellularly such as by direct injection into cells.

[00137] In another aspect, the pharmaceutical composition for use in preventing immune suppression comprises an effective amount of an RP105 protein or a nucleic

acid encoding an RP105 protein in admixture with a pharmaceutically acceptable diluent or carrier. Such compositions may be administered either alone or in combination with other active agents such as RP105 activators.

Diagnostic Assays

[00138] The finding by the present inventors that RP105 is involved in immune regulation allows the detection of conditions involving an increase or decrease in RP105 activity or expression resulting in an aberrant or inappropriate immune response. Such conditions include, but are not limited to, habitual fetal loss, autoimmune diseases, allergies, immune deficiency diseases, graft rejection, inflammatory conditions, wound healing, neurodegenerative diseases, stroke, spinal injury and conditions that lead to septic shock and organ dysfunction in critically ill patients.

[00139] Accordingly, the present invention provides a method of detecting a condition associated with increased or decreased RP105 expression or activity (including an absence) comprising assaying a sample for (a) a nucleic acid molecule encoding a RP105 protein or a fragment thereof or (b) an RP105 protein or a fragment thereof and comparing the amount of RP105 nucleic acid or protein detected with a suitable control.

Nucleic Acid Molecules

[00140] Nucleotide probes can be prepared based on the sequence of RP105 for use in the detection of nucleotide sequences encoding RP105 or fragments thereof in samples, preferably biological samples such as cells, tissues and bodily fluids. The

probes can be useful in detecting the presence of a condition associated with RP105 or monitoring the progress of such a condition. Accordingly, the present invention provides a method for detecting a nucleic acid molecules encoding RP105 comprising contacting the sample with a nucleotide probe capable of hybridizing with the nucleic acid molecule to form a hybridization product, under conditions which permit the formation of the hybridization product, and assaying for the hybridization product.

[00141] A nucleotide probe may be labeled with a detectable substance such as a radioactive label, which provides for an adequate signal and has sufficient half-life such as 32P, 3H, 14C or the like. Other detectable substances that may be used include antigens that are recognized by a specific labeled antibody, fluorescent compounds, enzymes, antibodies specific for a labeled antigen, and chemiluminescence. An appropriate label may be selected having regard to the rate of hybridization and binding of the probe to the nucleic acid to be detected and the amount of nucleic acid available for hybridization. Labeled probes may be hybridized to nucleic acids on solid supports such as nitrocellulose filters or nylon membranes as generally described in Sambrook *et al.*, 1989, Molecular Cloning, A Laboratory Manual (2nd ed.). The nucleotide probes may be used to detect genes, preferably in human cells, that hybridize to the nucleic acid molecule of the present invention preferably, nucleic acid molecules which hybridize to the nucleic acid molecule encoding RP105 under stringent hybridization conditions as described herein.

[00142] Nucleic acid molecules encoding a RP105 protein can be selectively amplified in a sample using the polymerase chain reaction (PCR) methods and cDNA or genomic DNA. It is possible to design synthetic oligonucleotide primers from the

nucleotide sequence of RP105 for use in PCR. A nucleic acid can be amplified from cDNA or genomic DNA using oligonucleotide primers and standard PCR amplification techniques. The amplified nucleic acid can be cloned into an appropriate vector and characterized by DNA sequence analysis. cDNA may be prepared from mRNA, by isolating total cellular mRNA by a variety of techniques, for example, by using the guanidinium-thiocyanate extraction procedure of Chirgwin *et al.*, Biochemistry, 18, 5294-5299 (1979). cDNA is then synthesized from the mRNA using reverse transcriptase (for example, Moloney MLV reverse transcriptase available from Gibco/BRL, Bethesda, MD, or AMV reverse transcriptase available from Seikagaku America, Inc., St. Petersburg, Fla.).

Proteins

[00143] The RP105 protein may be detected in a sample using antibodies that bind to the protein as described in detail above. Accordingly, the present invention provides a method for detecting a RP105 protein comprising contacting the sample with an antibody that binds to RP105 which is capable of being detected after it becomes bound to the RP105 in the sample.

[00144] Antibodies specifically reactive with RP105, or derivatives thereof, such as enzyme conjugates or labeled derivatives, may be used to detect RP105 in various biological materials, for example they may be used in any known immunoassays which rely on the binding interaction between an antigenic determinant of RP105, and the antibodies. Examples of such assays are radioimmunoassays, enzyme immunoassays (*e.g.* ELISA), immunofluorescence, immunoprecipitation, latex agglutination, hemagglutination and histochemical tests. Thus, the antibodies may be

used to detect and quantify RP105 in a sample in order to determine its role in particular cellular events or pathological states, and to diagnose and treat such pathological states.

[00145] In particular, the antibodies of the invention may be used in immuno-histochemical analyses, for example, at the cellular and sub-subcellular level, to detect RP105, to localize it to particular cells and tissues and to specific subcellular locations, and to quantitate the level of expression.

[00146] Cytochemical techniques known in the art for localizing antigens using light and electron microscopy may be used to detect RP105. Generally, an antibody of the invention may be labeled with a detectable substance and RP105 may be localized in tissue based upon the presence of the detectable substance. Examples of detectable substances include various enzymes, fluorescent materials, luminescent materials and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, biotin, alkaline phosphatase, b-galactosidase, or acetylcholinesterase; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; and examples of suitable radioactive material include radioactive iodine I-125, I-131 or 3-H. Antibodies may also be coupled to electron dense substances, such as ferritin or colloidal gold, which are readily visualized by electron microscopy.

[00147] Indirect methods may also be employed in which the primary antigen-antibody reaction is amplified by the introduction of a second antibody, having specificity for the antibody reactive against RP105. By way of example, if the antibody having

specificity against RP105 is a rabbit IgG antibody, the second antibody may be goat anti-rabbit gamma-globulin labeled with a detectable substance as described herein.

[00148] Where a radioactive label is used as a detectable substance, RP105 may be localized by autoradiography. The results of autoradiography may be quantitated by determining the density of particles in the autoradiographs by various optical methods, or by counting the grains.

[00149] The preferred embodiments are exemplified by the following examples.

EXAMPLES

[00150] Activation of Toll-like receptor (TLR) signaling by microbial signatures is critical to the induction of immune responses. Such responses demand tight regulation. RP105 is a TLR homolog, thought to be largely B cell-specific, which lacks a signaling domain. The present invention demonstrates that RP105 expression is wide, directly mirroring that of TLR4 on antigen presenting cells. Furthermore, RP105 is a specific inhibitor of TLR4 signaling in HEK293 cells, a function conferred by its extracellular domain. Notably, RP105 and its helper molecule, MD-1, interact directly with the TLR4 signaling complex, inhibiting the ability of this complex to bind microbial ligand. Finally, we demonstrate that RP105 is a physiological regulator of TLR4 signaling in dendritic cells, and of endotoxicity and *Leishmania major* infection *in vivo*.

[00151] Activation of TLR signaling by conserved microbial molecular signatures promotes the induction of both innate and adaptive immune responses. It has long

been clear that such immune responses need to be kept under tight control. Responses that are delayed or of insufficient vigor can lead to a failure to control infection. On the other hand, excessive or inappropriate inflammation can be harmful or even fatal. The hyper-inflammatory responses that characterize sepsis provide a paradigmatic example, as do the more localized inappropriate inflammatory processes leading to inflammatory bowel disease and arthritis.

[00152] Mammalian TLRs are characterized structurally by an extracellular leucine-rich repeat (LRR) domain, a conserved pattern of juxtamembrane cysteine residues, and an intracytoplasmic signaling domain (Toll/IL-1 resistance [TIR]) that is highly conserved across the TLRs as well as the receptors for IL-1 and IL-18. The TLR-like molecule RP105 was originally cloned as a B cell-specific molecule able to drive B cell proliferation. Like TLRs, RP105 has a conserved extracellular LRR domain and a TLR-like pattern of juxtamembrane cysteines. Unlike the TLRs, however, RP105 lacks a TIR domain, containing a mere 6-11 intracytoplasmic amino acids (depending upon the prediction algorithm). In parallel with TLR4, whose surface expression and signaling depends upon co-expression of the secreted extracellular protein MD-2, surface expression of RP105 is dependent upon the co-expression of the MD-2 homolog, MD-1.

[00153] Here, we show that RP105 is a specific homolog of TLR4. We further show that RP105 is not B cell-specific as originally proposed: RP105 protein expression directly mirrors that of TLR4 on antigen-presenting cells. In Toll and TLR4, mutation of the conserved juxtamembrane cysteine residues, or significant deletion of the extracellular portion, has been shown to result in a constitutively active molecule.

This suggests that Toll/TLR activation is normally restrained through extracellular protein/protein interactions, likely through the LRR domain. On the other hand, deletions or mutations in the TIR domain of Toll/TLRs can yield inactive or dominant negative molecules. Thus, RP105 has the apparent structure of an inhibitory TLR4 and RP105 is a physiological regulator of TLR4 signaling. RP105 is a specific inhibitor of TLR4 signaling, a function conferred by its extracellular domain. Without wishing to be bound by theory in any way, it is believed that the RP105/MD-1 complex interacts directly with TLR4/MD-2, inhibiting the ability of this LPS signaling complex to bind LPS. RP105 is a physiological regulator of TLR4 signaling in primary dendritic cells, and of responses to endotoxin as well as *Leishmania* infection *in vivo*.

[00154] RP105, a TLR4 homolog whose expression mirrors that of TLR4 on antigen presenting cells, is a negative regulator of TLR4 signaling. RP105 specifically inhibits TLR4 signaling when co-expressed in HEK293 cells. Further, RP105 is a physiological regulator of endotoxin-driven TLR4 signaling, as well as of endotoxicity *in vivo*. Finally, RP105-mediated counter-regulation is relevant in the context of infection with a well-described model (and human) pathogen: RP105 modulates the course of *L. major* infection, restraining both innate and adaptive immune responses. Although the activation of proinflammatory responses through TLRs is critical for host defense, excessive or inappropriate inflammation can itself be maladaptive. RP105 joins a growing list of molecules and processes that have been shown to inhibit TLR signaling. RP105 stands out for its apparent specificity for inhibition of TLR4 signaling. This specificity, together with the structural homologies between RP105 and TLR4 provides a mechanism of negative regulation of TLR4 signaling by RP105:

interference with TLR4 signaling through direct interactions of RP105/MD-1 with the TLR4/MD-2 cell surface signaling complex. Co-immunoprecipitation experiments revealed direct physical association between RP105/MD-1 and TRL4/MD-2, an association that inhibits LPS binding to this signaling complex. The exact stoichiometry of RP105/MD-1/MD-2/TLR4 interactions remains to be defined. We show that RP105 regulates LPS responses differently in myeloid cells and B cells. As noted, treatment with antibodies to RP105 does not activate human monocyte/macrophages, failing, for example, to drive proinflammatory cytokine production. As for LPS-driven B cell responses, while LPS-induced murine B cell proliferation is strictly dependent upon TLR4, B cells from RP105 knockout mice have reduced LPS-driven proliferative responses as well as diminished humoral immune responses when LPS is used as an adjuvant for vaccination with T cell-dependent antigens. It is reasonable to suspect that the dichotomous effects of RP105 on TLR4 signaling in B cells and myeloid cells are due to differential interactions with cell surface molecular partners in these different cell types. Widely differing levels of expression of TLR4 (significant on myeloid cells; hardly detectable on B cells) may provide the key. TLR4 multimerization appears to be necessary for signaling. While TLR4/MD-2 would be expected to have a higher affinity for homodimerization than for heterodimerization with RP105/MD-1, data presented here suggest the likelihood that both homo- and heterodimers can multimerize with further TLR4/MD-2 complexes. This suggests a model whereby: (a) when TLR4/MD-2 is highly expressed (e.g., on myeloid cells), lower affinity heterodimeric interactions inhibit TLR4 multimerization and signaling; but (b) when TLR4 is limiting (e.g., on B cells), such heterodimeric interactions serve to facilitate further TLR4 recruitment and signaling.

The fact that RP105 appears to promote B cell activation, while inhibiting DC activation, suggests the possibility that, overall, immunoregulation by RP105 leads to augmentation of humoral immunity (through effects on B cells) along with concomitant inhibition of cellular immune responses (through effects on DCs and macrophages).

