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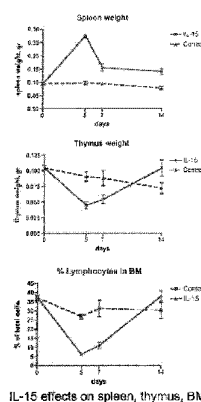
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Figure 2



IL-15 effects on spleen, thymus, BM

(57) Abstract: The present invention provides method for promoting the maturation and export of T cells from thymic tissue by contacting the thymic tissue with supraphysiological levels of interleukin (IL)-15. The present invention also provides methods for preventing, alleviating, reducing, and/or inhibiting lymphopenia or peripheral depletion of lymphocytes in a patient in need thereof by administering to the patient IL-15.

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USE OF IL-15 TO INCREASE THYMIC OUTPUT AND TO TREAT LYMPHOPENIA

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of U.S. provisional application no. 61/234,152, filed August 14, 2009; and U.S. provisional application no. 61/234,155, filed August 14, 2009. Each application is herein incorporated by reference.

FIELD OF THE INVENTION

[0002] The present invention provides compositions and methods for promoting the maturation and export of T cells from the thymus, *e.g.*, to peripheral lymphoid and non-lymphoid tissues by contacting the thymus tissue, *in vitro* or *in vivo*, with interleukin (IL)-15.

[0003] The invention additionally provides methods for preventing, alleviating, reducing, and/or inhibiting lymphopenia or depletion of lymphocytes in peripheral tissues in a patient in need thereof by administering IL-15 to the patient.

BACKGROUND OF THE INVENTION

[0004] Two common gamma-chain cytokines, IL-2 and IL-7 are currently approved or considered for both AIDS and cancer immunotherapy. *See*, Sportes, *et al.*, (2008) *J Exp Med* 205:1701-1714; Levy, Y. (2009) *J Clin Invest.* 119(4):997-100785; and Rosenberg, *et al.*, (2006) *J Immunother* 29:313-319. No clinical experience exists with the gamma-chain cytokine IL-15. *See*, Cheever, (2008) *Immunological Reviews* 222:357-368.

[0005] IL-15 is a non-redundant cytokine important for the development, survival, and proliferation of natural killer (NK) and CD8+ T-cells. It shares with IL-2 the same IL-2 beta gamma receptor and has many similar effects on lymphocytes, but unlike IL-2 is not produced by lymphocytes but by a plethora of other cells including, importantly, antigen presenting cells and macrophages, and stroma cells in several tissues. The biological effects of IL-2 and IL-15 at the level of the organism are dramatically different, as shown by work in knockout mice: lack of IL-15 causes immune system defects, whereas lack of IL-2 causes immune activation and severe autoimmunity. *See*, Waldmann, (2006) *Nat Rev Immunol* 6:595-601; and Ma, *et al.*, (2006) *Annu Rev Immunol* 24:657-679. Both cytokines are under tight and complex regulation at all steps of expression and secretion. The biological

differences of IL-2 and IL-15 are determined by their different production sites, their strength of association with membrane receptor proteins termed IL-2 Receptor alpha and IL-15 Receptor alpha (IL-15R α), respectively, and the regulation of these extra receptor molecules. IL-15 has been also reported to have a unique mechanism of action *in vivo* among the common gamma chain cytokines: IL-15 functions in a complex with IL-15R α and depends on the co-expression by the same cells of IL-15R α . See, Burkett, *et al.*, (2004) *J Exp Med* 200:825-834; Burkett, *et al.*, (2003) *Proc Natl Acad Sci USA* 100:4724-4729; Dubois, *et al.*, (2002) *Immunity* 17:537-547; Sandau, *et al.*, (2004) *J Immunol* 173:6537-6541; Schluns, *et al.*, (2004) *Blood* 103:988-994; Rubinstein, *et al.*, (2006) *Proc Natl Acad Sci USA* 103:9166-9171; Bergamaschi, *et al.*, (2008) *J Biol Chem* 283:4189-4199. IL-15 has non-redundant roles in the development and function of NK and intestinal intraepithelial lymphocytes (IELs). See, Cooper, *et al.*, (2001) *Blood* 97:3146-3151. It stimulates cytolytic activity, cytokine secretion, proliferation and survival of NK cells. See, Fehniger, *et al.*, (1999) *J Immunol* 162:4511-4520; Ross, *et al.*, (1997) *Blood* 89:910-918; and Carson, *et al.*, (1994) *J Exp Med* 180:1395-1403. IL-15 has a proliferative and survival effect on CD8 $^{+}$ memory T-cells and naive CD8 $^{+}$ T-cells. See, Tan, *et al.*, (2002) *J Exp Med* 195:1523-1532; Zhang, *et al.*, (1998) *Immunity* 8:591-599; Berard, *et al.*, (2003) *J Immunol* 170:5018-5026; and Alves, *et al.*, (2003) *Blood* 102:2541-2546.

[0006] Several studies have evaluated the effects of IL-15 administration *in vivo*. CD8 $^{+}$ memory T-cell proliferation increased after a single dose of IL-15 in normal mice. See, Zhang, *et al.*, (1998) *Immunity* 8:591-599. Administration of IL-15 to mice enhanced the antitumor activity after syngeneic bone marrow transplantation (BMT) and antigen-specific primary CD8 $^{+}$ T-cell responses following vaccination with peptide-pulsed dendritic cells. See, Rubinstein, *et al.*, (2002) *J Immunol* 169:4928-4935; Katsanis, *et al.*, (1996) *Transplantation* 62:872-875. IL-15 also enhanced immune reconstitution after allogeneic bone marrow transplantation. See, Alpdogan, *et al.*, (2005) *Blood* 105:865-873; and Evans, *et al.*, (1997) *Cell Immunol* 179:66-73. The ability of IL-15 to promote growth, survival and activation of key lymphocyte populations make it also an attractive candidate for supporting growth *in vitro* and *in vivo* of cells for adoptive cell therapy. See, Rosenberg, *et al.*, (2008) *Nat Rev Cancer* 8:299-308; and Berger, *et al.*, (2008) *J Clin Invest* 118:294-305.

[0007] We have demonstrated that efficient production of IL-15 requires the expression of IL-15 and IL-15 Receptor alpha (IL-15R α) in the same cell. See, Bergamaschi, *et al.*, (2008) *J Biol Chem* 283:4189-4199. Co-production leads to intracellular association of IL-15 and IL-15R α in the endoplasmic reticulum, stabilization of both molecules and efficient transport

to the cell surface (Figure 1). We showed that an additional critical step is the rapid cleavage and release of the IL-15/IL-15R α complex from the cell surface, both *in vitro* and *in vivo*, resulting in a soluble, systemically active form of IL-15/IL-15R α , in addition to the bioactive complex on the cell surface. See, Dubois, *et al.*, (2002) *Immunity* 17:537-547; Bergamaschi, *et al.*, (2008) *J Biol Chem* 283:4189-4199; and Budagian, *et al.*, (2004) *J Biol Chem* 279:40368-40375. Our experiments using IL-15 complexed to a deletion mutant of IL-15R α containing only the soluble Receptor alpha extracellular fragment demonstrated that this complex is bioactive *in vivo* in the absence of any membrane-bound form.

[0008] Therefore, we proposed that IL-15R α is part of a heterodimeric IL-15 cytokine, rather than functioning as a cytokine receptor. These results have been supported by other investigators, and provide the basis for a better understanding of IL-15 biology. See, Duitman, *et al.*, (2008) *Mol Cell Biol* 28:4851-4861; Mortier, *et al.*, (2008) *J Exp Med* 205:1213-1225. The results also provide the molecular basis to explain some intriguing observations, including the requirement of production of IL-15 and IL-15R α from the same cells for appropriate function *in vivo*. See, Sandau, *et al.*, (2004) *J Immunol* 173:6537-6541; and Koka, *et al.*, (2003) *J Exp Med* 197:977-984. Such results are fully explained by our finding that stabilization during co-expression in the same cell is required for physiological levels of IL-15 production. It has also been reported that the cells that physiologically express IL-15 also express IL-15R α , consistent with IL-15 production as a heterodimer in the body. See, Dubois, *et al.*, (2002) *Immunity* 17:537-547; Giri, *et al.*, (1995) *J Leukoc Biol* 57:763-766; and Ruckert, *et al.*, (2003) *Eur J Immunol* 33:3493-3503. Interpretation of all data available to date suggests that the main bioactive form of IL-15 is in a complex with the Receptor alpha either on the surface of the cells or in a soluble circulating form. It remains to be determined whether single-chain IL-15 is produced in the body in physiologically relevant levels and what is its exact function.

[0009] It has been previously reported that IL-15 secretion is inefficient. See, Bamford, *et al.*, (1998) *J Immunol* 160:4418-4426; Gaggero, *et al.*, (1999) *Eur J Immunol* 29:1265-1274; Kurys, *et al.*, (2000) *J Biol Chem* 275:30653-30659; Onu, *et al.*, (1997) *J Immunol* 158:255-262; and Tagaya, *et al.*, (1997) *Proc Natl Acad Sci USA* 94:14444-14449. We took a systematic approach to develop IL-15 expression vectors producing high levels of bioactive cytokine based on the observation that multiple regulatory steps during gene expression create bottlenecks of IL-15 production. See, Jalah, *et al.*, (2007) *DNA Cell Biol* 26:827-840; and Kutzler, *et al.*, (2005) *J Immunol* 175:112-123. We showed that combination of two approaches, namely mRNA optimization (RNA/codon optimization) of the IL-15 coding

sequences and substitution of the signal peptide with other efficient secretory signals resulted in synergistically improved expression and secretion of bioactive IL-15. *See, Jalah, et al., (2007) DNA Cell Biol* 26:827-840. Taking advantage of the stabilization of IL-15 by co-expression with IL-15R α described above, we produced equally optimized vectors for IL-15R α and combination vectors expressing both molecules, as well as combinations producing only the soluble heterodimeric cytokine. The final improvement in expression of secreted IL-15 was more than 1,000 fold compared to wt IL-15 cDNA, as determined by *in vitro* and *in vivo* experiments. We have produced similar vectors for mouse, macaque and human IL-15/IL-15R α .

[0010] Two forms of interleukin-15 (IL-15) are known, containing a long signal peptide (LSP) or a short signal peptide (SSP), respectively. The two forms are produced by alternatively spliced mRNAs and differ only in the length of their signal peptides, the 48 aa long signal peptide or the 21 aa short signal peptide (120, 121, 125-127). *See, Onu, et al., (1997) J Immunol* 158:255-262; Tagaya, *et al., (1997) Proc Natl Acad Sci USA* 94:14444-14449; Meazza, *et al., (1997) Eur J Immunol* 27:1049-1054; Meazza, *et al., (1996) Oncogene* 12:2187-2192; and Nishimura, *et al., (1998) J Immunol* 160:936-942. Whereas LSP IL-15 is secreted, SSP IL-15 remains exclusively intracellular and its function is not known. It has been proposed that SSP IL-15 may have a regulatory function since it was detected both in the cytoplasm and the nucleus of DNA-transfected cells. The SSP signal affects both stability and localization of IL-15, since lower levels of the SSP isoform were detected when the two isoforms were expressed from similar vectors. *See, Onu, et al., (1997) J Immunol* 158:255-262; Tagaya, *et al., (1997) Proc Natl Acad Sci USA* 94:14444-14449; and Bergamaschi, *et al., (2009) J Immunol*, 5:3064-72.

[0011] In Bergamaschi, we showed that, similar to LSP IL-15, SSP IL-15 is stabilized and secreted efficiently upon coexpression of IL-15R α in the same cell. *See, Bergamaschi, et al., (2009) J Immunol, supra.* Co-expression of SSP IL-15 and IL-15R α in mice showed increased plasma levels of bioactive SSP IL-15 and mobilization and expansion of NK and T cells. Therefore, SSP IL-15 is secreted and bioactive when produced as a heterodimer with IL-15R α in the same cell. The apparent stability of this complex both *in vitro* and *in vivo* is lower compared to LSP IL-15/IL-15R α complex, as revealed by direct comparisons. This results in lower production of secreted bioactive IL-15/IL-15R α . Thus, alternative splicing may provide the cell with the ability to produce different levels of bioactive IL-15. Since both forms of IL-15 may be produced in the same cell by alternative splicing, an additional level of regulation is possible. We showed that when both LSP IL-15 and SSP IL-15 are

produced in the same cell they compete for the binding to IL-15R α , resulting in lower levels of bioactive IL-15. Therefore, co-expressed SSP IL-15 acts as competitive inhibitor of LSP IL-15. This suggests that usage of alternative splicing is an additional level of control of IL-15 activity. Expression of both SSP and LSP forms of IL-15 appears to be conserved in many mammals, suggesting that SSP may be important for expressing a form of IL-15 with lower magnitude and duration of biological effects. The present invention is based, in part, on the discovery that SSP IL-15, which is produced in the thymus, is important for intra-thymic effects on lymphocyte differentiation and maturation.

BRIEF SUMMARY OF THE INVENTION

[0012] The present invention provides compositions and methods that promote the maturation of T cells in the thymus and the output or migration of mature and/or activated lymphocytes from a central lymphoid organ to peripheral tissues by administration of IL-15. The invention is based, in part, on the discovery that IL-15 promotes the migration of T cells out of the thymus and subsequently to peripheral lymphoid (*e.g.*, spleen and lymph node) and non-lymphoid tissues (*e.g.*, lung and liver). In some embodiments, the methods concurrently promote the maturation of lymphocytes in the bone marrow, *e.g.*, B cells and natural killer (NK) cells, and their migration to peripheral lymphoid and non-lymphoid tissues.

[0013] Accordingly, in one aspect, the invention provides methods of promoting T-cell maturation in thymic tissue comprising contacting the thymic tissue with IL-15.

[0014] The thymic tissue can be *in vivo* or *in vitro*.

[0015] In a related aspect, the invention provides methods of promoting the migration of lymphocytes from a central lymphoid tissue to one or more peripheral tissues in a subject in need thereof comprising administering to the subject IL-15.

[0016] With respect to the embodiments, in some embodiments, the lymphocytes are T cells and the central lymphoid tissue is thymus. In some embodiments, the lymphocytes are B cells and/or NK cells and the central lymphoid tissue is bone marrow.

[0017] In some embodiments, the lymphocytes migrating from the central lymphoid tissues are mature but not activated. In some embodiments, the lymphocytes migrating from the central lymphoid tissues are mature and activated. In some embodiments, the T cells migrating from the thymus are mature single positive (CD4⁺ or CD8⁺) T cells. The T cells induced to leave the thymus may be activated or not activated.

[0018] The invention additionally provides methods for preventing, treating, alleviating, reducing and/or inhibiting lymphopenia or depletion of lymphocytes in peripheral tissues by administration of IL-15. The present invention further provides methods for promoting the repopulation of peripheral tissues that have been depleted of lymphocytes and accelerating the recovery from lymphocyte depletion of peripheral tissues by the administration of IL-15.

[0019] Accordingly, in one aspect, the invention provides methods of preventing, reducing or inhibiting lymphopenia or depletion of lymphocytes in peripheral tissues in an individual in need thereof comprising systemically administering IL-15 to the individual.

[0020] In some embodiments, the lymphopenia or lymphocyte depletion of peripheral tissues is drug-induced. For example, the individual may be receiving anticancer drugs or antiviral drugs, or radiation therapy that induces lymphopenia or lymphocyte depletion of peripheral tissues.

[0021] In some embodiments, the IL-15 is co-administered with an agent that causes depletion of lymphocytes in peripheral tissues, *e.g.*, an anticancer or an antiviral agent. In some embodiments, the IL-15 is co-administered with radiation therapy.

[0022] In a related aspect, the invention provides methods of promoting or accelerating the repopulation of lymphocytes in peripheral tissues in an individual in need thereof comprising systemically administering IL-15 to the individual.

[0023] In some embodiments, the systemic administration of IL-15 prevents or reduces the depletion of or promotes or accelerates the repopulation of one or more of T cells, B cells or NK cells. In some embodiments, the systemic administration of IL-15 prevents or reduces the depletion of or promotes or accelerates the repopulation of one or more of CD4⁺ T cells or CD8⁺ T cells.

[0024] In some embodiments of the methods of the invention, the subject or patient is a mammal. In some embodiments, the subject or patient is a human.

[0025] When administered *in vivo* the IL-15 can be administered systemically, including without limitation, enterally (*i.e.*, orally) or parenterally, *e.g.*, intravenously, intramuscularly, subcutaneously, intradermally, intranasally, or inhalationally. In some embodiments, the IL-15 is administered locally, for example, intrathymically.

[0026] Systemic administration is at a dose that is sufficient to maintain IL-15 at supraphysiologic levels. For example, IL-15 DNA or protein can be administered at a dose sufficient to achieve plasma levels of IL-15 of about 1 to 1000 ng/ml, for example, plasma

levels of IL-15 of about 10 to 1000 ng/ml. The IL-15 and IL-15R α can be delivered in equimolar amounts. Such a range of IL-15 plasma concentrations can be achieved, *e.g.*, after intramuscular electroporation of about 0.1 mg IL-15/IL-15R α expressing DNA plasmid per kg body weight. Alternatively, an IL-15/IL-15R α protein complex can be administered at a dose of about 0.01 to 0.5 mg/kg. IL-15/IL-15R α polypeptides can be administered, *e.g.*, subcutaneously, intramuscularly, intraperitoneally or intravenously. *See, e.g.*, Rosati, *et al.*, *Vaccine* (2008) 26:5223-5229.

[0027] The IL-15 can be administered as a polypeptide or as a polynucleotide encoding IL-15. In some embodiments, the IL-15 is co-administered with IL-15R α , *e.g.*, as a heterodimer. The co-administered IL-15R α can be a polypeptide or a polynucleotide encoding IL-15R α . The co-administered IL-15R α can be in the same or different form as the IL-15. For example, both the IL-15 and the IL-15R α can be administered as polypeptides or as one or more polynucleotides encoding IL-15 and/or IL-15R α . Alternatively, one of the IL-15 and the IL-15R α can be administered as a polypeptide and the other as a polynucleotide encoding either IL-15 or IL-15R α . In some embodiments, the IL-15R α is a soluble IL-15R α . In some embodiments, the IL-15R α may be administered in the form of an Fc fusion protein or a polynucleotide that encodes an Fc fusion protein.

[0028] In some embodiments, the IL-15 and the IL-15R α are concurrently administered as one or more polynucleotides encoding IL-15 and/or IL-15R α . The polynucleotide encoding IL-15 and the polynucleotide encoding IL-15R α can be on the same or separate vectors, for example, single or multiple plasmid vectors. In some embodiments, the IL-15 and the IL-15R α polynucleotides are concurrently expressed from a plasmid vector of SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, or SEQ ID NO: 19.

[0029] In some embodiments, the polynucleotides encoding one or both of IL-15 and the IL-15R α are wild-type coding sequences. In some embodiments, the polynucleotide encoding IL-15 shares at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO:1. In some embodiments, the polynucleotide encoding IL-15R α shares at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO:5 or SEQ ID NO:7.

[0030] In some embodiments, the polynucleotides encoding one or both of IL-15 and the IL-15R α are codon optimized for improved expression over the wild-type coding sequences. In some embodiments, the polynucleotide encoding IL-15 shares at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO:3 or SEQ

ID NO:4. In some embodiments, the polynucleotide encoding IL-15R α shares at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO:9 or SEQ ID NO:11.

[0031] When expressed from a polynucleotide encoding IL-15, the coding sequence can have a native or a heterologous signal peptide. In some embodiments, the signal peptide is a native IL-15 signal peptide, for example, the native IL-15 long signal peptide or the native IL-15 short signal peptide. In some embodiments, the signal peptide is a heterologous signal peptide, for example, a signal peptide from granulocyte-macrophage colony stimulating factor (GM-CSF), tissue plasminogen activator (tPA), growth hormone, or an immunoglobulin.

[0032] In some embodiments, the peripheral tissue is a peripheral lymphoid tissue, including without limitation, spleen, lymph node, mucosal-associated lymphoid tissues (MALT), *e.g.*, tonsils and/or gut-associated lymphoid tissues (GALT), including Peyer's patches.

[0033] In some embodiments, the peripheral tissue is a peripheral non-lymphoid tissue, *e.g.*, lung, liver, kidney, heart, skin, *etc.*

[0034] Preferably, the IL-15 is administered without an antigen, *i.e.*, is not co-administered with an antigen.

[0035] In a related aspect, the invention provides a DNA vector encoding IL-15 and IL-15R α for use in promoting lymphocyte mobilization from central lymphoid tissue and migration to peripheral tissues.

[0036] In another aspect, the invention provides IL-15/IL-15R α for use in promoting lymphocyte mobilization from central lymphoid tissue and migration to peripheral tissues.

[0037] In a related aspect, the invention provides a DNA vector encoding IL-15 and IL-15R α for use in promoting the maturation and export of T cells from the thymus to peripheral tissues, including peripheral lymphoid and non-lymphoid tissues.

[0038] In another aspect, the invention provides IL-15/IL-15R α polypeptide complexes for use in promoting the maturation and export of T cells from the thymus to peripheral tissues, including peripheral lymphoid and non-lymphoid tissues.

[0039] In a related aspect, the invention provides a DNA vector encoding IL-15 and IL-15R α for use in promoting repopulation of depleted lymphocytes in peripheral tissues and/or preventing, reducing and/or inhibiting lymphopenia.

[0040] In another aspect, the invention provides IL-15/IL-15R α polypeptide complexes for use in promoting repopulation of depleted lymphocytes in peripheral tissues and/or preventing, reducing and/or inhibiting lymphopenia.

[0041] In another aspect, the invention provides stable cell lines that express IL-15/IL-15R α polypeptides. In some embodiments, the stable cell line expresses IL-15/IL-15R α in the form of a fusion protein. In some embodiments, the stable cell lines produce IL-15 and IL-15R α as different molecules. In some embodiments, the stable cell lines produce IL-15 and secreted IL-15R α deletions that lack the transmembrane anchor portion of the receptor. In some embodiments the stable cell lines produce IL-15 and fusions of IL-15R α to the an immunoglobulin Fc region. In some embodiments the stable cell lines produce IL-15 and IL-15R α fusions to polypeptides able to direct binding of the fusion to the cell surface of specific cell types. In some embodiments the stable cell lines produce IL-15 and IL-15R α fusions to polypeptides able to direct multimerization of the fusion.

