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(54) Title: METHOD AND COMPOSITION FOR ENHANCING AN IMMUNE RESPONSE

(57) **Abstract:** Provided is a method and composition for enhancing an immune response in a subject by administering to the subject an inhibitor of IL-13 or an inhibitor of an NK-T cell to the subject. The method can be used to prevent growth of a tumor in the subject, e.g., to inhibit tumor recurrence or metastasis. The method can also be used to enhance a response to a vaccine in a subject.

METHOD AND COMPOSITION FOR ENHANCING AN IMMUNE RESPONSE

Field of the Invention

5   The invention relates generally to methods and compositions for enhancing immune responses and more specifically to methods and compositions for inhibiting immune responses by inhibiting IL-13 expression or activity and/or NK-T cell activity.

Background of the Invention

10   The cytokine IL-13 has been implicated in several biological activities. These activities include, e.g., induction of IgG$_4$ and IgE switching, induction of germ line IgE heavy chain ε transcription, CD23 expression in normal human B cells, and induction of B cell proliferation in the presence of CD40L or anti-CD40 mAb.

        IL-13 shares some functional properties with the cytokine IL-4. While many reported
15   activities of IL-13 are similar to those of IL-4, IL-13 does not have growth promoting effects on activated T cells or T cell clones.

        IL-13 exhibits certain biological activities by interacting with an IL-13 receptor ("IL-13R") on the surface of target cells. IL-13R and the IL-4 receptor ("IL-4R") share a common component, which is required for receptor activation. However, IL-13 does not bind to cells
20   transfected with the IL-4 receptor.

Summary of the Invention

        The invention is based in part on the discovery that administration of an inhibitor of IL-13 to a subject, or disrupting NK-T cell function in a subject, inhibits recurrence of the
25   tumor. Accordingly, included in the invention is a method of inhibiting the growth of a tumor in a subject by administering an IL-13 inhibitor or an NK-T cell inhibitor to the subject.

        The IL-13 inhibitor is preferably a non-toxic inhibitor. For example, the inhibitor preferably lacks a cytotoxic moiety such as a Pseudomonas exotoxin, ricin, abrin and
30   Diphtheria toxin.

        In some embodiments, the inhibitor is a ligand for an IL-13 polypeptide. One example of a suitable IL-13 ligand is a polypeptide that includes an IL-13 binding region of an IL-13 receptor.

Preferably, the polypeptide also includes an immunoglobulin polypeptide. In some embodiments, the inhibitor is a fusion protein that includes a soluble mammalian (e.g., mouse or human) IL-13 receptor portion and a region of a human IgG₁ polypeptide. A preferred IL-13 ligand is sIL-13Rα2.Fc, which is described in Donaldson et al., J. Immunol. 161:2317-24,

5   1998.

Another suitable inhibitor is an IL-13 antagonist, e.g., an antagonistic antibody that binds to an IL-13 polypeptide. The antibody can be either a polyclonal antibody or a monoclonal antibody. Antibodies to IL-13 can be made using techniques known in the art. For example, an IL-13 polypeptide may be used to immunize animals to obtain polyclonal

10   and monoclonal antibodies which specifically react with the IL-13 protein, and which may inhibit binding of IL-13, or fragments thereof, to the receptor. Such antibodies may be obtained using the entire IL-13 as an immunogen, or by using fragments of IL-13, such as the soluble mature IL-13. Smaller fragments of IL-13 may also be used to immunize animals. The peptide immunogens additionally may contain a cysteine residue at the carboxyl

15   terminus, and are conjugated to a hapten such as keyhole limpet hemocyanin (KLH). Additional peptide immunogens may be generated by replacing tyrosine residues with sulfated tyrosine residues. Methods for synthesizing such peptides are known in the art, for example, as described in Merrifield, J. Amer. Chem. Soc. 85, 2149-2154, 1963.

Tumor growth or occurrence is also inhibited by blocking the activity of a CD1-

20   restricted NK-T cell. Preferably, the inhibitor binds to a CD1 molecule. Examples of CD-1 binding inhibitors include anti CD-1 antibodies or other soluble anti-CD1 ligands. Anti-CD1 antibodies are known in art and are described in, e.g., Roark et al., J. Imm. 160:3121-27, 1998.

In other embodiments, the inhibitor binds to an NK-T cell specific marker. An

25   example of an NK-T cell specific marker is a Vα24 polypeptide. An example of this inhibitor is an antibody to a Vα24 polypeptide. The antibody can be either a monoclonal antibody or a polyclonal antibody and can be made using the methods described above. Antibodies that recognize Vα24 polypeptide are described in, e.g., Prussin et al., J. Immunol.159, 5862-70, 1997.

30   The inhibitor can be administered to inhibit the growth of either a primary tumor, a metastatic tumor, or both. Thus, in some embodiments, the inhibitor is administered prior to detection of a primary tumor in the subject, e.g., to inhibit the growth of a primary tumor. In

other embodiments, the inhibitor is administered after detection of a primary tumor in the subject, *e.g.*, to inhibit the recurrence of a primary tumor or to inhibit metastasis.

In preferred embodiments, the inhibitor is administered in an amount sufficient to inhibit the growth of a tumor in the subject. For example, the inhibitor can be administered

5   in a therapeutically effective amount. By "therapeutically effective amount" is meant the total amount of each active component of the pharmaceutical composition or method that is sufficient to show a meaningful patient benefit, *e.g.*, amelioration of symptoms of, healing of, or a decrease in size or other property of a tumor.

The subject can be, *e.g.*, a mammal such as a human, dog, cat, horse, cow, pig, or

10   rodent (*e.g.*, mouse or rat).

The subject may be at an increased risk for developing a tumor. By "increased risk" is meant the subject is more likely to develop tumor than an age- matched control differing in one or more genetic factors or environmental factors, or both. For example, the subject may have an allele of a gene that predisposes the subject to increased risk of a tumor. The subject

15   may have inherited the allele, *i.e.*, the subject may have a familial history of increased risk for developing a tumor. The alleles may be found in protooncogenes or tumor suppressor genes of the subject. Examples of protooncogenes include, *e.g.*, ras genes and myc genes. Examples of tumor suppressor genes include, *e.g.*, a retinoblastoma gene, a p53 gene, a BRCA gene, an APC, and a DCC gene. Environmental conditions can include those that

20   expose the subject to known or suspected carcinogens.

In general, any type of tumor can be treated with the inhibitor. Preferably, the tumor is a recurring tumor, *i.e.*, a tumor that is subject to immunosurveillance. Examples of suitable tumor types include carcinomas, sarcomas, or cancers of the blood cells. Thus, the tumor can be a liver, melanoma, uterine, testicular, breast, colorectal, lung, prostate, renal cell, cervical,

25   pancreatic, ovarian, thyroid, nasopharyngeal tumor, a leukemia, and a lymphoma (such as Burkitt's lymphoma).

If desired, the inhibitor can be administered along with an additional treatment regimen or regimens that are intended to inhibit tumor growth. For example, the additional treatment regimen can include administration of a second agent that inhibits tumor growth. A

30   suitable second agent is one that increases levels of interferon gamma in the subject. The second agent can be interferon gamma itself.

The inhibitor and second agent (if desired) can be provided in the form of a pharmaceutical composition. Any suitable administration route and dosage schedule can be

used to deliver the inhibitor, or a pharmaceutical composition that includes the inhibitor. When the inhibitor is administered orally, the inhibitor can be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, the pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The

5   tablet, capsule, and powder contain from about 5 to 95% inhibitor, and preferably from about 25 to 90% inhibitor. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, or sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide

10   solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to 90% by weight of inhibitor, and preferably from about 1 to 50% inhibitor.

When a therapeutically effective amount of inhibitor is administered by intravenous, cutaneous or subcutaneous injection, the inhibitor will be in the form of a pyrogen-free,

15   parenterally acceptable aqueous solution. The preparation of such parenterally acceptable protein solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection should contain, in addition inhibitor, an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium

20   Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The pharmaceutical composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additives known to those of skill in the art.

The amount of the inhibitor in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the

25   nature of prior treatments which the patient has undergone. Ultimately, the attending

The duration of intravenous therapy using the pharmaceutical composition of the present invention will vary, depending on the severity of the disease being treated and the condition and potential idiosyncratic response of each individual patient.

Ultimately the attending physician will decide on the appropriate duration of

5   intravenous therapy using the pharmaceutical composition of the present invention. In some embodiments, the inhibitor is provided in a delayed-release composition. The timed-release composition can include a microparticle.