[00155] The apparent TLR4-specificity of RP105 raises another issue. Concurrent stimulation of TLR4 and other TLRs is able to effect reversal RP105-mediated inhibition of TLR4 signaling. Clearly, most microbes that express ligands that stimulate TLR4 signaling also express ligands that stimulate signaling through other TLRs. TLR4 is notable for leading to a more robust and complex response, both in terms of signaling and in terms of subsequent gene expression, than the other TLRs. TLR4 may also stand out among the TLRs for its ability to signal in response to a variety of endogenous "danger signals". The ability of TLR4 to recognize endogenous heat shock proteins and extracellular matrix components unmasked by tissue injury suggests the possibility RP105-mediated modulation of the *in vivo* response to *L. major* infection may not be a function of the expression of yet-to-be discovered leishmanial ligands for TLR4, but of the inflammation-induced expression of host-derived TLR ligands. More generally, such considerations raise the possibility that RP105 may well be of special importance in down-modulation of the injurious inflammatory responses observed in the systemic inflammatory response syndrome and in autoimmune diseases. In this regard, it is notable that RP105$^{-/-}$ mice spontaneously develop splenomegaly with age (data not shown).

Materials and Methods

[00156] *Reagents.* Zymosan A and *E. coli* K235 lipopolysaccharide (LPS) were from Sigma. Re-purified *E. coli* K235 LPS, free of contaminating TLR2 ligands, was generated as described previously (24). (Pam_3) Cys-Ser-$(Lys)_4$ [Pam_3Cys] was from EMC Microcollections. All restriction enzymes were from New England Biolabs. All culture media and reagents coming into contact with cultured cells were endotoxin-free to the limits of detection of the *Limulus* amebocyte lysate assay (Bio-Whittaker) at the concentrations employed, unless otherwise stated.

[00157] *Cloning and Expression Constructs.* Human RP105 and MD-1 were cloned from primary human monocytes isolated by countercurrent elutriation (25) using RT-PCR-mediated cloning techniques. Harvested RNA was treated with RNase-free DNase (Roche), and cDNA was synthesized using Superscript II RNase H$^-$ reverse transcriptase (Gibco). PCR primers were designed to amplify the entire coding regions of *LY64* and *LY86*, the genes encoding human RP105 and MD-1, respectively. Fragments of the anticipated size were amplified and cloned into pCR2.1-TOPO (Invitrogen). Nucleotide sequencing revealed 100% identity with the previously published sequences (11-13, 16). For expression in mammalian cells, MD-1 and RP105 were subcloned into pcDNA3.1 and pcDNA4/Myc-His (Invitrogen).

[00158] The HSV TK-Renilla luciferase reporter plasmid (pRL-TK) was from Promega; the NF-κB-Firefly Luciferase reporter plasmid (p-ELAM) has previously been described (26). cDNA for TLR4, a gift from A. Visintin, was subcloned into pcDNA3.1. The pEFBOS expression vector encoding MD-2 was a gift of K. Miyake.

[00159] *Cell lines.* HEK293 cell lines stably expressing CD14, CD14/TLR4, and CD14/TLR2 were cultured in RPMI 1640 (Gibco), supplemented with 10% FCS (Gibco), 2 mM L-glutamine (Sigma), 50 μg/ml gentamicin (Cellgro) and 5 μg/ml puromycin (Calbiochem). HEK293 cells stably expressing TLR4/MD-2 were cultured in DMEM (Gibco) supplemented with 10% FCS, 2 mM L-glutamine, 50 μg/ml gentamicin, 10 μg/ml ciprofloxacin (Bayer), and 0.5 mg/ml geneticin (Gibco). HEK293 cells stably expressing RP105/CD14/TLR4 were generated by transfecting linearized RP105 cDNA into CD14/TLR4 HEK293 cells. RP105-expressing clones were selected with 250 μg/ml Zeocin (Invitrogen) plus puromycin.

[00160] Transient transfections were performed using the PolyFect transfection reagent (Qiagen) according to the manufacturer's instructions. All plasmid DNA was isolated using the EndoFree plasmid maxi kit (Qiagen). Construct expression was quantified by flow cytometry (see below) using TLR4-PE, TLR2-FITC, RP105-PE mAb, (e-Bioscience) and CD14-FITC mAb (Becton Dickinson).

[00161] *In vitro stimulation.* HEK293 cells were cultured in 6-well plates (Costar) at 3 x 10^5 cells/ml (2 ml/well) for 24 h prior to transient transfection. 24 h after transfection, the culture media was changed and cells were stimulated (or mock stimulated) for an additional 24 h. Cell-free supernatants were collected and stored at –20°C until assayed for IL-8 production by ELISA (Pharmingen). To assay NF-κB-driven luciferase expression (26), cells were co-transfected with pELAM (0.5 μg) and pRL-TK (0.1 μg) plasmids for 24 h. After change of the culture media, cells were stimulated (or mock stimulated) for 5 h. Cells were subsequently lysed in passive lysis buffer (Promega), and luciferase activity was quantified on a Monolight 3010

luminometer (Pharmingen) using the dual luciferase reporter assay system (Promega). Firefly luciferase activity was normalized to Renilla luciferase activity.

[00162] *Mice.* RP105$^{-/-}$ mice, on a C57BL/6 background (> 10 generations) have been described previously (27). Mice were genotyped using standard PCR techniques. Mice were also phenotyped for RP105 surface expression on peripheral blood B cells by flow cytometry, as previously described (27) using B220-FITC mAb (Becton Dickinson) and RP105-PE mAb (e-Biosciences), along with isotype control antibodies from the respective companies (Fig. 10).

[00163] Resident peritoneal macrophages were harvested using standard techniques, and analyzed for RP105 expression by FACS (see below). Primary mouse DC subpopulations in spleen were analyzed by FACS after *in vivo* amplification of DC numbers through daily i.p. administration of 10 μg of flt3 ligand (Immunex/Amgen).

[00164] Mice were housed in a specific pathogen free facility in high-efficiency particulate-filtered laminar flow hoods with free access to food and water. Animal care was provided in accordance with National Institutes of Health guidelines. These studies were approved by the Cincinnati Children's Hospital Medical Center IACUC.

[00165] Ex vivo stimulation. Bone marrow-derived DC (BMDDC) generated using standard protocols (28), were cultured in 48-well plates at 2 x 10^6 cells/ml (500 μl/well) in RPMI 1640 supplemented with 10% FCS, 2 mM L-glutamine, and 50 μg/ml gentamicin. DC were stimulated with TLR ligands (or mock stimulated) for 24 h. Cell-free supernatants were collected and stored at –20°C until assay by ELISA for

TNF-α (Becton Dickinson), IL-12p70 and IL-6 (R&D Systems). DC surface phenotype was analyzed by flow cytometry before and after stimulation (see below).

[00166] *In vivo* stimulation. 6-8 week old $RP105^{-/-}$ and wild type littermate control mice were challenged i.p. with 25 μg of re-purified E. coli K235 LPS. One h later, serum was collected and stored at –20°C until analysis of TNF-α concentrations by ELISA.

[00167] *Human leukocytes.* PBMC were isolated from healthy volunteers by sedimentation over Ficoll/Hypaque gradients. Monocytes were purified by countercurrent elutriation from PBMC isolated by leukapheresis (25). Monocyte-derived DC were generated by standard protocols through culturing in media containing GM-CSF (500 U/ml) and IL-4 (1000 U/ml) [both from Peprotech] for 7-8 d. These studies were approved by the Cincinnati Children's Hospital Medical Center and the University of Cincinnati College of Medicine IRBs.

[00168] *Flow Cytometry.* Surface and intracellular protein expression by HEK293 cells was quantified by FACS techniques described previously (29). For surface protein expression, cells were harvested, washed in FACS staining buffer (PBS with 1% FCS and 0.05% sodium azide [Mallinckrodt]) and blocked in PBS containing 10% human AB serum for 30 min at 4°C. After a further wash, cells were incubated with directly labeled mAb for 30 min, washed, and fixed in 4% paraformaldehyde in PBS for 10-15 min. After further washing, cells were resuspended in FACS staining buffer. For intracellular protein expression, harvested cells were washed in FACS staining buffer and fixed and permeabilized using Fix&Perm (Caltag) according to the manufacturer's instructions, followed by incubation with mAb for 30 min. Cells were

subsequently washed in permeabilization buffer and resuspended in staining buffer. All incubations were done on ice. Flow cytometric analysis was performed using a FACSCalibur along with CellQuest Software (Becton Dickinson). At least 20,000 events were acquired for each data point.

[00169] RP105 expression (RP105-PE [e-Bioscience]) was analyzed on mouse splenic B-cells (B220-FITC [Becton-Dickinson]), BMDDC, and resident peritoneal macrophages (F4/80-allophycocyanin (APC) [e-Bioscience]). RP105 expression was also quantified on splenic DC subsets using directly labeled mAb to CD11c, CD11b, CD4, CD8α, B220, CD19 and GR-1 (all from Becton-Dickinson). I-A^b, CD80, CD86 and CD40 expression were quantified on BMDDC using directly labeled mAb (I-A^b from Becton-Dickinson; the rest from e-Bioscience). Cell death was monitored by FACS using propidium iodide (Sigma). FACS techniques were as above, except for a blocking step with mAb 24G2.

[00170] RP105 expression by human leukocytes was analyzed using RP105-PE (e-Biosciences). RP105 expression was examined on peripheral blood B cells (CD19-APC), myeloid DC (lineage mAb-FITC cocktail [CD3, CD14, CD19, CD20, CD56] negative; HLA-DR-PerCP and CD11c-APC positive), and plasmacytoid DC (lineage negative; HLA-DR-PerCP and BDCA4-APC positive) in PBMC. All mAb for these studies were from Becton-Dickinson, with the exception of that for BDCA4-APC (Miltenyi Biotech).

[00171] *Phylogenetic and domain analysis.* For phylogenetic analysis, sequence alignments were performed using the ClustalW (30) and Pileup (31) programs.

MEMSAT (32), SOSUI (33)and SABLE (34) software were used to predict the boundaries of the transmembrane domain of RP105.

[00172] *Statistical analysis*. Data analysis was performed using the unpaired Student's *t test*, or ANOVA with post-hoc analysis by Student's *t test*, as appropriate.

Results.

[00173] Phylogenetic analysis of the TLR family has revealed 5 TLR subfamilies: (1) TLR4; (2) TLR3; (3) TLR5; (4) TLR7, TLR8, TLR9; and (5) TLR 1 (2). Similar analytic techniques unequivocally place RP105 in the TLR4 subfamily (Fig. 1; Fig. 11). The domain structure of the 641 amino acid mature RP105 protein has previously been commented upon (10, 12, 13, 16). The extracellular portion of this type I transmembrane protein contains 22 leucine-rich repeats (repeats 7-10 being non-homologous), along with a pattern of juxtamembrane cysteines that is conserved among the Toll and TLR families. Prediction algorithms disagree on the C-terminal boundary of the transmembrane domain. Although the 6-11 amino acid intracellular domain of RP105 contains 1-2 tyrosine residues, it is devoid of recognized motifs that would suggest phosphorylation activity.

[00174] RP105 expression mirrors TLR4 expression on APC. RP105 has been thought to be a B cell-specific molecule in mice (10). Further, despite the fact that RP105 has been noted to be expressed at the mRNA level in primary human monocytic cells (11-13), the only published examination of RP105 protein expression in monocytic cells (by western blot analysis) was reported to be negative (13). We examined RP105 expression by FACS in human and murine monocytic cells. In neither species is

RP105 expression limited to B cells. In humans, RP105 is also expressed by human monocytes, as well as myeloid DC (Fig. 2a-c), cells that express considerably more TLR4 than do B cells (35) (and data not shown). Interestingly, RP105 is not expressed by human plasmacytoid DC (Fig. 2d), cells that also fail to express TLR4 (35-38) (and data not shown).