[0042] Further embodiments are as described herein.

DEFINITIONS

[0043] The term “central lymphoid tissue” or “central lymphoid organ” refers to specialized lymphoid tissues where the production of new lymphocytes, or lymphopoiesis, takes place. For example, T cells develop and mature in the thymus or thymic tissue. B cells and natural killer (NK) cells develop in bone marrow tissue. *See, e.g.,* Chapter 7 of Janeway, *et al., Immunobiology*, 2001, Garland Publishing, New York.

[0044] The term “peripheral lymphoid tissue” or “peripheral lymphoid organ” refers to peripheral tissues of highly organized architecture, with distinct areas of B cells and T cells. Newly produced lymphocytes leave the central lymphoid tissues, and are carried in the blood to the peripheral lymphoid tissues. Exemplary peripheral lymphoid tissues or organs include the spleen, lymph nodes, mucosal-associated lymphoid tissues (MALT), *e.g.,* tonsils and gut-associated lymphoid tissues (GALT), including Peyer’s patches.

[0045] The term “mature lymphocyte” refers to a lymphocyte that is undergone selection and development to maturity in the central lymphoid tissue sufficient to circulate to

peripheral lymphoid tissues. With respect to T cells, a mature T cell is characterized by the expression of either CD4 or CD8, but not both (*i.e.*, they are single positive), and expression of CD3. With respect to B cells, a mature B cell is characterized by VDJ rearranged immunoglobulin heavy chain gene, VJ rearranged immunoglobulin light chain gene, and the surface expression of IgD and/or IgM. The mature B cell may also express CD19 and the IL-7 receptor on the cell surface.

[0046] The term “activated lymphocyte” refers to lymphocytes that have recognized an antigen bound to a MHC molecule and the simultaneous delivery of a co-stimulatory signal by a specialized antigen-presenting cell. Activation of lymphocytes changes the expression of several cell-surface molecules.

[0047] With respect to T cells, resting naive T cells express L-selectin, and low levels of other adhesion molecules such as CD2 and LFA-1. Upon activation of the T cell, expression of L-selectin is lost and, instead, increased amounts of the integrin VLA-4 are expressed. Activated T cells also express higher densities of the adhesion molecules CD2 and LFA-1, increasing the avidity of the interaction of the activated T cell with potential target cells, and higher densities of the adhesion molecule CD44. Finally, the isoform of the CD45 molecule expressed by activated cells changes, by alternative splicing of the RNA transcript of the CD45 gene, so that activated T cells express the CD45RO isoform that associates with the T-cell receptor and CD4. Also, with respect to cytokine production, resting T cells produce little or no IL-2 and the β and γ subunits of the IL-2 receptor. In contrast, activated T cells produce significant amounts IL-2 along with the α chain of the IL-2 receptor.

[0048] With respect to B cells, activated B cells have undergone isotype switching and secrete immunoglobulin. Naive B cells express cell-surface IgM and IgD immunoglobulin isotypes. In contrast, activated or memory B cells express and secrete IgG, IgA or IgE immunoglobulin isotypes.

[0049] The terms “output” or “migration” from a central lymphoid tissue refers to migration or export of mature lymphocytes from a central lymphocyte tissue to a peripheral tissue, including lymphoid and non-lymphoid peripheral tissues. Output includes the migration of mature T cells from the thymus and the migration of mature B cells and NK cells from the bone marrow.

[0050] The terms “treating” and “treatment” refer to delaying the onset of, retarding or reversing the progress of, or alleviating or preventing either the disease or condition to which the term applies, or one or more symptoms of such disease or condition.

[0051] The terms “lymphopenia” or “lymphocytopenia” or “lymphocytic leucopenia” interchangeably refer to an abnormally small number of lymphocytes in the circulating blood or in peripheral circulation. Quantitatively, lymphopenia can be described by various cutoffs. In some embodiments, a patient is suffering from lymphopenia when their circulating blood total lymphocyte count falls below about 600/mm³. In some embodiments, a patient suffering from lymphopenia has less than about 2000/μL total circulating lymphocytes at birth, less than about 4500/μL total circulating lymphocytes at about age 9 months, or less than about 1000/μL total circulating lymphocytes patients older than about 9 months (children and adults). Lymphocytopenia has a wide range of possible causes, including viral (*e.g.*, HIV infection), bacterial (*e.g.*, active tuberculosis infection), and fungal infections; chronic failure of the right ventricle of the heart, Hodgkin’s disease and cancers of the lymphatic system, leukemia, a leak or rupture in the thoracic duct, side effects of prescription medications including anticancer agents, antiviral agents, and glucocorticoids, malnutrition resulting from diets that are low in protein, radiation therapy, uremia, autoimmune disorders, immune deficiency syndromes, high stress levels, and trauma. Lymphopenia may also be of unknown etiology (*i.e.*, idiopathic lymphopenia). Peripheral circulation of all types of lymphocytes or subpopulations of lymphocytes (*e.g.*, CD4+ T cells) may be depleted or abnormally low in a patient suffering from lymphopenia. *See, e.g.*, The Merck Manual, 18th Edition, 2006, Merck & Co.

[0052] The term “native mammalian interleukin-15 (IL-15)” refers to any naturally occurring interleukin-15 nucleic acid and amino acid sequences of the IL-15 from a mammalian species. Those of skill in the art will appreciate that interleukin-15 nucleic acid and amino acid sequences are publicly available in gene databases, for example, GenBank through the National Center for Biotechnological Information on the worldwide web at ncbi.nlm.nih.gov. Exemplified native mammalian IL-15 nucleic acid or amino acid sequences can be from, for example, human, primate, canine, feline, porcine, equine, bovine, ovine, rodentia, murine, rat, hamster, guinea pig, etc. Accession numbers for exemplified native mammalian IL-15 nucleic acid sequences include NM_172174.2 (human preproprotein); NM_172175 (human); NM_000585.3 (human preproprotein); U19843 (macaque); DQ021912 (macaque); AB000555 (macaque); NM_214390 (porcine); DQ152967 (ovine); NM_174090 (bovine); NM_008357 (murine); NM_013129 (rattus); DQ083522 (water buffalo); XM_844053 (canine); DQ157452 (lagomorpha); and NM_001009207 (feline). Accession numbers for exemplified native mammalian IL-15 amino acid sequences include NP_000576.1 (human preproprotein); NP_751914 (human preproprotein);

CAG46804 (human); CAG46777 (human); AAB60398 (macaque); AAY45895 (macaque); NP_999555 (porcine); NP_776515 (bovine); AAY83832 (water buffalo); ABB02300 (ovine); XP_849146 (canine); NP_001009207 (feline); NP_037261 (rattus); and NP_032383 (murine).

[0053] The term “interleukin-15” or “IL-15” refers to a polypeptide that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to a native mammalian IL-15 amino acid sequence, or a nucleotide encoding such a polypeptide, is biologically active, meaning the mutated protein (“mutein”) has functionality similar (75% or greater) to that of a native IL-15 protein in at least one functional assay. Functionally, IL-15 is a cytokine that regulates T cell and natural killer cell activation and proliferation. IL-15 and IL-2 share many biological activities, including binding to CD122, the IL-2 β /IL-15 β receptor subunit. The number of CD8⁺ memory cells is controlled by a balance between this IL-15 and IL-2. IL-15 induces the activation of JAK kinases, as well as the phosphorylation and activation of transcription activators STAT3, STAT5, and STAT6. IL-15 also increases the expression of apoptosis inhibitor BCL2L1/BCL-x(L), possibly through the transcription activation activity of STAT6, and thus prevents apoptosis. Two alternatively spliced transcript variants of the IL-15 gene encoding the same mature protein have been reported. Exemplified functional assays of an IL-15 polypeptide include proliferation of T-cells (*see, for example*, Montes, *et al.*, *Clin Exp Immunol* (2005) 142:292), and activation of NK cells, macrophages and neutrophils. Methods for isolation of particular immune cell subpopulations and detection of proliferation (*i.e.*, ³H-thymidine incorporation) are well known in the art. Cell-mediated cellular cytotoxicity assays can be used to measure NK cell, macrophage and neutrophil activation. Cell-mediated cellular cytotoxicity assays, including release of isotopes (⁵¹Cr), dyes (*e.g.*, tetrazolium, neutral red) or enzymes, are also well known in the art, with commercially available kits (Oxford Biomedical Research, Oxford, M; Cambrex, Walkersville, MD; Invitrogen, Carlsbad, CA). IL-15 has also been shown to inhibit Fas mediated apoptosis (*see*, Demirci and Li, *Cell Mol Immunol* (2004) 1:123). Apoptosis assays, including for example, TUNEL assays and annexin V assays, are well known in the art with commercially available kits (R&D Systems, Minneapolis, MN). *See also*, Coligan, *et al.*, *Current Methods in Immunology*, 1991-2006, John Wiley & Sons.

[0054] The term “native mammalian interleukin-15 Receptor alpha (IL15R α)” refers to any naturally occurring interleukin-15 receptor alpha nucleic acid and amino acid sequences of the IL-15 receptor alpha from a mammalian species. Those of skill in the art will appreciate that interleukin-15 receptor alpha nucleic acid and amino acid sequences are publicly

available in gene databases, for example, GenBank through the National Center for Biotechnological Information on the worldwide web at ncbi.nlm.nih.gov. Exemplified native mammalian IL-15 receptor alpha nucleic acid or amino acid sequences can be from, for example, human, primate, canine, feline, porcine, equine, bovine, ovine, rodentia, murine, rat, hamster, guinea pig, etc. Accession numbers for exemplified native mammalian IL-15 nucleic acid sequences include NM_172200.1 (human isoform 2); and NM_002189.2 (human isoform 1 precursor). Accession numbers for exemplified native mammalian IL-15 amino acid sequences include NP_751950.1 (human isoform 2); and NP_002180.1 (human isoform 1 precursor).

[0055] The term “interleukin-15 receptor alpha” or “IL15R α ” refers to a polypeptide that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to a native mammalian IL15R α amino acid sequence, or a nucleotide encoding such a polypeptide, is biologically active, meaning the mutated protein (“mutein”) has functionality similar (75% or greater) to that of a native IL15R α protein in at least one functional assay. IL15R α is a cytokine receptor that specifically binds IL15 with high affinity. One functional assay is specific binding to a native IL-15 protein.

[0056] The term “soluble IL-15 Receptor alpha” or “sIL-15 α ” refers to forms of IL-15 Receptor alpha lacking the transmembrane anchor portion of the receptor and thus able to be secreted out of the cell without being anchored to the plasma membrane. Exemplary sIL-15 α include aa 31-205 and aa31-185 of the native IL-15 Receptor alpha.

[0057] An “IL-15R α Fc fusion” or an “IL-15R α fused to an Fc region” as used herein refers to forms of IL-15R α in which the protein is fused to one or more domains of an Fc region of an immunoglobulin, typically of an IgG immunoglobulin. The Fc region comprises the CH2 and CH3 domains of the IgG heavy chain and the hinge region. The hinge serves as a flexible spacer between the two parts of the Fc-Fusion protein, allowing each part of the molecule to function independently. The use of Fc fusions is known in the art (see, *e.g.*, U.S. Patent Nos. 7,754,855; 5,480,981; 5,808,029; Wo7/23614; Wo98/28427 and references cited therein. Fc fusion proteins can include variant Fc molecules (*e.g.*, as described in U.S. Patent No. 7,732,570). Fc fusion proteins can be soluble in the plasma or can associate to the cell surface of cells having specific Fc receptors.

[0058] The term “nucleic acid” refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form. The term encompasses nucleic acids containing known nucleotide analogs or modified backbone residues or linkages, which

are synthetic, naturally occurring, and non-naturally occurring, which have similar binding properties as the reference nucleic acid, and which are metabolized in a manner similar to the reference nucleotides. Examples of such analogs include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, peptide-nucleic acids (PNAs).

[0059] Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions) and complementary sequences, as well as the sequence explicitly indicated. Degenerate codon substitutions can be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer *et al.*, *Nucleic Acid Res.* 19:5081 (1991); Ohtsuka *et al.*, *J. Biol. Chem.* 260:2605-2608 (1985); Rossolini *et al.*, *Mol. Cell. Probes* 8:91-98 (1994)). The term nucleic acid is used interchangeably with gene, cDNA, mRNA, oligonucleotide, and polynucleotide.

[0060] Degenerate codon substitutions for naturally occurring amino acids are in Table 1.

TABLE 1

1 st position (5' end)	2 nd position				3 rd position (3' end)
	U(T)	C	A	G	
U(T)	Phe	Ser	Tyr	Cys	U(T)
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	STOP	STOP	A
	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	U(T)
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U(T)
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U(T)
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A

1 st position (5' end)	2 nd position				3 rd position (3' end)
	U(T)	C	A	G	
	Val	Ala	Glu	Gly	G

[0061] The terms “identical” or percent “identity,” in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same (i.e., about 70% identity, preferably 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or higher identity over a specified region (e.g., of a IL-15 or IL-15R α sequence), when compared and aligned for maximum correspondence over a comparison window or designated region) as measured using a BLAST or BLAST 2.0 sequence comparison algorithms with default parameters described below, or by manual alignment and visual inspection (*see, e.g.*, NCBI web site or the like). Such sequences are then said to be “substantially identical.” This definition also refers to, or can be applied to, the complement of a test sequence. The definition also includes sequences that have deletions and/or additions, as well as those that have substitutions. As described below, the preferred algorithms can account for gaps and the like. Preferably, identity exists over a region that is at least about 25, 50, 75, 100, 150, 200 amino acids or nucleotides in length, and oftentimes over a region that is 225, 250, 300, 350, 400, 450, 500 amino acids or nucleotides in length or over the full-length of an amino acid or nucleic acid sequences.

[0062] For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared (here, an entire “native mammalian” IL-15 amino acid or nucleic acid sequence). When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Preferably, default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

[0063] A preferred example of algorithm that is suitable for determining percent sequence identity and sequence similarity are the BLAST algorithms, which are described in Altschul *et al.*, *Nuc. Acids Res.* 25:3389-3402 (1977) and Altschul *et al.*, *J. Mol. Biol.* 215:403-410 (1990), respectively. BLAST software is publicly available through the National Center for Biotechnology Information on the worldwide web at ncbi.nlm.nih.gov/. Both default

parameters or other non-default parameters can be used. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, M=5, N=-4 and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix (*see* Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915 (1989)) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands.

[0064] The term “GC content” refers to the percentage of a nucleic acid sequence comprised of deoxyguanosine (G) and/or deoxycytidine (C) deoxyribonucleosides, or guanosine (G) and/or cytidine (C) ribonucleoside residues.

[0065] The term “operably linked” refers to a functional linkage between a first nucleic acid sequence and a second nucleic acid sequence, such that the first and second nucleic acid sequences are transcribed into a single nucleic acid sequence. Operably linked nucleic acid sequences need not be physically adjacent to each other. The term “operably linked” also refers to a functional linkage between a nucleic acid expression control sequence (such as a promoter, or array of transcription factor binding sites) and a transcribable nucleic acid sequence, wherein the expression control sequence directs transcription of the nucleic acid corresponding to the transcribable sequence.

[0066] Amino acids can be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, can be referred to by their commonly accepted single-letter codes.

[0067] “Conservatively modified variants” as used herein applies to amino acid sequences. One of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a “conservatively modified variant” where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the invention.

[0068] The following eight groups each contain amino acids that are conservative substitutions for one another:

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

[0069] The terms “mammal” or “mammalian” refer to any animal within the taxonomic classification mammalia. A mammal can refer to a human or a non-human primate. A mammal can refer to a domestic animal, including for example, canine, feline, rodentia, including lagomorpha, murine, rattus, Cricetinae (hamsters), etc. A mammal can refer to an agricultural animal, including for example, bovine, ovine, porcine, equine, etc.

[0070] The term “therapeutically effective amount” refers to the dose of a therapeutic agent or agents sufficient to achieve the intended therapeutic effect with minimal or no undesirable side effects. A therapeutically effective amount can be readily determined by a skilled physician, e.g., by first administering a low dose of the pharmacological agent(s) and then incrementally increasing the dose until the desired therapeutic effect is achieved with minimal or no undesirable side effects.

[0071] The term “supraphysiologic levels” refers to levels of IL-15 in a particular tissue, e.g., blood, plasma, serum, thymus, that are above naturally occurring physiologic levels. Supraphysiologic levels of IL-15 in a tissue can also be achieved when the concentration of IL-15 in that tissue is sustained above naturally occurring levels for an extended period of time, e.g., for consecutive days or weeks or for the duration of therapeutic treatment. For example, IL-15 DNA or protein can be administered at a dose sufficient to achieve plasma levels of IL-15 of about 1 to 1000 ng/ml, for example, plasma levels of IL-15 of about 10 to 1000 ng/ml. The IL-15 and IL-15R α can be delivered in equimolar amounts. Alternatively, an IL-15/IL-15R α protein complex can be administered at a dose of about 0.01 to 0.5 mg/kg.

[0072] The term “co-administer” refers to the presence of two pharmacological agents, e.g., IL-15 and IL-15R α , in the blood at the same time. The two pharmacological agents can be administered concurrently or sequentially.

[0073] The term “consisting essentially of” refers to administration of the pharmacologically active agents expressly recited, *e.g.*, IL-15 and IL-15R α , and excludes pharmacologically active agents not expressly recited, *e.g.*, an antigen. The term consisting essentially of does not exclude pharmacologically inactive or inert agents, *e.g.*, physiologically acceptable carriers or excipients.

BRIEF DESCRIPTION OF THE DRAWINGS

[0074] Figure 1 illustrates a schematic of the mutual stabilization of IL-15 and IL-15 α .

[0075] Figure 2 illustrates the effects of systemic co-administration of polynucleotides expressing IL-15 and IL-15R α on spleen weight (top panel), thymus weight (middle panel) and percentage of lymphocytes in the bone marrow (bottom panel).

[0076] Figure 3 illustrates the effects of systemic co-administration of polynucleotides expressing IL-15 and IL-15R α on T cell maturation in the thymus. Double positive CD4+CD8+ T cells are decreased with a concomitant increase in CD3^{high} single positive T cells (*i.e.*, CD4+ or CD8+ T cells).

[0077] Figure 4 illustrates the migration of dividing carboxyfluorescein succinimidyl ester (“CFSE”)-loaded thymocytes to the lung in IL-15-treated and untreated control mice (upper panels). The lower panels show increased expression of CD122 (IL-2R β /IL-15R β) on lymphocytes, *e.g.*, total T cells and CD+ T cells, in the lung.

[0078] Figure 5 illustrates lymphocyte reconstitution in lung tissue of IL-15 knock-out (KO) mice treated with plasmid DNA encoding IL-15/IL-15R α compared to untreated control KO mice.

[0079] Figure 6 provides a schematic of the time course of a lymphodepletion experiment.

[0080] Figure 7 illustrates spleen weight over time after cyclophosphamide (Cyp) and Cyp + IL-15/IL-15R α administration.

[0081] Figure 8 illustrates the increase in lung NK cells after Cyp administration.

[0082] Figure 9 illustrates the increase in lung T cells in the presence of IL-15/IL-15R α .

[0083] Figure 10 illustrates that CD8+ T cells partially recover after IL-15/IL-15R α administration.

[0084] Figure 11 illustrates the increase in lung CD8⁺ T cells in the presence of IL-15/IL-15R α as reflected in the change of the ratio of CD8⁺ to CD4⁺ T cells after IL-15 administration.

[0085] Figure 12 illustrates a T cell analysis in the spleen after Cyp and IL-15/IL-15R α administration.

[0086] Figure 13 illustrates the full recovery of bone marrow T cells after IL-15/IL-15R α administration.

[0087] Figure 14 illustrates the IL-15/IL-15R α treatment protocol for lymphopenic mice used in Example 3.

[0088] Figure 15 illustrates that a single administration of IL-15/IL-15sR α -encoding DNA is sufficient for the complete recovery of NK cells in spleen and lung 5 days after DNA injection.

[0089] Figure 16 illustrates that IL-15/IL-15sR α administration promotes the recovery of CD8 T cells within 10 days after treatment, without significantly affecting the recovery of CD4 T cells.

[0090] Figure 17 illustrates that high levels of circulating IL-15/IL-15sR α promote a transient increase in the Teffector/Treg ratio after lymphoablation.

[0091] Figure 18 illustrates IL-15 levels in serum following hydrodynamic delivery of DNA vectors expressing different forms of IL-15.

[0092] Figure 19 illustrates CD25 expression on the surface of spleen T cells after IL-15/IL-15R α DNA delivery.

[0093] Figure 20 illustrates expression of CD62L on the surface of spleen T cells after IL-15/IL-15R α DNA delivery.

[0094] Figure 21 illustrates express of CD44 on the surface of spleen T cells after IL-15/IL-15R α DNA delivery.

[0095] Figure 22 illustrates a protocol (Example 5) for administration of purified IL-15/IL-15sR α *in vivo*.

[0096] Figure 23 illustrates that purified IL-15/IL-15R α is bioactive *in vivo*.

DETAILED DESCRIPTION

1. Introduction

[0097] The present invention is based, in part, on the surprising discovery that subjecting thymic tissue to supraphysiological levels of IL-15 promotes the maturation of T cells in the thymus from double positive CD4⁺CD8⁺ T cells to single positive (*i.e.*, CD4⁺ or CD8⁺) CD3^{high} T cells, decreases the frequency of apoptotic thymocytes, and increases the migration of mature T cells from the thymus to peripheral tissues, including lymphoid and non-lymphoid peripheral tissues.

[0098] The present invention is further based, in part, on the surprising discovery that systemic administration of supraphysiological levels of IL-15 promotes the maturation and export of lymphocytes from central lymphoid tissues (*e.g.*, in the thymus and bone marrow) to peripheral tissues, including lymphoid and non-lymphoid peripheral tissues.

2. Methods of Promoting Maturation of Lymphocytes in a Central Lymphoid Organ and the Migration of the Lymphocytes to Peripheral Tissues

[0099] The present invention provides methods of promoting T cell maturation in the thymus, decreasing apoptosis of T cells in the thymus and promoting migration or output of mature T cells from the thymus, by contacting the thymus tissue with supraphysiological levels of IL-15. The thymic tissue can be *in vivo* or *in vitro*.