Microparticles are known in the art and include those described in, *e.g.*, US Patent No.6,013,258. Suitable material to include in microparticles includes polymeric material

10   such as poly-lactic-co-glycolic acid (PLGA).

Also within the invention is a method of inhibiting the growth of a virus in subject by administering an IL-13 inhibitor or NK-T cell inhibitor to the subject. The IL-13 inhibitor or NK-T cell inhibitor can be any of the IL-13 inhibitors or NK-T cell inhibitors described herein.

15   In some embodiments, the inhibitor is used inhibiting a chronic infection of a mammal by a virus. The inhibitor is administered to a mammalian subject diagnosed as suffering from an acute infection by the virus.

Preferably, the inhibitor is administered before or during a primary exposure of the subject to the virus. In some embodiments, the virus causes, or is capable of causing, a

20   chronic infection in the subject. These viruses can include, *e.g.*, human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), cytomegalovirus (CMV), Epstein-Barr Virus (EBV), human T-cell leukemia virus (HTLV), and human papilloma virus (HPV) (such as HPV strain 16).

Also included in the invention is a method of enhancing an immune response in a

25   subject by administering an inhibitor of IL-13 or a NK-T cell to the subject. The inhibitor can include a ligand for an IL-13 polypeptide. In some embodiments, the inhibitor is added to enhance the response to a vaccine. The inhibitor can be administered prior to, concurrently with, or after, the vaccine. For example, the inhibitor can be administered between within about 30 days prior to and within about 30 days after introducing a vaccine

30   into said subject.

The vaccine can be directed to, *e.g.*, a tumor (such as the tumor types listed herein) or a pathogen. In some embodiments the pathogen is virus, a bacterium, or a eukaryotic cell.

The virus can be, e.g., human immunodeficiency virus (HIV). HIV vaccines used in the methods described herein can include vaccines described in, e.g., US Patent No. 5,932,318; Ahlers et al., J. Immunol. 150: 5647-65, 1993 (describing the construct PCLUS6-18IIIB); and Ahlers et al., AIDS Res. Hum. Retroviruses 12: 259-272, 1996. The peptide

5   vaccine preferably includes peptides of the HIV-1 envelope protein presenting multiple immune determinants. The peptide vaccine preferably elicits both humoral and cell-mediated immune responses in mice having a variety of MHC types. An example of such a peptide is PCLUS6.1-18IIIB (described in, e.g., US Patent No. 6,214,347).

The inhibitor may administered prophylactically or therapeutically and can be

10   administered in conjunction with prophylactic or therapeutic delivery of the vaccine.

If desired the inhibitor can be administered along with a second or additional agents that enhance an immune response. Additional agents include, e.g., adjuvants, cytokines (such as IL-12), and GM-CSF.

The inhibitor can additionally be a nucleic acid encoding an IL-13 inhibitor. Nucleic

15   acids encoding an IL-13 inhibitor can be administered using standard methods, e.g., those described in Felgner et al., US Patent No., 5,580,859.

It is expected that a dosage of approximately 1 to 200 μg of DNA, would be administered per kg of body weight. Where the patient is an adult human, vaccination regimens can include, e.g., intramuscular or subcutaneous administrations of 10-100 μg of nucleic acid when

20   delivered in a microparticle, or of about 100-1000 μg of naked DNA, repeated several times (e.g., 3-6 times).

Other standard delivery methods, e.g., biolistic transfer, or ex vivo treatment, can also be used. In ex vivo treatment, e.g., antigen presenting cells (APCs), dendritic cells, peripheral blood mononuclear cells, or bone marrow cells, can be obtained from a patient or an

25   appropriate donor and activated ex vivo with the immunogenic compositions, and then returned to the patient.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can

30   be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the

present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

5

Brief Description of the Drawings

FIGS. 1A and 1B are graphs showing the development of tumors in mice treated with anti-CD4 at different time periods.

FIGS. 2A and 2B are graphs showing the development of tumors in IL-4 KO, IL-4R, 10   and STAT6 KO mice.

FIGS. 3A-3C are graphs showing cytokine production of T cells from 5-12RM-injected BALB/c mice with anti-CD3 stimulation.

FIGS. 4A and 4B are graphs showing the effect of IL-13 inhibitor on tumor recurrence.

15   FIG. 5A is a representation of an electrophoretogram showing expression of IL-13 and IL-4 mRNA in $CD4^+$ cells.

FIGS. 5B and 5C are histograms showing production of IL-13 and IL-4 by $CD4^+NK1.1^+$ and $CD4^+NK1.1^-$ T cells.

FIGS. 6A-6C are histograms showing cytokine production of $CD4^+$ T cells from 15-
20   12RM-injected wild-type BALB/c mice and CD1 KO mice with anti-CD3 stimulation.

FIG. 7 is a graph showing the development of tumors in CD1 KO mice.

FIGS. 8A-8C are graphs showing CTL activity of CD1 KO mice injected with 15-12RM.

FIG. 9 is a graph showing CTL response in cells exposed to an HIV peptide vaccine,
25   GM-CSF, and either an IL-13 inhibitor or a control peptide.

Detailed Description of the Invention

The invention provides compositions and methods for inhibiting tumor growth in a subject. Also provided are methods and composition for treating viral infections, as well as
30   methods and compositions for enhancing immune responses in a subject.

Administration of a soluble form of an IL-13 receptor was found to inhibit tumor recurrence in a murine model system. Inhibition of NK-T cell activity has similarly been found to inhibit tumor recurrence in the model system. These results suggest that ligands for

IL-13 polypeptide and NK-T cells act to inhibit immune responses, such as those involved in tumor surveillance. Immune responses were enhanced by inhibiting IL-13 function and/or NK-T cell function.

5   The methods and compositions can be used to inhibit either primary tumor growth or secondary tumor growth (metastasis) in the subject. For example, the methods and compositions are suitable for use with a subject that has been diagnosed with a primary tumor in order to prevent additional growth of the primary tumor. Alternatively, or in addition, the methods and compositions disclosed herein can also be used to inhibit the development of metastases of the primary tumor.

10   The invention will be further illustrated in the following examples, which do not limit the scope of the appended claims.

Example 1. Identification of IL-13 inhibitor-mediated inhibition of tumor recurrence

This example describes studies identifying IL-13 as an important inhibitor of tumor

15   immunosurveilance. Studies are first presented showing that treatment of mice with antibodies to CD4 molecules inhibits cytotoxic T -cell (CTL) mediated tumor surveillance if administered within 10 days of injection of tumor cells. Next are presented studies demonstrating that that tumor recurrence did occur in IL-4 KO mice, but not in IL-4R KO mice or signal transducer and activator of transcription (STAT)6 KO mice. These results

20   suggest IL-13 is involved in tumor recurrence. These results are substantiated by showing that CD-4 depleted mice produced lower levels of IL-13, as well as data showing that administration of (sIL-13Rα2.Fc), an IL-13 inhibitor, inhibits tumor recurrence.

For the studies described herein, female BALB/c mice were purchased from Charles River Breeding Laboratories (Frederick, MD). IL-4 knockout (KO) mice (Noben-Trauth *et*

25   *al.*, Transgenic Res 5: 487-91, 1996) on a BALB/c background were obtained from the Jackson Laboratory (Bar Harbor, ME). The BALB/c mouse strain congenic for the C57BL/6 NK1.1 locus was provided by Drs. Anthony Scalzo and Wayne Yokayama (Scalzo, *et al.*, Immunogenetics 41: 148-51, 1995). C57BL/6 mice, IL-4R KO (Noben-Trauth *et al.*, Proc. Natl Acad. Sci. U S A 94: 10838-43, 1997), signal transducer and activator of transcription

30   (STAT)6 KO (Kaplan *et al.*, Immunity 4: 313-9, 1996) and CD1 KO (Smiley *et al.*, Science 275: 977-9, 1997) mice having the BALB/c background were bred under pathogen-free conditions. All the mice were maintained in a pathogen-free animal facility and were used at

6-10 weeks of age. Animal experiments were all approved by the National Cancer Institute
(NCI) Animal Care and Use Committee.