[00175] Similarly, mice express RP105 on resident peritoneal macrophages, splenic DC subsets, as well as bone marrow derived DC (Fig. 3). Interestingly, unlike those found in human peripheral blood, splenic plasmacytoid DC from flt3L-treated mice express both RP105 and TLR4 (Fig 3e; and data not shown). RP105 thus joins an elect list of "failed" B cell-specific molecules, including CD40 and NF-κB. Further, the expression of RP105 appears to directly mirror that of TLR4 on macrophages and DC.

[00176] *RP105 suppresses TLR4 mediated signaling in HEK293 cells.* HEK293 cells lack expression of endogenous TLR2, TLR4, TLR9, MD-2 and CD14 (26), as well as RP105 and MD-1 (data not shown). Their TLR signaling machinery is fully functional, however. As a result, HEK293 cells have been extensively employed for the *in vitro* analysis of TLR function (26, 39-46). Given the homology of RP105 to TLR4, we first examined whether RP105/MD-1 could act as a signaling receptor for LPS in HEK293 cells. HEK293 cells stably expressing CD14 were transiently transfected with cDNA encoding MD-1, MD-2, RP105, and/or TLR4. As shown in Figure 4a, while TLR4/MD-2 expression confers LPS-sensitivity, with resultant LPS-driven IL-8 production, RP105/MD-1 expression does not. As has been found

previously, *in vitro* overexpression of TLR4/MD-2 in HEK293 cells leads to baseline IL-8 production in the absence of stimulation.

[00177] The effects of RP105/MD-1 expression on LPS-driven TLR4 signaling were subsequently examined. Notably, RP105 expression inhibits LPS-driven IL-8 production by HEK293 cells in a dose-dependent manner (Fig. 4*b*). As might be expected, RP105-mediated inhibition of LPS-driven IL-8 production is associated with inhibition of LPS-driven NF-κB activation (Figure 4*c*).

[00178] The generality of RP105-mediated suppression of proinflammatory signaling was subsequently examined. As shown in Figure 5, RP105 does not inhibit IL-1- or TLR2-driven IL-8 production. Indeed, RP105 expression led to variable augmentation of TLR2-mediated IL-8 production in some experiments (shown). Thus, RP105-mediated suppression is TLR4-specific.

[00179] Finally, we formally examined the necessity for MD-1 expression in this system. As shown in Figure 6*a*, in the absence of MD-1 co-expression, RP105 does not inhibit TLR4 signaling. As previously reported (16), such MD-1 expression is required for surface expression of RP105 (Fig. 6*b*).

[00180] *RP105 regulates cytokine production in DC.* Expression studies in cell lines have considerable utility. They also have obvious drawbacks; principally, that overexpression may drive non-physiological interactions and processes. We thus examined $RP105^{-/-}$ mice, generated by targeted disruption of exon 3 of RP105 (27), backcrossed for more than 10 generations onto the C57BL/6 background. To define the effects of genetic deletion of RP105 on APC function, bone marrow-derived DC

were generated by standard techniques (47) from age-matched RP105$^{-/-}$ mice and wild-type littermate controls. No differences in baseline expression of CD80, CD86 or MHC class II were apparent in DC generated from mice of the different genotypes (data not shown). DC were subsequently stimulated with repurified *E. coli* LPS. DC from RP105$^{-/-}$ mice produced significantly more TNF-α, IL-12p70 and IL-6 than did DC from wild type controls (Fig. 7).

[00181] The biphasic dose-response for TNF-α, IL-12p70 and IL-6 production observed in DC from both wild type and knockout mice (Fig. 7) was of interest, as such biphasic dose response curves do not appear to have been reported previously in human or murine DC (or monocyte/macrophages) either by us or by others. Flow cytometric analysis revealed that this lower cytokine production at higher doses of LPS was not associated with greater DC apoptosis (data not shown). The use of non-repurified LPS, the usual reagent employed in both *in vitro* and *in vivo* studies, was revealing, however. Commercial, non-purified LPS is contaminated with lipopeptide ligands for TLR2 (24). As shown in Figure 8*a*, such LPS continues to drive increasing TNF-α production at higher doses (the very doses at which IL-8 production by "dirty" LPS is induced in HEK293 cells transfected with TLR2 [data not shown]). Notably, stimulation of DC with these higher doses of "dirty" LPS led to similar amounts of TNF-α production from RP105 sufficient and deficient DC (Fig. 8*a*). Indeed, stimulation with combinations of purified TLR4 and TLR2 agonists revealed that TLR2 agonists are able to effect functional reversal of RP105-mediated inhibition of TLR4 signaling (Fig. 8*b*). These data indicate that: (a) as with many biological agonistic responses, the response to a pure TLR4 ligand is biphasic, something

modifiable by secondary agonists; and (b) signaling through other TLRs can overcome RP105-mediated modulation of TLR4 signaling. These data further indicate that RP105-mediated suppression of TLR4 signaling is not merely an overexpression artifact in cell lines.

[00182] *RP105 regulates proinflammatory cytokine production in vivo.* Finally, LPS-driven cytokine production was characterized *in vivo* in order to examine whether RP105 plays a role in regulating systemic cytokine production. $RP105^{-/-}$ mice and wild type littermate controls were challenged intraperitoneally with re-purified *E. coli* LPS. $RP105^{-/-}$ mice produce significantly more TNF-α in response to LPS challenge (Fig. 9). Thus, RP105 is a negative regulator of TLR4 signaling *in vivo*.

Discussion

[00183] These results demonstrate that RP105, a TLR4 homologue whose expression mirrors that of TLR4 in antigen presenting cells, is a negative regulator of TLR4 signaling.

[00184] Without wishing to be bound by theory in any way, this specificity, along with the structural homologies between RP105 and TLR4, suggests an attractive hypothesis for the mechanism of negative regulation of TLR4 signaling by RP105: direct interference with TLR4 homoaggregation through the formation of RP105/TLR4 heterodimers. Other obvious mechanistic possibilities include more mediate interference with TLR4 aggregation via MD-1/MD-2 interactions, or signal transduction through the diminutive intracellular portion of RP105.

[00185] Mechanism aside, the fact that RP105 promotes B cell activation while inhibiting DC activation such that, immunoregulation by RP105 leads to augmentation of humoral immunity (through effects on B cells) along with concomitant inhibition of cellular immune responses (through effects on DCs).

[00186] RP105 is also of special importance in down-modulation of the injurious inflammatory responses observed in autoimmune diseases and the systemic inflammatory response syndrome (66).

[00187] The description fully satisfies the objects, aspects and advantages set forth. While the invention has been set forth in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications, and variations will be apparent to those skilled in the art in the light of the foregoing description. Accordingly, it is intended to embrace all such alternatives, modifications, and variations which fall within the spirit and scope of the following claims.

REFERENCES

1. Medzhitov, R., and C. Janeway, Jr. 2000. Innate immune recognition: mechanisms and pathways. *Immunol Rev* 173:89-97.

2. Takeda, K., T. Kaisho, and S. Akira. 2003. Toll-like receptors. *Annu Rev Immunol* 21:335-376.

3. Morrison, D.C., and J.L. Ryan. 1987. Endotoxins and disease mechanisms. *Annu Rev Med* 38:417-432.

4. Dinarello, C.A. 1991. The proinflammatory cytokines interleukin-1 and tumor necrosis factor and treatment of the septic shock syndrome. *J Infect Dis* 163:1177-1184.

5. Beutler, B., and E.T. Rietschel. 2003. Innate immune sensing and its roots: the story of endotoxin. *Nat Rev Immunol* 3:169-176.

6. Ardizzone, S., and G.B. Porro. 2002. Inflammatory bowel disease: new insights into pathogenesis and treatment. *J Intern Med* 252:475-496.

7. Bingham, C.O., 3rd. 2002. The pathogenesis of rheumatoid arthritis: pivotal cytokines involved in bone degradation and inflammation. *J Rheumatol Suppl* 65:3-9.

8. Barton, G.M., and R. Medzhitov. 2002. Toll-like receptors and their ligands. *Curr Top Microbiol Immunol* 270:81-92.

9. Miyake, K., Y. Yamashita, Y. Hitoshi, K. Takatsu, and M. Kimoto. 1994. Murine B cell proliferation and protection from apoptosis with an antibody against a 105-kD molecule: unresponsiveness of X-linked

immunodeficient B cells. *J Exp Med* 180:1217-1224.

10. Miyake, K., Y. Yamashita, M. Ogata, T. Sudo, and M. Kimoto. 1995. RP105, a novel B cell surface molecule implicated in B cell activation, is a member of the leucine-rich repeat protein family. *J Immunol* 154:3333-3340.

11. Miura, Y., K. Miyake, Y. Yamashita, R. Shimazu, N.G. Copeland, D.J. Gilbert, N.A. Jenkins, J. Inazawa, T. Abe, and M. Kimoto. 1996. Molecular cloning of a human RP105 homologue and chromosomal localization of the mouse and human RP105 genes (Ly64 and LY64). *Genomics* 38:299-304.

12. Fugier-Vivier, I., O. de Bouteiller, C. Guret, F. Fossiez, J. Banchereau, M.G. Mattei, S. Ait-Yahia, E. Garcia, S. Lebecque, and Y.J. Liu. 1997. Molecular cloning of human RP105. *Eur J Immunol* 27:1824-1827.

13. Roshak, A.K., K.M. Anderson, S.D. Holmes, Z. Jonak, B. Bolognese, J. Terrett, and L.A. Marshall. 1999. Anti-human RP105 sera induces lymphocyte proliferation. *J Leukoc Biol* 65:43-49.

14. Shimazu, R., S. Akashi, H. Ogata, Y. Nagai, K. Fukudome, K. Miyake, and M. Kimoto. 1999. MD-2, a Molecule that Confers Lipopolysaccharide Responsiveness on Toll- like Receptor 4. *J Exp Med* 189:1777-1782.

15. Miyake, K., R. Shimazu, J. Kondo, T. Niki, S. Akashi, H. Ogata, Y. Yamashita, Y. Miura, and M. Kimoto. 1998. Mouse MD-1, a molecule that is physically associated with RP105 and positively regulates its expression. *J Immunol* 161:1348-1353.

16. Miura, Y., R. Shimazu, K. Miyake, S. Akashi, H. Ogata, Y. Yamashita, Y. Narisawa, and M. Kimoto. 1998. RP105 is associated with MD-1 and transmits an activation signal in human B cells. *Blood* 92:2815-2822.

17. Nagai, Y., R. Shimazu, H. Ogata, S. Akashi, K. Sudo, H. Yamasaki, S. Hayashi, Y. Iwakura, M. Kimoto, and K. Miyake. 2002. Requirement for MD-1 in cell surface expression of RP105/CD180 and B-cell responsiveness to lipopolysaccharide. *Blood* 99:1699-1705.

18. Schneider, D.S., K.L. Hudson, T.Y. Lin, and K.V. Anderson. 1991. Dominant and recessive mutations define functional domains of Toll, a transmembrane protein required for dorsal-ventral polarity in the Drosophila embryo. *Genes Dev* 5:797-807.

19. Winans, K.A., and C. Hashimoto. 1995. Ventralization of the Drosophila embryo by deletion of extracellular leucine-rich repeats in the Toll protein. *Mol Biol Cell* 6:587-596.

20. Medzhitov, R., P. Preston-Hurlburt, and C.A. Janeway, Jr. 1997. A human homologue of the Drosophila Toll protein signals activation of adaptive immunity [see comments]. *Nature* 388:394-397.

21. Kobe, B., and A.V. Kajava. 2001. The leucine-rich repeat as a protein recognition motif. *Curr Opin Struct Biol* 11:725-732.

22. Vogel, S.N., D. Johnson, P.Y. Perera, A. Medvedev, L. Lariviere, S.T. Qureshi, and D. Malo. 1999. Cutting edge: functional characterization of the effect of the C3H/HeJ defect in mice that lack an lpsn gene: *In vivo* evidence for a dominant negative mutation [In Process Citation]. *J Immunol* 162:5666-5670.

23. Ronni, T., V. Agarwal, M. Haykinson, M.E. Haberland, G. Cheng, and S.T. Smale. 2003. Common interaction surfaces of the toll-like receptor 4 cytoplasmic domain stimulate multiple nuclear targets. *Mol Cell Biol* 23:2543-2555.