[0100] When the IL-15 is administered *in vivo*, it is provided to a subject or patient or individual in need thereof. The subject can be any mammal. In some embodiments, the mammal is a human or a non-human primate. Subjects who will benefit from the present methods have a deficiency of mature thymocytes and/or other lymphocytes in peripheral tissues, including lymphoid and non-lymphoid peripheral tissues. In some embodiments, the subject is immunodeficient or has lymphopenia. In some embodiments, the subject has a drug-induced immunodeficiency, *e.g.*, due to anticancer drugs. In some embodiments, the subject has an immunodeficiency secondary to a disease, *e.g.*, HIV infection. In some embodiments, the subject may have a genetic mutation that results in a non-functional IL-15 or non-functional IL-15 receptor subunit (*e.g.*, IL-15R α , IL-15R β , or IL-15R γ).

[0101] Sustained exposure of thymic tissue to supraphysiological levels of IL-15 promotes the maturation of double positive T cells. IL-15 promotes the terminal differentiation of the thymocytes to single positive T cells expressing either CD4 or CD8. The mature T cells also may express CD122 (also known as the beta subunit of IL-2/IL-15 receptor). The mature T

cells may also express high levels of the CD3 surface protein. IL-15-induced maturation of T cells also corresponds to a reduction in the frequency of immature T cells that undergo apoptosis. By contacting the thymic tissue with supraphysiologic levels of IL-15, the CD4⁺CD8⁺ double positive and CD3^{low} T cells can be substantially eliminated as the cells mature into single positive CD3^{high} T cells. After exposure to supraphysiologic levels of IL-15, at least 60%, 70%, 80%, 90%, 95% or more of the T cells are CD4⁺ or CD8⁺ single positive CD3^{high} T cells.

[0102] IL-15-induced maturation of T cells in thymus tissue also promotes the migration of the mature T cells to the peripheral tissues, including lymphoid and non-lymphoid peripheral tissues. The mature T cells leaving the thymus may or may not be activated. For example, after about 2, 3, 4, 5, 6, 7, 8, 9, 10 or more days exposure to supraphysiologic levels of IL-15, the thymus organ may have decreased in size, *e.g.*, by at least about 30%, 40%, 50%, or more, due to IL-15-induced thymic output.

[0103] Systemic administration of supraphysiologic levels of IL-15, *e.g.*, sustained over the course of *e.g.*, about 2, 3, 4, 5, 6, 7, 8, 9, 10 or more days, also promotes the maturation and migration of lymphocytes, including NK cells, from bone marrow. For example, after about 2, 3, 4, 5, 6, 7, 8, 9, 10 or more days exposure to supraphysiologic levels of IL-15, the percentage of lymphocytes in the bone marrow may have decreased, *e.g.*, by at least about 50%, 60%, 70%, 80%, or more, due to IL-15-induced lymphocyte output from bone marrow.

[0104] At the same time that the number of lymphocytes decrease in the central lymphoid tissues, *i.e.*, in the thymus and bone marrow, the number of lymphocytes in peripheral lymphoid tissues, *e.g.*, spleen, lymph node, mucosal-associated lymphoid tissues (MALT), *e.g.*, tonsils and/or gut-associated lymphoid tissues (GALT), including Peyer's patches, increases. Furthermore, the number of lymphocytes in peripheral non-lymphoid tissues, including the lung, liver, kidney, skin, and other tissues, also increases. In some embodiments, the administration of supraphysiologic levels of IL-15 increases the number of lymphocytes, including T cells, B cells and NK cells, in the blood.

3. Methods of treating lymphopenia

[0105] As explained above, in one aspect, the invention is based on the discovery that systemic administration of supraphysiological levels of IL-15 promotes the maturation and export of lymphocytes from central lymphoid tissues (*e.g.*, in the thymus and bone marrow) to peripheral tissues, including lymphoid and non-lymphoid peripheral tissues.

[0106] Accordingly, the invention provides methods for preventing, reducing and inhibiting the depletion of lymphocytes, including T cells, B cells and natural killer (NK) cells, in peripheral circulation or tissues by systemic administration of IL-15 to a subject in need thereof. The present invention also provides methods for accelerating the recovery from and shortening the time period of depletion of lymphocytes, including T cells, B cells and natural killer (NK) cells, in peripheral circulation or tissues by systemic administration of IL-15 to a subject in need thereof.

[0107] The subject, patient or individual can be any mammal. In some embodiments, the mammal is a human or a non-human primate. In some embodiments, the individual is a domestic mammal (*e.g.*, a canine or feline), a laboratory mammal (*e.g.*, a mouse, a rat, a rabbit, a hamster), or an agricultural mammal (*e.g.*, a bovine, a porcine, a ovine, an equine). Subjects who will benefit from the present methods either already have or will have (*e.g.*, as a result of a course of drug treatment) a deficiency of mature lymphocytes in peripheral circulation or tissues, including lymphoid and non-lymphoid peripheral tissues. In some embodiments, the subject is immunodeficient or has lymphopenia. For the purposes of treatment, the patient is already suffering abnormally low levels of circulating lymphocytes. For the purposes of prevention, the patient may have normal levels of peripheral lymphocytes and is likely to experience lymphodepletion, *e.g.*, as a result of a chemotherapeutic treatment.

[0108] Standards for diagnosing lymphopenia are known in the art, and can be made by any trained physician. In some embodiments, the patient has a circulating blood total lymphocyte count that is below about 600/mm³. In some embodiments, the patient has a circulating blood total lymphocyte count that is less than about 2000/μL total circulating lymphocytes at birth, less than about 4500/μL total circulating lymphocytes at about age 9 months, or less than about 1000/μL total circulating lymphocytes patients older than about 9 months (children and adults). *See, e.g.*, The Merck Manual, 18th Edition, 2006, Merck & Co.

[0109] The origins or etiology of the depletion or abnormally low can be for any reason. Lymphocytopenia has a wide range of possible causes, including viral (*e.g.*, HIV infection), bacterial (*e.g.*, active tuberculosis infection), and fungal infections; chronic failure of the right ventricle of the heart, Hodgkin's disease and cancers of the lymphatic system, leukemia, a leak or rupture in the thoracic duct, side effects of prescription medications including anticancer agents, antiviral agents, and glucocorticoids, malnutrition resulting from diets that are low in protein, radiation therapy, uremia, autoimmune disorders, immune deficiency syndromes, high stress levels, and trauma. The lymphopenia may also be of unknown etiology (*i.e.*, idiopathic lymphopenia).

[0110] The lymphocyte depletion may involve total lymphocytes (*e.g.*, T cells, B cells, and NK cells, etc.), or may only involve a subpopulation of total lymphocytes (one or more of T cells, CD4+ T cells, CD8+ T cells, B cells, NK cells).

[0111] In some embodiments, the patient has a disease that causes depletion of peripheral circulating lymphocytes. For example, the patient may suffer from a cancer, including Hodgkin's disease and cancers of the lymphatic system, leukemia; a viral infection, including HIV or hepatitis virus. In some embodiments, the patient is receiving chemotherapy, *e.g.*, an anticancer agent, an antiviral or antiretroviral agent, or a glucocorticoid, that causes depletion of peripheral circulating lymphocytes. Exemplary pharmacological agents that can cause lymphodepletion include without limitation vinblastine, fludarabine, aclarubicin, doxorubicin, exemestane, alefacept, alemtuzumab, chloramphenicol, pamidronate, idarubicin and cyclophosphamide.

[0112] In some embodiments, the subject may have a genetic mutation that results in a non-functional IL-15 or non-functional IL-15 receptor subunit (*e.g.*, IL 15R α , IL 15R β , or IL 15R γ).

4. IL-15

[0113] The IL-15 for use in the invention can be any physiologically active (*i.e.*, functional) IL-15. The IL-15 can be delivered as a polypeptide or a polynucleotide encoding IL-15. The IL-15 can be full-length or a physiologically active fragment thereof, for example, an IL-15 fragment that retains binding to IL-15R α and/or IL-15R β , or an IL-15 fragment that promotes proliferation and/or maturation of T cells. In some embodiments, the delivered or expressed IL-15 polypeptide has one or more amino acids that are substituted, added or deleted, while still retaining the physiological activity of IL-15. In some embodiments, the delivered or expressed IL-15 shares at least 90%, 93%, 95%, 97%, 98%, 99% or 100% amino acid sequence identity with a wild-type IL-15, *e.g.*, SEQ ID NO:2. In some embodiments, the polynucleotide encoding IL-15 shares at least 90%, 93%, 95%, 97%, 98%, 99% or 100% nucleic acid sequence identity with a wild-type IL-15 coding sequence, *e.g.*, SEQ ID NO:1.

[0114] The polynucleotide encoding IL-15 may have one or more codons altered for improved expression. In some embodiments, the polynucleotide encoding IL-15 shares at least 90%, 93%, 95%, 97%, 98%, 99% or 100% nucleic acid sequence identity with a wild-type IL-15 coding sequence, *e.g.*, SEQ ID NO:3. In some embodiments, the polynucleotide encoding IL-15 shares at least 96%, 97%, 98%, 99% or 100% nucleic acid sequence identity

with a wild-type IL-15 coding sequence, *e.g.*, SEQ ID NO:4. Polynucleotides encoding IL-15 which have altered codons for improved expression are described, *e.g.*, in WO 2007/084342 and in WO 2004/059556, the entire disclosures of each of which are hereby incorporated herein by reference for all purposes.

[0115] The polynucleotide encoding IL-15 can be operably linked to polynucleotide encoding a native signal peptide sequence, *e.g.*, the long IL-15 signal peptide sequence (LSP) or the short IL-15 signal peptide sequence (SSP). In some embodiments, the nucleic acid sequence encoding a native IL-15 signal peptide is replaced with a nucleic acid sequence encoding a signal peptide from a heterologous protein. The heterologous protein can be, for example, from tissue plasminogen activator (tPA), growth hormone, granulocyte-macrophage colony stimulating factor (GM-CSF) or an immunoglobulin (*e.g.*, IgE). An example of a human GMCSF-IL-15 fusion is provided in SEQ ID NO:18. In some embodiments, the nucleic acid encoding the IL-15 is operably linked to a nucleic acid encoding an RNA export element, for example a CTE or RTE_{m26}CTE.

[0116] Preferably, the IL-15 is administered as a heterodimer with IL-15R α . One or both of the IL-15 and the IL-15R α can be delivered as a polypeptide. One or both of the IL-15 and the IL-15R α can be delivered as a polynucleotide. In one embodiment, the IL-15 and the IL-15R α are co-administered as polypeptides. In one embodiment, an IL-15 polypeptide is co-administered with a polynucleotide encoding IL-15R α . In one embodiment, an IL-15R α polypeptide is co-administered with a polynucleotide encoding IL-15.

[0117] The administered IL-15R α can be any physiologically active (*i.e.*, functional) IL-15R α . The IL-15R α can be delivered as a polypeptide or a polynucleotide encoding IL-15R α . The IL-15R α can be full-length or a physiologically active fragment thereof, for example, an IL-15R α fragment that retains specific binding to IL-15. Further, the IL-15R α , *e.g.*, a fragment that retains specific binding to IL-15 and lacks the transmembrane anchor region, can be fused to an Fc region. In some embodiments, the delivered or expressed IL-15R α polypeptide has one or more amino acids that are substituted, added or deleted, while still retaining the physiological activity of IL-15R α . In some embodiments, the delivered or expressed IL-15 shares at least 90%, 93%, 95%, 97%, 98%, 99% or 100% amino acid sequence identity with a wild-type IL-15R α , *e.g.*, SEQ ID NO:5 or SEQ ID NO:7. In some embodiments, the polynucleotide encoding IL-15 shares at least 90%, 93%, 95%, 97%, 98%, 99% or 100% nucleic acid sequence identity with a wild-type IL-15 coding sequence, *e.g.*, SEQ ID NO:6 or SEQ ID NO:8.

[0118] The polynucleotide encoding IL-15R α may have one or more codons altered for improved expression. In some embodiments, the polynucleotide encoding IL-15R α shares at least 90%, 93%, 95%, 97%, 98%, 99% or 100% nucleic acid sequence identity with a wild-type IL-15R α coding sequence, *e.g.*, SEQ ID NO:9 or SEQ ID NO:11. Polynucleotides encoding IL-15R α which have altered codons for improved expression are described, *e.g.*, in WO 2007/084342.

[0119] The polynucleotide encoding IL-15R α can be operably linked to polynucleotide encoding a native signal peptide sequence. In some embodiments, the nucleic acid sequence encoding a native IL-15R α signal peptide is replaced with a nucleic acid sequence encoding a signal peptide from a heterologous protein. The heterologous protein can be, for example, from tissue plasminogen activator (tPA), growth hormone, granulocyte-macrophage colony stimulating factor (GM-CSF) or an immunoglobulin (*e.g.*, IgE). In some embodiments, the nucleic acid encoding the IL-15R α is operably linked to a nucleic acid encoding an RNA export element, for example a CTE or RTEM26CTE.

[0120] In some embodiments, the IL-15R α can be in the form of an Fc fusion protein. Examples of sIL-15R α polypeptide sequences are shown in SEQ ID NO:17 and SEQ ID NO:20. Typically, such proteins are secreted and can be found soluble in the plasma, or they can be associated with the surface of cells expressing the Fc receptor for the Fc region of the fusion protein. Different fragments of IL-15R α can be fused to the Fc region. Two examples of functional fusions are provided as SEQ ID NO:17 and SEQ ID NO:20, containing 205 or 200 amino acids within the IL-15R α region. In some embodiments, the IL-15R α region of the fusion protein can be released by proteolytic cleavage. In some embodiments, IL-15R α functional region of the protein is linked to a polypeptide that is able to bind specific cell types via surface receptors. In some embodiments, the IL-15-R α Fc fusion protein shares at least 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity with a polypeptide selected from the group consisting of SEQ ID NO:17 and SEQ ID NO:20.

[0121] In some embodiments, a polynucleotide encoding IL-15 is co-administered with a polynucleotide encoding IL-15R α . The polynucleotide encoding IL-15 and the polynucleotide encoding IL-15R α can be administered on the same vector or on separate vectors. Preferably the polynucleotide encoding IL-15 is co-administered with a polynucleotide encoding IL-15R α are on the same vector. An example of a plasmid that encodes an IL-15R α -Fc fusion having a polypeptide sequence of SEQ ID NO:17 and a human GM-CSF signal peptide-IL-15 of SEQ ID NO:18 is provided in SEQ ID NO:16. A

second example of a plasmid that encodes an IL-15R α -Fc fusion having a polypeptide sequence of SEQ ID NO:20 and a human GM-CSF signal peptide-IL-15 of SEQ ID NO:18 is provided in SEQ ID NO:19. In some embodiments, the administered vector shares at least 95%, 97%, 98%, 99% or 100% nucleic acid sequence identity with a plasmid vector selected from the group consisting of SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:19.

[0122] It is understood by one skilled in the art that expression vectors, promoters, polyadenylation signals, and secretory peptides alternatives to those in the example sequences provided herein can be used for the expression of the optimized IL-15 and IL-15 Receptor alpha.

[0123] For the purposes of the present methods, the IL-15 is not being used as an adjuvant to enhance the immune response against a particular antigen. Therefore, in the present methods, the IL-15 is administered without an antigen. Stated another way, the IL-15 is not co-administered with an antigen.

[0124] The IL-15 (and the IL-15R α) are administered at a dose sufficient to achieve supraphysiological levels of IL-15 systemically or in the target tissue, *e.g.*, thymus, for the desired time period. The desired time period can be hours, days, weeks, or longer if necessary. In some embodiments, supraphysiological levels of IL-15 are sustained throughout the duration of treatment or until a desired therapeutic endpoint is achieved, *e.g.*, the repopulation of peripheral tissues with lymphocytes. In some embodiments, the IL-15 is administered one time, as a bolus. In some embodiments, the IL-15 is administered two or more times. When administered multiple times, the IL-15 can be administered daily, weekly, bi-weekly, monthly, or as needed to sustain supraphysiological levels of IL-15 systemically or in the target tissue.

[0125] In embodiments where the IL-15 (and the IL-15R α) are administered as a polypeptide, typical dosages can range from about 0.1 mg/kg body weight up to and including about 0.5 mg/kg body weight. In some embodiments, the dose of polypeptide is about 0.01, 0.02, 0.05, 0.08, 0.1, 0.2, 0.3, 0.4, 0.5 mg/kg body weight.

[0126] In embodiments where the IL-15 (and the IL-15R α) are administered as a polynucleotide, dosages are sufficient to achieve plasma levels of IL-15 of about 1 to 1000 ng/ml, for example, plasma levels of IL-15 of about 10 to 1000 ng/ml. Such a range of plasma concentrations can be achieved, *e.g.*, after intramuscular electroporation of about 0.1

mg IL-15/IL-15sR α expressing DNA plasmid per kg body weight.. In some embodiments, the dose of nucleic acid is about 0.02, 0.05, 0.1, 0.2, 0.5 mg/kg body weight.

[0127] The IL-15 can be administered by a route appropriate to effect systemic supraphysiological levels of IL-15 or supraphysiological levels of IL-15 in the target tissue, *e.g.*, thymus. When co-administered with IL-15R α , the IL-15 and the IL-15R α can be administered via the same or different routes. In some embodiments, the IL-15 (and the IL-15R α) are administered systemically, including without limitation, enterally (*i.e.*, orally) or parenterally, *e.g.*, intravenously, intramuscularly, subcutaneously, intradermally, intranasally, or inhalationally. In some embodiments, the IL-15 (and the IL-15R α) are administered locally, for example, intrathymically or directly into the bone marrow.

[0128] For treatment of lymphopenia, systemic administration of IL-15 promotes and accelerates the repopulation of peripheral lymphocyte populations. After administration of IL-15, the peripherally circulating lymphocytes or lymphocyte subpopulations can be at least 80%, 85%, 90% or 95% of levels considered to be normal in a healthy individual. In some embodiments, the lymphocytes or lymphocyte subpopulations are completely repopulated to normal levels. In some embodiments, the repopulation of lymphocytes is days or weeks faster in an individual who received administration of IL-15 in comparison to an individual who did not receive administration of IL-15.

[0129] Systemic administration of IL-15 also prevents, reduces or inhibits lymphocyte depletion in peripheral circulation, *e.g.*, caused by chemotherapy or radiation therapy. After administration of IL-15, the peripherally circulating lymphocytes or lymphocyte subpopulations can be maintained at levels of at least 70%, 75%, 80%, 85%, 90% or 95% of normal levels. In some embodiments, the lymphocytes or lymphocyte subpopulations are maintained at normal levels.

[0130] In some embodiments, the IL-15 is co-administered with a chemotherapeutic agent that causes or may cause lymphopenia or lymphocyte depletion in peripheral tissues. The chemotherapeutic agent may be an anticancer agent or an antiviral agent. In some embodiments, the IL-15 is administered after a course of treatment with a chemotherapeutic agent that causes or may cause lymphopenia or lymphocyte depletion in peripheral tissues. In some embodiments, the IL-15 is administered prior to, during or after a course of radiation therapy.

EXAMPLES

The following examples are offered to illustrate, but not to limit the claimed invention.

Example 1: Systemic Administration of IL-15 Promotes Maturation of T cells in the Thymus and the Migration of T cells to Peripheral Tissues

[0131] IL-15/IL-15R α DNA was expressed systemically and locally at various levels in either normal or IL-15 knockout (KO) mice to further understand IL-15 biology. *See*, Bergamaschi, *et al.*, (2008) *J Biol Chem* 283:4189-4199. Supraphysiologic levels of IL-15/IL-15R α in normal mice have rapid and profound effects in many tissues. There is a rapid and reversible increase in the size of spleen, whereas the thymus becomes smaller and bone marrow lymphocyte numbers decrease (Figure 2). We have previously shown that spleen and lymph node size increase is proportional to the amount of IL-15 in the plasma. *See*, Bergamaschi, *et al.*, (2008) *J Biol Chem* 283:4189-4199. The kinetics and composition of lymphocytes in many tissues were studied using 10 parameter flow cytometry, as well as adoptive transfer of cells and *in vivo* labeling. Our results underscore the strong effects of IL-15 at all steps of lymphocyte development, as also suggested by many investigators. *Reviewed in*, *e.g.*, Boyman, *et al.*, (2007) *Curr Opin Immunol* 19:320-326; Sprent, *et al.*, (2008) *Immunol Cell Biol* 86:312-319; Sprent and Surh, (2003) *Immunol Lett* 85:145-149; Surh, *et al.*, (2006) *Immunol Rev* 211:154-163; Surh and Sprent, (2005) *Semin Immunol* 17:183-191; and Surh and Sprent, (2008) *Immunity* 29:848-862. However, prior to the present invention, the effects of IL-15 in the thymus have not been elucidated. Our results indicate that IL-15 stimulates the maturation of CD4⁺CD8⁺ double positive thymocytes into CD3^{high} single positive T cells (Figure 3) and accelerates their rapid migration to the periphery (Figure 4). Seven days after *in situ* labeling of thymocytes, IL-15/IL-15R α promoted their migration to the lung. In the presence of IL-15/IL-15R α the lymphocytes in the lung have higher levels of IL-2/IL-15R α (CD122, *see*, Figure 4, bottom) indicating that they are activated. These results are consistent with the notion that IL-15 promotes not only accelerated exit from the thymus, but also the migration to peripheral tissues and the activation of these lymphocytes.

[0132] Our results also show that, in addition to NK and memory CD8⁺ T cells that are profoundly affected, as expected, all lymphocytes including naïve and memory CD4 and CD8 cells, and B lymphocytes are also affected to either divide, migrate or be activated. This is in

agreement with the widespread (but not universal) expression of the IL-2/IL-15 betagamma receptor. The hierarchy of responsiveness of the lymphocyte subsets to IL-15 reflects the levels of CD122 (IL-2Rbeta) on their surface. *See, Bergamaschi, et al., (2008) J Biol Chem* 283:4189-4199.