15-12RM tumor cells were made by transfecting BALB/c 3T3 fibroblasts first with
HIV-1 IIIB gp160 and then with mutant ras and myc genes to make them tumorigenic
5   (Matsui *et al.*, J. Immunol. 163: 184-193, 1999). This and control BALB/c 3T3 cells
transfected with Neo only (18Neo) (Takahashi *et al.*, Proc. Natl. Acad. Sci. USA 85: 3105-
09, 1988) were maintained in complete T cell medium (CTM) which consisted of RPMI1640
with 10% FCS, L-glutamine, sodium pyruvate, nonessential amino acids, penicillin,
streptomycin and 5×10^{-5} M 2-mercaptoethanol, containing geneticin (200 µg/ml) (Sigma, St.
10 Louis, MO).

Purified rat anti-mouse CD4 mAb (GK1.5 (Wilde *et al.*, J. Immunol. 131: 2178-2183,
1983)) was obtained from the Frederick Cancer Research and Development Center, NCI
(Frederick, MD). IL-13 inhibitor, a fusion protein of murine IL-13Rα2 and human IgG1
(sIL-13Rα2.Fc), was made as described previously (Donaldson *et al.*, J Immunol 161: 2317-
15 24, 1998) and provided from the Research Support Team at Genetics Institute, Cambridge,
MA. Anti-CD3 (2C11(Leo *et al.*, Proc. Natl Acad. Sci. USA 84: 1374-78, 1987)), anti-CD28
(37.51), FITC-conjugated anti-CD4 (GK1.5) and PE-conjugated anti-NK1.1 (PK136) were
obtained from Pharmingen (San Diego, CA). Anti-Ia, anti-CD11b, anti-CD11c, anti-DX5,
anti-CD4 and anti-CD8 magnetic beads were purchased from Miltenyi Biotec Inc (Auburn,
20 CA). Recombinant mouse IL-2 was obtained from Pharmingen. Trizol, SuperScript cDNA
synthesis kit, and PCR SuperMixture were purchased from Life Technologies (Rockville,
MD).

One million 15-12RM cells in 200 µl of PBS were injected subcutaneously on the
right flank of the mouse. To deplete CD4^{+} cells *in vivo*, mice were inoculated
25 intraperitoneally with 0.2 ml of PBS containing 0.5 mg of anti-CD4 or control rat IgG
(Sigma, St. Louis, MO) as indicated. In some experiments, mice were treated every other day
for 8 days with 0.2 mg of IL-13 inhibitor (sIL-13Rα2.Fc) by intraperitoneal injection in 0.1
ml PBS.

For in vitro activation of T cells, CD4^{+} T cells and CD4^{+} NK-T cells, 96 well plates
30 were coated with anti-CD3 (10 µg/ml) over night at 4 °C. The plates were washed three
times with PBS before use. Single-cell suspensions of splenocytes from 15-12RM-injected
mice were prepared at different time points after the injection. T cells were negatively
selected from splenocytes by using magnetic beads coated with antibodies for Ia, CD11b,

CD11c and DX5. CD4^{+} T cells were obtained by depleting CD8^{+} cells from T cells by using anti-CD8 magnetic beads. The cells were cultured at a density of 1×10^5/well of a 96 well plate in 200 µl CTM. Two days after the stimulation 100 µl of culture medium was harvested from each well and stored at –70 °C until cytokine measurement. In some experiments,

5   CD4^{+} T cells (1×10^6/well) were stimulated with CD1-transfected L cells (Chen *et al.*, J Immunol 159: 2240-9, 1997) or L cells (2×10^4/well) with anti-CD28 (10 µg/ml) and IL-2 (20 units/ml). After one week, culture medium was harvested and stored at –70 °C until cytokine measurement.

Spleen cells of C57BL/6 mice or BALB/c mice congenic for the NK1.1 locus were

10   isolated by passing through a CD4-enrichment column (Cedar Lane, Westbury, NY) and then sorted for CD4^{+} NK1.1^{+} or CD4^{+} NK1.1^{-} T cells by using FACStar (Becton Dickenson, Mountain View, CA). To detect cytokine mRNA, the cells were cultured at a density of 1×10^5/well of a 96 well plate in 200 µl CTM. Eight hours after the stimulation cells were harvested from each well and stored at –70 °C until RNA extraction. For cytokine

15   measurement, 2×10^4 purified cells were stimulated in 96 well plates coated with 10 µg/ml each of anti-CD3 and anti-CD28 mAbs. Supernatants were collected at 48 hours and assayed for IL-4, IL-13 and IFN-γ.

The concentration of IL-4, IL-13 (R&D, Minneapolis, MA or Endogen, Woburn, MA), IFN-γ (R&D) in the culture supernatant were determined by ELISA kit according to the

20   manufacturer's instructions. All samples were assayed in triplicate.

Cytotoxic activity of CD8^{+} T cells against several target cells was measured by a 4 h-^{51}Cr release assay. CD8^{+} T cells were purified from splenocytes by depletion of B cells, macrophages, DC, NK cells and CD4^{+} cells using magnetic beads (Milteny Biotec Inc.). The percentage of specific ^{51}Cr release was calculated as follows:

25   100 × (experimental release – spontaneous release)/(maximum release – spontaneous release).

Maximum release was determined from supernatants of cells that were lysed by addition of 5 % Triton X-100. Spontaneous release was determined from target cells incubated without added effector cells.

30   Total RNA was extracted from sorted CD4^{+} NK-T cells with Trizol reagent. cDNAs were synthesized by using the Superscript cDNA synthesis kit according to manufacturer's instructions. PCR was done with Supermixture containing 0.2 µM primers by the GeneAmp

9700 PCR system (Perkin-Elmer, Norwalk, CT). The PCR was run for 28 cycles of 94 °C for

30 s, 55 °C for 1 min, 74°C for 1 min. The primers for IL-4 were purchased from Promega

(Madison, WI). The sequence of the primers used were follows: IL-13 (sense) 5'-

GACCCAGAGGATATTGCATG-3' (SEQ ID NO:1); IL-13 (antisense) 5'-

5   CCAGCAAAGTCTGATGTGAG-3' (SEQ ID NO:2); HPRT (sense) 5'-

GTTGGATACAGGCCAGACTTTGTTG-3' (SEQ ID NO:3); HPRT (antisense) 5'-

TCGGTATCCGGTCGGATGGGAG-3' (SEQ ID NO:4).

The data were analyzed for statistical significance using a Log-Rank test. The data

were considered significant at $P<0.05$.

10

Timing of anti-CD4 treatment to affect tumor recurrence

The effect of the timing of anti-CD4 treatment relative to tumor recurrence was

examined in the 15-12RM mouse model.

15-12RM tumor cells were injected subcutaneously (s.c.) in the right flank of

15   different groups of BALB/c mice at day 0. Anti-CD4 was administered to the different mice

in the groups for various times prior to or following implantation. The percentage of mice

developing tumors following administration of anti-CD4 was examined.

The results are presented in FIGS. 1A and 1B. FIG. 1A shows tumor growth in

groups of mice in which anti-CD4 was not administered (control), administered at day -3 to

20   50, day -3 to 30, or day -3 to 10.   FIG. 1B shows tumor growth in mice for which anti-CD4

was administered at day 1 to day 50, day 10 to day 50, and day 20 to day 50.

The mice of each group were intraperitoneally inoculated with 0.5mg of anti-CD4

mAb (GK1.5) during the indicated periods after 15-12RM injection. The antibody was

inoculated every day for the first 3 days and then twice a week. Five mice were used for each

25   group. $P<0.02$ by Log-Rank test between control and anti-CD4 day -3 to 10, 20 or day 1 to

50. Similar results were obtained in another experiment.

Tumors were detectable within 5 days (Matsui *et al.*, J. Immunol. 163: 184-193, 1999)

(FIG. 1A, control group). The tumors regressed and disappeared after 10-15 days. However,

recurrence of the tumors was observed between 20 and 40 days after inoculation and

30   recurrent tumors did not regress (FIG. 1A). Tumor recurrence observed in control mice

occurred because of incomplete elimination of tumor cells by $CD8^+$ CTL during the tumor

regression phase. Conversely, in anti-CD4-treated mice, tumor cells were completely

eliminated after the initial growth and did not recur (FIG. 1A and Matsui *et al.*, J. Immunol.

163: 184-193, 1999). These results suggested that CD4$^+$ T cells negatively regulated immunosurveillance against tumor cells by CTL.