24. Hirschfeld, M., Y. Ma, J.H. Weis, S.N. Vogel, and J.J. Weis. 2000. Cutting edge: repurification of lipopolysaccharide eliminates signaling through both human and murine toll-like receptor 2. *J Immunol* 165:618-622.

25. Karp, C.L., M. Wysocka, L.M. Wahl, J.M. Ahearn, P.J. Cuomo, B. Sherry, G. Trinchieri, and D.E. Griffin. 1996. Mechanism of suppression of cell-mediated immunity by measles virus [published erratum appears in Science 1997 Feb 21;275(5303):1053]. *Science* 273:228-231.

26. Latz, E., A. Visintin, E. Lien, K.A. Fitzgerald, B.G. Monks, E.A. Kurt-Jones, D.T. Golenbock, and T. Espevik. 2002. Lipopolysaccharide rapidly traffics to and from the Golgi apparatus with the toll-like receptor 4-MD-2-CD14 complex in a process that is distinct from the initiation of signal transduction. *J Biol Chem* 277:47834-47843.

27. Ogata, H., I. Su, K. Miyake, Y. Nagai, S. Akashi, I. Mecklenbrauker, K. Rajewsky, M. Kimoto, and A. Tarakhovsky. 2000. The toll-like receptor protein RP105 regulates lipopolysaccharide signaling in B cells. *J Exp Med* 192:23-29.

28. Lutz, M.B., N. Kukutsch, A.L. Ogilvie, S. Rossner, F. Koch, N. Romani, and G. Schuler. 1999. An advanced culture method for generating large quantities of highly pure dendritic cells from mouse bone marrow. *J Immunol Methods* 223:77-92.

29. Atabani, S.F., A.A. Byrnes, A. Jaye, I.M. Kidd, A.F. Magnusen, H. Whittle, and C.L. Karp. 2001. Natural measles causes prolonged suppression of interleukin-12 production. *J Infect Dis* 184.

30. Jeanmougin, F., J.D. Thompson, M. Gouy, D.G. Higgins, and T.J. Gibson. 1998. Multiple sequence alignment with Clustal X. *Trends Biochem Sci* 23:403-405.

31. Devereux, J., P. Haeberli, and O. Smithies. 1984. A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res* 12:387-395.

32. Jones, D.T., W.R. Taylor, and J.M. Thornton. 1994. A model recognition approach to the prediction of all-helical membrane protein structure and topology. *Biochemistry* 33:3038-3049.

33. Hirokawa, T., S. Boon-Chieng, and S. Mitaku. 1998. SOSUI: classification and secondary structure prediction system for membrane proteins. *Bioinformatics* 14:378-379.

34. http://folding.chmcc.org/.

35. Jarrossay, D., G. Napolitani, M. Colonna, F. Sallusto, and A. Lanzavecchia. 2001. Specialization and complementarity in microbial molecule recognition by human myeloid and plasmacytoid dendritic cells. *Eur J Immunol* 31:3388-3393.

36. Kadowaki, N., S. Ho, S. Antonenko, R.W. Malefyt, R.A. Kastelein, F. Bazan, and Y.J. Liu. 2001. Subsets of human dendritic cell precursors express different toll-like receptors and respond to different microbial antigens. *J Exp Med* 194:863-869.

37. Krug, A., A. Towarowski, S. Britsch, S. Rothenfusser, V. Hornung, R. Bals, T. Giese, H. Engelmann, S. Endres, A.M. Krieg, and G. Hartmann. 2001. Toll-like receptor expression reveals CpG DNA as a unique microbial stimulus for plasmacytoid dendritic cells which synergizes with CD40 ligand to induce high amounts of IL-12. *Eur J Immunol* 31:3026-3037.

38. Ito, T., R. Amakawa, T. Kaisho, H. Hemmi, K. Tajima, K. Uehira, Y. Ozaki, H. Tomizawa, S. Akira, and S. Fukuhara. 2002. Interferon-alpha and interleukin-12 are induced differentially by Toll-like receptor 7 ligands in human blood dendritic cell subsets. *J Exp Med* 195:1507-1512.

39. Espevik, T., E. Latz, E. Lien, B. Monks, and D.T. Golenbock. 2003. Cell distributions and functions of Toll-like receptor 4 studied by fluorescent gene constructs. *Scand J Infect Dis* 35:660-664.

40. Fitzgerald, K.A., D.C. Rowe, B.J. Barnes, D.R. Caffrey, A. Visintin, E. Latz, B. Monks, P.M. Pitha, and D.T. Golenbock. 2003. LPS-TLR4 signaling to IRF-3/7 and NF-kappaB involves the toll adapters TRAM and TRIF. *J Exp Med* 198:1043-1055.

41. Sandor, F., E. Latz, F. Re, L. Mandell, G. Repik, D.T. Golenbock, T. Espevik, E.A. Kurt-Jones, and R.W. Finberg. 2003. Importance of extra- and intracellular domains of TLR1 and TLR2 in NFkappa B signaling. *J Cell Biol* 162:1099-1110.

42. Kurt-Jones, E.A., L. Popova, L. Kwinn, L.M. Haynes, L.P. Jones, R.A. Tripp, E.E. Walsh, M.W. Freeman, D.T. Golenbock, L.J. Anderson, and R.W. Finberg. 2000. Pattern recognition receptors TLR4 and CD14 mediate response to respiratory syncytial virus. *Nat Immunol* 1:398-401.

43. Mambula, S.S., K. Sau, P. Henneke, D.T. Golenbock, and S.M. Levitz. 2002. Toll-like receptor (TLR) signaling in response to Aspergillus fumigatus. *J Biol Chem* 277:39320-39326.

44. Flo, T.H., L. Ryan, E. Latz, O. Takeuchi, B.G. Monks, E. Lien, O. Halaas, S. Akira, G. Skjak-Braek, D.T. Golenbock, and T. Espevik. 2002. Involvement of toll-like receptor (TLR) 2 and TLR4 in cell activation by mannuronic acid polymers. *J Biol Chem* 277:35489-35495.

45. Lien, E., J.C. Chow, L.D. Hawkins, P.D. McGuinness, K. Miyake, T. Espevik, F. Gusovsky, and D.T. Golenbock. 2001. A novel synthetic acyclic lipid A-like agonist activates cells via the lipopolysaccharide/toll-like receptor 4 signaling pathway. *J Biol Chem* 276:1873-1880.

46. Latz, E., A. Visintin, E. Lien, K.A. Fitzgerald, T. Espevik, and D.T. Golenbock. 2003. The LPS receptor generates inflammatory signals from the cell surface. *J Endotoxin Res* 9:375-380.

47. Belkaid, Y., C.A. Piccirillo, S. Mendez, E.M. Shevach, and D.L. Sacks. 2002. CD4+CD25+ regulatory T cells control Leishmania major persistence and immunity. *Nature* 420:502-507.

48. Abreu, M., P. Vora, L. Thomas, E. Arnold, and M. Arditi. 2001. Decreased expression of Toll-like receptor-4 and MD-2 correlates with intestinal epithelial cell protection against dysregulated proinflammatory gene expression in response to bacterial lipopolysaccharide. *J Immunol* 167:1609-1616.

49. Naik, S., E.J. Kelly, L. Meijer, S. Pettersson, and I.R.

Sanderson. 2001. Absence of Toll-like receptor 4 explains endotoxin hyporesponsiveness in human intestinal epithelium. *J Pediatr Gastroenterol Nutr* 32:449-453.

50. Cario, E., and D.K. Podolsky. 2000. Differential alteration in intestinal epithelial cell expression of toll-like receptor 3 (TLR3) and TLR4 in inflammatory bowel disease. *Infect Immun* 68:7010-7017.

51. Cross, A.S. 2002. Endotoxin tolerance-current concepts in historical perspective. *J Endotoxin Res* 8:83-98.

52. Wysocka, M., S. Robertson, H. Riemann, J. Caamano, C. Hunter, A. Mackiewicz, L.J. Montaner, G. Trinchieri, and C.L. Karp. 2001. Il-12 suppression during experimental endotoxin tolerance: dendritic cell loss and macrophage hyporesponsiveness. *J Immunol* 166:7504-7513.

53. Wald, D., J. Qin, Z. Zhao, Y. Qian, M. Naramura, L. Tian, J. Towne, J.E. Sims, G.R. Stark, and X. Li. 2003. SIGIRR, a negative regulator of Toll-like receptor-interleukin 1 receptor signaling. *Nat Immunol* 4:920-927.

54. Kobayashi, K., L.D. Hernandez, J.E. Galan, C.A. Janeway, Jr., R. Medzhitov, and R.A. Flavell. 2002. IRAK-M is a negative regulator of Toll-like receptor signaling. *Cell* 110:191-202.

55. Burns, K., S. Janssens, B. Brissoni, N. Olivos, R. Beyaert, and J. Tschopp. 2003. Inhibition of interleukin 1 receptor/Toll-like receptor signaling through the alternatively spliced, short form of MyD88 is due to its failure to recruit IRAK-4. *J Exp Med* 197:263-268.

56. Zhang, G., and S. Ghosh. 2002. Negative regulation of toll-like receptor-mediated signaling by Tollip. *J Biol Chem* 277:7059-7065.

57. Yamashita, Y., K. Miyake, Y. Miura, Y. Kaneko, H. Yagita, T. Suda, S. Nagata, J. Nomura, N. Sakaguchi, and M. Kimoto. 1996. Activation mediated by RP105 but not CD40 makes normal B cells susceptible to anti-IgM-induced apoptosis: a role for Fc receptor coligation. *J Exp Med* 184:113-120.

58. Yazawa, N., M. Fujimoto, S. Sato, K. Miyake, N. Asano, Y. Nagai, O. Takeuchi, K. Takeda, H. Okochi, S. Akira, T.F. Tedder, and K. Tamaki. 2003. CD19 regulates innate immunity by the toll-like receptor RP105 signaling in B lymphocytes. *Blood* 102:1374-1380.

59. Chan, V.W., I. Mecklenbrauker, I. Su, G. Texido, M. Leitges, R. Carsetti, C.A. Lowell, K. Rajewsky, K. Miyake, and A. Tarakhovsky. 1998. The molecular mechanism of B cell activation by toll-like receptor protein RP-105. *J Exp Med* 188:93-101.

60. Vogel, S.N., K.A. Fitzgerald, and M.J. Fenton. 2003. TLRs: Differential adapter utilization by Toll-like receptors mediates TLR-specific patterns of gene expression. *Mol Interventions* 3:466-477.

61. Ohashi, K., V. Burkart, S. Flohe, and H. Kolb. 2000. Cutting edge: heat shock protein 60 is a putative endogenous ligand of the toll-like receptor-4 complex. *J Immunol* 164:558-561.

62. Okamura, Y., M. Watari, E.S. Jerud, D.W. Young, S.T. Ishizaka, J. Rose, J.C. Chow, and J.F. Strauss, 3rd. 2001. The extra domain A of fibronectin activates Toll-like receptor 4. *J Biol Chem* 276:10229-10233.

63. Termeer, C., F. Benedix, J. Sleeman, C. Fieber, U. Voith, T. Ahrens, K. Miyake, M. Freudenberg, C. Galanos, and J.C. Simon. 2002.

Oligosaccharides of Hyaluronan activate dendritic cells via toll-like receptor 4. *J Exp Med* 195:99-111.

64. Johnson, G.B., G.J. Brunn, Y. Kodaira, and J.L. Platt. 2002. Receptor-mediated monitoring of tissue well-being via detection of soluble heparan sulfate by Toll-like receptor 4. *J Immunol* 168:5233-5239.

65. Smiley, S.T., J.A. King, and W.W. Hancock. 2001. Fibrinogen stimulates macrophage chemokine secretion through toll-like receptor 4. *J Immunol* 167:2887-2894.

66. Johnson, G.B., G.J. Brunn, and J.L. Platt. 2004. Cutting Edge: An endogenous pathway to systemic inflammatory response syndrome (SIRS)-like reactions through Toll-like receptor 4. *J Immunol* 172:20-24.

CLAIMS

What is claimed is:

1. A method of using of an activator of RP105 to prepare a medicament to suppress an immune response.

2. The method according to claim 1 wherein the medicament is used to prevent or treat an autoimmune disease.

3. A use according to claims 1 or 2 wherein the activator of RP105 is an oligonucleotide that activates the expression of RP105.