[0133] Our observations are further supported by experiments performed in an IL-15 KO model, to correct the lymphocyte defects by administering plasmid DNA encoding IL-15/IL-15R α heterodimer. IL-15 KO mice are characterized by a decrease in total T cell count that preferentially affects CD8⁺ T cells, which are almost completely absent in peripheral tissues. We show that IL-15/IL-15R α is able to repopulate non-lymphoid organs, such as lungs, with both mature CD4 and CD8 T lymphocytes. The increase in CD4 T cells upon IL-15/IL-15R α treatment is 10-fold, while the increase in the CD8⁺ population is significantly greater, reaching 100-fold (Figure 5). These results underscore the feasibility of using IL-15/IL-15R α DNA to correct defects associated with lymphopenia (*e.g.*, caused by total absence of IL-15 or of another etiology). Analysis of lymphocytes migrating in different organs in the presence of IL-15 suggests that many acquire rapidly a memory phenotype in the absence of antigen recognition and that IL-15 promotes re-entry of some lymphocytes into the thymus. The issue of lymphocyte re-entry in the thymus is controversial, and the study of IL-15 effects may contribute to the understanding of this phenomenon. *See, Sprent and Surh (2009) Immunol Cell Biol* 87:46-49; *Bosco, et al., (2009) Immunol Cell Biol* 87:50-57; *Agus, et al., (1991) J Exp Med* 173:1039-1046. Our preliminary data indicate that transfer of CFSE loaded thymocytes into normal mice results in homing into the thymus only in animals receiving IL-15.

[0134] We have found that IL-15 decreases the frequency of apoptotic thymocytes, mainly by promoting their terminal differentiation into mature single positive T cells. Our results after intrathymic injection of CFSE indicate that IL-15 increases thymic output, as reflected by the higher frequency of fully mature CFSE labeled T cells in the spleen and lung of IL-15 treated mice.

[0135] We have further observed that the enlarged spleen size upon IL-15 treatment is partially due to increased frequency of B lymphocytes, either by local proliferation, B cell migration from other compartments, or both. In addition, during *in vivo* experiments with adoptive transferred CFSE-labeled splenocytes we observed IL-15-induced proliferation of both CD4 naïve and memory T cells. In contrast to CD8⁺ T cells, which almost universally proliferate in the presence of IL-15, the CD4⁺ T cell responses appear to be restricted to a subset of cells.

Example 2: Correction of Cyclophosphamide-Induced Lymphopenia by IL-15/IL-15R α
DNA Administration

Summary

[0136] The present example shows the reversal of cyclophosphamide-induced lymphopenia in normal young mice by systemic administration of IL-15. One or two high doses of IL-15 were administered two (2) days (or two (2) and twelve (12) days) after cyclophosphamide by hydrodynamic DNA injection. The results show that mice recover faster from lymphopenia after IL-15 administration in comparison to control mice with cyclophosphamide-induced lymphopenia that did not receive IL-15. Lymphocytes recovered faster in peripheral tissues after IL-15 administration. NK cells were the first to recover, whereas T cells recovered in approximately one month. In the course of these studies, we discovered that two administrations of IL-15 improved T cell recovery over a single administration of IL-15. In addition, low and sustained levels of IL-15 provides for a more efficient repopulation of lymphocytes to the peripheral tissues in comparison to a single high dose. These results demonstrate that IL-15 is useful in treating and/or preventing lymphopenia.

Methods

Cyclophosphamide administration

[0137] Six-to-eight week old female Balb/c mice were obtained from Charles River Laboratory (Frederick, MD). Cyclophosphamide (Sigma) was dissolved in pyrogen-free saline and injected intra-peritoneally (i.p.) at a dose of 200 mg/kg of body weight. Two treatments with cyclophosphamide were performed at day -4 and -2.

DNA Injection

[0138] On day 0, hydrodynamic injection of either a control vector or IL-15 and IL-15R α expression plasmid into cyclophosphamide treated mice was performed. Empty vector DNA was also administered to the cyclophosphamide-untreated mice, as control. Briefly, 0.2 μ g to 2 μ g of DNA in 1.6 ml of sterile 0.9% NaCl were injected into mice through the tail vein within 7 seconds using a 27.5 gauge needle. Highly purified, endotoxin-free DNA plasmids were produced using Qiagen EndoFree Giga kit (Qiagen, Hilden).

Lymphocyte analysis

[0139] Mice were sacrificed at different time points (days 2-26) after DNA injection and serum, bone marrow, thymus, spleen, liver and lungs were collected for analysis.

[0140] For bone marrow lymphocyte isolation, left and right femurs were collected and centrifuged at 13,000 for 5 min, re-suspended, and centrifuged again (total of 3 times). Collected cells were re-suspended in RPMI containing 10% fetal calf serum and viable cells were counted using Acridine Orange (Molecular Probes)/Ethidium Bromide (Fisher) dye.

[0141] For splenocyte or thymocyte isolation, spleens or thymi were gently squeezed through a 100 μ m Cell Strainer (Thomas) and washed in RPMI (Gibco) to remove any remaining lymphocytes from the organ stroma. After centrifugation, the cells were re-suspended in RPMI containing 10% fetal calf serum and counted.

[0142] To isolate lymphocytes from livers or lungs, the tissues were minced and incubated with 200 U/ml of collagenase (Sigma) and 30 U/ml of DNase (Roche) for 1 h at 37°C, then single cells were collected, centrifuged and re-suspended in complete RPMI with 10% fetal calf serum.

[0143] For phenotyping, the cells were incubated with the following mix of directly conjugated anti-mouse antibodies (BD Pharmingen): CD3-APC, CD4-PerCP, CD8-PECy7, CD44-APC, CD49b-FITC, CD19-PE, CD62L-PE. Labeled cell samples were analyzed by flow cytometry using an LSR II Flow Cytometer (BD) and were analyzed using FlowJo software (Tree Star, San Carlos, CA).

[0144] Lymphocytes of the different group of mice were counted and compared. Statistical analyses were performed using the Prism Software Program. Comparisons of two groups were performed by non-parametric Mann-Whitney t test. Confidence intervals were 0.05, and all p values were two-tailed.

Results

[0145] Two injections of cyclophosphamide at days -4 and -2 were used to generate lymphodepleted mice. At day 0 (and also, for some mice at day 10) IL-15/15R α DNA expression vector was injected in the tail vein, which generated high systemic levels of bioactive IL-15/15R α , as published (Bergamaschi, *et al.*, *J Biol Chem.* (2008) 283(7):4189-99). The biological effects after injection of IL-15/15R α DNA were compared to the injection of a non-producing DNA (vector BV) as negative control in cyclophosphamide-treated animals.

[0146] Different tissues, including lung, liver, spleen, thymus and bone marrow, were extracted from mice sacrificed at days 2-26 from DNA injection and the lymphocyte populations were studied.

[0147] Cyclophosphamide treatment had strong effects on lymphocytes, as reflected in the increased spleen weight of treated animals (Figure 7). Four animals per time point were sacrificed and the spleen weight was monitored. The two groups treated with cyclophosphamide (CP+vector, treated with a non-producing DNA vector; CP+IL-15) had a smaller spleen at day 2 after DNA treatment (4 days after cyclophosphamide). At this early point and also at day 5 the IL-15 treated animals showed a statistically significant difference in spleen size, indicating accelerated recovery by IL-15.

Lung

[0148] We also analyzed lymphocyte numbers and subsets in different tissues to evaluate the effects of IL-15/15R α administration. These experiments were performed after one or two IL-15/15R α DNA administrations (at days 0 and 10).

[0149] Lung lymphocytes were evaluated in order to determine the effects of IL-15/15R α on a peripheral site, where lymphocytes need to function. IL-15 is known to affect strongly CD8⁺ T cells and NK cells. High levels of IL-15 (achieved with two injections of 2 μ g DNA at days 0 and 10), favors lymphocyte recovery in the lung after Cyp treatment.

Effects on Natural Killer (NK) cells:

[0150] Mice were treated at days -4 and -2 and injected with DNA at day 0. Two groups of mice were injected with either BV negative control DNA or with IL-15/IL-15R α DNA. The IL-15/IL-15R α -treated animals had a trend for higher NK numbers for all time points. At day 14, comparison of the group receiving empty vector with the group of 2x IL-15/IL-15R α administration (DNA injections at days 0 and 10) showed that IL-15/15R α significantly increased lung NK cell recovery ($p=0.03$).

[0151] The lymphocyte population that recovers first is the NK cells. In our experiments after cyclophosphamide treatment the NK cells recovered partially in the absence of any other intervention. IL-15/15R α administration accelerated this recovery. The best recovery was observed after two IL-15 injections at days 0 and 10. Examination at day 14 showed a significant increase in NK by IL-15 compared to Cyp ($p=0.03$). See, Figure 8.

Effects on Lung T cells

[0152] In contrast to NK cells, lung T cells do not recover as fast. The mice were treated and analyzed as above. Lung T cells were enumerated at day 14 after the first DNA injection. It was found that total T cells increased at day 14 after two IL-15/R α administrations at days 0 and 10, compared to the Cyp treated animals. See, Figure 9.

[0153] The lung T cells were also distinguished according to expression of CD4 or CD8 and compared among different groups of mice. It was found that the CD8⁺ T cells increased preferentially after IL-15/15R α administration at day 14 ($p=0.0357$). Moreover, at days 6 and 14 the CD8/CD4 ratio was increased, demonstrating the preferential stimulation of CD8⁺ T cells by IL-15. The ratio returns to normal by day 26, in the group that received IL-15/15R α . See, Figures 10 and 11.

Spleen

[0154] In the spleen, we also found that T cells recover faster after two injections of IL-15/15R α ($p=0.0357$). Similar to the results in the lung, two doses of IL-15/15R α (days 0 and 10) were able to increase spleen lymphocytes after Cyp ($p=0.03$). See, Figure 12.

Bone Marrow

[0155] Sustained high level of IL-15 (achieved with two injections of 2 μ g DNA at days 0 and 10) resulted in T cell recovery in bone marrow by day 14 after the first DNA injection (Figure 13). IL-15 affected both CD4 and CD8 compartments. Treatment with two administrations of IL-15/15R α resulted in high levels of bone marrow T cells at day 14 compared to Cyp treated animals.

Example 3: Therapeutic effects of IL-15 on lymphopenia in two different mouse strains

[0156] This example also employed Black6 mice to analyze therapeutic effects of various forms of IL-15 on lymphopenia. Two different mouse strains, BALB/c and Black6, were used in these experiments. Both strains showed accelerated lymphocyte reconstitution upon treatment with IL-15/IL-15R α .

Treatment of lymphoablated mice with IL-15 DNA

[0157] Female Balb/c or Black6 mice 6-8 weeks in age were treated intra-peritoneally with a dose of 200 mg/kg of body weight of cyclophosphamide (CYP, Figure 14). Two injections of CYP were performed at day -4 and day -2. At day 0 and day 5, hydrodynamic injection of either a control DNA or DNA expressing IL-15/IL-15sR α soluble molecule was performed. Control vector was also delivered in CYP-untreated mice as control. Mice were sacrificed at different time points: day -1 to assess the CYP-induced lymphoablation and day 5, 10, 17 and 24 to follow immune reconstitution in presence or absence of exogenous IL-15. Different tissues (spleen, thymus, bone marrow, lung and liver) were harvested and analyzed for the presence of different lymphocyte subsets. Analysis was performed by flow cytometry after staining the cells with fluorescent-labeled antibodies.

[0158] For flow analysis, isolated cells were incubated with the following directly conjugated anti-mouse antibodies (BD Pharmingen) in appropriate combinations according to the objectives of the experiment:

CD3-APC or CD3-APC-Cy7, CD4-PerCp, CD8-Pacific Blue, CD44-APC, CD62L-PE, CD19-APC-Cy7 or CD19-PeCy7, CD49b-FITC, CD25-APC-Cy7, CD122-PE. T cells were defined as CD3⁺ cells in the lymphocyte gate; NK cells were defined as CD3⁻CD49b⁺ cells.

[0159] For identification of Treg population (T CD4⁺CD25⁺FoxP3⁺ cells), the cells were fixed and permeabilized (eBioscience), and incubated with anti-mouse FoxP3-PeCy7 antibody (eBioscience). T effector cells were defined as CD3⁺FoxP3⁻ lymphocytes. Therefore, the term “Teffector” as used in here refers to all T cells except Treg.

[0160] Figure 15 shows the reconstitution of NK cell compartment in spleen and lung after CYP treatment. CYP-untreated mice were used as baseline control (squares). Two injections of CYP resulted in a drastic reduction of the absolute number of NK cells in both spleen and lung (day -1). NK cells spontaneously recover between day 10 and day 14 days after control DNA injection (triangles). One single administration of IL-15/IL-15sR α DNA was able to promote a full recovery of NK within 5 days after DNA injection. The second IL-15/IL-15sR α expressing DNA injection resulted in an even further expansion of NK cells in both spleen and lung (circles).

[0161] Figure 16 shows the reconstitution of T cell compartment in spleen and lung after CYP treatment. CYP-untreated mice were used as baseline control (squares). Two injections of CYP resulted in a 4 fold reduction in the level of splenic T cells and in 10 fold reduction in the level of T cells residing in the lung (day -1). The spontaneous recovery of T cells appeared to be slower in comparison with the recovery of NK cells and was still incomplete at day 24 after control DNA injection. The kinetics of spontaneous recovery of T CD8 and T CD4 was similar in both spleen and lung (triangles). Two injections of DNA expressing IL-15/IL-15sR α were able to fully reconstitute the T cell numbers within 10 days after DNA administration in both spleen and lung. IL-15 promoted mainly the expansion of T CD8 cells that reached normal level at day 5 after DNA injection and were boosted over normal level at day 10 after DNA injection. IL-15 did not significantly affect the recovery of T CD4 and B cells.

[0162] In addition, T cells recovering in the presence of high level of IL-15/IL-15sR α show increased T effector (Teff)/T regulatory (Treg) ratio and increased ability to secrete IFN γ and greater degranulation after in vitro stimulation. Figure 17 is an analysis of

the Teff/Treg ratio after CYP treatment for lymphodepletion and during the recovery phase. The Teff/Treg ratio increased significantly at day 10 after IL-15/15sR α DNA injection.

Example 4. DNA delivery for IL-15 to treat lymphopenia

[0163] In these examples, three preferred DNA vector combinations are evaluated for the therapeutic delivery of IL-15 to treat lymphopenia:

- 1 Co-delivery in the same cells, using preferably optimized expression plasmids expressing IL-15 and essentially full-length IL-15R α , such as SEQ ID NO:13 and SEQ ID NO:14.
- 2 Co-delivery in the same cells, using preferably optimized expression plasmids expressing IL-15 and soluble (s) IL-15R α , such as SEQ ID NO:15.
- 3 Co-delivery in the same cells, using preferably optimized expression plasmids expressing IL-15 and IL-15R α fusions to the constant region of an immunoglobulin molecule (Fc) such as SEQ ID NO:16 and SEQ ID NO:19. The construction of Fc fusion proteins is known in the art. Such constructs have been used in *in vivo* experiments in mice to show that IL-15 and IL15R α -Fc fusion heterodimers are active *in vivo*.

[0164] Delivery of IL-15/IL-15R α heterodimer by approach (1) above leads to expression of both plasma membrane-bound and secreted IL-15/IL-15R α . Delivery by approach (2) leads to exclusively secreted IL-15/IL-15R α heterodimer. Delivery by approach (3) leads to a secreted bioactive heterodimer, which is then bound to cells expressing the Fc Ab receptor on their surface. These cells can present the IL-15/IL-15R α Fc heterodimer to neighboring cells, resulting in activation.

[0165] The three types of vectors have been tested in mice and have been shown to produce systemically bioactive levels of IL-15/IL-15R α (see Figure 18, showing expression of the three types of complexes). Because the localization, trafficking and stability of the different types of complexes vary, the biological effects on lymphocytes is also variable. Figure 18 shows expression of different IL-15/IL-15R α heterodimeric forms in mice by hydrodynamic injection of DNA vectors. Mice were injected at the tail vein (hydrodynamic delivery) with 0.1 μ g of DNA expressing the different forms of IL-15/IL-15R α . Plasma levels of IL-15 were measured at days 1 and 2.5 by R&D Quantiglo ELISA. Measurement of plasma levels of IL-15 produced by the different vectors showed that the highest plasma levels were achieved by the DNA vector producing IL-15/IL-15R α Fc fusion. The stability of the produced proteins was also different, with the IL-15/IL-15R α Fc and the IL-15/IL-15R α full

length showing the greatest stability. The IL-15/sIL-15R α that is not cell associated was less stable.

[0166] Table 2 shows the CD4/CD8 ratios measured in the spleen and lung of mice treated with different IL-15/IL-15R α heterodimeric forms, 2 ½ days after hydrodynamic injection of 0.1 µg of DNA vector (see Figure 17).

<u>VECTOR</u>	<u>Spleen</u>	<u>Lung</u>
IL-15/IL-15R α (full length)	1.36	0.8
IL-15/sIL-15R α (soluble)	0.81	0.24
IL-15/IL-15R α Fc fusion to Fc	0.63	0.52
DNA vector control	2	1.61

[0167] In these experiments, it was discovered that the different molecules have differential effects on lymphocytes. Therefore, the different IL-15 complexes can be used alone or in combinations for the most beneficial treatment under specific conditions. For example, delivery of combinations of IL-15/sR α soluble complex and IL-15/15R α Fc fusion complex provides the opportunity to deliver both soluble and cell-bound IL-15 (through the Fc receptor) at different levels and proportions.

[0168] In addition to the different ratios of CD4/CD8 cells (as shown in Table 1), the different IL-15 heterodimers also showed differences in the effects on other surface markers of lymphocytes. Figure 19 shows that IL-15/15R α Fc expression induced high levels of CD25 (IL-2 Receptor alpha) on both T CD4 and T CD8 cells, whereas the other forms of IL-15/IL-15R α heterodimers did not affect CD25 expression strongly.

[0169] Figure 20 shows that IL-15/IL-15R α Fc increased the levels of CD62L on the surface of spleen T cells, whereas the other forms of IL-15/IL-15R α either did not affect or decreased average levels of CD62L on spleen T cells. In contrast, IL-15/IL-15R α Fc was less effective in increasing CD44 on spleen T cells compared to either IL-15/IL-15R α full-length or IL-15/IL-15sR α (Figure 21).

Example 5. Protein Delivery

[0170] As an alternative method to provide IL-15, delivery of purified protein can be used. Protein purification from cell lines over-producing IL-15/IL-15R α complexes has been achieved. Similar to DNA, different forms of the heterodimer can be used alone or in combinations for obtaining the appropriate effects:

1 Delivery of purified IL-15/soluble (s) IL-15R α , such as SEQ ID NO:10 and SEQ ID NO:12.

2 Delivery of purified IL-15/IL-15R α Fc fusion protein (fusion to the constant region of an immunoglobulin molecule, such as SEQ ID NO:17 and SEQ ID NO:20)

[0171] IL-15/sIL-15R α was purified from overproducing human 293 cells and delivered into lympho-ablated mice. The results showed that this heterodimer is bioactive and that it promoted the proliferation of adoptively transferred lymphocytes (T cells, NK cells, but not B cells).

[0172] Experimental procedure (Figure 22): Mice were treated with Cyclophosphamide (Cyp) and two days later they were given 3 μ g of HPLC-purified IL-15/s15Ra protein intraperitoneally for 6 days. Splenocytes were purified from young Bl/6 mice, labeled with CFSE, and 10⁷ cells were injected by the IV route to the lympho-ablated animals. Proliferation of the adoptively transferred cells was followed by CFSE dilution.

[0173] Thus, these results indicate that different forms of IL-15/IL-15R α heterodimer have different stability, interactions in the body, processing and stability. This offers the opportunity to exploit such properties for using these cytokines to provide maximal benefit. Accordingly, the different forms can be combined in different ratios and administration schedules. Different forms can be administered either simultaneously or sequentially.

[0174] IL-15R α – Fc fusions previously employed have been used with various degrees of effectiveness. The studies exemplified in Figure23 show that the Fc fusion we used has greater plasma half-life compared to IL-15/s15R α .

[0175] In the examples of sequences, described herein, the 205FC fusion (SEQ ID NO:17) contains the natural processing site generating the s15R α from the membrane-bound form, whereas the 200FC fusion (SEQ ID NO:20) does not have an intact processing site. These are examples of Fc fusions that may be processed differently to generate non-cell associated forms after cleavage between the 15R α region and the antibody constant region. Additional molecules can be generated having processing sites for cleavage and generating both cell associated and soluble forms of the cytokine. Additional methods for cell attachment, other than the Fc region are known in the art and can also be employed.