To examine when CD4$^+$ T cells regulate CTL specific for tumor cells, mice were treated with CD4 antibody over different intervals after 15-12RM injection (Fig. 1A). All
5   mice in the groups which started to receive CD4 antibody 3 days before 15-12RM injection and stopped receiving antibody at day 10 or day 20 were resistant to tumor recurrence in a similar fashion to the mice treated from day –3 to the end of the experiment. Although initiation of anti-CD4 treatment could be delayed until day 1, starting treatment on day 10 was too late to have any impact on the late recurrence (FIG. 1B). This suggests that
10   immunosurveillance of CTL against tumor cells is regulated by CD4$^+$ T cells at an early tumor growth phase. Depletion of CD4$^+$ T cells during the first 10 days was both necessary and sufficient to prevent this regulation.

Down-regulation of CTL immunosurveillance through IL-4R-STAT6

15   IL-4 KO and IL-4R KO mice were used to clarify whether IL-4 and its signaling pathway are involved in down-regulation of immunosurveillance by CTL. The results are shown in FIGS. 2A and 2B. FIG. 2A shows the results obtained in control mice or mice treated with anti-CD4 antibody or BALB/c background IL-4 KO, IL-4R KO, or heterozygote littermate mice were injected subcutaneously with 1 × 10^6 15-12RM cells. The mice in the
20   anti-CD4-treated group were treated with anti-CD4 mAb every day from 3 days before 15-12RM injection until the day of the injection and twice a week thereafter. Five mice were used for each group except for the IL-4R +/- group in which 7 mice were used. $P<0.05$ by Log-Rank test between the control group and the IL-4R-/- group. The result shown is a representative of two experiments with similar results.

25   For the results shown in FIG. 2B, BALB/c-STAT6 KO mice or wild-type BALB/c mice either untreated or treated with anti-CD4 were injected subcutaneously with 1 × 10^6 15-12RM cells. The mice in anti-CD4-treated group were treated with anti-CD4 mAb every day from 3 days before 15-12RM injection and twice a week thereafter. Five mice were used for each group. $P<0.02$ by Log-Rank test between the control group and the STAT6 KO group.
30   One representative experiment of three is shown.

Tumor recurrence occurred in IL-4 KO mice; indeed, the tumor growth pattern was not significantly different from that of control mice (FIG. 2A and Matsui *et al.*, J. Immunol. 163: 184-193, 1999). In contrast, in IL-4R KO mice, tumors did not recur after the initial

growth and regression, although in heterozygous littermates they recurred as in wild-type mice (FIG. 2A).

STAT6 activation is known to be necessary for signaling through the IL-4R signal pathway (Shimoda *et al.*, Nature 380: 630-3 1996; Takeda *et al.*, Nature 380: 627-30, 1996;

5   and Kaplan *et al.*, Immunity 4: 313-9, 1996). Accordingly, to investigate whether the protection observed in IL-4R KO mice but not in IL-4 KO mice was dependent on the IL-4R signal pathway, STAT6 KO mice were inoculated with 15-12RM (FIG. 2b). The STAT6 KO mice were as resistant to tumor recurrence as CD4-depleted mice. This suggested that immunosurveillance by CTL was limited through the IL-4R-STAT6 signaling pathway, but

10   this regulation did not require IL-4 itself. Therefore, IL-13, the only other cytokine known to signal through the IL-4R-STAT6 signaling pathway, is likely to play a significant role in this limitation.

Production of cytokines in CD4-depleted mice

15   Because T cells are major producers of IL-13 and IL-4, *in vitro* production of both cytokines by T cells from 15-12RM-injected mice was measured by ELISA after anti-CD3 stimulation. The results are shown in FIG. 3. On days 0, 2, 4, 6, 10 after injection of 15-12RM cells, two mice per group were sacrificed and their spleen cells were harvested. A fraction of the cells was used to purify T cells. One hundred thousand freshly isolated T cells

20   from 15-12RM-injected mice were cultured in an anti-CD3 coated plate for 48 h. The culture supernatant was collected and concentration of each cytokine was determined by ELISA. The figure shows the average value of two mice. Similar results were obtained in an additional experiment.

Both IL-13 and IL-4 production in T cells from control mice are elevated as early as 2

25   days after 15-12RM injection. The IL-13 and IL-4 production reached a maximum on days 2 and 4, respectively, and then dropped to baseline by day 6 after injection. In the T cells from CD4-depleted mice, the absolute amount of IL-13 was 50-75 % less than that of the cells from 15-12RM injected control mice, even though production of IL-13 was up-regulated on day 2. Additionally, IL-4 production was not up-regulated even after 15-12RM injection.

30   Thus, splenic T cells from CD4-depleted mice, which are resistant to tumor recurrence, produced less IL-13 and IL-4.

Production of IFN-γ, which plays a protective role against tumor growth in this system (Matsui *et al.*, J. Immunol. 163: 184-193, 1999), was measured. In contrast to IL-13

and IL-4 production, T cells from anti-CD4-treated mice produced 25-100 % more IFN-γ

than the cells from control mice. These results suggested that removing CD4$^+$ T cells

suppresses production of IL-13 and IL-4 but allows more IFN-γ production, which was

shown previously (Matsui *et al.*, J. Immunol. 163: 184-193, 1999) to be necessary for the

5   immunosurveillance.

An IL-13 inhibitor (sIL-13Pα2.Fc) inhibits tumor recurrence

The results obtained above were compatible with either IL-13 alone being necessary

for down-regulation of immunosurveillance or the possibility that either IL-4 or IL-13 was

10   sufficient and eliminating both would be necessary to prevent recurrence. To distinguish

these possibilities, the effect of IL-13 inhibitor (sIL-13Rα2.Fc) in both wild-type and IL-4

KO mice was tested. The results are shown in FIGS. 4A and 4B. Control BALB/c mice,

mice treated with either anti-CD4 or IL-13 inhibitor (sIL-13Rα2.Fc) (FIG. 4A) or BALB/c-

IL-4 KO mice either untreated or treated with IL-13 inhibitor (FIG. 4B) were injected

15   subcutaneously with 1×10^6 15-12RM cells. The anti-CD4 (0.5 mg) was inoculated on day

0, 1, 2, 6 and 10 after 15-12RM. The mice in IL-13 inhibitor-treated groups were treated with

sIL-13Rα2.Fc (0.2 mg) every other day from day 0 to day 8. Five mice were used for each

group. *P*<0.01 by Log-Rank test between the control group and the IL-13 inhibitor group.

P<0.02 by Log-Rank test between the IL-4 KO group and the IL-4KO + IL-13 inhibitor

20   group. One representative experiment of two is shown.

The IL-13 inhibitor was inoculated every other day from day 0 to day 8 after 15-

12RM injection, but did not prevent the initial growth and regression of the tumor. However,

when IL-4 KO mice, which are not resistant to tumor recurrence, were treated with IL-13

inhibitor, they were completely protected from the recurrence. When wild-type mice were

25   treated with the IL-13 inhibitor, they also behaved like CD4-depleted mice and IL-13

inhibitor-treated IL-4 KO mice. These results showed that in this biphasic tumor model, IL-

13 is necessary for the tumor recurrence, regardless of the capacity of the mice to make IL-4.

Example 2. Inhibition of tumor growth by disrupting NK-T cell function

This example presents data showing that IL-13 is produced by NK-T cells, and that CD1 KO mice, which lack NK-T cells, produce low levels of IL-13. Also illustrated in this example is inhibition of tumor growth in CD1 KO mice.

5

NKT cells can produce IL-13

Treatment during the first 8 days with IL-13 inhibitor protected mice from tumor recurrence (FIGS. 4A and 4B). CD4$^+$NK1.1$^+$ T cells are among the earliest cells to produce a large amount of IL-4 upon TCR activation (Chen *et al.*, J Immunol 159: 2240-9, 1997).

10   Accordingly, the ability of CD4$^+$ NKT cells to produce IL-13 was examined. Since BALB/c mice do not express the NK1.1 allele recognized by available mAbs, CD4$^+$ NKT cells from C57BL/6 mice or BALB/c mice congenic for the NK1.1 locus were sorted by purifying CD4$^+$ T cells and sorting for CD4$^+$NK1.1$^+$ and CD4$^+$NK1.1$^-$ populations, and stimulated these with anti-CD3 antibody.

15   Production of IL-13 mRNA and protein was measured. The results are shown in FIGS. 5A-5C. Freshly sorted splenic CD4$^+$ NK-T cells from C57BL/6 mice were stimulated with plate-bound anti-CD3 (2C11) for 8 h. The cells were harvested and mRNA for IL-13 and IL-4 was detected by RT-PCR. FIG. 5A shows expression of mRNA of IL-13 and IL-4 in CD4$^+$ NK-T cells after anti-CD3 stimulation.