4. A use according to any one of claims 1 or 2 wherein the activator of RP105 is an antibody that binds to RP105.

5. A use according to any one of claims 1 to 4 wherein the medicament further comprises an ORP105 protein or a nucleic acid molecule encoding an RP105 protein.

6. A use of an RP105 protein or a nucleic acid sequence encoding an RP105 protein to prepare a medicament to inhibit an immune response.

7. A pharmaceutical composition for use in suppressing an immune response comprising an activator of RP105 in admixture with a suitable diluent or carrier.

8. A composition according to claim 7 wherein the activator is an antibody that binds RP105.

9. A composition according to claim 7 wherein the activator is an oligonucleotide

that is complimentary to a nucleic acid sequence from an RP105 gene.

10. A pharmaceutical composition for use in preventing immune suppression comprising an RP105 protein or a nucleic acid encoding an RP105 protein in admixture with a suitable diluent or carrier.

11. A method of identifying substances that can activate RP105, comprising the steps of: (a) reacting RP105 and a test substance, under conditions which allow for formation of a complex between the RP105 and the test substance, and (b) assaying for complexes of RP105 and the test substance, for free substance or for activation of RP105.

12. A method of identifying substances that can activate RP105, comprising the steps of: (a) reacting RP105 and a test substance, under conditions which allow for the activation of the RP105, and (b) assaying for activation of RP105.

13. A method of identifying substances that can activate RP105, comprising the steps of: (a) reacting RP105 and a test substance, under conditions which allow for the activation of the RP105, and (b) assaying for the inhibition of the activity or expression level of TLR4.

14. A method of using a compound identified according to claim 11 to prepare a medicament to modulate an inflammatory or immune response.

15. A method of using a compound identified according to claim12 to prepare a medicament to suppress an inflammatory or immune response.

16. A method of using a compound identified according to claim 13 to prepare a

medicament to suppress an inflammatory or immune response.

17. A method of treating a TLR-4 mediated disease or pathology in a subject comprising administering a pharmaceutically effective amount of an activator of RP105 to the subject.

18. The method according to claim 17, wherein the TLR-4 mediated disease or pathology is selected from the group consisting of psoriasis, atopic dermatitis, asthma, COPD, adult respiratory disease, arthritis, inflammatory bowel disease, Crohn's disease, ulcerative colitis, septic shock, endotoxic shock, gram negative sepsis, toxic shock syndrome, stroke, cardiac and renal reperfusion injury, glomerulonephritis, thrombosis, Alzheimer's disease, graft vs. host reaction, allograft rejections, malaria, acute respiratory distress syndrome, delayed type hypersensitivity reaction, atherosclerosis, cerebral and cardiac ischemia, osteoarthritis, multiple sclerosis, restinosis, angiogenesis, osteoporosis, gingivitis, respiratory viruses, herpes viruses, hepatitis viruses, HIV, Kaposi's sarcoma associated virus, meningitis, cystic fibrosis, pre-term labor, cough, pruritis, multi-organ dysfunction, trauma, strains, sprains, contusions, psoriatic arthritis, herpes, encephalitis, CNS vasculitis, traumatic brain injury, CNS tumors, subarachnoid hemorrhage, post surgical trauma, interstitial pneumonitis, hypersensitivity, crystal induced arthritis, acute and chronic pancreatitis, acute alcoholic hepatitis, necrotizing enterocolitis, chronic sinusitis, angiogenic ocular disease, ocular inflammation, retinopathy of prematurity, diabetic retinopathy, macular degeneration with the wet type preferred and corneal neovascularization, polymyositis, vasculitis, acne, gastric

and duodenal ulcers, celiac disease, esophagitis, glossitis, airflow obstruction, airway hyperresponsiveness, bronchiectasis, bronchiolitis, bronchiolitis obliterans, chronic bronchitis, cor pulmonae, cough, dyspnea, emphysema, hypercapnea, hyperinflation, hypoxemia, hyperoxia-induced inflammations, hypoxia, surgical lung volume reduction, pulmonary fibrosis, pulmonary hypertension, right ventricular hypertrophy, peritonitis associated with continuous ambulatory peritoneal dialysis (CAPD), granulocytic ehrlichiosis, sarcoidosis, small airway disease, ventilation-perfusion mismatching, wheeze, colds, gout, alcoholic liver disease, lupus, burn therapy, periodontitis and early transplantation.

19. The method according to claim 17, wherein the compounds of the present invention are administered in conjunction with one or more drugs, agents or therapeutics selected from the group consisting of: glucocorticoids, 5-lipoxygenase inhibitors, β-2 adrenoreceptor agonists, muscarinic M1 and M3 antagonists, muscarinic M2 agonists, NK3 antagonists, LTB4 antagonists, cysteinyl leukotriene antagonists, bronchodilators, PDE4 inhibitors, PDE inhibitors, elastase inhibitors, MMP inhibitors, phospholipase A2 inhibitors, phospholipase D inhibitors, histamine H1 antagonists, histamine H3 antagonists, dopamine agonists, adenosine A2 agonists, NK1 and NK2 antagonists, GABA-b agonists, nociceptin agonists, expectorants, mucolytic agents, decongestants, antioxidants, anti-IL-8 anti-bodies, anti-IL-5 antibodies, anti-IgE antibodies, anti-TNF antibodies, IL-10, adhesion molecule inhibitors, and growth hormones.

20. The method according to claim 17, wherein the compounds of the present invention are administered in conjunction with one or more therapeutic steroids.

21. The method according to claim 17, wherein the one or more therapeutic steroids are selected from the group consisting of corticoids, glucocorticoids, dexamethasone, prednisone, prednisalone, and betamethasone.

22. A method of treating a condition associated with cytokine production in a subject comprising administering a pharmaceutically effective amount of an activator of RP105 to the subject.

23. A method of using an activator of RP105 in the manufacture of a medicament for the treatment of a condition associated with cytokine production, wherein the condition is rheumatoid arthritis or a condition resulting from an infection.

24. The method according to claim 22 wherein the condition is a tumor necrosis factor (TNF) associated condition.

25. The method according to claim 24 wherein the TNF is TNF-α.

26. The method according to claim 22 wherein the condition is an interleukin-1 (IL-1) associated condition.

27. The method according to claim 26 wherein the IL-1 is IL-1β.

28. The method according to claim 22 wherein the condition is sepsis.

29. The method according to claim 22 wherein the condition is septic shock.

30. The method according to claim 22 wherein the condition is systemic inflammatory response syndrome

31. The method according to claim 17 or 22 wherein the condition is induced by a Toll Related Receptor (TRR) ligand.

32. The method according to claim 17 or 22 wherein the condition is induced by lipopolysaccharide (LPS).

33. The method according to claim 17 or 22 wherein the condition is induced by Gram-negative bacteria.

34. The method according to claim 17 or 22 wherein the activator is specific for RP105.

35. The method according to claim 17 or 22 wherein the activator is a chemical activator.

36. The method according to claim 17 or 22 wherein the activator is an antibody or a fragment thereof that is capable of binding specifically to RP105 or a fragment thereof.

37. The method according to claim 17 or 22 wherein the activator is a nucleic acid capable of increasing the expression of RP105.

38. A method for identifying an activator of RP105, which activator is suitable for use in the treatment of a condition associated with cytokine production wherein the condition s marked by pathogenic inflammatory or immune responses, the method comprising: (a) providing, as a first component, a cell capable of TLR4 or TLR ligand-induced cytokine production; (b) providing, as a second component, a TLR ligand or TLR4 activator; (c) contacting the first and second components in the presence of a test agent; (d) determining

whether the test agent is able to increase RP105-mediated downregulation of TLR4 activity; thereby to determine whether the test agent acts as an inhibitor of cytokine production.

39. The method according to claim 38 further comprising the step of determining whether the activator is specific for RP105.

40. The activator of TLR4-induced expression of cytokines identified or identifiable by a method as defined in claim 38.

41. A pharmaceutical formulation comprising a pharmaceutically acceptable carrier and an activator of RP105 identifiable by a method according to claim 38.

42. A method of using an activator of RP105 to study the inhibition of sepsis and/or septic shock caused by a TLR4, *in vitro*.