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be

suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

EXAMPLES OF SEQUENCES

SEQ ID NO:1

Human wild-type IL-15 nucleic acid sequence

ATGAGAATTTTCGAAACCACATTTGAGAAGTATTTCCATCCAGTGCTACTTGTGTTTACTTCT
AAACAGTCATTTTCTAACTGAAGCTGGCATTCATGTCTTCATTTTGGGCTGTTTCAGTGCAG
GGCTTCCTAAAACAGAAGCCAACTGGGTGAATGTAATAAGTGATTGAAAAAATTGAAGAT
CTTATTCAATCTATGCATATTGATGCTACTTTATATACGGAAAGTGATGTTACCCCCAGTTG
CAAAGTAACAGCAATGAAGTGCTTTCTCTTGGAGTTACAAGTTATTTCACTTGAGTCTGGAG
ATGCAAGTATTCATGATACAGTAGAAAATCTGATCATCCTAGCAAACAACAGTTTGTCTTCT
AATGGGAATGTAACAGAATCTGGATGCAAAGAATGTGAGGAAGTGGAGGAAAAAATATTAA
AGAATTTTTCAGAGTTTTGTACATATTGTCCAATGTTTCATCAACACTTCTTGA

SEQ ID NO:2

Human wild-type IL-15 amino acid sequence

M	R	I	S	K	P	H	L	R	S	I	S	I	Q	C	Y	L	C	L	L	L
N	S	H	F	L	T	E	A	G	I	H	V	F	I	L	G	C	F	S	A	G
L	P	K	T	E	A	N	W	V	N	V	I	S	D	L	K	K	I	E	D	L
I	Q	S	M	H	I	D	A	T	L	Y	T	E	S	D	V	H	P	S	C	K
V	T	A	M	K	C	F	L	L	E	L	Q	V	I	S	L	E	S	G	D	A
S	I	H	D	T	V	E	N	L	I	I	L	A	N	N	S	L	S	S	N	G
N	V	T	E	S	G	C	K	E	C	E	E	L	E	E	K	N	I	K	E	F
L	Q	S	F	V	H	I	V	Q	M	F	I	N	T	S	•					

SEQ ID NO:3

Human improved IL-15 nucleic acid sequence (opt1)

ATGCGGATCTCGAAGCCGCACCTGCGGTGCGATATCGATCCAGTGCTACCTGTGCCTGCTCCT
GAACTCGCACTTCCTCACGGAGGCCGGTATACACGTCTTCATCCTGGGCTGCTTCTCGGCCG
GGCTGCCGAAGACGGAGGCGAACTGGGTGAACGTGATCTCGGACCTGAAGAAGATCGAGGAC
CTCATCCAGTCGATGCACATCGACGCGACGCTGTACACGGAGTCGGACGTCCACCCGTCGTG
CAAGGTCACGGCGATGAAGTGCTTCCTCCTGGAGCTCCAAGTCATCTCGCTCGAGTCGGGGG
ACGCGTCGATCCACGACACGGTGGAGAACCTGATCATCCTGGCGAACAACCTCGCTGTCGTG
AACGGGAACGTACGGAGTCGGGCTGCAAGGAGTGCGAGGAGCTGGAGGAGAAGAACATCAA
GGAGTTCCTGCAGTCGTTGTCGTCACATCGTCCAGATGTTTCATCAACACGTCGTGA

SEQ ID NO:4

Human improved IL-15 nucleic acid sequence (opt2)

ATGAGGATCAGCAAGCCCCACCTGAGGAGCATCAGCATCCAGTGCTACCTGTGCCTGCTGCT
GAACAGCCACTTCCTGACCGAGGCCGGTATACACGTGTTTCATCCTGGGCTGCTTTAGCGCCG
GACTGCCCAAGACCGAGGCCAATTGGGTGAACGTGATCAGCGACCTGAAGAAGATCGAGGAC

CTCATCCAGAGCATGCACATCGACGCCACCCTGTACACCGAGAGCGATGTGCACCCCAGCTG
 TAAGGTGACCGCCATGAAGTGCTTTCTGCTGGAGCTGCAAGTGATCAGCCTGGAGAGCGGCG
 ACGCCAGCATCCACGACACCGTGGAGAACCTGATCATCCTGGCCAACAACAGCCTGAGCAGC
 AACGGCAATGTGACCGAGAGCGGCTGTAAGGAGTGTGAGGAGCTGGAGGAGAAGAACATCAA
 GGAGTTTCTGCAGAGCTTCGTGCACATCGTGCAGATGTTTCATCAACACCAGCTGA

SEQ ID NO:5

**Homo sapiens interleukin 15 receptor, alpha (IL15RA),
 transcript variant 1, mRNA - GenBank Accession No. NM_002189**

```

1  cccagagcag cgctcgccac ctccccccgg cctggggcagc gctcgccccg ggagtcacagc
61 ggtgtcctgt ggagctgccg ccatggcccc gcggcggggc cgcggtgcc ggacctcgg
121 tctccccggc ctgtactgct tgtgtgtgt cgggcggcg ggcagcggg gcacacgtg
181 ccctcccc atgtccgtg aacacgcaga catctgggtc aagagctaca gcttgactc
241 cagggagcgg tacatttgta actctggttt caagcgtaaa gccggcagc ccagcctgac
301 ggagtgcgtg ttgaacaagg ccacgaatgt cgccactgg acaaccccc gtctcaaatg
361 cattagagac cctgccctgg ttcaccaaag gccagcgcca ccctccacag taacgacggc
421 aggggtgacc ccacagccag agagcctctc cccttctgga aaagagccc gagcttcac
481 tcccagctca aacaacacag cggccacaac agcagctatt gtcccgggc cccagctgat
541 gccttcaaaa tcaccttcca caggaaccac agagataagc agtcatgagt cctccacgg
601 caccctct cagacaacag ccaagaactg ggaactcaca gcatccgcct cccaccagcc
661 gccaggtgtg tatccacagg gccacagcga caccactgtg gctatctcca cgtccactgt
721 cctgctgtgt gggctgagcg ctgtgtctct cctggcatgc tacctcaagt caaggcaaac
781 tccccgctg gccagcgtt aaatggaagc catggaggct ctgccggtga cttgggggac
841 cagcagcaga gatgaagact tggaaaactg ctctcaccac ctatgaaact cggggaaacc
901 agcccagcta agtcgggagt gaaggagcct ctctgcttta gctaaagacg actgagaaga
961 ggtgcaagga agcgggctcc aggagcaagc tcaccaggcc tctcagaagt cccagcagga
1021 tctcacggac tgccgggtcg gcgcctcctg cgcgaggag caggttctcc gcattcccat
1081 gggcaccacc tgccctgcctg tctgtccttg gaccagggc ccagcttccc aggagagacc
1141 aaaggcttct gagcaggatt tttatttcat tacagtgtga gctgcctgga atacatgtgg
1201 taatgaaata aaaaccctgc ccgaatctt cgtccctca tccaaacttt cagttcacag
1261 agaaaagtga catacccaaa gctctctgtc aattacaagg cttctcctgg cgtgggagac
1321 gtctacaggg aagacaccag cgtttgggct tctaaccacc ctgtctccag ctgtctgca
1381 cacatggaca gggacctggg aaaggtggga gagatgctga gccacggaa tctctccat
1441 tgaaggattc aggaagaaga aaactcaact cagtgccatt ttacgaatat atgcgtttat
1501 atttatactt ccttgtctat tatactata cattatata tatttgtatt ttgacattgt
1561 accttgtata aacaaaataa aacatctatt ttcaatattt ttaaatgca

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SEQ ID NO:6

**interleukin 15 receptor, alpha isoform 1 precursor [Homo
 sapiens] - GenBank Accession No. NP_002180**

```

1  maprrargcr tlglpallll lllrppatrg itcpppmsve hadiwvksys lysreryicn
61 sgfkrkagts sltecvluka tnvahwttps lkciirdpalv hqrpappstv ttagvtpqpe
121 slspsgkepa asspsnnta attaaivpgs qlmpskspst gtteisshe shgtpsqtta
181 knweltasas hqppgvypqg hsdttvaist stvllcglsa vsllacylks rgtpplasve
241 meamealpvt wgtssrdedl encshhl

```

SEQ ID NO:7

**Homo sapiens interleukin 15 receptor, alpha (IL15RA),
 transcript variant 2, mRNA - GenBank Accession No. NM_172200**

```

1  caggaattcg gcgaagtggc ggagctgggg ccccagcggg cgccgggggc cgcgggagcc
61 agcaggtggc gggggctgcg ctccggcccg gccagagcgc accaggcagg tgcccgcgc
121 tccgcaccgc ggcgacacct ccgcgggcac tcacccaggc cgcccgctca caaccgagcg
181 cagggccgcg gagggagacc aggaagaccg aaggcggagc agctggaggc gaccagcgcc
241 gggcgaggtc aagtggatcc gagccgcaga gagggctgga gagagtctgc tctccgatga
301 ctttgccac tctcttcgca gtggggacac cggaccgagt gcacactgga ggtcccagag
361 cacgacgagc gcggaggacc gggaggctcc cgggcttgcg tgggcatcac gtgcctccc
421 cccatgtccg tggaacacgc agacatctgg gtcaagagct acagcttgta ctccagggag

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481  cggtagacattt  gtaactctgg  tttcaagcgt  aaagccggca  cgtccagcct  gacggagtgc
541  gtgttgaaaca  aggccacgaa  tgtcgccac  tggacaaccc  ccagtctcaa  atgcattaga
601  gaccctgccc  tggttcacca  aaggccagcg  ccaccctcca  cagtaacgac  ggcagggggtg
661  accccacagc  cagagagcct  ctccccttct  ggaaaagagc  ccgcagcttc  atctcccagc
721  tcaaacaaca  cagcggccac  aacagcagct  attgtcccgg  gctcccagct  gatgccttca
781  aaatcacctt  ccacaggaac  cacagagata  agcagtcatt  agtcctccca  cggcaccccc
841  tctcagacaa  cagccaagaa  ctgggaactc  acagcatccg  cctcccacca  gccgccaggt
901  gtgtatccac  agggccacag  cgacaccact  gtggctatct  ccacgtccac  tgtcctgctg
961  tgtgggctga  gcgctgtgtc  tctcctggca  tgctacctca  agtcaaggca  aactcccccg
1021 ctggccagcg  ttgaaatgga  agccatggag  gctctgccgg  tgacttgggg  gaccagcagc
1081 agagatgaag  acttggaaaa  ctgctctcac  cacctatgaa  actcggggaa  accagcccag
1141 ctaagtccgg  agtgaaggag  cctctctgct  ttagctaaag  acgactgaga  agaggtgcaa
1201 ggaagcgggc  tccaggagca  agctcaccag  gcctctcaga  agtcccagca  ggatctcacg
1261 gactgccggg  tcggcgccct  ctgctcgagg  gagcagggtt  tccgcattcc  catgggcacc
1321 acctgcctgc  ctgtcgtgct  ttggaccag  ggcccagctt  cccaggagag  accaaaggct
1381 tctgagcagg  atttttatct  cattacagt  tgagctgctt  ggaatacatg  tggtaatgaa
1441 ataaaaaccc  tgccccgaat  cttccgtccc  tcctcctaac  tttcagttca  cagagaaaag
1501 tgacataccc  aaagctctct  gtcaattaca  aggcttctcc  tggcgtggga  gacgtctaca
1561 gggaagacac  cagcgtttgg  gcttctaacc  accctgtctc  cagctgctct  gcacacatgg
1621 acagggacct  gggaaagggt  ggagagatgc  tgagcccagc  gaatcctctc  cattgaagga
1681 ttcaggaaga  agaaaactca  actcagtgcc  attttacgaa  tatatgcgtt  tatatttata
1741 cttccttgct  tattatatct  atacattata  tattatttgt  attttgacat  tgtaccttgt
1801 ataaacaaaa  taaaacatct  attttcaata  tttttaaaat  gca

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SEQ ID NO:8

**interleukin 15 receptor, alpha isoform 2 [Homo sapiens] -
GenBank Accession No. NP_751950**

```

1  msvehadiwv  ksyslysrer  yicnsgfkrk  agtssltecv  lnkatnvahw  ttpsllkcird
61  palvhqrpap  pstvttagvt  pqpeslpspg  kepaasspss  nntaattaai  vpgsqlimpsk
121 spstgtteis  shesshgtps  qttaknwelt  asashqppgv  ypqghsdttv  aiststvllo
181 glsavslilac  ylkstrqtppl  asvemeamea  lpvtwgtssr  dedlencshh  l

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SEQ ID NO:9

**Improved human interleukin 15 (IL-15) receptor alpha (IL15Ra),
transcript variant 1 (OPT)**

```

atggccccga  ggcggggcgc  aggctgcccg  accctcggtc  tcccggcgct  gctactgctc  60
ctgctgctcc  ggccgcccgc  gacgcggggc  atcacgtgcc  cgccccccat  gtccgtggag  120
cacgcagaca  tctgggtcaa  gagctacagc  ttgtactccc  gggagcggta  catctgcaac  180
tcgggtttca  agcggaaagg  cggcacgtcc  agcctgacgg  agtgcgtgtt  gaacaaggcc  240
acgaatgtcg  cccactggac  gacccccctc  ctcaagtgca  tccgcgaccc  ggccctgggt  300
caccagcggc  ccgcgccacc  ctccaccgta  acgacggcgg  gggtgacccc  gcagccggag  360
agcctctccc  cgtcgggaaa  ggagcccggc  gcgtcgtcgc  ccagctcgaa  caacacggcg  420
gccacaactg  cagcgatcgt  cccgggctcc  cagctgatgc  cgtcgaagtc  gccgtccacg  480
ggaaccacgg  agatcagcag  tcatgagtc  tcccacggca  ccccctcgca  aacgacggcc  540
aagaactggg  aactcacggc  gtcgcctccc  caccagccgc  cgggggtgta  tccgcaaggc  600
cacagcgaca  ccacggtggc  gatctccacg  tccacggctc  tgctgtgtgg  gctgagcgcg  660
gtgtcgctcc  tggcgtgcta  cctcaagtgc  aggcagactc  ccccgctggc  cagcgttgag  720
atggaggcca  tggaggctct  gccgggtgac  tgggggacca  gcagcaggga  tgaggacttg  780
gagaactgct  cgcaccacct  ataatga  807

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**SEQ ID NO:10 - improved human interleukin 15 (IL-15) receptor
alpha (IL15Ra), transcript variant 1 (OPT)**

Met Ala Pro Arg Arg Ala Arg Gly Cys Arg Thr Leu Gly Leu Pro Ala

1 5 10 15

Leu Leu Leu Leu Leu Leu Arg Pro Pro Ala Thr Arg Gly Ile Thr

20 25 30

Cys Pro Pro Pro Met Ser Val Glu His Ala Asp Ile Trp Val Lys Ser
 35 40 45
 Tyr Ser Leu Tyr Ser Arg Glu Arg Tyr Ile Cys Asn Ser Gly Phe Lys
 50 55 60
 Arg Lys Ala Gly Thr Ser Ser Leu Thr Glu Cys Val Leu Asn Lys Ala
 65 70 75 80
 Thr Asn Val Ala His Trp Thr Thr Pro Ser Leu Lys Cys Ile Arg Asp
 85 90 95
 Pro Ala Leu Val His Gln Arg Pro Ala Pro Pro Ser Thr Val Thr Thr
 100 105 110
 Ala Gly Val Thr Pro Gln Pro Glu Ser Leu Ser Pro Ser Gly Lys Glu
 115 120 125
 Pro Ala Ala Ser Ser Pro Ser Ser Asn Asn Thr Ala Ala Thr Thr Ala
 130 135 140
 Ala Ile Val Pro Gly Ser Gln Leu Met Pro Ser Lys Ser Pro Ser Thr
 145 150 155 160
 Gly Thr Thr Glu Ile Ser Ser His Glu Ser Ser His Gly Thr Pro Ser
 165 170 175
 Gln Thr Thr Ala Lys Asn Trp Glu Leu Thr Ala Ser Ala Ser His Gln
 180 185 190
 Pro Pro Gly Val Tyr Pro Gln Gly His Ser Asp Thr Thr Val Ala Ile
 195 200 205
 Ser Thr Ser Thr Val Leu Leu Cys Gly Leu Ser Ala Val Ser Leu Leu
 210 215 220
 Ala Cys Tyr Leu Lys Ser Arg Gln Thr Pro Pro Leu Ala Ser Val Glu
 225 230 235 240
 Met Glu Ala Met Glu Ala Leu Pro Val Thr Trp Gly Thr Ser Ser Arg
 245 250 255
 Asp Glu Asp Leu Glu Asn Cys Ser His His Leu
 260 265

**SEQ ID NO:11 - improved human soluble interleukin 15 (IL-15)
receptor alpha (IL-15sRa) (OPT)**

atggccccga ggcgggcgcg aggctgccgg accctcggtc tcccggcgct gctactgctc 60
 ctgctgctcc ggccgcccgc gacgcggggc atcacgtgcc cgccccccat gtccgtggag 120
 cacgcagaca tctgggtcaa gagctacagc ttgtactccc gggagcggta catctgcaac 180
 tcgggtttca agcgggaaggc cggcacgtcc agcctgacgg agtgcggtt gaacaaggcc 240
 acgaatgtcg cccactggac gacccccctcg ctcaagtgc tccgcgaccc ggccctgggt 300
 caccagcggc ccgcgccacc ctccaccgta acgacggcgg gggtagcccc gcagccggag 360
 agcctctccc cgtcgggaaa ggagcccgcc gcgtcgtcgc ccagctcgaa caacacggcg 420
 gccacaactg cagcgatcgt cccgggctcc cagctgatgc cgtcgaagtc gccgtccacg 480
 ggaaccacgg agatcagcag tcatgagtcc tcccacggca ccccctcgca aacgacggcc 540
 aagaactggg aactcacggc gtccgcctcc caccagccgc cgggggtgta tccgcaaggc 600
 cacagcgaca ccacgtaatg a 621