20   FIG. 5B demonstrates production of IL-13 and IL-4 by CD4$^+$NK1.1$^+$ and CD4$^+$NK1.1$^-$ T cells. Freshly sorted splenic CD4$^+$NK1.1$^+$ and CD4$^+$NK1.1$^-$ T cells from BALB/c mice congenic for the NK1.1 locus were stimulated with plate-bound anti-CD3 (2C11) and anti-CD28 (37.51) for 48 h. Supernatants were collected at 48 h and assayed for IL-4 and IL-13 by ELISA.

25   Production of IL-13 and IL-4 by CD4$^+$ T cells from 15-12RM-injected mice stimulated with CD1-transfected L cells is shown in FIG. 5C. CD4$^+$ T cells purified from spleen cells from normal and 15-12RM-injected (on day 3 after injection) BALB/c mice were stimulated with CD1-transfected L cells or non-transfected L cells for a week. Anti-CD28 mAb (10 μg/ml) and IL-2 (20 units/ml) were also added in the culture. The culture

30   supernatant was collected and concentration of IL-13 and IL-4 were determined by ELISA. Asterisks indicate a level under the detection limit (<15.6 pg/ml).

IL-13 was clearly induced in CD4$^+$NK1.1$^+$ T cells by anti-CD3 stimulation, whereas CD4$^+$NK1.1$^-$ T cells made little (FIGS. 5A and 5B). Furthermore, when CD4$^+$ T cells from

15-12RM-injected BALB/c mice were stimulated with CD1-transfected L cells, they

produced more IL-13 and IL-4 than the cells from non-tumor-bearing mice (FIG. 5C) but did

not produce detectable IFN-γ (data not shown). The lower level of IL-4 in FIG. 5C than in

Fig. 5B may be due to consumption during the 7-day culture in the former, *versus* 48 h in the

5   latter. In contrast, because the purified CD4$^+$ T cells do not have IL-13 receptors, that

cytokine should not have been consumed. Thus, CD4$^+$ NKT cells can indeed make IL-13,

and are a primary source of this cytokine. Moreover, this activity appears to be enhanced in

tumor-bearing animals.

10   *IL-13 production is markedly decreased in CD1 KO mice*

If NKT cells are a major source of IL-13, then their absence in CD1 KO mice should

markedly reduce IL-13 production. Accordingly, the *in vitro* production of IL-13, IL-4 and

IFN-γ in CD4$^+$ T cells of 15-12RM-injected CD1 KO mice was examined. These mice lack

15   CD1-restricted NKT cells, including CD4$^+$NKT cells, but retain conventional MHC class II-

restricted CD4$^+$ T cells.

The results are shown in FIG 6. Two mice per group were sacrificed and their spleen

cells were harvested on days 0, 2, 4, 6, 10 after injection of 15-12RM cells. A fraction of the

cells was used to purify CD4$^+$ T cells. One hundred thousand freshly isolated T cells from

20   15-12RM-injected mice were cultured in an anti-CD3 coated plate for 48 h. The culture

supernatant was collected and concentration of each cytokines was determined by ELISA.

The each value shows the average of two mice. The results are representative of two

independent experiments. Asterisks indicate a level under the detection limit (<7.8 pg/ml).

CD4$^+$ T cells purified from spleen cells of CD1 KO mice produced only a slight

25   amount of IL-13 and IL-4 after 15-12RM injection, whereas CD4$^+$ T cells from wild-type

mice up-regulated the production of these cytokines on days 2, 4 and 10 after tumor

inoculation. This indicates that the majority of IL-13 production was from or dependent on

NKT cells. Conversely, the IFN-γ produced was even higher in the cells of CD1 KO mice

than in those of wild-type mice.

30

CD1 KO mice show low levels of tumor recurrence

Because CD4$^+$ NKT cells can produce IL-13 and indeed are responsible for much of

the IL-13 production, and these cells would be depleted by anti-CD4 treatment in addition to

conventional CD4$^+$ T cells, it was hypothesized that NKT cells play a critical role in down-regulation of tumor immunosurveillance by CTL. To test this hypothesis, CD1 KO mice were inoculated with 15-12RM and the effect of tumor growth with or without and-CD4 treatment was measured.

5   The results are shown in FIG. 7. BALB/c background CD1 KO mice and wild-type BALB/c mice either untreated or treated with anti-CD4 were injected subcutaneously with 1 × 10^6 15-12RM cells. The mice in the anti-CD4-treated group were treated with anti-CD4 (0.5 mg) starting 3 days before 15-12RM injection and twice a week thereafter. Five mice were used for each group. $P<0.02$ by Log-Rank test between the control group and the CD1

10   KO group. One representative experiment of four is shown. In the CD1 KO mice, tumors grew initially and regressed in the same time period as in wild-type mice. However, CD1 KO mice were as resistant to tumor recurrence as anti-CD4-treated wild-type mice. Thus, CD1-restricted NKT cells appeared to be necessary for the down-regulation of immunosurveillance.

15   The activity of CD8$^+$ T cells purified from splenocytes of anti-CD4-treated (FIG. 8A), CD1 KO (FIG. 8B) and control mice (FIG. 8C) examined for CTL activity at 12 days after 15-12RM injection against 18Neo targets pulsed with 1 μM of P18IIIB or unpulsed, without any *in vitro* stimulation. One representative experiment of three is shown in FIG. 8A-8C, respectively .

20   In CD4-depleted mice, protection against tumor recurrence appeared to be associated with higher CTL activity of CD8$^+$ T cells immediately *ex vivo* without restimulation than in control mice (FIG. 8A and Matsui *et al.*, J. Immunol. 163: 184-193, 1999). To determine whether the same applied to CD1 KO mice, the CTL activity of purified CD8$^+$ T cells from 15-12RM-injected CD1 KO mice was compared with that of the CD8$^+$ T cells from tumor

25   injected control and anti-CD4-treated mice (FIG. 8B). Freshly isolated CD8$^+$ T cells from CD1 KO (FIG. 8B) lysed P18IIIB-pulsed 18Neo at a low level without *in vitro* stimulation, whereas CD8$^+$ T cells from intact tumor-bearing mice did not kill P18IIIB-pulsed 18Neo without restimulation (FIG. 8C). Thus, the presence of NKT cells led to reduced CTL activity immediately *ex vivo*.

30   Taken together, these results demonstrate that IL-13 produced either by CD1-restricted CD4$^+$ NKT cells, or possibly by other cells dependent on NKT cells, at an early growth phase of the tumor (within 10 days) triggers negative regulation of CTL

17

immunosurveillance, and that this negative regulation is mediated through the IL-4Rα-STAT6 pathway.

Example 3.   An IL-13 inhibitor enhances a CTL response to a synthetic peptide HIV
5   **vaccine**

The ability of an IL-13 inhibitor for enhancing a cytotoxic T cell lymphocyte (CTL) response was examined.

BALB/c mice were immunized subcutaneously with a synthetic peptide vaccine
10   PCLUS6.1-18IIIB (20 nmoles) (US Patent No. 5,932,218) that was mixed with GM-CSF (5 ug) in an emulsion adjuvant, Montanide ISA-51 on day 0.   On each of days 0, 1, 2, 4, and 6, 200 ug of the IL-13 inhibitor IL-13Ra2-Fc was administered intraperitoneally. Controls received a control IgG instead of IL-13 inhibitor.

The subsequent CTL response in the spleens of the test and control mice, harvested 14
15   days later and stimulated for 1 week in culture, was measured and is plotted in the graph shown in FIG. 9.   Targets cells were coated with P18IIIB peptide.

Significantly higher CTL-mediated lysis was observed when the IL-13 inhibitor was administered along with a synthetic peptide HIV vaccine to mice. These results demonstrate that an increased the cytotoxic T lymphocyte (CTL) response in the presence of the inhibitor
20   as compared to the vaccine without the IL-13 inhibitor.

<u>Other Embodiments</u>

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not
25   limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

What is claimed is:

1.   A method of inhibiting the growth of a tumor in a subject, the method comprising administering to said subject an IL-13 inhibitor, wherein said inhibitor comprises an IL-13 ligand.

5   2.   The method of claim 1, wherein said inhibitor is administered in an amount sufficient to inhibit the growth of a tumor in said subject.