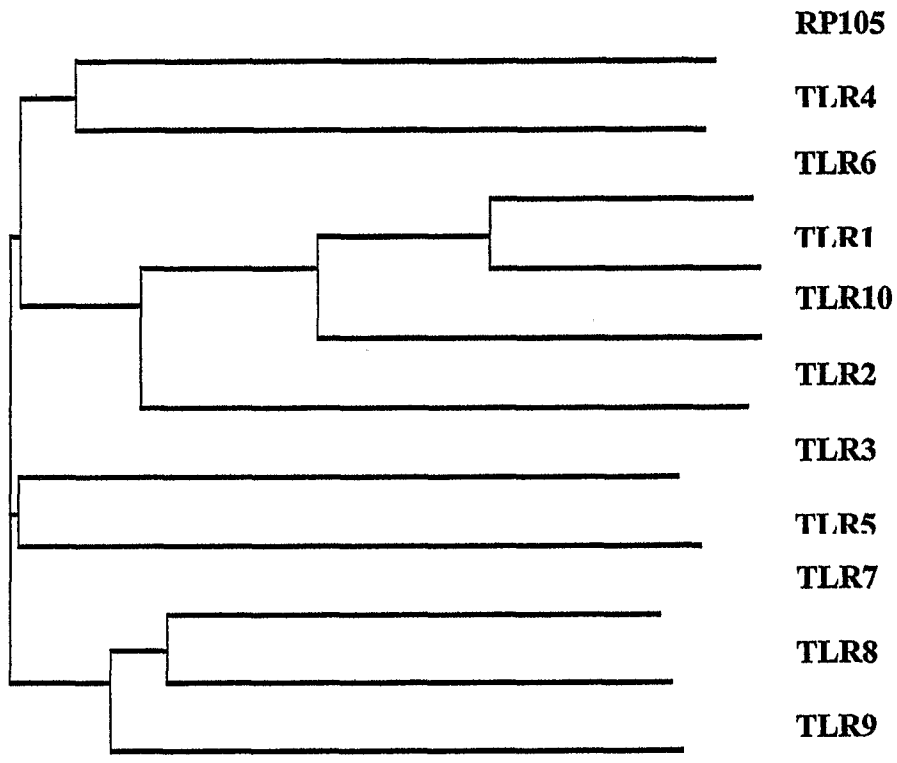


Figure 1

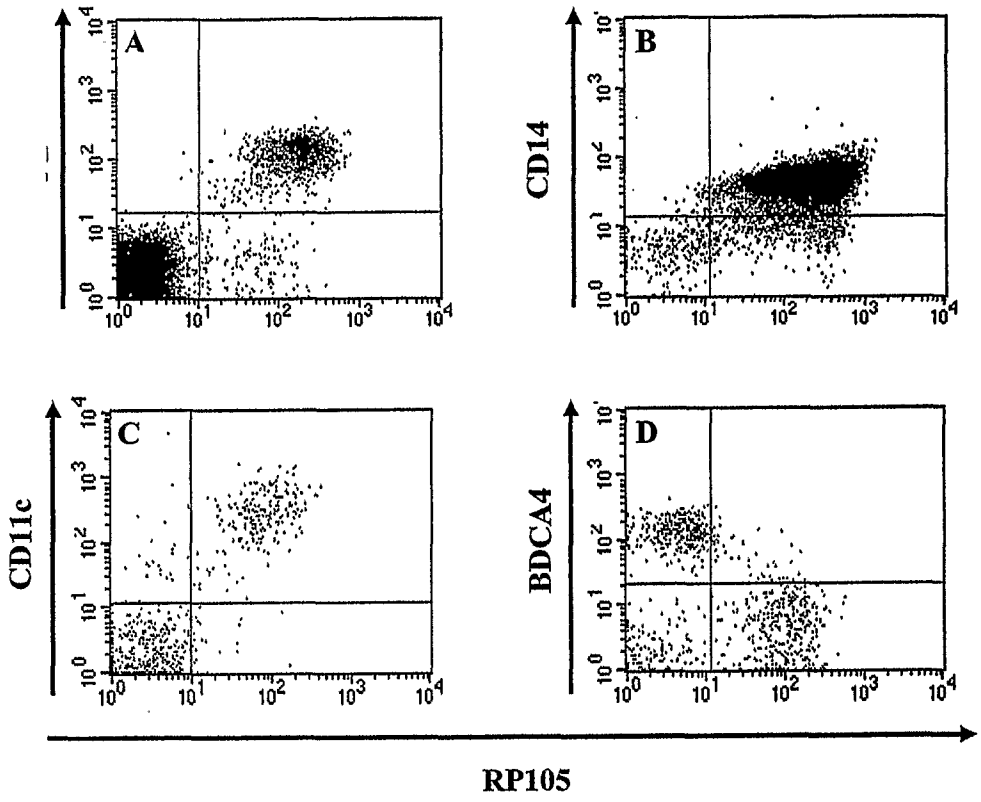


Figure 2

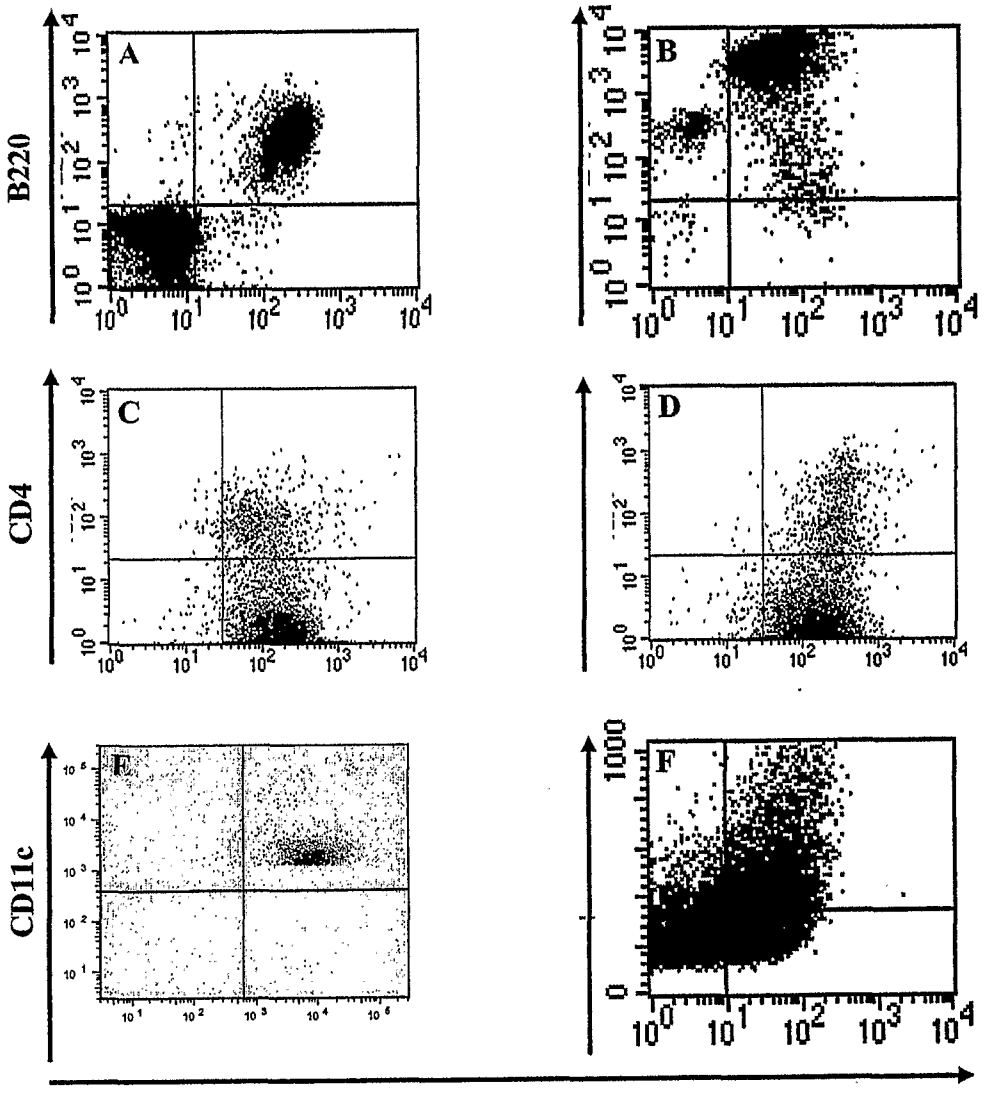


Figure 3

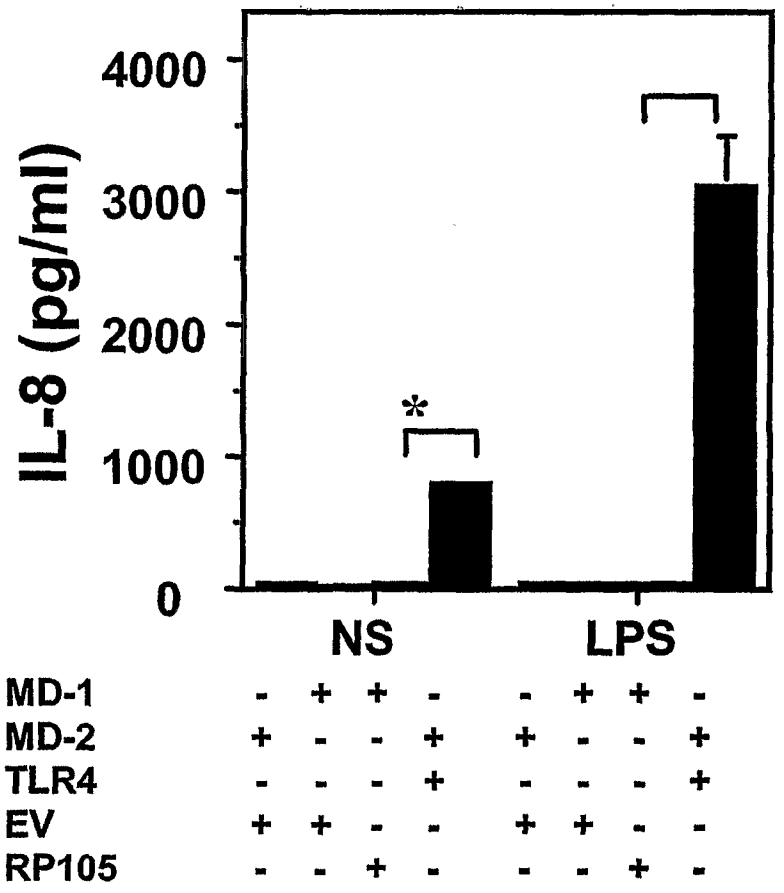


Figure 4a

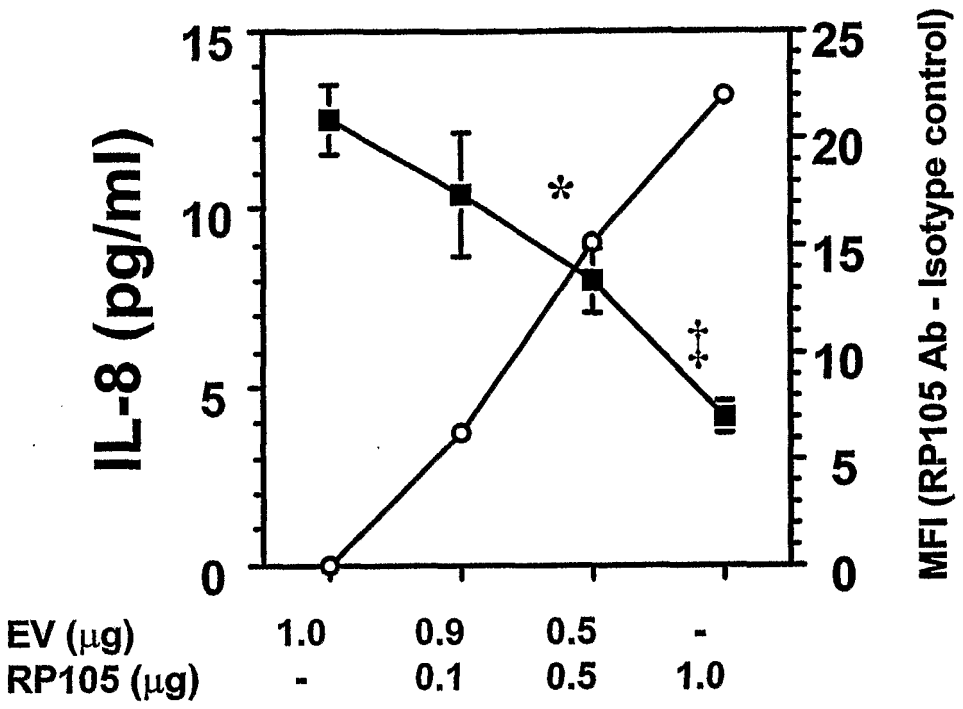

IL-8 (pg/ml)
MFI (RP105 Ab - Isotype control)
15
10
5
0
25
20
15
10
5
0
*
‡
EV (μg) 1.0 0.9 0.5 -
RP105 (μg) - 0.1 0.5 1.0

Figure 4b

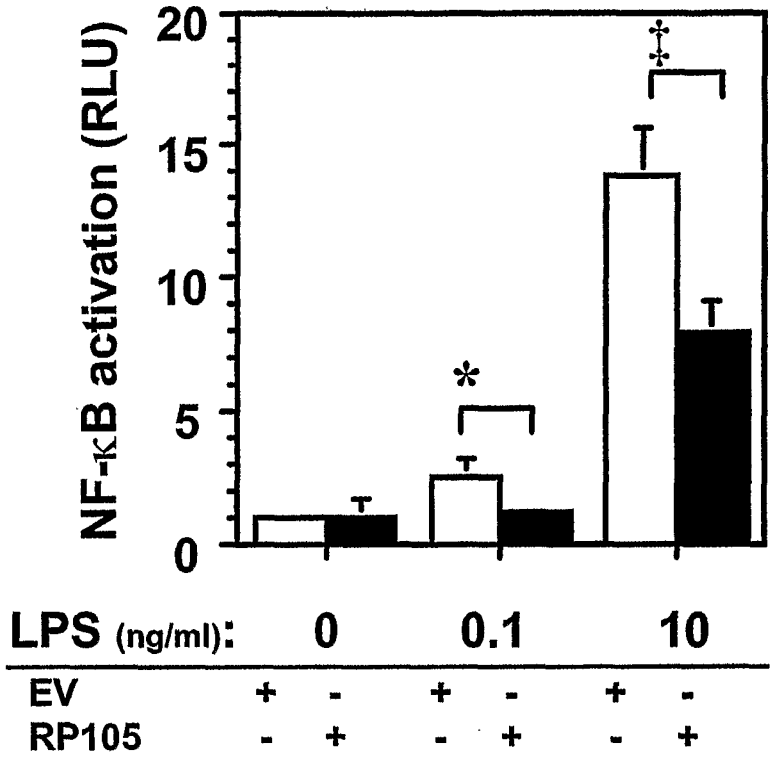
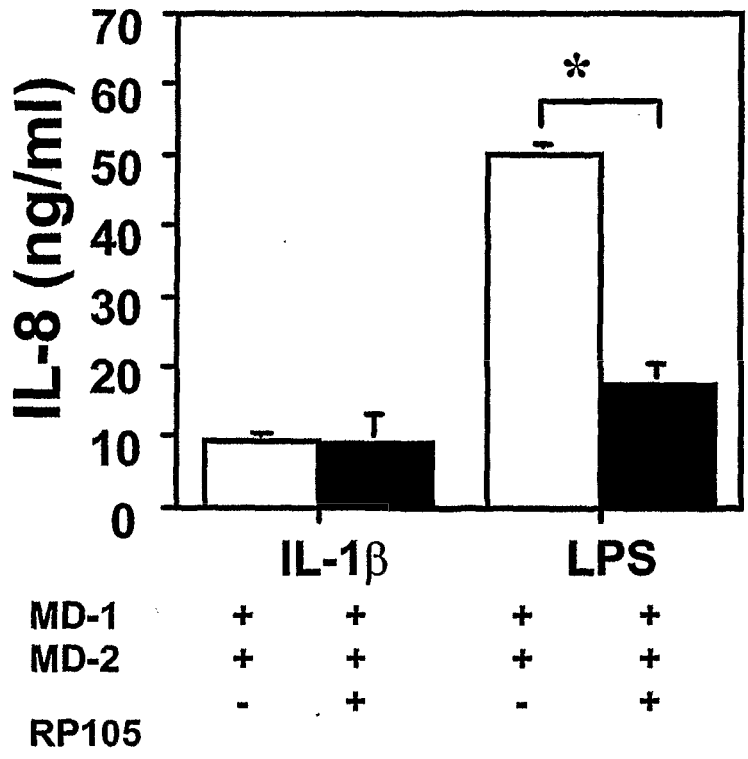