**SEQ ID NO:12 - improved human soluble interleukin 15 (IL-15)
receptor alpha (IL-15sRa) (OPT)**

```

Met Ala Pro Arg Arg Ala Arg Gly Cys Arg Thr Leu Gly Leu Pro Ala
  1             5             10             15
Leu Leu Leu Leu Leu Leu Arg Pro Pro Ala Thr Arg Gly Ile Thr
      20             25             30
Cys Pro Pro Pro Met Ser Val Glu His Ala Asp Ile Trp Val Lys Ser
      35             40             45
Tyr Ser Leu Tyr Ser Arg Glu Arg Tyr Ile Cys Asn Ser Gly Phe Lys
      50             55             60
Arg Lys Ala Gly Thr Ser Ser Leu Thr Glu Cys Val Leu Asn Lys Ala
      65             70             75             80
Thr Asn Val Ala His Trp Thr Thr Pro Ser Leu Lys Cys Ile Arg Asp
      85             90             95
Pro Ala Leu Val His Gln Arg Pro Ala Pro Pro Ser Thr Val Thr Thr
      100            105            110
Ala Gly Val Thr Pro Gln Pro Glu Ser Leu Ser Pro Ser Gly Lys Glu
      115            120            125
Pro Ala Ala Ser Ser Pro Ser Ser Asn Asn Thr Ala Ala Thr Thr Ala
      130            135            140
Ala Ile Val Pro Gly Ser Gln Leu Met Pro Ser Lys Ser Pro Ser Thr
      145            150            155            160
Gly Thr Thr Glu Ile Ser Ser His Glu Ser Ser His Gly Thr Pro Ser
      165            170            175
Gln Thr Thr Ala Lys Asn Trp Glu Leu Thr Ala Ser Ala Ser His Gln
      180            185            190
Pro Pro Gly Val Tyr Pro Gln Gly His Ser Asp Thr Thr
      195            200            205

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SEQ ID NO:13

Dual expression plasmid human IL15Ra+IL15

CCTGGCCATTGCATACGTTGTATCCATATCATAATATGTACATTTATATTGGCTCATGTCCA
 ACATTACCGCCATGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTC
 ATTAGTTCATAGCCCATATATGGAGTTCGCGTTACATAACTTACGGTAAATGGCCCGCCTG
 GCTGACCGCCCAACGACCCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACG
 CCAATAGGGACTTTCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGC
 AGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGC
 CCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTAC
 GTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATA
 GCGGTTTGACTCACGGGGATTTCCAAGTCTCACCCCCATTGACGTCAATGGGAGTTTGT
 GGCACCAAATCAACGGGACTTTCCAAATGTCTGTAACAACCTCCGCCCCATTGACGCAAATG
 GGCGGTAGGCGTGACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGAT
 CGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCC
 TCCGCGGGCGCGCTCGAGGAATTCGCTAGCAAGAAATGGCCCCGAGGCGGGCGGAGGCTG

CCGGACCCTCGGTCTCCCGGCGCTGCTACTGCTCCTGCTGCTCCGGCCGCCGGCGACGCGGG
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CCTGACGGAGTGCGTGTGTAACAAGGCCACGAATGTCGCCCACTGGACGACCCCTCGCTCA
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CGTCGAAGTCGCCGTCCACGGGAACCACGGAGATCAGCAGTCATGAGTCTCCACGCGCACC
CCCTCGCAAACGACGGCCAAGAAGTGGGAACCTCACGGCGTCCGCTCCACAGCCGCGGGG
GGTGTATCCGCAAGGCCACAGCGACACCACGGTGGCGATCTCCACGTCCACGGTCTGCTGT
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SEQ ID NO:15**Dual expression plasmid human IL15sRa(soluble)+IL15tPA6**

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**SEQ ID NO:17--huIL15sRa205-Fc--underlined region is IL15sRa
sequence**

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L	L	R	P	P	A	T	R	G	I	T	C	P	P	P	M	S	V	E	H	A
D	I	W	V	K	S	Y	S	L	Y	S	R	E	R	Y	I	C	N	S	G	F
K	R	K	A	G	T	S	S	L	T	E	C	V	L	N	K	A	T	N	V	A
H	W	T	T	P	S	L	K	C	I	R	D	P	A	L	V	H	Q	R	P	A
P	P	S	T	V	T	T	A	G	V	T	P	Q	P	E	S	L	S	P	S	G
K	E	P	A	A	S	S	P	S	S	N	N	T	A	A	T	T	A	A	I	V
P	G	S	Q	L	M	P	S	K	S	P	S	T	G	T	T	E	I	S	S	H
E	S	S	H	G	T	P	S	Q	T	T	A	K	N	W	E	L	T	A	S	A
S	H	Q	P	P	G	V	Y	P	Q	G	H	S	D	T	T	P	K	S	C	D
K	T	H	T	C	P	P	C	P	A	P	E	L	L	G	G	P	S	V	F	L
F	P	P	K	P	K	D	T	L	M	I	S	R	T	P	E	V	T	C	V	V
V	D	V	S	H	E	D	P	E	V	K	F	N	W	Y	V	D	G	V	E	V
H	N	A	K	T	K	P	R	E	E	Q	Y	N	S	T	Y	R	V	V	S	V
L	T	V	L	H	Q	D	W	L	N	G	K	E	Y	K	C	K	V	S	N	K
A	L	P	A	P	I	E	K	T	I	S	K	A	K	G	Q	P	R	E	P	Q
V	Y	T	L	P	P	S	R	D	E	L	T	K	N	Q	V	S	L	T	C	L
V	K	G	F	Y	P	S	D	I	A	V	E	W	E	S	N	G	Q	P	E	N
N	Y	K	T	T	P	P	V	L	D	S	D	G	S	F	F	L	Y	S	K	L
T	V	D	K	S	R	W	Q	Q	G	N	V	F	S	C	S	V	M	H	E	A
L	H	N	H	Y	T	Q	K	S	L	S	L	S	P	G	K					

SEQ ID NO:18--huGMCSF-IL15

M	W	L	Q	S	L	L	L	L	G	T	V	A	C	S	I	S	N	W	V	N
V	I	S	D	L	K	K	I	E	D	L	I	Q	S	M	H	I	D	A	T	L

Y	T	E	S	D	V	H	P	S	C	K	V	T	A	M	K	C	F	L	L	E
L	Q	V	I	S	L	E	S	G	D	A	S	I	H	D	T	V	E	N	L	I
I	L	A	N	N	S	L	S	S	N	G	N	V	T	E	S	G	C	K	E	C
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F	I	N	T	S																

SEQ ID NO:19--AG256DPhuIL15sRa200FC+huGMIL15— The capitalized, bolded region is the coding region for the IL-15Receptor alpha 200FC fusion

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SEQ ID NO:20--huIL15sRa200-Fc

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D	I	W	V	K	S	Y	S	L	Y	S	R	E	R	Y	I	C	N	S	G	F
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The claims defining the invention are as follows.

1. A method of preventing or reducing drug-induced lymphopenia in a human patient treated with an anti-cancer agent or who will be treated with the anti-cancer agent, the method comprising systemically administering a heterodimer comprising (i) an IL-15 and (ii) an IL-15R α -Fc fusion protein that comprises a soluble IL-15R α that lacks the transmembrane anchor portion fused to an Fc region to the human patient at a dose that maintains supraphysiological plasma levels of IL-15 for an extended period of time of about 2, 3, 4, 5, 6, 7, 8, 9, 10 or more days.

2. The method of claim 1, wherein the IL-15R α -Fc fusion protein comprises an amino acid sequence that has at least 95% amino acid sequence identity to the IL-15R α -Fc fusion protein sequence of SEQ ID NO:17 or SEQ ID NO:20.

3. A method of treating or preventing lymphopenia in an individual in need thereof comprising systemically administering to the individual a heterodimer comprising (i) an interleukin-15 (IL-15) and (ii) an IL-15R α -Fc fusion protein that comprises a soluble IL-15R α fused to an Fc region; wherein the IL-15 has at least 95% amino acid sequence identity to the region of SEQ ID NO:2 that corresponds to mature IL-15; and the IL-15R α -Fc fusion protein comprises an amino acid sequence that has at least 95% amino acid sequence identity to the IL-15R α -Fc fusion protein sequence of SEQ ID NO:17 or SEQ ID NO:20;

wherein the heterodimer is administered at a dose that maintains supraphysiological plasma levels of IL-15 for two or more days.

4. The method of claim 3, wherein the individual is a human.

5. The method of claim 3 or 4, wherein the lymphopenia is drug-induced lymphopenia.

6. The method of claim 5, wherein the individual is receiving or will receive an anti-cancer agent that induces lymphopenia.

7. The method of any of claims 1, 2, or 6, wherein the anti-cancer agent is a chemotherapeutic agent that induces lymphopenia.

8. The method of any of claims 1, 2, 6, or 7, wherein the heterodimer is co-administered with the anti-cancer agent.
9. The method of any of claims 1, 2, 6, or 7, wherein the heterodimer is administered after a
course of treatment with the anti-cancer agent.
10. The method of any of claims 1 to 9, wherein the IL-15 comprises the region of SEQ ID NO:2 that corresponds to mature IL-15 and the IL-15R α -Fc fusion protein comprises the IL-15R α -Fc fusion protein sequence of SEQ ID NO:17 or SEQ ID NO:20.
11. The method of any of claims 1 to 10, wherein the supraphysiological levels of IL-15 are sustained for a time period that promotes the migration of mature T cells to peripheral tissues.
12. The method of any of claims 1 to 11, wherein the supraphysiological levels of IL-15 are sustained for a time period that promotes the repopulation of lymphocytes in peripheral tissues.
13. The method of any of claims 1 to 12, wherein the heterodimer is administered subcutaneously.
14. The method of any of claims 1 to 13, wherein the heterodimer is administered at a dose sufficient to achieve plasma levels of IL-15 of about 1 to 1000 ng/ml.
15. The method of any of claims 1 to 14, wherein the heterodimer is administered two or more times.
16. The method of any of claims 1 to 15, wherein the heterodimer is not co-administered with an antigen.
17. The method of any of claims 1 to 16, wherein the heterodimer is delivered as a polypeptide complex.