3.   The method of claim 1, wherein said inhibitor is administered prior to detection of a primary tumor in said subject.

4.   The method of claim 1, wherein said inhibitor is administered after detection
10   of a primary tumor in said subject.

5.   The method of claim 1, wherein said subject is at increased risk for developing said tumor.

6.   The method of claim 1, wherein said subject has an allele of a gene that increases the risk of developing said tumor in said subject.

15   7.   The method of claim 6, wherein said gene is a tumor suppressor gene selected from the group consisting of a retinoblastoma gene, a p53 gene, a BRCA gene, a APC, and a DCC gene.

8.   The method of claim 1, wherein said inhibitor is administered prior to treatment of a tumor in said subject.

20   9.   The method of claim 1, wherein said inhibitor is administered to said subject within two weeks prior to surgical resection of a tumor in said subject.

10.   The method of claim 9, wherein said inhibitor is administered to said subject within two weeks after surgical resection of a tumor in said subject.

11.   The method of claim 10, wherein said inhibitor is administered concurrently
25   with treatment of a tumor in said subject.

12.   The method of claim 1, wherein said inhibitor is administered following treatment of a tumor in said subject.

13.   The method of claim 1, wherein said inhibitor comprises an IL-13 binding region of an IL-13 receptor.

5   14.   The method of claim 13, wherein said inhibitor further comprises an immunoglobulin polypeptide.

15.   The method of claim 1, wherein said inhibitor is a fusion protein comprising a murine IL-13α 2 polypeptide and an Fc region of a IgG_1 polypeptide or a fusion protein comprising a human IL-13α 2 polypeptide and an Fc region of a IgG_1 polypeptide.

10   16.   The method of claim 1, wherein said inhibitor is an antibody which binds to IL-13.

17.   The method of claim 16, wherein said antibody is a polyclonal antibody.

18.   The method of claim 16, wherein said antibody is a monoclonal antibody.

19.   The method of claim 1, wherein said inhibitor is an IL-13 antagonist.

15   20.   The method of claim 1, wherein said subject is a human.

21.   The method of claim 1, wherein said tumor is a carcinoma, sarcoma, or cancer of a blood cell.

22.   The method of claim 1, wherein said tumor is a recurring tumor.

23.   The method of claim 1, wherein said tumor is selected from the group
20   consisting of breast cancer, liver cancer, melanoma, uterine cancer, colorectal cancer, lung cancer, prostate cancer, renal cell cancer, cervical cancer, renal cell carcinoma, pancreatic cancer, ovarian cancer, thyroid cancer, nasopharyngeal cancer, leukemia and lymphoma.

24.   The method of claim 1, wherein said tumor is selected from the group consisting of renal cell carcinoma, melanoma, testicular cancer, leukemia, and lymphoma.

25

25.   The method of claim 1, further comprising administering to said subject a second agent that inhibits tumor growth.

26.   The method of claim 25, wherein said second agent increases interferon gamma levels in said subject.

5   27.   The method of claim 1, wherein said inhibitor is administered systemically to said subject.

28.   The method of claim 1, wherein said inhibitor is administered topically to said subject.

29.   The method of claim 1, wherein said inhibitor is administered by a route

10   selected from intravenous, subcutaneous, and intramuscular delivery.

30.   The method of claim 1, wherein said inhibitor is administered by subcutaneous delivery.

31.   The method of claim 1, wherein said inhibitor is administered in a delayed-release composition.

15   32.   The method of claim 31, wherein said delayed-release composition comprises a microparticle

33.   The method of claim 32, wherein said microparticle is poly-lactic-co-glycolic acid (PLGA).

34.   A method of inhibiting the growth of a tumor in a subject, the method

20   comprising administering a non-cytotoxic inhibitor of IL-13.

35.   The method of claim 34, wherein said inhibitor inhibits IL-13 gene expression in said subject.

36.   The method of claim 34, wherein said inhibitor is an anti-sense IL-13 nucleic acid or an IL-13 ribozyme.

25   37.   The method of claim 34, wherein said inhibitor inhibits IL-13 activity in said subject.

38.   The method of claim 34, wherein said inhibitor is an IL-13 receptor antagonist.

39.   The method of claim 38, wherein said antagonist is an antibody which binds to an IL-13 receptor.

5   40.   The method of claim 34, wherein said inhibitor comprises an IL-13 binding region of an IL-13 receptor.

41.   The method of claim 34, wherein said inhibitor further comprises an immunoglobulin polypeptide.

42.   The method of claim 34, wherein said inhibitor is an antibody which binds to
10   IL-13.

43.   The method of claim 42, wherein said antibody is a polyclonal antibody.

44.   The method of claim 42, wherein said antibody is a monoclonal antibody.

45.   A method of inhibiting the growth of a tumor in a subject, the method comprising administering to said subject a NK-T cell inhibitor in an amount sufficient to
15   inhibit the growth of a tumor in said subject.

46.   The method of claim 45, wherein said cell binds to an antigen-presenting cell that expresses a CD1 polypeptide.

47.   The method of claim 46, wherein said inhibitor binds to a CD1 molecule.

48.   The method of claim 45, wherein said inhibitor comprises a ligand for a Vα24
20   polypeptide.

49.   A method of inhibiting the growth of a virus in subject, the method comprising administering an IL-13 inhibitor to said subject, wherein said inhibitor comprises an IL-13 ligand.

50.   The method of claim 49, wherein said inhibitor is administered before or
25   during a primary exposure of said subject to said virus.

51.   The method of claim 49, wherein said virus is selected from the group consisting of human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), human papilloma virus (HPV), human T-cell leukemia virus (HTLV), cytomegalovirus (CMV), and Epstein-Barr Virus (EBV).

5   52.   A method of inhibiting a chronic infection of a mammal by a virus, comprising administering to said mammal an IL-13 inhibitor, said inhibitor comprising an IL-13 ligand, wherein said inhibitor is administered to a mammal is diagnosed as suffering from an acute infection by said virus.

53.   The method of claim 52, wherein said virus is selected from the group
10   consisting of human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), human papilloma virus (HPV), human T-cell leukemia virus (HTLV), cytomegalovirus (CMV), and Epstein-Barr Virus (EBV).

54.   A method of inhibiting the growth of a virus in subject, the method comprising administering to said subject an agent that inhibits activity of a NK-T cell.

15   55.   The method of claim 54, wherein said cell is a CD1-restricted cell.

56.   The method of claim 55, wherein said inhibitor binds to CD1.

57.   The method of claim 54, wherein said inhibitor comprises a ligand for a $V\alpha24$ polypeptide.

58.   The method of claim 54, wherein said virus is selected from the group
20   consisting of human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), human papilloma virus (HPV), human T-cell leukemia virus (HTLV), cytomegalovirus (CMV), and Epstein-Barr Virus (EBV).

59.   A method of enhancing an immune response in a subject, the method comprising administering an inhibitor of IL-13 to said subject, wherein said inhibitor
25   comprises a ligand for an IL-13 polypeptide.

60.   The method of claim 59, further comprising introducing a vaccine into said subject.

23

61.   The method of claim 60, wherein said inhibitor is administered within 30 days prior to introducing a vaccine into said subject.

62.   The method of claim 60, wherein said inhibitor is administered within 30 days after introducing a vaccine into said subject.

5   63.   The method of claim 60, wherein said vaccine is directed to a tumor.

64.   The method of claim 60, wherein said vaccine is directed to a pathogen.

65.   The method of claim 64, wherein said pathogen is selected from the group consisting of a virus, a bacterium, and a eukaryotic cell.

66.   The method of claim 60, wherein said vaccine is administered prophylactically

10   to said subject.

67.   The method of claim 60, wherein said vaccine is administered therapeutically to said subject.

68.   The method of claim 60, wherein said virus is human immunodeficiency virus (HIV).

15   69.   The method of claim 68, wherein said vaccine elicits a cytotoxic T cell (CTL)-response in said host.

70.   The method of claim 60, wherein said vaccine is a PCLUS6.1-18IIIB peptide.

71.   The method of claim 69, wherein said vaccine is a PCLUS6.1-18IIIB peptide.

72.   The method of claim 60, further comprising administering to said subject a

20   second agent that enhances an immune response.

73.   The method of claim 72, wherein said agent comprises IL-12.

74.   A method of enhancing an immune response in a subject, the method comprising administering an inhibitor of an NK-T cell to said subject.

75.   The method of claim 74, further comprising introducing a vaccine into said

25   subject.