Figure 4c

A


IL-8 (ng/ml)
70
60
50
40
30
20
10
0
*
IL-1β
LPS
MD-1 + + + +
MD-2 + + + +
RP105 - + - +

B

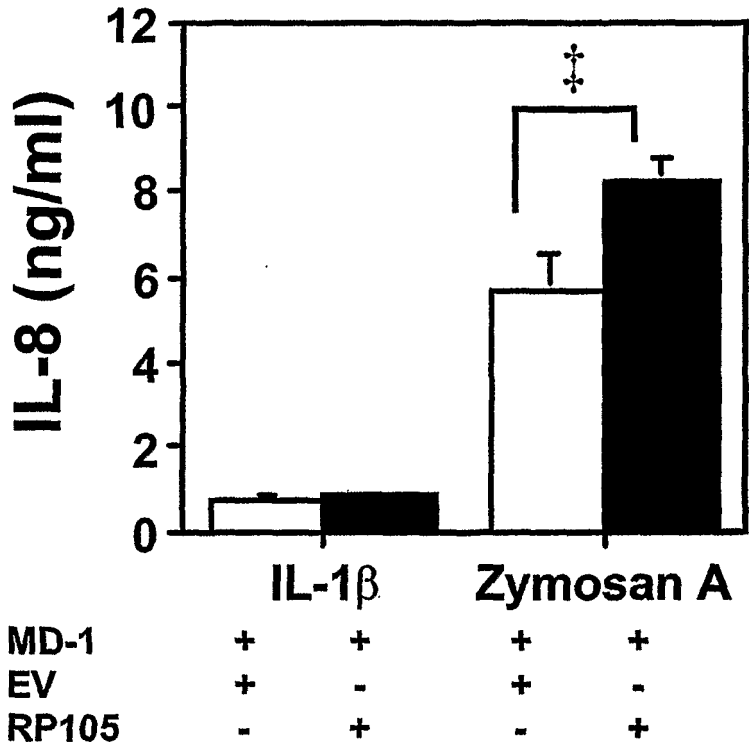

IL-8 (ng/ml)
12
10
8
6
4
2
0
‡
IL-1β
Zymosan A
MD-1 + + + +
EV + - + -
RP105 - + - +

Figure 5

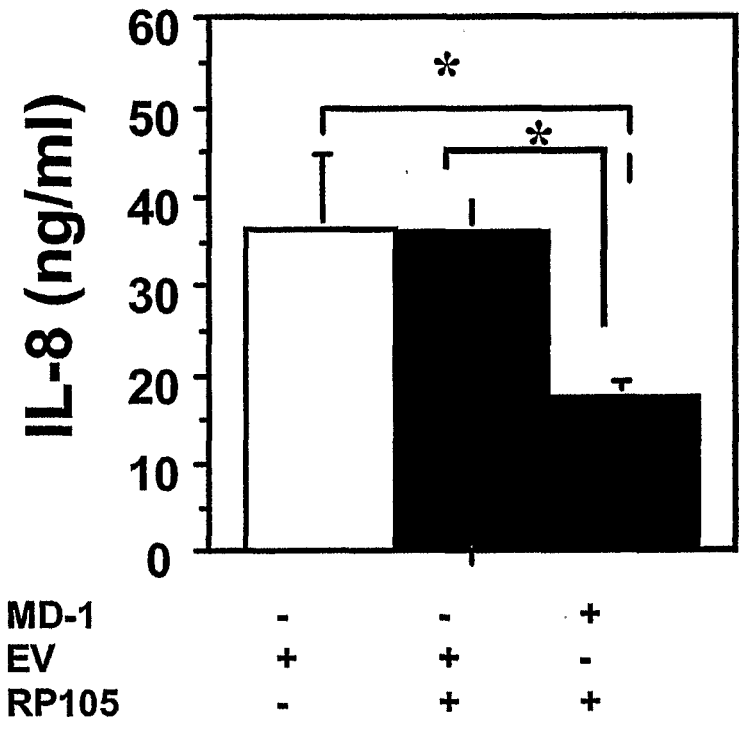


Figure 6a

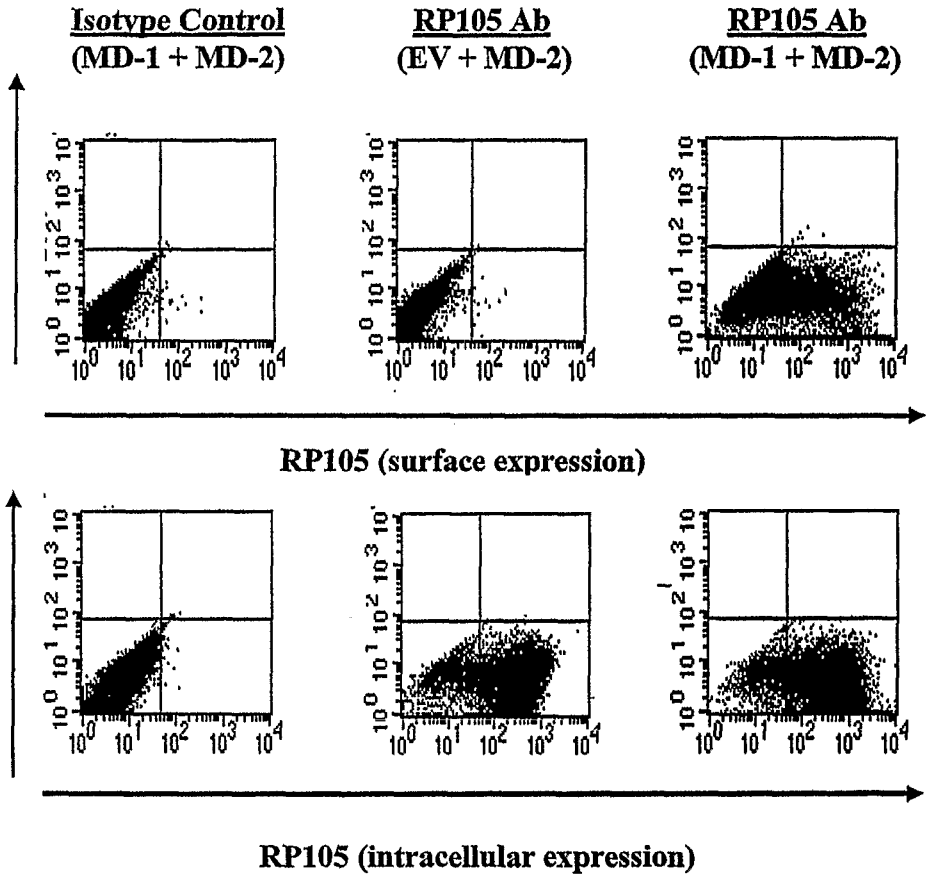
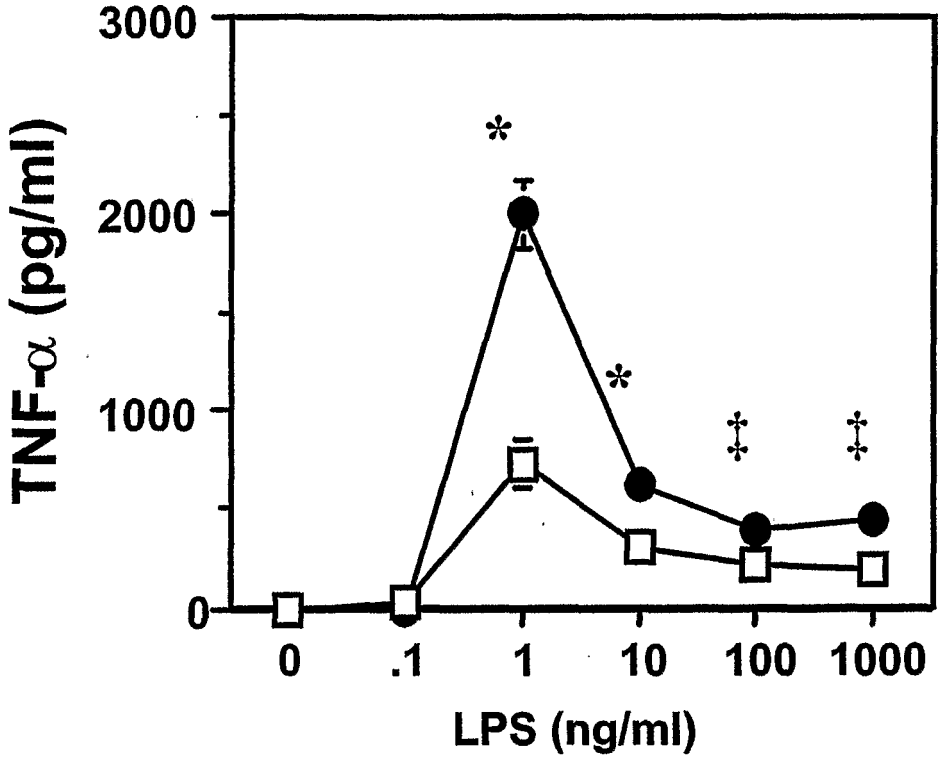

Isotype Control
(MD-1 + MD-2)
RP105 Ab
(EV + MD-2)
RP105 Ab
(MD-1 + MD-2)
RP105 (surface expression)
RP105 (intracellular expression)

Figure 6b

A


3000
2000
1000
0
TNF-α (pg/ml)
*
*
‡
‡
0
.1
1
10
100
1000
LPS (ng/ml)

B

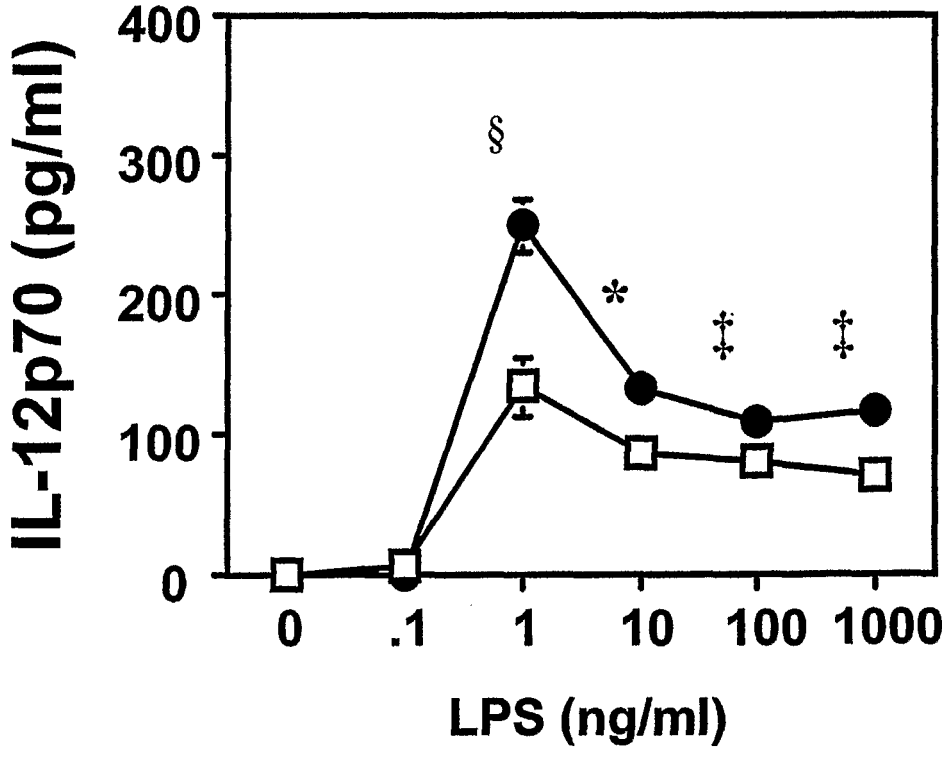

400
300
200
100
0
IL-12p70 (pg/ml)
§
*
‡
‡
0
.1
1
10
100
1000
LPS (ng/ml)