18. The method of any of claims 1 to 16, wherein the heterodimer is delivered as a nucleic acid, and the IL-15 and the IL-15R α -Fc fusion protein are co-expressed

from a single vector comprising a nucleic acid sequence that encodes the IL-15 and a nucleic acid sequence that encodes the IL-15R α -Fc fusion protein.

19. The method of any of claims 1 to 16, wherein the heterodimer is delivered as a nucleic acid, and the IL-15 and the IL-15R α -Fc fusion protein are expressed from separate vectors, wherein one vector comprises the nucleic acid sequence that encodes the IL-15 and a separate vector comprises the nucleic acid sequence that encodes the IL-15R α -Fc fusion protein.

20. The method of claim 18 or 19, wherein the nucleic acid encoding IL-15 and/or the nucleic acid encoding the IL-15R α -Fc fusion protein encodes a heterologous signal peptide.

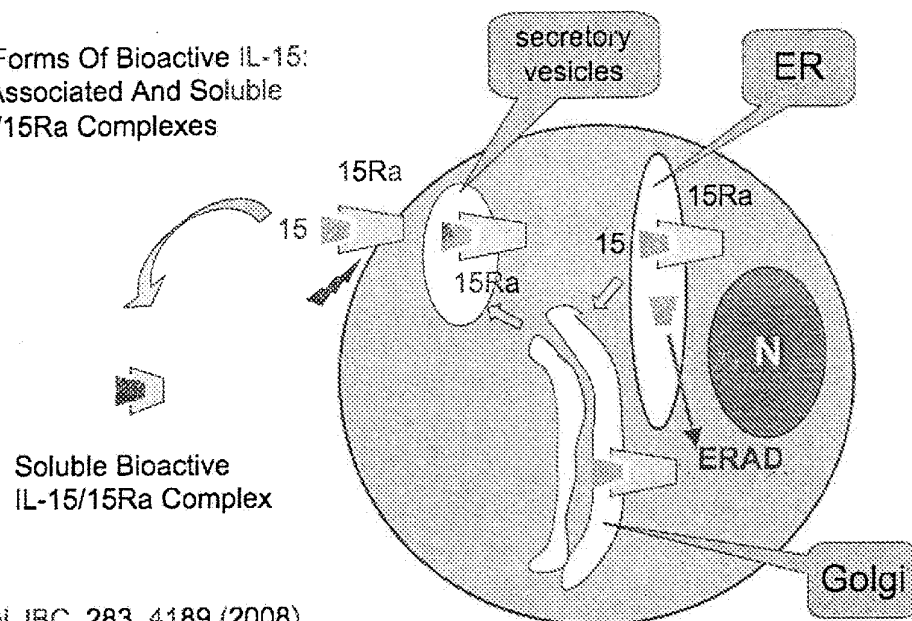
21. The method of claim 18, 19, or 20, wherein the nucleic acid sequence that encodes IL-15 comprises the region of SEQ ID NO:3 or SEQ ID NO:4 that encodes mature IL-15; and the nucleic acid sequence that encodes the IL-15R α -Fc fusion protein comprises the nucleic acid sequence of SEQ ID NO:16 or SEQ ID NO:19 that encodes the IL-15R α -Fc fusion protein.

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Figure 1

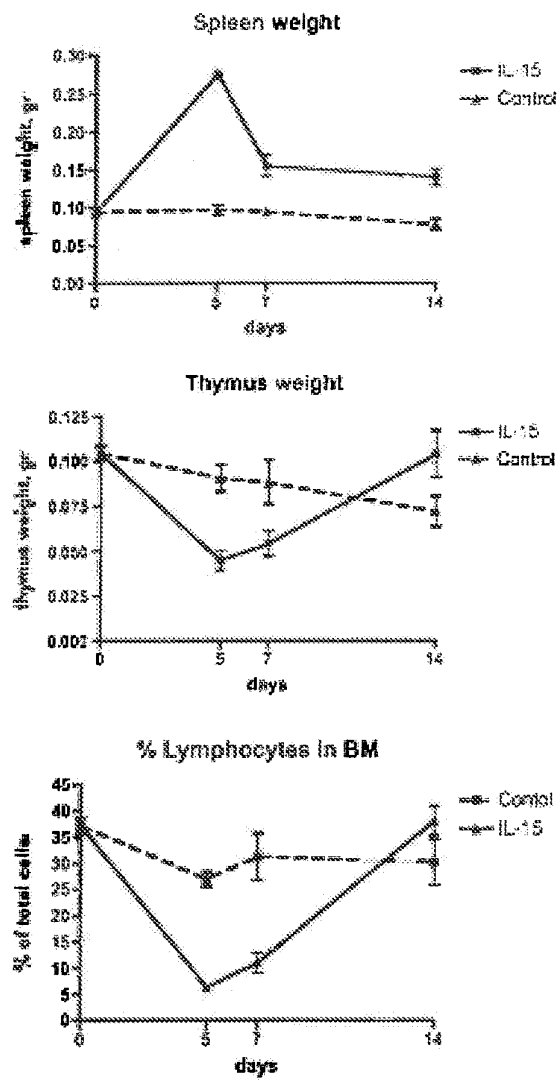
Mutual Stabilization of IL-15 and IL-15 Ra

Two Forms Of Bioactive IL-15:
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IL-15/15Ra Complexes

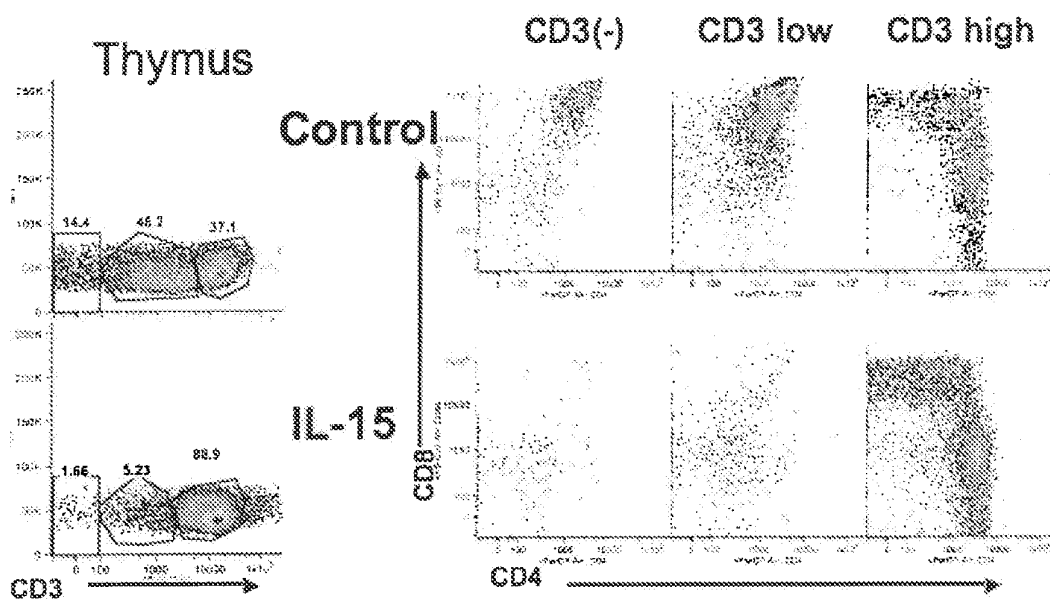


Bergamaschi JBC, 283, 4189 (2008)

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Figure 2**IL-15 effects on spleen, thymus, BM**

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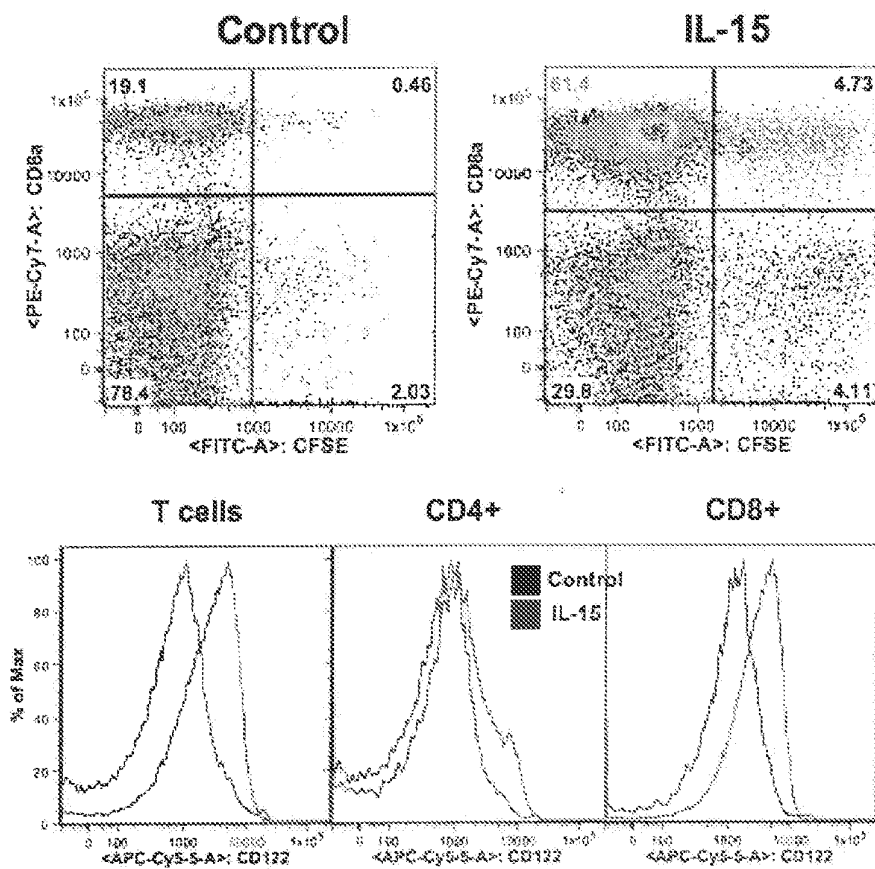
Figure 3

Effects of IL-15 on the thymus. Double positive cells are eliminated.

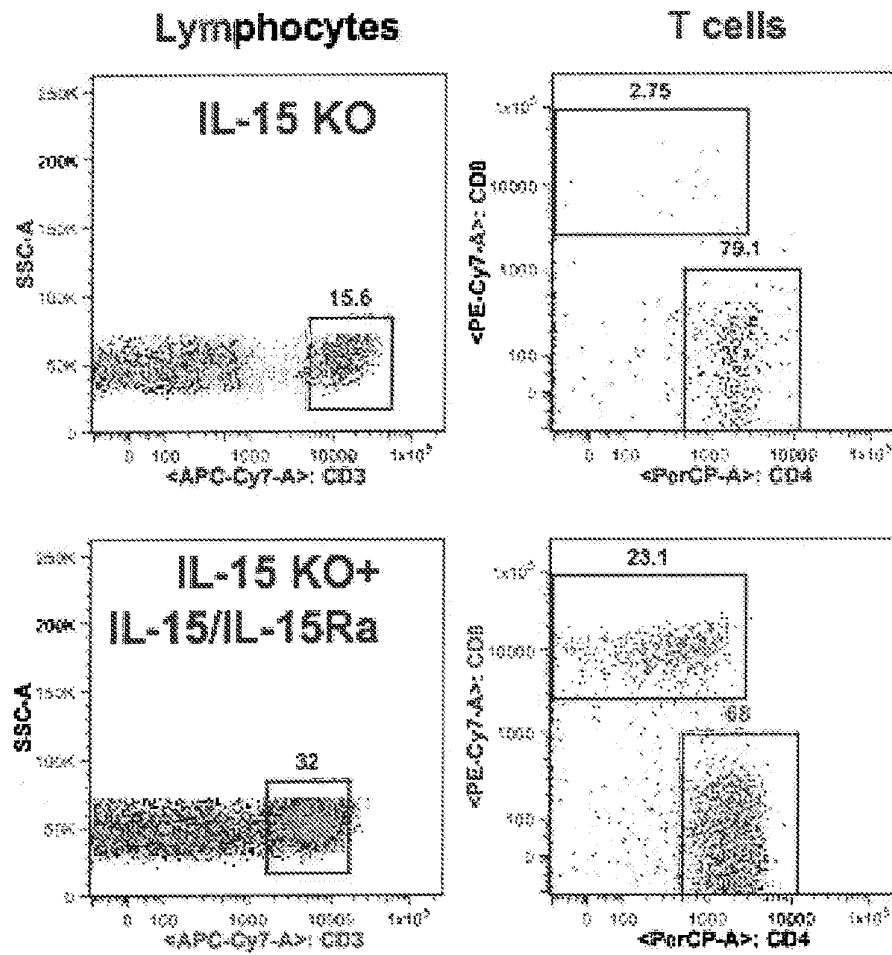
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Figure 4

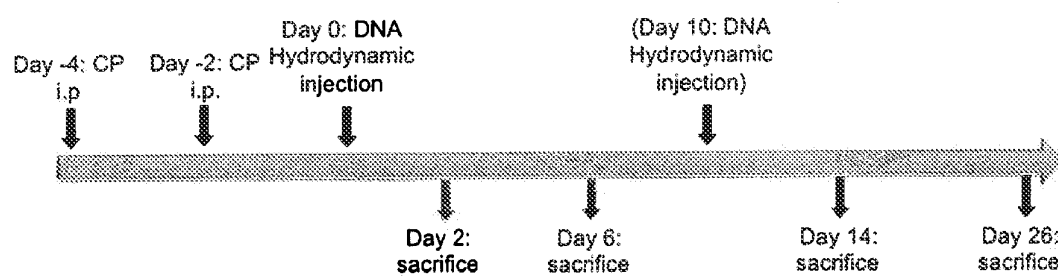
**Migration of Dividing CFSE-loaded Thymocytes
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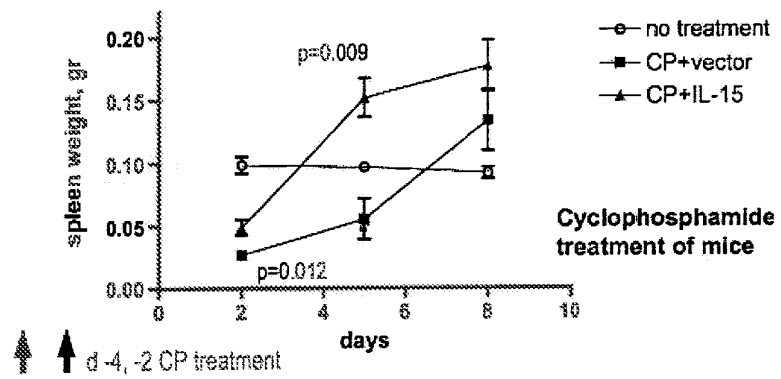
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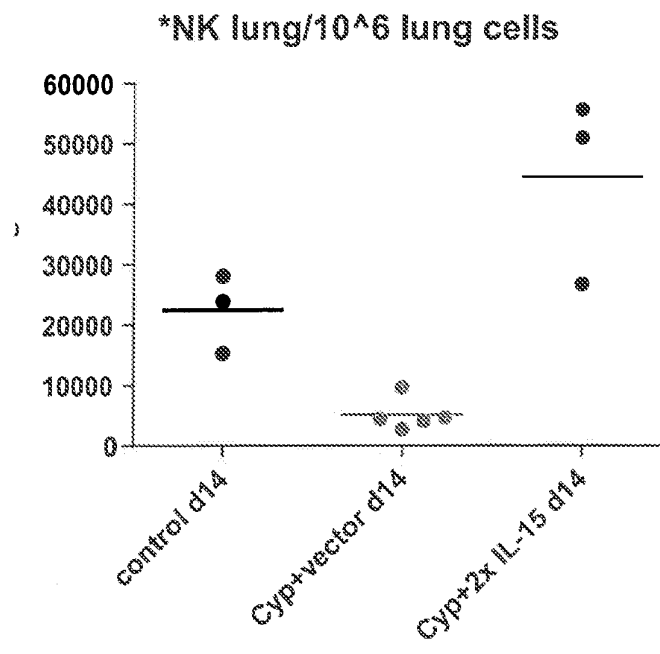
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Figure 6

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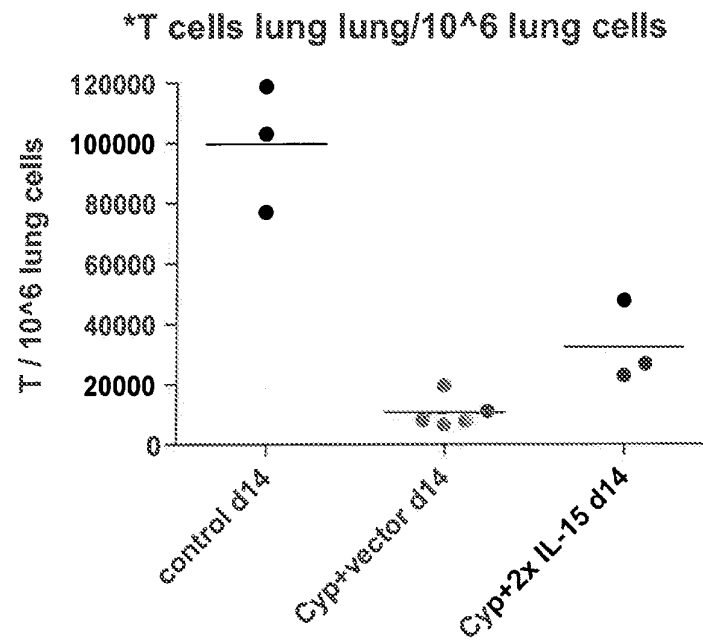
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8/23

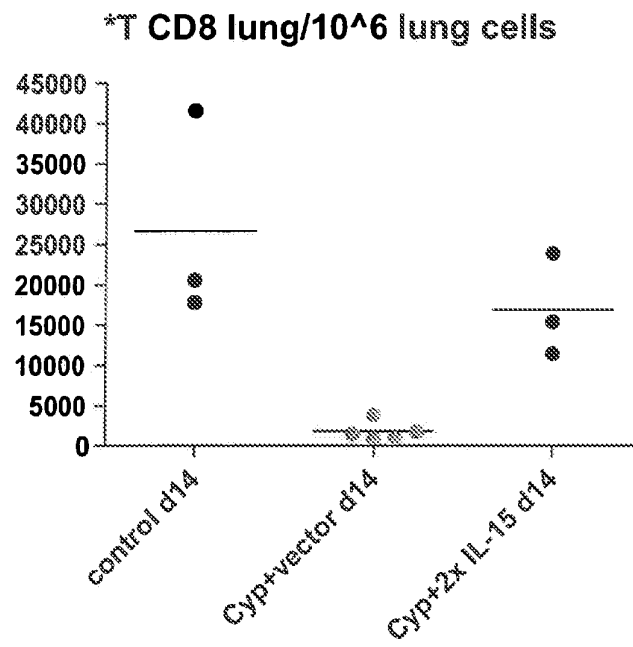
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9/23

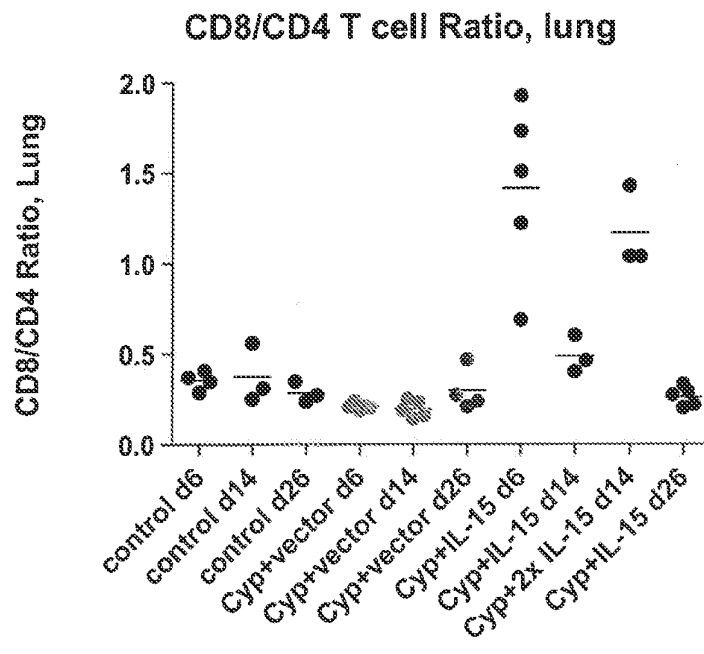
Figure 9



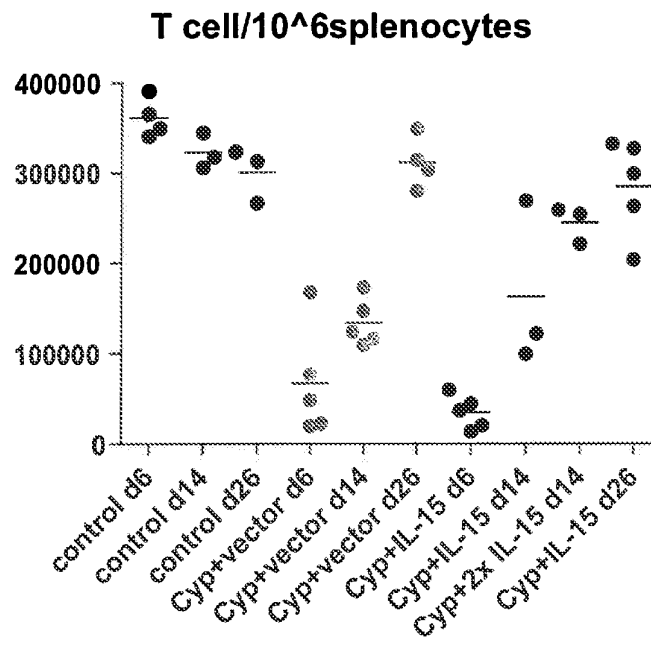
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Figure 10

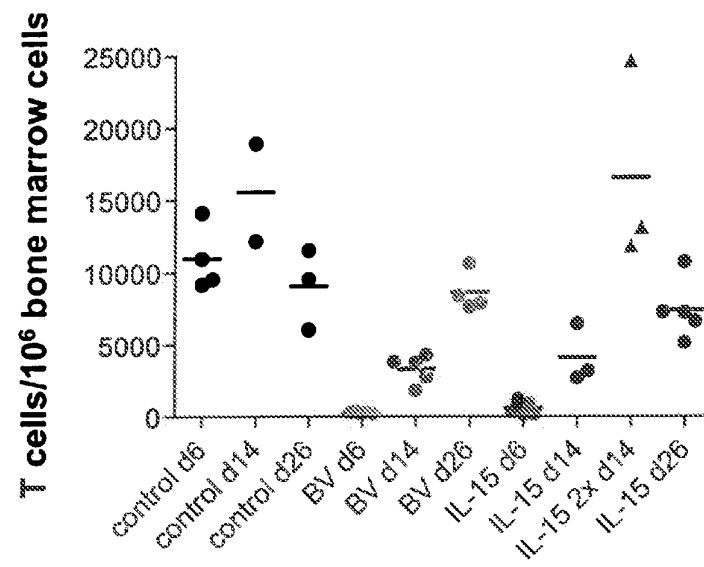
11/23

Figure 11

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Figure 12

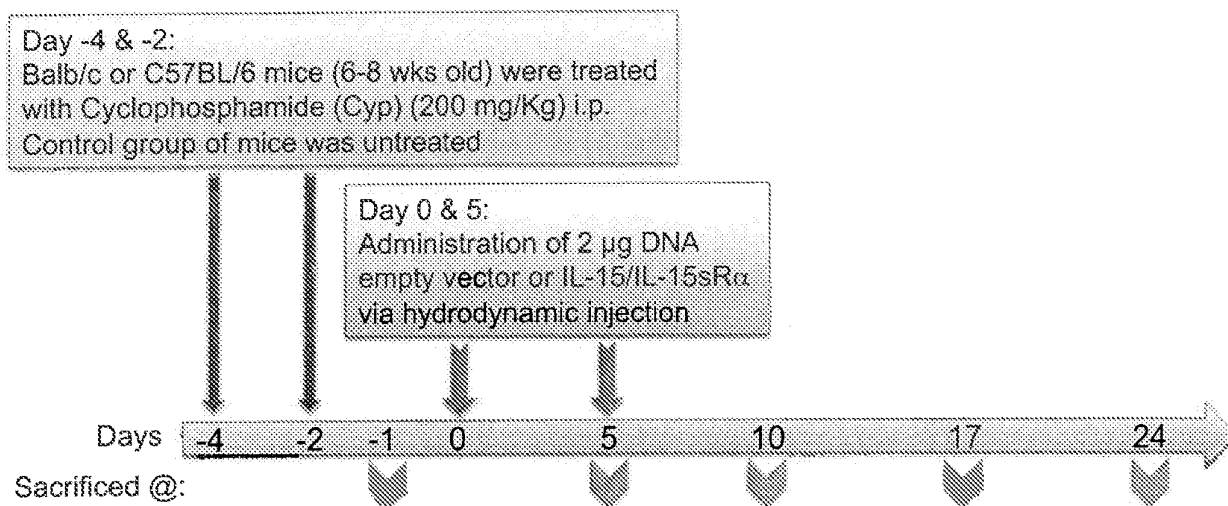
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Figure 13

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Figure 14

IL-15/R α Treatment Of Lymphopenic Mice (Cyclophosphamide-treated)

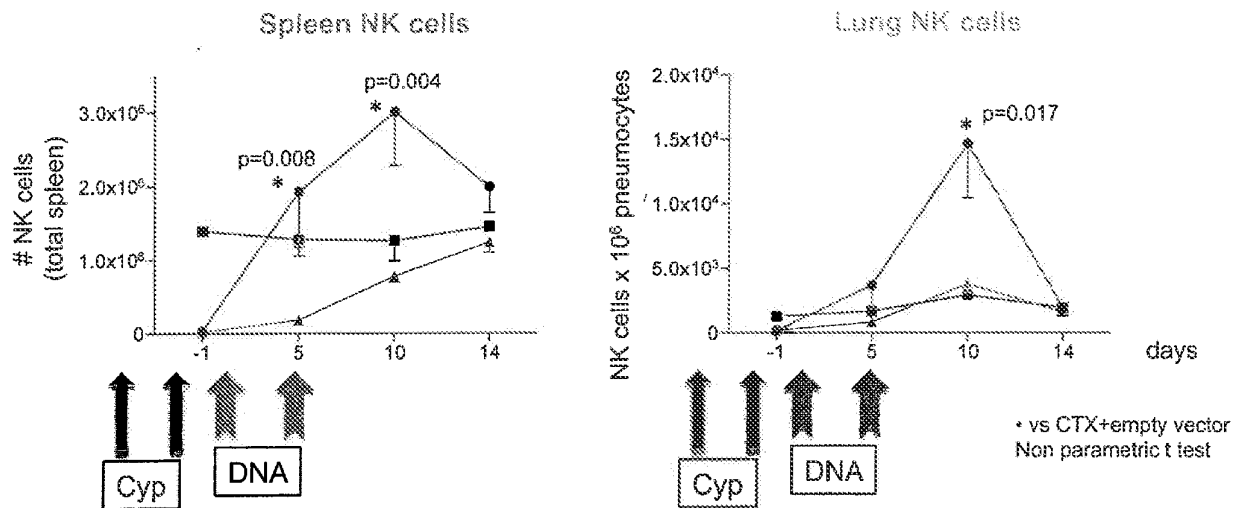


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Figure 15

Recovery of NK cells

—■— control —▲— Cyp+empty vector —●— Cyp+IL-15/IL-15sR α



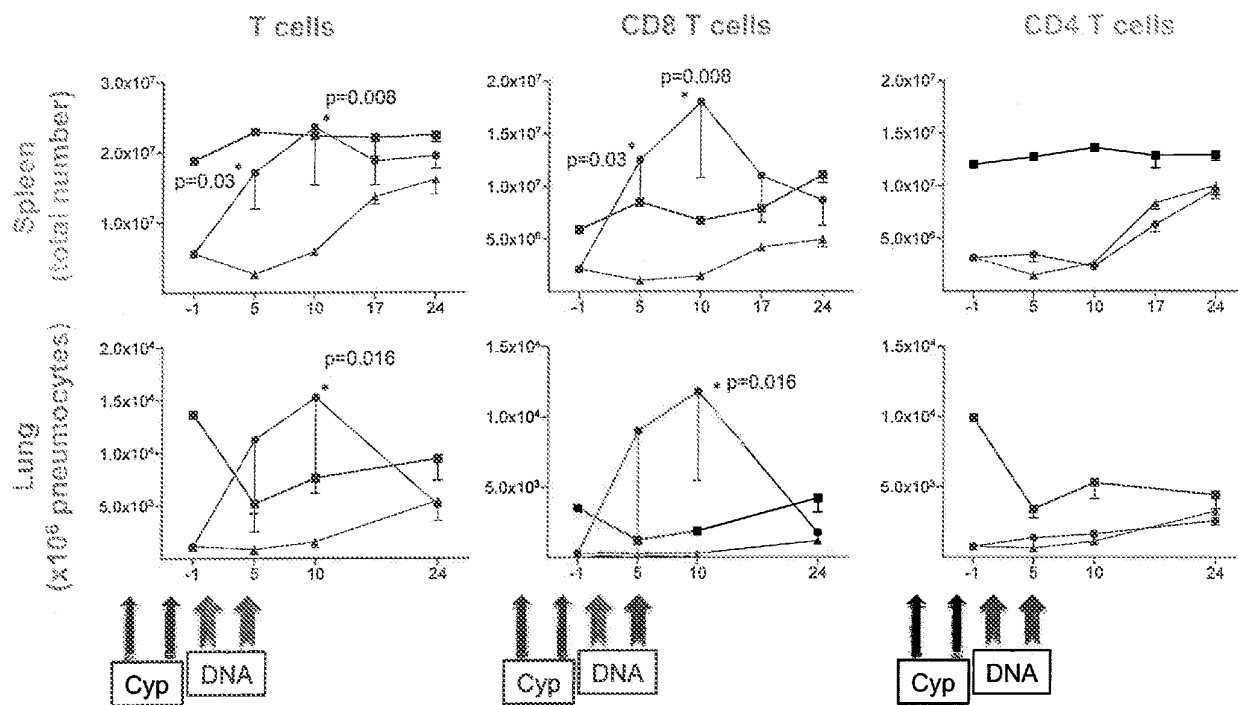
Single administration of IL-15/IL-15sR α -encoding DNA is sufficient for the complete recovery of NK cells in spleen and lung 5 days after DNA injection

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Figure 16

Recovery of T cells

■ control ▲ Cyp+empty vector ◆ Cyp+IL-15/IL-15sR α

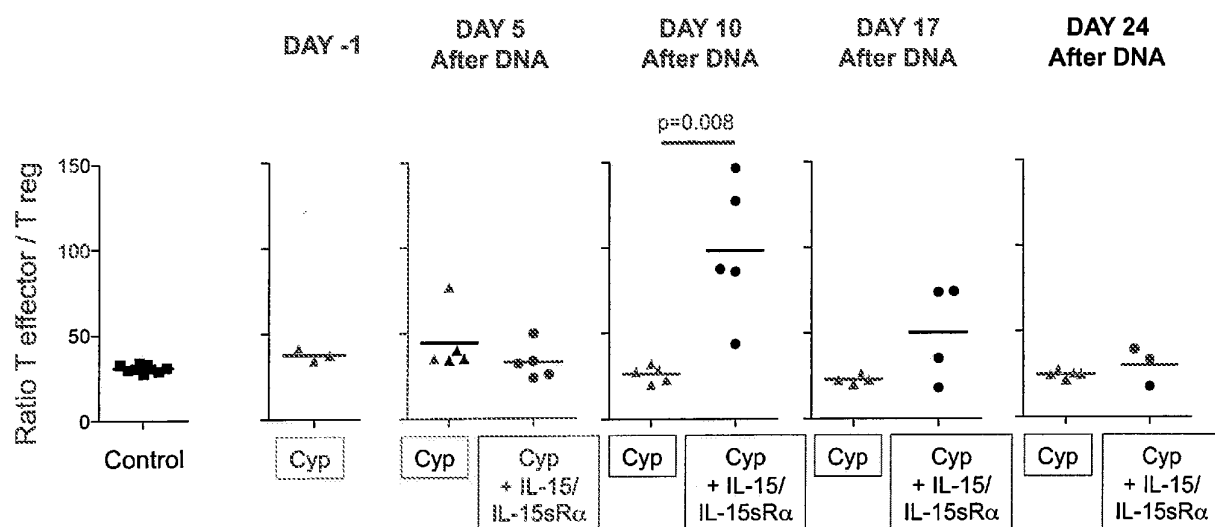


IL-15/IL-15sR α administration promotes the recovery of CD8 T cells within 10 days after treatment, without affecting significantly the recovery of CD4 T cells

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Figure 17

Ratio Of T effector / Treg after Lymphoablation

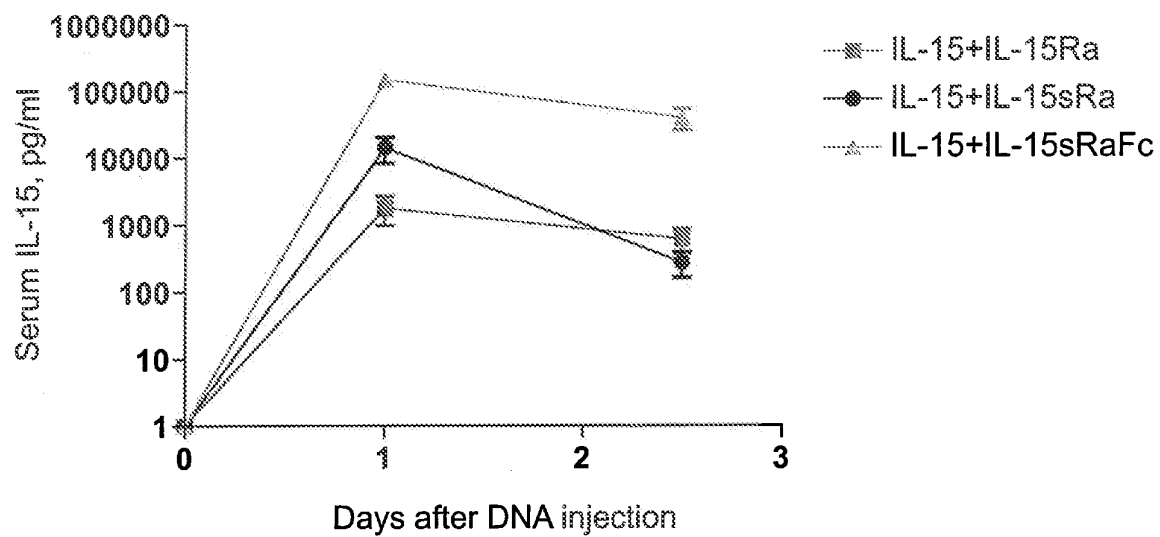


- High levels of circulating IL-15/IL-15sRα promote a transient increase in the Teffector/Treg ratio

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Figure 18

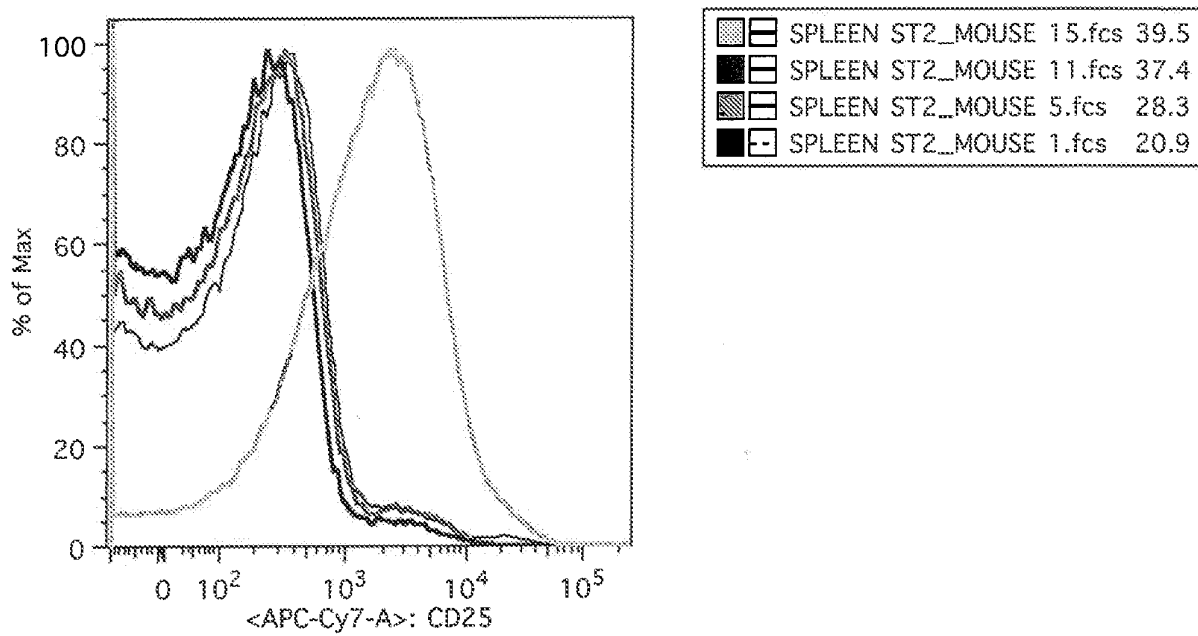
Hydrodynamic Delivery of DNA vectors expressing
different forms of IL-15



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Figure 19

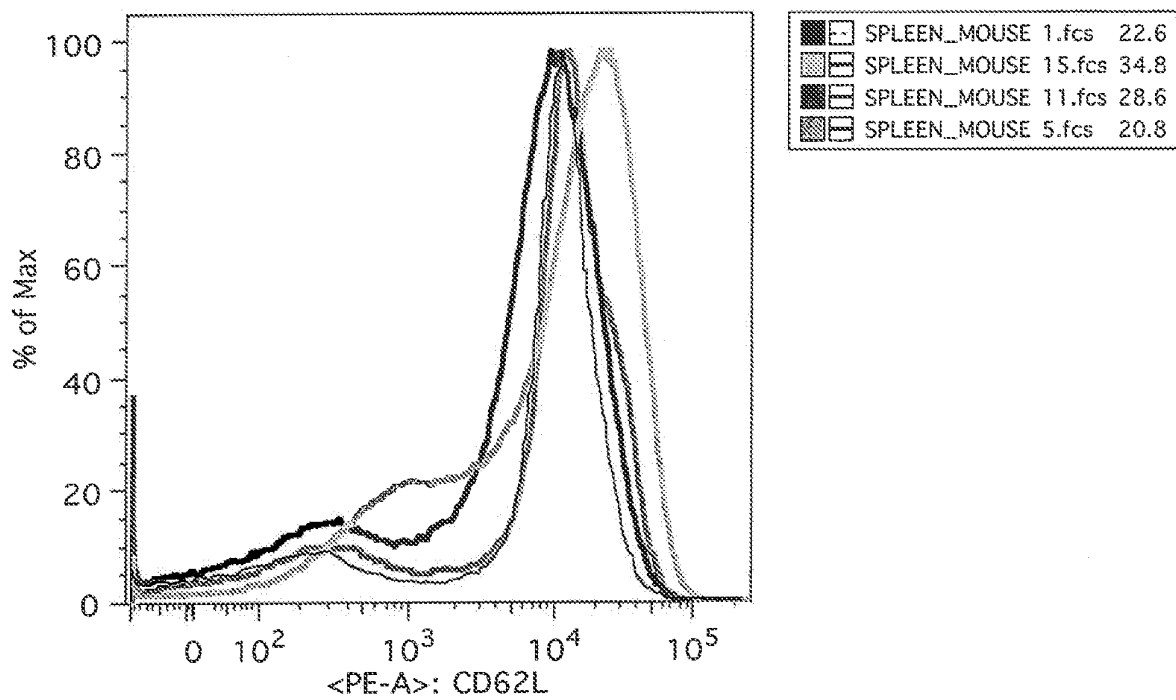
CD25 Expression in Spleen T cells After IL-15/15Ra DNA Delivery



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Figure 20

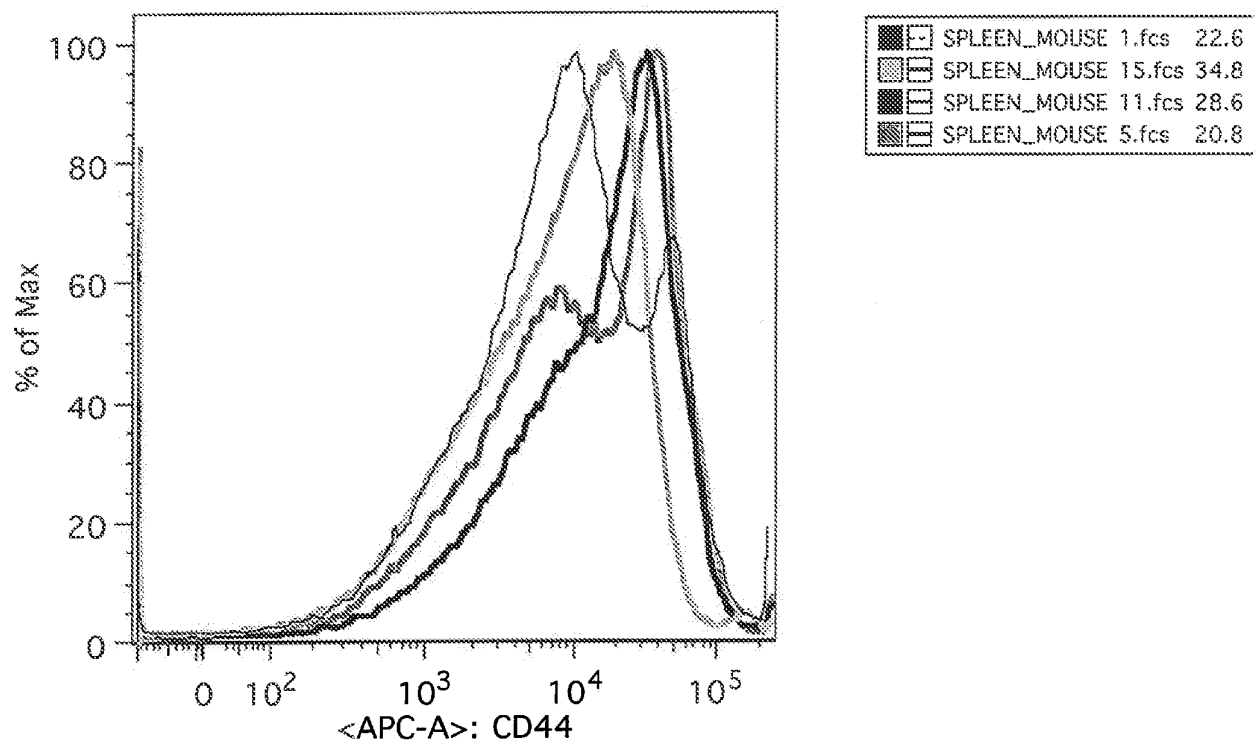
Expression of CD62L On The Surface Of Spleen T Cells
After IL-15/15Ra DNA Delivery



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Figure 21

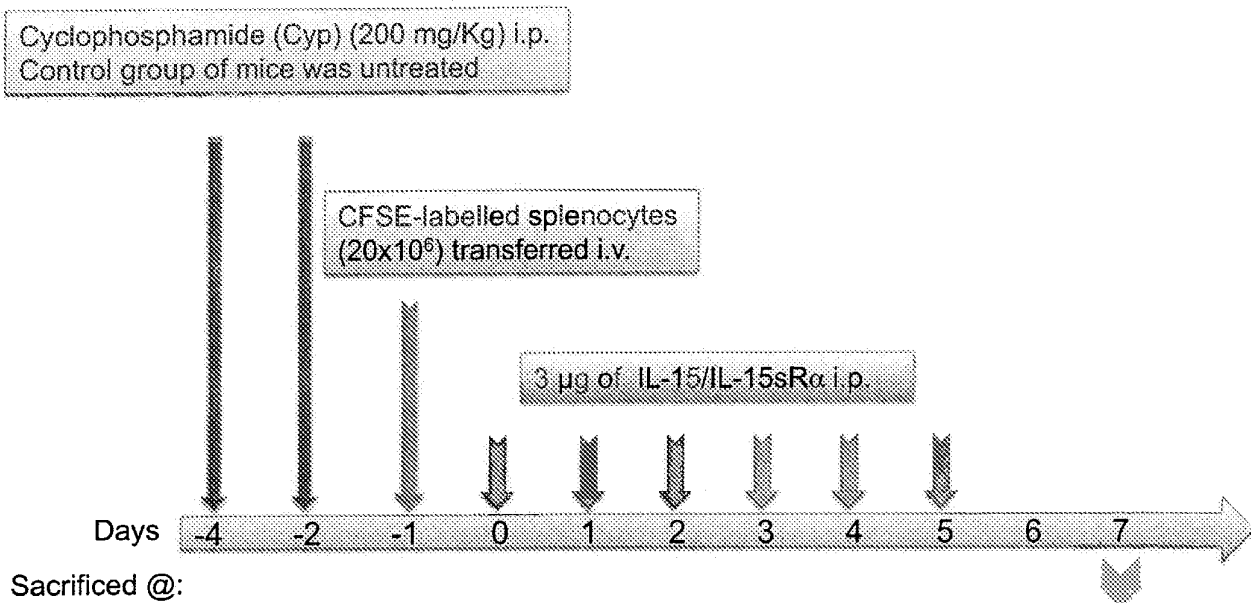
Expression of CD44 On The Surface Of Spleen T Cells
After IL-15/15Ra DNA Delivery



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Figure 22

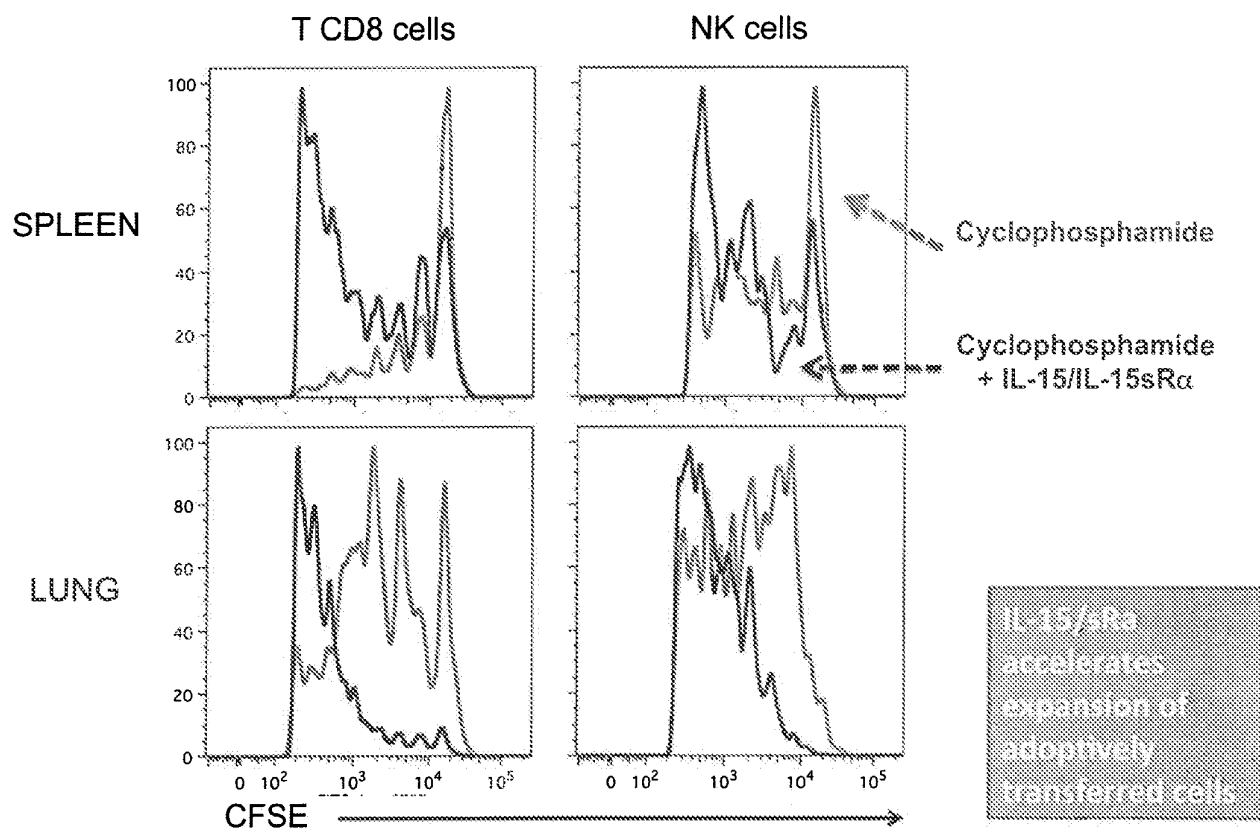
Purified IL-15/IL-15sR α Is Bioactive in vivo



23/23

Figure 23

Purified IL-15/IL-15sR α Is Bioactive in vivo



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Felber, Barbara K.
Valentin, Antonio
Bergamaschi, Cristina
The Government of the United States of America
as represented by The Secretary of the
Department of Health and Human Services

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<223> human interleukin-15 receptor, alpha (IL15RA),
transcript variant 2 cDNA

<220>
<221> CDS
<222> (424)..(1119)
<223> interleukin-15 receptor, alpha (IL15RA), isoform 2

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tccgcaccgc ggcgacacct ccgcgggcac tcacccaggc cggccgctca caaccgagcg 180
cagggccgcg gagggagacc aggaaagccg aaggcggagc agctggaggc gaccagcgcc 240
gggcgaggtc aagtggatcc gagccgcaga gagggctgga gagagtctgc tctccgatga 300
ctttgccac tctcttcgca gtggggacac cggaccgagt gcacactgga ggtcccagag 360
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cccatgtccg tggaaacacg agacatctgg gtcaagagct acagcttgta ctccaggag 480
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gtgttgaaca aggccacgaa tgtcgcccac tggacaaccc ccagtctcaa atgcattaga 600
gacctgccc tggttcacca aaggccagcg ccacctcca cagtaacgac ggcaggggtg 660
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<210> 8
<211> 231
<212> PRT
<213> Homo sapiens

<220>
<223> human interleukin-15 receptor, alpha (IL15RA),
isoform 2

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20 25 30
Thr Ser Ser Leu Thr Glu Cys Val Leu Asn Lys Ala Thr Asn Val Ala
35 40 45
His Trp Thr Thr Pro Ser Leu Lys Cys Ile Arg Asp Pro Ala Leu Val
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50	His	Gln	Arg	Pro	Ala	Pro	55	Pro	Ser	Thr	Val	Thr	60	Thr	Ala	Gly	Val	Thr
65	Pro	Gln	Pro	Glu	Ser	Leu	70	Ser	Pro	Ser	Gly	Lys	75	Glu	Pro	Ala	Ala	80
				85	Asn	Asn	Thr	Ala	Ala	Thr	Thr	Ala	Ala	Ile	Val	Pro		
	Ser	Pro	Ser	100	Met	Pro	Ser	Lys	105	Pro	Ser	Thr	Gly	110	Thr	Thr	Glu	
	Gly	Ser	Gln	115	His	Glu	Ser	Ser	120	His	Gly	Thr	Pro	Ser	Gln	Thr	Ala	
	Ile	Ser	Ser	130	Trp	Glu	Leu	Thr	135	Ala	Ser	Ala	Ser	His	Gln	Pro	Pro	
	Lys	Asn	Trp	145	Gly	His	Ser	Asp	150	Thr	Thr	Val	Ala	Ile	Ser	Thr	Ser	
	Tyr	Pro	Gln	165	Cys	Gly	Leu	Ser	170	Ala	Val	Ser	Leu	Leu	Ala	Cys	Tyr	
	Val	Leu	Leu	180	Thr	Pro	Pro	Leu	185	Ala	Ser	Val	Glu	Met	Glu	Ala	Met	
	Lys	Ser	Arg	195	Val	Thr	Trp	200	Gly	Thr	Ser	Ser	Arg	Asp	Glu	Asp	Leu	
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	Glu	Asn	Cys	225														

<210> 9
 <211> 807
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> synthetic improved human interleukin-15 (IL-15)
 receptor alpha (IL15Ra), transcript variant 1 (OPT)

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	cacgcagaca	tctgggtcaa	gagctacagc	ttgtactccc	gggagcggta	catctgcaac	180	
	tcgggtttca	agcgggaagg	cggcacgtcc	agcctgacgg	agtgcgtgtt	gaacaaggcc	240	
	acgaatgtcg	cccactggac	gacccctc	ctcaagtga	tccgcgaccc	ggccctgggt	300	
	caccagcggc	ccgcgccacc	ctccaccgta	acgacggcgg	gggtgacccc	gcagccggag	360	
	agcctctccc	cgtcgggaaa	ggagcccgc	gcgtcgtcgc	ccagctcgaa	caacacggcg	420	
	gccacaactg	cagcgatcgt	cccgggtcc	cagctgatgc	cgctgaagtc	gccgtccacc	480	
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	aagaactggg	aactcacggc	gtccgcctcc	caccagccgc	cggggggtgta	tccgcaaggc	600	
	cacagcgaca	ccacggtggc	gatctccacg	tccacggtcc	tgctgtgtgg	gctgagcgcg	660	