76.   The method of claim 75, wherein said inhibitor is administered within 30 days prior to introducing a vaccine into said subject.

77.   The method of claim 75, wherein said inhibitor is administered within 30 days after introducing a vaccine into said subject.

5   78.   The method of claim 75, wherein said vaccine is directed to a tumor.

79.   The method of claim 75, wherein said vaccine is directed to a pathogen.

80.   The method of claim 79, wherein said pathogen is selected from the group consisting of a virus, a bacterium, and a eukaryotic cell.

81.   The method of claim 75, wherein said vaccine is prophylactic.

10   82.   The method of claim 75, wherein said vaccine is therapeutic.

83.   The method of claim 75, further comprising administering to said subject a second agent that enhances an immune response.

84.   The method of claim 83, wherein said agent comprises IL-12.

85.   A method of inhibiting the growth of a tumor in a subject, the method
15   comprising administering to said subject a nucleic acid encoding an IL-13 ligand.
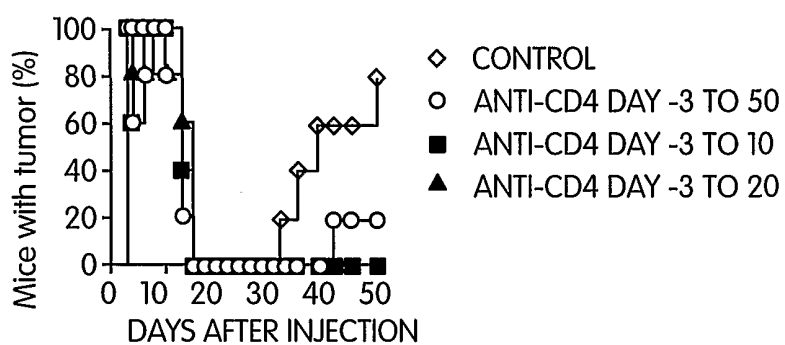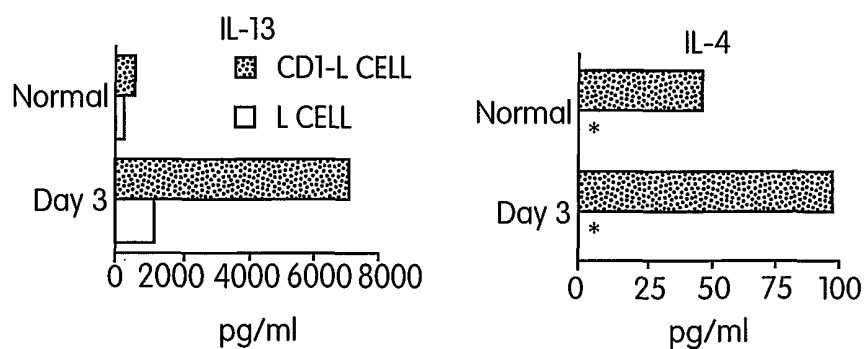
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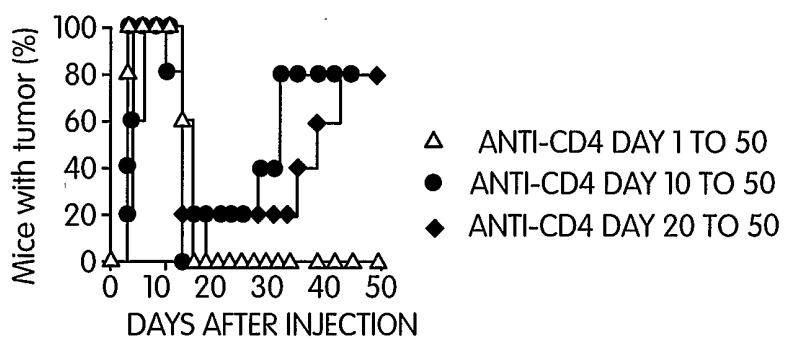
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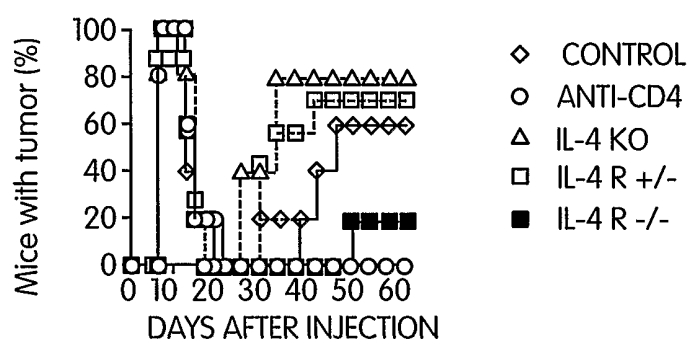
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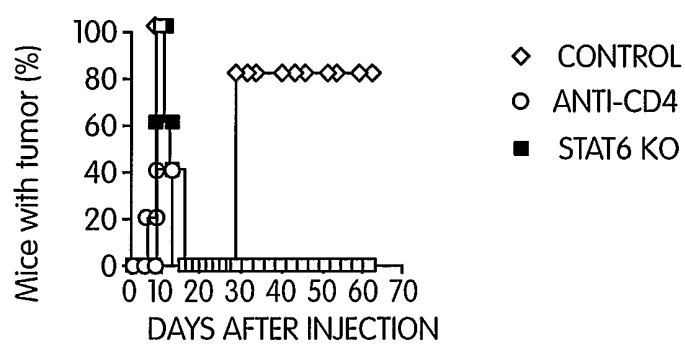
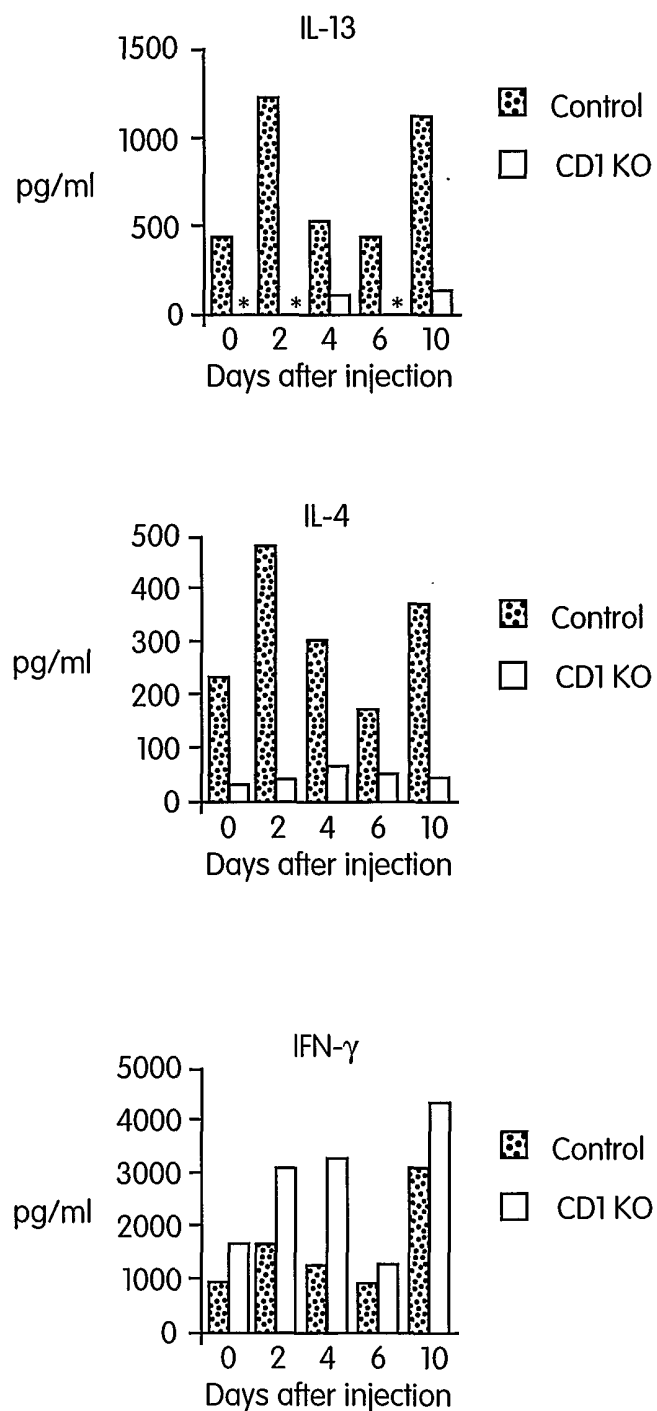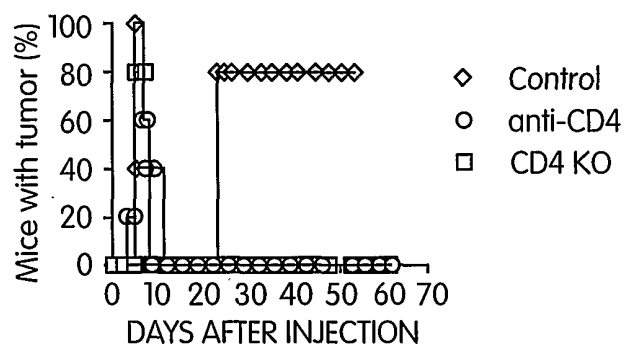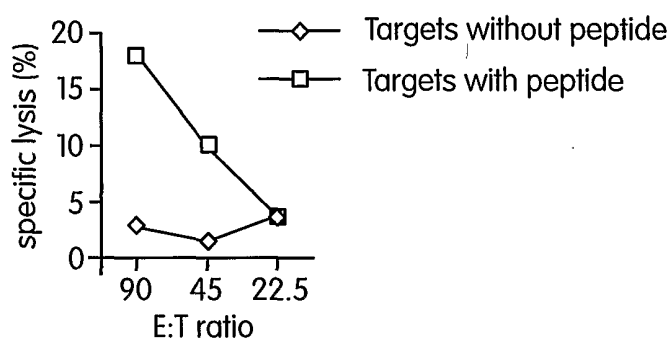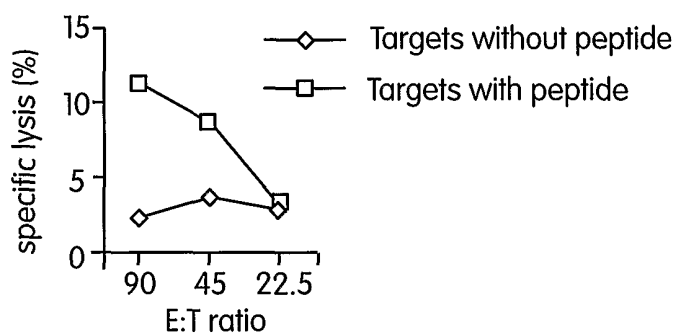