Figure 7

C

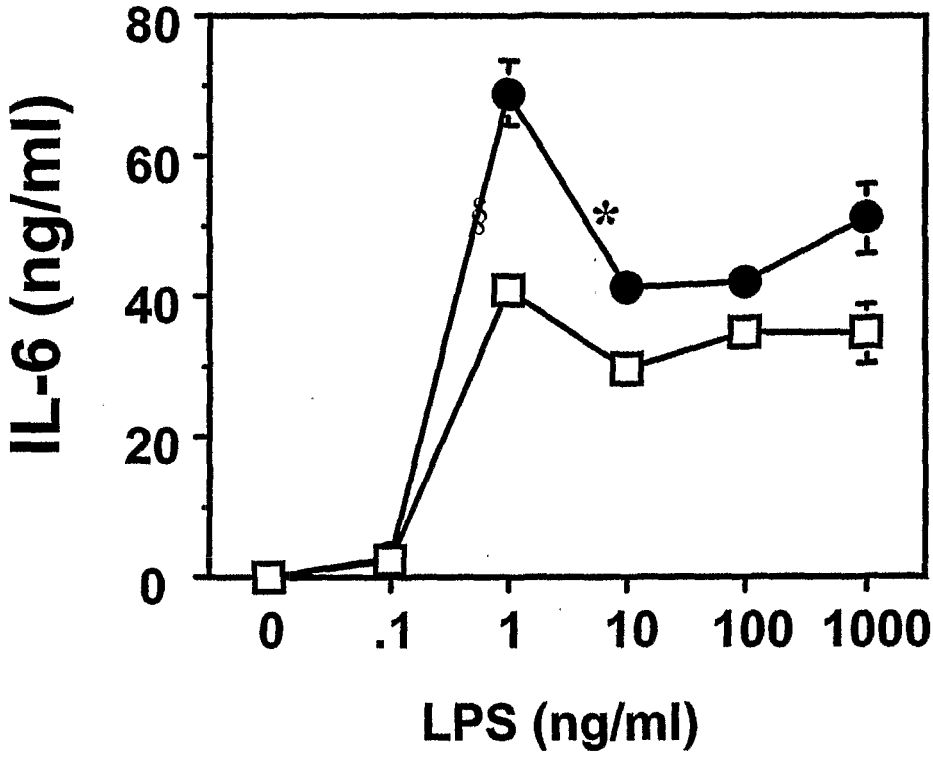
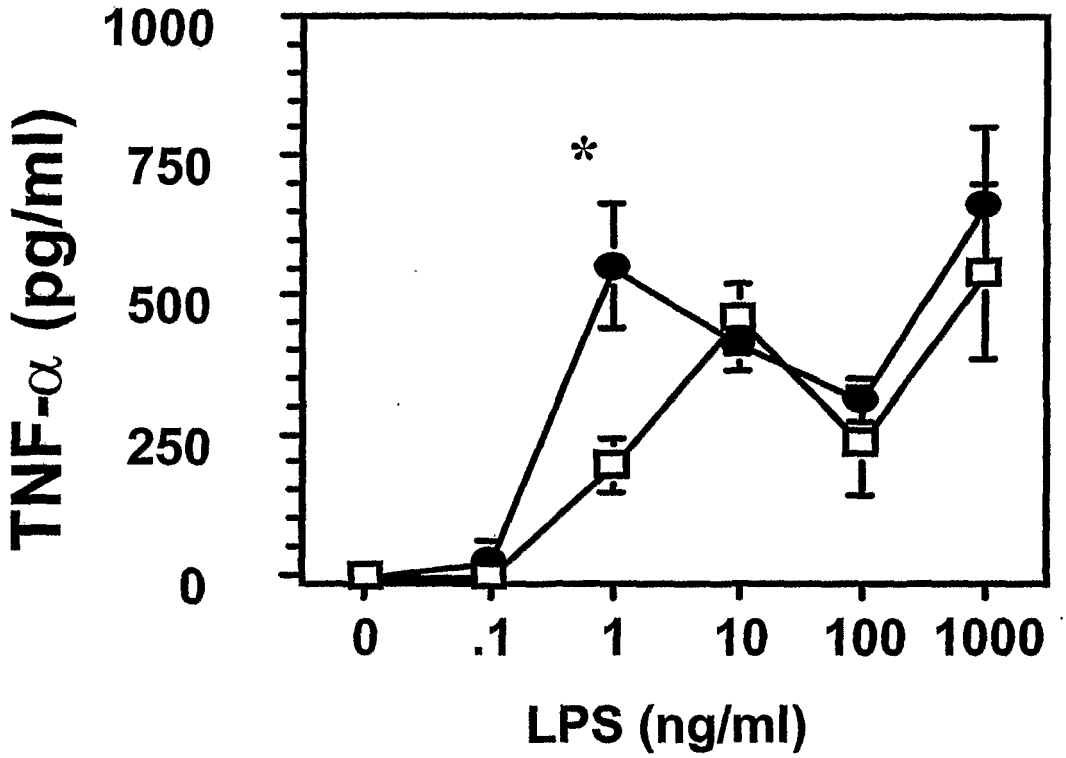


Figure 7

A


1000
750
500
250
0
TNF-α (pg/ml)
*
0
.1
1
10
100
1000
LPS (ng/ml)

B

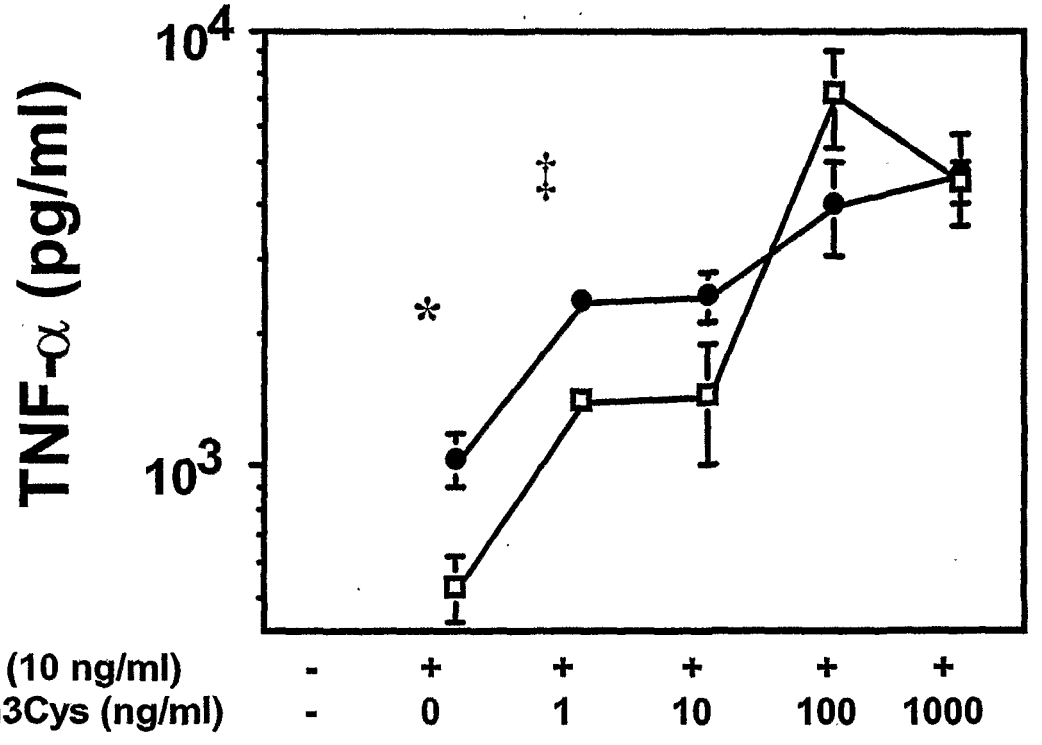

10^4
10^3
TNF-α (pg/ml)
*
‡
LPS (10 ng/ml) - + + + + +
Pam3Cys (ng/ml) - 0 1 10 100 1000

Figure 8

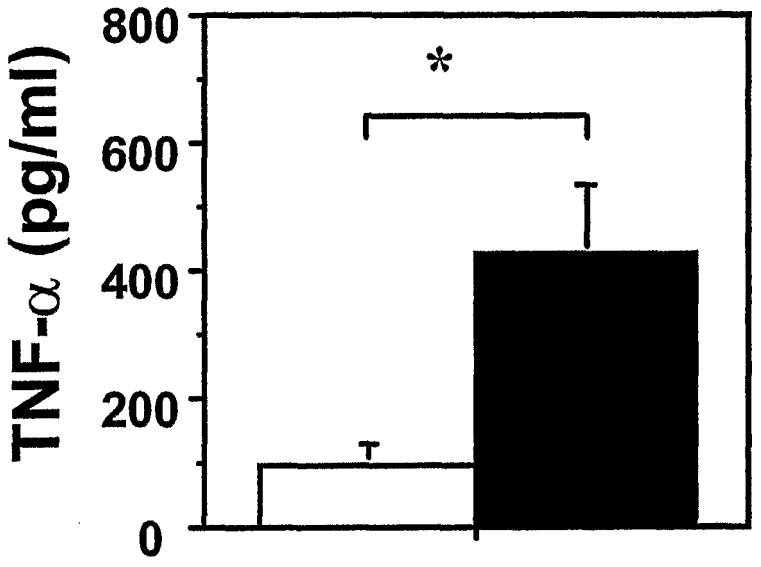
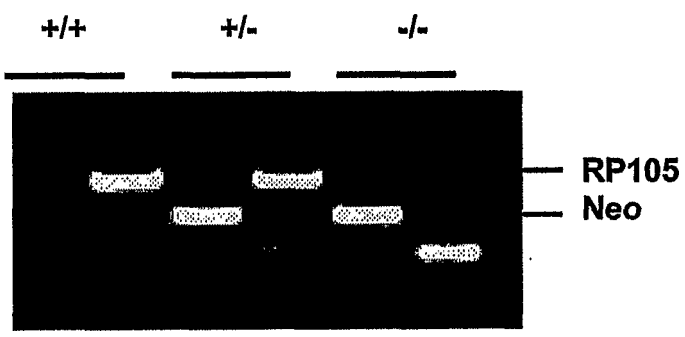


Figure 9

Figure 10.

A



B

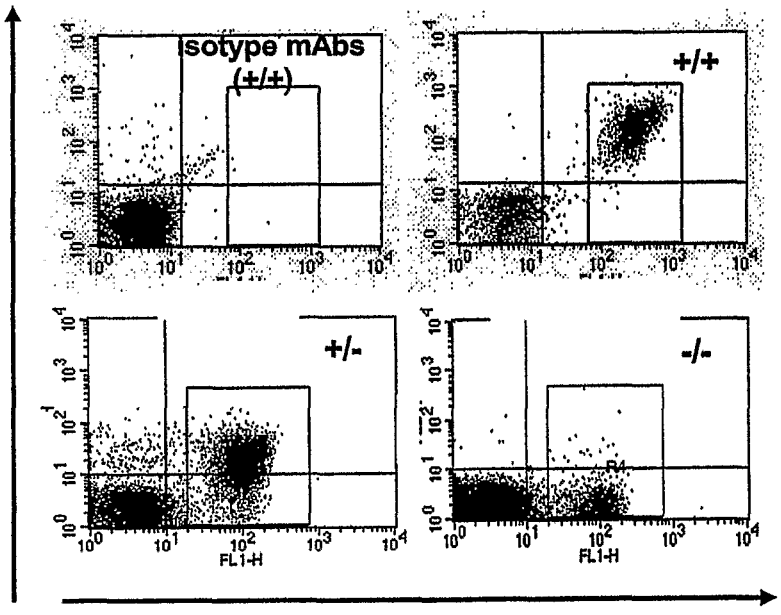


Figure 11.