	gtgtcgtccc	tggcgtgcta	cctcaagtgc	aggcagactc	ccccgctggc	cagcgttgag	720	
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<210> 10
 <211> 267
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> synthetic improved human interleukin-15 (IL-15)
 receptor alpha (IL15Ra), isoform 1 (OPT)

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	Leu	Leu	Leu	Leu	Leu	Leu	Leu	Arg	Pro	Pro	Ala	Thr	Arg	Gly	Ile	Thr
			20					25					30			
	Cys	Pro	Pro	Pro	Met	Ser	Val	Glu	His	Ala	Asp	Ile	Trp	Val	Lys	Ser
		35						40					45			
	Tyr	Ser	Leu	Tyr	Ser	Arg	Glu	Arg	Tyr	Ile	Cys	Asn	Ser	Gly	Phe	Lys
	50						55					60				

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Arg	Lys	Ala	Gly	Thr	Ser	Ser	Leu	Thr	Glu	Cys	Val	Leu	Asn	Lys	Ala
65					70				75						80
Thr	Asn	Val	Ala	His	Trp	Thr	Thr	Pro	Ser	Leu	Lys	Cys	Ile	Arg	Asp
				85					90					95	
Pro	Ala	Leu	Val	His	Gln	Arg	Pro	Ala	Pro	Pro	Ser	Thr	Val	Thr	Thr
			100					105					110		
Ala	Gly	Val	Thr	Pro	Gln	Pro	Glu	Ser	Leu	Ser	Pro	Ser	Gly	Lys	Glu
		115					120					125			
Pro	Ala	Ala	Ser	Ser	Pro	Ser	Ser	Asn	Asn	Thr	Ala	Ala	Thr	Thr	Ala
	130					135					140				
Ala	Ile	Val	Pro	Gly	Ser	Gln	Leu	Met	Pro	Ser	Lys	Ser	Pro	Ser	Thr
145					150					155					160
Gly	Thr	Thr	Glu	Ile	Ser	Ser	His	Glu	Ser	Ser	His	Gly	Thr	Pro	Ser
				165					170					175	
Gln	Thr	Thr	Ala	Lys	Asn	Trp	Glu	Leu	Thr	Ala	Ser	Ala	Ser	His	Gln
			180					185					190		
Pro	Pro	Gly	Val	Tyr	Pro	Gln	Gly	His	Ser	Asp	Thr	Thr	Val	Ala	Ile
		195					200					205			
Ser	Thr	Ser	Thr	Val	Leu	Leu	Cys	Gly	Leu	Ser	Ala	Val	Ser	Leu	Leu
	210					215					220				
Ala	Cys	Tyr	Leu	Lys	Ser	Arg	Gln	Thr	Pro	Pro	Leu	Ala	Ser	Val	Glu
225					230					235					240
Met	Glu	Ala	Met	Glu	Ala	Leu	Pro	Val	Thr	Trp	Gly	Thr	Ser	Ser	Arg
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Asp	Glu	Asp	Leu	Glu	Asn	Cys	Ser	His	His	Leu					
			260					265							

<210> 11
 <211> 621
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> synthetic improved human soluble interleukin-15
 (IL-15) receptor alpha (IL-15sRa) (OPT)

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 cacgcagaca tctgggtcaa gagctacagc ttgtactccc gggagcggta catctgcaac 180
 tcgggtttca agcgggaaggc cggcacgtcc agcctgacgg agtgcggtgt gaacaaggcc 240
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 caccagcggc ccgcgccacc ctccaccgta acgacggcgg gggtagcccc gcagccggag 360
 agcctctccc cgtcgggaaa ggagcccgcc gcgtcgctcg ccagctcgaa caacacggcg 420
 gccacaactg cagcgatcgt cccgggctcc cagctgatgc cgtcgaagtc gccgtccacg 480
 ggaaccacgg agatcagcag tcatgagtcc tcccacggca cccctcgca aacgacggcc 540
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<210> 12
 <211> 205
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> synthetic improved human soluble interleukin-15
 (IL-15) receptor alpha (IL-15sRa) (OPT)

<400> 12
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 20 25 30
 Cys Pro Pro Pro Met Ser Val Glu His Ala Asp Ile Trp Val Lys Ser
 35 40 45
 Tyr Ser Leu Tyr Ser Arg Glu Arg Tyr Ile Cys Asn Ser Gly Phe Lys
 50 55 60

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Arg	Lys	Ala	Gly	Thr	Ser	Ser	Leu	Thr	Glu	Cys	Val	Leu	Asn	Lys	Ala
65					70				75						80
Thr	Asn	Val	Ala	His	Trp	Thr	Thr	Pro	Ser	Leu	Lys	Cys	Ile	Arg	Asp
				85					90					95	
Pro	Ala	Leu	Val	His	Gln	Arg	Pro	Ala	Pro	Pro	Ser	Thr	Val	Thr	Thr
			100					105					110		
Ala	Gly	Val	Thr	Pro	Gln	Pro	Glu	Ser	Leu	Ser	Pro	Ser	Gly	Lys	Glu
		115					120					125			
Pro	Ala	Ala	Ser	Ser	Pro	Ser	Ser	Asn	Asn	Thr	Ala	Ala	Thr	Thr	Ala
	130					135					140				
Ala	Ile	Val	Pro	Gly	Ser	Gln	Leu	Met	Pro	Ser	Lys	Ser	Pro	Ser	Thr
145					150					155					160
Gly	Thr	Thr	Glu	Ile	Ser	Ser	His	Glu	Ser	Ser	His	Gly	Thr	Pro	Ser
			165					170						175	
Gln	Thr	Thr	Ala	Lys	Asn	Trp	Glu	Leu	Thr	Ala	Ser	Ala	Ser	His	Gln
			180					185					190		
Pro	Pro	Gly	Val	Tyr	Pro	Gln	Gly	His	Ser	Asp	Thr	Thr			
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<210> 13

<211> 5958

<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic dual expression plasmid vector human
IL15Ra+IL15

<400> 13

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<211> 5918

<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic dual expression plasmid vector human
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-581-1PC

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<210> 16

<211> 6369

<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic sIL-15Ralpha-Fc fusion protein plasmid
vector DPhuIL15sRa205FC+huGMIL15

<400> 16

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-581-1PC

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<210> 17

<211> 436

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic sIL-15Ralpha-Fc fusion protein
huIL15sRa205-Fc

<400> 17

-581-1PC

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			20					25					30		
Cys	Pro	Pro	Pro	Met	Ser	Val	Glu	His	Ala	Asp	Ile	Trp	Val	Lys	Ser
		35					40					45			
Tyr	Ser	Leu	Tyr	Ser	Arg	Glu	Arg	Tyr	Ile	Cys	Asn	Ser	Gly	Phe	Lys
	50					55					60				
Arg	Lys	Ala	Gly	Thr	Ser	Ser	Leu	Thr	Glu	Cys	Val	Leu	Asn	Lys	Ala
65					70					75					80
Thr	Asn	Val	Ala	His	Trp	Thr	Thr	Pro	Ser	Leu	Lys	Cys	Ile	Arg	Asp
				85					90					95	
Pro	Ala	Leu	Val	His	Gln	Arg	Pro	Ala	Pro	Pro	Ser	Thr	Val	Thr	Thr
			100					105					110		
Ala	Gly	Val	Thr	Pro	Gln	Pro	Glu	Ser	Leu	Ser	Pro	Ser	Gly	Lys	Glu
		115					120					125			
Pro	Ala	Ala	Ser	Ser	Pro	Ser	Ser	Asn	Asn	Thr	Ala	Ala	Thr	Thr	Ala
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Ala	Ile	Val	Pro	Gly	Ser	Gln	Leu	Met	Pro	Ser	Lys	Ser	Pro	Ser	Thr
145					150					155					160
Gly	Thr	Thr	Glu	Ile	Ser	Ser	His	Glu	Ser	Ser	His	Gly	Thr	Pro	Ser
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Gln	Thr	Thr	Ala	Lys	Asn	Trp	Glu	Leu	Thr	Ala	Ser	Ala	Ser	His	Gln
			180					185					190		
Pro	Pro	Gly	Val	Tyr	Pro	Gln	Gly	His	Ser	Asp	Thr	Thr	Pro	Lys	Ser
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Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu
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Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser
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His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu
			260					265					270		
Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr
		275					280					285			
Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn
	290					295					300				
Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro
305					310					315					320
Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln
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Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val
			340					345					350		
Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val
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Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro
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Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr
385					390					395					400
Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val
				405					410					415	
Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu
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Ser	Pro	Gly	Lys												
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<210> 18

<211> 131

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic human GMCSF-IL15 fusion protein
huGMCSF-IL15

<400> 18

-581-1PC

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Ser	Asn	Trp	Val	Asn	Val	Ile	Ser	Asp	Leu	Lys	Lys	Ile	Glu	Asp	Leu
			20					25					30		
Ile	Gln	Ser	Met	His	Ile	Asp	Ala	Thr	Leu	Tyr	Thr	Glu	Ser	Asp	Val
		35					40					45			
His	Pro	Ser	Cys	Lys	Val	Thr	Ala	Met	Lys	Cys	Phe	Leu	Leu	Glu	Leu
	50					55					60				
Gln	Val	Ile	Ser	Leu	Glu	Ser	Gly	Asp	Ala	Ser	Ile	His	Asp	Thr	Val
65					70					75					80
Glu	Asn	Leu	Ile	Ile	Leu	Ala	Asn	Asn	Ser	Leu	Ser	Ser	Asn	Gly	Asn
				85				90					95		
Val	Thr	Glu	Ser	Gly	Cys	Lys	Glu	Cys	Glu	Glu	Leu	Glu	Glu	Lys	Asn
			100					105					110		
Ile	Lys	Glu	Phe	Leu	Gln	Ser	Phe	Val	His	Ile	Val	Gln	Met	Phe	Ile
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<210> 19

<211> 6354

<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic IL-15Ralpha-Fc fusion protein plasmid
vector AG256DPhuIL15sRA200FC+huGMIL15

<400> 19

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