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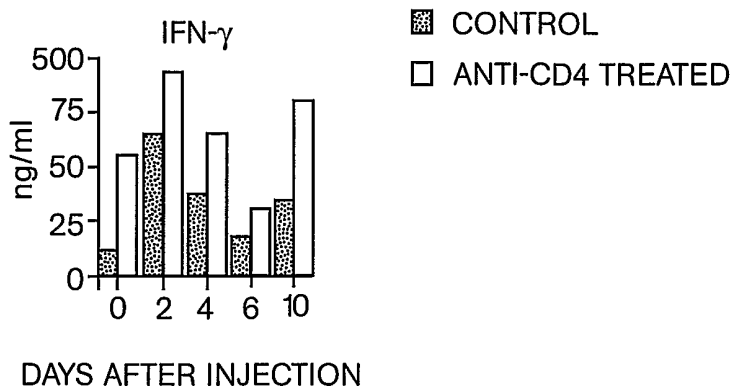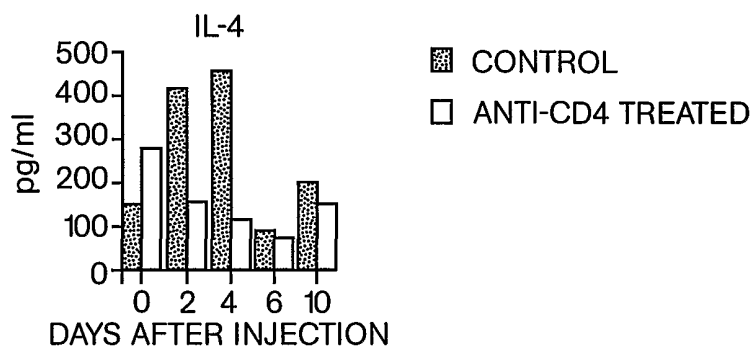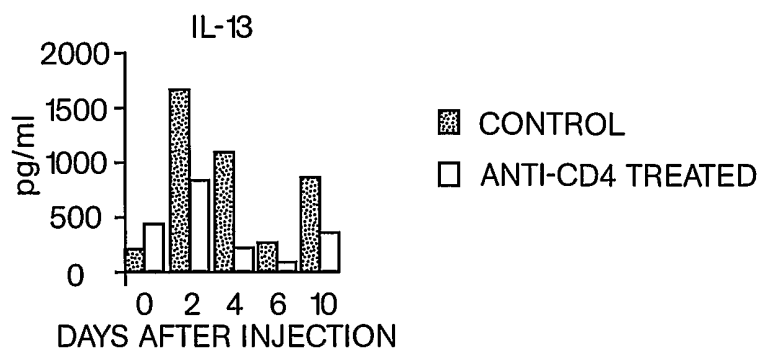
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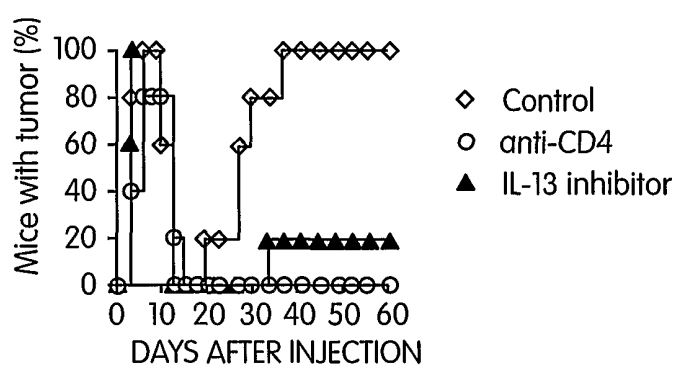
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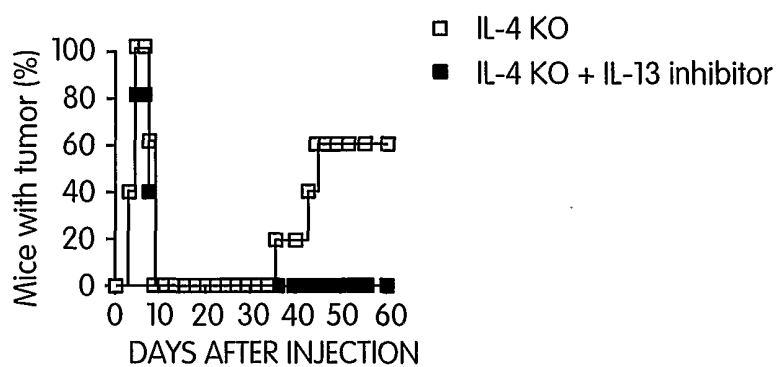
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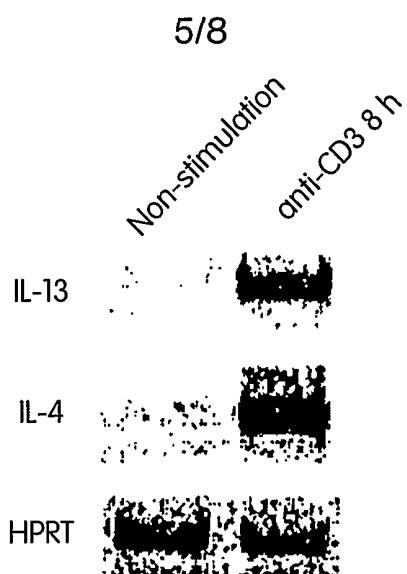
Fig. 5a
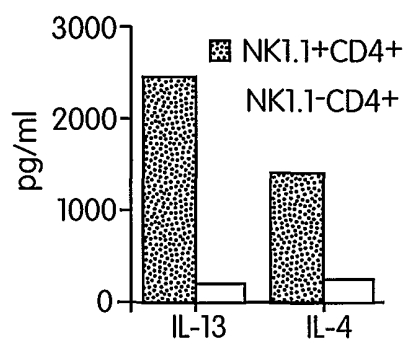
Fig. 5b

Fig. 5c

Fig. 6

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Fig. 7

Fig. 8a

Fig. 8b

Fig. 8c

Fig. 9